EUROMAR 2012

EUROMAR 2012

Magnetic Resonance Conference

1-5 July
University College Dublin
Ireland

COST Spin Hyperpolarisation
29th June -1st July

XeMat 2012
27-29 June

EUROMAR

UCD DUBLIN


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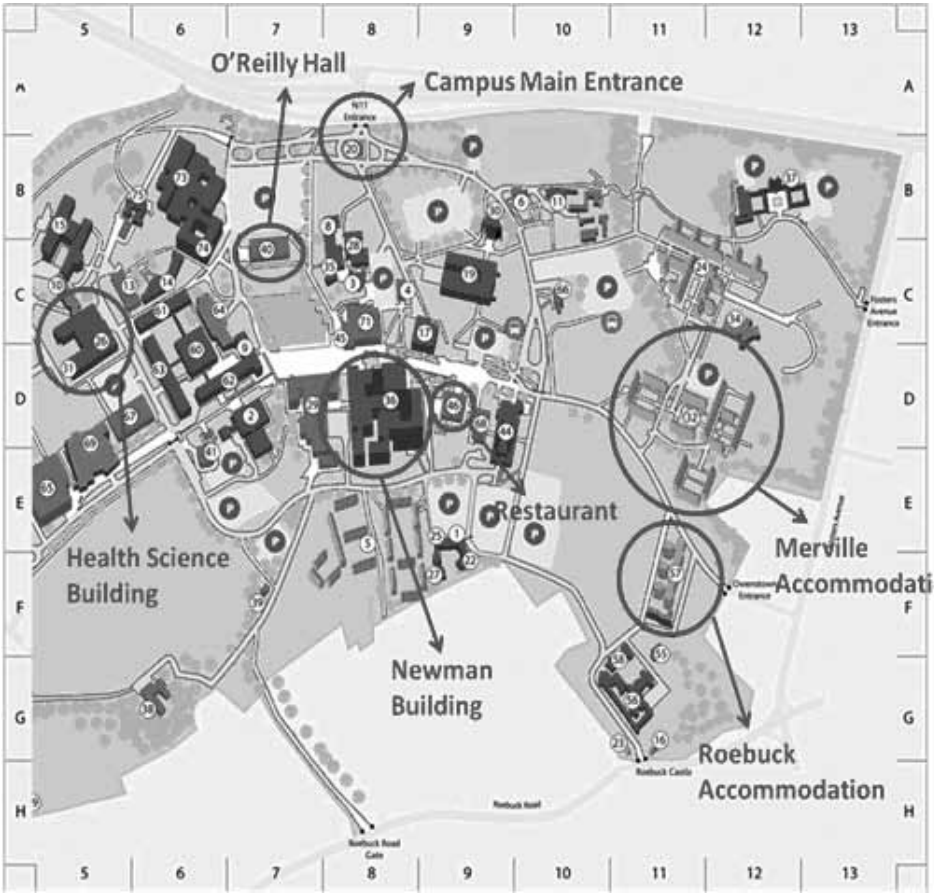
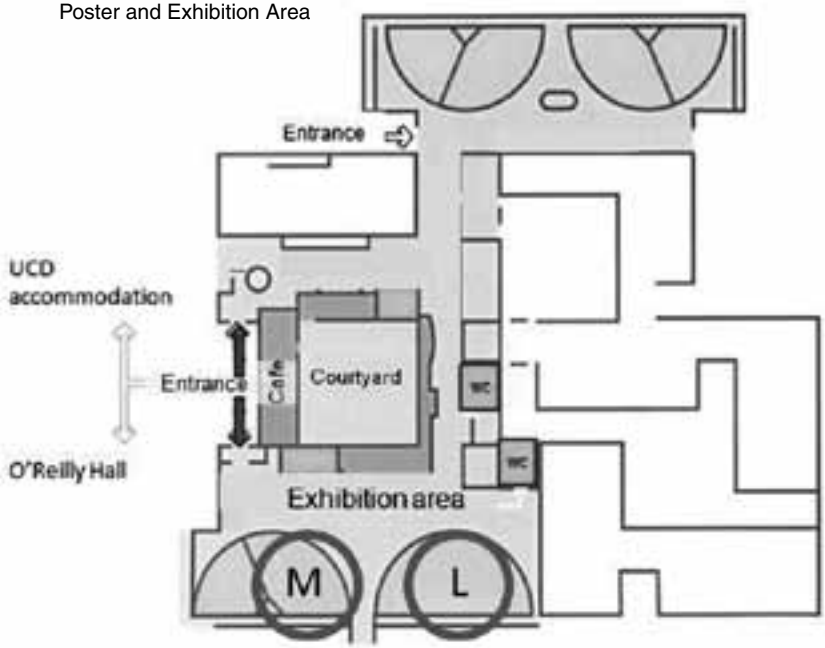
UCD Campus Map

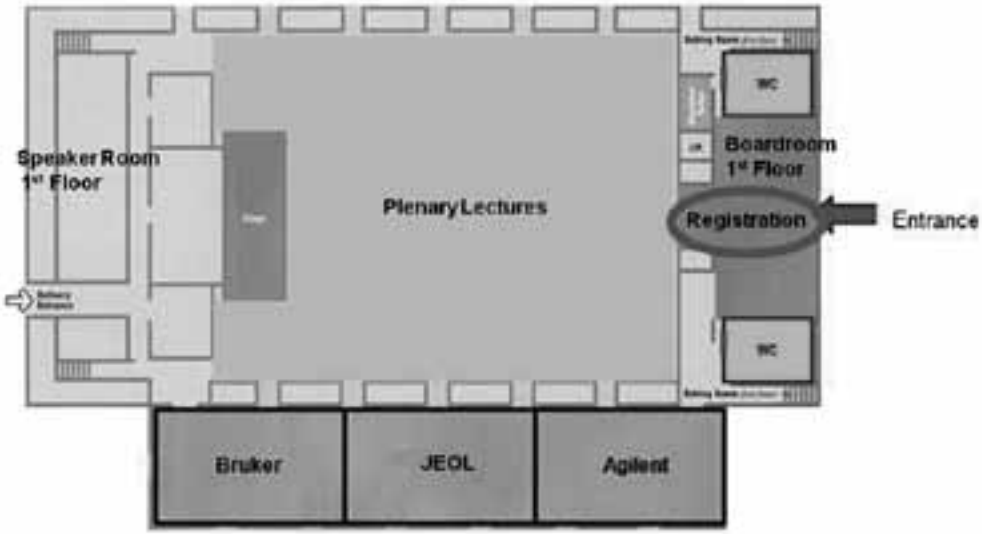


Registration –	**O' Reilly Hall**
Tutorial Lectures –	**Health Science Building**
Welcome Reception –	**O'Reilly Hall**
Hospitality Suites –	**O'Reilly Hall**
Plenary Lectures –	**O'Reilly Hall**
Parallel Session Lectures –	**Newman Building**
Poster Presentations –	**Newman Building**
Exhibitors and Vendors –	**O'Reilly Hall and Newman Building**

Newman Building
Parallel Sessions: Theatre L & M
Poster and Exhibition Area



O'Reilly Hall
Opening and Plenary Lectures
Hospitality Suites, Conference Dinner
Boardroom, Speaker Room 1st Floor



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Fáilte romhat chuig EUROMAR 2012 i mBaile Átha Cliath

Welcome to EUROMAR 2012 in Dublin

EUROMAR is well-established as the premier annual international conference in the field of Magnetic Resonance. Therefore it is not surprising that it is attended by world-class scientists, decision makers, postdoctoral and postgraduate scientists, publishers and leading companies in the area of magnetic resonance research and its applications. EUROMAR emerged in 2004 after merging three major NMR conferences, the European Experimental NMR conference, the AMPERE congress and the United Kingdom RSC NMR Discussion Group. These groups had established individual magnetic resonance meetings for several decades dating back to the early 1950s.

The International Program Committee of EUROMAR has put together an exciting scientific program that reflects the vitality and diversity of the current magnetic resonance activities in the field of magnetic resonance spectroscopy. The Scientific presentations at EUROMAR 2012 cover a wide range of magnetic resonance spectroscopy. This includes scientific and technical advancements as well as novel and innovative applications in the life sciences, physical sciences and medical research.

EUROMAR 2012 will be held in Ireland which is known as the land of Saints and Scholars. The capital city, Dublin, lies at the mouth of the river Liffey near the Irish Sea. It is a city rich in historical and cultural attractions including the unique Book of Kells, the famous Guinness brewery, the neighbouring hills of Tara and the monastic village of Glendalough. The conference will take place at University College Dublin, one of the largest academic institutions in Ireland. There will be an opportunity to sample the history and culture of Dublin and its surroundings as excursions are planned throughout the conference.

We are delighted that this year, there are two Satellite Meetings, the XeMat meeting and the EU COST action in Hyperpolarisation Spin Physics and Methodology in NMR and MRI that are scheduled back to back with the EUROMAR conference. The XeMat 2012 covers a number of common interests in the DNP, para-hydrogen and hyperpolarized noble gas community. The XeMat meeting is scheduled for the 27th-29th June and the COST Network action meeting is scheduled for the 29th June – 1st July and is followed by the EUROMAR 2012 conference from 1st-5th July. Sponsorship from various bodies has helped to provide generous support for postgraduate students and young emerging postdoctoral scientists.

Finally, the scientific program committee and the local organising committee hope to make the XeMat, the COST Network Action and the EUROMAR 2012 conference exciting and memorable scientific event. You will have the ample opportunities to visit a hospitable and attractive city and we hope you enjoy both the science and Dublin.

Chandralal Hewage
Chairman
EUROMAR 2012 conference

General Information

Registration Desk & Conference Office

The registration desk and conference office are located in the O'Reilly Hall and will be open Sunday the 1st of July at 10:00 and remain open till the end of the conference.

Location of Lectures and Sessions

All sessions will take place in University College Dublin, Belfield, Dublin 4.

Tutorial sessions will take place in the Health Science Building

The plenary lectures will be held in the O'Reilly Hall.

The parallel session lectures will be held in the Newman Building, theatres L and M. The theatres are situated next to each another in the Newman building and notifications will be present outside the lecture theatre for identifying the session.

The poster presentations will be held along the Newman building concourse.

Name Badges

All delegates are kindly asked to wear the provided badges through the conference when on campus.

Speakers

Stewards will be available to assist speakers in transferring their presentations to provided computers or setting up personal computers. Speakers are kindly asked to be present in the lecture hall 20 minutes before the session starts.

Speaker Room

The O'Reilly hall conference room will be available for speakers only; with provided laptops and internet connection should any last minute changes be required for their talks, and is located on the first floor of the O'Reilly Hall.

Lunch and Tea/Coffee

Lunch boxes will be provided daily between 12.45 and 13.15. Tea and Coffee stations will be located in several areas of the Newman building and O'Reilly Hall throughout the conference. There are also several on campus cafes and shops available.

General Information

Welcome Reception and Conference Dinner

The welcome reception will be held in the O'Reilly hall on Sunday, July 1st, at 19.30-21.30 and we invite you all to join us for some food and refreshments.
The conference dinner will be held on Thursday, July 5th in the O'Reilly Hall at 19.45.

Tickets will be required for admission to the conference dinner.

Public Transportation

UCD student accommodation is located on campus; however several bus routes serve the university. A bus stop located at the main entrance of the University on the N11 is serviced by bus numbers 39a, 145 and 46a which arrive every 10/20 minutes and all travel through Dublin city centre. A return ticket from UCD to St Stephens Green, city centre, costs €2.15 (exact fare only; no change given).Additional information can be found at www.dublinbus.ie.

Vendor User Meetings and Activities

Bruker and Agilent Technologies host their user meetings on Sunday, July 1st, in the Health Science building on campus between 14.00-17.00. Bruker and Agilent will host their hospitality suites in the O'Reilly Hall. JEOL will hold lunch time seminars during the conference in the Newman Building, Theatre M.

Internet

The UCD wireless opened WaveLAN network is available on campus including accommodation and throughout the Newman building and O'Reilly Hall. To connect:

- Turn on Wi-Fi
- Select the Wi-Fi icon located in the bottom left side of the screen
- Select WaveLAN Network and connect

Usernames and passwords will also be provided for access to the local campus computers present in the Newman Building.

Tours

All tours will depart from and return to O'Reilly Hall, UCD. Please note the departure time of the tours below. Tours will depart on time and no refunds will be offered to late-comers.

We request that attendees are in the foyer of O'Reilly Hall 10 mins before the departure time.

Monday 2nd July 09:00 – 13:00 - Dublin City Tour

Tuesday 3rd July 13:00 – 18:00 - Glendalough & Powerscourt House and Gardens

Wednesday 4th July 13:00 – 17:30 - Old Jameson Distillery and the Guinness Storehouse

Friday 6th July 09:00 – 16:00 - Newgrange

Special Meetings (Invited only)

The following meetings will be held each day from Monday the 2nd of July to Thursday the 5th, in the O'Reilly Hall Boardroom (1st Floor) at lunch time 12.45-13.45.

Monday:	EUROMAR Board of Trustees meeting
Tuesday:	AMPERE Bureau meeting
Wednesday:	EUROMAR 2013 program committee meeting
Thursday:	EUROMAR 2012 local committee meeting

Other Meetings (Invited only)

AMPERE Committee Meeting;
Tuesday at 18.45 in the Boardroom, O'Reilly Hall

AMPERE General Assembly;
Wednesday at 18.45 in the Plenary Lecture Theatre, O'Reilly Hall

Future EUROMAR conferences

The 9th EUROMAR conference will be held in Hersonissos, Crete, Greece, 30th of June- 5th July, 2013.

Poster Presentations

Categories	Poster Numbers
Low-Field NMR	200-208
Computation	209-221
In Vivo and MRI	222-236
Biological Advances	237-239
Metabolomics	240-261
Liquid state Methods	262-299
Instrumentation	300-308
Membrane Proteins	309-319
Biosolids	320-329
Soluble Proteins	330-343
Paramagnetic Systems	344-355
In Cell and Natively Unstructured Proteins	356-360
Hyperpolarisation	361-399
Nucleic Acids	400-407
Relaxation and Dynamics	408-432
Other	433-449
Materials and Polymers	450-486
EPR	487-514
Small Molecules and Pharmaceuticals	515-552
Solid-State NMR Methods	553-574

Poster Sessions

There are four poster sessions, from Monday the 2nd of July to Thursday the 5th of July between 13.45 and 15.45 each day. Authors are asked to be present at their posters on the day they have been allocated. Poster session allocations are as follows:
Each abstract is assigned a poster number which indicates its category (see previous page) and also has a two letters code to reflect the day of presentation, by the author. **MO-Monday, TU-Tuesday, WE-Wednesday and TH-Thursday.**

Examples:
253TU, would be a poster in the Metabolomics category and be presenting on Tuesday the 3rd of July.
326WE, is in the Biosolids category and would be presenting on Wednesday the 4th of July.

Set-up and removal

Authors are kindly asked to have their posters put up before 12.45 on Monday the 2nd of July, and leave them on display for the duration of the conference. Poster areas are separated according to category and all boards will be labelled with the individual abstract code which can be found in this book in the list of abstracts. Posters should be removed before 16:00 on Thursday the 5th of July; any unclaimed posters will be discarded.

PROFESSOR ROBERT BLINC

On September 26, 2011, Professor Robert Blinc passed away. Robert was not only a gifted scientist and creative mind, but a highly educated man with broad interests and a dear friend to many of us. He was always willing and ready to provide service for the NMR community. He was the president of the groupement AMPERE between 1990–1996 and he was active in AMPERE until his death. Amongst many other things, has helped to shape EUROMAR.

Robert Blinc was born on October 31, 1933, in Ljubljana, Slovenia. He graduated in 1958 and completed PhD with Professor Hadži in 1959 in physics at the University of Ljubljana. After a postdoctoral year spent in the group of Professor John Waugh at M.I.T., Cambridge, Mass., Robert Blinc was appointed as a professor of physics at the University of Ljubljana where he also initiated the NMR laboratory at the Jožef Stefan Institute in Ljubljana. He was a lead in the field of magnetic resonance of ice, ferroelectric materials, liquid crystals, incommensurate dielectrics, pseudospin glasses, relaxor ferroelectrics, fullerenes, and fullerene nanomagnets. He detected solitons and phasons in incommensurate systems using NMR, determined the Edwards-Anderson order parameter in proton and deuteron glasses, and discovered the origin of giant electromechanical effect in PMN-PZT relaxors via the existence of critical end point.

Robert Blinc authored or coauthored over 700 original research paper and three books. *Advanced Ferroelectricity* (Oxford Science Publications) appeared in August 2011, shortly before he passed away.

Robert Blinc was a member (and vice-president in the years 1980–1999) of the Academy of Science and Arts of Slovenia. He was a member of seven foreign Academies of Sciences and has received numerous national and international scientific prizes.

We, his colleagues, former students and coworkers, owe him a debt of gratitude for his enthusiasm and dedication to the phenomena of solid-state physics and magnetic resonance spectroscopy and his great commitment for the international NMR community. He will remain in our memories.

Beat Meier (President of AMPERE)
Janez Dolinšek (Vice-President of AMPERE)

SIR PAUL T. CALLAGHAN

Sir Paul T. Callaghan passed away on March 24, 2012 in Wellington, New Zealand. He was born August 19, 1947 in Wanganui, New Zealand and graduated from Victoria University of Wellington. He obtained his DPhil in physics studying anisotropic gamma ray emission from oriented nuclei at Clarendon Laboratory at Oxford University.

Paul returned to New Zealand in 1984 where he started a program at Massey University to apply NMR to study soft matter. His prolific career led to over 240 scientific articles including his first NMR book *Principles of Nuclear Magnetic Resonance Microscopy* and *Translational Dynamics and Magnetic Resonance: Principles of Pulsed Gradient Spin Echo NMR,* finished in 2011.

Paul's contributions in NMR include pioneering the concept of q-space diffraction and imaging, performing modulated gradient spin echo experiments with Janez Stepisnik, developing the science of using NMR to study rheology, development of compact NMR instruments, and performing novel NMR experiments on the Antarctic sea ice.

Paul left Massey in 2001 to become the Alan MacDiarmid Professor of Physical Sciences at Victoria University of Wellington where he was the inaugural director of the MacDiarmid Institute for Advanced Materials and Nanotechnology. He was only the 36th New Zealander to be elected as a fellow of the Royal Society of London. He helped establish Magritek in 2004 to commercialize small-scale NMR instruments. Paul's many awards and prizes include his appointment as a Principal Companion of the New Zealand Order of Merit in 2006 and, when the traditional honors were restored, was knighted in 2009. He also won the AMPERE Prize (2004) and the Günther Laukien Prize for Magnetic Resonance (2010).

Paul was also active in the public domain, initially to impart more knowledge of science to the public but later in trying to influence New Zealand's economic policy towards higher per-capita GDP and improved living standards via the development of small high-tech and high-value industries such as Magritek. He participated in radio and TV programs and delivered many public lectures on the role of science in the New Zealand economy as well as on "science, life, and universe." *He co-authored As Far as We Know: Conversations about Science, Life and the Universe and Are Angels OK?: The Parallel Universes of New Zealand Writers and Scientists and authored Wool to Weta: Transforming New Zealand's Culture and Economy.* He championed Zealandia, a refuge in Wellington that aims to restore the habitat prior to the introduction of mammals. For such public service and scientific accomplishments, he was named New Zealander of the Year for 2011.

He is survived by his wife Miang Lim, daughter Catherine and son Christopher, three siblings, two grandsons, many cousins and other relatives. He also leaves behind a grateful nation, the world-wide NMR community, many former students and postdocs, and many good friends.

Eiichi Fukushima

Awards and Prizes

The Raymond Andrew Prize of the AMPERE Group

Galia Debelouchina, Princeton University, USA
"Amyloid Fibril Structure of Peptides and Proteins by Magic Angle Spinning NMR Spectroscopy and Dynamic Nuclear Polarization"

AMPERE Prize

Lyndon Emsley, Université de Lyon, France
"NMR Crystallography"

The MRC awards for Young Scientists by John Wiley & Sons

Till Biskup, University of Oxford, UK
"Cryptochromes; Potential compass molecules with an unexpected variety of electron transfer pathways"

Jean-Nicolas Dumez, Weizmann Institute of Science, Israel
"Multidimensional pulses and spatially encoded magnetic resonance"

Katja Petzold, University of Michigan, USA
"Excited States in RNA Using Relaxation Dispersion NMR – a General Behaviour?"

SUNDAY 1ST JULY

10.00-16.30	**Registration**
	Health Science Building Tutorial Lectures Chair: Thomas Meersman
14.00-14.45	**Lorraine Brennan** Current Trends in Metabolomics
14.45-15.30	**Philip Grandinetti** Quadrupolar NMR in Solids
15.30-16.15	**Kurt Zilm** The Inner Workings of NMR Probes – How to Get More from CPMAS
	O'Reilly Hall
16.30-16.45	**Welcome Remarks**
	Chandralal Hewage **Lucio Fyrdman** **Beat Meier**
16.45-17.00	**Remembrance**
	Robert Blinc by **Beat Meier** Paul Callaghan by **Andrew Coy**
17.00-18.30	Chair: **Hans-Wolfgang Spiess** The Raymond Andrew Prize **PL1 - Galia Debelouchina** Amyloid Fibril Structure of Peptides and Proteins by Magic Angle Spinning NMR Spectroscopy and Dynamic Nuclear Polarization
	The AMPERE Prize **PL 2 - Lyndon Emsley** NMR Crystallography
18.30-19.15	Chair: **Lucio Frydman** Keynote Lecture **PL3 - Robert Tycko** Biomolecular Solid State NMR: Getting Better All the Time
19.15-22.15	WELCOME MIXER

MONDAY 2ND JULY

	Chair: **Lyndon Emsley**	
8.30-9.15	**PL 4 - Jeremy Nicholson** Spectroscopy and systems medicine in the real world	
9.15-10.00	**PL 5 - Hartmut Oschkinat** Structural Biology by DNP MAS NMR and Investigations on the Transport Cycle of an ABC Transporter	
10.00-10.45	Coffee	
	Theatre M	**Theatre L**
	Chair: **PK Madhu** New Methods in Solids and Oriented Media	Chair: **L Brennan / P Crowley** Metabolism & In-cell NMR
10.45-11.20	**PS 101 - Matthias Ernst** Decoupling and Recoupling Using Phase-Alternating Pulse Sequences	**PS 105 - Julian Griffin** Greater than the sum of the parts : Using Data fusion to improve the sensitivity of ^{1}H HR-MAS NMR spectroscopy in Breast Cancer
11.20-11.45	**PS 102 - Yusuke Nishiyama** $^{1}H^{14}N$ HMQC above 110 kHz MAS	**PS 106 - Mika Ala-Korpela** High-Throughput Serum NMR – The New Era in Epidemiology & Genetics
11.45-12.10	**PS 103 - Christina Thiele** Fast access to Residual Dipolar Couplings by single-scan 2D NMR in oriented media	**PS 107 - Philipp Selenko** In-cell NMR in Mammalian Cells
12.10-12.45	**PS 104 - Niels Nielsen** Efficient Coherence Transfer Methods for Biological Solid-State NMR	**PS 108 - Gary Pielak** Macromolecular Crowding & Protein Chemistry: Views from Inside & Outside Cells
12.45-13.45	Lunch	
13.45-15.45	Poster Presentation (MO) and Tea	
	Theatre L	**Theatre M**
	Chair: **Paul Malthouse** Bioliquids NMR I	Chair: **Daniella Goldfarb** EPR I
15.45-16.20	**PS 109 - Michael Sattler** NMR Studies of Molecular Recognition and Dynamics of (Large) Protein Complexes in Solution	**PS 113 - Christopher Kay** From Solid State Physics to Structural Biology: Putting a Spin on it with EPR Spectroscopy
16.20-16.45	**PS 110 - Michael Overduin** Structural Mechanism of Calmodulin Activation and Autoinhibition of CaMK1 Kinase	**PS 114 - Peter Roberts** Investigation of Electron Spin Relaxation and Spectral Diffusion using Two-Dimensional Inverse Laplace Transforms
16.45-17.10	**PS 111 - Shin-ichi Tate** Functionally detuning motion for the hydride transfer step, which is intrinsically encoded in the active loop dynamics of dihydrofolate reductase, DHFR	**PS 115 - Till Biskup** Cryptochromes; Potential compass molecules with an unexpected variety of electron transfer pathways
17.10-17.45	**PS 112 - Juli Feigon** The Architecture of Telomerase	**PS 116 - Marina Bennati** Distance Measurements and Dynamic Nuclear Polarization at 9 and 94 GHz EPR Frequencies
	Chair: **Göran Karlsson**	
17.55-18.40	**PL 6 - Gerhard Wagner** New NMR Approaches for Challenging Proteins	

Tuesday 3rd July

	Chair: **Miquel Pons**	
8.30-9.15	**PL 7 - Malcolm Levitt** Singlet NMR	
9.15-10.00	**PL 8 - Sabine Van Doorslaer** Gaining insight in (bio)inorganic chemistry using EPR and DFT	
10.00-10.45	Coffee	
	Theatre M	**Theatre L**
	Chair: **Anja Böckmann** Biosolids NMR I	Chair: **Kenneth Mok** Liquid State Methods
10.45-11.20	**PS 117 - Melinda Duer** Heavy mice and lighter things: using NMR to elucidate molecular structures in tissues	**PS 121 - Hanudatta Atreya** Novel NMR Methods with High Resolution and Sensitivity: from Protein Structures to Nanotubes
11.20-11.45	**PS 118 - Shenlin Wang** High-resolution structure of a seven-helical membrane protein determined by solid-state NMR	**PS 122 - Warren Warren** Revisiting Decades-Old Spin Physics to Improve Modern Magnetic Resonance Imaging
11.45-12.10	**PS 119 - Jean-Philippe Demers** Solid-state NMR reveals the structural architecture of Shigella flexneri Type-III Secretion Needles	**PS 123 - Hans Kalbitzer** Detection of excited states of proteins by high pressure NMR spectroscopy - a new strategy for rational drug design
12.10-12.45	**PS 120 - Francesco Ravotti** Pushing for resolution in ^{13}C spectra of uniformly labelled proteins	**PS 124 - Gareth Morris** Controlling J modulation: new spin echo and pure shift NMR techniques
12.45-13.45	Lunch	
13.45-15.45	Poster Presentation (TU) and Tea	
	Theatre L	**Theatre M**
	Chair: **Michael Williamson** Computational	Chair: **Janez Dolinšek** Materials
15.45-16.20	**PS 125 - Peter Güntert** Reliable and flexible automated assignment of NMR spectra	**PS 129 - Denis Arčon** Superconductivity competing with an antiferromagnetic Mott-insulating state in alkali-doped fullerides
16.20-16.45	**PS 126 - Jochen Balbach** Dynamic Inter-Domain Crosstalk Determines Enzyme Activity	**PS 130 - Stephen Cottrell** Kinetics of Hydrogen Abstraction in Propane studied by Muon Spin Resonance (μSR)
16.45-17.10	**PS 127 - Patrick Giraudeau** Fast 2D and 3D NMR tools for metabolic flux analysis in complex biological mixtures	**PS 131 - Marianne Giesecke** Electrokinetic NMR (eNMR) as a tool to study new energetic materials
17.10-17.45	**PS 128 - Michael Nilges** Structures of large complexes from heterogeneous data and Bayesian data analysis	**PS 132 - Michael Deschamps** Supercapacitor electrodes and solid-state electrolytes studied by NMR
	Chair: **Muriel Delepierre**	
17.55-18.40	**PL 9 - Michele Vendruscolo** Characterization of free energy landscapes of proteins using NMR spectroscopy	

WEDNESDAY 4TH JULY

	Chair: **Beat Meier**	
8.30-9.15	**PL 10 - Anne Lesage** Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy	
9.15-10.00	**PL 11 - Martin Blackledge** Towards an atomic resolution description of functionally important motions in folded and unfolded proteins using high resolution NMR spectroscopy	
10.00-10.45	Coffee	
	Theatre L	Theatre M
	Chair: **Clare Grey** Nuclei and Electrons	Chair: **Gil Navon** In vivo and MRI
10.45-11.20	**PS 133 - Christopher Jaroniec** Protein fold determined by paramagnetic magic-angle spinning solid-state NMR spectroscopy	**PS 137 - Yoram Cohen** Single and Double-PFG NMR and MRI: From Model Systems to Imaging of the CNS
11.20-11.45	**PS 134 - Bela Bode** PELDOR distance measurements in homo-oligomeric systems	**PS 138 - Jean-Nicolas Dumez** Multidimensional pulses and spatially encoded magnetic resonance
11.45-12.10	**PS 135 - Thorsten Maly** An Integrated Terahertz Gyrotron for DNP-NMR Spectroscopy	**PS 139 - Alexej Jerschow** Long Lived Coherent Response Signal in Bone
12.10-12.45	**PS 136 - Dany Carlier** NMR spectroscopy combined with DFT calculations to study paramagnetic materials for Li-ion batteries	**PS 140 - Klaas Nicolay** Multi-parametric MR imaging and spectroscopy of cardiovascular disease in small animals
12.45-13.45	Lunch	
13.45-15.45	Poster Presentation (WE) and Tea	
	Theatre L	Theatre M
	Chair: **Christian Griesinger** Bioliquids NMR II	Chair: **Walter Köckenberger** Hyperpolarisation
15.45-16.20	**PS 141 - Ramakrishna Hosur** Protein NMR - Stretching the Limits	**PS 145 - Nicholas Kuzma** Dynamic nuclear polarization of frozen gases
16.20-16.45	**PS 142 - Jordan Chill** NMR Study of Structure and Dynamics in the Intrinsically Disordered C-terminal Domain of WASp-Interacting Protein	**PS 146 - Kent Thurber** Dynamic Nuclear Polarization (DNP) with MAS at low temperature (25 K)
16.45-17.10	**PS 143 - Dominique Frueh** Transient Substrate and Domain Interactions in Non-Ribosomal Peptide Synthetases	**PS 147 - Christian Hilty** Investigation of Protein Folding using Dissolution DNP
17.10-17.45	**PS 144 - Joshua Wand** Unraveling Protein Motion and Hydration	**PS 148 - Simon Duckett** Signal amplification via reversible interaction with parahydrogen: Opportunities for NMR
	Chair: **Gunnar Jeschke**	
17.55-18.40	**PL 12 - Jörg Wrachtrup** Seeing spins at the nanoscale	

Thursday 5th July

	Chair: **Geoffrey Bodenhausen**	
8.30-9.15	**PL 13 - Harald Schwalbe** RNA regulation elements studied by NMR spectroscopy	
9.15-10.00	**PL 14 - Kevin Brindle** Imaging metabolism – Watching tumours gasp and die with hyperpolarized MRI	
10.00-10.45	Coffee	
	Theatre L	**Theatre M**
	Chair: **Bernhard Blümich** Emerging Areas	Chair: **Alexej Jerschow** Biosolids NMR II
10.45-11.20	**PS 149 - Jamie Walls** Improving resolution in NMR using pathway selective pulses	**PS 153 - Daniel Huster** Solid-State NMR Studies of Aβ Protofibrils and Mature Fibrils
11.20-11.45	**PS 150 - Vikram Bajaj** NMR and MRI at the Microscale	**PS 154 - Henrik Müller** Towards structural comparison of spontaneously formed and prion-seeded full-length recombinant PrP-fibrils by solid-state NMR
11.45-12.10	**PS 151 - Vasiliki Demas** Magnetic Resonance for in vitro diagnostics: from detecting pathogens to characterizing and monitoring the blood physiology	**PS 155 - Umit Akbey** Solid-State NMR Studies of Deuterated Proteins: Higher Resolution and Better Sensitivity
12.10-12.45	**PS 152 - Stephan Appelt** The physics of PHIP hyperpolarized low field NMR	**PS 156 - Chad Rienstra** Solid State NMR of Fibrils and Membrane Proteins
12.45-13.45	Lunch	
13.45-15.45	Poster Presentation (TH) and Tea	
	Theatre L	**Theatre M**
	Chair: **Ad Bax** Biomacromolecular Assemblies	Chair: **Sabine Van Doorslaer** EPR II
15.45-16.20	**PS 157 - Stanley Opella** Structure Determination of Membrane Proteins in Phospholipid Bilayers	**PS 161 - Aharon Blank** Nonlinear Induction Detection of Electron Spin Resonance
16.20-16.45	**PS 158 - Katja Petzold** Excited States in RNA Using Relaxation Dispersion NMR – a General Behaviour?	**PS 162 - Gunnar Jeschke** Fitting of protein structural transitions with EPR distance constraints: Optimization of algorithms
16.45-17.10	**PS 159 - Jason Schnell** Structural Studies of Oligomeric TatA, the Pore Component of the Twin Arginine Translocase	**PS 163 - Christopher Wedge** Chemical Engineering of Molecular Qubits
17.10-17.45	**PS 160 - Philipp Neudecker** NMR Solution Structure of an Invisible Protein State at the Edge between Folding and Aggregation into Amyloid Fibrils	**PS 164 - Vladimir Dyakonov** Application of Electron Paramagnetic Resonance to Study Fundamentals Processes in Organic Photovoltaic Materials and Devices
	Chair: **Georgios Papavassiliou** (Theatre L)	
17.55-18.40	**PL 15 - Thomas Prisner** New methods for EPR and NMR	

Plenary Lectures

PL 01

Amyloid Fibril Structure of Peptides and Proteins by Magic Angle Spinning NMR Spectroscopy and Dynamic Nuclear Polarization

Galia Debelouchina[1], Marvin Bayro[1], Geoffrey Platt[2], Anthony Fitzpatrick[3], Melanie Rosay[4], Werner Maas[4], Sheena Radford[2], Christopher Dobson[3], Robert Griffin[1]

[1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Astbury Centre for Structural Molecular Biology and Institute of Molecular and Cellular Biology, University of Leeds, Leeds, UK, [3]Department of Chemistry, University of Cambridge, Cambridge, UK, 4Bruker BioSpin, Billerica, MA, USA

Amyloid fibrils are insoluble, non-crystalline protein filaments associated with a number of diseases such as Alzheimer's and Type II diabetes. They can have a functional role in different organisms and many proteins and peptides have been found to form amyloid fibrils *in vitro*. We have used magic angle spinning (MAS) NMR spectroscopy to investigate the structure of two amyloid fibril systems – an 11-residue segment from the disease-related protein transthyretin (TTR); and β_2-microglobulin (β_2m), a 99-residue protein associated with dialysis-related amyloidosis. The TTR(105-115) case exemplifies our efforts to characterize the hierarchy of structures present in the fibril form, including the organization of the β-strands into β-sheets (tertiary structure), the β-sheet interface that defines each protofilament (quaternary structure), and the protofilament-to-protofilament contacts that lead to the formation of the complete fibril. Our efforts were guided by information obtained from other methods such as cryo-electron microscopy and resulted in the very first atomic resolution structure of a complete amyloid fibril. We have extended the methods used in the TTR(105-115) structure determination procedure to the fibrils formed by β_2m, a process complicated not only by the much larger size of the protein involved but also by the high degree of dynamics exhibited in these fibrils. Nevertheless, we were able to characterize the secondary structure of the protein in the fibril form, and the tertiary and quaternary interactions within the fibrils. Our work on amyloid fibrils has also benefited extensively from the development of dynamic nuclear polarization, a method used to enhance the sensitivity of MAS NMR experiments, leading to unprecedented gains in signal-to-noise ratios and acquisition times.

PL 2

NMR Crystallography

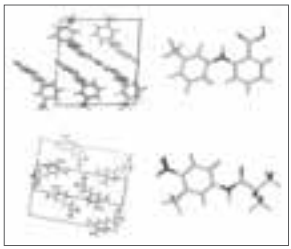
Lyndon Emsley

Ecole normale supérieure de Lyon, Lyon, France

Structural characterization is one of the key challenges for modern chemistry, especially for powders. Our aim is to establish a protocol for natural abundance NMR crystallography for crystal structure elucidation of powdered solids, particularly of pharmaceutical relevance.

Towards this end we explore the possibility of complete *ab initio* structure determination in molecular crystals using combined 1H NMR and computationally based structure prediction techniques. We combine molecular modeling and plane wave DFT calculations of NMR parameters with high-resolution solid-state 1H NMR experiments (and powder X-ray diffraction). We illustrate the feasibility of this method in several examples.

The figure shows the crystal structures obtained from this method for powder samples of flufenamic acid (upper) and flutamide (lower), superimposed with the structures obtained by X-ray diffraction from single crystal samples. (They are essentially identical).



One of the barriers to further progress with this approach is the possibility to measure proton chemical shifts in powders. Towards this end we will present our most recent progress with homonuclear dipolar decoupling strategies. Notably, we will introduce a new parameterisation scheme for eDUMBO type decoupling, we will provide a framework which covers both PMLG and DUMBO decoupling, and we will explore the differences and similarities of the two schemes.

Finally, we will show how one of the key limitations to acquiring multi-dimensional NMR spectra from small molecules (which often have prohibitively long T1 relaxation times) can be overcome by using dynamic nuclear polarisation.

PL 3

BIOMOLECULAR SOLID STATE NMR: GETTING BETTER ALL THE TIME

Robert Tycko, Wei Qiang, Junxia Lu, Marvin Bayro, Alexey Potapov, Eric Moore, Wai-Ming Yau, Kent Thurber
National Institutes of Health, Bethesda, MD, USA

The field of biomolecular solid state NMR has expanded enormously over the past 15 years, as techniques and technology have improved and as an increasing number of research groups have explored an increasing variety of biologically and biophysically interesting systems. This lecture will include recent results from projects in my lab in which we are applying solid state NMR methods to specific protein systems and/or developing new methods with potentially general utility. In the area of amyloid fibril research, we have recently characterized the molecular structure of metastable, neurotoxic protofibrils formed by the disease-associated Asp23-to-Asn mutant of the 40-residue beta-amyloid peptide (D23N-Ab40). The D23N-Ab40 protofibrils contain a novel double-layered antiparallel cross-beta structure, which contrasts with the parallel cross-beta structure that is commonly observed in mature beta-amyloid fibrils. We are also in the process of characterizing beta-amyloid fibril structures that develop in brain tissue of Alzheimer's disease patients. This work is aided by new dipolar recoupling techniques for quantitative measurements of 13C-13C and 15N-15N distances in uniformly 15N,13C-labeled samples, and by computer-aided resonance assignment methods. In the area of technology, we are constructing equipment for magic-angle spinning and dynamic nuclear polarization at temperatures below 25 K. The rationale for these efforts and recent results will be described. Finally, I will mention new research directions that we hope to pursue in the near future.

PL 4

SPECTROSCOPY AND SYSTEMS MEDICINE IN THE REAL WORLD

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Systems biology tools are now being applied at the individual and population level utilizing analytical and statistical methods that report on integrated biological functions non-invasively. Metabolic phenotyping offers an important window on integrated system function and both NMR and mass spectrometric (MS) methods have been successfully applied to characterize and quantify a wide range of metabolites in biological fluids and tissues to explore the biochemical sequelae of human disease processes (1-3). A major feature of human biology that has recently been recognised is the extensive interaction with the gut microbiome at the metabolic control and signalling level (4,5). These symbiotic supraorganismal interactions greatly increase the degrees of freedom of the system and there is extensive transgenomic control of metabolism that poses a significant challenge to current analytical and modelling approaches and indeed our fundamental notions of the diseased state and the etiopathogenesis of many common diseases. We have developed new scalable and translatable strategies utilising NMR and MS methods for "*phenotyping the hospital patient journey*" (6) capitalising on the use of metabolic modelling and pharmaco-metabonomics (7,8) for diagnostic and prognostic biomarker generation to aid clinical decision making at point-of-care. Such diagnostics (including those for near real-time applications as in surgery and critical-care 6) can be extremely sensitive for the detection of diagnostic and prognostic biomarkers in a variety conditions and are a powerful adjunct to conventional procedures for disease assessment. Many biomarkers have deeper mechanistic significance and may also generate new therapeutic leads or metrics of efficacy for clinical trial deployment. Furthermore the complex ands subtle gene-environment interactions that generate disease risks in the general human population also express themselves in the metabolic phenotype (9) and as such the Metabolome Wide Association Study approach (10) gives us a powerful new tool to generate disease risk biomarkers from epidemiological sample collections and for assessing the health of whole populations. Such population risk models and biomarkers an also feedback to individual patient healthcare models thus closing the personal and public healthcare modelling triangle.

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PL 5

Structural Biology by DNP MAS NMR and Investigations on the Transport Cycle of an ABC Transporter

Hartmut Oschkinat[1], Arne Linden[1], Sascha Lange[1], Umit Akbey[1], Trent Franks[1], Barth van Rossum[1], Britta Kunert[1], Anja Voreck[1], Robert G. Griffin[2], Erwin Schneider[3], Vivien Lange[1], Edgar Specker[1]

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Solid-state NMR enables the investigation of heterogeneous, complex biological samples at high resolution. A major factor enabling such investigations is dynamic nuclear polarisation (DNP), which was introduced to increase signal-to-noise by one or two orders of magnitude. The application of dynamic nuclear polarisation (DNP) requires further optimization of samples, experimental parameters and concepts. Here, spectra with reasonable line width are obtained on membrane-integrated complexes. During the DNP process, the electron polarization is transferred to the surrounding core nuclei and subsequently to the bulk nuclei. This process depends on several factors such as; relaxation behavior, proton concentration, spin-diffusion and type of the nucleus. As a result, for each nucleus to be polarized there is a characteristic exponential polarization build-up behavior, with different time constant (τ_B) for each nucleus of interest. A systematic investigation of the polarization build-up behavior for different nuclei (^{1}H, ^{2}H, ^{13}C, ^{15}N) is presented. One of the applications of DNP involves cell membranes of the electric organ of the electric ray Torpedo californica. They are densely packed with acetyl choline receptors. Cobra neurotoxin (NTII), which binds very tightly to them, can be isotopically labeled and overexpressed and is investiated in the bound state. A major application of DNP involves investigations of the nascent chain within and directly after leaving the ribosome. The initial folding events are not yet well understood, and prefolding saves several chaperone steps and protects against enzymatic degradation, so it is important to understand this mechanism. Unlabeled ribosomes with a labeled nascent chain peptide are prepared, then chemical shift analysis is performed on the nascent chain signals. Investigations of membrane proteins may be facilitated by extensive deuteration, and subsequent detection of protons under magic-angle spinning conditions. An ABC transporter reconstituted into native lipid bilayers is investigated and spectra of different functional states will be presented together with initial assignments. In essence, the solid-state NMR data enable the draft of a new transport cycle.

PL 6

New NMR Approaches for Challenging Proteins

Gerhard Wagner[1], Sven Hyberts[1], Alexander Milbradt[1], Dominique Frueh[3], Haribabu Arthanari[1], Sebastian Hiller[4], Gregory Heffron[1], Tsyr-Yan Yu[1], Paul Coote[2], Navin Khaneja[2], Koh Takeuchi[5], Ichio Shimada[5], Maayan Gal[1], Alexander Koglin[6], Scott Robson[1], Christian Wasmer[1], Katherine Edmonds[1], David Jones[7], Bernhard Geierstanger[7]

[1]Harvard Medical School, Boston, Massachusetts, USA, [2]Harvard University, Cambridge, Massachusetts, USA, [3]Johns Hopkins University, Baltimore, Maryland, USA, [4]University of Basel, Basel, Switzerland, [5]National institute of Advanced Industrial Science and Technology, Tokyo, Japan, [6]Los Alamos National Laboratory, Los Alamos, Ne Mexico, USA, [7]Novartis Genomics Institute, San Diego, USA

While NMR studies of small proteins has become near routine it still remains time consuming for more challenging proteins. We claim that the power of modern spectrometers is underutilized in common routine procedures, and much progress can be made. Attempts of such efforts will be presented with new ^{1}H detected experiments. In addition, we have explored ^{13}C and ^{15}N direct detection for assigning proteins. Due to the slow transverse relaxation of carbon and nitrogen signals, pulse sequences become very efficient and compensate for the low inherent sensitivity.

Resolution and sensitivity of these experiments can be enhanced dramatically with non-uniform sampling (NUS) and suitable processing methods. This allows an efficient use of the resolution power of modern high-field instruments in multidimensional NMR experiments. This is in contrast to uniform sampling where only a small part of the indirect dimensions can be covered, which largely underutilizes the power of modern high-field instruments. Processing methods for NUS data have advanced dramatically so that we can now process high-resolution sparsely sampled spectra up to three indirect dimensions within a few hours. In this way the resolution in the indirect dimensions can approach that in the direct dimension. We can record 4D NOESY spectra by sampling less than 0.3% of the Nyquist grid with faithful reconstruction of peak heights. Thus, the high resolution in 3D and 4D spectra obtainable yields precise measurements of peak positions. However, this is of moderate impact if peak positions vary between instruments and experiments, due to sample temperature variation depending on the input of rf power. This can be overcome with a newly available T-lock device.

PL 7

Singlet NMR

Malcolm Levitt

University of Southampton, Southampton, UK

Singlet nuclear spin states are quantum states of a nuclear spin-1/2 pair that are antisymmetric with respect to spin exchange, and which have total spin zero. They are therefore non-magnetic and protected against many important relaxation mechanisms. These states often exhibit long relaxation times which may exceed the normal relaxation time T1 by an order of magnitude or even more. In the special case of the 15N spin pair in 15N-labelled nitrous oxide, the nuclear singlet lifetime can be as long as 25 minutes.

I will discuss the phenomenon of singlet nuclear spin order in a variety of contexts - including gas-phase parahydrogen, small molecules in solution, and also some examples of nuclear singlet states in solids. I will discuss how nuclear singlet spin order may be generated from magnetization, how it is maintained, and how it is converted back into observable magnetization.

Our latest work in the field includes the generation of hyperpolarized nuclear singlet order using dynamic nuclear polarization. We have been particularly interested in the properties of nearly equivalent spin pairs, where the hyperpolarized singlet order is long-lived in high magnetic field, even without any external intervention. We have designed and demonstrated molecular systems that exhibit 13C singlet lifetimes of more than 10 minutes.

Together with many collaborators, we have also studied the nuclear singlet states of parawater molecules trapped inside fullerene cavities, using neutron scattering and infrared spectroscopy, as well as NMR.

PL 8

Gaining Insight in (Bio)Inorganic Chemistry using EPR and DFT

Sabine Van Doorslaer

University of Antwerp, Antwerp, Belgium

Multi-frequency EPR in combination with DFT is a versatile tool to characterize paramagnetic transition-metal complexes. In this talk, I will demonstrate how modern-day EPR can be used to elucidate different questions in (bio)inorganic chemistry. In the context of bioinorganic chemistry, I will focus on the study of heme proteins (globins, aromatase and chlorite dismutase) and show how EPR is one of the important analytical tools in this field of protein science. It will be shown that after decades of EPR research into ferric heme proteins, these biomolecules still put methodological EPR challenges. Furthermore, it will be shown how pulsed EPR in combination with DFT can be used to understand the complex reactions of cancerogenic Cr(VI) with biosystems.

In a second part of the talk, I will move to the field of inorganic chemistry, elucidating how a combined EPR-DFT approach can reveal unprecedented unique information on homogeneous catalysts (using the example of the so-called Jacobsen catalyst, a cobalt salen complex) and on heterogeneous catalysts (using the examples from different zeolitic and titania materials).

In all cases, the advantages, but also the limits and challenges, of the spectroscopic approaches will be discussed.

PL 9

Characterization of Free Energy Landscapes of Proteins Using NMR Spectroscopy

Michele Vendruscolo

University of Cambridge, Cambridge, UK

The dynamics of proteins play a crucial role in many of their biological functions, including ligand binding and enzyme catalysis. It is therefore of great importance to be able to characterise such dynamics with high accuracy in order to obtain a better understanding of the mechanisms by which proteins perform their activities. I will describe recent advances in the development of procedures to include NMR information about dynamics in the process of protein structure determination. In this context, I will show how the incorporation of NMR measurements in molecular dynamics simulations as replica-averaged structural restraints can provide ensembles of conformations that represent with accuracy the free energy landscapes of proteins.

PL 10

Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy

Anne Lesage

University of Lyon, Villeurbanne, France

NMR spectroscopy (often in conjunction with diffraction methods) is the method of choice for characterizing surfaces whenever possible, but the detection limit of NMR is far too low to allow many modern materials to be examined. Because it provides dramatic sensitivity enhancement, solid-state Dynamic Nuclear Polarization (DNP) NMR is currently emerging as a powerful tool to study samples previously inaccessible to NMR. In 2010, our group has shown how DNP could be used to selectively enhance the NMR signals from surfaces (DNP SENS).[1] This approach provides remarkable signal enhancements (currently up to ~> 100 for both 29Si and 13C nuclei) and consequently leads to large reduction in experimental times (currently up to a factor ten thousand)[2]. Two-dimensional ^{1}H-^{13}C or ^{1}H-^{29}Si correlation spectra under DNP conditions have thus been applied to determine in an expeditious way the bonding topologies and local conformations of functional groups incorporated in hybrid mesoporous materials.[3] More recently, we have shown that DNP experiments can be performed using non-aqueous solvents, which in combination with the biradical bTbK, provides signal enhancements similar to that obtained using aqueous solutions.[4] The latest developments in this field will be presented, including the introduction of new polarizing agents and the first applications of DNP SENS to heterogeneous organometallic catalysts.

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PL 11

Towards an Atomic Resolution Description of Functionally Important Motions in Folded and Unfolded Proteins Using High Resolution NMR Spectroscopy

Valery Ozenne, Robert Schneider, Guillaume Communie, Jie-rong Huang, Luca Mollica, Elise Delaforge, Jaka Kragelj, Damien Maurin, Paul Guerry, Loic Salmon, Malene Ringkjøbing Jensen, Martin Blackledge

Institut de Biologie Structurale CEA, CNRS, UJF, Grenoble, France

Proteins are inherently flexible, displaying a broad range of dynamics over a hierarchy of time-scales from pico-seconds to seconds. This plasticity enables conformational changes that are essential for biomolecular function. NMR is sensitive to all conformational fluctuations occurring up to the millisecond and we have developed robust methods to quantitatively describe these molecular motions. We combine analytical and numerical approaches to develop a self-consistent representation of all motions occurring in proteins on timescales from the picosecond to the milliseconds,[1-3] and compare these motions in the free and bound forms of the proteins.[4]

Intrinsically disordered proteins (IDPs) represent extreme examples where protein flexibility plays a determining role in biological function. The development of meaningful descriptions of the behaviour of IDPs is a key challenge for contemporary structural biology, due to their inherent conformational disorder[5] Due to the increase in available degrees of freedom compared to a static picture, the description of accurate protein conformational ensembles requires the development of robust approaches to determine the significance and uniqueness of any proposed equilibrium.[6] I will present new techniques to determine the level of intrinsic structure, dynamics and interaction kinetics in IDPs and apply these to pre-recognition state of active sites of viral proteins in their physiological context.[6-8]

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PL 12

Seeing Spins at the Nanoscale

Joerg Wrachtrup

Institute of physics, University of Stuttgart, Stuttgart, Germany

Spins are a key element in quantum technology. Either they serve as efficient hubs, memories or processing units in quantum registers or constitute building blocks for novel ultrasensitive sensor devices. Particularly versatile in this respect are spin systems which couple to the light field and thus allow for readout of very few and even single spins. Besides diamond defect spins rare earth elements belong to this class of quantum systems. The talk will describe how to use such systems to probe fundamental properties of entangled multipartied spin clusters and for magnetic resonance imaging with unprecedented sensitivity

PL 13

RNA Regulation Elements Studied by NMR Spectroscopy

Jörg Rinnenthal[1], Hannah Steinert[1], Dominic Wagner[1], Anke Reining[1], Boris Fürtig[1], Janina Buck[1], Anna Wacker[1], Senada Nozinovic[1], Thorsten Marquardsen[2], Frank Engelke[2], Robert Hänsel[1], Volker Dötsch[1], Karl von Laer[3], Beatrix Suess[3], Harald Schwalbe[1]

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In the recent past, the importance of RNA-based regulation has become increasingly recognized. Regulation is based on structural transitions of RNA modules located in the 5' untranslated region of mRNA. The regulation mechanism act on the level of transcription as well as translation. Riboswitches sense changes in the concentration of small molecule metabolites while RNA thermometers respond to temperature changes.

In this contribution, we will describe novel NMR methods to monitor such structural transitions at atomic resolution. Elucidation of the coupling of binding or melting into allosteric structural transitions at the heart of the regulation events will be discussed with the examples of translational riboswitches.

PL 14

Imaging Metabolism – Watching Tumours Gasp and Die with Hyperpolarized MRI

Kevin Brindle

University of Cambridge, Cambridge, UK

Patients with similar tumour types can have markedly different responses to the same therapy. The development of new treatments would benefit, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment [1].

We have been developing methods for detecting the early responses of tumours to therapy, including magnetic resonance (MR) imaging of tumour cell metabolism using hyperpolarized ^{13}C-labelled cellular metabolites (reviewed in [2,3]). Nuclear spin hyperpolarization can increase sensitivity in the MR experiment by >10,000x. This has allowed us to image the location of labelled cell substrates and, more importantly, their metabolic conversion into other metabolites. These substrates include pyruvate, lactate, glutamine, glutamate, fumarate, bicarbonate and ascorbate. We have shown that exchange of hyperpolarized ^{13}C label between lactate and pyruvate can be imaged in animal models of lymphoma and glioma and that this flux is decreased post-treatment. We showed that hyperpolarized [1,4-^{13}C]fumarate can be used to detect tumour cell necrosis post treatment in lymphoma and that both the polarized pyruvate and fumarate experiments can detect early evidence of treatment response in a breast tumour model and also early responses to anti-vascular and anti-angiogenic drugs. We have shown that tissue pH can be imaged from the ratio of the signal intensities of hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ following intravenous injection of hyperpolarized $H^{13}CO_3^-$ and that tumour redox state can be determined by monitoring the oxidation and reduction of [1-^{13}C]ascorbate and [1-^{13}C]dehydroascorbate respectively.

We have recently obtained funding for clinical trials with polarised pyruvate and fumarate to detect treatment response in lymphoma, glioma and breast cancer patients.

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2. Brindle, K.M., *et al. Magn Reson Med* **66**, 505-519 (2011).
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PL 15

New Methods for EPR and NMR

Thomas Prisner

Institute of Physical and Theoretical Chemistry, Center of Biomolecular Magnetic Resonance Goethe University, Frankfurt, Germany

Efficient excitation of electron spin resonance transitions is hampered in many cases, because of broad linewidth and short relaxation times. This reduces the efficiency of many pulse EPR experiments as well as efficient polarization transfer to nuclear spins in ENDOR or DNP applications, especially at high magnetic fields. In this presentation we will show new methodological and technical approaches to overcome some of these limitations in EPR and DNP spectroscopy. We developed a fast pulse shaping unit allowing 1 ns time resolution and arbitrary phases for microwave EPR pulses, which allows for the first time to excite electronic spins with optimum control pulses (1). Furthermore, new double resonance structures working at 400 MHz for proton and 260 GHz for electron spins (corresponding to a magnetic field of 9.2 T) allow efficient excitation and detection of EPR and NMR transitions in liquids (2). First applications to pulsed EPR and DNP will be shown and the potential of these new methods discussed.

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2) V. Denysenkov, & T. Prisner, (2012) Liquid State Dynamic Nuclear Polarization Probe with Fabry-Perot Resonator at 9.2 Tesla *J. Magn.Reson.*, **217**, 1-5

Parallel Session Lectures

PS 101

Decoupling and Recoupling Using Phase-Alternating Pulse Sequences

Kong Ooi Tan, Vipin Agarwal, Anders Nielsen, Matthias Ernst
ETH Zürich, Zürich, Switzerland

Using phase-alternating pulse sequences in recoupling or decoupling experiments opens up new possibilities due to the fact that they have additional degrees of freedom. The location of potential resonance conditions is only determined by the modulation frequency while the rf-field amplitude determines the strengths of the various resonance conditions. In cw irradiation, the strength of the resonance condition is constant and the location is determined by the rf-field amplitude. Using an interaction-frame picture, one can determine the Fourier coefficients that characterize the strength of the resonance conditions analytically and numerically and use Floquet theory to understand the sequences.

In my presentation I will show two examples where replacing cw irradiation by phase-alternating irradiation allows either the implementation of new experiments or improving existing experiments. The first example concerns the PAIN/PAR second-order recoupling sequences. [1] In the heteronuclear PAIN experiment, one always has simultaneous PAR polarization transfer. This can be beneficial and used in novel experiments for simultaneously recording homonuclear and heteronuclear correlation experiments to obtain structural restraints. [2] On the other hand, it is also often unwanted since the homonuclear transfer leads to relay peaks that make the heteronuclear experiment less useful. Using a heteronuclear version of the RESORT experiment, we are able to suppress the homonuclear polarization transfer to a large extent while at the same time kepp the heteronuclear transfer. The second example concerns decoupling during a C9-based TOBSY sequence for J-coupling polarization transfer. [3] Using phase-alternating decoupling sequences, it is possible to improve the polarization-transfer efficiency and also make the selection of the decoupling rf-field amplitude more straightforward than in cw decoupling.

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[3] E. Hardy, R. Verel, B. Meier, Journal of Magnetic Resonance. 148 (2001) 459–464.

PS 102

^{1}H{overtone-^{14}N} HMQC above 110 kHz MAS

Yusuke Nishiyama, Yuki Endo, Takahiro Nemoto
JEOL RESONANCE Inc., Tokyo, Japan

The ^{1}H{overtone-^{14}N} HMQC correlation at very fast MAS is presented. The pulse sequence is the same as standard HMQC but the ^{14}N pulses are irradiated with the frequency twice the ^{14}N Larmor frequency. The ^{14}N DQ coherence is directly manipulated by these overtone-^{14}N irradiations. The ^{1}H{overtone-^{14}N} HMQC at very fast MAS has several advantages over ^{1}H{double-quantum (DQ) / single-quantum (SQ) ^{14}N} HMQC and direct overtone-^{14}N observation.

1) The overtone ^{14}N spectra are free from the first order quadrupolar broadening thus the fine adjustment of the magic angle is not required.
2) The indirect detection of overtone-^{14}N spectra do not show spinning frequency dependent shifts which is observed in the direct observation of overtone-^{14}N MAS spectra [1].
3) The ^{1}H{overtone-^{14}N} HMQC gives higher sensitivity than ^{1}H{DQ ^{14}N} HMQC.
4) The arbitrary spectral width of the overtone-^{14}N dimension can be used, since there is no need to synchronize the indirect dimension to sample spinning.
5) The very fast MAS greatly improved the efficiency of ^{1}H{overtone-^{14}N} HMQC.[2]

Overtone-^{14}N is the second order process and thus requires strong rf irradiation. The recently introduced 0.75 mm MAS system helps us to efficiently observe ^{1}H{overtone-^{14}N} HMQC spectra with the help of micro-coil system and very fast MAS above 110 kHz.

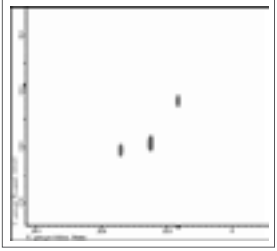

Figure ^{1}H{overtone-^{14}N} HMQC of L-histidine at 70 kHz MAS

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PS 103

Fast Access to Residual Dipolar Couplings by Single-Scan 2D NMR in Oriented Media

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The utility of residual dipolar couplings for determining the structure of small organic molecules is increasingly recognized[1]. The most frequently used RDCs are $^{1}D_{CH}$, which can be measured either by F_2- or F_1-coupled HSQC experiments. While the efficiency of RDCs is highly recognized, these approaches suffer from the long experiment times characterizing 2D NMR experiments, due to the need to record numerous t_1 increments to sample the indirect dimension. If one would want to investigate transient species, these measurement times need to be reduced significantly.

NMR spectroscopists have designed several approaches dealing with the time drawback of multi-dimensional NMR experiments. One of the most impressive and efficient is probably the "ultrafast" 2D NMR methodology, recently proposed by L. Frydman and co-workers [2], capable of providing a complete 2D correlation in a single scan, ie in a fraction of a second. In the last five years, we have significantly improved the performances (sensitivity, resolution, lineshape, etc.) of this approach [3], thus increasing its range of applications. Here, we present an original NMR method to measure Residual Dipolar Couplings in a very short time, by ultrafast 2D NMR experiments performed for the first time in oriented media.

We designed an ultrafast coupled-HSQC pulse sequence capable of directly giving access to carbon-proton couplings in a single scan The dipolar couplings extracted from these experiments are in very good agreement with those obtained from conventional experiments. These results highlight the potentialities of ultrafast 2D NMR as a tool for the structural study of organic compounds, and open promising perspectives in the field of small molecule NMR analysis.

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PS 104

Efficient Coherence Transfer Methods for Biological Solid-State NMR

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Biological solid-state NMR relies on samples with many combinations of ^{15}N, ^{13}C, and ^{2}H labelling in addition to varying amounts of ^{1}H spins. Combined with multiple-dimensional experiments these samples offer multiple opportunities for assignments and establishment of structural constraints. Such experiments use numerous coherence transfer elements bringing magnetization from spin to spin. Also different spin species gives different possibilities to exploit polarization from different sources. With many elements involved, it is important that losses in the transfer processes are minimized as much as possible in each individual to maintain high sensitivity.

In this presentation, we address attention to systematic design of improved homo- and heteronuclear coherence transfer elements for biological solid-state NMR developed using optimal control and analytical methods. We present optimal control and analytical pulse sequences offering sensitivity-enhanced heteronuclear coherence transfer in ^{1}H-^{15}N, ^{1}H-^{13}C, ^{15}N-^{13}C spin-pair[1,2] and ^{2}H-^{13}C and ^{2}H-^{15}N spin-pair[2] systems. For homonuclear transfers, we address attention to resolution and sensitivity enhancements through spin-state-[4] and coherence-order selective[5] transfer. Finally, we demonstrate sensitivity enhancement by simultaneous[6] or interleaved[7] sampling of spectra provided through different polarization sources or different coherence transfer pathways.

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PS 105

Greater than the Sum of the Parts : Using Data Fusion to Improve the Sensitivity of 1H HR-MAS NMR Spectroscopy in Breast Cancer

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The aim of the METAcancer project (www.metacancer-fp7.eu) is to investigate metabolites that can be used as prognostic and predictive biomarkers by applying different metabolic profiling technologies (i.e. NMR, GC-MS and LC-MS) to maximize the coverage of the breast cancer metabolome. Data fusion strategies can be applied to elucidate the combined potential of the multiple profiling techniques for the diagnosis and classification of breast cancer. One commonly used strategy consists of building separate classification models for the individual platforms and subsequently aggregating the individual model outcomes. By doing so it facilitates a comparison and ranking of the diagnostic potential of each platform. However, this fusion on the "model level" provides only limited insight into the relative importance of individual metabolites with regard to the disease and is furthermore unable to elucidate the interrelationship amongst the variables, or the shared common structure across the different platforms. An alternative approach is to use multi-block (MB) based approaches. Here we present the use of MB-Principal components analysis and MB-partial least squares analysis to create data fusion models that dramatically improve the predictive capability of the high resolution MAS 1H NMR spectra collected of the breast tumours.

PS 106

High-Throughput Serum NMR – The New Era in Epidemiology & Genetics

Mika Ala-Korpela

Computational Medicine, University of Oulu, Oulu, Finland

Comprehensive approaches to gain insights into metabolic variation are becoming increasingly popular in order to understand disease aetiologies and to develop metabolic phenotyping for holistic risk assessment and diagnostics. Towards these goals, we have set-up an automated high-throughput platform for serum NMR metabolomics (1) that has now (May 2012) been operational for over 3 years with around 90,000 samples from a wide variety of epidemiological studies analysed. The methodology enables absolute quantification of specific molecular identities and we have recently presented genome-wide association and heritability results for 117 directly detected metabolic measures and 99 variables derived from these measures (2). The primary metabolic information includes extensive characterisation of 14 lipoprotein subclasses and their lipid constituents together with various low-molecular-weight metabolites. These molecular data relate to multiple biological pathways and metabolic functions in health and in disease. Consequently, combined with genome-wide and gene expression data at the population level, this comprehensive metabolic information has started to trigger detailed systems-level findings (3-5). This novel line of systems epidemiology is anticipated to grow rapidly and to allow a more thorough molecular understanding of biochemical pathways and disease pathologies. Yet metabolomics will be truly useful in epidemiology or in genetic studies only if quantitative data on specific, identified metabolites are available. During the talk the technological as well as the metabolic characteristics of the new platform will be presented and discussed in relation to epidemiology and genetics (6,7).

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PS 107

In-Cell NMR in Mammalian Cells

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Protein structures and dynamics are intricately connected to a biomolecule's physiological environment. While most proteins function inside cells, our knowledge about protein structure and dynamics is usually obtained from artificial experimental setups that bear little resemblance to the physical and biological properties of live cells. Modern methods in high-resolution in-cell NMR spectroscopy now offer the possibility to correct those deeds.

Here, we present initial sets of multi-dimensional in-cell NMR measurements of protein structures and dynamics in five different mammalian cell types.

PS 108

Macromolecular Crowding and Protein Chemistry: Views from Inside and Outside Cells

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I will discuss the successes and challenges of in-cell protein NMR and the quantification of protein folding, stability, and diffusion under crowded conditions. One challenge is the inability to observe ^{15}N-^{1}H HSQC spectra from most globular proteins in cells. To understand this problem we turned to in vitro experiments, using synthetic polymers, globular proteins, and cell lysates to assess how these crowding agents affect protein diffusion. To examine stability, we used NMR-detected amide-proton exchange to quantify the opening free energy of test proteins in the presence of crowding agents. To examine folding in cells, we applied ^{19}F NMR to an unstable test protein. Our results highlight the differences between crowding agents and shed light on the biological effects of crowding.

PS 109

NMR STUDIES OF MOLECULAR RECOGNITION AND DYNAMICS OF (LARGE) PROTEIN COMPLEXES IN SOLUTION

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Eukaryotic multi-domain proteins play crucial roles in the regulation of gene expression and cellular signalling. As their conformations often depend on dynamic domain rearrangements it is important to use solution techniques for their structural analysis [1]. Examples will be presented that highlight the role of conformational dynamics during molecular recognition: i) the recognition of poly-pyrimidine tract RNA by the essential splicing factor U2AF65 during spliceosome assembly and ii) the recognition of dimethyl-arginines by Tudor domains in snRNP maturation.

We have developed a versatile and efficient protocol for determining the quaternary structure of multi-domain proteins and protein complexes in solution by combining experimental data derived from solution state NMR as well as Small Angle X-ray and/or Neutron Scattering (SAXS/SANS) experiments [2-4]. Information about the relative orientation of domains or subunits is obtained from NMR residual dipolar couplings (RDCs). Long-range (up to 20Å) distance restraints are obtained from paramagnetic relaxation enhancements (PRE) using spin-labeled proteins and/or RNA. In addition, solvent PRE data can be used for structural refinement of molecular interfaces. The RDCs, PREs (from covalent spin labels), solvent PREs and SAS data are jointly used for structure calculation in ARIA/CNS supplemented with additional information from chemical shift perturbation or biochemical data. We employ this protocol for studying large protein complexes that are linked to the recognition of pre-mRNA during the assembly of the spliceosome. Current progress with these studies will be presented.

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PS 110

STRUCTURAL MECHANISM OF CALMODULIN ACTIVATION AND AUTOINHIBITION OF CAMK1 KINASE

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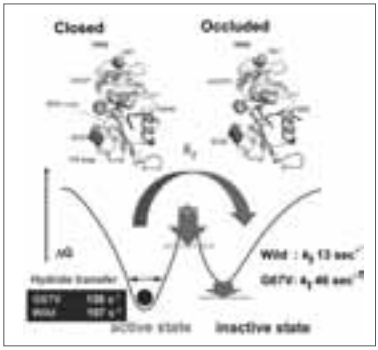
The structure of CaMK1 has been characterized in its monomeric and dimeric states by NMR spectroscopy and X-ray crystallography, respectively, revealing an unprecedented N-terminal strand exchange. The activation loops of the autoinhibited enzyme are seen by NMR to form an open, unstructured ensemble, permitting only millimolar affinity ATP interaction, despite induction of chemical shift perturbations across the two lobes. Calmodulin dislodges the regulatory C-terminal helix from its autoinhibitory, pseudo-substrate position in a stepwise fashion. This culminates in an activated state which adopts a novel, in-line three-lobed complexed structure as resolved by small-angle X-ray scattering. Engagement of the active site by a small molecule inhibitor engages a regulatory helix, and induces perturbations across both kinase lobes. The diversity of calmodulin-mediated regulatory states adopted in kinase structures is discussed, as is the utility of regulatory helix clampdown for inhibitor design.



PS 111

Functionally Detuning Motion for the Hydride Transfer Step, Which Is Intrinsically Encoded in the Active Loop Dynamics of Dihydrofolate Reductase, DHFR

Shin-ichi Tate

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Elucidation of the functional role of the protein structural dynamics is essential for expanding our understanding how protein works. In exploring the functional significance of the specific protein motion, carefully designed site-directed mutagenesis is needed; it changes the motion but keeps the active site structure. Given that type of mutants become available, we would get clear insights into the functional role of protein dynamics *per se* with distinguished it from the structural factors. Here, we report the case study using such elaborate mutant to see the role of structural dynamics.

My group has been studying the functional effects of the distal site mutation from the active site of dihydrofolate reductase, DHFR. Among the collected data, we found that amino acid changes to G67 caused substantial modulations to the enzyme activities. G67 is placed in the flexible loop far distant from the active site of DHFR. One remarkable mutant, G67V, showed about 20% reduction in the hydride transfer rate relative to the wild-type. This observation inspired us to think the G67V mutant is the one to change the functionally relevant structural dynamics in the active site with keeping the structure intact.

NMR spectral comparison between the wild-type and G67V confirmed that the mutation caused no apparent structural changes on the active site. R_2 relaxation-rate dispersion experiments, however, showed that G67V mutant has the three-time more frequent structural transition from the ground-state to the excited-state structures. The results showed that DHFR has an intrinsic motion to detune the hydride-transfer step, which is associated with the large-amplitude loop structural change between the closed and occluded forms. The possible biological significance of the functionally impairing mechanism encoded in DHFR structure dynamics will be discussed.

PS 112

The Architecture of Telomerase

Juli Feigon

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Telomerase is a ribonucleoprotein complex essential for maintenance of telomere DNA at the ends of linear chromosomes. We are investigating the structure, function, and protein interactions of verterbrate and ciliate telomerase RNA.

The catalytic core of Tetrahymena telomerase comprises a ternary complex of telomerase RNA (TER), telomerase reverse transcriptase (TERT), and the essential La family protein p65. We have used a combined NMR and X-ray crystallography approach to determine the structure of p65 C-terminal domain and its complex with stem IV of TER. The structure reveals that RNA recognition is achieved by a novel combination of single- and double-strand RNA binding, which induces a dramatic bend in TER. The domain is a cryptic, atypical RNA recognition motif with a disordered C-terminal extension that forms a α-helix in the complex necessary for hierarchical assembly of TERT with p65-TER. This work provides the first structural insight into biogenesis and assembly of TER with a telomerase-specific protein. Additionally, our studies define a structurally homologous domain in genuine La and LARP7 proteins and suggest a general mode of RNA binding for biogenesis of their diverse RNA targets.

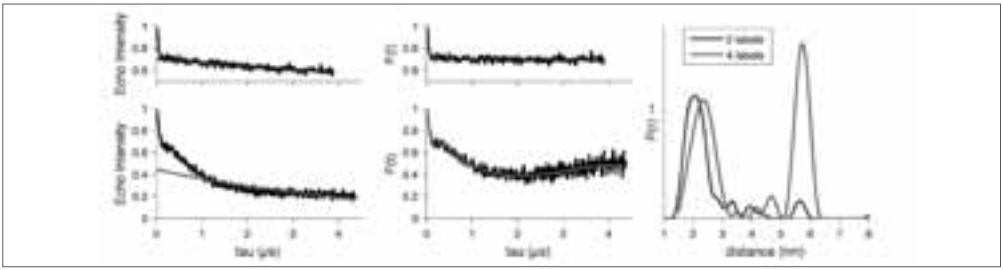
Human telomerase RNA contains a catalytic core including a conserved pseudoknot essential for function. We have determined the structures and investigated the dynamics of sub-domains of this catalytic core using NMR, and modeled the structure of the complete ~150 nucleotide core domain.

PS 113

FROM SOLID STATE PHYSICS TO STRUCTURAL BIOLOGY: PUTTING A SPIN ON IT WITH EPR SPECTROSCOPY

Chris Kay
University College London, London, UK

We are using pulsed EPR spectroscopy to solve problems ranging from physics to biology and in this talk I will give a few examples of our ongoing work in which either intrinsic electron spin centers such as group V dopants in silicon [1] or nitroxide spin labels engineered into proteins by site-directed mutagenesis [2] allow exquisite structural information to be extracted from the sample. The hyperfine interaction between an electron and a neighbouring nucleus, which may be determined by ENDOR, gives information on the spatial distribution of the wavefunction and allows interactions with $I \neq 0$ nuclei in the vicinity of a spin to be explored, while in structural biology, measuring spin-spin interactions between pairs of electrons by ELDOR allows distances of up to 7 nm to be determined.



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PS 114

INVESTIGATION OF ELECTRON SPIN RELAXATION AND SPECTRAL DIFFUSION USING TWO-DIMENSIONAL INVERSE LAPLACE TRANSFORMS

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Solid state dynamic nuclear polarisation (DNP) can be described using the solid effect, cross effect and thermal mixing mechanisms. A current area of interest is an extension of our theoretical understanding of these mechanisms, and in particular an improvement of simulations used to describe DNP experiments [1]. Electron spin relaxation and spectral diffusion play a fundamental role in the mechanisms of DNP, particularly for radicals with inhomogeneously broadened EPR lines. Therefore, a better understanding of these processes and experimental characterisation of the corresponding time constants is essential for a complete understanding of DNP enhancement.

To investigate the behaviour of electron spins during and after constant microwave (MW) irradiation, three-dimensional 'pump-recovery' experiments were performed as follows: the MW field was switched on for a time t_{pump}, off for a time t_{rec}, and the electron magnetisation remaining after these two time intervals was measured using longitudinal detection. t_{pump} and t_{rec} were incremented along the first two dimensions. MW pumping was done at a frequency close to the centre of the inhomogeneously broadened EPR line, while detection frequency was incremented along a third dimension.

Data were processed using a home-written two-dimensional inverse Laplace transform tool: the data were fitted to exponential decay kernels to extract distributions of the electron T_1 and T_{pump}, where T_{pump} is a phenomenological time constant describing the decay of the electron polarisation during the MW irradiation. The algorithm, which will be presented in some detail, does not use a non-negativity constraint, and therefore does not make any assumptions regarding the signs of the different signal components.

All time constant distributions contained at least two peaks with opposite signs at similar T_1 values but different T_{pump} values. The results of experiments conducted at different 4-Amino-TEMPO radical concentrations, temperatures, and microwave pump frequencies will be presented. Implications for the optimisation of DNP experiments will be discussed.

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PS 115

Cryptochromes: Potential Compass Molecules with an Unexpected Variety of Electron Transfer Pathways

Till Biskup[1,2], Asako Okafuji[2], Bernd Paulus[2], Kenichi Hitomi[3], Elizabeth D. Getzoff[3], Erik Schleicher[2], Stefan Weber[2]

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Cryptochromes (Cry) and their siblings, the photolyases (PHR), occur in all three kingdoms of life. Whereas PHRs repair specific UV-induced DNA damages, the Crys' functions are diverse ranging from controlling plant growth and development and entraining the circadian clock to being a candidate magnetoreceptor in, e.g., migratory birds. PHRs are well-known to readily form radical pairs (RP) between flavin (FAD) and a tryptophan (Trp) upon illumination with blue light if starting from fully oxidised FAD. In analogy, this reaction is discussed as main primary photoreaction in Crys, and it is these RPs that are at the heart of the postulated magnetoreception capabilities of those proteins.

Using time-resolved EPR (TREPR) on a Cry from Xenopus laevis, we could for the first time demonstrate that Crys indeed form spin-correlated RPs between FAD and Trp upon blue-light excitation [1]. Additionally, we assigned the RP partners and showed that the magnetic coupling parameters of these RPs are in favour of sensing weak magnetic fields. Furthermore, with a multi-frequency TREPR approach we could unequivocally demonstrate the formed spin-correlated RPs to originate from a pure singlet-precursor state [2]

Extending our studies to another Cry, from *Synechocystis* sp., revealed a striking diversity in electron transfer (ET) pathways in Crys [3]: Although the conserved ET chain of three Trps (TrpA to C), common to most PHRs and Crys, is clearly present, instead of the terminal TrpC an alternative TrpC' further apart from TrpB than TrpC gets used as final electron donor, as we showed by applying TREPR to wild-type and mutant proteins [3].

Finally, we could show that blocking the ET pathway in *X. laevis* Cry at the first or second position (TrpA or TrpB) leads to an alternative ET involving two tyrosines (Tyr). This demonstrates that TREPR even at X-band can clearly distinguish between FAD-Trp and FAD-Tyr RPs. Taken together, these results reveal an unexpected diversity in ET pathways in Crys and show that EPR is clearly suited to investigating biological ET.

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PS 116

Distance Measurements and Dynamic Nuclear Polarization at 9 and 94 GHz EPR Frequencies

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Pulsed EPR techniques, in particular the two-frequency DEER or PELDOR method, have been established over the past years as a valuable tool to obtain distances and their distributions between paramagnetic centers in biological systems. At high EPR frequencies ($\nu \geq 90$ GHz) the EPR spectrum of spin labels is dominated by g anisotropy and, because of the selective excitation of molecular orientations, the experiments can deliver information not only about the distance but also about the relative orientation of the labels. The technical issues related with the performance of these experiments at high EPR frequencies will be discussed and a new experimental design is presented that provides the possibility of performing these experiments with nitroxide radicals. Another technique that has experienced a renaissance in the past years is dynamic nuclear polarization. The method could provide a means to overcome the current sensitivity limits in solution and solid state NMR. Enhancement of the nuclear spin polarization via DNP requires optimized pumping (saturation) of the electron spins with highly efficient microwave irradiation, suitable polarizing agents and knowledge about electron-nuclear spin relaxation. In the past years, we have performed extensive studies on DNP in aqueous solutions at 9 and 94 GHz using different polarizers. These studies have allowed to quantitatively rationalize the observed enhancements in terms of the Overhauser mechanism. Furthermore, they were employed to design and optimize a shuttle spectrometer for liquid DNP that opens the door to applications in NMR spectroscopy of biological samples.

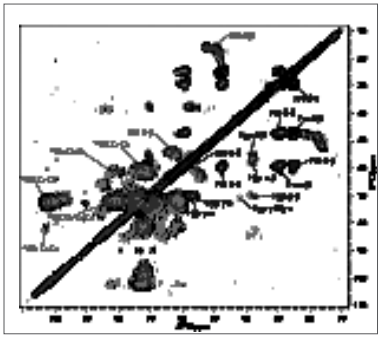
PS 117

Heavy Mice and Lighter Things: Using NMR to Elucidate Molecular Structures in Tissues

Melinda Duer[1], Wing Ying Chow[1], David Reid[1], Roger Brooks[1], Rakesh Rajan[1], Dominique Bihan[1], Richard Farndale[1], David Slatter[1], Jeremy Skepper[1], Cathy Shanahan[2]

[1]University of Cambridge, Cambridge, UK, [2]Kings College London, London, UK

At the molecular level, it is the extracellular matrix (ECM) that forms the basic substance of tissues. The ECM gives tissues their mechanical strength and provides the environment for cells to function, including providing essential pathways for cell signalling. However, how the ECM provides its various mechanical and biological functions remains largely elusive, primarily because of a lack of structural information for most of the molecules involved and even less on how they interact. The ECM is primarily composed of fibril-forming ***collagen proteins***, interspersed by a mesh of proteoglycans and other molecules. Collagens are triple helical proteins with of order 1000 residues per chain. An X-ray diffraction structure reveals the molecular packing within a pure collagen fibre, but not the atomic positions. In biology, however, collagens are not present as fibres of pure material but rather form microfibrils in which the collagen triple helices are glycosylated and interacting with a wide range of other molecules. The structure of collagens in their native environments are not currently known, yet it is this structure that confers the material properties of the tissue and that cells of the tissue interact with.



Solid-state NMR can in principle reveal molecular structure information for proteins in heterogeneous environments, but the necessary multidimensional experiments are fraught with difficulties at natural abundance levels of ^{13}C and ^{15}N.

This talk will describe recent developments in the Duer group that allow the way forward for detailed structure determination work of collagens in native tissues and in in vitro models for native tissues. This then opens the possibility of examining molecular structures in diseased tissues or tissues affected by diseases such as diabetes, in order to understand the pathology at a molecular level for the purposes of designing highly targeted, possibly even individual, therapeutic strategies.

PS 118

High-Resolution Structure of a Seven-Helical Membrane Protein Determined by Solid-State NMR

Shenlin Wang[1], Lichi Shi[1], Izuru Kawamura[2], So-Young Kim[3], Kwang-Hwan Jung[3], Leonid S. Brown[1], Vladimir Ladizhansky[1]

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Solid state NMR (ssNMR) spectroscopy is emerging as a powerful technique for studying structures of membrane proteins and provides the unique opportunity in investigating membrane protein structures in native-like membrane-mimicking environments. Recent advancements of ssNMR have demonstrated the feasibility of obtaining high resolution 3D structures of large membrane proteins. In this presentation, I will show our recently solved ssNMR structure of a 28 KDa lipid-embedded seven-helix transmembrane (7TM) photosensor, sensory rhodopsin from Aneabena sp. PCC 7120 (ASR). The structure was determined using distance restraints obtained from analysis of carbon-carbon spin-diffusion spectra of alternately labeled samples, together with dihedral angle restraints obtained from chemical shifts, and further refined using long range distance restraints derived from measurements of paramagnetic relaxation enhancement in single mutant ASRs containing covalently-attached paramagnetic tag. The overall fold of ASR contains seven well-defined α-helices forming a helical bundle, sharing a similar architecture with G-protein couple receptors and other bacterial rhodopsins with known structures. A short β-hairpin was found to link the second and the third helix of ASR in ssNMR structure, while this region is disordered in the X-ray structure. The site-specific dynamic properties and light-induced conformational changes of ASR in lipid environment were also studied by ssNMR. We found that the cytoplasmic halves of several helices undergo significant structural rearrangement upon illumination. The structural and dynamic properties characterized here provided hints for understanding the photosensory function of ASR. The developed methodology can be applied to other 7TM proteins, e.g., those of the family of G protein–coupled receptors, in the future.

PS 119

SOLID-STATE NMR REVEALS THE STRUCTURAL ARCHITECTURE OF *SHIGELLA FLEXNERI* TYPE-III SECRETION NEEDLES

Jean-Philippe Demers[1], Nikolas Sgourakis[2], Antoine Loquet[1], Karin Giller[1], Stefan Becker[1], David Baker[2], Adam Lange[1]

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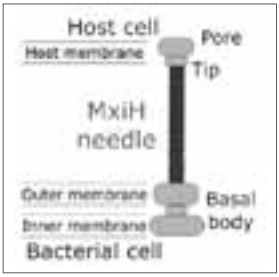

Type-III Secretion Systems (T3SS) are present in many gram-negative bacteria, where they are an essential determinant for bacterial infection. T3SS are formed of ~25 proteins and consist of a hollow needle and a basal body which anchors the needle to both bacterial membranes. The needle extends into the extracellular space where it makes contact with the host cell. Upon contact, translocator proteins form a pore through which effector proteins enter and alter the function of the host cell during infection.

Based on 2D solid-state NMR experiments recorded on uniformly, [1-^{13}C]-glucose and [2-^{13}C]-glucose labeled proteins, we have obtained the 95%-complete assignment of ^{13}C and ^{15}N chemical shifts for the needle of *Shigella flexneri* bacteria which cause bacillary dysentery in human. Very sharp ^{13}C line-widths are observed, ranging from 0.3 to 0.6 ppm in the uniformly labeled sample and from 0.1 to 0.4 ppm in sparsely labeled samples. Only the first 3 N-terminal residues are flexible; in contrast to the crystal structure of MxiH monomer where the first 14 N-terminal residues are disordered (Deane et al, PNAS, 2006). The secondary structure consists of a long α-helix (L12 to A38), a loop (E39 to N43) and a second α-helix which extends up to the C-terminus (P43 to R83). Both α-helices are kinked, respectively at residues T23 and Q64. Although rigid, the N-terminal segment from S2 to T11 does not adopt any conventional secondary structure. The solid-state chemical shifts are incompatible with the possibility of a 13-residue-long protrusion within the second α-helix, which was proposed from 3D image reconstruction of the Shigella needle by electron cryo-microscopy (Fujii et al, PNAS, 2012). The secondary structure elements are highly similar to those identified in the homologous needle protein of *Salmonella typhimurium* (Loquet et al, Nature, 2012; Loquet et al, JACS, 2011), allowing us to propose a general structural architecture of T3SS needle assemblies.

PS 120

PUSHING FOR RESOLUTION IN ^{13}C SPECTRA OF UNIFORMLY LABELLED PROTEINS

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While the resolution in ^{13}C spectra of uniformly labelled proteins has continuously improved over the last decades, further improvements are still badly needed. The presence of scalar homonuclear ^{13}C - ^{13}C J couplings, which are often only partially resolved, limits the resolution and increases peaks overlap. Here we demonstrate significant improvements in resolution for a ^{13}C - ^{13}C correlation experiment by J-decoupling in both the indirect and direct dimension. Using S3E (Spin State Selective Excitation), developed by Sørensen and coworkers [1], for the direct dimension and optimized decoupling we obtained high-resolution C_{alpha} - CO correlation spectra, we achieve a line width of 16 Hz in the indirect dimension and 20 Hz in the direct one for ubiquitin.

The contribution will discuss the experimental conditions and requirements and also compare the results obtained with other studies of J-decoupling in solid-state NMR.

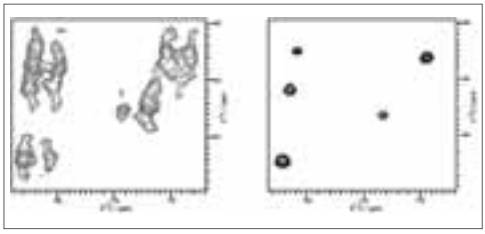

Fig: 50 ms MIRROR experiment on Ubiquitin without (left) and with (right) applying J decoupling. Spectra were measured on 500MHz spectrometer, at 25 kHz MAS and applying 120 kHz XiX decoupling.

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PS 121

Novel NMR Methods with High Resolution and Sensitivity: from Protein Structures to Nanotubes

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New ^{1}H and ^{13}C-detected NMR methods in solution and solid-state to unravel structural, dynamic and functional roles of biomolecules in high-resolution will be presented. These experiments provide chemical shift correlations of backbone, side chain and methyl groups with high resolution, selectivity and sensitivity. Some of the experiments facilitate fast data acquisition and are augmented by novel isotope labelling schemes. These methods have been applied to different biological systems of which one particular system involves the insulin-like growth factor binding proteins (IGFBPs), a family of 6 homologous proteins about 30 kDa in size and implicated in different types of cancers. IGFBPs bind insulin-like growth factors (IGF) strongly and regulate the activity of the latter. We recently discovered that a C-terminal fragment of IGFBP-2 self-assembles spontaneously and reversibly into soluble nanotubular structures several micrometers long via a mechanism involving inter-molecular disulphide bonds and exhibiting enhanced fluorescence. In addition to this system, application of the new methods for structural studies of paramagnetic proteins and intrinsically disordered systems will be presented.

PS 122

Revisiting Decades-Old Spin Physics to Improve Modern Magnetic Resonance Imaging

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This talk will re-examine a variety of magnetic resonance effects which were assumed to be well characterized decades ago, but which in fact were not completely understood; the consequences of this re-examination is prediction and demonstration of new sources of contrast, and an improved quantitative understanding of imaging effects. First, we will show that the classical treatment of dipolar effects in solution, as used by numerous groups (including ours) over the last few decades, contains some important oversimplifications; when corrected, this leads to new pulse sequence effects (secular Hamiltonian proportional to Ix; homonuclear decoupling with gradient pulses alone; large frequency shifts from unmodulated, spherical magnetization). Second, we have found experimentally that equal spacing between multiple echo pulses (the traditional Carr-Purcell-Meiboom-Gill sequence) often does not provide optimal refocusing in imaging applications, particularly with fatty tissue. Many effects contribute, but the most important (and frequently overlooked) is that such sequences combine with J couplings and small chemical shift differences to take spins out of the first-order limit, even at high fields. Surprisingly, a specific nonintuitive set of delays originally derived for quantum computing often gives more signal than CPMG, for the same total delay and total number of pulses, and provides new sources of contrast based on fat composition. Finally, Hyperpolarized imaging shows great promise for clinical applications, but a major challenge is short T1 relaxation times. T1 relaxation was thought to be uncontrollable by pulse sequences, until work by Levitt on singlet states between inequivalent spins showed that longer lived states are accessible. We have generalized this to show that pairs of equivalent spins permit storage of polarization in molecular states with extremely long values of T1. We will discuss recently developed methods to store and extract population from these states, with or without chemical transformation. For example, in an A2X2 spin system, the “triplet-triplet” state X has dipole allowed transitions from many other states, but the “singlet-singlet” state does not, and they can be interconverted with specific pulse sequences. We discuss the classes of molecules which have long-lived states of this form, evaluate the lifetime using the SPINACH program, and present experimental data on several such molecules.

PS 123

DETECTION OF EXCITED STATES OF PROTEINS BY HIGH PRESSURE NMR SPECTROSCOPY - A NEW STRATEGY FOR RATIONAL DRUG DESIGN

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Protein-protein interactions are usually assumed to be difficult to address since the interaction sites are not suited for designing low-molecular mass compounds that selectively recognize the interaction site and can compete with the protein-protein interaction. Here, we describe a general strategy for drug design that is based on the detection of rare "excited" states by high-pressure NMR spectroscopy and their use for a direct or competitive or the allosteric modulation of characteristic properties (as protein-protein interactions or enzymatic activity). 1D and 2D dynamic pressure perturbation spectroscopy can be used to characterize the kinetic properties of the candidate compounds [3].

We show the fundamentals of the proposed strategy on the Ras protein complexed with GTP analogue GppNHp. The interaction of activated Ras (Ras.Mg^{2+}.GppNHp) with the effector Raf-kinase can effectively be inhibited by the small compounds such as Zn^{2+}-cyclen [1,2] that stabilise conformational state 1(T) of the protein that has a low affinity for effectors. With Zn2+-BPA we could identify a compound that binds distant from the active centre of Ras but nevertheless allosterically inhibits the the Ras interaction.

In Ras activated by GppNHp binding we could identify four different conformational states by high pressure NMR spectroscopy that are in principle drugable. Compared to the ground state 2(T) they are characterized by smaller specific volumes, the specific volume change ΔV are - 18, -43.7, and - 46.9 ml mol^{-1}.

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PS 124

CONTROLLING J MODULATION: NEW SPIN ECHO AND PURE SHIFT NMR TECHNIQUES

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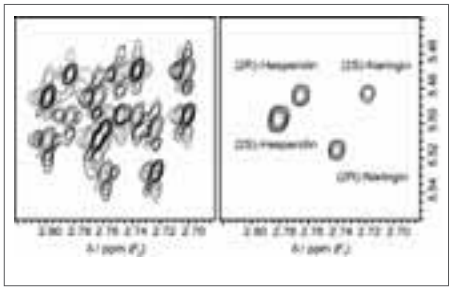

Scalar coupling is a key source of information on chemical structure, but is also an unwelcome source of complications. J modulation of spin echoes can severely complicate their use for T_2 measurement or filtration, while homonuclear multiplet structure greatly reduces the spectral resolution of proton NMR. It has been known since 1983 that J modulation in an AX spin system can be reversed by a quadrature 90° pulse[1], and refocused in a "perfect echo"[2], but the method is in fact general for short echo times, allowing efficient suppression of J modulation at low RF duty cycle in CPMG-type experiments[3], for example in metabolomics. Broadband homonuclear decoupling methods have been sought since the early days of NMR, but are only now becoming practical tools[4]. Proton multiplet structure can be suppressed by a variety of methods, in both 1D and 2D experiments. The figure to the right, for the 3QF-COSY spectrum of a mixture of natural products, illustrates the improvement in resolution obtainable.

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PS 125

Reliable and Flexible Automated Assignment of NMR Spectra

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The new FLYA algorithm for automated NMR resonance assignment determines chemical shift assignments on the basis of peak lists from any combination of multidimensional through-bond or through-space NMR experiments for proteins. Backbone and side chain assignments can be determined. All experimental data is used simultaneously, thereby exploiting optimally the redundancy present in the input peak lists and circumventing potential pitfalls of assignment strategies in which results obtained in a given step remain fixed input data for subsequent steps. Instead of prescribing a specific assignment strategy, the FLYA algorithm requires only experimental peak lists and the primary structure of the protein, from which the peaks expected in a given spectrum can be generated by applying a set of rules, defined in a straightforward way by specifying through-bond or through-space magnetization transfer pathways. The algorithm determines the resonance assignment by finding an optimal mapping between the set of expected peaks that are assigned by definition but have unknown positions and the set of measured peaks in the input peak lists that are initially unassigned but have a known position in the spectrum. Using peak lists obtained by purely automated peak picking from the experimental spectra of three proteins, the FLYA algorithm assigned correctly 96–99% of the backbone and 90–91% of all resonances that could be assigned manually. Systematic studies quantified the impact of various factors on the assignment accuracy, namely the extent of missing real peaks and the amount of additional artifact peaks in the input peak lists, as well as the accuracy of the peak positions. Comparing the resonance assignments from FLYA with those obtained from two other existing algorithms showed that using identical experimental input data these other algorithms yielded significantly (40–141%) more erroneous assignments than FLYA. The FLYA algorithm thus has the reliability and flexibility to replace most manual and semi-automatic assignment procedures for NMR studies of proteins.

PS 126

Dynamic Inter-Domain Crosstalk Determines Enzyme Activity

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The catalytic efficiency of enzymes such as prolyl and disulfide isomerases increases by orders of magnitude, if the enzymes contain additional chaperone domains. We studied the molecular enzyme mechanism of the two-domain metallochaperone and PPIase SlyD [6], which is involved in twin-arginine translocation and nickel metabolism [1], by combining various biophysical methods including dynamic and real-time NMR [5, 3], stopped-flow fluorescence, single-molecule FRET [2] and SAXS [6]. The dynamic crosstalk between the PPIase and chaperone domain could be studied on a ps-to-ns time scale (Lipari-Szabo analysis) and µs-to-ms time scale (R_2 dispersion) and modulated by binding of immunosuppressiva and point mutations. Single-molecule FRET revealed large scale motions of the two domains, which were silent in NMR. Together with real-time NMR during actual catalysis of protein substrates, a molecular interpretation of the Michaelis-Menten parameter K_M and k_{cat} was possible. Opening and closing of the two domains facilitates sampling of an optimal substrate conformation for the catalytic center at a rate of about 100 s^{-1}. Only one out of 100 closings is productive for substrate release with about 1 s^{-1} for k_{cat}. The Michaelis-Menten SlyD-substrate complex could be structurally characterized by 2D and 3D real-time BEST NMR for fast data acquisition.

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PS 127

FAST 2D AND 3D NMR TOOLS FOR METABOLIC FLUX ANALYSIS IN COMPLEX BIOLOGICAL MIXTURES

Patrick Giraudeau[1], Edern Cahoreau[2], Stéphane Massou[2], Meerakhan Pathan[1], Jean-Charles Portais[2], Serge Akoka[1]

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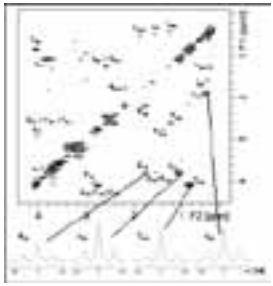
The determination of specific ^{13}C isotopic enrichments (IE) in complex mixtures of ^{13}C-labeled metabolites is a powerful tool for studying metabolic fluxes in living systems. Existing methods rely on homonuclear 2D NMR experiments where IE are measured from ^{13}C satellites. [1]However, these methods suffer from long acquisition times, thus limiting their use as a quantitative tool for fluxomics.

We designed **an ensemble of methods for measuring specific ^{13}C-enrichments in a very fast and accurate way**, by using experiments based on ultrafast 2D NMR. [2] This approach is capable of providing a complete 2D correlation in a single scan. Strategies based on ultrafast heteronuclear J-resolved spectroscopy [3] and ultrafast COSY and zTOCSY [4] will be presented, all of them characterized by excellent analytical performances.

Ultrafast and conventional 2D methods are still limited by overlap due to ^{1}H-^{13}C splittings, thus limiting the metabolic information accessible for complex biological mixtures. To bypass this limitation, we propose **a fast 3D NMR method, UFJCOSY, based on ultrafast spatially-encoded NMR**, which gives unambiguous access to isotopic enrichments in biological mixtures in a few minutes. [5] The ^{1}H-^{13}C couplings are tilted in a third dimension, and the full 3D spectrum is recorded in a few minutes with an hybrid conventional-ultrafast strategy.

The principles and the analytical evaluation of these methods will be presented, as well as **their application to the measurement of ^{13}C-enrichments on a biomass hydrolyzate obtained from *E. Coli* cells**.



COSY plane from a 3D UFJCOSY NMR spectrum recorded in 22 min on a biomass hydrolyzate from E. coli cells grown on 50% of [U-^{13}C]-glucose and 50% of n.a. glucose. Isotopic enrichments are measured from columns perpendicular to the COSY plane (bottom). Recording a conventional 3D spectrum equivalent to this dataset would have required ca. 24 hours acquisition time.

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PS 128

STRUCTURES OF LARGE COMPLEXES FROM HETEROGENEOUS DATA AND BAYESIAN DATA ANALYSIS

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To characterize 3D structures of the large and often flexible macromolecular complexes that govern cellular processes, high-resolution structure determination is the exception, and multiple sources of structural data at multiple resolutions need to be used. Integrating these data into one consistent picture poses particular difficulties: data are much more sparse than in high resolution methods; data sets from heterogeneous sources are of highly different and unknown quality and may be mutually inconsistent; data are in general averaged over large ensembles and long measurement times. Also pre-existing structural knowledge is of different quality, ranging from high-resolution structures over homology models to low resolution models.

Despite substantial efforts to address this data highly complex integration problem, no completely satisfying solution exists. In this paper, we will outline a general framework, principally based on Bayesian probability theory. Appropriate models for the major data types used in hybrid approaches (electron microscopy, cross-linking/ mass spectrometry, various spectroscopy techniques, SAXS, ...) need to be developed, as well as representations to include structural knowledge for individual components of the complexes. The ultimate goal will be a multi-scale version of the approach we introduced for NMR (Rieping et al., Science 309, 303-305, 2005), implemented in the program ISD. Currently, our approach uses elements from a complete Bayesian treatment, and pays particular attention to the appropriate modeling of outliers. We present examples with data from PRE and from chemical crosslinking / mass spectrometry.

PS 129

Superconductivity Competing with an Antiferromagnetic Mott-Insulating State in Alkali-Doped Fullerides

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Superconductivity in alkali fullerides (A_3C_{60} where A = alkali metal) was for many years discussed within the BCS theory where high-energy intra-molecular phonons are responsible for the Cooper pairing with s-wave symmetry. This view has been challenged recently by us with the discovery that cubic Cs_3C_{60} [1-3], which retain the threefold degeneracy of the electronically active t_{1u} orbitals, is under ambient pressure conditions insulator. In this compound, the electronic correlations win over the kinetic energy due to the electronic delocalisation and are responsible for the antiferromagnetic insulating (AFI) ground state with T_N = 46 K in the A15 [1,2] and $T_N \approx 2$ K in the fcc polymorph [3]. With the application of pressure, A15 Cs_3C_{60} undergoes a metal-insulator transition (MIT) and the superconductivity is restored at the surprisingly high temperature of T_C = 38 K at 0.79 GPa [1]. The highest superconducting transition temperature in fcc phase is 35 K [3].

In this contribution we report on our low-temperature and high-pressure dependent local probe NMR investigations of both cubic Cs_3C_{60} phases. In particular we trace the pressure dependence of metal-insulator and superconducting transitions and discuss the unconventional nature of both the normal as well as superconducting state. Measurements are extended to a new family of materials where chemical pressure plays a similar role as physical pressure.

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PS 130

Kinetics of Hydrogen Abstraction in Propane Studied by Muon Spin Resonance (μSR)

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Hydrogen abstraction reactions are key steps in free radical mechanisms that dominate the combustion and atmospheric chemistry of the alkane hydrocarbons[1] and, as such, merit study for their relevance to current environmental concerns. The lower mass alkanes also provide important 'test cases' of H-atom reaction rate theory for polyatomic systems[2], and their study through *ab initio* methods is an active area of research.

Muon spin resonance[3] provides a novel experimental technique for studying such H-atom abstraction reactions. Positive muons of energy ~4.1MeV are stopped in a target cell containing propane/nitrogen gas mixtures, with a fraction forming muonium, Mu, a bound μ+e- atom[3]. With a mass of 0.113 amu, Mu may be considered the lightest isotope of hydrogen. The abstraction reaction $Mu+C_3H_8$ at 300K is followed by measuring the slow formation of the diamagnetic product MuH using resonance techniques and the methods of Morozumi *et al*[4] for analysis. Measurements were carried out for number of propane partial pressures to yield an average rate constant, k_{Mu}, of $(7.2\pm0.8)\times10^{-16}$ cm^3s^{-1} at 300K. Rates obtained from Mu reactions may readily be compared with those determined for incident H and D atoms to reveal novel kinetic isotope effects; surprisingly Mu abstraction is only about a factor of three slower than that for $H+C_3H_8$, suggesting a large contribution from quantum tunnelling.

The experimental setup presents a considerable challenge. A gas pressure cell capable of operating at ~50 bar was designed with a thin (175μm) window to permit beam entry. The short muon lifetime (~2.2μs) requires the application of large radio frequency fields that need to be homogeneous over a comparatively large volume (~8cm^3) to encompass the profile of the implanted muons. Both the setup and results obtained will be discussed.

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Ps 131

Electrokinetic NMR (eNMR) as a Tool to Study New Energetic Materials

Marianne Giesecke[1], Matilda Klett[2], Andreas Nyman[2], Fredrik Hallberg[1], Rakel Wreland Lindström[2], Göran Lindbergh[2], István Furó[1]

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Electrokinetic NMR (eNMR) observes molecular displacement induced by electric field. The mechanism can, for example, be electrophoresis or electroosmosis. Here we present two examples of how eNMR can provide information about potential materials for electrolytes in batteries.

Polyethylene oxide (PEO) is a promising candidate for solid polymer electrolytes as it can solvate and transport lithium ions (Li^+). It is therefore important to understand which parameters control binding cations to PEO. Complex formation between monodisperse PEO chains and a large set of cations in methanol was studied by estimating the acquired effective charge of the PEO by a combination of diffusion NMR and electrophoretic NMR experiments. The relative strength of binding of monovalent cations varied along the ion size as $Li^+ < Na^+ < K^+ \approx Rb^+ \approx Cs^+$. All polyvalent cations were found to bind very weakly, except for Ba^{2+} which exhibited binding in strength similar to that of monovalent ions. Considering all cations, we find that binding occurs below a critical surface charge density.

Poor mass transport in the electrolyte of Li-ion batteries causes large performance loss in high power applications, such as in vehicles. Determination of the critical transport properties under or near operating conditions is of great importance for the development of Li-ion battery technology. By obtaining temporally and spatially resolved concentration data, NMR imaging is demonstrated as a versatile tool for the investigation of mass transport in electrochemical systems. We use *in-situ* ^{7}Li NMR imaging to directly capture the gradual build-up of concentration gradients in a battery electrolyte under current applied. An effective salt diffusivity and Li^+ transport number is obtained by using a physical mass transport model.

PS 132

Supercapacitor Electrodes and Solid-State Electrolytes Studied by NMR

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Supercapacitors made of nanoporous carbons, with a 1M solution of tetraethylammonium tetrafluoroborate in acetonitrile as electrolyte, have been studied by NMR. With ^{13}C and ^{11}B *ex situ* MAS-NMR, a new insight on the molecular mechanisms at work inside supercapacitors electrodes is easily obtained, providing critical information for the development of supercapacitors with enhanced performance. First, we observe that, in activated carbons soaked with an electrolyte solution, two distinct adsorption sites are detected, both undergoing chemical exchange with the free electrolyte molecules. Second, MAS-NMR provides the direct experimental evidence that upon charging, anions are substituted by cations in the negative carbon electrode and cations by anions in the positive electrode. It also shows that acetonitrile molecules are expelled from the porosity at the negative electrode only. Moreover, the NMR signatures of the electrolyte species (chemical shifts) seem to be affected both by the graphene layers and the electronic charges carried by the same graphene layers. Moreover, the electrochemical properties of the supercapacitors can be correlated with the NMR signatures of the nanoporous carbons.

In solid-state electrolyte, recent advances on Pulsed Field Gradient probes have allowed recording lithium self-diffusion coefficients in materials such as Li_3N, taking into account the anisotropy of diffusion. We measured the diffusion coefficients on a wide range of temperatures, up to 800K using laser heating and BN crucibles. The activation energies for diffusion can be compared to those obtained for conductivity offering interesting insights on the molecular mechanisms at work in these materials.

PS 133

Protein Fold Determined by Paramagnetic Magic-Angle Spinning Solid-State NMR Spectroscopy

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Paramagnetic solid-state NMR structural studies of the 56-residue B1 immunoglobulin binding domain of protein G (GB1) will be presented. Using a set of GB1 variants modified to contain EDTA-Cu(II) tags at positions 8, 19, 28, 42, 46 or 53 we have determined over 200 longitudinal ^{15}N paramagnetic relaxation enhancement (PRE) restraints by multidimensional NMR methods. These data yield ^{15}N-Cu^{2+} distances in the ~10-20 Å regime that are inaccessible by conventional approaches. We show that this set of backbone ^{15}N PREs (~4-5 restraints per residue) combined with backbone dihedral angle restraints derived from ^{13}C and ^{15}N chemical shifts and, importantly, in the absence of the usual solid-state NMR distance restraints is sufficient to determine the protein backbone fold which is in agreement with the high-resolution X-ray structure. We will also present the results of recent studies aimed at: (i) evaluating the influence of intermolecular electron-nucleus couplings and secondary protein Cu(II) binding sites on the accurate measurement of ^{15}N PREs and (ii) the development of Cu(II)-binding tags for the structural analysis of proteins by solid-state NMR.

PS 134

PELDOR Distance Measurements in Homo-Oligomeric Systems

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Pulse EPR distance measurements have evolved to a standard tool for generating long-range constraints for structural modelling. Especially the pulsed electron–electron double resonance (PELDOR or DEER) [1] method is becoming increasingly applied [2,3]. Especially for structural studies on different functional states of membrane proteins which are difficult to access by most biophysical methods PELDOR is very promising.

However, in samples of membrane proteins several approximations commonly made in data analysis might not be well met. The effect of the finite sample concentration is not necessarily as straightforward as in homogeneous solutions. In phospholipid vesicle membranes this might lead to stretched exponential functions and rather challenging signal decay times [4]. In detergent-solubilised samples it can be debated whether the distribution of spin centres is homogeneous at all [5]. Furthermore, systems of singly-labelled monomers forming trimers or higher oligomers not only allow extracting the oligomerisation state from the modulation depth [6], but also exhibit multi-spin effects hampering the distance analysis [7] which are often neglected. These multi-spin effects should increase significantly with oligomeric state.

In this contribution we will address these problems and present the validation of an alternative approach to data analysis in symmetric multi-spin systems.

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PS 135

An Integrated Terahertz Gyrotron for DNP-NMR Spectroscopy

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Dynamic Nuclear Polarization (DNP) is a method to boost signal intensities of NMR signals by several orders of magnitude; therefore experiments that typically require days to weeks of acquisition time can be performed in minutes with DNP. In a DNP experiment the large thermal polarization of a paramagnetic polarizing agents is transferred to surrounding nuclei by irradiating the EPR transition of the polarizing agent with THz radiation.

DNP-enhanced solid-state NMR experiments are typically performed at T<90 K and to efficiently saturate the corresponding EPR transitions, several watts of high-power, high-frequency THz radiation are required. At high magnetic fields (>9.4 T, >400 MHz 1H, > 263 GHz e-) currently the gyrotron oscillator is the only demonstrated device capable of generating sufficient THz power. However, a gyrotron operating in the fundamental cyclotron harmonic requires a separate superconducting magnet of approximately the same magnetic field strength that is required for the NMR experiments to generate the corresponding high-power THz radiation. Therefore, most gyrotrons that are used in DNP-NMR experiments operate in the second harmonic and only half of the magnetic field strength of the NMR experiment is required. This significantly reduces the cost of the system but second harmonic operation is technically very challenging and often shows poor THz generation efficiency.

Here we present a new approach that incorporates the gyrotron into the NMR magnet thus eliminating the need for an additional superconducting magnet for the gyrotron while permitting operation in the efficient fundamental cyclotron harmonic. In addition, no THz transmission line is required, further reducing the overall cost of the system. We will present first experimental results of a prototype operating at 198 GHz that is currently developed for DNP-NMR experiments at 300 MHz (^{1}H).

PS 136

NMR Spectroscopy Combined with DFT Calculations to Study Paramagnetic Materials for Li-Ion Batteries

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Nuclear Magnetic Resonance, through hyperfine coupling, is a powerful technique to probe the local electronic environment of a given nucleus in a paramagnetic compound. It is thus commonly used to probe Li environment in intercalation electrode materials for Li-ion battery application [1]. These compounds are often paramagnetic, and their NMR shifts are consequently mainly due to the presence of electron spin density at the site of the nucleus of interest that induces a local magnetization which directly results in the so-called Fermi contact shift. This density of electron spin is transferred to the nucleus probed by NMR from the d orbitals of the transition metal.

For some compounds, the ^{6,7}Li Magic Angle Spinning (MAS) NMR spectra are very complex to interpret because of the presence of several Li environments (different Li sites and/or different paramagnetic ions distribution around a given crystallographic site). In order to assign the Li signals, some of us have been using DFT calculations for some years [2]. Recently, we developed a quantitative approach and applied it successfully on several transition metal oxides and phosphates compounds [3-4]. In some cases, the spin transfer mechanisms from the transition metal ion to the probed nucleus was analyzed though DFT calculations and allows us to better understand the nature of the chemical bonds in the phases [2]. ^{7}Li MAS NMR and DFT calculations can also be applied to study the Li intercalation/deintercalation processes and Li diffusion.

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PS 137

SINGLE AND DOUBLE-PFG NMR AND MRI: FROM MODEL SYSTEMS TO IMAGING OF THE CNS

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Single-pulsed-field-gradient (s-PFG) MR methodologies, i.e. sequences which utilize only one pair of diffusion sensitizing gradient pulses, have been by far the most widely diffusion NMR methods used to study chemical, physical, geological and biomedical systems.[1-2] In neuroscience, diffusion tensor imaging (DTI) has provided an excellent means to study coherently placed anisotropic structures[2], and the q-space approach allows one to extract compartmental dimensions.[3] Indeed, when mono-disperse and coherently placed compartments are present, diffusion-diffraction minima can be observed at high q-values s-PFG MR experiments, from which accurate compartment dimensions can be extracted.[1,3] Although s-PFG NMR and MRI techniques have been widely employed, these methodologies suffer from several inherent limitations that limit our ability to obtain micro-structural information, especially when the compartments in which the diffusion process occurs are characterized by size distribution and are randomly oriented in space, as frequently encounter in Nature. In such systems double-PFG (d-PFG) MR sequences, i.e. sequences which uses *two pairs* of diffusion sensitizing gradient pulses, can be used.[4] The d-PFG MR experiment, first proposed by Cory et al.[6a] is an extension of s-PFG sequence, and employs two gradient pairs $\mathbf{G}_1$ and $\mathbf{G}_2$ which are separated by a mixing time (t_m). Another variant of d-PFG MR experiment was recently introduced, in which the middle gradients are superimposed, yielding tm=0ms, a desirable property for some applications.[4b] The angular d-PFG MR experiment, in which the angle ψ between the gradients is varied at a defined q-value, has been predicted to result in a bell-shaped function from which the size of the compartment could be extracted, even at low q-values.[4c] Recently, the full theory of such experiments was developped.[4e-f]

The phenomenon of diffusion-diffraction in s-PFG MR[5a,b] will be presented and we will demonstrate how it can be used to study compartment size in coherently organized mono-disperse samples where ensemble anisotropy exist (eA).[5c] Following that we will demonstrate the added values of using d-PFG methodologies.[8] First the ability of d-PFG MR to provide structural information on controlled systems, i.e. systems in which the ground truth is know a priori, will be provided.[9a,b] We will show that this methodology works even in systems with size distribution which are randomly oriented i.e. systems in which there is only microscopic anisotropy (μA) and no ensemble anisotropy (eA).[9c,d] Next we will show that angular d-PFG MR can be used to obtain compartment size and shape in randomly oriented systems such as emulsions and yeast cells using relatively weak magnetic pulse gradients.[9e,f] Finally, if time permit, we will show that d-PFG MRS can provide signatures for white and gray matter, and then recently obtained d-PFG MR images of rat brain, both in vitro and in vivo, will be presented.[10,a,b]

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PS 138

MULTIDIMENSIONAL PULSES AND SPATIALLY ENCODED MAGNETIC RESONANCE

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The spatial encoding of NMR interactions makes it possible to acquire multidimensional spectra in a single scan. Spatial encoding has also been shown to provide an alternative imaging modality, which benefits from a high robustness against chemical shift artefacts and susceptibility effects, thus promising access to hitherto invisible areas.

We are exploring ways in which the concepts of spatial encoding can be used to design multidimensional pulses (*n*D), i.e., pulses that are selective in two dimensions or more simultaneously, Such *n*D pulses could for example be used to provide slice-selective spatially encoded MRI sequences and localised spectroscopy with arbitrarily shaped voxels.

Spatial encoding most often relies on a sequential excitation of the spins using a linearly frequency-swept or chirp pulse. Starting from a chirp pulse, a 2D pulse can be obtained using a separable design. The resulting "hybrid" pulse can be seen as performing a walk in a hybrid direct and reciprocal excitation space. Analysis and numerical simulation reveal the properties of sampled chirp pulses, in particular regarding excitation sidebands and ways to suppress them

As we illustrate through experiments and simulations, hybrid 2D pulses can be used to address arbitrary 2D patterns and can be included in either spatially encoded (Fig. 1) or Fourier imaging sequences. They can also benefit from a higher robustness against inhomogeneities of the static magnetic field B0.

Fig. 1: Selective excitation of a star-shaped region using a hybrid 2D pulse.

PS 139

LONG LIVED COHERENT RESPONSE SIGNAL IN BONE

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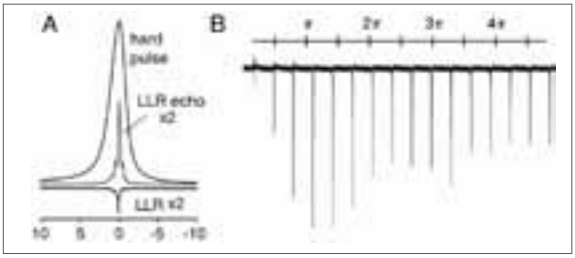
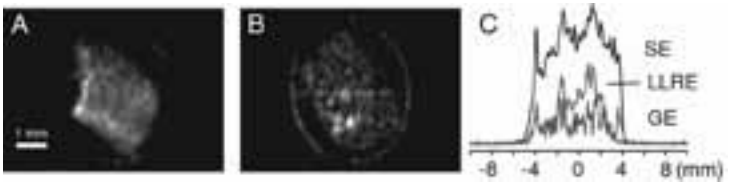
While it is generally assumed that one cannot excite narrow lines in a homogeneously-broadened spin system, recent demonstrations have proven the contrary. In some cases, signals can be excited which substantially exceed the lifetime of the regular FID. The theoretical origin of the phenomenon is currently not understood.

Here, we demonstrate that long-lived signals can be excited in cortical bone samples. In addition, it is shown that the signals originate from the bound water fraction in bone, and, most importantly, strong evidence is provided that dipolar coupling complexity is at the origin of the phenomenon. This result is further supported by quantum and classical spin simulations.

The use of such long-lived signals would enhance the ability to visualize rigid tissues and solid samples with high sensitivity, resolution, and specificity via MRI.



Long-lived response (LLR) signal in cortical bone and nutation curves.



PS 140

MULTI-PARAMETRIC MR IMAGING AND SPECTROSCOPY OF CARDIOVASCULAR DISEASE IN SMALL ANIMALS

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Cardiovascular (CV) diseases are major causes of mortality and morbidity in modern society and therefore there is a continuous need for more effective CV disease detection and therapy monitoring. NMR imaging (MRI) and spectroscopy (MRS) have much to offer to translational biomedical research that aims to improve the diagnostics and treatment of CV disorders. This lecture highlights recent MR technology advances from studies of mouse and rat models of CV disease. MRI provides excellent tools for measuring the function of rodent myocardium over time. The main challenges of such measurements are related to the small size and high heart rate of mice and this has prompted a lot of research into the acceleration of the MRI procedures. MRI and MRS are capable of quantifying several other key aspects of CV disease development, including the perfusion and metabolic status of the heart. Perfusion can be determined via first-pass tracking of the passage of a contrast agent bolus. This scan type represents a major challenge in terms of imaging speed in case of mouse myocardium. Cardiac metabolic status was deduced among others from the conversion of ^{13}C-MRS of hyperpolarized enriched pyruvate. Recently, several novel MRI approaches have been introduced that are aimed to enhance the specificity of the MRI read-out. These include ultra-short echo-time MRI for the visualization of cardiac fibrosis. Fibrotic tissue has very short T2's and escapes detection by traditional spatial encoding procedures. In addition, considerable progress has been made with the development of MRI contrast agents that bind specifically to tissue-associated biomarkers and thereby improve the sensitivity as well as the specificity of disease detection. Examples for the illustration of the wide range of capabilities of MRI and MRS in CV research will be drawn from studies in our lab on rat and mouse models of type 2 diabetes (a major risk factor for CV disease), atherosclerosis, thrombus formation and myocardial infarction. Attention will also be paid to exciting developments of hybrid imaging approaches, in which the strengths of different modalities are combined. Many of the techniques presented allow for a relatively straightforward translation from the small animal to the human setting and can therefore be expected to contribute to the improved clinical management of human cardiovascular disease patients in the future.

PS 141

PROTEIN NMR - STRETCHING THE LIMITS

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Nuclear Magnetic Resonance (NMR) spectroscopy as a technique has been undergoing unparalleled development. Much of this in the recent years has been driven by applications in structural biology, especially to proteins. The enormous sensitivity and resolution enhancements achieved during the last two decades in combination with developments in recombinant technology in biology are opening up new avenues for hitherto inaccessible investigations. New methodological developments are continuously happening pushing the frontiers further and further.

We have designed new NMR methods and protocols for rapid chemical shift assignment of protein backbone ($^{1}H^{N}$, ^{15}N, $^{13}C\alpha$, and $^{13}C'$) resonances. The assignment protocols are based on (a) adequate number of check points (glycines, alanines, and serines/threonines) identified from a few HSQC type spectra and (b) sequential (^{15}N, $^{13}C\alpha$, and $^{13}C'$) correlations derived either from 2D spectra (in case of small well folded proteins of MW <12kDa) or 3D spectra (in case of unfolded/unstructured proteins or proteins of higher MW). The 2D-NMR based assignment protocol provides the fastest and cost-effective method of sequential assignment of backbone (^{1}HN, ^{15}N, $^{13}C\alpha$, and $^{13}C'$) resonances. The protocol has been automated and the algorithm has been named as AUTOBA. We have also designed a web-based server (http://www.tifr.res.in/~hosur/autoba) for making this whole assignment procedure simple and easy. The latest addition in these endeavours is the use of multiple receivers which add a totally new dimension to NMR methodology developments. We believe that the methods and protocols described here would be of immense value for routine use in structural and functional proteomics research by NMR. Specific applications to small and large protein systems will be presented.

PS 142

NMR Study of Structure and Dynamics in the Intrinsically Disordered C-terminal Domain of WASp-Interacting Protein

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WASp-interacting protein (WIP) is a 503-residue proline-rich polypeptide expressed in human T cells. The WIP C-terminal domain binds to the Wiskott-Aldrich syndrome protein (WASp) and regulates its activation and degradation, and the WIP/WASp interaction has been shown as critical for actin polymerization and implicated in the onset of WAS and X-linked thrombocytopenia. However, as an intrinsically disordered protein WIP has defied structural characterization in its unbound state. Here we use nuclear magnetic resonance (NMR) to investigate the biophysical behavior of WIP^C, a C-terminal domain fragment of WIP including residues 407-503 and containing the WASp binding site. Protonless ^{13}C-detected NMR experiments were employed to perform resonance assignment in light of the poor spectral dispersion exhibited by WIP^C and the high occurrence (25%) of proline residues. A combination of 3D- and 5D-NMR experiments, the latter with non-uniform sampling and optimized for the WIP^C sequence, was sufficient to accomplish full resonance assignment. Secondary chemical shift analysis revealed a propensity for helical conformation in the 446-456 segment, a short capped helix in the 473-478 segment, both corresponding to WASp binding epitopes, and a polyproline II conformations for the proline stretches of WIP^C. These structural propensities were confirmed by measuring ^{15}N relaxation rates, protection from solvent exchange, and circular dichroism. We conclude that the disordered WIP^C is comprised of regions with latent structure connected by flexible loops, and suggest potential roles for this arrangement of structural motifs.

PS 143

Transient Substrate and Domain Interactions in Non-Ribosomal Peptide Synthetases

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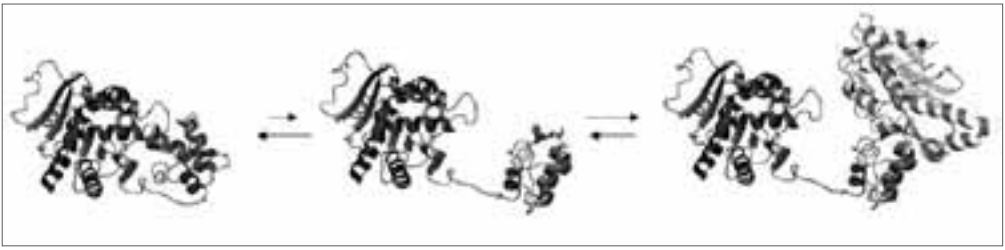
Non-ribosomal peptide synthetases (NRPSs) are multi-module, multi-domain enzymes, that synthetize important natural products in bacteria and fungi, many of which with pharmaceutical applications (e.g. antibiotics, antitumor agents, immunosuppressants). NRPSs use an assembly line organization to covalently load chemical substrates onto each module and catalyze peptide bond formation between substrates loaded on adjacent modules. These multiple catalytic steps require a series of sequential domain/domain and domain/substrate interactions, which are currently poorly understood. We have used NMR to study transient interactions between domains and between domains and chemical substrates. We show that many NRPS domains recognize both chemical and protein substrates and we discuss structural and dynamics effects during molecular interactions. Understanding the dynamic mechanism of NRPS domain communication may open the venue to efficient NRPS assembly line reprogramming and the production of novel pharmaceuticals.



PS 144

Unraveling Protein Motion and Hydration

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The nature of internal motion of proteins is of considerable interest but remains largely uncharacterized. Protein motion on a wide range of timescales has been intimately linked to a number of protein functions including conformational selection, catalytic activity and, through the conformational entropy that it represents, binding affinity and allosteric regulation. Here we use high-pressure perturbation to illuminate the coupling of side chain motion within the interior of human ubiquitin. In contrast to the main chain, the motions of the methyl bearing side chains have a large and variable pressure dependence. The classic three Gaussian distribution of methyl axis L-S generalized order parameters is maintained at all pressures with shifting of the relative populations to the more restricted motional modes. Between 1.6 and 2.5 kbar there is a qualitative change in the distributions. Within the core of the protein, the pressure sensitivity of methyl motion correlates with the magnitude of motion at ambient pressure. Spatial clustering of the dynamic response to applied hydrostatic pressure is also seen indicating limited cooperativity of motion on the sub-nanosecond time scale and suggesting regions of variable compressibility. Using recently developed approaches for detecting protein-water contacts, we find that water does not penetrate the protein even at the highest pressures employed (2.5 kbar). These and other features indicate that the native ensemble contains a significant fraction of members with characteristics ascribed to the recently postulated “dry molten globule.” The coupling of the motion of water at the surface and in the bulk has long been thought to “slave” the motions of the protein. Site-resolved measurement of this has been lacking. Here we show that the confinement of ubiquitin within the high-viscosity water core of a reverse micelle affects internal protein motion in only small and subtle ways. This may require a revision of the “solvent slaving” model for protein motion. Supported by the NIH and the NSF.

PS 145

Dynamic Nuclear Polarization of Frozen Gases

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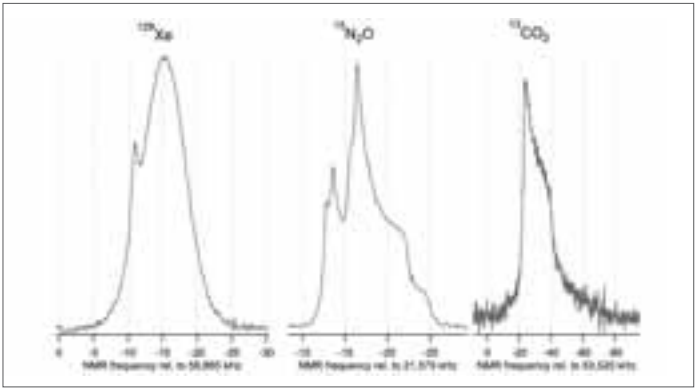
Achieving hyperpolarization of gases using dynamic nuclear polarization (DNP) in the frozen mixtures at 5 T, 1.4 K offers a number of potential advantages as well as challenges. Here we present our solid-state NMR data and models on hyperpolarized ^{129}Xe, $^{15}N_2O$, and $^{13}CO_2$. Each system offers unique insights that help understand similar phenomena in seemingly unrelated settings, with a number of new findings, surprizes, and unexpected results. These include thermally-activated clustering of pure gases in otherwise well-mixed

gas/glassing agent/radical mixtures, utility of dipolar-enhanced chemical-shift-anisotropy powder-patterns for establishing absolute polarization of $^{15}N_2O$, as well as peculiar dependence of T_1 relaxation rates on cluster size, temperature, and molecular orientation.



PS 146

Dynamic Nuclear Polarization (DNP) with MAS at Low Temperature (25 K)

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Dynamic nuclear polarization (DNP) can provide large sensitivity enhancements of solid state NMR. We have constructed a novel probe for DNP-enhanced solid state MAS NMR at 20-25 K and 9.4 Tesla, with DNP enhancement provided by a low power microwave source (30 mW) at 264 GHz. The DNP-MAS probe uses helium to cool the sample, while the rotor is spun with nitrogen gas. The probe design is similar to our earlier work on MAS at low temperature (Thurber et al., J. Magn. Reson. 2008), but includes a waveguide for microwave irradiation. We find that low-power microwave irradiation can increase 13C CP signals by up to 30X relative to signals without microwaves at 21 K, in frozen glycerol/water solutions containing the triradical dopant DOTOPA-TEMPO, with MAS at 7 kHz. Dependences of DNP on dopant, temperature, MAS frequency, and deuteration level will be discussed, and our most recent results for 1D and 2D solid state NMR of 13C-labeled peptides will be presented. Theoretical calculations and simulations of cross-effect DNP with MAS will also be described, for the case of biradicals with a large electron g-anisotropy, as is appropriate for the nitroxide radicals that we use in experiments. If T1e is comparable to or greater than the MAS rotation period, the cross-effect can be viewed as two separate events: 1. Saturation of one electron spin by the microwaves; 2. Three-spin interaction among two electrons and one nucleus, which can transfer polarization to the nuclear spin, if the electrons have a polarization difference. Interestingly, this theory predicts that MAS alone, without microwave irradiation, can affect nuclear spin polarizations in doped samples. Such effects are observed experimentally.

PS 147

Investigation of Protein Folding using Dissolution DNP

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Biomolecular NMR in the vast majority of cases is applied for the determination of high-resolution structures and dynamics under equilibrium conditions, where extensive signal averaging can be used. For NMR to be amenable to the investigation of non-equilibrium processes, alternative strategies have to be employed in order to obtain sufficient signal intensities. Solid-to-liquid state dynamic nuclear polarization (DNP) provides large signal enhancements and enables monitoring of transient processes in real-time. Up to present, however, solid-to-liquid state DNP has been applied primarily in conjunction with small molecules, due to challenges including sample dissolution and spin relaxation prior to the acquisition of an NMR spectrum. In the present work, we use rapid sample injection to extend the reach of this technique to macromolecules, and permit the study of the protein folding process. Unfolded polypeptide of the 96 amino-acid ribosomal protein L23 from *T. Thermophilus* is polarized on ^{13}C in the solid state and dissolved under denaturing conditions at low pH. During injection of the hyperpolarized sample into an NMR tube, a pH-jump is applied to trigger re-folding of the protein. Subsequent acquisition of a series of NMR spectra shows the folding process in real time. The spectrum from each scan can be decomposed into a linear combination of contributions from folded and unfolded protein species. This analysis yields the fractions of folded and unfolded populations at each measured time point. The kinetic parameters of the folding reaction are then obtained by fitting the relative populations of folded and unfolded forms of the protein to a kinetic model, while at the same time accounting for the effects of spin relaxation. This folding rate constant obtained can be verified using fluorescence spectroscopy. However, in contrast to optical techniques, the DNP-NMR spectra contain chemical shift resolution, therefore allowing the simultaneous observation of multiple sites from uniformly or selectively isotope enriched protein. These results demonstrate that dissolution DNP can be used to further extend the applicability of NMR in observing dynamic processes on the sub-second time scale, involving proteins or other biological macromolecules.

PS 148

Signal Amplification via Reversible Interaction with Parahydrogen: Opportunities for NMR

Simon Duckett, Ryan Mewis, Marianna Fekete, Lyrelle Lloyd, Louise Highton, Gary Green, Alex Hooper, Richard Green, Majid Khan, Kevin Atkinson

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Hyperpolarization methods are used to produce magnetic states whose populations can be several orders of magnitude larger than would be otherwise attainable in a typical magnetic field associated with a high field spectrometer. Such non-equilibrium magnetic states have been created using techniques as diverse as DNP, optical pumping and hydrogenative parahydrogen induced polarization (PHIP). These methods typically deliver a burst of the hyperpolarized substrate molecules which must be rapidly monitored. Like PHIP, Signal Amplification by Reversible Exchange (SABRE) is able to produce a hyperpolarized substrate in a few seconds, however, unlike PHIP it does not require the incorporation of parahdrogen into it. Instead, SABRE uses a labile complex which possesses two hydride ligands and a weakly bound substrate to produce a platform through which polarization is transferred at low magnetic field from parahydrogen to the substrate. Equilibration of the free and bound substrate molecules, over a period of a few seconds, produces hyperpolarized material in solution. This technique therefore offers the potential to rapidly produce hyperpolarized samples without their chemical modification.

This talk will illustrate, how the SABRE derived hyperpolarization technique enables the rapid completion of a range of NMR measurements. These include ^{13}C, $^{13}C\{^{1}H\}$ and nOe data, in addition to more complex 2D-COSY, ultra-fast-2D-COSY and 2D-HMBC spectra. These observations are made possible by the use of a flow probe and external sample preparation cell to re-hyperpolarize the substrate and hence allow repeat measurements to be made within seconds. The potential benefit of the combination of SABRE and 2D NMR methods to rapidly characterize low concentration analytes are therefore illustrated.

PS 149

Improving Resolution in NMR Using Pathway Selective Pulses

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University of Miami, Coral Gables, FL, USA

Determining the availability of evolution pathways in quantum systems, which is important to a variety of spectroscopies, is often accomplished with a pathway selection scheme (PSS). Recently [1], we introduced a method of converting a PSS into a pathway selective pulse (PSP) which selectively excites spin systems **only if** certain evolution pathways are available. In designing a PSP, perfect time-reversal is required, which, unfortunately, can only be achieved in very simple spin systems. In this presentation, we study the effects of imperfect time-reversal due to homonuclear spin-spin couplings, field inomogeneity, and relaxation on the performance of PSPs. Finally, we experimentally demonstrate that PSPs can be used to improve spectral resolution by reducing NMR line widths by roughly 10-20%, as shown below in Figure 1 on a 2-^{13}C glucose sample.

[1] C.D. Ridge and J.D. Walls, "Pathway Selective Pulses", *J. Phys. Chem. Lett.* **2**, 2478-2482 (2011).

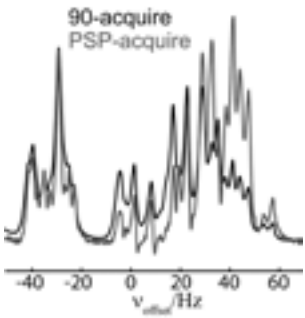

Figure 1: Comparison of the spectrum for a 90-acquire (black) experiment compared with a PSP-acquire (red) experiment on 2-^{13}C glucose on a 500 MHz spectrometer. A reduction of line width of approximately 10-205 is found in the PSP experiment.

PS 150

NMR and MRI at the Microscale

Vikram Bajaj

University of California, Berkeley, Berkeley, California, USA

I will discuss recent advances that enhance the capabilities of NMR and MRI in **portable chemical analysis**. These include:

(1) **Optical detection of NMR with alkali vapor and diamond magnetometers.** Optical spectroscopic methods can detect single molecules, but they lack the chemical specificity of NMR. Using engineered systems whose optical and magnetic degrees of freedom are coupled, we record NMR spectra with optical sensitivity. Applications include low field relaxometry for chemical analysis, and microfluidic NMR. The polarization of optical photons can also be transferred to nuclei. Examples include a microfabricated xenon gas polarizer for microfluidic, hyperpolarized NMR, and polarization transfer from NV- centers in diamond to coupled nuclei.

(2) **MRI Chemical Sensing.** We have developed xenon-based MRI contrast agents for hyperpolarized molecular recognition and detection in vivo and in vitro. By covalently attaching individual sensor molecules to self-assembling scaffolds, we have increased their environmental compatibility, robustness, and sensitivity, demonstrating their ability to detect molecules in femtomolar concentrations. We have also developed a combinatorial library process to produce sensors targeted to desired analytes. These have been applied *in vivo* as molecular imaging agents and reporters, and *in vitro* for chemical sensing in complex mixtures.

(3) **Remotely Detected MRI:** MRI can elucidate the interior structure of an optically opaque object in unparalleled detail but is ultimately limited by the need to enclose the object within a detection coil; imaging of microscopic features within macroscopic objects occurs with low sensitivity because these features occupy only a small fraction of the detector's volume. We overcome this limitation using *remotely detected MRI*: images of fluids flowing in an object are encoded into the phase and intensity of their NMR signals and decoded by a single volume-matched detector after they flow out of the sample. Using remote detection and compressive sampling, we have obtained microscopic (up to 10 μm) images of flow and velocity distributions in microfluidic devices, packed bead microreactors, polymer monolith chromatography columns, and other structures.

PS 151

Magnetic Resonance for In Vitro Diagnostics: From Detecting Pathogens to Characterizing and Monitoring the Blood Physiology

Vasiliki Demas

T2 Biosystems, Lexington, MA, USA

Advances in the field of portable NMR over the past few decades have changed the perception of NMR as a specialized, expensive, bulky, laboratory, hospital or industrial system, and enabled a new era of non-invasive, compact and inexpensive systems for applications, from art preservation to benchtop analysis and homeland security, medical imaging, biomolecule sensing and other diagnostics applications.

T2 Biosystems has been combining portable NMR with magnetic relaxation switch technology to create diagnostic sensors for rapid, sensitive, selective diagnosis in point of care settings. Magnetic relaxation switch assays consist of superparamagnetic nanoparticles that transition between dispersed and clustered states due to the presence of a target substance, and affect the T2 relaxation times of surrounding water molecules in the specimen under investigation. Analyte sensitivity is achieved by functionalizing the nanoparticles with a binding agent to combine with the analyte of interest. Examples of detection of analytes such as viruses, bacterial cells, proteins, nucleic acids, and small molecules in a variety of samples from buffer to serum, plasma and whole blood have been demonstrated at T2Biosystems as well as the laboratories of the company's founders.

The core of Biosystem's technology is the T2MR, a compact, custom NMR system based on a permanent magnet and single board spectrometer

In addition to the magnetic relaxation switch technology, T2 Biosystems has recently introduced the use of T2MR to measurement signal changes in connection to blood coagulation parameters and platelet activity.

The work presented is based on contributions by several individuals at T2 Biosystems

PS 152

The Physics of PHIP Hyperpolarized Low Field NMR

Johannes Colell[1], Stefan Gloeggler[1], Pierre Tuerschmann[1], Bernhard Blümich[1], Stephan Appelt[2]
[1]RWTH Aachen University, Aachen, NRW, Germany, [2]Forschungszentrum Juelich GmbH, Juelich, NRW, Germany

In this contribution the fundamental differences between PHIP hyperpolarized and thermally prepolarized NMR spectroscopy in high and low magnetic fields will be presented. For thermally prepolarized (eg. 2T field) homonuclear *J*-coupled ^{1}H spins the signal to noise ratio drops with decreasing measurement field strengths. In small fields, where the chemical shift difference (in Hz) is smaller than the *J*-coupling (the inverse weak coupling regime), both chemical shift and the homonuclear *J*-coupling information is lost. For the case of PHIP hyperpolarized NMR spectroscopy in the inverse weak coupling regime the structure of the NMR spectral lines retain both the *J*-coupling and the chemical shift information, although compared to high field in a completely different way.

The second fundamental difference between PHIP and thermally hyperpolarized NMR appears if hetero nuclei (samples with natural abundance) are present while the molecule is hydrogenated with para-hydrogen. In the case of thermally prepolarized NMR multiplet lines arising from heteronuclear *J*-couplings are more than two orders of magnitude smaller compared to the central line. For the PHIP enhanced spectrum the amplitude of the multiplet associated with the hetero- and homonuclear *J*-coupling network is enhanced by nearly two orders of magnitude compared to the central lines.

Both principles mentioned above were experimentally verified with a newly developed low-field NMR spectrometer and by measuring a chemical compound developed by our group that forms a “two ^{1}H spin system” after hydrogenation (Silylynolether). Our current results show, that it is possible to determine the complete set of homo- and heteronuclear J-coupling constants including chemical shifts at low fields (10 mT) and with a single scan.

PS 153

Solid-State NMR Studies of Aβ Protofibrils and Mature Fibrils

Holger A. Scheidt[1], Isabel Morgado[2], Juliane Adler[1], Daniel Huster[1]
[1]University of Leipzig, Leipzig, Germany, [2]Martin Luther University, Halle, Germany

Aβ(1-40) is the major fibril-forming peptide from Alzheimer’s disease. Several techniques provided a wealth of structural information on mature Aβ(1-40) fibrils. Yet, these fibrils are the products of a complex formation mechanism that is, from a structural point of view, not well understood. We used solid-state NMR spectroscopy to elucidate the structure of Aβ protofibrils. This analysis was possible because binding of the antibody B10AP prevents the conversion of these metastable intermediates into mature fibrils. A set of eight peptides with varying isotope labeling schemes was obtained from chemical synthesis. The labels cover 30 residues that are distributed over the entire peptide sequence. ^{13}C CPMAS spectra were recorded for unambiguous assignment of all carbons. From the conformation dependent chemical shifts we could identify peptide segments of stable secondary structure and evaluated the backbone structure using TALOS. Based on the chemical shift data, Aβ protofibrils encompass residues 16-22 and 30-36 in β-sheet conformation. Further, three structural regions of the protofibrils present random coil-like chemical shifts. One encompasses residues 23-26 and forms an intermediate segment in between the adjacent β-strands. The other two regions occur at the peptide N-terminus and within a small C-terminal segment. Further information about the dynamics of these regions is provided by order parameter measurement through dipolar couplings. We found that protofibrils show high order parameters (>0.8) within the β-strand regions, while the measured S values are below 0.8 at the termini. Thus, significant structural order exists also within those sequence segments that have chemical shift values corresponding to random coil. We further studied tertiary contacts in Aβ protofibrils and compared them to mature Aβ fibrils. While intraresidue contacts between Glu 22 and Ile 31 were found in Aβ protofibrils, these contacts were completely absent in mature Aβ fibrils. As those intramolecular contacts have also been reported in Aβ oligomers, our measurements suggest that Aβ protofibrils are structurally more closely related to oligomers than to mature fibrils. This suggests that some structural alterations have to take place on the pathway from Aβ oligomers/ protofibrils towards mature fibrils.

PS 154

Towards Structural Comparison of Spontaneously Formed and Prion-Seeded Full-Length Recombinant PrP-fibrils by Solid-State NMR

Henrik Müller[1], Timo Piechatzek[1], Oleksandr Brener[2], Henrike Heise[1]

[1]Research Center Jülich (FZJ), Jülich, Germany, [2]Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

In contrast to other neurodegenerative diseases, transmissible spongiform encephalopathies may be acquired by transmission of proteinaceous infectious particles (prions) which are formed by β sheet rich amyloid fibrils. Due to the failure of biophysical structure analysis on insoluble, non-crystalline, and heterogeneous protein fibrils, not amenable to X-ray crystallography and liquid-state NMR, the detailed structural architecture of prions is unknown. Because all model concepts available to date are based on low resolution data, it is a current matter of intense debate in which prion protein (PrP) sequence segment the β sheet core is situated, whether prions are β helices[1] or β sandwiches[2], and how the presence of infectious as well as non-infectious PrP fibrils can be explained structurally. As demonstrated recently[3], solid-state NMR proved to be valuable for structural characterisation of PrP fibrils.

In our contribution, we will report on our experimental progress in using high resolution solid-state NMR to structurally characterise amyloid fibrils of full-length ovine recombinant (rec) PrP comprising residues 25-233. Our first focus will be on explaining the generation of amyloid fibrils formed spontaneously as well as by seeding with pre-formed recPrP fibrils or scrapie sheep brain-derived prions in NMR-sufficient yields. Based on biophysical characterisation we will demonstrate that our in vitro-derived recPrP fibrils closely emulate prions in living organisms. Thereafter, we would like to present our solid-state NMR data about resonance assignment and secondary structure of recPrP fibrils as well as comparing fingerprint spectra of recPrP fibrils formed spontaneously or by seeding. We will demonstrate that our recPrP fibril preparations are characterised by enough homogeneity to draw first structural conclusions such as where the β sheet core as well as a remaining α helical region appears to be situated and which influence seeding with highly infectious prions seems to exert.

1. Govaerts et al., PNAS 2004; 101: 8342-7.
2. Cobb et al., PNAS 2007; 104: 18946-51.
3. Tycko et al., Biochemistry 2010; 49: 9488-97.

PS 155

Solid-State NMR Studies of Deuterated Proteins: Higher Resolution and Better Sensitivity

Umit Akbey, Hartmut Oschkinat

Leibniz Institute für Molekulare Pharmakologie, Berlin, Germany

Protein deuteration applied to solid-state NMR experiments results in high sensitivity and resolution,[1,2] leading to spectra similar to solution-state. To maximize sensitivity optimum proton concentration is determined at various MAS frequency. At 60 kHz MAS 100 % of exchangeable protons can be utilized without sacrificing resolution,[3] whereas at 24 kHz only 30-40 % is practical. New types of proton detected experiments will be demonstrated.

Despite the progress in the proton detected experiments, there has been a lack of demonstrations for the heteronucleus-detected experiments. As a result, achieving sufficient initial polarization ad spin-diffusion between heteronuclei in deuterated systems is a major issue. Here, we explain our tool-package which we have recently shown to be very successful to be used on perdeuterated proteins, to achieve superior initial magnetization and to sufficiently distribute magnetization between heteronuclei. This tool-package contain experimental techniques such as; double nucleus enhanced recoupling (DONER) method,[4,5] which re-introduces the nearly-collapsed spin-diffusion process by the use of proton and deuterium spins as well as triple cross-polarization (TCP) for increasing overall sensitivity,[6] optimal-control (OC) based schemes to increase performance (RESPIRATION),[7] RAPID acquisition techniques and more.[8]

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2. Akbey, U.; Lange, S.; Franks, W. T.; Linser, R.; Rehbein, K.; Diehl, A.; Rossum, B. J.; Reif, B.; Oschkinat, H. J Bio NMR 2010, 46 (1), 67.
3. Lewandowski, J. R.; Dumez, J. N.; Akbey, U.; Lange, S.; Emsley, L.; Oschkinat, H. JPC Lett 2011, 2 (17), 2205.
4. Akbey, U.; Oschkinat, H.; Rossum, B. J. JACS 2009, 131 (47), 17054.
5. Akbey, U.; Rossum, B. J.; Oschkinat, H. JMR. 2012, 217, 77-85.
6. Akbey, U.; Camponeschi, F.; Rossum, B. J.; Oschkinat, H. ChemPhysChem 2011, 11, 2092.
7. Wei, D. X.; Akbey, U.; Paaske, B.; Oschkinat, H.; Reif, B.; Bjerring, M.; Nielsen, N. C. JPC Lett. 2011, 2 (11), 1289.
8. Bjerring, M.; Paaske, B.; Oschkinat, H.; Akbey, U.; Nielsen, N. C. JMR. 2011, 214, 324.

PS 156

Solid State NMR of Fibrils and Membrane Proteins

Chad Rienstra

University of Illinois at Urbana-Champaign, Urbana, IL, USA

I will present applications of magic-angle spinning solid-state NMR spectroscopy to fibrils and membrane proteins, leveraging new methods developed with microcrystalline proteins. This talk will focus on the following recent publications and recent, yet-to-be-published results from these ongoing projects.

"Structured Regions of alpha-Synuclein Fibrils Include the Early-Onset Parkinson's Disease Mutation Sites", Comellas, G.; Lemkau L.R.; Nieuwkoop A. J.; Kloepper K.D.; Ladror D.T.; Ebisu, R.; Woods, W.; Lipton, A.S.; George, J. M.; Rienstra, C.M. *J. Mol. Biol.*, **2011**, *411*, 881-895.

"Structural intermediates during alpha-synuclein fibrillogenesis on phospholipid vesicles", Comellas G.; Lemkau L.R.; Zhou, D.H.; George J.M.; Rienstra, C.M. *J. Am. Chem. Soc.,* **2012,** *134*, 5090-5099.

"Ultra high resolution protein structures using NMR chemical shift tensors", Wylie, B.J.; Sperling, L.J.; Nieuwkoop A.J.; Franks, W.T.; Oldfield, E.; Rienstra, C.M. *Proc. Natl. Acad. Sci. USA*, **2011**, *108*(41), 16974-16979.

"High-resolution membrane protein structure by joint calculations with solid-state NMR and X-ray experimental data", Tang, M.; Sperling, L.J.; Berthold, D.A.; Schwieters, C.D.; Nesbitt, A.E.; Nieuwkoop A.J.; Rienstra, C.M. *J. Biomol. NMR*, **2011**, *51(3)*, 227-233.

PS 157

Structure Determination of Membrane Proteins in Phospholipid Bilayers

Stanley Opella

University of California, San Diego, La Jolla, California, USA

Membrane proteins are strongly influenced by their phospholipid bilayer environment. Therefore, it an important goal to study them in this environment, even though it presents challenges for the most commonly used methods of structure determination. Indeed, much of the effort in NMR spectroscopy has been devoted to identifying suitable sample conditions. We have taken the opposite approach and have developed instrumentation, experimental methods, and calculations tailored for membrane proteins in a near-native environment. Our approach merges the methods of oriented sample (OS) solid-state NMR and magic angle spinning (MAS) solid-state NMR and relies on the intrinsic rotational diffusion of membrane proteins about the bilayer normal to motionally average heteronuclear dipolar and chemical shift anisotropy powder patterns. The resulting axially symmetric, narrowed powder patterns yield exactly the same orientational information as obtained from stationary aligned samples. The benefits are that uniformly $^{13}C/^{15}N$ labelled samples can be used, which increases sensitivity through ^{13}C (instead of ^{15}N) detection, and provides spin ½ nuclei at all backbone sites for systematic assignment schemes. With sufficient angular constraints, we use a combination of Rosetta and Xplor-NIH to obtain final refined structures with backbone RMSDs < 2 A. Examples with one, two, and seven trans-membrane helices will be shown, including the three-dimensional structure of the chemokine receptor CXCR1, which is a G-protein coupled receptor with 350 residues and 7 trans-membrane helices. Our approach is general and can be applied to a wide range of membrane proteins including those in the alpha helical and beta barrel classes.

PS 158

Excited States in RNA Using Relaxation Dispersion NMR – a General Behaviour?

Katja Petzold, Hashim M. Al-Hashimi
University of Michigan, Ann Arbor, MI, USA

The dimerization initiation site (DIS) is a regulatory RNA of the HIV-virus. Upon viral maturation DIS undergoes dimerization, which is essential for the production of viruses. The mechanism of this secondary structure rearrangement – a complete exchange of strands – remains unknown (Fig 1A), but the bulge and its motions are highly conserved and crucial. We investigated the role of µs- to ms-motions using relaxation-dispersion NMR measurements to reveal possible dimer-forming transition states.

We determined the excited state chemical shifts and their corresponding secondary structures, and confirmed those results by mutagenesis. The excited states biological relevance was demonstrated by biochemical experiments - upon quenching bulge motions a reduction in dimerization potential follows (publication in submission).

Finding these excited states, which differ from proteins or base flipping in DNA (Nikolova et al. 2011 Nature), we consequently wanted to study the universality of this mechanism. Database analysis revealed potential candidates, which we then studied it by $R_{1\rho}$ relaxation dispersion measurements. This experiment allows us to characterize the population, lifetime and chemical shift of the excited state (Fig. 1B) and allows therefore the estimation of the structure of the excited state.

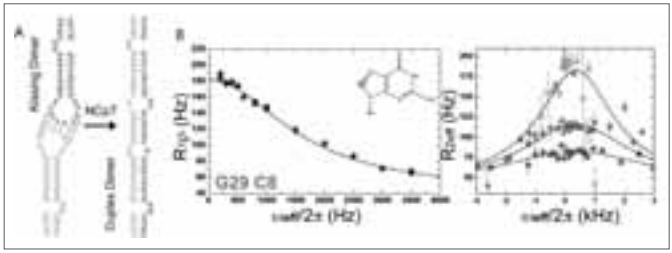


Figure 1: (A) Model Dimerization Initiation Site RNA kissing complex – left and elongated comples - right. (B) Example for R1rho relaxation dispersion profile, left – on-resonance, right – off-resonance, which allow for extraction of k_{EX}, pb and CS_b.

PS 159

Structural Studies of Oligomeric TatA, the Pore Component of the Twin Arginine Translocase

Jason Schnell, Ben Berks, Fernanda Rodriguez
University of Oxford, Oxford, UK

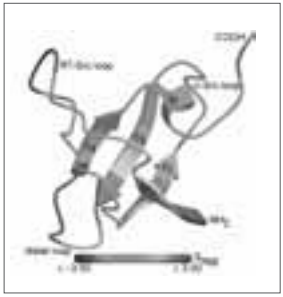
Nature has evolved two fundamentally different methods for translocating proteins out of the cytoplasm. In the Sec translocase, proteins are "threaded" across the membrane in an unfolded form. In a second structurally and mechanistically unrelated pathway, cytoplasmic proteins are targeted to the Twin Arginine Translocase (Tat) pathway and translocated in a *fully folded* form. The Tat pathway is found in bacteria, archaea and plant chloroplasts, and is required for important bacterial cellular processes including respiratory and photosynthetic energy metabolism. In *E. coli*, the Tat pathway requires three proteins, of which TatA is the pore component. TatA is a small transmembrane protein that assembles into ring-like oligomers of variable size, however the mechanism of assembly and the translocation pathway are unknown. We have determined the high resolution structure of the TatA pore by solution NMR using a novel "bottom up" approach that takes advantage of a conformationally locked TatA mutant that permits assembly of the relatively large pore complex from sparse intermolecular restraints. The results indicate an unexpected mode of assembly, with obvious implications for folded-protein translocation as well as regulation of the TatA assembly. The methodology is likely to be generally applicable to a wide range of weakly assembling membrane protein complexes.

PS 160

NMR SOLUTION STRUCTURE OF AN INVISIBLE PROTEIN STATE AT THE EDGE BETWEEN FOLDING AND AGGREGATION INTO AMYLOID FIBRILS

Philipp Neudecker[1], Philipp Neudecker[2], Philipp Neudecker[3], Paul Robustelli[4], Andrea Cavalli[4], Patrick Walsh[1], Patrick Walsh[5], Patrik Lundström[1], Arash Zarrine-Afsar[1], Simon Sharpe[1], Simon Sharpe[5], Michele Vendruscolo[4], Lewis Kay[1], Lewis Kay[5]

[1]University of Toronto, Toronto, ON, Canada, [2]Heinrich-Heine-Universität, Düsseldorf, Germany, [3]Forschungszentrum Jülich, Jülich, Germany, [4]University of Cambridge, Cambridge, UK, [5]Hospital for Sick Children, Toronto, ON, Canada



Protein folding intermediates have been implicated in amyloid fibril formation involved in neurodegenerative disorders. However, the structural mechanisms by which intermediates initiate fibrillar aggregation have remained largely elusive. To gain insight, we used CPMG relaxation dispersion NMR spectroscopy to determine the atomic-resolution three-dimensional solution structure of a 2% populated, on-pathway folding intermediate of the A39V/N53P/V55L Fyn SH3 domain. To this end, we used the backbone chemical shifts and RDCs/RCSAs of the "invisible" intermediate reconstructed from CPMG experiments as experimental input for structure calculations based on chemical shift restrained replica exchange molecular dynamics simulations via the CamShift approach (1). The COOH-terminus remains disordered in this intermediate (2), thereby exposing the aggregation-prone NH_2-terminal beta-strand (Figure, structure color-coded according to the surface aggregation propensity score, S_{agg}). Accordingly, mutants lacking the COOH-terminus and thus mimicking the intermediate fail to safeguard the folding route and spontaneously form beta-sheet-rich fibrillar aggregates with a diameter of several nanometers and an affinity for the dye Congo red. The structure provides a detailed characterization of the non-native interactions stabilizing an aggregation-prone intermediate under native conditions and insight into how such an intermediate can derail folding and initiate fibrillation.

(1) P. Robustelli, K. Kohlhoff, A. Cavalli & M. Vendruscolo: Using NMR Chemical Shifts as Structural Restraints in Molecular Dynamics Simulations of Proteins, *Structure* **18**, 923-933 (2010)

(2) P. Neudecker, P. Robustelli, A. Cavalli, P. Walsh, P. Lundström, Arash Zarrine-Afsar, S. Sharpe, M. Vendruscolo & L. E. Kay: Structure of an Intermediate State in Protein Folding and Aggregation, *Science* **336**, 362-366 (2012)

PS 161

NONLINEAR INDUCTION DETECTION OF ELECTRON SPIN RESONANCE

Gil Bachar[1], Oren Suchoi[1], Oleg Shtempluck[1], Aharon Blank[2], Eyal Buks[1]

[1]Department of Electrical Engineering, Technion, Haifa, Israel, [2]Schulich Faculty of Chemistry, Technion, Haifa, Israel

We present a new approach to the induction detection of electron spin resonance (ESR) signals exploiting the nonlinear properties of a superconducting resonator. Our experiments employ a yttrium barium copper oxide (YBCO) superconducting stripline microwave (MW) resonator integrated with a microbridge (a narrow, sub-micron sized stricture placed in the middle of the stripline). The resonator is operated at liquid He temperatures in continuous wave mode. When exceeding a threshold in the injected microwave power, a strong nonlinear response of the resonator is thermally activated in the microbridge. This non-linearity is manifested through large changes in the frequency of the reflected signal. The responsivity factor characterizing the ESR-induced change in the system's output signal is about 100 times larger when operating the resonator near the instability input power threshold, compared to the value obtained in the linear regime of operation. Preliminary experimental results, together with a theoretical model of this phenomenon are presented. Under appropriate conditions nonlinear induction detection of ESR can potentially greatly improve upon the current capabilities of conventional linear induction detection ESR.

PS 162

FITTING OF PROTEIN STRUCTURAL TRANSITIONS WITH EPR DISTANCE CONSTRAINTS: OPTIMIZATION OF ALGORITHMS

Gunnar Jeschke

ETH Zurich, Zurich, Switzerland

By combining site-directed spin labelling and EPR-based distance measurements it is possible to obtain distance constraints on length scales that match the size of proteins and protein complexes. Such methodology works independently of the size and environment of the protein. It is often used to characterize structural changes that result from addition of substrates or ATP or from hydrolysis of ATP, for instance, for transport proteins located in membranes. The main disadvantage of the approach is the only moderate number of distance constraints, typically in the range from 10 to 100, which is less than the number of degrees of freedom of the protein backbone. Such sparse constraints cannot provide a unique model for the structural transition without taking into account further information. Even then, the model is necessarily coarse-grained, also because of the limited accuracy in predicting spin label side chain conformations.

Additional information, based exclusively on the topology of the (known) initial structure of the transition, can be included by an anisotropic elastic network model. Algorithms for fitting the final state of the transition from the initial structure and sparse distance constraints between Cα atoms and for selecting the best site pairs were suggested by Zheng and Brooks [1] and recently adapted to spin label pairs [2]. Here I report on optimization of the two algorithms with the aim to obtain better fits of the final structure at given number of constraints. The fit algorithm is stabilized and the problem of overfitting strongly reduced by considering each iteration step as a thermal excursion of the network, based on the equipartition theorem. Furthermore, it helps to gradually extend the size of the active space of normal modes during fitting. Site pair selection can be improved by separating the two conflicting criteria of maximal distance change and maximal linear independence of the distance change vectors and by determining an optimum relative weighting of these criteria. I also consider how results are influenced by parametrization of the elastic network model.

[1] Zheng, W.; Brooks, B.R. *Biophys. J.* **2006**, 90, 4327-4336.
[2] Jeschke, G. J. *Chem. Theory Comput.* **2012**, in press.

PS 163

CHEMICAL ENGINEERING OF MOLECULAR QUBITS

Christopher Wedge[1], Grigore Timco[2], Amy Webber[1], Eike Spielberg[1], Floriana Tuna[2], Eric McInnes[2], Richard Winpenny[2], Stephen Blundell[1], Arzhang Ardavan[1]

[1]CAESR, Clarendon Laboratory, University of Oxford, Oxford, UK, [2]School of Chemistry & Photon Science Institute, University of Manchester, Manchester, UK

We show that the electron spin phase memory time, the most important property of a molecular nanomagnet from the perspective of quantum information processing, can be improved dramatically by chemically engineering the molecular structure to optimise the environment of the spin. We vary systematically each structural component of the class of antiferromagnetic Cr_7Ni rings to identify the sources of decoherence. The optimal structure exhibits a phase memory time exceeding 15 μs [1].

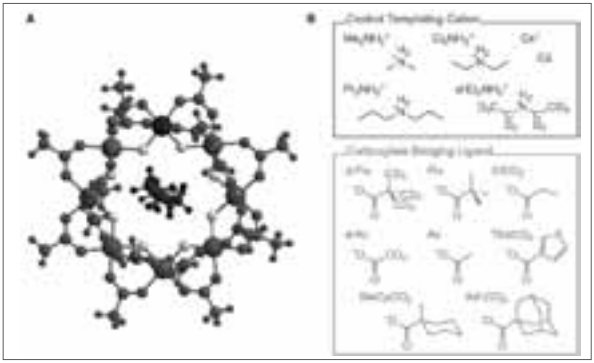


Figure 1: (A) Crystal structure of $[^nPr_2NH_2][Cr_7NiF_8Ac_{16}]$ (B) Chemical structures of possible variants of the two key molecular building blocks.

[1] C. J. Wedge et al., Phys. Rev. Lett., **108**, 107204 (2012).

PS 164

Application of Electron Paramagnetic Resonance to Study Fundamentals Processes in Organic Photovoltaic Materials and Devices

Vladimir Dyakonov

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Power conversion efficiencies of nanostructured polymer solar cells are exceeding 10% on a lab scale, making them ready for commercial introduction. The mechanisms governing the charge carrier generation yield appear to be very complex, as they imply the presence of a number of intermediate excited states, neutral and charged ones /1,2/. Recent investigations indicate that on illumination of polymer:fullerene blends, apart from mobile charges also charge transfer and triplet states are formed /3/. With respect to triplets, it is unclear how these excited states are generated, via inter-system crossing, or via back transfer of an electron from fullerene to polymer. In both pathways, triplet formation may be considered as charge carrier loss factor. On the other hand, the fusion of two triplets may lead to a formation of singlet excitons. Alternatively, the splitting of singlets may lead to two triplet excitons. In these cases, a generation of charges by utilizing of the so far unused photons will be possible. At the same time, however, the intrinsic stability of polymer solar cells has only scarcely been addressed. The oxygen induced degradation may also involve triplet excited states. Although fusion of two triplets may lead to a formation of a singlet exciton, triplets are also prone to react with oxygen resulting in degradation of the polymer.

In all the above reactions and scenarios, a reliable experimental tool of probing triplet and charge transfer states seems to be essential. In this contribution we will discuss the results of the complementary optical, electrical and spin sensitive measurements on the novel high-performance polymer-fullerene blends as well as real solar cells to address the question on the role of triplet excitons and charge transfer states in the transformations occurring in the device active layers.

1. C. Deibel, T. Strobel, V. Dyakonov, Adv. Mater. 22, 4097 (2010).
2. C. Deibel, V. Dyakonov, Rep. Prog. Phys. 73, 096401 (2010).
3. M. Liedtke, A. Sperlich, H. Kraus, A. Baumann, C. Deibel, M.J.M. Wirix, J. Loos, C. M. Cardona, V. Dyakonov, JACS 133, 9088 (2011).

Poster Presentations

Categories	Poster Numbers
Low-Field NMR	200-208
Computation	209-221
In Vivo and MRI	222-236
Biological Advances	237-239
Metabolomics	240-261
Liquid state Methods	262-299
Instrumentation	300-308
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Small Molecules and Pharmaceuticals	515-552
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200MO

Imaging of Weak Magnetic Implants Using the Low Field MRI

Daniel Gogola, Pavol Szomolanyi, Ivan Frollo

Institute of measurement science Slovak academy of sciences, Slovakia, Slovakia

Samples made of weak magnetic materials cause local inhomogeneities near of these samples in the main field of MR tomograph. These inhomogeneities lead to loss of phase coherence and thus rapid loss of signal in the area. In our research we investigated inhomogeneous field of weak magnetic materials in low field MR tomography, as the susceptibility effects are minimal at low fields. Our goal was to find the possibility for imaging some clinically used magnetic implants by low field MR tomograph 0.18T.

Fig.1. Left: Two images of planar phantoms, ring and grid which was made of very weak magnetic materials (low magnetic tapes) were imaged using plastic holder with the liquid contained 5 mM NiCl2 + 55 mM NaCl in distilled water. Images of these samples were obtained by the GRE sequence with the following setting: TE 10 ms, TR 800 ms, slice thickness 3 mm. Right: Two pictures of elbow with weak magnetic metallic implants. To minimize the susceptibility effects, these images were obtained by the SE sequence with the following setting: TE 26 ms, TR 2460 ms, slice thickness 2 mm.

We investigated properties of GRE and SE imaging sequences with a goal to find the best setup of their parameters with a help of two planar phantoms: ring and grid. Based on obtained findings, MR images of elbow with wire implants were realized, right side in Fig.1. These implants were imaged few weeks after surgery. Our study shows that imaging of weak magnetic metallic implants are feasible at low field MR tomograph 0.18 T without using post-processing techniques (e.g. IDEAL).

201TU

Single-Sided NMR in Characterization of Master Paintings

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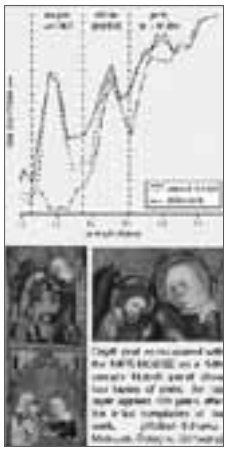
We report progress in using single-sided, low-field NMR measurements to analyze master paintings. While single-sided NMR is typically insensitive to chemical properties of samples, it can detect changes indicative of the physical state of a sample, often by measuring the relaxation times, T_1 and T_2. By measuring T_2 values stepwise through the depth of a triptych panel (right), a layer structure is seen where an additional layer was applied 100 years after the initial work was completed (in collaboration with the Wallraf-Richardz-Museum, Cologne, Germany). In addition, the complex chemistry of the curing of oil paint occurs over centuries, propagating physical changes in paint that can be measured by low-field NMR. Many such measurements, made on master paintings from the 14th through 20th centuries, have shown a correlation between the age of the painting and T_1, creating a rudimentary "dating curve." This curve allows approximate dating of paintings using contact-free, non-destructive NMR measurements; studies to improve the accuracy of such dating are underway. These and related measurements have obvious applications in art history and analysis, and have recently distinguished forged from original paintings by early 20th century artists. These results have been achieved with attention to the color of a given pigment, but not necessarily the chemical composition of the paint.



202WE

In-Line MR Imaging with a Mobile Tomograh

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Conventional MRI magnets use expensive and sophisticated superconducting coils to generate strong and homogeneous magnetic fields. In general they are bulky units that require safety features and permanent maintenance with cryogenic coolants, which complicate the use of such magnets on the factory floor. Recently a method has been worked out to shim NMR magnets made from blocks of permanent magnet material to generate highly homogeneous magnetic fields across the volume of the sample [1,2]. Thanks to this approach spectroscopy and imaging methods can be implemented with magnets with extremely favorable ratios of sample size to magnet size. Desktop MRI systems are particularly attractive for the rubber industry to mesaure in-line geometrical and physical parameters like cross-link density and the elastic modulus.

In previous work we have demonstrated that the internal and external contours of rubber profiles can be reconstructed with high spatial precision by applying edge-detection algorithms to low-resolution MR images measured with a mobile tomograph implemented on samples at rest [3]. In this work we discuss the potential application of this image processing approach for in-line quality control of extruded rubber materials in short experimental times. The in-line application of the method requires measuring moving samples passing through the magnet at considerable speed. The length of the sample used to reconstruct the complete 2D image depends on the slice thickness, velocity of the extrudate and on the total acquisition time. The latter depends on the pulse sequence employed to acquire the 2D image and the delay between scans. We analyze different pulse sequences that minimize the acquisition time and alternative methods to reduce distortions introduced by lateral sample vibration.

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203TH

MOBILE High-Resolution NMR Sensor for Biodiesel Transesterification Monitoring and Quality Control

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In the last decades, the request for alternative and renewable sources of energy to diminish the dependence on fossil fuel has caused fuels derived from biological sources to be increasingly scrutinized and utilized. Biodiesel [1], defined as the mono-alkyl esters of vegetable oils or animal fats [2], derived from these feedstocks by transesterification, is probably the most commonly used biofuel as a replacement for petroleum-derived diesel fuel.

High-field NMR spectroscopy has been employed in the past for monitoring the transesterification reaction and the quality control of biodiesel. However, the use of conventional high-resolution NMR relies on expensive and sophisticated superconducting magnets. The development towards the highest achievable magnetic fields has pulled NMR spectrometers away from the chemistry hoods in the synthesis laboratories and restricts the use of NMR to experimental conditions more demanding, than those acceptable in industrial environments, where cryogenic liquids often cannot be used. In these cases, small and robust magnets that provide a good share of the performance of large magnets could be installed in chemistry labs to monitor reactions in real time, or mounted in the production line of chemical plants to improve the product quality.

During the last years important progress has been achieved in the development of small, robust magnets [3, 4]. Today it is possible to obtain proton NMR spectra of different compounds contained in a 5mm NMR tube with resolutions better than 0.15 ppm using a magnet that is shorter than the sample tube [3]. In this work we show the performance of this new generation of magnets, working at a magnetic field strength of 1 Tesla. These high-resolution systems allow us to follow the rate of conversion during the biodiesel transesterification process and to quantify the concentration via line integration. We also show the application of this small magnet to determine physical properties of the biofuel such as the self-diffusion coefficient from which the viscosity can be determined. The diffusion coefficient was measured with pulsed field gradients benefitting from the excellent switching times of the magnet.

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204MO

The Use of Unilateral NMR to Study the Effect of Biodiesel/ Diesel under Different Elastomers

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Biodiesel is an energetic matrix with several advantages when compared to fossil fuels. However, the compatibility of biodiesel with materials from automobile engines, especially elastomers, is a growing concern. Thus, the aim of this work was to use low-field unilateral NMR to study the effect of biodiesel/diesel in commercial rubbers. For this purpose, natural rubber (NR), ethylene-propylene-diene rubber (EPDM) and nitrilic rubber (NIT) was immersed for 28 days at 25˚C in a B20 mixture (20% of biodiesel in 80% of diesel). Rubber before immersion (BI) and after immersion (AI) in B20 mixture were analyzed in a NMR Tecmag instrument equipped with a home-made low-field unilateral magnet, operating at 14.3 MHz. Transverse relaxation times (T_2) were obtained through Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence at 23˚C. Data were processed by using both NTNMR and Microcal Origin 6.0 softwares. Thereby, information about molecular mobility could be assessed since polymeric chains perform movement from one equilibrium position to another. To NR-BI a 2.6 ms of transverse relaxation time T_2 was observed and after immersion in biodiesel solution the transverse relaxation tends to 11.2 ms. The increasing tendency is observed to other rubbers which T_2 relaxation to EPDM-BI is 5.7 and EPDM-AI is 11.6 ms and NIT-BI is 1.6 ms and NIT-AI is 3.6 ms. In general, the disparity of relaxation times for different elastomers is related to changes in their microstructure and for harder rubbers a shorter T_2 is obtained. Thus, the increase of relaxation time to all studied rubbers denotes a softening of system and as the biggest variation is to NR, it means that this rubber is most susceptible to solution of biodiesel (B20) while NIT exhibited less variation of relaxation times, indicating more resistance. Besides, in general, with shortening of T_2, glass transition temperature (T_g) increases. This occurs as a result of a major cross links between chains that is correlated with lower molecular mobility and steric hindrance of segments. Therefore, the increase of T_2 in the studied rubbers reveals the rubber degradation at lower temperatures which is related to the decreasing in T_g. It must be considered that less number of cross links can also be related to the increasing of the porosity of the material and the B20 mixture can penetrates in these porous, as observed by high resolution NMR and diffuse reflectance infrared spectroscopy.

205TU

An Algorithm for Image Reconstruction in Non-Linearly Varying Magnetic Fields

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Processing industries are becoming increasingly reliant on advanced sensing technologies to improve efficiency. In the mining and food industry the on-line analysis and imaging of key parameters such as specific elements, minerals or moisture, improves the characterization of raw materials on conveyor belts and is necessary for manufacturing and process quality assurance. To make electrically conductive (water-saturated) raw materials in large sample volumes accessible by NMR, one has to operate at low frequencies due to the electromagnetic skin effect. The main objective of the work reported here is to propose a numerical algorithm for on-line NMR imaging in spatially non-linear static magnetic fields at low strength. Wideband spin-echo pulse sequences are employed for signals generation. We study the resolution and sensitivity of this non-conventional imaging configuration where the sample moves through the static field of a Helmholtz coil. To localize the origin of the NMR signal in the sample volume we do not employ additional magnetic field gradients as in conventional imaging devices, but utilize the natural spatially dependent inhomogeneity of RF and DC fields generated by surface and volume coils. Detailed modelling of realistic on-line geometries will be described.

206WE

LOW-FIELD RELAXATION IMAGING AND CROSS-RELAXATION INVESTIGATIONS OF BOVINE ARTICULAR CARTILAGE

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The layered structure of mammal articular cartilage is reflected by a pronounced depth dependence of T_2, which is a consequence of different degrees of order of the collagen fibers but also of a gradient of water and glycosaminoglycan (GAG) concentration, respectively. The orientational order results in an angular dependence of T_2 that becomes less pronounced at greater distance from the joint surface. T_1 at conventional laboratory fields does show little variation in comparison.

In this study, the dependence of relaxation times in bovine articular cartilage is investigated at a low magnetic field strength of 0.27 T using a portable scanner. While a systematic variation of T_2 is found that is in agreement to similar mammalian cartilage observed at high fields, T_1 also shows a strong depth dependence that correlates with the separation of the tissue into three distinct zones. This pronounced effect is explained by the increased T_1 contrast commonly found towards smaller magnetic field strengths, a consequence of slow and anisotropic molecular reorientations that dominate the relaxation dispersion at small Larmor frequencies.

The same experiment has been repeated after saturating the samples in 0.8 mM aqueous Gd-complex solutions that reduce T_1 of water protons. If a charged complex such as gadopentetic acid $(Gd(DTPA)^{2-})$ is used, the T_1 change is found to be depth-dependent and can be exploited for a routine assessment of damaged tissue as it is found for a number of degenerative diseases; neutral Gadoteridol (Gd(HPDO3A)), on the other hand, gives rise to a uniform T_1 change. As a complementary but non-invasive approach, the ^{1}H-^{14}N cross-relaxation rate at the characteristic frequencies of the nitrogen quadrupolar nucleus is suggested as an evidence for a variation in overall protein (GAG) concentration and mobility.

E. Rößler, C. Mattea, A. Mollova, S. Stapf, *Low-field one-dimensional and direction-dependent relaxation imaging of bovine articular cartilage*, J. Magn. Reson. **213**, 112-118 (2011).

207TH

NUCLEAR MAGNETIC RELAXATION IN WATER AT ULTRA LOW MAGNETIC FIELDS

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Nuclear magnetic relaxation of water has been studied since the early times of NMR and is understood within the well known relaxation theory given by Bloembergen, Purcell and Pound (BPP). The range of low Larmor frequencies was studied using field cycling techniques, which have some limitations for the field range of a few microtesla. Here, we focus on this range by making use of a home made superconducting quantum interference device (SQUID) based NMR-spectrometer inside a heavily magnetically shielded room, which enables us to measure the spin dynamics of liquid water below the Earth´s geomagnetic field down to 100 nanotesla. Our data for the T_1- and T_2-relaxation rates of pure water are in line with the BPP relaxation theory except for the field range below 20 microtesla (see figure 1). Both T_1- and, much more dramatically, T_2-relaxation rates of water are increased with respect to the theoretical expectation, while towards zero field both relaxation rates nicely converge. We further show that this behaviour depends on the pH-value and is proportional to the content of the hydrogen isotope 17O of the investigated water samples.

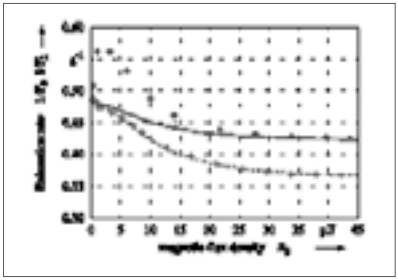

Figure1: Measured T_1- (red) and T_2-relaxation rates (blue) in dependency to the Larmor frequency. The dotted red line shows the fitted T_1- relaxation theory, the dashed blue line the expected appearance of the T_2-relaxation rate.

208MO

^{1}H DQ NMR in Vulcanized Natural Rubber Filled with Ferroelectric Ceramic Nanoparticles

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Time domain NMR has become a major method for dynamic and structural characterization of polymer networks. Network chains are formed by repetitive units in dynamically restrict regions whose length scale is inversely related to the intensity of residual dipolar couplings, which relates to the density of cross-links in the network. Among the methods currently used in polymer network studies, ^{1}H double-quantum (DQ) NMR is one of the most versatile and robust, providing a reliable estimation of the average dipolar couplings values as well as their distribution. In this study ^{1}H double-quantum (DQ) NMR was used to investigate vulcanized natural rubber filled with ferroelectric ceramic nanoparticles of potassium niobate strontium $KSr_2Nb_5O_{15}$ (KSN) (20 nm). Such filler polymers have attracted scientific and technological interest because of their potential use in flexible electronic devices and electromagnetic wave absorbing materials. Aiming to investigate the effect of the filler on the network formation during the vulcanization process, samples with different concentration of nanoparticles (0, 1, 2, 3, 4, 5, 10, 20 and 50 phr) and thickness (0.2 mm, 2 mm and 6 mm) were compared. The preliminary results showed an increase in the residual dipolar coupling upon filler addition, which was found to be thickness dependent, but independent of the nanoparticles concentration. This behavior was attributed to the higher thermal gradient during the sample preparation in thinner samples, providing stronger cross-linked networks. Interestingly, the 2 mm thick sample filled with 3 phr of KSN showed increased of the average dipolar coupling as compared with the other filled samples, which resulted in a higher glass transition and enhanced mechanical properties of filled vulcanized natural rubber.

209TU

The Density Matrix Theory of the COSY and the DQF-COSY NMR Experiments

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Two steps in the phase cycle are necessary in the COSY NMR experiment to achieve phase modulation of the signal during t_1 and eight steps in the phase cycle are needed in the DQF-COSY NMR experiment to achieve double quantum filtration and phase modulation of the signal during t_1. This means that one needs to make the calculation ten times if one wants to simulate the COSY and DQF-COSY NMR spectra of any spin system by using either the density matrix or the product operator formalism. By using the density matrix, it will be shown in this presentation that one needs to calculate only one set of coefficients to simulate both the COSY and the DQF-COSY NMR spectra of an AX system of any spins. This contributes to a significant simplification of the calculations compare to a full density matrix treatment. The expressions found in the case of an AX system of any spins are also valid for an AMX system of three spins I=7/2. Some theoretical simulations for such a system in the case of slow, intermediate and fast relaxing quadrupolar nuclei will be presented to illustrate the theory.

210WE

An Ant Colony Optimization Based Approach for NMR Structure Based Assignment Problem

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Nuclear Magnetic Resonance (NMR) Spectroscopy is an important technique that allows determining protein structure in solution. An important problem in protein structure determination using NMR spectroscopy is the mapping of peaks to corresponding nuclei. Structure Based Assignment (SBA) is an approach to solve this problem using a template structure that is homologous to the target. Previously we have developed an approach based on integer linear programming to solve this problem within NVR framework. This approach computed the optimal solution that minimized a scoring function according to distance (NOE) constraints. However, it was unable to solve the assignments for larger proteins. NVR-TS is an alternative approach that applies a tabu search approach to this problem and computes the optimal solutions for small proteins. NVR-TS can also compute a solution for larger proteins. In this paper, we propose an ant colony optimization based approach to this prob-lem. Our method finds optimal solutions for small proteins and achieves higher accuracies on larger proteins compared to NVR-TS.

211TH

Sequence-Specific Mapping of the Interaction between Urea and Unfolded Ubiquitin from Ensemble Analysis of NMR and Small Angle Scattering Data

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The molecular details of how urea interacts with, and eventually denatures proteins, remain largely unknown. In this study we have used extensive experimental NMR data, in combination with statistical coil ensemble modelling and small angle scattering, to analyze the conformational behavior of the protein ubiquitin in the presence of urea. In order to develop an atomic resolution understanding of the denatured state, conformational ensembles of full-atom descriptions of unfolded proteins, including sidechain conformations derived from rotamer libraries, are combined with random sampling of explicit urea molecules in interaction with the protein. We demonstrate that the direct-binding model of urea to the protein backbone is compatible with available experimental data. In the context of this model we find that in the presence of 8M urea approximately 40% of the backbone peptide groups bind a urea molecule, independently reproducing results from a model-free analysis of small angle neutron and X-ray scattering data. Crucially, this analysis also provides sequence specific details of the interaction between urea and the protein backbone. The pattern of urea-binding along the amino-acid sequence reveals a higher level of binding in the central part of the protein, a trend which resembles independent results derived from chemical shift mapping of the urea-protein interaction. Together these results substantiate the direct-binding model and provide a framework for studying the physical basis of interactions between proteins and solvent molecules.

212MO

Quantum Chemical Calculations of Zero-Field Splitting in Nickel(II) Complexes: Can We Trust the DFT?

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The zero-field splitting (ZFS) is an important quantity in the electron spin Hamiltonian for S=1 or higher. During recent years, we could see a rapid development of quantum chemical methods to calculate this property, led by Frank Neese and his group[1]. Here, we report calculations of the ZFS in some six- and five-coordinated Ni(II) complexes (S=1), using different levels of theory within the framework of the ORCA program package[2]. We compare the high-end *ab initio* calculations (CASSCF, NEVPT2), making use of both the second-order perturbation theory and the quasi-degenerate perturbation approach, with DFT methods using different functionals. The pattern of results obtained at the *ab initio* levels is quite consistent and in reasonable agreement with experimental data. The DFT-calculated ZFS are very strongly functional-dependent. The functionals BLYP and PBE, recently used in a study of hydrated Ni(II)3 [3] do not seem to function very well for our systems.

We wish to thank Professor Frank Neese for providing us the ORCA program. This work was partially supported by Swedish Research Concil and by funds for science in years 2009-2012, as research project No N N202 105936, Polish Ministry of Science and Education.

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213TU

Quantum Mechanical Simulation of Cross-Effect DNP with Krylov-Bogolyubov Time Averaging and State Space Restriction

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Dynamic nuclear polarization is a technique that enhances the NMR signal by transferring spin polarization from paramagnetic centers to the surrounding nuclei. To gain more insight, a careful analysis of quantum-mechanical simulations should be carry out. However, due to the high dimension of the problem within the full Liouville space formalism one is restricted to a relatively small number of spins [1],[2].

Cross-effect DNP involves the interaction of two coupled electrons with neighboring nuclear spins. The matching conditions require the difference in electron Zeeman frequencies to be close to the nuclear Larmor frequency. When the electronic dipole-dipole interaction strength is sufficiently large CE DNP leads to very effective polarization build-up, which is widely used in experiments with bi-radical molecules. We apply an averaging technique proposed by Krylov and Bogolyubov to restrict the evolution of the spin system to a subspace that consists only of a set of states with zero and single-quantum coherence order, profoundly reducing the dimension of the required state space. In the presence of the relaxation, higher spin correlation order states are weakly populated and, in general, without a significant loss of the accuracy, can be omitted leading to a further reduction of the dimension for these calculations [4]. The relaxation superoperator in our strategy is derived for the full set of pure electronic and nuclear Zeeman states avoiding the need to diagonalize the Hamilton. Our strategy makes it possible to increase the number of spins by a factor of two and substantially reduces the required computational time.

A set of numerical results are presented using our strategy to simulate polarization transfer in multi-spin solid-state sample containing 2 electron spins and up to 11 nuclear spins. The detailed analysis of the error caused by the averaging technique as well as a validation of the relaxation pathways is presented. The influence of microwave power and electronic dipolar interaction strength on the final level of polarization is discussed. We also discuss the perspective for increasing the number of spins in the simulation even further.

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214WE

CHEMICAL SHIFT PREDICTION FOR PROTEIN STRUCTURE CALCULATION AND QUALITY ASSESSMENT USING AN OPTIMALLY PARAMETERIZED FORCE FIELD

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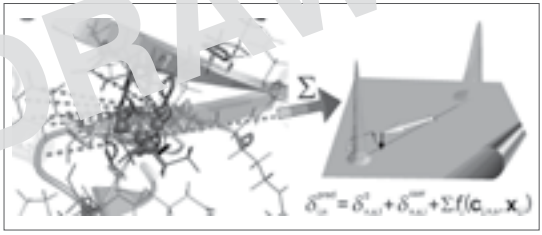

The exquisite sensitivity of chemical shifts as reporters of structural information, and the ability to measure them routinely and accurately, gives great import to formulations that elucidate the structure-chemical-shift relationship. Here we present a new and highly accurate, precise, and robust formulation for the prediction of NMR chemical shifts from protein structures. Our approach, shAIC (shift prediction guided by Akaikes Information Criterion), capitalizes on mathematical ideas and an information-theoretic principle, to represent the functional form of the relationship between structure and chemical shift as a parsimonious sum of smooth analytical potentials which optimally takes into account short-, medium-, and long-range parameters in a nuclei-specific manner to capture potential chemical shift perturbations caused by distant nuclei. shAIC outperforms the state-of-the-art methods that use analytical formulations. Moreover, for structures derived by NMR or structures with novel folds, shAIC delivers better overall results; even when it is compared to sophisticated machine learning approaches. shAIC provides for a computationally lightweight implementation that is unimpeded by molecular size, making it an ideal for use as a chemical shift force field.

215TH

NOE-NET : USE OF NOE NETWORKS FOR THE NMR RESONANCE ASSIGNEMENT OF PROTEINS WITH KNOWN 3D-STRUCTURE

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CNRS, Gif sur Yvette, France

Structural genomics programs today yield an increasing number of protein structures, obtained by X-Ray diffraction, whose functions remain to be elucidated. NMR plays here a crucial role through its ability to readily identify binding sites in their complexes or to map dynamic features on the structure.

An important NMR limiting step is the often fastidious assignment of the NMR spectra. For proteins whose 3D structures are already known, the matching of experimental and back-calculated data allows a straightforward assignment of the NMR spectra.

We developed NOE*net*, a structure-based assignment approach (1). It is based on a complete search algorithm, robust against assignment errors, even for sparse input data. It allows functional studies, like modeling of protein-complexes or protein dynamics studies for proteins as large as 28 kD.

Almost any type of additional restraints (chemical shifts, dipolar couplings…) can be used as filters to speed up the procedure or restrict the assignment ensemble. NOEnet being mainly based on NMR data (NOEs) orthogonal to those used in triple resonance experiments (J-couplings), its combination even with a low number of ambiguous J-coupling based sequential connectivities yields a high precision assignment ensemble.

The efficiency and the limits of this approach will be discussed on a series of experimental exemples and applications.

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Robust structure-based resonance assignment for functional protein studies by NMR.

J. Biomol. NMR. (2010) **46**, 157-73.

The software is available at the following address : www.icsn.cnrs-gif.fr/guittet

216MO

PHOSPHORUS CHEMICAL SHIFTS IN DREW-DICKERSON DODECAMER AND DNA HAIRPIN FROM MD-DFT CALCULATIONS: NMR BASED FORCE FIELD VALIDATION

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Structural determination of nucleic acids from nuclear magnetic resonance spectroscopy (NMR) relies on sufficient number or NMR restrains and an adequate force field (FF). An important open point here is the possible use of isotropic ^{31}P chemical shifts (δP) as structural restrains. At the same time, attempts have been made recently (*J. Mol. Biol.* **2008**, 382, 956) to use experimental δP for FF validation in terms of populations of the BI state (both torsion angles at the phosphate atom in +gauche region) and the BII state (one torsion angle +gauche, the other trans). Our work studies the relationships between δP and structure as well as the possibility to validate FFs by means of comparing δP from quantum chemistry and from experiment. δP for a canonical B-DNA with the sequence [d(CGCGAATTCGCG)]2 and a DNA hairpin with the sequence d(GCGAAAGC) have been computed using density functional theory. The calculations have been performed on dimethyl-phosphate (DMP) and isopropyl-ethyl-phosphate (IPEP) with geometries cut out of snapshots of classical molecular dynamics simulation of the two DNAs. The molecular dynamics (MD) / density functional theory (DFT) values of δP reported in this work are capable of reproducing qualitative trends in sequence dependence of experimental P provided that MD is capable of determining the correct ratio of % of BII for the sugar-phosphate-sugar steps in question. The quantitative trends are in theory overestimated by a factor of ca. 1.7. When scaled by the inverse of the latter factor, theoretical δP for the BI and BII subparts of the MD trajectory can be used to determine the expected % of BII in time so that the experimental value of δP is matched.

The results of the simulation have been further analyzed in terms of the torsion angle dependence of δP. The dependences of the shifts on torsion angles α and ζ found in our earlier work are preserved upon extending the model from DMP to IPEP. The chemical shift maps for DD and HP demonstrate that δP is by far not determined solely by the BI/BII ratio for the particular step, but that both of torsion angles α and ζ are important for δP. Especially the BII values are sensitive to the particular combination of α and ζ . The unusually high value of δP for the step G3pA4 of HP seems to arise due to the torsion angle β flipping from its usual trans to the +gauche region, which implicates that also the value of the angle β must be considered for δP if in a given step significantly deviating from its conventional value. Finally, phosphorus chemical shift in DMP has been studied by means of varying torsion angles α and ζ on a grid over the whole range of 360o, as well as by means of calculations on snapshots from Car-Parrinello molecular dynamics. Our MO analysis of contributions to δP implicates that the difference in δP of the gauche, gauche vs. the gauche, trans conformations arises due to stereoelectronic effects involving the P-$O_{anjonic}$ bonds. The findings made in this work provide transparent structure - δP relationships for possible use as NMR restrains and suggest that NMR calculations on MD snapshots can be in the future employed for the validation of newly developed FFs.

217TU

CLEAN PROCESSING OF RANDOMLY SAMPLED MULTI-DIMENSIONAL NMR DATA SETS

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We show that the CLEAN processing [1] that originally was introduced for reconstructing multidimensional spectra from projections is also useful for processing randomly sampled data sets. The technique was tested using randomly sampled 2D and 3D data sets for both, liquids and solids samples. The time saving factor of 4 to 6 was achieved in 2D applications while in 3D experiments a factor of 20 to 30 is readily achievable.

The 'CLEAN' algorithm was first devised to correct problems that arise in radio astronomy [2,3]. This is a general iterative procedure designed to separate a particular physical response from its associated artefacts provided that the form of both are known a priori. In radio astronomy it is employed to suppress distortion of the image caused by known deficiencies in the antenna array. In NMR spectroscopy it can remove the characteristic dispersion-mode components from the phase-twist lineshape [4]. The CLEAN algorithm was then reinvented in multi-dimensional NMR [1], where limited radial sampling creates well-defined ridge-like artefacts. It searches for the tallest peak in the reconstructed spectrum and then subtracts that response along with its associated artifacts, storing the intensity and frequency co-ordinates in a table. The next stage considers the next tallest peak and removes it from the spectrum. Each new iteration subtracts an additional set of the offending artifacts, thus reducing the number of residual false crosspeaks ('false positives'). The algorithm continues until the search routine has found all responses that lie just above the baseplane noise. The same algorithm was later adapted to reduce artefacts generated by other types of non-uniform sampling.

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218WE

Closing the Simulation Loop: Direct Fitting of Atomic Coordinates of Radicals to Experimental ESR Data

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We report geometric structures of several organic radicals, both in solution and in pro-tein powders, determined by direct variation of atomic coordinates against the experimental ESR data. In the fitting procedure, we take advantage of the recently introduced large-scale spin dynamics simulation algorithms [1] and of the fact that the accuracy of quantum mechanical calculations of magnetic parameters has improved to the point of quantitative correctness – it is now possible (although still expensive from the supercomputing resource perspective) to reproduce experimental ESR spectra by varying atomic coordinates in the DFT input [2].

Solutions or workarounds are presented for the three significant difficulties encountered in such schemes – the local minimum problem in spectral fitting, the lack of vibrational averaging in the quantum mechanical calculations of magnetic parameters and the lack of analytical derivative algorithms for magnetic parameters.

For tyrosyl radicals in protein environment, the fitting runs require around 50 BFGS iterations with four-point central finite-difference g-tensor and hyperfine tensor gradients computed at each step for 63 coordinates – to a total of 25,200 independent GIAO B3LYP/EPR-II calculations, consuming about 10,000 core-hours on our SGI Altix 4700 supercomputer. The cost of the same number of spin dynamics simulations is much smaller. It is likely that the fitting times would be reduced once the analytical gradients of magnetic parameters become available.

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219TH

DFT Calculations of NMR Parameters in Biologically Active Saccharide Derivatives

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DFT calculations were aimed at analysis of 3D structure and NMR parameters (chemical shifts and coupling constants) in oligosaccharide derivatives bearing sulphate groups. Both B3LYP/6–311++G** and M05-2X/6–311++G** methods have been used for geometry optimization evaluating explicit solvent molecules. Optimized geometries showed considerable influences of counterions (Na^+ and Ca^{2+}) upon pyranose rings and the glycosidic linkage conformation. Solvent had only a limited influence upon magnitudes of proton-proton spin-spin coupling constants. Interatomic distances, bond and torsion angles indicated that the structure of the 2-O-sulfated iduronic acid residue influenced geometry of the N,6-sulfated glucosamine residue. Three-bond proton-proton spin-spin coupling constants agreed well with experimental data for both Na^+ and Ca^{2+} counterions. Analysis also showed that the Fermi contact term was not always dominant and that paramagnetic and diamagnetic contributions considerably influenced magnitudes of proton-proton spin-spin coupling constants.1

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220MO

Calculation of NMR Chemical Shifts for Xe Guests in MIL-53

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X-ray diffraction is a well established and standard technique in solving crystal structures of powders as well as single crystals of organic and inorganic materials. Yet, for investigating local structural features, other methods have to be considered, as diffraction requires long-range order.

Here we present structural investigations on MIL53[1], a mesoporous metal-organic framework. MIL53 can incorporate various guest molecules, such as water, acetone, or xenon. Of particular interest is the adjustment of the network to the nature of the guest molecule and the respective loading by breathing (guest shape responsive fitting). This behavior can be further altered by functionalising the organic linker.

We are investigating the local geometry and interaction strength of the host-guest interactions combining DFT calculations under periodic boundary conditions and solid state NMR. The first step is to search for model structures using DFT methods. Different guest molecules were placed in the MIL53 network, to search for binding sites and probe the breathing mechanism. Molecular dynamics simulations were used to support geometry optimisations and test the stability of binding sites. We could show, that for sufficiently high quality settings, the breathing can be reproduced for water and xenon as guests. This is particularly interesting, as the breathing is caused by building a hydrogen bond network in the case of water, and by dispersion forces in the case of xenon. Chemical shift calculations were then used to judge the quality of structure proposals out of the DFT calculations, and explain chemical shift ranges especially for ^{129}Xe. Here temperature effects have to be considered, as xenon wanders through the cavity.

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221TU

The Indirect Spin-Spin 13C-13C Coupling Constants in Polycyclic Aromatic Compounds and Their Correlation to Structural Parameters

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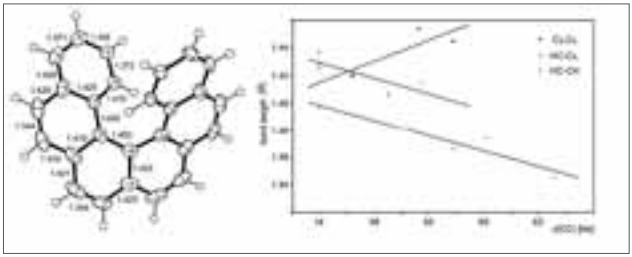
The polycyclic aromatic compounds provided many interesting physical properties. The unusual optical and electronic properties have origin namely in the conjugated π-π aromatic system. These unique systems are mostly studied by theoretical approaches. Here we would like to present interconnection between the structural parameters gained by theoretical approaches with the experimentally accessible data. Especially, we focus on the indirect spin-spin ^{13}C-^{13}C interaction constants, that represent unique source of information on the electron distribution within involved atoms and reflect the carbon-caron bonding environment as the measurement of the ^{n}J(C-C) at natural abundance became feasible using the modern NMR instruments in a reasonable time.

The theoretical prediction of the ^{n}J(C-C) coupling constants was done using DFT calculation (B3PW91/6-311++G(d,p)) which provides an excellent agreement with the experimental values. The experimental values are further correlated to the carbon-carbon bond lenghts and angles, that are accessible by single crystal X-ray crystallography.



The financial support is provided by Technology Agency of the Czech Republic (grant no. TA01010646).

222WE

Non-Invasive Measurements of Water Transport and Optical Properties in the Human Eye

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We have employed MRI to study properties of the human eye that are not readily measurable by standard techniques of optometry. The eye lens has no vasculature, so transport of nutrients and waste products into and out of the lens, which is important for maintaining lens health and preventing development of cataract, relies primarily on passive diffusion. We measured water transport in the human eye lens in vitro using both isotope (D_2O) substitution and diffusion tensor imaging (DTI). The results showed that a barrier to diffusion develops around the lens nucleus with age, which may inhibit transport of antioxidants (glutathione) into the nucleus and contribute to the onset of senile cataract.

Unlike a conventional glass or plastic lens, where refraction of light takes place only at the surfaces, the eye lens exhibits a refractive index distribution, so that refraction occurs continuously through the lens. Using optical methods, to date it has not been possible to measure the refractive index distribution non-invasively without making assumptions about the form of the distribution. We have developed an MRI technique that for the first time allows us to map the refractive index distribution of human eye lenses both in vitro and in vivo and to investigate changes with age and state of accommodation. The results have provided new insights into the aging of the lens and the origins of presbyopia - the loss of the ability to focus on near objects (i.e. to 'accommodate') with age.

We have also shown that NMR micro-imaging of the human eye can provide useful biometric data of value in developing new methods for restoring accommodation and the treatment of refractive errors such as myopia (short sightedness). Conventional optical methods such as slit lamp photography and phakometry are unable to fully characterise changes in lens shape and equatorial radius with age and state of accommodation due to their inability to image behind the iris. Accurate measurements of lens thickness and the axial length of the eye (important in myopia) are also subject to uncertainties arising from incomplete knowledge of the refractive index distribution.

223TH

^{27}Al NMR Thermometry of an Operating Catalytic Reactor

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An NMR based technique capable of providing the quantitative temperature mapping of an operating catalytic reactor has been developed. The technique is based on the detection of the ^{27}Al NMR signal of a solid phase and its temperature dependence. The ^{27}Al NMR signal of a solid phase (support of a heterogeneous catalyst, γ-Al_2O_3) has been detected on a common liquid phase NMR imaging instrument, without application of the special NMR solid state hardware and techniques. The developed NMR thermometry technique has been applied to get the quantitative information on the temperature distribution in a single-pellet operating catalytic reactor in the course of a highly exothermic gas phase catalytic reaction of hydrogen oxidation. The 2D NMR images of a catalyst pellet (4%Pt/γ-Al_2O_3) have been detected using the ^{27}Al NMR signal of a solid phase (γ-Al_2O_3) in the regimes with various hydrogen flow rates and a constant oxygen flow rate characterized by the different average temperature of the catalyst pellet. The detected 2D ^{27}Al NMR images have shown a significant non-uniformity of the NMR signal inside the catalyst pellet. A special calibration binding the ^{27}Al NMR signal intensity of the solid phase and the catalyst temperature has been performed and then used to recalculate the 2D ^{27}Al NMR images of the catalyst pellet detected in the various regimes of the catalytic reactor operation into the temperature maps. The obtained 2D temperature maps have shown a significant temperature gradient inside the catalyst pellet in a radial direction bound with a non-symmetrical placement of the catalyst pellet in the reactor (the pellet has been glued on the reactor wall).

The obtained results show the perceptivity of the application of the developed technique for the quantitative investigations of the heat transfer processes in the operating catalytic reactors, namely in situ.

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224MO

In Vivo Single-Scan 3D Spectroscopic Imaging by Spatiotemporal Encoding

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A method for the acquisition of quality single-scan spectroscopically-resolved 2D spatial images in a single scan is introduced and illustrated. By contrast to the majority of single-shot spectroscopic imaging sequences developed so far, the method here discussed is not based on the acquisition of echo planar data in the k/t-space – but rather on the use of recently proposed spatiotemporal encoding (SPEN) methods. These techniques provide a robust alternative to classical echo-planar spectroscopic imaging (EPSI) techniques, as they can scan two spatial plus one spectral dimension by oscillating a single imaging gradient. Whereas this feature was demonstrated in the past on simple phantoms, it was achieved at the expense of a number of challenges that remained to be overcome. These including compromises in the spatial/spectral characteristics, and an incompatibility with multi-slice operations. Since that original proposal studies have emerged for dealing independently with such limitations –including the use of super resolution algorithms and of inversion (as opposed to excitation-) encoded sequences. This work extends these formulations to achieve an improved spectroscopic imaging experiment capable of resolving multisliced 2D images according to the chemical shifts, in a single <1sec scan. *In vivo* results of fat and water separation on abdominal imaging on mice at 7T and on human breast imaging at 3T are presented.

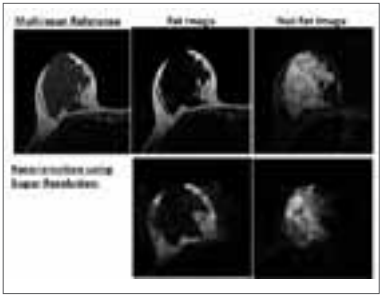

Figure: Comparison between spectroscopic images obtained from a single-scan SPEN sequence (bottom; 1 of 5 slices illustrated) and multi-scan reference images collected with and without fat suppression (top, resolution = 0.8x0.8 mm^2). The SPEN images arose from a single 170 ms long scan; super-resolution processing clearly resolved the fat and water (connective tissue) images at a 2.0x1.33 mm^2 resolution.

225TU

Hyperpolarized Xenon-129 T1 Relaxation Rate Dependence on the Partial Pressure of Oxygen,pO2, in Porcine Lungs

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The ability to map fractional oxygen concentrations across the human lung providing information on regional perfusion and ventilation is extremely valuable in diagnosing lung diseases such as COPD. Due to its non-invasive nature and high sensitivity, regional pO_2 measurements using hyperpolarized (HP) gas MRI has quickly become a popular means of investigating lung function. By monitoring the local relaxation of inhaled ^{129}Xe it is possible to estimate regional pO_2. Several groups have reported pO_2 variations across the human lung [1,2] based on the assumption that changes in T1 are almost wholly due to oxygen concentration. In an excised system it is possible to simulate a breath hold at a near to zero pO_2 to investigate the accuracy of this notion. This allows for T1 mapping of uniform distributions and controlled partial pressures of oxygen well below 20%; a difficult if not impossible study in humans. Regional ^{129}Xe T1 data were measured from a porcine lung set for varying pO_2 which showed homogeneous distributions of relaxation rates across the lungs as well as a significant reduction in T1 for increased values of pO_2, Regional calculations of the Relaxivity of xenon in the presence of oxygen were performed. An average Relaxivity of K= (1.6 +/- 0.3)*10^{-6} s^{-1} Pa^{-1} was found. Relative to an inert glass phantom, there is a finite rate at zero pO_2 indicative of an additional relaxation mechanism. We ascribe this to surface relaxation at the large area of the lung tissue. This surface interaction is homogenous with the exception of the trachea where tissue interaction is expected to be less. In summary, we have measured the Relaxivity of oxygen in porcine lungs and identified two distinct relaxation mechanisms; one proportional to the partial pressure of oxygen and the other we believe due to a surface relaxation process. This is an important step towards quantifying the basic relaxation mechanisms of ^{129}Xe in lung tissue.

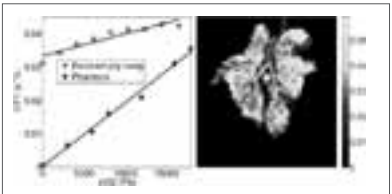

Figure 1 a) Relaxivity plot b) Intercept map,s^{-1}

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226WE

Brain Metabolites Change During Visual Sexual Stimulation in Healthy Women Using Functional MR Spectroscopy

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Numerous functional magnetic resonance imaging (fMRI) studies demonstrated the key brain areas associated with visual sexual arousal. However, the changes in brain metabolites involved in visual sexual stimuli have not been reported. This study utilized functional MR spectroscopy (fMRS) to evaluate the changes in brain metabolites associated with sexual arousal induced by stimulation with erotic video clips in healthy women.

Twenty-three healthy, right-handed women (38.4±10.0 years) participated in ^{1}H-fMRS and fMRI studies. T1 and T2 MR images were used for voxel localization of the anterior cingulate gyrus, which is one of the most important key centers associated with sexual arousal. The single-voxel ^{1}H MRS measurements were performed using a point-resolved spectroscopy (PRESS) sequence with TR/TE = 2000/30 ms, 96 acquisitions (scanning time 3 minutes 20 seconds), 1200 Hz spectral width, 1024 data points, and 7.2 cm^3 (18 × 20 × 20 mm) voxel size. The changes of brain metabolites were measured during the time- frames: "before-during-after" visual sexual stimulation.

During visual sexual stimulation with erotic video clips, the average score for the perceived sexual arousal was 3.13 ± 0.76 (mean ± S.D) on the 5-point Likert scale. The subjective perceptual response to sexual arousal "during" visual stimulation was significantly different from both of "before" and "after" conditions (ANOVA test; F=182.15, p<0.001). "During" the visual sexual stimulation, concentrations of α-Glx/Cr, β·γ-Glx/Cr, Cho/Cr and Lac/Cr increased significantly as compared to "before" or "after" sexual stimulation (repeated-measures ANOVA, p<0.05). After visual stimulation, however, the levels of most metabolites were recovered to the equilibrium state in which no significant difference of metabolites concentration between "before" and "after" time frames was observed (p>0.05).

In conclusion, ^{1}H-fMRS, for the first time, was applied to assess the brain metabolic changes at the different time frame of "before-during-after" visual sexual stimulation. The fMRS outcomes in relation to functional MRI data will be more useful to understand the neural mechanism associated with sexual arousal.

227TH

Optic Nerve: Separating Compartments Based on ^{23}Na and ^{7}Li TQF Spectra and TQF-Diffusion Anisotropy

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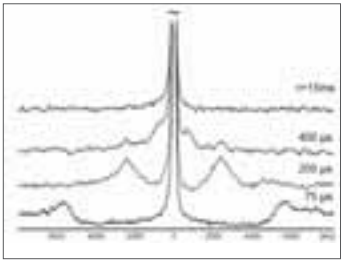

Sodium ions play an important role in physiology and body homeostasis. In connective tissues they were used to characterize structure on the basis of residual quadrupolar interaction that reflects local order and the extent of binding of the sodium ions to macromolecules. In the current study we present results that demonstrate that in optic nerve there are several compartments where the quadrupolar interaction is not averaged to zero indicating that sodium ions motion is anisotropic. In the figure we show results of triple quantum filtered (TQF) NMR spectra of Na measured by the pulse sequence 90-τ-90-tTQ-90-τ-90-t_{LM}-90-Acq. At least three different splittings are clearly observed at 10, 5 kHz and one with a hardly resolved splitting smaller than 2 kHz. Their intensities reach a maximum with 1/τ of the order of the quadrupolar splitting. In order to determine whether the signal measured at τ=200μs (5kHz splitting) and that observed with τ>12ms stem from compartments with different geometries, diffusion measurements were conducted. It was found that the diffusion anisotropy of the 5 kHz splitting is much smaller than that of the peak observed with τ>12ms i.e D_{zz}/D_{xx} is 1.9 and 4.5 respectively. These results suggest that the larger splitting is mostly due to the extracellular matrix while the latter is due to compartments such as axons and cells. The above results are consistent with previous results reported for D_2O in optic and sciatic nerves. The binding of the sodium to macromolecules is further supported by measurements of longitudinal relaxation using TQF, where bi-exponential decay was found for the 10 and 5 kHz splittings. Similar trends were observed for samples where sodium was replaced by lithium. From the shifts of ^{7}Li TQF spectra susceptibility differences in different compartments are evident.

228MO

Monitoring Hydrogenation of Propene in Microfluidic Chips by Remote Detection MRI

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Microfluidic devices provide capabilities that exceed those of large scale systems. NMR spectroscopy would be an almost perfect method for monitoring processes in microfluidic devices as it is non-invasive and provides versatile spatial, dynamic and spectroscopic information. However, small amount of fluid and extremely low fluid filling factors in microfluidic devices make conventional NMR experiments using a large coil around a microfluidic device difficult or even impossible, especially when dealing with gases having density of several orders of magnitude lower than liquids' density.

Remote detection (RD) NMR method, in which the encoding of spin coherences of fluid flowing through a microfluidic chip is performed by a large coil around the chip and the signal is detected by another, much smaller and sensitive coil outside the chip, provides an elegant solution to the sensitivity issue [1,2]. In addition, the method enables one to measure time-of-flight flow images. In this work, we monitor hydrogenation of propene gas in microfluidic chips containing several parallel channels coated with platinum. We show that RD MRI provides detailed information about active reaction regions, reaction kinetics and fluid dynamics in chips. Hydrogenation of propene using parahydrogen did not result in parahydrogen-induced polarization (PHIP), implying that correlation of hydrogen atoms of a parahydrogen molecule is lost due to the fast diffusion of hydrogen atoms on the Pt surface. Earlier, we showed that combined RD MRI and PHIP methods allow gas flow profiling in microfluidic chips [3,4] and detailed analysis of performance of microfluidic packed-bed reactors [5].

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229TU

Flow Propagators in a Micromixer by NMR Imaging

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A chemical micromixer was studied by pulsed-field-gradient-spin-echo (PGSE) nuclear magnetic resonance (NMR) imaging and velocity distributions (so called propagators) with two dimensional spatial resolution were measured. In order to gain a better signal-to-noise ratio (SNR) a surface coil matching the volume of interest was built enabling the acquisition of velocity maps with very high spatial resolution. The experimental data is also compared with theoretically derived distributions and a good agreement was found. The results show that the propagator data can provide rich information about flow behaviour and the information is essential for evaluating the performance of a micromixer. The data reveals, for example, deviations in the shape and size of the channel structures and multicomponent flow velocity distribution of overlapping channels. In the latter case the propagator data efficiently compensates the lack of spatial resolution in the third dimension (which is typical for velocity imaging by NMR imaging).

230WE

^{2}H Solid-State NMR Study of the Effect of Antimicrobials on Intact Non-Mutated E. coli

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Considering the complexity of their composition, especially the presence of lipopolysaccharides (LPS) in their external membrane, the interaction of molecules with Gram-negative bacteria should ideally be studied on intact cells. Therefore, the aim of this work was to facilitate the ^{2}H solid-state (SS) NMR study of membrane interactions on intact bacteria by deuterating phospholipids in non-mutated *Escherichia coli.* ^{2}H labeling of the lipid acyl chains does not modify the membrane properties and its signal is sensitive to changes in the membrane organization and dynamics. Bacteria were grown in the presence of deuterated palmitic acid. Fatty acid analyses reveal that 76% of the phospholipids have deuterated acyl chains. The responsiveness of these E. coli was evaluated using molecules with reported antimicrobial activity. ^{2}H SS-NMR spectra obtained on intact bacteria in the presence of the antibiotic polymyxin B (PxB), fullerenol nanoparticles (NPs) and the detergent cetyltrimetylammonium chloride (CTAC) revealed in all cases an increased proportion of lipids in the gel phase. This effect is in agreement with an insertion of PxB lipid tail in the bacterial membrane[1] while an interaction of the NPs with LPS in *E. coli's* outer membrane would induce a tighter phospholipids packing. Interestingly, lipid segregation and a disordering effect on DPPG acyl chains is seen with model DPPC/DPPG membranes[2]. This difference will be discussed. CTAC has the strongest effect, killing most of the bacteria by rigidifying their membrane via insertion of the detergent hydrophobic chain[3]. Finally, we studied the effect on *E. coli* membranes of the external form of the blue pigment marennine produced by marine microalgae (*Haslea provincialis*). This polyphenol has a molecular weight of ~10 kDa and shows activity against marine bacteria[4]. Our results revealed a greater proportion of lipids in the gel phase induced by the pigment and suggest some affinity for the LPS. In conclusion, our study demonstrates successful deuteration of the membrane phospholipids of *E. coli* without requiring any bacteria mutation and their application to the ^{2}H NMR study of various antimicrobial types. [1]Delcour (2009) Biochim. Biophys. Acta 1724, 808 [2]Brisebois, Arnold, Chabre, Roy & Marcotte (2012) Eur. Biophys. J. in press [3]Beyer (1986) Biochim. Biophys. Acta 855, 365 [4]Gastineau *et al.* (2012) *J. Agric. Food Chem.* in press J

231TH

The Emergence of Evanescent Wave Coupling and Wave Interference Phenomena at NMR Frequencies

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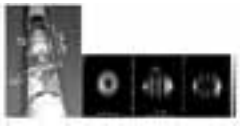
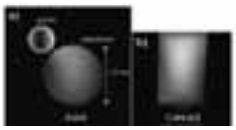
At high-field strength (> 4 T), the propagation wave vector of the excitation field can no longer be ignored as the wavelength becomes comparable to the imaging volume, particularly if the medium's dielectric constant is large. We present our latest developments on a travelling wave transmission system that demonstrates, for the first time in NMR, wave interference fringe recording at 16.4T. This result is remarkable as it shows interference effects with more than a single fringe within a FOV that utilizes whole length of the gradient insert (~11 cm). The transmission system consists of a metal bore of the magnet and an acrylic tube filled with deionized water. The excitation of a waveguide is achieved through a transmit-receive loop coil probe placed at one or both ends of the guide. We describe different EM modes propagating inside the dielectric based on the orientation of the loop coil relative to the guide. Another remarkable wave propagation phenomena demonstrated at 3T is evanescent wave coupling and excitation.



On the left, waveguide setup with acrylic tube filled with Di-water + loop-coil. MR images of Di-water phantom: axial (a), coronal off-axis (b) and on-axis (c). Dark spot in the center (a) and horizontal strip (c) is the null of TE_{01} mode. Vertical stripes (b, c) are fringes with period $\lambda/2$=3 cm.



The phantom was placed collinearly with the guide and 29 cm away from the coil. Remarkably, the evanescent field from the propagating mode generates uniform B_1 field inside a phantom with a usable SNR. (b) shows coronal slice of evanescently detected MR signal.

232MO

Analysis of Image Heterogeneity Using 2D Minkowski Functionals Detects Tumour Responses to Treatment

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Tumour responses to treatment in the clinic are conventionally assessed from CT or MR imaging measurements of decreases in tumour size (1). However, for some drugs, tumour shrinkage may take many weeks to become manifest, while for others there may be no change in tumour size despite a positive response to treatment. A characteristic of tumours is their heterogeneous appearance in MR images due to their irregular and uncoordinated growth and a chaotic and intermittent blood supply. The resulting areas of necrosis and hemorrhage can lead to hyper- and hypointensity, respectively, in T_2-weighted images and may also be influenced by treatment, where successful treatment can result in a change in these areas.

Minkowski Functionals (MFs), have been widely employed in cosmology as precise morphological and structural descriptors for the study of the evolution and morphology of galaxies (2,3). Tumour cell death in subcutaneous EL4 murine lymphomas was induced either by treatment with etoposide, a cytotoxic drug, or with a vascular disrupting agent combretastatin A4-phosphate, which produced hemorrhagic necrosis in the absence of any significant change in tumour size. T_2-weighted MR images were acquired pre- and post-treatment, and changes in image heterogeneity were quantified using 2D MFs. We show here that MFs can detect the morphological changes that accompany tumour cell death following drug treatment in the absence of any exogenous contrast agent.

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233TU

Biotransformation of Methanol and Formaldehyde by Bacteria Isolated from Clouds. Comparison with Radical Chemistry.

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Solar light is considered as the predominant catalyst for chemical reactions occurring in the atmosphere. It is generally admitted that in the atmospheric aqueous phase of clouds the reactivity of organic compounds is driven by the presence of free radicals (•OH, NO_3•) produced mainly by photochemical processes. However, it was shown that living and active microorganisms are present there and they could play an active role in cloud chemistry.

In this study biodegradation kinetics of methanol and formaldehyde (two important polutants of the atmosphere) by 4 bacterial strains (*Pseudomonas* spp., *Bacillus* sp. and *Frigoribacterium* sp.) isolated from cloud water have been investigated by NMR at 5 °C and 17 °C. The biodegradation was observed at both temperatures with rates ranged from 10^{-19} to 10^{-21} mol cell-1 s-1 for formaldehyde, and from 10^{-21} to 10^{-23} mol $cell^{-1}$ s^{-1} for methanol. C3 Compounds (glycerol, 1,2- and 1,3-propanediol) were identified as metabolic intermediates from formaldehyde by *Bacillus* sp.

Extent of microbiological oxidation of organic compounds, as an alternative route to radical chemistry in clouds, was considered by comparison of biodegradation rates with those related to the reactivity of organic species with free radicals •OH (daytime chemistry) and NO3• (nighttime chemistry). Our results give a clear evidence about the same range of magnitude of biological and chemical reaction rates and their relative contribution under tested scenarios, including the temperature of the clouds (5 or 17 °C), the category of the clouds (urban and remote) and the diurnal cycle (day and night time). They also show that biotransformation processes could be the main sink for C1 compounds in liquid clouds during the night in both, polluted and non polluted clouds.

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234WE

Non-Invasive Analysis of *In Vivo* Redox Status on Cisplatin Induced Nephrotoxicity using Overhauser-MRI

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Regulation of tissue redox status is an important factor to maintain normal physiological conditions in the living body. Breakdown of redox homoeostasis may lead to oxidative stress and can induce many pathological conditions such as neurological disorders, inflammation, cancer, and ageing. Therefore, imaging of tissue redox status could have one of the markers for drug discovery and clinical applications for novel diagnosis. Overhauser-MRI (OMRI) enables visualization of tissue redox status in animals based on dynamic nuclear polarization (DNP) using nitroxyl radical as a redox-sensitive probe non-invasively. Cisplatin, major anti-cancer agent, is known to often cause nephrotoxicity, suggesting the involvement of reactive oxygen species (ROS). In this presentation, the evaluation of cisplatin-induced nephrotoxicity and protective effects of antioxidants was performed by OMRI using nitroxyl radicals.

Cisplatin solution was administrated to C57/BL6 mice intraperitoneally. The in vivo redox status in kidney was measured from enhanced OMRI intensity decay rate of intravenously injected nitroxyl probe (membrane permeable and non-permeable one). The decay rate of cisplatin-treated rats was slower than that of control, suggesting that cisplatin causes a change of redox status even after a 3-hr administration. In addition, decay rate in cisplatin-treated group was recovered after treatment of antioxidants such as tempol or N-Acetyl Cysteine (NAC). On the other hand, image intensity of T2 weighted MRI in kidney was increased from 24-hr after injection of cisplatin. In addition, blood urea nitrogen (BUN) level was increased from 72-hr. These results suggested tissue redox status was changed before detection of conventional methods.

235TH

Visualization of Paramagenetic Nitroxyl Probes for *In Vivo* Redox Imaging Using Mass Spectrometry Imaging and Overhauser-MRI

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Nitroxyl probes are redox-active and paramagnetic species which can be oxidized or reduced by the corresponding reactants in vivo. Nitroxyl probes were shown to undergo oxidation to the corresponding oxoammonium by various oxidants such as hypervalent heme, HO_2., CO_3-., and NO_2. radicals. In addition, Nitroxyl probes have been used as redox sensitive probes for electron paramagnetic resonance (EPR) and Overhauser MRI (OMRI) to elucidate redox status in many disease models. In vivo OMRI imaging provides unique functional information such as tissue oxygen, pH and global tissue redox status and shows anatomical structure by high field type of that. On the other hand, mass spectrometry imaging(MSI) utilized matrix assisted laser desorption/Ionization (MALDI) has emerged as a useful technique for the direct observation of various biomolecules in a tissue section. Therefore, combination analysis of OMRI and MSI should be powerful tool to clarify the redox mechanisms on in vivo redox imaging data. In this study, visualization of various kinds of nitroxyl probe (redox probe) by MSI was tested. To demonstrate the possibility of visualization of nitroxyl probe, CAT-1, on in vivo OMRI and MSI imaging using same animal was performed.

Seven nitroxyl probes were measured by MSI with 9-Aminoacridine(9-AA) for negative ion mode and 2,5-dihydroxybenzoic acid(DHB) for positive ion mode as matric to observed MS signal. We observed strong MS signal from ^{14}N-CAT1 and ^{15}N-CAT nitroxyl probes.

In vivo OMRI image of mouse upper abdomen after intravenous injection of CAT-1 showed high intensity area in the hear region. One of the nitroxyl probes, CAT-1 had a strong MS peak (as a reduced form) and was visualized by MSI with high-image resolution (50 um) in mouse heart and kidney tissue sections. These data suggested that OMRI/MSI technique has a possibility for redox analysis with high quality image resolution simultaneous analysis of both alteration of tissue redox status and distribution of metabolites.

236MO

Plasma-Membrane Permeability of S. cerevisiae by PGSE NMR

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The plasma membrane is an essential component of all living cells. It is involved in cell integrity and volume maintenance, metabolism, signal regulation and transport. It is fully permeable for small uncharged molecules like water.

The widely used and well established NMR method for measurement of exchange over plasma membrane utilizes doping of the extracellular compartment by paramagnetic agents which enhance relaxation of extracellular molecules [1, 2]. Alternatively, the difference in apparent translational diffusion coefficients can be used as a "labeling" method to distinguish extra- and intracellular water NMR signal. Such method was proposed and applied to monitor the temperature dependent plasma-membrane fluidity of bakers yeast [3]. The Filter Exchange spectroscopy (FEXSY) experiment comprises two building blocks formed by pulsed gradients of magnetic field. The amplitude and spacing of gradient pulses in first block are set to a fixed value to effectively suppress the NMR signal originating from extracellular water molecules. After short delay (10 – 400 ms) called mixing time, during which the water molecules exchange over plasma membrane, the second block is applied. Here the amplitude of gradient pulses is gradually incremented in sixteen steps in order to perform a standard NMR experiment for measurement of translational diffusion. The most interesting parameter – the intracellular water life-time – is then obtained by fitting the theoretical equations (Eq. 2-12 in [3]) to experimental signal intensities.

We studied three *S. cerevisiae* deletion mutant strains (erg2, erg4 and erg6) which lack genes involved in the synthesis of ergosterol, the most important steroid compound present in the plasma membrane.

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237TU

Vivaldi: A Visualisation and Validation Resource at PDBe

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We describe a new service that aids in the analysis, visualisation and validation of NMR structures present in the Protein Data Bank (PDB). It provides access to experimental NMR data and model coordinates, using interactive visualisation tools, and augmented with structural annotations and model validation information.

The service, called Vivaldi (Visualisation and Validation Display; http://www.pdbe.org/vivaldi), presents information about the modelled NMR ensemble, validation of experimental chemical shifts, residual dipolar couplings and distance restraints and validation scores based on empirical knowledge and databases. Vivaldi was designed for both expert NMR spectroscopists and casual non-expert users who wish to obtain a better grasp of the information content and quality of NMR structures in the public archive.

Vivaldi displays clustering information, high-level conformational analysis results and validation data calculated by a variety of programs; it makes this information available through interactive and mutually linked 3D views, graphs, tables and plain text panels.

238WE

Saturation Transfer Difference and WaterLOGSY Nuclear Magnetic Resonance Studies on the Specific Binding between Different Oligosaccharides from Neisseria meningitidis and the Mannose Binding Lectin

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Sanofi Pasteur, Marcy L'Etoile, France

Mannose Binding Lectin (MBL) is a key protein of the innate immune system and most especially of the Lectin Complement Pathway[1] (LCP). This calcium-dependant lectin can bind specifically mannose and N-acetylglucosamine (GlcNAc) residues and more particularly the ones present on the surface of a wide range of pathogens such as viruses, bacteria, fungi, protozoa. The complex formed can activate either the complement system or act directly as an opsonin[2], to finally cause lysis or phagocytosis of the pathogen.

Lipooligosaccharide (LOS) is the main component of the outer membrane of *Neisseria meningitidis*, a Gram-negative diplococcus, which is a causative agent of bacterial meningitis. The oligosaccharidic component of the LOS is the major exposed and immunodominant part of the LOS, and contains, among other monosaccharides, GlcNAc residues[3].

Previous works done in our laboratory have reported the binding capacity of MBL with some immunotypes of *N. meningitidis*. To understand the interaction mechanism and the immunotype specificity observed, we investigated the interaction at the molecular level. The study was performed using purified oligosaccharides obtained by acidic hydrolysis from several meningococcal LOS immunotypes and commercially available MBL.

The protein-ligand interaction was confirmed using Nuclear Magnetic Resonance spectroscopy, especially Saturation Transfer Difference (STD-NMR[4]) and WaterLOGSY[5] sequences.

The implementation of these powerful sequences to our study case demonstrates the key role of the GlcNAc residue along with its position in the oligosaccharide for the interaction with the MBL.

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239TH

Selectively Labeled Ubiquitin from [1-13C]- or [2-13C]-Glucose Sources: Labeling Patterns and Spectroscopic Features

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The spectral resolution in uniformly ^{13}C labeled proteins is limited by ^{13}C-^{13}C J-couplings and residual dipolar interactions. Furthermore, the weak ^{13}C-^{13}C dipolar couplings are truncated by the strong couplings between directly bonded ^{13}C when measuring dipolar distance restraints.

The use of either [1-13C]- or [2-13C]-glucose is expected to lead to a labeling pattern with magnetically active nuclei separated by at least two covalent bonds (P.Lundström et al, J.Biomol NMR, 2007). More specifically, [2-13C]-glucose is supposed to result in labels at Cα while Cβ and CO are not labeled for most of the amino acids (P.Lundström et al, J.Biomol NMR, 2007).

We present a quantitative analysis of the labeling scheme of Ubiquitin synthesized using [1-13C]- or [2-13C]-glucose. The analysis was performed in solution by using 1H-13C HSQC spectra as well as in the solid state. NCA spectra of crystallized Ubiquitin have a linewidth of 18 Hz in the carbon dimension and demonstrate that even though two adjacent carbons are visible in the HSQC spectra, they are most of the time not coupled and therefore not both labeled in the same molecule.

Furthermore, Lundström et al.have found that the labeling pattern for the amino acids derived from the citric acid cycle depends on the number of passes through this cycle. In Ubiquitin, the duration of the expression (in the range from 1.5 to 4 hours) of the protein did not influence its labeling pattern.

Finally, we used this labeling technique to record PDSD spectra with long mixing time (up to 2 seconds) in order to analyse the long-range cross-peaks and investigate the information contents for structural investigations.

240MO

Statistical Signal Recovery of Low Intensity Signals: Uncovering Fossils from the Data Set

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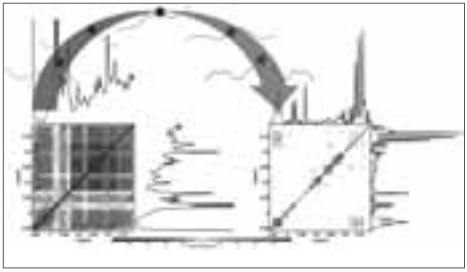
Advances in automated reproducible measurements of ^{1}H-NMR spectra have made it possible to run increasingly large metabolic profiling studies. In large-scale molecular epidemiology studies, such as the INTERMAP study, with intra- and inter-population differences it is not uncommon to find specific metabolite signals present in only a small fraction of the data, very close to the baseline/noise level or both. With higher magnetic field strengths, more of these low-intensity signals become distinguishable from noise and uncovering their identity can be of great importance in understanding the metabolic features in sub-populations. In recent years, statistical total correlation spectroscopy (STOCSY) has proven to be a useful tool in metabolic profiling. It constructs a pseudo selective TOCSY from multiple 1H-NMR spectra. However, a selective pseudo-spectrum of a low-intensity signal can be statistically confounded by spectra without the signal and thus failing to show anything meaningful. We propose using subset selection to extract spectra that contain the signal of interest and to consecutively perform a STOCSY analysis on this subset alone. This will provide an unobstructed view of the hidden 'fossil' in the data set. Subset optimization by reference matching using an iterative learning algorithm (STRATA) has been applied to data from an on-going metabolome-wide association study on obesity in 1880 non-diabetic US individuals from the INTERMAP study. As a new tool in statistical spectroscopy, STRATA helps in elucidating structures of unknown compounds.



241TU

Insulin Deprivation Affects Metabolism and Metabolism-Associated Gene Transcript Levels of In Vitro Cultured Human Sertoli Cells

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Successful spermatogenesis is dependent on several important functions conducted by Sertoli cells. These cells metabolize glucose, producing lactate for developing germ cells. As insulin regulates glucose uptake and its disturbance/insensitivity is associated with diabetes mellitus, we aimed to determine the effect of insulin deprivation in human Sertoli cells (hSCs) metabolism and metabolism-associated gene expression.

hSCs primary cultures were maintained in the absence/presence of insulin and metabolite variations were determined by ^{1}H-NMR. mRNA expression levels of glucose transporters (GLUT1, GLUT3), lactate dehydrogenase (LDHA) and monocarboxylate transporter (MCT4) were determined by RT-PCR.

Insulin deprivation resulted in decreased lactate production and in a decrease of glucose consumption that was completely reverted after 6h. Cells of both groups consumed similar amounts of glucose. In insulin-deprived cells, transcript levels of genes associated to lactate metabolism (LDHA and MCT4) were decreased. Transcript levels of genes involved in glucose uptake exhibited a divergent variation: GLUT3 levels were decreased while GLUT1 levels increased.

Insulin-deprived hSCs exhibited: (1) altered glucose consumption and lactate secretion; (2) altered expression of metabolism-associated genes involved in lactate production and export; (3) an adaptation of glucose uptake by modulating the expression of GLUT1 and GLUT3.

This is the first report regarding the effect of insulin-deprivation on hSCs metabolism.

242WE

Improving the Preprocessing of NMR Spectral Data Before Data Analysis in Metabolomic Studies by Reference Deconvolution

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NMR data, to be submitted to multivariate data processing, require adequate preprocessing of the data. Most common is the process of bucketing or binning, but in this process the information of minor peaks is easily lost. Alternative methods, such as peak picking or deconvolution also present specific problems.
Ideally, the NMR data should be presented in its basic form representing each signal with the chemical shift, signal form and intensity. This form of presenting is independent of the magnetic field and would permit comparison of all different types of NMR spectra.

In reference deconvolution the nmr spectra are corrected for imperfections in the lineshape, especially caused by shimming problems. Inadequate shimming can cause imperfections ranging from increases in the line-width, up to signal distortion or even duplication of the signals.

A software has been developed which automatically uses the signal from an internal standard for the determination of the experimental peak shape. Subsequently all signals in the spectrum are corrected for this peak shape and peaks are reduced to single lines. These data are much more adequate for the subsequent data analysis in metabolomics.

A great reduction in data points is obtained and furthermore small signals are preserved, even when they are not resolved from major peaks.
The spectral data after passing through reference deconvolution present an increase in resolution which facilitates the recognition of the form of the signals from individual hydrogen atoms, as being doublets, triplets or more complex multiplets.

243TH

Nuclear Magnetic Resonance (NMR)-Based Metabonomic Studies of Horse Serum

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Nuclear magnetic resonance (NMR)-based metabonomic studies use multivariate statistical analyses of NMR spectra from biological samples to detect metabolic profiles (metabonomes) associated with diseases, toxins or genetic variations. Osteochondrosis dissecans (OCD) is a developmental orthopedic metabolic disease that leads articular cartilage lesions and is of particular concern in horses. The radiographic lesions become apparent in young horses between 3 to 12 months of age, although clinical signs may become evident only when the animals are put into training. OCD in horses represents an economic disadvantage, due to the cost of the surgical correction and the depreciation of the value of the affected horses. OCD may be heritable in some breeds of horses, but the metabonome associated with it has not yet been identified. I will present our recent findings in NMR metabonomic analysis of horse serum. The results show distinct metabolic profiles of horses affected by OCD versus siblings that didn't develop the disease. It appears that a distinct OCD metabonome does exist. More analysis is underway to fully characterize the significantly contributing metabolites and possibly utilize this knowledge for nutritional/supplemental treatment of the condition.

244MO

Ex vivo HR-MAS NMR, in vivo MRS-MRI and Multivariate Analysis to Highlight Biomarkers in Gliomas

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Gliomas account for about 40% of total primitive brain tumors, and discrimination between high and low glioma grade remains a vital diagnostic decision, determining the most effective treatment and having an important impact on patient management and outcome. The in vivo MRS is considered a tool able to help the diagnosis based on MRI in the evaluation of several human pathologies, including cancer. *In vivo* MRS provides the spectra of living tissues, directly correlated to their chemical composition, but it can be used when the molecular markers of tissues are well established by means of a detailed biochemical picture. This last can be derived from the spectroscopic analysis of *ex vivo* biopsy samples using High Resolution Magic Angle Spinning (HR-MAS) NMR technique. The *ex vivo* HR-MAS NMR spectra provide more details about metabolites (aminoacids, carbohydrates, osmolites, organic acids, mobile lipids) than *in vivo* MRS and permits to produce a metabolic picture of the tissues. Accurate biochemical assignment of metabolites will improve our interpretation of HR-MAS data and the translation of NMR tumor biomarkers to *in vivo* studies. 1D and 2D HR-MAS NMR experiments were used to determine metabolites of brain tumor (astrocytoma grade II, grade III gliomas, glioblastomas). We developed this project on gliomas with the aim to gain a better insight into the discrimination among different grades and subtypes using *ex vivo* HR-MAS NMR, *in vivo* MRS, MRI, clinical data and statistical analysis. We report experiments performed on 15 specimen already collected from different grade glioma. Different amount of some small metabolites such as alanine, lactate, glutamine, glutamate, myo-inositol and glycine in two different *ex vivo* high grade glioma samples. The *ex vivo* spectra obtained on samples from different locations, line-broadened in order to be compared with the *in vivo* MR spectrum, obtained from the same selected voxel. A number of metabolites have been identified as potential biomarkers of tumor type; now we need to combine all the *in vivo*, *ex vivo*, histological and clinical data to obtain a unique tumor fingerprints. Results gathered from this study should lead to the development of tools that can facilitate the distinction of tumor types and grade that cannot be readily distinguished by histopathology or by routine neuroimaging.

245TU

Metabolomics Analysis of Targeted Multi-Compartmental Samples for the Diagnosis of Thyroid Cancer

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The occurrence of thyroid nodules increases significantly since 1990 due to the improvement of the detection techniques (ultrasound-guided fine-needle aspiration technique) [1]. However, the correct diagnosis of thyroid lesions remains challenging since up to 30% of thyroid nodules are classified as “indeterminate” after cytological examination. In this context, a surgical excision is necessary before a definite diagnosis of the thyroid lesions is obtained. Up to about 85% of the patients with indeterminate nodules are finally diagnosed as benign and undergo unnecessary surgery [2]. It is thus important to develop new approaches, which would help in reducing the number of surgical intervention.

Assuming that metabolic variations would pre-empt the development of morphologic modifications associated with malignancy, we have evaluated the potential of NMR-based metabolomics techniques as a complementary tool for thyroid cancer diagnosis.

Our approach focuses on targeted multi-compartmental samples, i.e. tissues excised from thyroid lesions and plasma from peripheral blood, collected from patients with benign and malignant “indeterminate”, as well as well-differentiated malignant tumors. Using high-resolution magic angle spinning (HR-MAS) and liquid-state NMR spectroscopy in combination with statistical multivariate analysis (OPLSDA), we obtained distinct biochemical and metabolic profiles from excised tissue and peripheral blood. For both sample types, a clear discrimination between malignant and benign samples was achieved, leading to statistical models with good prediction efficiency [3]. In addition, complementary sets of markers were characterized providing additional information about thyroid cancer metabolic characteristics. Finally, we have explored multidimensional 1H and 13C slow HR-MAS NMR spectroscopy of different types of tissues [4] to improve the preservation of tissue integrity during HR-MAS NMR experiments.

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246WE

BIRD'S EYE VIEWING OF METABOLIC REACTIONS IN BIOCONSORTIA AND HOST AS A SUPERORGANISM

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The biological evolutionary development of host- microbial symbiosis leads to extensive 'transgenomic' modulation of metabolism and physiology as a superorganism. The composition of the gut microbiome is highly variable, and its diversity can be significantly affected by alterations in diet. The 'transgenomic' modulations of individuals reflected by variations in the microbial symbionts are likely to impact host health and disease. Here I show, with combination of murine gnotobiote model system and multi-omics-based approach, that prior existence of acetate produced by bifidobacteria in the distal colon can prevent death from following enterohemorrhagic Escherichia coli O157:H7 infection, through the enhancement of gut epithelial barrier function [1]. Next, I show an approach to evaluate the intestinal variation and to predict metabolic pathways of major microbial symbionts affected by the variation of food intakes. The covariance of structural variation in gut microbiome and host metabolism was visualized based on the correlation with the denaturing gradient gel electrophoresis (DGGE) and NMR profiles from feces [2]. We improved the DGGE-NMR correlation analysis and the multiple sample collection of a single subject, and integrated the metabolic information, gene expression profiles and microbial community structure in intravital systems. Our approach provides a foundation for evaluation of systemic effects of diet [3] that are of relevance to personal and public heath care solutions. Furthermore, it will open up a new avenue that will clear up metabolic dynamics in the complex microbial community in the gut, not only mammalian, but also fishes and insect systems, along with environmental metabonomics data.

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247TH

THE RELATIONSHIP BETWEEN FITNESS LEVELS AND METABOLOMIC PROFILES IN HEALTHY ADULTS

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Metabolomics is the study of metabolites in biological samples and its application in the field of nutrition and health is increasing exponentially. While much effort has been invested in understanding factors that influence the metabolomic profile there is relatively little known about the effect fitness level has on the metabolite composition of biofluids. This study aimed to establish the relationship between fitness level, substrate oxidation rates and the metabolomic profile.

214 healthy adults aged 18-60 years were recruited as part of a metabolic challenge study. The volunteers underwent a sub maximal 4 stages VO_2 test and had detailed body composition analysis performed. Fasting urine and blood samples were collected. A cohort of 67 subjects (35 male, 32 female) were selected based on their estimated VO_{2max} levels and metabolomics was used to analyse their biofluid samples. The levels of various biochemical markers were determined using an immunoassay. The subjects were split into fitness groups according to their VO_{2max} levels (ml/kg/min).

Statistical analysis of the data revealed significant differences in normalised fat and carbohydrate oxidation levels between the fitness groups. Analysis of the oxygen kinetic data also revealed significant differences between the groups.

Metabolomic analysis of the urine samples revealed significantly different profiles in the fitness groups with the differences being more pronounced in the females. A total of 20 amino acids and derivatives were significantly lower in females in the high fitness group. For males, only 4 amino acids were significantly different. These differences were mirrored in the biochemical analysis of the urine samples which revealed significant differences in the levels of C peptide, IL6, insulin and leptin in the female fitness groups and a significant difference in the leptin levels between the male fitness groups. In conclusion this study demonstrates a relationship between fitness level and the metabolomic profile. Moreover, the metabolite changes show that a reduced excretion of amino acids in adults is associated with increased fitness levels and an increased fat oxidation rate during exercise.

NOT TO BE DRAWN

248MO

^{1}H-NMR in Inborn Errors of Metabolism: Salla Disease and Guanidinoacetate Methyltransferase Deficiency

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Inborn errors of metabolism are genetically determined diseases caused by deficiency of specific proteins – emzymes, transporters, receptors and others. During the last years, enormous progress in diagnostic of inherited metabolic diseases (IMDs) has been observed. A total number of IMDs has been increasing and more than 1000 well defined diseases are known at present. New IMDs due to disorders in various metabolic pathways have been disclosing permanently.

Proton NMR spectroscopy is a special analytical technique playing an important role in the diagnostic. In the diagnostic of hereditary metabolic diseases this is a great advantage compared to other techniques. NMR spectroscopy of body fluids may be considered as an alternative analytical approach for known diagnosing, but also as yet unknown, inborn errors of metabolism. However, as the NMR technique requires no derivatization or extraction there is any loss of metabolites in sample pretreatment. Sample preparation is limited to the addition of an internal standard and pH standardization of samples, because of proton chemical shifts of most metabolites are pH dependent.

In our laboratory ^{1}H NMR spectroscopy of urine samples was used for the detection and diagnosing of two inborn errors of metabolism in children patients - Salla disease and Guanidinoacetate methyltransferase deficiency.

Acknowledgements
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249TU

HR-MAS NMR of Fruits of Plants Belonging to the Rutaceae Family

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Citrus, as one of the worldwide favourite fruits, draws more attention recently due to its therapeutic potentials. In animal models, d-limonene and structural analogues have demonstrated strong chemopreventive effects in lymphomas, mammary, gastric, liver, and lung cancers. [P.L. Crowell, M.N. Gould, Crit. Rev. Oncog. 5, 1, 1994] Limonoids, citrus secondary metabolites, seem to have multiple bioactive functions, including anticancer, reducing cholesterol, anti-anxiety, antimicrobial, and antiviral activities. [G. D. Manners, J. Agric. Food Chem. 2007, 55, 8285.]

This is a HR-MAS NMR study on samples from exocarp, mesocarp, endocarp and seeds of *Citrus Medica* and *Citrus Limon* fruits aimed at to the evaluation of the potentialities of the technique in the detection and identification of terpenes in different parts of the fruit.

1D (water-presaturated, spin-echo and diffusion-filtered spectra) as well as 2D NMR experiments (COSY, TOCSY and HSQC) are carried out at 4 °C on different regions of the commercial fruit cut in pieces that can fit the HR-MAS 4 mm rotor and directly put into it.

Attempts at gaining some insight into the dimensions of oil bodies in the flavedo through diffusion experiments will be also presented.

250WE

Murine Melanoma´s Metabolic Profile Studied by Nuclear Magnetic Resonance

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Melanomas are tumours derived from melanocytes, the pigment-producing cells of the skin. Cells that undergo the carcinogenesis process present intracellular changes, such as immortalization, independence of growth signals and metabolic changes that significantly modify the intracellular and the tumor microenvironment [1]. One of these changes was first described by Otto Warburg, who observed modifications in the metabolism of intracellular glucose. He observed a curious process in which tumor cells start to produce intense aerobic glycolysis, and pyruvate generated by glucose metabolism is reduced to lactate. This process appears to be disadvantageous because glycolysis generates 18 times less ATP than oxidative phosphorylation, in addition, the production of lactate generates an exacerbated acidosis in the tumor microenvironment that is harmful to normal cells. A model of murine melanoma progression [2], called tm-1, from a murine melanocytes strain (non-tumorigenic) was produced in our laboratory by repeated cycles of prevention of cell adhesion. Previous studies indicate that these strains are more pro-oxidant than the melan-a (non-tumorigenic), and survive in conditions of oxidative stress [3]. In cells capable to generating tumours in mice, our group observed the silencing of galectin-3 gene (*gal-3*), a multifunctional endogenous lectin which seems to act by modulating the mitochondrial response to different types of stress and conditioning the cell death. In order to understand the impact of galectin-3 expression in tumor cell metabolism, the Metabolomics approach is being used to map quantitatively the intermediates of glucose metabolism comparing their concentrations in different cell lines at different tensions of oxygen and glucose. As the first step in this work, results indicated that Metabolomics approach can be used to distinguish strains of cells in non-tumorigenic and tumorigenic cells as well as give us clues of which metabolites have been changed between them. New samples at different tensions of oxygen (normoxia and hypoxia) are still been measured and processed to understand in a systematic way as how the glycolytic pathway is being changed.

251TH

Using Filter Diagonalization Method to Process HR-MAS Spectra of Cancer Cells

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High-resolution magic angle spinning (HR-MAS) has been a promising tool to study metabolic profile of intact cancer cells and tissues. Although MAS technique strongly improves the spectra resolution of small molecules, it is not fast enough to reduce the line width of large molecules and assemblies signals. Therefore, they have been eliminated by a T_2 filter, based on Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Moreover, HR-MAS experiments with T_2 filter have to be preceded by water suppression procedure to avoid signal overlap and dynamic range problems. Although most of the HR-MAS experiments are focused in sharp signals of small molecules, information contained in broad signals may also be relevant. Despite the great success of this methodology, other techniques such as Filter Diagonalization Method (FDM) can be applied to the same purpose. In essence, FDM is a parametric non-linear method for fitting time-domain signals. Among other practical applications, the FDM has been recently used to selectively remove uninterested and corrupted solvent broad signals from complex NMR spectra without disturbing overlap or nearby narrower signals. They have shown that FDM can efficiently model broad signals in time domain for posterior subtraction from the original transient signal, resulting in an objective separation of the underlying structured spectrum. In this work we describe that the procedures of water suppression and T_2 or diffusing filters are unnecessary steps when the FDM is used to process the full time domain HR-MAS NMR signals obtained from breast cancer cells. Results demonstrate the efficiency of the FDM post-acquisition processing to obtain high resolution ^{1}H NMR spectra of heterogeneous biological materials, like cancer cells, even by using HR-MAS probe without water suppression and T_2 filter.

252MO

1H Nuclear Magnetic Resonance Spectroscopy Based Metabolomics from Human Serum to Clinical Science

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Metabolomics is a rapidly progressing topic in clinical science because the metabolite composition is influenced by diseases, the diet and life style of an individual.

We have assessed ultrafiltrated human serum samples, an obvious clinical utility for metabolites to help diagnose disease at an early stage and to understand metabolites mechanism. Our samples were collected from North Estonian Regional Hospital and measured by micro-probe of 600 MHz NMR spectroscopy.

Our results confirm that NMR based about 53 metabolites such as glucose, lactate, and creatinine etc. and successfully be identified. We discuss also score plots produced by Principal Component Analysis (PCA), which revealed difference in the metabolic profile between the age and others.

253TU

Seasonal Variation of Seaweed Components by Physicochemical Profiling

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The seaweed components are assembled by various beneficial polysaccharides, such as alginic acid, fucoidan, and agarose, and have an attractive attention as biomass resources. Although a small portion of these beneficial polysaccharides have been used in both manufacturing and pharmaceutical products such as food additives and medical products, the vast majority remains unexploited for their potential industrial applications because of the lack of evaluation and characterization techniques for the utility, applicability, and availability of these natural products. In addition, it is known that the biomass components of seaweed have different compositions and structures between inter-spices, inter-growing area, and seasonal changes. For example, it is known that ratio of mannuronic acid and guluronic acid in alginic acid is changed among seasons. Therefore, it is important to evaluate the compositional variations of seaweed within seasons sampled from constant area. Here, we described an analytical strategy for seasonal variation of seaweed by multiple physicochemical profiling. Natural brown algae, Hizikia fusiformis, were collected through a year from intertidal area at Aburatsubo in Japan. To evaluate the components of water-soluble, macromolecular, and mineral fractions, solid- and solution-NMR measurements such as cross polarization magic angle spinning (CP-MAS) and ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC), attenuated total reflectance-Fourier transform infrared (ATR-FTIR), and inductively-coupled plasma optical emission spectrometry (ICP-OES) were performed. In addition, Thermogravimetric-differential thermal analysis (TG-DTA) was used to evaluate the physicochemical variations within seasons. All obtained data were then digitized and statistically computed to evaluate by multivariate statistical analysis such as principal component analysis (PCA) and correlation analysis. In the result of PCA by ATR-FTIR spectra, compositional profiles in summer and in winter were characteristically clustered, respectively. In addition, the other analysis such as NMR and TG-DTA showed differential chemical compositions and thermodynamic properties between summer and winter. Therefore, our analytical strategy by multiple physicochemical profiling was possible to evaluate and characterize the seasonal variations of seaweed biomass.

254WE

Investigating of Metabolic Profiling Technique for the Detritus Analysis toward Estuarine Ecosystems Evaluations

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Estuarine environments are accumulated a large quantity of the organic matter, consumed by a detritus cycle, from the land deposits. The estuarine environments exist in rich biodiversity, and human society receives a great benefit from its ecosystem services. However, the estuarine environments have complicated aqueous ecosystems, thus the comprehensive evaluation of the biotic interaction and stability are difficult by the conventional bottom-up approach. In order to advance the accumulation of knowledge about conservation, regeneration, and exploitation of the ecosystem service, the development of analytical valuation by bird's eye viewing approach is expected. Here, we described metabolic, mineral, and microbial community profiling strategy for evaluation of the estuarine environment. We targeted the sediment (the source of the detritus cycle) and water in the estuarine and coastal environments using 10 sampling points in Kanto and Tohoku region in Japan. To cover environmental information for a wide range, we used a lab made collection device for sampling of the bottom sediment, and obtained it ten times from surface mud in the range of about 10 m^2 per one points. Organic matter was extracted from sedimentation by NaOH treatment, and was dissolved in DMSO for NMR measurement. In addition, we analyzed the microbial community profiles and mineral profiles by denaturing gradient gel electrophoresis (DGGE) and inductively-coupled plasma optical emission spectrometry (ICP-OES), respectively. The NMR and DGGE data were processed by binning and matrixing, and the all obtained data were evaluated with multivariate statistical analysis such as principal components analysis (PCA). The organic matter in the sediments was successfully characterized by NMR measurements with multivariate statistical analysis. The organic matter information of collected sediment contributes to an evaluation of the estuarine environment in this our sampling strategy. In addition, microbial community and mineral profiles obtained from DGGE and ICP measurements provided the information about characteristic community and mineral compositions among each sampling point. Therefore, our sampling strategy was possible to extract the information about the characteristics of individual estuarine environments.

255TH

The Potential Use of NMR as a Method for Phenotyping Differentiating Mesenchymal Stem Cells

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Stem cell based strategies to produce engineered tissues promise to combat disease and alleviate suffering. The ability of adult mesenchymal stem cells (MSCs) to differentiate along multiple lineages lends great promise for future regenerative therapies. Such therapies, however, require scientific challenges to be met before translation for patient benefit. One challenge is the need for a non-invasive method for stem cell phenotyping prior to (or even during) their use in clinical applications. This work describes the use of NMR spectroscopy as a non-invasive analytical tool to monitor the proliferation and subsequent differentiation of MSCs derived from human dental pulp (HDPSCs). HDPSCs, isolated from extracted teeth, were induced along an osteogenic pathway, growing either in a monolayer or following seeding on to 3D scaffolds made from electrospun collagen or hydroxyapatite. 1d proton spectra were obtained at several time points over a 6 week period. Spectra were processed as a time series, filtered and clustered using peak analysis or metabolite profile in order to identify trends or possible biomarkers responsible for cell phenotype. Validation of cell differentiation using histological staining and biochemical assays was carried out in parallel studies. Data showed a trend in NMR spectral pattern/metabolite shift after three weeks in culture associated with a corresponding shift towards osteoblast phenotype. This suggests that NMR spectroscopy may be used as a non-invasive method for MSC phenotyping to accelerate the route to cell based therapies and potentially act as a quality control tool at pre-implantation.

256MO

Metabolic Profiling of Chicorium endivia by NMR Aimed at Cultivar Traceability and Valorisation

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NMR has become a high-throughput, well-established and effective analytical technique to assess and monitor the identity, traceability and nutritional value of cultivated vegetables. Indeed, the NMR profiles provide comprehensive spectroscopic/structural information on a wide range of metabolites simultaneously and in an unbiased manner [1, 2]. The Italian CISIA-VISPLP project exploits the NMR metabolic profiles of endive (*Chicorium endivia* var. *crispum*) and escarole (*Chicorium endivia* var. *latifolium*) aimed at traceability and value increase of patented, local and novel varieties. Synchronised cultivars of endive (E02.7162 and Myrna) and escarole (Confiance, Flester) were examined at the transplant and harvesting phases (22 and 86 days after sowing, respectively). Focussing on the leaf hydro-soluble fraction, more than thirty primary (e.g.: organic acids, sugars, amino acids) and secondary metabolites (e.g.: phenolics, inositols, ethanolammine) were identified. The multivariate statistical analysis of NMR profiles pointed at the leaf developmental stage as the major factor of metabolite variability, independently from the cultivar. As for traceability, PCA allowed cultivar discrimination with high and moderate significance at transplant and harvesting, respectively. Concerning valorisation, the NMR profiles provided useful info for market-oriented and nutrition quality choices.

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257TU

Chemical Profiling of Sediment Ecosystems in the Deep-Sea Area with Whale Fall

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The oceans cover over two-thirds of our planet with an average depth of about 3,800 m. The deep-sea area which occupies the great portion of the oceans has basically nutrient- and mineral-poor environment. When whales die and sink to the seafloor in deeper area, their decaying carcasses form "oases" at the bottom of the ocean, and provide sustenance and energy source over periods of decades for a complex localized ecosystem that are often highly specific to these unusual and ephemeral habitats. The whale falls are usually investigated in a biological viewpoint such as community members of the ecosystem, thus little information about chemical profiles and their relations to complex microbial ecosystem are available. To evaluate the chemical profiles in an environment, a nuclear magnetic resonance (NMR)-based metabolomics approach including our previously developed approach is a powerful tool for comprehensively evaluating the metabolic profiles of biochemical complexes in natural ecosystems and has been used extensively to study metabolites from a wide range of biological systems in various environments [1]. Therefore, we applied our developed approaches to sediments in the deep-sea area including a whale fall for characterization and evaluation of the chemical profiles. The metabolomic, ionomic, and microbial community profiles at the whale fall sediment were characteristically observed by using NMR-based metabolomics approach, inductively-coupled plasma optical emission spectrometry (ICP-OES)-based ionomic analysis, and pyrosequencing-based approach, respectively. Our chemical profiling approach was successfully evaluated the sediments in deep-sea area including the whale fall, and suggested that the microbial community and chemical profiles were closely linked to each other.

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258WE

^{1}H NMR Based Metabonomics as a Tool in Anti-Doping Control Strategies

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Metabonomics is well established as a powerful method for the evaluation of the metabolic profile of an organism, proffering a holistic view of an organism's response to different exogenous factors. The aim of this work was to examine whether the NMR based metabonomics approach could be used as complementary tool to the existing methods on the basis of a non-targeted metabolic profiling able to capture the biochemical alterations after the abuse of exogenous steroids. The applicability of the method was tested in a cohort of 263 human urine samples of both men and women athletes targeted to doping controls. Among them, 59 had been reported as positive by the official doping controls for the application of exogenous anabolic steroids. The study included the NMR measurements of all samples and the chemometric analysis of the resulted spectroscopic data set. The latter consisted of complex 1H NMR spectra containing hundreds of signals from both endogenous and exogenous metabolites and was analyzed by multivariate statistical tools (PCA, PLS-DA, OPLS-DA, iECVA). The developed models exhibited partial grouping between the originally classified groups, highlighting significant differences of the metabolites, like creatine, creatinine, hippurate, and acetate among the groups of athletes. It is concluded that the NMR based metabonomics approach could be used as an ultra fast and cost effective predictive tool in anti-doping control that could highlight those samples suspected to originate from doped athletes on the basis of their metabolic fingerprint analysis. In order for this method to be fully applicable, the samples collection should be accompanied with extended metadata information that could be utilized in the multivariate statistical analysis.

259TH

^{1}H NMR Profile for Normal and Diabetes Groups in Relationship with the Geographic Region

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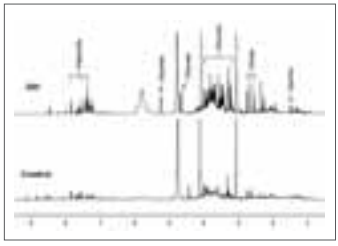
The metabolic profile of urine from a control group has been obtained by ^{1}H-NMR spectroscopy at 400 MHz. Data have been processed both as absolute (mmol/L) and relative (mmol/mol of creatinine) concentrations. The normal values have been compared with data from type II diabetes mellitus (DM II). Both groups belong to a population located in Romania (Eastern Europe). The average concentrations of various metabolites in urine for normal and DM II subjects are presented.



Our data are in good agreement with some previously reported data but they are not identical. Possible explanations for the small variations are discussed in terms of NMR experimental parameters and lifestyle differences. The present study indicates that when both the control and DM groups are chosen from the same geographical region, the tendencies can be interpreted with confidence.

260MO

Software for MFA and Application in Model Cancer Systems

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Metabolomics and metabolic flux analysis (MFA) are increasingly used to study metabolic pathways. NMR spectroscopy together with the use of selectively 13C labelled metabolic precursors is a powerful tool to study intracellular metabolic pathways. Using this approach the fate of individual carbon can be followed revealing the metabolic pathways involved in metabolisation of the 13C enriched precursors fed to the cells. However the success of such an analysis critically depends on the quality of resonance assignments which can be quite difficult in complex mixtures such as cell extracts. Here we present NMRLab/MetaboLab, a Matlab based software which contains a module that specifically addresses the challenges involved in assigning complex mixtures of biological extracts [2]. A spectral library of more than 150 metabolites is included in order to enable efficient assignment of 2D-HSQC resonance lines. Tight links to online resources such as the human metabolome data base (HMDB) and the small molecule pathway data base (SMPDB) enable the user to directly link the obtained isotopomer distribution to the usage of specific metabolic pathways. MFA has been applied to AML cancer cells, which were fed for 24 hours with either unlabelled, 13C(1,2)-enriched glucose or 13C(3)-enriched glutamine. The cells were either untreated or treated with a combination of Bezafibrate (BEZ) and Medroxyprogesterone Acetate (MPA) [3]. In the past it was shown that the combination of the lipid-lowering drug BEZ and the sex hormone MPA has a potent anti-leukaemic effect, associated with the induction of high ROS. The data presented here show a strong influence on the citric acid cycle and cell salvage pathway caused by the drug treatment, especially on pyrimidine metabolism and on the use of pentose-phosphate derived carbons.

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261TU

Metabolomic Analyses of Urine Samples Obtained from Humans Diagnosed with T2DM and IGT and Treated with Yerba-Mate Infusions Using Nuclear Magnetic Resonance

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The aim of this work was to apply the concept of metabolomic analyses in urine samples obtained from humans diagnosed with type 2 diabetes mellitus (T2DM) and impaired glucose tolerance (IGT) and treated with yerba-mate (*Ilex paraguariensis*) infusions using nuclear magnetic resonance. The use of yerba-mate as a functional food is relatively recent and earlier studies have shown that aqueous extracts of *Ilex paraguariensis*, which are rich in phenolic compounds, are capable of inhibit the formation of the advanced glycation end-products. Nuclear magnetic resonance (^{1}H NMR and two-dimensional experiments) was capable of identify and quantify the major metabolites present in the human urine samples diagnosed with T2DM and IGT. Figure 1 shows the PCA score plot of urine samples from humans diagnosed impaired glucose tolerance (IGT) and treated with yerba-mate. PCA score plot shows that the urine of humans treated with yerba-mate is different from the urines of humans non-treated. The relatively quantification of the metabolites alanine, DMA, citrate, creatinine, betaína and TMAO showed that the intake of yerba-mate infusions could assist in the treatment of DM.

Figure 1 – PCA score plot of urine samples from humans diagnosed impaired glucose tolerance (IGT). Red - treated with yerba-mate

262WE

Sensitivity - Optimized Fast - Pulsing Multidimensional NMR Techniques, Versatile Tools for Detecting Low Concentrated Molecules Containing Low Abundant Nuclei

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Homo and hetero-multidimensional NMR (nD NMR) are usually required for structural elucidation of unknown molecules. nD NMR allows bursting spectral information along numerous dimensions, and thus making easier the spectral analysis. Moreover, sensitive ^{1}H detected nD NMR experiments are also suitable techniques to sensitively detect low abundant heteronuclei. In the context of bio-macromolecules, Brutscher and al. have developed new fast and sensitive techniques like SOFAST [1] and BEST [2] methods for recording multidimensional ^{1}H-X (X=^{13}C, ^{15}N) experiments in order to follow rapid events in proteins [3] and RNAs [4]. In the field of small molecules, new fast heteronuclear nD NMR techniques introduced by Freeman et al. [5] and Furrer [6] have permitted to gain in sensitivity and in time for detecting low naturally abundant nuclei.

Fast-pulsing NMR techniques are based on shortening the time between two consecutive experiments (recycling time) allowing for recovery of longitudinal magnetization. Thus, it becomes possible to increase the repetition rate of a given experiment and also the sensitivity.

For extending the field of application of rapid repetition techniques, they have newly been implemented for detecting ^{29}Si nuclei in silylated derivatives, for probing H bonds in peptides, and for characterizing polymer - supported products.

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263TH

Multiplex Acquisition of Multiple Quantum Spectra

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In the quest for reducing the time requirements for coherence selection without discarding valuable information or sensitivity our group has recently been targeting homonuclear multiple quantum spectra. The acquisition of multiple quantum spectra of higher orders is time consuming due to the long phase cycles necessary for coherence selection together with quadrature detection. The alternative solutions using gradient selection techniques involve a loss of (per root scan) sensitivity.
By applying the multiplex phase cycling approach [1] the coherence selection is deferred to the processing. In principle different coherence orders can be selected [2], by linear combinations of FIDs stemming from separate phase steps. But in standard multiple quantum excitation sequences it is only possible to generate either odd or even coherence orders. We implemented various multiplex experiments using a 45 degree phase shift in the excitation sandwich to allow both odd and even order quantum coherences and multiplex phase cycling. To achieve quadrature detection in the indirect dimension, sine and cosine modulated data sets are generated using multiplex quadrature detection (MQD) [3]. This approach yields a time saving of e.g. up to 45 % for pure absorptive 5Q spectra, and at the same time pure phase 2Q, 3Q and 4Q spectra are also obtained through different linear combinations. To save further time this approach can be combined with other time saving techniques such as sparse sampling.

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Acknowledgement: The experiments were performed at the Austro-Czech NMR Research Center in Linz co-financed by the European Union in the context of the project RERI-uasb, EFRE RU2-EU-124/100-2010 (ETC Austria-Czech Republic 2007-2013)

264MO

Exploring Crown Ether and Cyclodextrin as a Resolving Agent for Diffusion Ordered SpectroscopY (DOSY)

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Diffusion Ordered Spectroscopy (DOSY) generally fails to separate the isomeric species possessing identical molecular weight and similar hydrodynamic radii. The present study demonstrates the resolution of isomers using crown ether and α/β Cyclodextrin as a resolving agent in Diffusion Ordered Spectroscopy. The resolution of isomers has been achieved by measuring the significant differences in the diffusion rates between the positional isomers of aminobenzoic acids, benzenedicarboxylic acids and between the geometric isomers, fumaric acid and maleic acid by Cyclodextrin. The isomers of Chloroaniline and complex mixture of organic molecules resolved by crown ether.

265TU

Ultra High-Resolution NMR in Inhomogeneous Magnetic Fields:Two-Dimensional Long-Lived Coherence Correlation Spectroscopy

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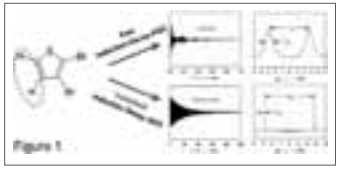
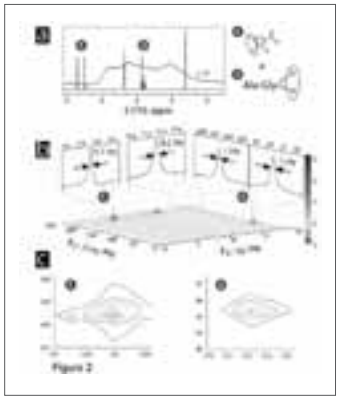
[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Indian Institute of Technology, New Delhi, India, [3]University of Southampton, Southampton, UK



Long-lived coherences (LLC's) [1,2] involving pairs of chemically inequivalent spins can be excited and sustained during protracted radio-frequency irradiation periods that alternate with brief windows for signal observation [3]. Fourier transformation of the "sustained induction decays" recorded in a single scan yields NMR spectra with line-widths in the range $10 < \Delta\nu < 100$ mHz. (Fig. 1)



We designed a method to excite and detect LLC's in a broadband manner, allowing one to record two-dimensional NMR spectra that probe chemical shift differences in one dimension and scalar couplings in the other with unprecedented resolution. Such spectra recorded irrespective of the magnetic field homogeneity (even if the homogeneity is deliberately degraded to $\Delta B_0/B_0 = 10$ ppm) (Fig. 2). In contrast to other techniques [4], our method does not require any sophisticated gradients or adiabatic frequency-swept pulses.

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266WE

BOOSTING SENSITIVITY OF LIGAND-PROTEIN SCREENING BY NMR OF LONG-LIVED STATES

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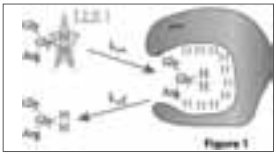
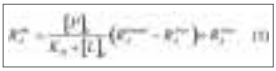
EPFL, Lausanne, Switzerland

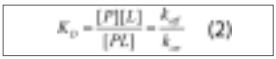
The binding affinity of a ligand to a protein is a key parameter in pharmaceutical research and drug development. NMR has shown to be a technique of choice to measure dissociation constants K_D.



This constant can be extracted by titration of the ligand, and if the protein-ligand complex is in fast exchange on the NMR time scale, any observed NMR parameter (A) can be described by:

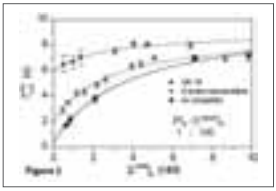


We have developed a new method that exploits the unusual lifetime $T_{LLS} > T_1$ of Long Lived States (LLS) [1]. The proposed method benefits from a greatly enhanced contrast between the bound and free ligands LLS relaxation rates R_{LLS}^{bound} and R_{LLS}^{free}. The LLS method permits to dramatically lower the protein-ligand ratio needed to measure the dissociation constant K_D (1:100 in figure 2). This opens the way either to a decrease in protein concentration, to a gain in experimental time, or to a better contrast.



Either direct titration of the ligand under investigation or competition experiments can be monitored by LLS relaxation.

LLS can readily be excited and sustained in virtually any peptide containing at least one glycine, thus offering a broad choice of inexpensive weak 'test' ligands without requiring any isotopic labeling.



Our method is illustrated by screening of inhibitors of a prototypical target for cancer therapy, the urokinase-type plasminogen activator (uPA) [2] (Fig. 2)

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267TH

STRUCTURAL VARIATIONS OF PRION PROTEINS CAUSED BY MUTATIONS IN HUMAN GENOME

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The development of transmissible spongiphorm encephalopathies (TSE) is associated with the conversion of the cellular prion protein (PrP^C) into the misfolded, pathogenic isoform (PrP^{Sc}). In human genetic forms of these diseases, mutations in the globular C-terminal domain of PrP^C are hypothesized to favor spontaneous generation of PrP^{Sc} in specific brain regions, leading to neuronal cell degeneration and death. Approximately 10-15 % of TSEs are associated with mutations. In our recent works, we have determined the NMR solution-state structures of the truncated recombinant human (Hu) PrPs carrying the pathological Q212P (90-231, M129) mutation and V210I (90-231, M129) polymorphism. While Q212P mutation is linked Gerstmann-Sträussler-Scheinker syndrome (GSS) the V210I mutation is linked to genetic Creutzfeldt-Jakob disease (CJD). In order to determine high-resolution structures triple resonance (^1H, ^{13}C and ^{15}N) NMR experiments were performed by 800 MHz NMR spectrometer. The determined structures of both mutants consist of unstructured N-terminal part (residues 90-124) and well-defined C-terminal domain (residues 125-228). The C-terminal part contains three α-helices (residues 144-156, 173-193 and 200-230) and a short, antiparallel β-sheet (residues 129-130 and 162-163). Detailed analysis and comparison with the structure of the WT Hu-PrP revealed that although structures share similar global fold, mutations introduces some local structural differences. The observed variations are mostly clustered at the α2-α3 inter-helical interface and in the β2-α2 loop region. The alteration of conformation of the β2–α2 loop region and the subsequent changes in hydrophobic cluster facilitates intermolecular interactions between PrPs. The high-resolution NMR structures offer new clues on the earliest events of the pathogenic conversion process and could be used for the development of antiprion drugs.

268MO

Noise and Spin-Noise: Cable Dependence of Optimal Tuning/Matching Conditions

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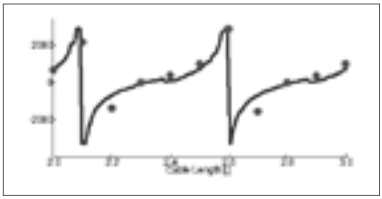
Previous studies have shown that tuning/matching conditions optimized for transmission and detection can be significantly different for a variety of commercial NMR probes.

In addition, it was also shown that by optimizing reception tuning (as opposed to typical transmission or reflection tuning) one may in some cases obtain sensitivity enhancements by as much as 25-50%. In earlier work, spin-noise and absorbed circuit noise signals have also been used to characterize reception optima. In this work, we show how the length of the coaxial transmission line cable between the pre-amplifier and the probe affects the positions of the reception tuning optimum, the radiation damping strength, induced frequency shifts, as well as, the shape of the spin-noise and absorbed circuit noise line shapes.



Spin-noise tuning optimum offset from conventional tuning optimum as a function of coaxial cable length in units of wavelength. Measured values (green), and simulated curve (blue).

269TU

Conformational Analysis of New Rotameric Molecular Clips Using Variable Temperature NMR Study

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Molecular clips are well-known artificial receptors for dihydroxyaromatics, e.g. resorcinol and other biomolecules bearing a dihydroxyaromatic unit. Two xylylene walls and two carbonyl groups, making a pre-organized cavity, are the key features which bind the substrate via two aryl-stacking & two hydrogen-bonding interactions[1] (Fig. 1).

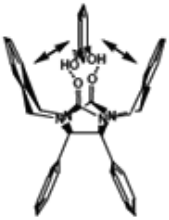

Figure 1: a molecular clip binds resorcinol

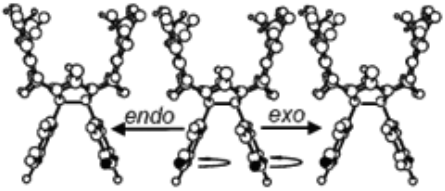

Figure 2: interconversion from *cis* (middle) to *trans* (left and right)

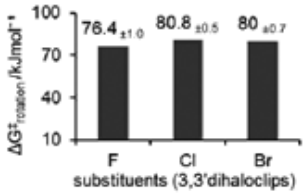

Figure 3: the free energy was found to be same

Recently we have prepared[2] new clips which exist as “rotamers” (isomers interconverted by rotation about a single bond; Fig 2). A VT-NMR study shows that the barrier for the interconversion of these rotamers, together with free energy calculations, is almost the same for any substituents at 3,3-positions of the locking phenyl (Fig 3). The energy barrier was found to be too high to access by VT for 2,2-disubstituted clip.

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[2] Kozien, M. PhD Thesis, ITT Dublin, 2009.

270WE

A Generalised Way to Represent the Stejskal-Tanner Equation

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Diffusion NMR or DOSY has become more and more widely applied over the years, having found its way to the NMR toolbox applied by researchers in different fields of chemistry. The widely known Stejskal-Tanner equation is central to any diffusion NMR experiment, describing the signal attenuation due to loss of coherence caused by diffusion as a function of the experimental parameters. What is less widely known is that the equation itself is not invariable when applying different pulse sequences or different gradient pulse shapes and should thus be modified accordingly. Despite DOSY having become such a common technique, this is often overlooked. Here, we represent the Stejskal-Tanner equation in a novel way, leading to expressions that do not assume any gradient shape beforehand, leaving only a few number of parameters that need to be filled in depending solely on the choice of gradient shape. This new approach to represent the Stejskal-Tanner equation increases awareness of its dependence on gradient shape. Moreover, it relieves designers of future diffusion NMR pulse sequences of struggling with the cumbersome task of deriving and reporting the equation for each gradient shape separately. A quick reference table of the Stejskal-Tanner equation for the most basic diffusion NMR pulse sequences for any gradient shape will be presented.

Reference:
Sinnaeve D, *Concepts in Magnetic Resonance Part A*, 40A, 39-65 (2012)

271TH

Blind Source Separation for the Processing of Highly Overlapping DOSY Datasets

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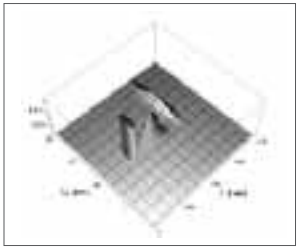
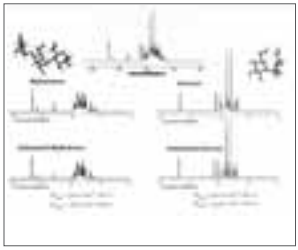


One way to identify molecules in a mixture is the use of PFGSE ("DOSY") experiments, which provide a means of tagging the components by their mobilities, if the spread of these latter is sufficiently large and the processing effective.[1] We explored here the separation performance of Blind Source Separation (BSS),[2] an approach that allows estimating the spectra of N unknown sources (the spectra of the pure components) from a series of v mixed spectra[3] (the single traces of the DOSY dataset in this case). We explored two different BSS approaches, Independent Component Analysis[4] and NNSC,[5] which uses non-negative matrix factorization (NMF) with an additional sparse coding (SC). We did not introduce any parametric modeling of the sources or of the mixing matrix (e.g. the exponential behavior of the PFGSE decay). The results (***estimated sources*** and ***diffusion coefficients***) on the mixture of *sucrose* and *maltotriose*, characterized by a high level of spectral overlap, are shown in the Figure (for NNSC). Thus, hyperparametric BSS can provide excellent separation and calculation of the diffusion constants. The effect of hyperparametrization on the separation, the limitations of the methods and their and applications to two more complex mixtures will be illustrated.

Acknowledgment Grants ANR-08-BLAN-273 ; Region PACA (APO-G-2009)



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272MO

NMR Pulse Gradient Spin Echo Measurement of Self-Diffusion in Nano-Pores

Janez Stepišnik, Aleš Mohorič, Bernd Fritzinger, Ulrich Scheler

[1]University of Ljubljana, FMF, Ljubljana, Slovenia, [2]Leibniz-Institut für Polymerforschung, Dresden, Germany, [3]Institut Jožef Stefan, Ljubljana, Slovenia

NMR pulse gradient spin echo is the most efficient method for non-invasive elucidation of molecular transport in heterogeneous media. With a proper interpretation of experimental data, the method can also be applied to investigate molecular self-diffusion in nano-pores. We show it by the analysis of restricted self-diffusion measurement of water molecules trapped in a porous polyamide membrane. PGSE measurement gives the spin echo dependence on the magnetic field gradient that exhibits the diffraction undulation of the decay that prevents the use of the inverse-Laplace transform method to extract the pore size distribution. The q-space cosine Fourier transform of data gives the propagator in the form of the sum of normal distributions[1]. Its decomposition gives three propagators with the amplitudes that decay due to the spin relaxation and with the second moments that remain fixed as the interval between gradient pulses increases. This indicates a motional narrowing regime of measurements, in which the size of pores can be obtained from the fourth root of the second moments[2]. 3-D plot of the spin-relaxation rate and pore size distributions shows the prevailing share of pores with the radius r = 100(1±0.1) nm (70%) and pores with the radius r = 175(1±0.17) nm (20%). Water in these two types of pores has almost identical spin relaxation T_2=10(1±0.2) ms, while water in the pores with the radius r = 282(1±0.03) nm (5%) have a broader distribution of relaxation times, T_2=14(1±0.36) ms. This approach[3] exposes the NMR PGSE technique as a useful tool in the nanotechnology.

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273TU

SelEXSIDE - An Incredibly Fast, Easy and Reliable Method for the Measurement of Long-Range ^{13}C-^{1}H Coupling Constants

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[1]University of Bristol, Bristol, UK, [2]Agilent Technologies UK Ltd, Yarnton, Oxford, UK

The application of 3-bond ^{13}C-^{1}H scalar coupling constants has seen a tremendous growth in elucidation of 3-dimensional structures of organic molecules. Unfortunately, in practical terms $^{3}J_{CH}$ values are difficult to extract – they are relatively small and of same magnitude as $^{3}J_{HH}$ coupling constants but also made more complicated by the low sensitivity of the ^{13}C nucleus. Many new experiments for simplifying the measurement of $^{3}J_{CH}$ have been reported in literature and their main setbacks are that the interpretation of the resulting spectra is not straightforward, long selective pulse sequences lead to the loss of signal due to t2 relaxation and high-resolution 2-dimensional methods typically require extended experiment times. A new, fast, easy to interpret approach is reported for determination of long-range ^{13}C-^{1}H coupling constant where EXSIDE is converted to be doubly-selective in both the ^{13}C and ^{1}H domains. This sequence termed SelEXSIDE, is easy to interpret and reduces a multi-hour (> 6 hrs) experiment to a matter of minutes (as short as 4 mins) for each coupling constant measured.

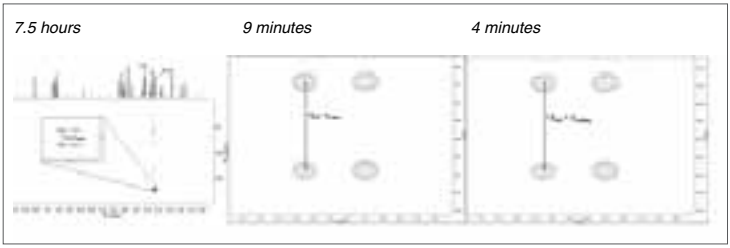

Figure 1: (a) EXSIDE spectrum (~7.5 hours) for H11b-C10 of strychnine with 200 ppm (spectral width, 1675 t1 increments, 4 scans/ inc. (b) SelEXSIDE spectrum (9 mins) for H11b-C10 of strychnine with 4 ppm (500 Hz) ^{13}C spectral width, 32 t1 increments, 4 scans/ inc. (c) SelEXSIDE spectrum (4 mins) for H11b-C10 of strychnine with 4 ppm (500 Hz) ^{13}C spectral width, 32 t1 increments, 2 scans/inc.

274WE

Extra Large Solvent Effects on One-Bond Spin-Spin Coupling Constants in Organo-Mercury Compunds and Their Possible Use as Solvent Effect References

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Indirect spin-spin coupling constants (SSCC), like many other molecular parameters measured in dense media, are perturbed by bulk medium and/or specific intermolecular interactions. This phenomenon, generalized under the term *solvent effect*, has hindered the usage of SSCCs and can potentially cause significant errors, if the value measured in one medium is used, without precautions, for further interpretations of spectra measured in other media.

To estimate the magnitude and type of solvent effects, it is practical to find out a convenient reference molecule that is subject to a strong solvent effect. Based on our recently found correlation of solvent sensitivities of SSCCs with their solvent effect-free values, such a molecule could be searched among the ones with large values of SSCCs. As possible reference candidates, we have chosen organo-mercury compounds, dimethylmercury (DMM) and methyl mercury chloride (MMCl) in particular.

We observed linear correlation of SSCCs and so called **f(ε)=2(ε-1)/(2ε+1)** solvent "reaction field" values, shown earlier only for $^1J_{CH}$ of several substituted methanes. In this work, we have verified the existence of similar correlation for different pairs of coupled nuclei, such as C-H, C-Hg and C-C. The changes of $^1J_{HgC}$ SSCC measured in chloroform (**ε=4.8, f(ε)=0.7**) and DMSO (**ε=46.7, f(ε)=0.97**) are 60.7 and 262.8 Hz for DMM and MMCl molecules correspondingly (or 8.8% and 18.5%). Temperature dependence of SSCCs in these molecules is also substantial, about 0.6 Hz per 1 K.

Besides deriving a solvent effect correction for SSCCs, we foresee many other applications of reference molecules that are super-reflective for the solvent effects. In particular, the "reaction field", hence dielectric permittivity, of the solution can be easily measured. We have also determined the corrected values of dipolar couplings and anisotropies of indirect coupling constants in DMM and MMCl based on our data and the measurements available in the literature.

275TH

Protein Thermodynamics Monitored by High Pressure NMR Spectroscopy

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Using high pressure for protein unfolding studies is an elegant way to investigate both structure and thermodynamics of proteins [1]. Amongst other techniques to monitor unfolding events of proteins, pressure induced protein unfolding is a reversible method, which avoids denaturing agents.

In the present work we use a high pressure system in combination with high resolution NMR spectroscopy to characterize different protein states of the cold shock protein from Bacillus subtilis (BsCspB) [2] and a temperature sensitive variant of the cold shock protein from Bacillus caldolyticus (BcCsp R3E L66E) [3]. For that purpose we set up an NMR pressure device, which works up to 2500 bar. Pressure, heat and cold induced unfolding has been monitored for both proteins. Changes in the proteins structure were analyzed by recording 1H-15N HSQC spectra as well as 1H spectra. Out of this data we determined the change in heat capacity, thermal expansion, compressibility and the change in volume between the native and the unfolded state.

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276MO

The Comparison of Convex and Non-Convex Compressed Sensing Applied in Multidimensional NMR

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The resolution of multidimensional NMR spectra can be severely limited when the regular sampling based on the Nyquist-Shannon theorem is used. The theorem binds the sampling rate with a bandwidth of a sampled signal and thus implicitly creates a dependence between the line width and the time of experiment, often making the latter one very long. Recently, the non-linear sampling theorem has been formulated by Candes, Romberg and Tao, that determines the required number of sampling points to be dependent rather on the number of peaks in a spectrum than on its size. In order to apply the new method, referred to as compressed sensing, the sampling should be non-uniform and the spectrum has to be reconstructed by iterative minimization of its lp-norm, where $0< p \leq 1$. Compressed sensing has quickly found the applications in many branches of science. In our previous work, we have introduced it to multidimensional NMR and have shown the examples of reconstruction of two-dimensional spectra. In the present study we discuss in details the accuracy and robustness of two compressed sensing algorithms: convex (iterative soft thresholding) and non-convex (iteratively re-weighted least squares with local l0 -norm) in application to two- and three-dimensional datasets. We show that the later method is more effective, which is in line with the recent works on the theory of compressed sensing.

277TU

Broadband Homonuclear Decoupling in the F1 Dimension of 2D Experiments

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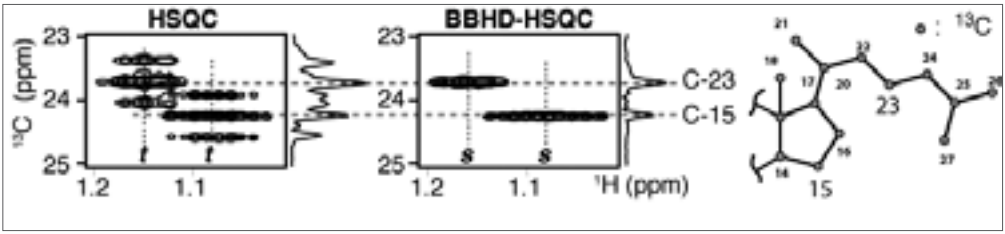
Scalar coupling constants can give insights about the structures of molecules but they often reduce the sensitivity of the experiments by spreading the signals in the form of complex multiplet structures. Heteronuclear decoupling techniques have been available for quite some time, but eliminating homonuclear interactions is a much more difficult challenge. The first broadband homodecoupling based on spatial encoding was introduced by Zangger and Sterk for proton spectra and extended to COSY and DOSY experiments by James Keeler and Gareth Morris respectively.

We introduced a modified broadband homonuclear decoupling schemes (BBHD) to eliminate the coupling patterns in the indirect carbon dimension (F1) of 2D NMR experiments. It has been implemented in CC-COSY and heteronuclear HSQC experiments to record the spectra of 92%-^{13}C enriched cholesterol. Solutions to overcome the intrinsically low sensitivity of spatially encoded experiments and modifications involving selective “re-coupling” (BBDec-SRec) to measure coupling constants are presented.



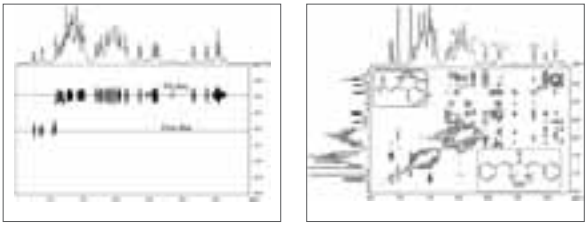
278WE

Classification of Pd-Based Catalytic System by Modern NMR Techniques.

Sergey Zalesskiy, Valentin Ananikov
N.D. Zelinsky Institute of Organic Chemistry RAS, Moscow, Russia

Pd_2dba_3 (tris(dibenzylideneacetone)dipalladium) is a commonly used precursor of Pd^0 for catalytic reactions and transition metal chemistry. However, a little was known about the structure of the complex and it's stability. Utilizing 2D NMR techniques together with diffusion-ordered spectroscopy we were able to determine the state of complex in solution and provide complete assignment of ^1H NMR spectrum.

We investigated possible conformations of the dba ligand with labile diene fragment by means of nuclear Overhauser effect spectroscopy and found all three ligands in complex to exist in s,*cis*-s,*cis* conformation. On the basis of NMR data we suggested a simple and reliable formula to determine the target complex purity. Beyond that, the analysis of complex stability and decomposition (SEM, ICP-MS) was carried out resulting in improved method for synthesis of Pd_2dba_3 with higher yields and purity. The present study has clearly shown that the content and naure of catalytically active species in Pd_2dba_3 may significantly vary (see: S.S. Zalesskiy, V.P. Ananikov *Organometallics* **2012**, *31*, 2302).



^1H DOSY and ^1H-^1H NOESY spectra of Pd_2dba_3 in $CDCl_3$

279TH

Multiple-Quantum Edited DOSY for Improved NMR Analysis of Small-Molecule Mixtures

G N Manjunatha Reddy, Stefano Caldarelli
University of Aix-Marseilles, Marseille, Bouches de Rhone, France

Pulsed field gradient (PFG) NMR is a common tool for the NMR analysis small-molecule mixtures. [1,2] This approach is quite robust in identifying large variations in the molecular weight. However, common mixtures usually involve molecules of similar size, as other natural forces induce or simplify the separation of objects largely different in mass or size. To solve this case, improved experimental/processing protocols have been proposed, one notable example being the addition of an agent that enhances the differences in molecular mobility (matrix assisted dosy).[3,4] A variant of this latter, for example, makes use of immobilized phases of the kind normally used in HPLC experiments. [5] An alternative way of enancing the differences in observed mobilities is the use of multiple-quantum coherences, p, as the apparent diffusion coefficient has a p^2 dependence. Moreover, MQ-filtering as the property of simplifying the NMR spectrum, a fact that has been exploited recently for identifying the components of complex mixtures. [5] An example of MQ-encoded DOSY of mixtuers is illustratrated in Figure 1, in which the enhancement of the separation induced in going from 1Q to 4Q is clearly demonstrated.

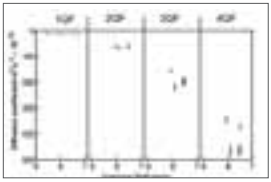


Figure 1:
Demonstration of MQ-encoded DOSY NMRfor the mixture of naphthalene and anthracene, 20 mg ml^{-1} in cdcl3. Notice the enhanced difference in the apparent diffusion coefficients as going to high-quantum orders.

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280MO

NMR Experiments for the Structural Characterisation of ^{13}C Methylated Compounds. Application to the Analysis of Humic Substances.

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The University of Edinburgh, Edinburgh, UK

Humic substances (HS) are complex super mixtures containing thousands of organic compounds, which are produced via microbial degradation of plant and animal residues. HS play crucial roles in global biogeochemical cycles. However, their structural composition is poorly understood and the lack of this knowledge is considered to be the rate-determining-step in elucidating the roles of HS in environmental processes. NMR spectroscopy together with FT-ICR mass spectrometry are two of the most promising high-resolution techniques for the structural characterisation of HS.

HS are composed from carbon- and oxygen-rich compounds that contain numerous OH groups (aliphatic, phenolic, and carboxylic). By methylating OH moieties with ^{13}C labelled methyl groups, we introduce an NMR active nucleus that allows us to filter out the vast majority of resonances and to detect signals only from the immediate neighbourhood of the $^{13}CH_3O$ groups. Comparing the obtained ^{1}H and ^{13}C chemical shifts with database information we can suggest structural fragments present in these compounds.

We have developed several n-dimensional NMR experiments that use proton-carbon and carbon-carbon couplings or the NOE to transfer the magnetisation from $^{13}CH_3O$ groups to neighbouring carbon or proton atoms. We demonstrate these new NMR experiments on a model mixture of methylated organic compounds and also present our first results for a particular HS operational fraction, fulvic acid, isolated from a Scottish peat bog.

281TU

Pulse Design for Broadband Homonuclear Cross-Polarization across Weak J-Couplings

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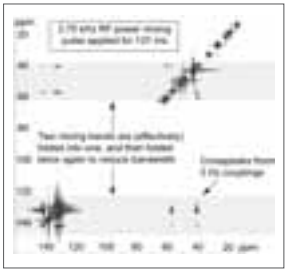
Broadband homonuclear mixing pulses are required for protein spectroscopy; however, they must be used at sufficiently low power and for sufficiently short times to avoid probe damage and sample heating.

We present several new mixing pulses for resolving weak J-couplings (e.g. 2-5 Hz) between spins separated by large bandwidths (up to tens of kHz). These pulses are of low enough power that they can safely be run for long mixing times – allowing for magnetization transfer across small J-couplings. We have designed these pulses analytically (rather than by numerical optimization) by constructing a series of interaction frames. We show how to choose pulse parameters in successive frames to effectively *fold up* a large chemical shift bandwidth arbitrarily many times, while maintaining couplings between spins. In the final interaction frame the effective bandwidth is small relative to the coupling strength.



Our designs are validated by several carbon-channel TOCSY experiments on 500 MHz and 800 MHz spectrometers. We have used mixing pulses with power levels between 2 and 3 kHz for up to 200 ms to resolve couplings with J < 5 Hz, despite large separation in chemical shift frequencies.

We present a variety of experimental spectra, the pulses we have used, and our analytical pulse design methods.

282WE

High-Level Geometric Control of New Rotameric Molecular Clip Receptors: NMR Studies of Rotamer Interconversion and Binding to Dihydroxybenzene Guests

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Institute of Technology Tallaght, Dublin, Ireland

Molecular clips are well-known artificial receptors for dihydroxyaromatics, e.g. resorcinol. Two xylylene walls & two carbonyls, making a pre-organized cavity, are key features which bind the substrate via two aryl-stacking & two hydrogen-bonding interactions[1] (Fig. 1).

Recently we have prepared[2] new clips which exist as rotamers (Fig 2). VT-NMR shows that the barrier for the interconversion of the rotamers is the same for any substituents at the 3,3'-positions of the locking phenyl. The energy barrier was found to be too high to access by VT for 2,2'-disubstituted clip. The values of binding constants were found to be comparable with those presented in the literature for analogous molecular clips.[1][2]

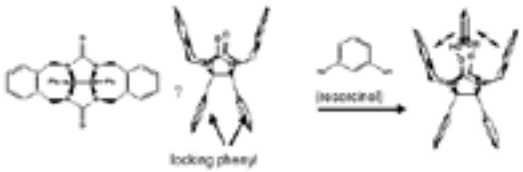

Figure 1: a molecular clip binds resorcinol via aryl stacking and H-bonding

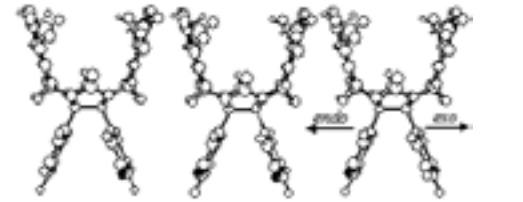

Figure 2: interconversion from cis (middle) to trans (left and right)

283TH

Polyisocyanides as a New Alignment Medium to Measure RDCs for Small Organic Molecules

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Residual dipolar couplings (RDCs) are an important parameter in organic structure determination. RDCs can be observed by NMR when molecules are anisotropically oriented in a solution. For high resolution structure calculation the degree of anisotropy should be small. For water insoluble organic molecules, stretched polymer gels and liquid crystals have been used as alignment media. Extraction of RDCs in these liquid crystal alignment media happens to be complicated, due to the relatively large degree of orientation in these media.

Here we are investigating Polyisocyanides as alignment medium. Helically chiral Polyisocyanides can form liquid crystals in Chloroform solution. These Polyisocycanide liquid crystals were found to give anisotropic molecular alignment in the magnetic field and are useful to measure residual dipolar couplings (RDCs) from analytes e.g. strychnine. They show less quadrupolar splitting of the deuterated solvent signal compared with other liquid crystal systems such as Poly-γ-benzyl-L-glutamate (PBLG) and hence less undesired line broadening.

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284MO

Application of Chemometrics and ^{1}H NMR Analysis to Compositional Study of Treated and Untreated Sewage

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The compositional study of sewage is very important since it can make direct influence on the design of wastewater treatment station (WTS) and even can indicate whether the treatment is effective or if irregular discharges occur. Chemometric technique (Principal Component Analysis-PCA) was employ to analyze ^{1}H NMR data set of domestic sewage (treated and untreated) in order to identify chemical compounds variation and factors that affect the wastewater composition according to discharge. Thereby, samples were collected weekly in a period from June 2011 to May 2012 which an aliquot of 20 ml was filtered (4.5 µm filter) and dried in a speed vacuum. To get ^{1}H NMR data, sample was solubilized in 600 µl of D_2O/TMSP-d4 (0.16 mg.ml^{-1}) and a quintuplicate was acquired. To perform chemometric analyze, a bucket table was done employing the software Amix-Viewer (Bruker Biospin) and media centering as processing. The analysis provided relevant information on the samples composition that has predominance of short-chain organic compounds which were similar to all studied period. Multivariate technique emphasizes variations involving major constituents and successfully described trends among the sewages on the basis of its organic compounds. PCA for untreated sewage (US) showed temporal trends (seasonal influence) (91.6% total variance) which lower concentrations of organic compounds were found in winter period probable due to the less microbial activity. Besides, PCA revealed irregular discharge (possibly industrial waste) with high pollution load. PCA of treated sewage (TS) (94.6% total variance) showed one principal grouping leading to conclude a homogeneity of treatment in WTS. The examination of PC1, PC2, PC3 and PC4 loadings to US and TS suggested that separations mainly occurred due to spectral position at δ 2.73 (dimethylamine) and δ 1.94 (acetic acid) and to irregular discharge, δ 5.08 (possibly urea). Thus, seasonal or irregular variations in wastewater composition showed by ^{1}H NMR can be a guiding factor for determining treatment parameters (to safe disposal) and a good predictor of illegal industrial discharge. In addition, was performed quantitative analysis of the characterized compounds that further contributed to this study (acetate, acetone, alanine, dimethylamine, leucine, formate, propionate and butyrate) using TMSP-d4 as internal standard.

285TU

Iterative Thresholding Algorithm for Multi-Exponential Decay Applied to PFG NMR Data

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Diffusion ordered spectroscopy (DOSY) is a well-known technique for determination of diffusion coefficients from a PFG (Pulsed Field Gradient) NMR experiment [1]. Various techniques have been introduced to process the diffusive decay of a PFG NMR experiment. However, many of these techniques fail in resolving diffusion coefficients of a multi-exponential decay. A multi-exponential decay may arise from multi-component mixtures with overlapping signals in the frequency dimension. Thus, there is a need for new algorithms allowing for multi-exponential processing where the maximum entropy method (MEM) is one example of such an algorithm [2].

Here, we introduce a new method to process DOSY data. Our approach combines the MEM method with the l1-norm penalty function that is commonly used in the so-called Compressed Sensing [3] technique. We implemented the l1-norm minimization using the Fast Iterative Shrinkage Thresholding Algorithm (FISTA) [4]. This allows us to obtain a computationally cheap and reliable method referred to as Iterative Thresholding Algorithm for Multi-exponential Decay (ITAMeD).

We compared our method with the MEM method on simulated data sets and as well on experimental DOSY data for mixtures of Polyethylene glycol (PEG) polymers with various molecular weights (1080 g/mol, 11840 g/mol, 124700 g/mol). For PEG solutions containing only one PEG, a single-exponential decay was obtained compared to PEG mixtures that revealed multi-exponential behaviour for one signal in the frequency dimension. In all cases ITAMeD showed a significant improvement in the computational time and precision of the results comparing to the MEM approach.

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286WE

^{2}H NMR and IRMS of Some Romanian Wines

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Isotopic analyses are now official or standard methods in Europe and North America for routine use in testing the authenticity of several food products. These methods are based on the measurement of stable isotope content (^{2}H, ^{13}C, ^{18}O) of the product or of a specific component such as an ingredient or target molecule of the product. The determinations carried out using nuclear magnetic resonance (NMR), and Isotopic Ratio Mass Spectrometry (IRMS), provide information on the botanical and geographical origin of the food product.

A deuterium natural abundance quantitative NMR method (SNIF-NMR: Site -specific Natural Isotope Fractionation) was developed as an efficient and powerful tool capable of characterizing the chemical origins of organic molecules and distinguishing their biological and geographical origin. The SNIF method is based on the measurement of deuterium / hydrogen (D/H) ratios at the specific sites of the ethanol. Using these methods, we present the obtained results for a series of Romanian wines. Our results may be use like reference data set for authenticity and origin control of wines.

NMR measurements were performed the BRUKER Avance III 500 UltraShield NMR spectrometer equipped with a special probehead (SEX 500 MHz S2 10 mm) for recording ^{2}H NMR spectra, proton decupling and lock on ^{19}F.

The measurements of ^{18}O/^{16}O and ^{13}C/^{12}C ratio were performed by Delta V Advantage Isotope Ratio Mass Spectrometer. ^{18}O/^{16}O isotopic ratio of the water from wine was determined by Isotopic Ratios Mass Spectrometry (IRMS) using the ions having the mass of 46 (^{12}C^{16}O^{18}O) and 44 (^{12}C^{16}O$_2$). The intensities were obtained on carbon dioxide equilibrated isotopically together with the water from wine according to the isotopic exchange reaction. The ^{13}C/^{12}C isotope ratio was expressed by its deviation from a working reference.

Acknowledgments: This work was supported by CNCSIS-UEFISCSU, project number:
PN II-RU PD 130/2010.

287TH

Parallel Ultra-Fast NMR Spectroscopy

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In this work we demonstrate the emerging capabilities of analytical ultrafast NMR spectroscopy, and its combination with the state-of-the-art electronics and hardware & software in particular. In particular, a novel approach – parallel ultra-fast spectroscopy (UF PANSY) combining the ultra-fast (UF) NMR methodology with use of multiple receivers and parallel acquisition dramatically increases the amount of information that can be obtained from a single measurement in a fraction of a second.

The Initial targets of this new approach involve the parallel collection of “classic” 2D NMR experiments, UF PANSY-COSY (CORrelation SpectroscopY) , UF PANSY-HETCOR (HETeronuclear CORrelation spectroscopy), UF PANSY-TOCSY (TOtal Correlation SpectroscopY) in a single transient. A number of new data acquisition and processing strategies have been developed to enable the practical implementation of the UF PANSY technique. The experiments have been complemented with graphical user interface for ultimate ease of use. Currently we pursue further extensions of this technique to incorporate new NMR experiments, nuclei other than ^{19}F, ^{13}C and ^{1}H, and higher dimensionalities. We are confident that a successful implementation of the UF PANSY experiments will provide a new impetus for pursuing a number of new projects, in particular, for analytical and bio-molecular applications including studies of rapidly-changing systems, such as conformational changes in polypeptides, metabolic processes, structural analysis of unstable molecules and similar processes proceeding in the minute timescales.

288MO

Modified Polystyrene Gels as Chiral Alignment Media

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New polystyrene (PS)-based gels were synthesized and their ability to act as chiral alignment media for high-resolution NMR has to be tested. In a three-step synthesis p-vinyl benzoic acid was obtained as basis for further modification of the latter obtained polystyrene. Various alcohols, such as L- and D menthol[1] or (S)-(+)- and (R)-(-)-2-butanol, can be coupled easily to the styrene monomer by esterification of the benzoic acid moiety. For polymerisation the chiral functionalized monomer was mixed with 0.3% divinylbenzene as well as 0.25 % azoisobutyronitrile and heated to 60 °C for several days in sealed glass tubes. The resulting solid material was swollen in standard 5mm-NMR-tubes to give stretched gels.[2]

As a test molecule strychnine was diffused into the L-menthol-polymer gels and gated-decoupled ^{13}C-spectra were recorded at 700 MHz with and without alignment. Residual dipolar couplings up to 26 Hz and line widths below 25 Hz were obtained. Since the alignment strength and resolution of the polymer sticks is promising their power to discriminate enantiomers is tested by measuring either pairs of enantiomers in one type of gel or by measuring one enantiomeric form of the analyte in complementary gels, i.e. L- and D-menthol modified PS.

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289TU

Qualitative, Quantitative and Microstructural Characterization of Polyisoprene-Polybutadiene High Cis Copolymers Using Liquid-State NMR

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Block and random Polyisoprene-Polybutadiene high cis copolymers at different compositions have been synthesized. Qualitative, quantitative and microstructural characterization is very important to understand which synthesis mechanisms and operating parameters (catalyst, temperature, etc) affect the structural characteristics and therefore mechanical properties. NMR spectroscopy is the most appropriate analytical technique to characterize these copolymers from a quantitative and microstructural point of view. In this respect a NMR method to determine molar composition in terms of polyisoprene and polybutadiene content and of comonomers distribution (dyads) along macromolecular chains has been developed. Characteristic peaks of polyisoprene 3,4 and 1,4 (cis/trans) units and polybutadiene 1,2 and 1,4 (cis/trans) units have been assigned by 2D-HMQC (Heteronuclear Multiple Quantum Correlation) analysis. Isomeric ratio between these monomeric units was determined employing ^{13}C-NMR, by integrating the area of methyl and methylene groups. Results obtained confirmed a prevalence of polyisoprene-polybutadiene cis-1,4 units. Analysis of ^{13}C-DEPT135 spectrum allowed identification of methylene carbons in isoprene and butadiene, located in the range from 33 to 26 ppm. Comonomers distribution along polymeric chain in terms of random (IB+BI) and block (BB, II) dyads has been calculated by integrating the area of characteristic peaks. It has been confirmed that random copolymers have a higher concentration of dyads IB+BI than block copolymers, which instead show a prevalence of dyads II and BB. Molar composition has been calculated from sequences distribution using the following relationship:

PIs = II + 0,5*(IB+BI) ; PBu = BB + 0,5*(IB+BI)

Polyisoprene and polybutadiene content obtained by dyads distribution is in agreement with percentage of comonomers weighted in reactor feed. These results have shown the correct assignment of ^{13}C-NMR peaks to the corresponding dyads. In conclusion NMR characterization allowed us to obtain detailed information about microstructure and isomeric composition of block and random Polyisoprene-Polybutadiene copolymers.

290WE

SIMULTANEOUS QUANTITATIVE AND QUALITATIVE ANALYSES OF COMPONENTS ACTIVES AND EXCIPIENTS IN DRUG FORMULATIONS BY ^{1}H NMR

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The need for effective and reliable quality control in final products and/or raw materials from pharmaceutical becomes the analyses of its actives components and constituents very important to manufacturers, as well as to users of these products. For this purpose, standard methods, such as pharmacopoeia, and from governmental agencies are used. The most commonly used techniques are the chromatographic and spectroscopic, such as UV Visible and Infrared. The Nuclear Magnetic Resonance spectroscopy (NMR), which is an important qualitative analytical tool, is rarely used in quantitative measurements. In this context, the objective of this work was to demonstrate the viability of ^{1}H NMR for simultaneous qualitative and quantitative analyses of active components and excipients in drugs formulations. Two commercial drugs samples were examined in triplicates, using dimethyl sulfone compound (CRM traceable to NIST) 99.65 ± 0.08%, as an internal standard (IS), and deuterated dimethylsulfoxide as a solvent. All analyses were performed in an Inova 400 Varian spectrometer, with the validated ^{1}H qNMR method. From the ^{1}H NMR spectra, it identified three active components (paracetamol, caffeine and chlorpheniramine maleate) and three excipients compounds (ethyl alcohol, propylene glycol and methylparaben) presents in the drug formulations. The content of each compound was obtained using the following equation:

$$Content_x(mg/mL) = \frac{Area_x \times n^o of\ nucleus\ absorbent_{IS} \times molecular\ weight_x(g) \times weighed\ mass_{IS}(g) \times Purity_{IS} \times 10^3}{Area_{IS} \times n^o of\ nucleus\ absorbent_x \times molecular\ weight_{IS}(g) \times V_{dissolution}(mL)}$$

In one drug we obtained 421 mg/mL of paracetamol and 65.1 mg/mL of caffeine. In the second drug, 96.8 mg/mL of paracetamol and 2.04 mg/mL of chlorpheniramine maleate. These results are reliable because they were obtained by validated ^{1}H qNMR methodology. Furthermore, the active components content determinate from simultaneous quantification is in according to described values in their bulls. Therefore, the same can be done to obtain the content of the excipients identified. It concluded that a single NMR measurement provides structural and quantitative information of active components and excipients in the sample and, thus, contributes to an efficient, simple and fast quality control.

291TH

EVALUATION OF PHASE ALTERNATION STEADY-STATE FREE PRECESSION PULSE SEQUENCES FOR FAST HIGH RESOLUTION NMR ACQUISITION

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Steady State Free Precession (SSFP) sequences have been used to enhance signal to noise ratio in high resolution NMR spectrum but it introduces strong phase and amplitude anomalies. These distortions are essentially caused by the truncation of the signal and the strong interaction between the free induction decay (FID) and echo component. To reduce these distortions we have been testing SSFP sequences with phased alternation. To understanding the effect of the phase alternation in SSFP signals we have compared the experimental results with the numerical simulations using Bloch equations. The ^{1}H and ^{13}C experiments have been performed in an Inova 400 Varian spectrometer. Phase alternation SSFP sequences such as (x –x) and (x x -x –x) has been tested and compared with conventional SSFP sequence, without phase alternation. The FID and echo signals for on resonance signals for x-x sequence have the same phase (fig. 1a), conversely to the conventional SSFP, which are dephased by 180º (fig. 1b). Therefore the addition of the conventional and x -x SSFP signals, can produces a FID without the presence of the echo and consequently, suppressing these anomalies. The SSFP x x-x-x sequence (fig 1c) produces a more complicated results and it varies from maximum to minimum amplitudes depending on the frequency. Theoretical results are in excellent agreement with experimental results.

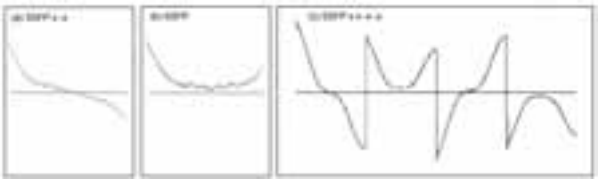

Figure 1. ^{1}H NMR signals: a) SSFP x-x; b) SSFP; c) SSFP x x-x-x.

292MO

Real-Time NMR Spectroscopy of the *In Situ* Partial Unfolding of a Protein: Glucono-Delta-Lactone-Based Conversion of a-Lactalbumin to its Molten-Globule Form

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α-Lactalbumin (α-LA) is a small globular whey protein, and has been the focus of much scientific interest of late due to its role in formation of a protein-fatty acid complex exhibiting remarkable cytotoxic properties in a wide range of tumour cell lines as well as in human/animal tumour models. This nanoparticle, known as HAMLET / BAMLET (Human / Bovine Alpha-lactalbumin Made LEthal to Tumour cells), contains two major constituents in milk - α-LA and oleic acid (OA) – where the protein moiety is partially-unfolded. The physiologically acidic conditions within the stomach of nursing infants are favourable for the conversion of native α-LA to its partially unfolded molten globule state, prompting speculation that HAMLET-like complexes may be spontaneously formed in the gastro-intestinal tract of infants. In order to effectively mimic the changing stomach environment of infants, glucono-delta-lactone (GDL) was used to gradually decrease the pH of the protein solution with/without oleic acid over a period of 90 minutes in-situ. Real time monitoring of ^{1}H spectra were performed every 5 minutes using a 600 MHz NMR spectrometer. In particular, the loss of native structure as a function of pH could be monitored by analysis of the upfield region of the spectra (-2.5 – 0.0 ppm) that reflects the native-state packing of aliphatic side chains. Quantification of the oleic acid peak showed there was a stoichiometry of 4.8 between oleic acid and protein in solution. The state of binding between the partially-unfolded protein with fatty acid was clearly demonstrated with pulsed-field gradient diffusion NMR experiments. Under acidic conditions, the rates of signal decay as a function of gradient strength were not coincident, however, upon recovery to neutral pH, the rates of decay became in good agreement, suggesting that both components were bound and behaving as a single particle in solution. It therefore appears that the formation of active HAMLET / BAMLET complexes require a return to native-like environment, possibly to allow 'closure' of the exposed hydrophobic region and assisting in the harbouring of the weakly-bound fatty acid moieties.

293TU

High Resolution NMR Spectroscopy for Monitoring of Biomass Conversion in Ionic Liquid Media

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Continuous efforts have focused on the conversion of various carbohydrates into a single renewable biomass-derived building block, as the effective way of atmospheric carbon dioxide fixation. One of actual chemical processes observed in ionic liquids, is conversion of carbohydrates into 5-hydroxymethylfurfural, which has diverse range of chemical and industrial applications.

A new NMR procedure has been developed to investigate molecular structures and chemical reactions directly in ionic liquids. The elimination of micro heterogeneity by mechanical stirring allowed to record high-resolution 1D and 2D NMR spectra for a broad range of IL systems. This approach was useful for conducting mechanistic studies in native-state ionic liquids.

The mechanism of the conversion of carbohydrates to 5-hydroxymethylfurfural (5-HMF) was studied at the molecular level with the detection of anomers and intermediate species.

Alpha-glucopyranose-1,2-borate intermediate complex was characterized by 1D and 2D NMR methods.

Different monosaccharides, like pento- and hexoaldoses, and, especially, hexoketoses revealed significant difference of their anomeric composition dependant on the solvent.
Fructose in ionic liquids appeared in all possible anomeric forms (α,β-furanose, α–,β–pyranose and even open ketose form), the equilibrium depended on sample temperature.

A new NMR procedure that has been developed, allowed to investigate molecular structures and chemical reactions of carbohydrate coversion into 5-HMF directly in ionic liquids.

294WE

The Sequential Analysis of Intrinsically Disordered Proteins Using a Set of Multidimensional (5D) NMR Experiments Acquired with Non-Uniform Sampling

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The backbone and side chain assignment of intrinsically disordered proteins (IDPs) can be performed using multidimensional (5D) NMR experiments with non-uniform sampling (NUS) of the evolution time space. Smaller IDPs can be conventionally assigned using a standard set of triple-resonance NMR experiments. However as the size of IDP increases, the structural disorder in combination with high sequential repeats results in severely crowded spectra. We used a set of 5D experiments[1,2] with long evolution times (aided by NUS). The three protein samples used were: H6cBASP1 (Chicken Brain Acid-Soluble Protein) , H6hBASP1 (Human Brain Acid-Soluble Protein) and Intrinsically disordered MYC, Human Osteopontin (assignment in progress) . The experiments we used were: 5D HNCOCACB , 5D HabCabCONH , 5D HN(CA)CONH, 5D (HACA)CON(CA)CONH , 5D (H)NCO(NCA)CONH , 5D HC(CC-TOCSY)CONH , 3D HNCO and 2D ^{15}N-HSQC. All the spectra were processed using multidimensional Fourier transform (MFT)[3], and for some spectra, the artifacts cleaning algorithms[4,5] were used for solving ambiguities with weak or overlapped peaks. The strategy of signal assignment was based on the analysis of two-dimensional cross-sections obtained from 5D spectra by the sparse MFT procedure[6]. High dimensionality of the experiments and high resolution of the obtained spectra allowed us to efficiently assign the backbone and side chains of large IDPs.

295TH

Singlet NMR Signal-To-Noise Enhancement Using Multiple Spin-Echo Signal Acquisition

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Singlet states of coupled pairs of spin-1/2 nuclei may display lifetimes that are much longer than conventional T1 relaxation times. Such long-lived states find application in exploring slow motional processes and as a means of storing hyperpolarized spin order. A promising tracer for hyperpolarized MRI is $^{15}N_2O$, whose ^{15}N singlet lifetime in solution exceeds several minutes, even in blood [1, 2].

In practice, the relatively low ^{15}N gyromagnetic ratio and the limited solubility of $^{15}N_2O$ makes difficult to observe its NMR signals at ordinary thermal polarisation levels.

In this work, we present a method to improve the limited sensitivity of ^{15}N detection by continuous refocusing and acquisition of the signal using multiple spin-echo trains. This method is used widely in solid state NMR for quadrupolar nuclei [3], but less commonly in solution NMR [4].

We show that the multiple spin-echo signal acquisition allows up to an order of magnitude signal-to-noise enhancement in a single-scan. The achievable enhancement depends on the values of T_2 and T_2^* time constants, spacing of refocusing pulses and acceptable degradation in digital resolution of the Fourier-transformed spectrum. The method is used to study the low-field singlet relaxation of $^{15}N_2O$ dissolved in DMSO.

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296MO

HR-HMBC: Measuring Heteronuclear One-Bond Couplings with Enhanced Resolution

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Heteronuclear one-bond couplings have a variety of applications and their accurate determination is the basis for obtaining specific structural information of mostly small organic compounds. In this context, it is of utmost importance to reduce signal overlap to a minimum and a number of techniques has been introduced during the last decades. Here, we introduce a modified version of the HR-HMBC (*Magn. Reson. Chem.* **2010**, 48, 179-183) for heteronuclear one-bond measurements with improved resolution due to the J-resolved-like tilt of corresponding multiplet patterns. The pulse sequence is introduced and its performance is compared to a standard ω_2-coupled HSQC experiment. Two real life examples provide evidence for its resolving power.

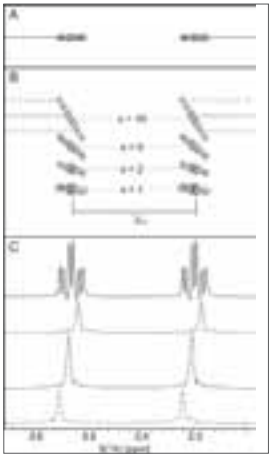

Figure: Comparison of the resolution of the CLIP-HSQC and the modified HR-HMBC acquired on a sample of menthol in $CDCl_3$. (A) A CLIP-HSQC with 512 increments and (B) HR-HMBCs with different scaling values (κ) and with 512 increments are shown. From bottom to top: a conventional HMBC (κ = 1), two-fold (κ = 2), five-fold (κ = 5) and ten-fold (κ = 10) scaling in the indirect dimension. (C) Various slices of the signal at approximately 71.6 ppm (^{13}C) are shown. In the CLIP-HSQC (top) the complete multiplet pattern is present. The slices of the modified HR-HMBC (second to fourth spectra, κ = 10) show only a singlet-like residual signal for the α- and β-components with respect to the $^1J_{CH}$ coupling, making it easier to determine the corresponding coupling constant. Slices are marked with lines of matching style in the 2D spectra (A and B).

297TU

Application of Optimal Control Pulses to the Removal of Zero Quantum Artifacts

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Among the possible artifacts in NMR spectroscopy, those resulting from homonuclear zero quantum coherences belong to the most persistent ones as conventional magnetic field gradients do not affect corresponding coherences. Applying special optimal control derived inversion pulses with a quadratic phase of the rotation axis in the xy-plane in the presence of a magnetic field gradient, these coherences can be dephased, reducing or removing zero quantum artifacts.

We present an implementation of the zero quantum suppression scheme introduced by Thrippleton and Keeler where substituting the adiabatic pulses allows for finer control over pulse properties and shorter pulse lengths.

298WE

Cross Relaxation Rates from Pure Shift NOESY Spectra

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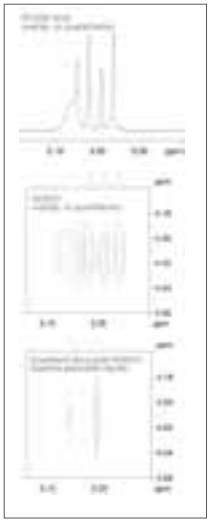
In proton NMR scalar couplings contain a wealth of useful information for chemical structure elucidation and signal assignment. However suppressing the signal multiplet structure by pure-shift techniques may also be of great interest as this can drastically reduce signal overlap and simplify the interpretation of one- and two-dimensional spectra.[1] In NOESY spectra scalar couplings remain an unwanted feature as they often complicate or inhibit the extraction of the desired cross relaxation rates. Very recently it has been shown that in these spectra the multiplet structure of the individual signals can be suppressed thereby simplifying the resulting spectra.[2]



We show that both one dimensional proton spectra using a Zangger Sterk broadband decoupling element and F_2 broadband-decoupled NOESY spectra can be used to obtain quantitative information on processes such as dipolar interactions or chemical exchange. As baseline separation of the individual signals is a prerequisite for the quantification the resulting singlet line shape in F_2 proves very useful, especially in busy regions of the spectra. The figures show two NOESY cross peaks in the strychnine spectrum that can only easily be quantified separately if scalar couplings are suppressed.

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299TH

BUBI: Concurrent Broadband Refocusing and Broadband Inversion in $^{1}H,^{13}C$ Spin Systems

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As the field strength of NMR magnets steadily increases, signal to noise and spectral resolution improves on one hand, but uniform inversion requires shorter and shorter hard pulse lengths that cannot always be provided by the available hardware. State-of-the-art experiments are therefore based on broadband inversion and refocusing pulses with lower rf-amplitude demands but longer pulse lengths.

Conventional broadband shaped pulses are optimized using single uncoupled spins that do not take into account the effect of coupling evolution. As can be shown by algebraic considerations, corresponding pulse shapes perform well in coupled spin pairs as long as no other pulse is applied simultaneously. However, if two pulses are applied concurrently, spectral artefacts are observed frequently that must be attributed to the evolution of couplings.

Here, we show the derivation of a pulse sandwich for $^{1}H,^{13}C$ correlation experiments which covers the most common simultaneous application of pulses, the refocusing on ^{1}H and inversion on ^{13}C, using optimal control derived algorithms. For the optimization, a novel concept for combined cost functions needed to be derived.

Although the resulting BUBI pulse shows only slight numerical improvement compared to individually optimized broadband pulses, the experimental verification demonstrates a significant improvement in the context of avoidance of spectral artifacts. Especially the application within heteronuclear CPMG-type sequence elements appears to give a drastic gain in performance.

300MO

Wavelet Analysis of NMR Logging Data

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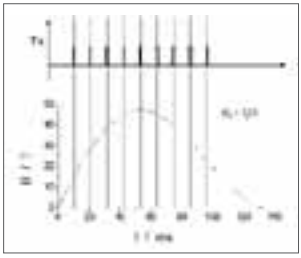
NMR(Nuclear Magnetic Resonance) logging is important tool for evaluating the oil and gas production potential of subsurface formations. The main obstacle of NMR logging instrument is its very low signal to noise ratio as well as its overlapping resonances with different transverse relaxation time (T_2) values. Noise in NMR logging instrument is mainly due to thermal noise induced by the movement of charged particles in the radio frequency coils and the small anomalies in the preamplifiers. The estimations of bulk volume irreducible , permeability, and fluid type depend on the accurate measurement of the spin-spin relaxation time (T_2) . Thus denoising should be performed to improve the signal to noise ratio of the raw logging data. NMR signals consist mainly of harmonics with different durations, i.e., they have time-dependant spectra. Therefore, a Time-Frequency representation is needed for describing both the time and the frequency characteristics simultaneously. Wavelet transform is an effective time-frequency representation for noise reduction.A novel method ***wavelet transform*** (WT),called mathematical microscope, is employed for ***resolution*** of an overlapping NMR spectrum. Wavelet energy spectra of NMR signals carry some crucial information and can be regarded as a microscope expressing the characteristics of signals. The resolution of an ***NMR spectrum*** is largely determined by the stability of the external magnetic field it experiences. The resolution will become poor if the magnetic field is unstable. It is difficult to extract effective information from such a spectrum.Wavelet is an analysis tool well-suited to the study of multiscale, nonstationary processes occurring over finite spatial and temporal domains. NMR is very efficient for characterization of reservoir,petrophysical imaging and Gas Dynamics in Gas Shale Nanopores.

301TU

NMR in Pulsed High-Field Magnets

Holger Stork, Philippe Bontemps, Geert Rikken

Laboratoire National des Champs Magnétiques Intenses, Toulouse, France



Despite pulsed magnets offer the highest available fields (non-destructively up to 100 T so far) only quite recently efforts are performed to introduce NMR in that environment. NMR measurements at magnetic fields significantly exceeding 45 T (as currently can be produced in continuous mode) can potentially provide new insights into a diversity of field-driven phenomena including the low temperature normal state of certain high-temperature superconductors.

The basic method of NMR in pulsed fields is sketched in the figure. Note that the bottom graph shows the magnetic field evolution while the graph on the top represents the receiver channel. During the field pulse, RF pulses are applied to the resonant circuit containing the sample coil. Every time the magnetic field matches the Larmor condition, a NMR signal can be detected. Due to the detailed knowledge of the magnetic field evolution the effect of field change during detection on spectra can be entirely removed. Because of the short field pulse the method is limited to systems with sufficiently fast spin-lattice relaxation.

In our contribution we will present the state-of-the-art of NMR in pulsed fields at LNCMI including an optimized magnet and probe and we will show first results.

302WE

Developing a Novel Rapid Microfluidic Freeze-Quench Device for Trapping Intermediates of Enzymatic Reactions for EPR Analysis

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Rapid freeze quench electron paramagnetic resonance (RFQ)-EPR is a method for trapping short lived intermediates in chemical reactions. The trapped intermediates are then subjected to EPR spectroscopy, investigation that provides information on their structure. In RFQ-EPR two (or more) reacting components are mixed at room temperature and after some delay the mixture is sprayed into a cold trap and transferred into the EPR tube. A major difficulty in using commercial RFQ-EPR is the relatively large amount of sample needed for each time point, a large part of which is wasted in the dead volume of the tubes and mixer. The small sample volume (~2 µl) needed for our high field (W-band) EPR spectrometer calls for the development of a microfluidic based RFQ-EPR device. This is particularly important in light of the cost and the difficulties of producing large amounts of spin labeled RNA and proteins which are currently studied in our laboratory. Microfluidics freeze quench for EPR is not commercially available and has been reported mainly by Gerfen and coworkers. Here we present a dedicated microfluidic based RFQ-EPR apparatus for W-band measurements suitable for the small sample size designed in our laboratory. Our mixer is based on a recently published design with a modified cold trap that allows collecting all time points in one go. The reduction of nitroxide radicals using dithionite, employing the signal of Mn^{2+} as an internal standard is used to test and calibrate the microfluidic RFQ.

303TH

Energy Harvesting towards Autonomous MRI Detection

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We present a first prototype of an energy harvesting coil made from insulated copper wire (thickness = 0.1 mm, 5000 turns) wound on a PMMA holder, designed to fit smoothly inside the bore of a Bruker AVANCE III MRI spectrometer. The harvesting coil scavenges power from gradient switches and RF pulses and converts these AC signals into a DC voltage suitable for driving the coil's preamplifier. To limit power consumption, we used a monolithic IC preamplifier manufactured at the Fraunhofer IAF III-V foundry [1] with a power consumption of only 10 mW.

The current harvesting coil is able to output a power level of 2 mW during a standard FLASH sequence. In order to meet the necessary power requirements for the preamplifier, it is only switched on during the actual data acquisition, which takes 10 ms per measurement cycle. This interval is determined using a microcontroller.

Several measurements were performed to compare images taken with a variety of surface coils, with and without a preamplifier, and with and without using the energy harvesting power supply, in order to validate that the concept of an autonomous power source inside the bore actually works and to exclude any disturbance of the FID acquisition process.

In [2,3] completely wireless MR systems were suggested. However the power supply necessary for such a system was not addressed in that work. This observation lead to the idea that we present here, i.e., to use a coil in the vicinity of the MR sensitive volume to extract energy from gradient and RF-fields that are always present for image encoding. This work is a first step towards a future completely autonomous energy-self-sufficient MR system.

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304MO

A Modular Microcoil Device Realized with Conductive Interconnect Stripes

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We demonstrate a new and straightforward method to electrically connect a microcoil to a tuning & matching circuit. For convenient use of the device at different B_0-fields, thus different Larmor frequencies, the microcoil and the tuning & matching circuit are on separate and exchangeable substrates. In order to realize this modular concept, we use conductive interconnect stripes to electrically connect both the microcoil and the tuning & matching substrates. The interconnect stripes are clamped inbetween the substrates and can be easily released without the application of heat, mechanical pressure or chemicals in order to attach a different tuning & matching substrate to the device.

The microcoil substrate is manufactured using a Microsystem Technology process as recently described by our group.[1,2] The fabrication of the microcoil requires the use of a heat-sensitive photoresist material. As a consequence, critical heating of the microcoil substrate due to a soldering process should be avoided. By using conductive interconnect stripes to electrically contact the microcoil to the tuning & matching substrate, our approach completely circumvents this heating issue.

The modular microcoil device was electrically characterized and tested by imaging in a 9.4T Bruker BioSpec system. The S_{11} parameter of the device was measured to be -34dB at 400MHz, which indicates negligible power reflection losses. The microcoil inductance was 23nH and the Q-factor was 41 at 400MHz. The ohmic resistance between the microcoil and the tuning & matching substrate due to the conductive interconnect stripe was measured to be 1.4Ω at 400MHz. This resistance value can be reduced by increasing the size of the contact pads on the microcoil substrate. The MRI experiment achieved a spatial resolution of 33µm×33µm×100µm (x-y-z). An SNR of 31 was obtained from a 3.5 nL deionized water sample within a total scan time of 51s.

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305TU

PIC Microcontroller Based External Fast Analog to Digital Converter to Acquire Wide-Line Solid NMR Spectra by BRUKER DRX Spectrometer

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Eötvös Loránd University, Budapest, Hungary

The aim was to digitize FIDs of quadrupole nuclei by our BRUKER DRX 500 NMR spectrometer up to 10 MHz sampling rates at an extremely low budget. This sampling rate provides a spectrum width much wider than achieved by the built-in HADC unit with its maximal 100 kHz spectrum width.
A high speed dual channel analog to digital conversion was accomplished by a home built “PIC-ADC” module, consisting only of a few commercial components: operational amplifiers, ADC ICs (10 MSPS, 12 bit), high speed static RAMs (256kx16 bit), a PIC 18F4520 microcontroller, an USB-UART converter and a few other digital ICs.
In order to record the FIDs with reproducible phases the controller is synchronized with the 40 MHz output of TCU and with the pulse program software. The desired sampling rate is generated by dividing the 40 MHz clock signal by 2N. The experiments are started with the pulse program which waits for the trigger signal of PIC-ADC on the TRIG1 line of the TCU. When the negative trigger signal arrives, the pulse sequence is applied and finally the external ADC conversion begins simultaneously with the internal HADC on the falling edge of the RCUGO of RCU. The internal logic increments the address, starts the data sampling and writes out the result of the conversion to the data bus. As the preset number of points was acquired, the microcontroller reads out the content of the two buffer RAMs, and sends out the data to the PC via USB. A new acquisition cycle is started by a new TRIG1 signal.
The complex FID’s are acquired, added controlled by phase cycling, and converted to BRUKER fid file format by PC software written in Java. Only the ##$SW_h line of the acqus file must be modified before the standard TopSpin data processing to obtain wide-line NMR spectra.

Wide-line 27Al MAS NMR spectra of polycrystalline aluminium sulphate were measured at 11.744 Tesla with 1.25 MHz spectrum width to demonstrate the usefulness of PIC-ADC.

306WE

Towards an Ultra High Sensitivity 5mm-Cryogenic NMR Probe with High Temperature Superconducting (HTS) RF Coils -Effect of Diamagnetic Magnetization of the HTS-

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An ultra-high sensitivity cryogenic NMR probe with a high temperature superconductor (HTS) RF coil is being developed as a national project supported by the Japan Science and Technology Agency; the project comprises the development of an HTS NMR magnet and the HTS cryogenic probe. The HTS NMR magnet uses ReBCO-coated tape conductors, which have a higher critical current density at high magnetic field compared with low temperature superconductors (LTS); it enables us to achieve a high magnetic field with a smaller magnet volume. The high sensitivity of the NMR probe is achieved by using YBCO HTS thin films, which are 20-fold smaller in RF surface resistance compared with a conventional copper foil; it corresponds to an enhancement in NMR sensitivity by a factor of 4 compared with conventional cryogenic probes.

W. W. Brey et al. (J. Magn. Reson. 179(2006) 290-293) have already developed an YBCO HTS-NMR cryogenic probe for a 1mm NMR sample tube. This poster deals with the HTS NMR probe, applicable to a more common 5 mm NMR sample tube. Therefore, the effect of diamagnetic property of the HTS coil is much more serious.

Thus, we are following three steps to develop the 5mm-HTS NMR probe. Firstly, the effect of HTS films, 25mmx25mm, installed and cooled at the RF coil position is determined and formulated by experiments using a microcoil NMR. Secondly, the actual HTS RF coil will be manufactured and the magnetic field inhomogeneity along the magnet axis measured by a microcoil NMR, so that we can determine whether the field inhomogeneity due to diamagnetism is limited to less than the shim coil ability. Finally, tuning and matching circuits will be attached and NMR measurements using the cryogenic HTS probe will be achieved.

This poster presents an experimental procedure and experimental results for the first step of the development. Both the temperature dependence of the field distortion along the NMR magnet axis and a temporal magnetic field fluctuation with a time interval of 1hr will be reported.

307TH

Development of Triple Resonance Solution NMR Probe for a Low Temperature Superconducting/ High Temperature Superconducting (LTS/HTS) 1.03 GHz NMR Magnet

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An Ultra-high magnetic field, such as > 23.5 T (1GHz), is useful to achieve high NMR sensitivity and high resolution in protein NMR measurements. However, a Low Temperature Superconducting (LTS) NMR magnet is unable to exceed 23.5 T due to the size of its critical magnetic field. We have commenced a project to replaces an innermost Nb_3Sn coil of the current 920 MHz NMR with a Bi2223 High Temperature Superconducting (HTS) coil in order to a exceed 1 GHz field, i.e. 1.03GHz.

Fig.1 1.03GHz NMR magnet installed in NIMS.

Fig.2 1.03GHz triple resonance NMR probe

This poster presents the development of a triple resonance solution NMR probe for the 1.03GHz LTS/ HTS NMR magnet; the probe was originally manufactured for the 920MHz NMR, being reformed for the 1.03GHz NMR. For the 1.03 GHz triple resonance probe, there is a trade-off between the 1H resonance frequency and the ^{1}H sensitivity. For instance, the resonance frequency is enhanced with an increase in the quartz tube thickness as the RF coil inductance is reduced, while the B1 intensity per unit current, corresponding to the NMR sensitivity, decreases at the same time. We have developed a new RF coil design, realizing the coexistence between a high 1.4 GHz frequency and high 1H sensitivity. In this poster, both 3D electromagnetic field simulation results and experimental results for the 1.03GHz RF coil will be reported. Based on these results, the triple-resonance NMR probe will be developed in our laboratory, and will be installed in the 1.03 GHz magnet. The NMR system is expected to be operational by the end of March, 2013.

308MO

A Rapid Injection Apparatus in Fast Chemical Reactions

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The detection and characterisation of intermediate states in chemical reactions are very important for understanding the mechanisms of products or side products formation. Many reaction pathways have been explained using NMR-spectroscopy, but in the majority of studies, results have been limited to medium-fast to slow chemical reactions. In cases of rapid chemical reactions where the intermediate states are prohibitively short-lived, special equipment is required to allow reactions be initiated in situ within the NMR magnet. A new NMR rapid injection device is proposed here with semi-automated double pumping system adaptable to conventional 5mm probeheads. The major advantages of this apparatus compared to other systems are: 1) the reagent solutions are pre-equilibrated within the magnet before mixing which allows for a wide range of reactions temperatures, 2) the sealed system allows for the performance of air-sensitive reactions and 3) the injection and flushing system enables reliable and reproducible repetition of reaction and accumulation of experiment for detecting weak signals. We will show one example in an application...

309TU

Paramagnetic Enzyme Meets Paramagnetic Substrate

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EPR is well suited to solve some protein problems that elude X-ray structure studies. Questions about how substrate finds the catalytic iron center in lipoxygenases illustrate the point. Lipoxygenases are involved in inflammatory cascades and are pharmaceutical targets. With M Bradshaw and S Frausto (FSU), a lysolecithin substrate analog with a spin label on the polar end is shown to bind at the basic pH optimum of the enzyme and to be an inhibitor. To determine the binding site of the polar end of the lysolecithin, the 100 kDa protein was also spin labeled at five introduced cysteines, each > 20 Å from iron. Modeling spin label sites with PRONOX predicts good fits within the low-pH X-ray structure. With P Borbat and J Freed (Cornell), distances between spins in ten double-labeled lipoxygenases have been determined by pulsed dipolar EPR spectroscopy (PDS). The fit of the experimental distances to those predicted by PRONOX was optimized to represent the protein as a five-sided polyhedron of spins. To find the binding site of the substrate analog, additional PDS distances from each point of the polyhedron to the lysolecithin spin were determined. Trilateration provides well defined coordinates that place the spin at a possible entrance to the substrate cavity, a position that leads to follow-up mutations to understand how substrate opens the cavity entrance.

A second problem about the lipoxygenase iron center is to understand the significance of two high-spin ferric signals reflecting different symmetry. With A Garreta and A Manresa (Barcelona) a bacterial lipoxygenase that reacts slowly with substrate helps to define the first-formed ferric intermediate in catalysis. Isotopic labeling provides information on the line shape contributions from nitrogen hyperfine and distribution in ZFS parameters.

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310WE

High-Resolution Solid-State NMR Studies on BamA - A Large Membrane Protein Machine

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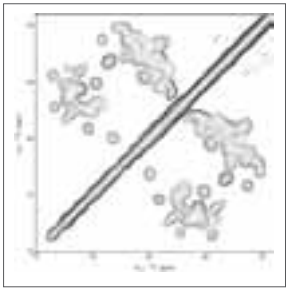
Solid-state NMR (ssNMR) provides unique opportunities to study integral membrane proteins in their native environment in atomic detail, yet sensitivity and resolution remain critical factors.[1] Here we report high-resolution ssNMR studies on the 790-residue outer membrane protein BamA, which forms the central component of the β-barrel assembly machinery (BAM) in gram-negative bacteria. Although structures of the five soluble domains that constitute the periplasmic extension of BamA have been reported, the structure of the integral transmembrane domain and of the full-length protein machine have so far remained elusive.[2]

We succeeded in reconstituting full-length BamA in lipid bilayers at relatively low lipid-to-protein ratio (LPR), allowing ssNMR experiments in a functional environment (see ref. 3). Using a truncated BamA construct lacking four of the soluble domains, the LPR could be further reduced yielding a >10-fold increase in signal-to-noise ratio. Concomitantly, we observed an improved resolution in ^{13}C and ^{15}N dimensions in the latter sample type, indicative of sizable protein motion in a more dilute lipid environment.



These advancements have enabled us to apply state-of-the-art ssNMR methods for spectral assignment and structure elucidation of BamA. In addition, we have explored proton-detected experiments on highly deuterated samples, which point to excellent proton linewidths of around 100-150 Hz at moderate MAS rates. In this contribution, we report on our most recent findings that provide novel insights into the structural organization and the molecular plasticity of this large integral membrane protein in its native-like environment.

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311TH

Towards an Understanding of the Mechanism of Interaction of Alpha – Synuclein with Lipid Bilayers

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Substantial evidence links alpha synuclein (a-syn), a small highly conserved pre-synaptic protein with unknown function, to both the familial and sporadic forms of Parkinson's disease (PD). Rare familial cases of PD are associated with missense point mutations (A30P, E46K, and A53T) in α–syn, or with the hyper-expression of the wild type (WT) protein due to its gene duplication/triplication. When isolated in solution, the protein is intrinsically disordered, but in the presence of lipid surfaces a-syn adopts a highly helical structure that is believed to mediate its normal function(s), e.g. synaptic plasticity, presynaptic vesicle pool size and neurotransmitter release. The interaction between a-syn and lipid bilayers seems to be highly dependent on their composition and structure and the binding mode of the disease variants of a-syn seems to differ from the one of the WT. Understanding the difference between the lipid binding mechanisms of α-syn and its mutants is crucial to explaining the increase in toxicity of the latter. Moreover, a-syn oligomers and/or aggregates have been proposed to affect vesicle trafficking processes via interactions with both Rab and SNAREs proteins, mainly involved in vesicles docking and fusion.

Here, we propose a multi-faceted experimental approach to investigate the mechanism of interaction between a-syn and membrane bilayers by characterizing at atomic detail the interactions between a-syn and lipids, using membrane mimetic environments of gradually increasing complexity; i.e. ranging from simple detergent micelles to lipid bilayers of biologically relevant compositions. Membrane perturbation upon a-syn binding and the conformational changes of a-syn induced by lipid binding is investigated using a large range of spectroscopic techniques including circular dichroism and heteronuclear multidimensional NMR spectroscopy. Our results suggest that the apparent binding affinity of α–syn to lipids as well as the mechanism of interaction differ considerably for the different membrane mimetic environments. The structural properties of a-syn fully bound to the different membrane mimetic environments are compared. This broad strategy is essential to characterize how membrane curvature and composition influence the behaviour of a-syn at the atomic level and to better understand its physiological role as well as its role in the pathogenesis of PD. In addition, extending these studies to tertiary systems involving membrane, α-syn and either Rab or SNAREs proteins would lead to a better understanding of the interactions responsible for the loss of the biological function of these proteins leading to synucleinopathies.

312MO

Interactions of Small Molecular Ligands with Membrane Proteins

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Structure determination of membrane proteins using X-ray crystallography and NMR is often difficult due to the intrinsic dynamics of membrane proteins. But some ligands that exhibit high-affinity might stabilize the membrane protein fold upon binding and thus allow structure determination of the resulting ligand-protein complex. A biologically highly important membrane protein, for which the high-resolution structure is not yet known due to its complex dynamic nature, is the mitochondrial 18 kDa translocator protein (1,2). We demonstrate that the tertiary fold of the translocator protein might be stabilized by selected natural and synthetic compounds such as cholesterol, etifoxin and PK11195 (1) as monitored by solution-state NMR. On the basis of 2D [^{1}H,^{15}N] HSQC spectra of ^{15}N-labelled translocator protein in the presence of ligands we estimate theirs suitability for structural studies by means of solution NMR techniques.

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313TU

Refined Structure and Topology by Combined Solid-State and Solution NMR : A Membrane-Anchor Domain of Huntingtin

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^{15}N chemical shift and ^{2}H solid-state NMR spectroscopy on oriented phospholipid bilayers was used to determine precisely the topology of Htt17, the N-terminal membrane anchor of huntingtin. Peptide motions were taken into account including wagging and azimuthal fluctuations. The helical tilt angle (~77o) of this amphipathic polypeptide was found nearly constant in all membranes investigated with a hydrophilic side of the helix being exposed into membrane water interface. Furthermore, by comparing the solution and solid-state NMR data in detail it is possible to select from the solution NMR conformational ensemble a small subset of conformations that fits best the orientational restraints derived from supported bilayer environments, thereby delineating a simple-to-implement method which allows one to refine the polypeptide structures obtained by multidimensional solution NMR spectroscopy in their native planar lipid bilayer environment.

In addition, valuable information on the rotational diffusion constants of the peptide in the membrane is obtained. The decrease in bilayer order parameters observed in ^{2}H solid-state NMR spectra of fatty-acid deuterated membranes due to Htt17 domain agrees with models where amphipathic helices disrupt the bilayer packing thereby causing membrane thinning.

The N-terminal domain of huntingtin (Htt17), located immediately upstream of the decisive polyglutamine tract, strongly influences important properties of this large protein and thereby the development of Huntington's disease. Htt17 markedly increases polyglutamine aggregation rates and huntingtin's interactions with biological membranes. Thereby, understanding the structure and membrane-anchor function of Htt17 is an important prerequisite to design new avenues to easy of prevent outbreak of the disease.

314WE

Solid State NMR Studies of Light Capturing and Light Protected States of Light Harvesting Complexes II of Chlamydomonas reinhardtii

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The photosynthetic antenna of plants and green algae captures sunlight energy and transfers it to the reaction center. But under excess sunlight conditions, it switches into a photo protective state in which excess light energy is safely dissipated as heat, called Non Photochemical Quenching. This state can be mimicked in vitro by self-aggregation of isolated light harvesting complexes. Labeled Arginine was incorporated into Light harvesting complexes by using CC-424 *Chlamydomonas reinhardtii* auxotrophic strain. Light harvesting complexes prepared in light-capturing and in photoprotective states were studied by Solid State NMR. ^{13}C CPMAS spectra of the light capturing state shows three new peaks compared to photoprotective state which suggests conformational changes during this transition. ^{13}C-^{15}N Arginine incorporation in *Chlamydomonas reinhardtii* cells using auxotrophic strains has proven successful and a promising way to probe structural changes in antenna proteins.

315TH

Investigation of Human VDAC2 with Solid-State NMR and MD

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Voltage-dependent anion channels (VDACs), also known as mitochondrial porins, are 30-35 kDa pore-forming proteins found in the mitochondrial outer membrane (MOM) of eucariots. To date, multiple VDAC isoforms (VDAC1, VDAC2, and VDAC3) have been identified in a variety of organisms, including yeast, plants, mouse, and humans. Although all VDACs are highly preserved in all eucariotic kingdoms some studies revealed important differences in the regulatory functions within the different cell types. For example, genetic studies have shown that VDAC2 knockout mice are embryonic lethal, whereas both VDAC1 and VDAC3 knockout mice are viable, which might suggest that VDAC2 has some different functions from VDAC1 and VDAC3.

The main interest of this study is the determination of the structure of the N-terminal part of hVDAC2 and the study of its dynamics in a lipid bilayer. Although the N-teminal part of hVDAC2 is for 11 amino acids longer than of hVDAC1, the part where the α-helix is expected in hVDAC2 has more than 90% similarity with hVDAC1 so we expect that the N-terminal part of hVDAC2 will be similar to the N-terminal part of hVDAC1 due to this high sequence similarity.

The results obtained by solid state NMR experiments and molecular dynamics simulations on homology model of VDAC2 will be presented.

316MO

Characterization of the T-Cell Receptor Transmembrane Domain by Advanced EPR Techniques

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The T-cell receptor complex (TCR-CD3) serves a critical role in protecting the organism from infectious agents. TCR is composed of a heterodimer of α and β chains, which are responsible for antigen recognition and interact with the Major Histocompatibility Complex (MHC) molecules of the antigen presenting cells (APC) as well as the CD3γε, CD3δε, and ζζ signaling modules. The latter are responsible for coupling ligand binding to the signaling pathways that result in T-cell activation. A charged region critical for TCR assembly and function has been identified to reside in the transmembrane region of the TCR-α chain. This region consists of a stretch of nine amino acids, two of which are hydrophilic (lysine and arginine) and the rest are hydrophobic (GLRILLLKV). Previous studies have shown that a synthetic peptide corresponding to this sequence, termed core peptide (CP), can suppress the immune response in animal models of T-cell-mediated inflammation. The aim of this work was to characterize the CP in terms of structure, oligomerization state, and orientation with respect to the membrane using pulse EPR (Electron Paramagnetic Resonance) techniques. ESEEM (electron spin-echo envelope modulation) method was used to probe the position and orientation of peptides within membranes. This method is based on the measurement of weak dipolar interactions between nitroxide spin labels attached to the peptide and water molecules isotopically labeled with deuterium (D_2O). DEER (Double Electron Electron Resonance) measurements were used to determine the conformation and oligomarization state of CP within the membrane. A 15-residues long segment of the TCR-α, containing the CP was labeled using standard site directed spin labeling technique at positions 2, 3, 9, 13 and 14. The measurements were performed on model membranes of egg PC/Cholesterol (9:1 mol/mol) in D2O solutions. It was found that the peptide is localized to the membrane and is in general perpendicular to the membrane normal. Furthermore, it was shown that the peptide has an α-helix conformation and it tends to form aggregates/ oligomers in the membrane. These results can shed light on the mechanism of the interaction of the CP within the membrane milieu and also serve as a basis for further research of using CP as a potential therapeutic agent in human inflammatory and autoimmune disorders.

317TU

Labeling Peptides for Solid State ^{19}F-NMR Structure Analysis: a Polar Substituent for Serine/Threonine

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Solid state ^{19}F-NMR is a powerful tool to study membrane-associated peptides in their native membrane-bound state [Koch *et. al* (2012) Top Curr Chem]. The measurement of selective orientational constraints in macroscopically oriented samples reveals the peptide structure, its membrane alignment, and dynamic behaviour. A major advantage of using fluorine labels - compared to conventional ^{15}N ^{13}C and ^{2}H isotopes - is the high sensitivity and the lack of natural abundance background [Ulrich (2005) Prog NMR Spectr 46:1].

Single residues of the peptide need to be substituted one by one using specific CF_3-labeled amino acids, with as little perturbation as possible. Several such ^{19}F-labeled peptide analogues have to be prepared to perform a full structure analysis, which requires at least four different orientational constraints to describe one helical segment. A current limitation of this labeling approach is the poor repertoire of suitable amino acids, which limits the sites on the peptide that can be ^{19}F-labeled. So far, we have rationally designed several hydrophobic CF_3-substituted α-amino acids as ^{19}F-labels for structural studies: CF_3-Bpg as a suitable substituent for aliphatic Leu/Ile/Met/etc. [Mikhailiuk *et. al* (2006) Angew Chem Int Ed, 45:5659, Afonin *et. al* (2007) J Pep Sci 13:614], and various Pro analogues [Mykhailiuk *et. al* (2008) Angew Chem Int Ed, 47:5765]. Here, we report a new CF3-labeled amino acid with a polar side chain as a surrogate for Ser or Thr (CF_3-homoserine: "CF_3-Hse").

To examine the utility of CF_3-Hse as a ^{19}F-NMR label for structure analysis, we incorporated it into several natural antimicrobial peptides which have already been comprehensively characterized. Analogues of Magainin 2, Temporin A, PGLa were all synthesized with either CF_3-Bpg or CF_3-Ser in place of the native Ser. We have analyzed the respective CF_3 dipolar couplings in these analogues in order to compare the resulting orientational constraints directly with one another and with the previous results of the full structural analysis. This way, we have been able to demonstrate that the novel amino acid CF_3-Hse is well suited for the solid state ^{19}F-NMR approach. This is the first designated polar ^{19}F-label for solid state NMR structure analysis of membrane-bound peptides.

318WE

Solution Structure of Monomeric Hepatitis C Virus p7 in a Native, Drug Binding, Hairpin Conformation

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Interferon-sparing treatments for the ~170 million chronic carriers of hepatitis C virus (HCV) infection will require novel inhibitors of multiple virus-specific processes for use in combination. In this regard, the p7 ion channel protein plays a critical role during the production of infectious virions and represents an exciting new antiviral target.

We present the solution structure for monomeric p7 from HCV genotype 1b, calculated using both NOE and chemical shift-based methods. P7 forms a distinctive hairpin structure with significant differences to previously reported models, including an extended inter-helical loop projecting perpendicular to the trans-membrane helices. This hairpin is stabilised through multiple inter-helix contacts and supported biophysically by Paramagnetic Enhancement (PRE) measurements.

The value and relevance of this structure was confirmed by its specific interaction with the prototype p7 inhibitor, rimantadine, in solution. Chemical shift changes observed in the presence of drug correspond to the location of resistance mutations observed in clinical trials and engineering one such resistance mutation into the p7 sequence abrogated drug-induced chemical shift changes.

Furthermore the monomeric hairpin structure could be used to successfully create a model of the heptameric ion channel complex. This was subsequently used to perform virtual high throughput screening. Promising candidates were tested both in an *in vitro* dye release assay and in virus culture. This has resulted in the identification of novel p7 specific inhibitors which are active in the nM range.

We will also report on the progress of solution NMR with the native heptameric ion channel solubilised in DHPC micelles.

319ATH

Helix-Bundle Architecture of Na^+/Proline Symporter from Coarse-Grained Modelling Constrained by EPR Distance Data

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The large size, intrinsic conformational flexibility, and sensitivity to environmental conditions of membrane proteins often prevent successful application of established experimental techniques as X-ray diffraction and NMR for structure/function studies. To obtain at least coarse information on structure or structural transitions, site-directed spin labeling combined with long-range distance measurements by pulse EPR may present an important and sometimes a unique alternative. Here we present determination of the relative arrangement of transmembrane helices - a helix bundle - of the secondary Na^+/proline symporter PutP from sparse EPR distance constraints by using coarse-grained modelling based on the matrix geometry approach. The modelling procedure is supported by information from structure templates that were derived from proteins that are supposed to share the same LeuT fold. PutP itself could not yet be crystallized, despite some attempts were made.

Our computationally efficient approach results in a stable helix bundle architecture of PutP, despite the fact that the number of about 80 distance constraints is not sufficient to uniquely position the 13 transmembrane helices in space. Although we use template information from related proteins to make up for the insufficient number of constraints, we can show that the existing constraints are sufficient to discriminate against wrong templates. Furthermore, only 10 out of the 13 transmembrane helices belong to the core of the LeuT fold that is shared between proteins of this class of transporters. Our approach allows for locating the remaining 3 helices with respect to the core.

319BTH

In Situ Solid-State NMR Study of the Baseplate Antenna Complex of Chlorobaculum tepidum Located in the Lipid Envelope

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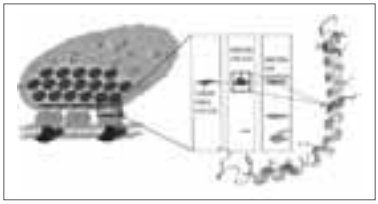
We present *in situ* solid-state NMR analysis of CsmA proteins in their native membrane heterogeneous environment in the photoreceptor of *Chlorobaculum tepidum*. Using different combinations of 2D and 3D solid-state NMR spectra, we have assigned 90% of the CsmA resonances and the most resonances of bacteriochlorophyll a (BChl a) pigment. Based on chemical shift data we provide information about the structure and conformation of CsmA in the baseplate antenna complex. Secondary structure analysis indicated a canonical α-helix for CsmA and an overall data analysis reveal high symmetry of the proteins molecules in the baseplate. We compare solid-state data in membrane heterogeneous environment with liquid-state chemical shifts for pure protein to extract the information about conformational difference. Comparison provides the clue for tertiary structure of the CsmA and interaction with BChl a ligand.



320MO

Unscrambling Solid-State NMR Parameters in Heterogeneous Model Membranes

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Solid-state NMR is ideal for studying membranes and membrane-associated proteins and peptides that are difficult to study by other means. Disorder and heterogeneity that are typical of these types of samples, however, makes analysis of the results challenging. Examples include: (a) heterogeneous distributions of ^{31}P chemical shift tensor parameters that report on phospholipid headgroup disorder; and (b) distributions of internuclear distances measured by rotational-echo double-resonance (REDOR), for example, of heteronuclear pairs within membrane-associated peptides. A method that uses an adaptation of Boltzmann statistics maximum entropy for a model-free approach to analysis of this type of troublesome data provides the means to characterize the distributions of such heterogeneous systems.

In the case of REDOR data, the method can reveal multiple distances with relatively few data points, which is of particular benefit in application to biological systems where signal lifetimes are limited by relaxation. This has been recently extended to include spin systems for which the observe nucleus is dipolar coupled to two or three dephasing nuclei. The method reverses the practice where preconceived internuclear distance models are slowly optimised to better 'fit' experimental REDOR data and, instead, provides the information necessary to construct models based on unbiased data analysis. Examples include application to membrane-associated Alzheimer's β peptide. In the case of chemical shift tensor analysis, we have applied the method to arbitrarily complex phospholipid mixtures for analysis of subtle perturbations, for example, by association of antimicrobial peptides with model membrane systems.

321TU

Solid-State NMR Investigations of Structure and Dynamics of Different Molecular Species along Amyloid b Fibrillation Pathway

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Protein aggregates composed of mature Aβ fibrils represent the end product of a long, complex, and not well understood fibrillation process. Using solid state NMR we determine secondary structure and molecular dynamics in Aβ protofibrils, which were stabilized by the antibody B10AP, and mature Aβ fibrils at a single residue level.

The well known two β-strands of the mature fibrils are already preformed in protofibrils but these regions have to elongate during the conversion into mature fibrils, especially for the residues 10-16. Overall the secondary structure of Aβ protofibrils is closer related to Aβ oligomers than to mature Aβ fibrils.

In addition, we investigate interresidual contacts between amino acid in the two β-strands. We observe a contact between Glu 22 and Ile 31 in Aβ protofibrils, which is absent for mature Aβ fibrils. Such a close proximity between Glu 22 and Ile 31 is also observed for Aβ oligomers. Therefore some structural changes have to occur during the conversion from protofibrils to mature fibrils. A rearrangement of the intramolecular hydrogen bonds present in oligomers to the intermolecular hydrogen bonds of the cross–β-structure of mature fibrils during this process as suggested before seems probable.

Both Aβ protofibrils and mature Aβ fibrils are very rigid and show a quite similar backbone dynamic. The β-strand segments and the turn linking these two β-strands exhibit order parameter (measured in DIPSHIFT experiments) between 0.8 and 0.95. The first ~8 N-terminal and the last C-terminal residues exhibit lower order parameters between ~0.4 and 0.8. Interestingly, the order parameters increase again for the first two residues Asp 1 and Ala 2, suggesting that this part is less mobile than typically assumed and might be worth some additional attention. The somewhat lower order parameters for Aβ protofibrils for residue 12 and 16 can be explained by the β-sheet structure which is present in mature Aβ fibrils in this region.

322WE

Peptides, Cell Cultures and a Heavy Mouse: Solid-State NMR Tools for Collagen Matrices

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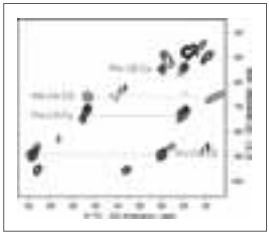
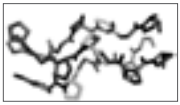
The extracellular matrix (ECM) plays an essential role in survival and function of cells in all body tissues such as bone, skin, and blood vessels. While there is a good appreciation of the components present in a functional matrix, such as collagen proteins, proteoglycans and protein aggregates, the arrangements of these components at the molecular level is still not well understood.



Solid-state NMR (ssNMR) is an increasingly important method for direct detection of molecular interactions in native tissues. We present a systematic toolkit where we use three types of carbon-13 and nitrogen-15 enriched samples: model synthetic peptides, osteoblast cell cultures, and tissue from a mouse fed on an isotope-enriched diet.

With these samples in hand, we carried out 2D experiments including SQ-DQ correlations (via POST-C7), PDSD, and double CP. We achieved highly resolved spectra of the enriched mouse bone. The direct comparison of the spectra of mouse bone to model peptides strongly indicates that that highly idealised peptides are in fact poor models of native collagen in the ECM, and at best, only represent a subset of the overall collagen structural diversity.

The osteoblast cultures are a more tunable expression system than a living mammal. By feeding the culture with specifically enriched nutrients, we found signals which are likely to correspond to a protein-glycan interaction. This open up the possibility of studying the glycosylation, glycation and sugar metabolism in mammalian cells by ssNMR.



323TH

Improved Solid-State NMR Methods for the Analysis of Nitrogen-14 in Biosolids

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Nitrogen is one of the most abundant elements and plays a key role in the chemistry of biological systems. Despite its widespread distribution the study of the naturally occurring isotope of nitrogen, nitrogen-14 (99.6%), has been relatively limited as it is a spin-1 nucleus that typically exhibits a large (>MHz) quadrupolar interaction. Accordingly most studies of nitrogen sites in biomolecules have been performed on samples enriched with nitrogen-15. Such approaches limit the application of NMR to samples where isotope enrichment can be performed, precluding the analysis of naturally occurring samples (e.g. naturally occurring proteins/environmental samples) or molecules where isotope enrichment is either impossible or financially intractable. Furthermore, the replacement of nitrogen-14 with nitrogen-15 also removes the large quadrupolar interaction that can provide a wealth of structural and dynamic information about the system.

Recently a series of experiments have been developed which permit the characterisation of nitrogen-14 sites through their interaction with neighbouring 'spy' nuclei (reviewed in Cavadini 2010). These techniques rely on HMQC style experiments where coupling between the nitrogen-14 and spy nuclei is mediated by a residual dipolar splittings (RDS). Here we demonstrate a novel experiment whereby recoupling of the interactions between the nitrogen-14 site and the spy nucleus is mediated by the application of a moderate rf field to the nitrogen-14. The resulting $^{13}C/^{14}N$ spectra show good sensitivity on natural abundance (25% vs CP) and labelled materials (15% vs CP); whilst the nitrogen-14 lineshapes observed show good agreement with the previously reported spectral properties of the nitrogen-14 sites indicating a quantitative analysis of the quadrupolar interaction is possible. We are currently utilizing this approach to gain insights into the structure and dynamics of biomolecules and investigating how such approaches may be utilized to study where to date sensitivity has precluded the use of nitrogen-14 and isotope enrichment has not been tractable.

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324MO

Probing Water – Protein Interactions in Filamentous Bacteriophage *pf1* by Solid State NMR Spectroscopy

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Water plays an important role in the biological process. Important biological molecules such as proteins are uniquely adapted to use their aqueous environment to facilitate their functions. The surface hydration of protein is the manifestation of their local stereochemistry. Solid state NMR spectroscopy has recently contributed insights into the structure and dynamics of water in and around microcrystalline proteins[1 - 3], We will be presenting experimental results of interaction of water molecules with uniformly ^{13}C and ^{15}N labelled fully hydrated form of filamentous bacteriophage pf1. We measure site-specific interaction of water molecules by ^{1}H / ^{15}N MELODI HETCOR4 experiment. We find that residues Ser-10, Gln-16, Asp-18 and Arg-44 are the residues interacting with water molecules out of 46 residues (monomer) of coat protein of bacteriophage *pf1*. Bacteriophage pf1 is a well-studied example of biological supramolecular assemblies and there is no earlier report on the water interaction in this system. Interestingly, out of the four residues showing interaction with water, Arg-44 has also been reported to interact with the DNA bases in *pf1*. The present experimental results may have profound implications in unraveling the role of water at the interface in biological supramolecular assemblies.

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325TU

Probing Hydrogen Bonding Network in Bone by Solid State NMR Spectroscopy

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Water is one of the important components of bone along with inorganic phosphates and organic proteins. It acts as pore-filling fluid in bone between organic and inorganic part. The possible role of water in bone structure has not been fully explored[1,2]. We will be presenting the possible ultra-structural changes in bone by solid state NMR (SSNMR). In our study, we used four bone samples with different strength of hydrogen bonding network and water content. ^{1}H high-resolution high-speed magic-angle spinning solid-state NMR provides information on the effect exerted by various water level contents present in bone. We have measured ^{1}H chemical shifts of collagen side chains by 1D and 2D correlation spectroscopy. Four samples of various degrees of dehydration and H/D exchanged bone samples are compared and the possible change in ^{1}H chemical shift at higher spinning speed will be presented. These study shows effect of hydration on the molecular mobility of collagen in cortical bone samples.

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326WE

Structural Study of Mussel Anchoring Protein Fibers

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In the marine environment, mussels anchor to solid surfaces using their byssus – a beard-like series of high-performance protein-based fibres which combination of strength and extensibility is only surpassed by silk. These unique mechanical properties prevent the molluscs from being washed away by tides and currents. Byssal threads are composed of an extensible proximal (P) part close to the mussel shell and a tough distal (D) region glued to substrates. According to the current structural model byssal threads are made of three collagen-rich copolymers called Pre-Cols. Each collagen domain in the PreCols would be flanked by regions akin to elastin (Pre-Col P), silk (Pre-Col D) and plant cell wall (Pre-Col NG, all along the fibre). These PreCols are ended by histidine-rich domains reticulated by metal ions. The fibres are coated by a protein (mefp-1). The molecular structure of the byssus protein domains in the core and cuticle needs to be ascertained experimentally in order to explain the byssus' remarkable mechanical properties. We therefore have studied the structure of the blue mussel (*Mytilus edulis*) byssal threads by ^{13}C-^{13}C solid-state NMR using magic-angle spinning on high (600 MHz) and ultrahigh field (900 MHz) spectrometers. Acquisition of high-resolution two-dimensional spectra was enabled by an optimized ^{13}C enrichment of the byssus. The experimental linewidths (below 1.5 ppm) reveal a well-ordered structure. By comparing the measured chemical shifts to literature values and chemical shift predictions from the software ShiftX, our results confirm that the collagen triple helix is the prevailing conformation in which amino acids are found in the filaments. In addition, a torsion angle pair which best defines this specific triple helix could be determined. Alanines were observed in five conformational domains including antiparallel β–sheets and β–turns that would dominate in the silk-like regions. Finally, according to the chemical shift analysis of amino acids mainly found in the cuticle, the fibre coating would be unstructured. In conclusion, a complete refined structural model of byssal threads can now be proposed.

327TH

Molecular Level NMR Elucidation of Inorganic-Bioorganic Interfaces in Biominerals and Model Composites

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The interfaces between bio-organic molecules and inorganic solid are abundant in living organisms (biomineralization) and in diverse technological applications (sensors, nano-electronics, catalysis) and play a critical role in determining the properties and functionality of the resulting composites. As such, their characterization at the molecular level is essential both to understand the principles of their mode of functionality and to design new materials. Additional to being minor components, these interfaces lack long range order and are therefore difficult to characterize. This is addressed in this study primarily by exhaustive combination of multinuclear solid state NMR techniques (REDOR, TEDOR, 2D-HETCOR, SLF) applied to both biominerals and model systems. First shown is the mechanism by which bioavailable calcium is biomineralsized into storage organs, gastroliths, by fresh water crayfish. Solution NMR is applied to the decalcified gastroliths, identifying vast presence of inorganic phosphate (Pi) and two primary metabolites - phosphoenolpyruvate (PEP) and citrate. Solid state NMR applied to the intact gastroliths exposes their functional structure, showing that Pi and PEP are molecularly dispersed within the entire amorphous $CaCO_3$ (ACC) as a solid solution; all citrate molecules are interact tightly with Ca^{2+} ions together with a P-moiety. Their vast abundance clearly identifies them as the major factor in stabilizing the bioavailable form. Second, the binding specificity of non-polar amino acids to silica surfaces is shown (Ala and Gly on SBA-15 and MCM-41). In spite of the apparent simplicity of the system, no conclusive experimental evidence on the mode of binding existed. Our solid state NMR measurements reveal a general geometric-dynamic structure, where the amino acids interact via their charged ammonium moiety with 3-4 surface silanols hydroxyls at specific relative geometry. Increasing the water content we observe the processes that weaken the binding: the pendent carboxylate end of the bound amino acid undergoes larger reorientation amplitude; hydrogen exchange occurs between the ammonium and the adjacent hydroxyls. Finally isotropic motion, dissolution, is reached with as few as three water molecules at the binding site.

328MO

Understanding Biomineralization: Characterization of Porous Silica/Peptide Hybrid Materials by NMR

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Biomineralization is one of the most interesting topics for material scientists. Nature manages to create hybrid materials made of an inorganic matrices and organic fibers to combine most desired and often reverse properties.

In the case of mammal bones for example, a material made of hydroxyl apatite and collagen, this means a combination of properties like the robustness caused by apatite and the flexibility caused by collagen leading to a material with unique features.

To copy these properties, material scientists must understand how the two materials are linked to each other and how they arrange to give these sorts of materials and consequently show these properties.

Extensive studies have already been done to understand the role of apatite, the collagen structure and Biomineralization processes in general [1-2].

In the present work model systems containing porous silica based materials linked to collagen-like peptides are synthesized and studied by high resolution solid-state NMR (^{29}Si, ^{13}C, ^{1}H, ^{15}N) MAS and REDOR NMR, liquid-state NMR, MS, HPLC and sorption measurements.

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329TU

Characterization of Aggregation Dynamics of the Alzheimer's Peptide Amyloid-beta 40 in the Presence of Small Inhibitory Molecules

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The Alzheimer's peptide amyloid-beta 40 (Aβ40) is a 40 amino acid long peptide and one of the main constituents of amyloid plaques, which cause neurodegeneration in the course of Alzheimer's disease. Aβ40 forms oligomeric and fibrillar aggregates *in vivo*. We investigate interactions between Aβ40 and small inhibitory compounds, such as the non-steroidal anti-inflammatory drug (NSAID) sulindac sulfide. Our results demonstrate that these compounds highly influence the solubility and aggregation properties of Aβ40 and produce insoluble structured species. This project focuses on the determination of ligand binding and aggregation dynamics using solution-state NMR, as well as a conformational analysis of Aβ40 ensembles in solution. In addition, we present a characterization of Aβ40 aggregates in the presence and absence of the inhibitors, using both solution- and MAS solid-state NMR spectroscopy.

330WE

Probing the Intermolecular Interactions between Human γS- and αB-crystallins by Solution NMR

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Crystallins play a vital role in maintaining the transparency and high refractivity of the eye lens. Damaged or misfolded crystallins can form insoluble fibrous aggregates that can result in opacification of the eye lens, commonly known as cataract. Presently, the only available treatment is the surgical replacement of the cataractous eye lens with an intraocular lens. Development of non-surgical alternatives for the prevention or reversal of cataract growth is mired by limited knowledge of the mechanisms that govern its formation.

Three major classes of crystallins, α-, β-, and γ-crystallins, exist in mammalian eye lenses. α-crystallins are molecular chaperone proteins that function to solubilize misfolded β- and γ- crystallins however, α-crystallins are unable to refold misfolded proteins. β- and γ-crystallins primarily function as structural proteins. Insoluble aggregates of all three forms of crystallins can form light scattering aberrations that leaves to loss of clarity in the eye lens.

γS-crystallin is the most highly expressed crystallin in the cortex of the eye lens during development, and is the most highly conserved γ-crystallin among mammalian species. Recently, the γS-G18V point mutation has been linked to the formation of progressive cortical cataracts and provides a useful model system to investigate cataract formation.

Of particular interest are the intermolecular interactions between γS-crystallin and αB-crystallin as well as with itself as elucidating these interactions will help understanding the pathways of cataract formation and may lead to the design of small molecule drug treatments.

331TH

Structural Insights into Human S100A1 Protein S-Nitrosylation

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S100A1 is a small, acidic homodimer that is a member of the Ca^{2+}-binding S100 protein family. It is expressed in brain and heart tissue where it plays a crucial role as a modulator of Ca^{2+} homeostasis, energy metabolism, neurotransmitter release and contractile performance. Understanding the factors regulating human S100A1 function is of special importance, since it is a marker of several human diseases and a heart failure therapy target molecule. Post-translational modifications are one of the ubiquitous ways of increasing the functional diversity of proteins. S100A1 belongs to a subclass of S100 proteins that possess a conserved cysteine residue in the C-terminal part of the protein that is hyperreactive towards nitric oxide donors. Previous studies demonstrate that post-translational modifications of this unique Cys85, such as S-nitrosylation, result in increase of Ca^{2+} affinity and notable structural changes of S100A1 protein. Therefore, the effects of S-nitrosylation on Ca^{2+} binding and on the high-resolution 3D structure of human apo-S100A1 protein were studied. We have determined the high-resolution 3D structure of human apo-S100A1 protein with S-nitrosylated C-terminal Cys85 (*apo*-S100A1-*NO*) in solution by NMR spectroscopy and compare it to that of apo-S100A1 protein structure. Although, modification does not influence tight homodimeric packaging, S-nitrosylation still induces notable changes in interhelical angles. The structural alterations were also observed for the residues subsequent to Cys85. This part of structure is often involved in Ca^{2+}-dependent interaction of S100A1 with target proteins and small molecules. Moreover, cysteine in a hydrophobic environment is often engaged in electrostatic interactions with aromatic residues. The biggest change of aromatic side-chain orientation upon S-nitrosylation in S100A1 was observed for Phe44. Covalent addition of NO to the thiol group changes the geometry of the thiol–aromatic interaction. The obtained results suggest that change in the mutual arrangement of the Cys85–Phe44 pair can work as a molecular switch that initiates transduction of an NO-related redox signal. Reshaping of the pre-existing aromatic–thiol interactions may be one of the mechanisms by which S-nitrosylation alters protein structure and function.

332MO

NMR Study of Geobacter Respiratory Chains: Mapping Interactions between Redox Partners

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Extracellular electron transfer is one of the physiological capability of *Geobacter sulfurreducens (Gs)*, allowing these bacteria to reduce toxic and/or radioactive metals and grow on electrode surfaces [1]. These competences make this microorganism very promising for biotechnological applications in the bioremediation and electricity fields. The bacterium *Gs* has a large number and diversity of *c*-type cytochromes that have been identified as key components of the extracellular electron transfer respiratory chains by extensive gene knockout and proteomic studies [1]. However, the shortage structural data obtained at conditions close to physiological environment has retarded the understanding of the functional organization of these respiratory chains and the elucidation of extracellular electron transfer mechanisms. The first solution structure of a *Gs* electron transfer protein named PpcA was recently determined [2]. Using PpcA enriched ^{15}N stable isotope, the ^{1}H and ^{15}N backbone and side chain signals were used to map interactions between this protein and a putative redox partner. In order to study the complex interactions between redox partners (PpcA-redox ligand), a series of 2D-^{1}H-^{15}N HSQC NMR spectra were recorded at increased concentrations of ligand. The dissociation constant value obtained from the fitting of the ligand-induced chemical shift perturbation curves suggests a formation of a low affinity complex. Indeed to allow a fast electron transfer a typically weak complex is expected between redox partners to ensure fast dissociation rate constants [3]. The mostly affected signals were essentially confined to the neighborhood of heme IV, which have been previous identified as the putative entry gate for electrons into PpcA [4]. In this work a molecular interaction was identified for the first time between PpcA and a redox partner constituting the first step toward the rationalization of the *Gs* respiratory chains.

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333TU

Interaction of Monomeric Tau and Aggregation Inhibitors

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In many neurodegenerative disorders, like tauopathies wrongly folded species of proteins form abnormal deposits in the brain. Here focus on the intrinsically disordered protein Tau (1), which is known to be the main agent associated with Alzheimer disease. The physiological role of Tau is the stabilization and regulation of microtubules and the support of the outgrowth of axons (2,3). An extensively phosphorylated Tau no longer binds to microtubules and aggregates into intracellular neurofibrially tangles, which is believed to be a molecular base of Alzheimer disease (AD). Finding small molecules that can inhibit tau aggregation process would lead to understand their mechanism of action together with molecular bases of tauopathies. We probed the impact of a selected tau aggregation inhibitor on the conformational sampling of the tau backbone using residual dipolar couplings (RDCs) and chemical shifts perturbations (CSPs). Quantitative analysis of $N\text{-}H^N$, Cα-Hα, CO-Cα, $CO\text{-}H^N$ RDCs provided detailed insights into its mechanism of action.

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334WE

NMR-Optimized Cell-Free Protein Expression

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Today, advanced labelling is almost mandatory for successful NMR studies of proteins. Over the last years, cell-free protein expression has shown to be a versatile tool for this purpose. Sufficient amounts of protein are readily produced but incorporation efficiency rarely exceeds 10% in a continuous exchange reaction and 30% in a batch reaction. We have previously applied rational design principles to identify additives that improve the performance of cell-free protein expression in batch mode (1). However, in a batch reaction, eventually at least one component will become limiting for further synthesis, either due to scarcity or accumulation. We have now used several methods, including NMR, to monitor turnover of select metabolites during cell-free protein synthesis, in order to better understand the limitations of cell-free protein production. Based on these findings, we developed an enhanced protocol to achieve an average amino acid incorporation efficiency exceeding 50%. This is an important step towards a more general use of site-specifically modified amino acids required in the more advanced labelling strategies.

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335TH

Comprehensive Determination of Protein Tyrosine pKa Values Using Indirect 13C NMR Spectroscopy: an Application to Photoactive Yellow Protein

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The accurate determination of side chain charge states is essential for our understanding about protein function, stability, and mechanism. Additionally, accurate experimental data are a requirement for developing and testing electrostatic theories. In recent years, 2D and 3D heteronuclear NMR spectroscopy have become powerful tools to determine protonation state for individual titrable group, by utilizing the exquisite sensitivity of ^{13}C and ^{15}N nuclei to follow these protonation events. Methods are now available to study routinely the side chain pKa values of Arg, Lys, His, Asp, Glu, and the N- and C-termini of small globular proteins. Importantly, the large and specific response of the heteronuclear chemical shifts allows the unambiguous separation of charges developing within a side chain from those in its vicinity, and these approaches exceed the use of ^{1}H NMR to study electrostatic interactions in proteins.

However, an analogues method that would enable the comprehensive study of tyrosine (Tyr) (de)protonation in uniformly enriched proteins was still missing. In this present study, we have successfully identified the protonation behavior of all tyrosine residues in photoactive yellow protein (PYP). To achieve this goal, we developed several NMR pulse sequences that monitor different ^{13}C chemical shifts in the phenolic ring. For reasons discussed in this presentation, only one of these NMR experiments showed the necessary sensitivity and resolution to follow all tyrosine residues for PYP. Using this method, we can follow the effect of electrostatic interactions on the pKa constants of surface exposed Tyr residues, partially buried side chain and deeply buried residues in the hydrophobic core. Of special interest in the case of PYP, Tyr-42 donates a short hydrogen bond to the para-coumaric acid (pCA) chromophore. We find that this hydrogen bond is very persistent and can only be disrupted by unfolding of the protein, or hydrolysis of the thioester linkage to *p*CA at high pH. We also demonstrate that the pH-dependence of the PYP photocycle is not affected tby the Tyr-42 protonation state in the electronic ground state.

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336MO

Protein Folding Topography and Dynamics of Calmodulin via ^{19}F-NMR

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Calmodulin (CaM) is a ubiquitous calcium sensor protein which binds and activates a variety of enzymes involved in cell signaling pathways. In its calcium loaded state, CaM is extremely resistant to heat denaturation, with a melt temperature (T_m) of around 115 °C. In this study, *Xenopus laevis* CaM was prepared such that each of the eight phenylalanine residues was substituted with 3-fluoro phenylalanine. ^{19}F NMR studies then focused on defined topologies associated with the folding process at temperatures near the regime where the protein is completely folded. Near 70°C, near-UV circular dichroism and ^{1}H NMR-based measurements of protein diffusion rates reveal the onset of a stable, compact, near-native folding intermediate. ^{19}F NMR solvent isotope shifts reveal a gradual loss of water from the hydrophobic core with increasing temperature, until the point at which the near-native intermediate state is attained. At this point, water is observed to enter the hydrophobic core and stabilize the protein. Paramagnetic shifts from dissolved oxygen reveal an increase in oxygen accessibility with temperature until the near-native intermediate is reached, whereupon oxygen solubility decreases. Taken together, we conclude that hydrophobicity of the protein interior increases with temperature, until the near-native state is established, where water cooperatively enters and stabilizes the hydrophobic core. ^{19}F CPMG experiments provide a measure of the interconversion between the folded state and the near-native hydrated intermediate; at higher temperatures, folding rates are on the order of 10,000 Hz. Moreover, as temperature is lowered, folding rates increase, presumably because the effect of off-pathway misfolding events on the exchange process is diminished.

PREVIEW

337TU

Structural Studies on CPEB1/4

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The Cytoplasmic Polyadenylation Element Binding (CPEB) proteins are RNA binding proteins, which have been implicated in cell cycle control, cellular senescence and in the formation of memory through synaptic plasticity.

The CPEB family is constituted of four members. Independently of the single CPEB protein, isoform or organism, all proteins of the CPEB family are characterized by the presence of two RNA Recognition Motifs (RRM domains) located in the carboxy terminal region and followed by a highly conserved zinc finger domain. The amino terminal portion of the protein features no obvious structural motif, however, it is the site for protein-protein interactions and specific phosphorylation, key to the regulation of CPE containing mRNAs. The Cytoplasmic Polyadenylation Element (CPE) present in the 3' UTR of the majority of target mRNAs is the main binding platform for the CPEB family of proteins. Within that family CPEB2, 3 and 4 form one group due to their high sequence conservation, whereas CPEB1 features two insertions in the first RRM domain. In order to investigate the impact of the differences between the two sub-groups within the CPEB-family, we have chosen one representative for each: CPEB 1 and CPEB4. The focus of the work is to obtain structural information of the RNA binding regions of CPEB1 and CPEB4 applying solution NMR and X-Ray Crystallography.

In order to gain insight into the interaction- mechanisms of CPEB4 with its binding platform CPE in the mRNAs, we expressed the RRM domains of CPEB4 single and as a pair. Various multidimensional NMR experiments have been acquired and we are currently analysing the spectra in order to map the binding site in the RRM domains. Using 15N-HSQC – based titration experiments, interactions between the RRM1-RRM2 tandem of CPEB4 and a RNA template containing a CPE (consensus sequence: UUUUUAU) have been detected. Additionally, EMSA assays have also shown binding of CPEB4 RRM1 and the RRM1-RRM2 tandem with the CPE-containing RNA.

Crystallization trials were set up with unbound CPEB4 RRM1-RRM2 pair as well as in the complex with RNA. For both samples crystals were obtained which are currently being optimized.

338WE

Molecular Recognition of Cell Surface Glycosaminoglycans by Eosinophil Cationic Protein. Structural Clues for its Cytotoxic Activity.

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Eosinophil Cationic Protein is a cytotoxic RNase present in large amounts in the eosinophil granules. During inflammation, it is secreted and plays a role in host defence with bactericidal, antiviral, and antiparasitic activities. Cationic and aromatic residues have been reported to be essential for its interaction with membranes[1] and glycosaminoglycans (GAGs)[2], suggesting that recognition of heparan sulphates exposed at the mammalian cellular surfaces may drive and modulate its cytotoxicity[3]. To get deeper insight into the cytotoxic process we have explored the interactions of ECP with a GAG mimetic. By using NMR spectroscopy and MD simulations we have determined the 3D structure of ECP in complex with a representative trisaccharide heparin-derivative. We have also estimated its binding affinity (μM range).

The complex structure reveals that the carbohydrate binds at the catalytic site and that the charged sulphate and carboxylate groups are clustered with well-defined orientations. Charged and polar residues and the conserved W10 of ECP are involved in the recognition. The pyranose ring of IdoA adopts the skew-boat $^{2}S_{0}$ conformation as observed in other complexes[4]. This recognition event may constitute the first step of the ECP's cytotoxic mechanism of action by facilitating contacts with the membrane that would then trigger membrane destabilization and cell death.

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339TH

Identifying Functionally Important Residues of ARKADIA E3 Ubiquitin Ligase Ring Domain in E2 Recruitement and E3-E2 Interaction

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E3 ubiquitin ligases play a key role in the proteolytic degradation of proteins through the Ubiquitin-Proteasome pathway [Hershko A & Ciechanover A, Annu Rev Biochem 1998, 67, 425]. ARKADIA is the first example of an E3 ligase that positively regulates TGF–β family signaling through its C-terminal RING finger domain [Episkopou V et al. PLoS Biol 2007, 5, e67].

The ARKADIA RING finger, was cloned and expressed in its zinc-loaded form and studied through multi-nuclear and multi-dimensional NMR Spectroscopy [Kandias NG et al. BBRC 2009, 378, 498]. The 3D NMR solution structure of ARKADIA RING finger was determined and deposited in PDB (2KIZ). NMR-driven titration studies were also performed to probe the interaction interface of ARKADIA RING and the partner E2 (UbcH5B) enzyme and the RING-E2 complex was constructed through an NMR-driven docking protocol (Chasapis CT et al. Proteins 2012).

Additionally, this study resulted to the identification of ARKADIA RING functionally important residues, such as the conserved, in many RING domains, Trp972. Trp972 is considered as one of the key residues for E2 recognition and binding [Huang A, et al. J Mol Biol 2009, 385, 507]. According to recent experimental evidence, the mutation of the Trp972 to Arg abolishes the ability of Arkadia to amplify TGF-b-Smad2/3 signaling responses in tissue culture transcription assays [Episkopou V, et al. Cancer Res. 2011, 71, 6438] suggesting that this residue is essential in the ubiquitin ligase enzymatic activity, consistent with the E2 recruitment. Various ARKADIA Trp mutants were prepared and are studied through NMR spectroscopy in order to obtain an atomic-level insight about the structural base of ARKADIA RING capability to select and bind the appropriate E2 in order to exhibit its ubiquitin ligase activity.

340MO

NMR Investigation of a Novel Pex5-Pex14 Interaction in Peroxisomal Import

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Protein import into peroxisomes is a multi-step process which depends on a complex and dynamic network of protein protein interactions. It involves the recognition of peroxisomal targeting signals (PTS) by import receptors, docking of the receptor-cargo complex at the peroxisomal membrane, translocation of the cargo across the membrane and release of the cargo and receptor. Pex5 is a cycling import receptor which binds PTS1 containing matrix proteins at its C-terminus and interacts with the proteins of the docking complex at the N terminus. An important step in the peroxisomal import pathway is the interaction of the import receptor Pex5 with Pex14, a central component of the docking complex. We have previously reported the NMR structure of a conserved domain of human Pex14 bound to a Pex5 WxxxF/Y motif peptide. Here we have identified a novel Pex14 binding motif in the N-terminus of Pex5 and determined the NMR structure of this novel Pex14-Pex5 complex. The structure is based on distance restraints derived from inter-molecular NOEs and PRE restraints. Our structural data is complemented with functional studies which show the importance of the novel Pex5-Pex14 interaction in peroxisomal import.

341TU

Aprataxin: A Recovery Enzyme for Single Strand Break Repair

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Genetic defects in the repair of DNA damage are implicated in a number of diseases, which include neurological dysfunction and cancer. Single strand breaks (SSBs) are the most frequent type of damage [1] (>10.000 per cell and day). Inefficient repair of SSBs in postmitotic cells such as neurons may lead to neurodegenerative diseases, e.g. ataxia oculomotor apraxia (AOA1). AOA1 is linked to mutations in aprataxin (APTX), which functions in concert with PARP-1, XRCC1 and PNKP during SSB repair by removing obstructive adenylate 5'-ends [2]. APTX comprises two structural domains: an N-terminal XRCC1–binding FHA domain and a C-terminal catalytically active HIT domain including a zincfinger. We established recombinant expression, purification for both domains and the in vitro production of the natural substrate (rAppDNA). The HIT domain alone is sufficient for multi-turnover deadenylation catalysis, indicating that the structure of this domain will be of functional significance. We will present the current status of the ongoing solution state NMR-based structure determination, which includes also non-conventional labelling strategies.

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342WE

The C-Terminal Domain of the Archeal MCM Protein Adopts a 'Truncated' Winged-Helix Fold

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The minichromosome maintenance complex (MCM) plays a central role in the replication of DNA in eukaryotes and archaea. The MCM complex serves as helicase and forms a hexameric ring-shaped complex on the DNA to be replicated. The MCM in eukarya consists of six closely related, non-identical proteins whereas the archeal MCM constitutes a homohexameric assembly. The high sequence homology between the MCM of both kingdoms suggests that they are derived from a common ancestor. This renders the archeal MCM a valuable model to study the structure-function relationship of this essential component of the replication machinery. Even though considerable structural information on the archeal MCM is available through X-ray [1] and cryo-electronmicroscopy [2] studies [3], such information was missing on their very C-terminal domain. Here we present the solution structures of the C-terminal MCM domains derived from *M. thermoautotrophicus* and *S. solfataricus*. The structures were solved in a semi-automated fashion from heteronuclear NMR spectroscopy data using the UNIO environment. These domains adopt a winged-helix fold preceeded by a less ordered N-terminal extension. However, their C-terminal Wing 1 element is shortened and the Wing 2 element appears missing. A detailed comparison with structures of other winged-helix domains will be presented.

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343TH

Identification of the Previously Unknown Actin Binding Site of Heart Disease-Related Protein MS1

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MS1 is a 375 amino acid cytoskeletal protein (Arai et al., 2002). The upregulation of MS1 in response to increased blood pressure has been implicated in the development of the heart disease, left ventricular hypertrophy (Mahadeva et al., 2002). It has previously been found to contain two actin binding sites (Fogl et al., 2011). Using a variety of biophysical techniques including NMR and circular dichroism spectroscopy, the first of the two actin binding domains was found to be unstructured (Fogl et al., 2011). However, the second actin binding site, the C-terminal domain of ms1, was shown to be structured, which lead to further investigation.

In this poster, we discuss the use of NMR-based structure-aided mutagenesis to identify the site of actin binding in the second, C-terminal, actin-binding domain. It was possible to combine the information gained from determining the 3D structure of the second actin-binding domain with data about the conservation of the amino acid sequence of this domain in order to perform rational mutagenesis experiments. The mutants were studied using NMR and actin co-sedimentation assays to judge if they were folded and if they bound actin. These experiments led to the identification of the actin-binding site of this domain.

344MO

Evaluation of Different Strategies to Discriminate Covalent and Dipolar Metal-Ligand Interactions in Trivalent Lanthanide and Actinide ^{n}PrBTP-complexes by NMR Investigations

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Partitioning and transmutation (P&T) is a strategy of reducing the long term radiotoxicity of spent nuclear fuel by separating the actinides (An) and converting them into shorter-lived or stable fission products. Due to the chemical similarity between lanthanides (Ln) and An, this is a demanding task. It can be performed by liquid-liquid extraction using selective N-donor extracting ligands. BTP-type (alkylated bis-triazinyle pyridine) ligands were found to have high separation factors (>100) for Am(III) over Eu(III). However, little is known about the molecular origin of their selectivity.

Previous studies have shown that BTP complexes of An(III) and Ln(III) are isostructural, allowing a comparative study of the different binding. Variation of the metal ion type will lead to changes in electron density distribution on the ligand, measured by NMR as the overall chemical shift.

Separation of the purely paramagnetic chemical shift into two parts, a dipolar part (pseudo-contact shift, PCS) and a part due to electron transfer via covalent bonds (Fermi-contact shift, FCS), can be achieved by several methods utilizing different structural and electronic parameters. Measuring the chemical shift over a range of temperatures, as suggested by Bleaney, does not rely on such structural and electronic parameters, yet the theoretical basis for this method remains doubtful. Reilley proposed a correlation of the chemical shifts for all lanthanide complexes to spin expectation values and Bleaney parameters. The latter imply structural properties of lanthanide ions. Unfortunately, Reilley's method is presently applicable only to lanthanide complexes since the respective values and parameters for actinide ions are unknown. Furthermore a large number of complexes must be evaluated, which is problematic especially for An. We have experimentally compared the two methods and report on evaluation of the resulting inaccuracies in application of the temperature-dependent method. This enables us to present conclusions for $Am(^{n}PrBTP)_3(NO_3)_3$, which has been studied for the first time with this method.

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345TU

NMR-Investigations on Paramagnetic and Anionic Effects in nPrBTP Complexes of Trivalent Lanthanides and Americium

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World-wide efforts are being made to optimize separation technologies for removing trivalent actinides (An) from the chemically similar lanthanides (Ln) in nuclear waste streams. This is a crucial step in the partitioning and transmutation (P&T) strategy for reducing the long term radiotoxicity of spent nuclear fuel. Separation of An from Ln in nitric acid solution can be performed by liquid-liquid extraction using highly selective N-donor ligands, e.g., alkylated bis-triazinyle pyridines (BTP) in organic solvents. However, the molecular reason for their selectivity is not yet known but has great importance for further improvements of extraction agents.

NMR experiments utilizing the paramagnetism of the such Ln and An-complexes are ideally suited to obtain an in-depth understanding of their differences,. Am(III) has a diamagnetic ground state that is separated from neighbouring paramagnetic excited states by more than 3100 cm^{-1}. This supports the notion that Am-complexes are diamagnetic. NMR investigations at KIT-INE on $Am(nPrBTP)_3^{3+}$ complexes, however, show that there is a small paramagnetic relaxation enhancement and a temperature dependent paramagnetic shift in ^{1}H, ^{13}C and ^{15}N spectra. Furthermore, strong chemical shifts on the coordinating nitrogen atom in the pyridine moiety indicate that the binding mode in the americium complexes is vastly different than in the isostructural lanthanide complexes.

Previous studies show that An- and Ln-BTP-complexes exhibit an application-relevant dependency of the separation factor on the anion. This leads to the expectation that there might be an associated interaction between the ligand and the counterion of a yet unknown nature. NMR-investigations on lanthanide complexes with coordinating nitrate ions, weakly coordinating chloride ions and non-coordinating triflate ions show that these only slightly affect the electron density distribution. In diamagnetic cases results correspond to theoretically predicted values and the change in chemical shift is caused by a change in ligand-metal bond-length. Investigations on $Am(nPrBTP)_3^{3+}$ complexes prove that there is no interaction between aromatic ϖ-systems and the anions.

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346WE

Sodium-Dependent Movement of Covalently Bound FMN Residue(s) in Na^+-Translocating NADH:Quinone Oxidoreductase

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Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) is a component of respiratory electron-transport chain of various bacteria, generating transmembrane electrochemical Na+ potential. The species producing the EPR signal in Na^+-NQR are flavin mononucleotide residues (FMN) bound to the NqrB and NqrC subunits (FMN_{NqrB} and FMN_{NqrC}, respectively) and [2Fe-2S] cluster. These species are the main candidates for the role of the intermediates of transmembrane electron transport.

We found that the change in Na^+ concentration in the reaction medium has no effect on the thermodynamic properties of prosthetic groups of Na^+-NQR from *Vibrio harveyi*, as was revealed by the anaerobic equilibrium redox titration of the enzyme's EPR spectra. On the other hand, the change in Na^+ concentration strongly alters the EPR spectral properties of the radical pair formed by the two FMN residues bound to the NqrB and NqrC subunits [1]. At the same time, no Na^+-dependent change in pulse ENDOR spectra of FMN radicals of Na^+-NQR is detected. Therefore, no substantial spin density redistribution within FMN radical occurs. In the presence of Na^+, the interspin distance between FMN_{NqrB} and FMN_{NqrC} of about 21 Å was determined from pulse X-band ELDOR [2]. The ELDOR trace change dramatically upon Na^+ removal, and dipolar modulation disappears. From the simulation of X- and Q-band EPR spectra the distance between FMN_{NqrB} and FMN_{NqrC} 15.5 Å in the absence of Na+ was estimated. Thus the distance between the covalently bound FMN residues can vary on more than 5 Å upon changes in Na^+ concentration. Using these results, we proposed a scheme of the sodium potential generation by Na^+-NQR based on the redox- and sodium-dependent conformational changes in the enzyme.

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347TH

Magnetic Susceptibility Investigations on Aqueous Actinide Cations

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The systematic determination of actinide paramagnetic behavior provides information about the number of unpaired electrons and their electronic states. In reprocessing schemes, these electrons are of great interest because being unpaired they are potentially more readily available to interact with donor atoms of complexing ligands or extractant molecules. Therefore, an increased understanding of actinide paramagnetism would greatly aid in understanding the processes at work in liquid-liquid solvent extraction systems.

Although the paramagnetism of actinide solids has been the subject of several investigations, work in the solution state is limited and dated, performed at the dawn of the actinide hypothesis. Only few magnetic studies of the light actinides in solution were performed under the auspices of the Manhattan District Project and published in the post-war period.
In this work, we investigated magnetic susceptibilities of all of the readily accessible actinide cations, from uranium to californium, in perchloric media by use of the NMR Evans' method. Significant deviations were observed from analogous lanthanides, and from previous solid and solution actinide studies. The influence on magnetic susceptibility of more complexing media, namely chloride and nitric media, were also studied in some cases.
To our knowledge, it is the first comprehensive examination of actinide ion susceptibility in solution using NMR.

348MO

Theory of EPR Lineshape in Samples Concentrated in Paramagnetic Spins: Effect of Enhanced Internal Magnetic Field on High-Field High-Frequency (HFHF) EPR Lineshape

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A theoretial treatment is provided for the calculation of EPR (electron paramagnetic resonance) lineshape as affected by interactions with of paramagnetic ions in the vicinity. The internal fields seen by the various paramagnetic ions due to interactions with paramagnetic ions in their vicinity, as well as the resulting lineshapes, become quite significant at high magnetic fields required in high-frequency (HFHF) EPR. The resulting EPR signals for the various ions are therefore characterized by different g-shifts and lineshapes, so that the overall EPR lineshape, which is an overlap of these, becomes distorted, or even split in HFHF EPR, from that observed at lower frequencies. The observed EPR lineshapes in $MnSO_4.H_2O$ powder and K_3CrO_8 single-crystal samples have been simulated here taking into account g-shifts and modified lineshapes. These simulations show that in these samples, concentrated in paramagnetic spins, the position and lineshapes of EPR signals are significantly modified in HFHF EPR involving very high magnetic fields.

349TU

THE INFLUENCE OF COATING ON THE RELAXIVITY OF SUPERPARAMAGNETIC NANOPARTICLES AS MRI CONTRAST AGENTS

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A series of iron oxide nanoparticles (USPIO NPs) with a constant superparamagnetic core coated with a silica shell with increasing thicknesses has been exploited to understand the influence of a coating on the relaxivity of superparamagnetic NPs and to validate the existing theoretical models. [1] The core-shell γ-Fe_2O_3@SiO_2 NPs have been synthesized by co-precipitation and stabilized by a Stöber process. The iron oxide core has a diameter of 9.6 nm and the silica shell thickness varies from 0.6 to 71 nm as confirmed by TEM pictures. [2] The NPs were studied by magnetometry and relaxometry. As expected for particles built up on the same magnetic core, the size and the saturation magnetization obtained by magnetometry remain almost constant. Their relaxometric behavior shows a strong decrease of the r1 and r2 relaxivities of their aqueous suspensions on increasing the coating thickness of the particles. All NMRD profiles have been fitted by the model developed by Roch et al. [1]. This model provides a good fitting of the NMRD curves for particles covered with the thinnest silica layers. Nevertheless, with the increase in the coating, the diamagnetic contribution of the silica cannot be neglected in the fitting of the NMRD profile and must be added to that of the magnetic particles alone. A linear relationship between the sizes measured by electron microscopy and those obtained by the fitting of the NMRD profiles shows that these are significantly lower than the former. Their magnetizations measured by relaxometry also decrease relative to the values values of M_{sat} obtained by magnetometry, which correspond to the core. However, this "magnetic dilution" is smaller than expected if the entire silica shell was water impermeable. Both results indicate that a significant part of the silica coating is permeable to water. [3] The adequate silica shell thickness may, thus, be tuned to allow for both, a sufficiently high response as MRI CA, and an adequate grafting of targeted biomolecules for molecular imaging. The NPs also do not show in vitro toxicity towards microglial cells [2].

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350WE

^{1}H RELAXATION IN SOLUTIONS OF NITROXIDE RADICALS - HYPERFINE COUPLING, ELECTRON SPIN RELAXATION AND ECCENTRICITY EFFECTS

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Nuclear spin relaxation in paramagnetic solutions is enhanced due to strong nuclear spin-electron spin dipole-dipole (DD) interactions; this is referred to as paramagnetic relaxation enhancement (PRE). As the PRE for solutions of nitroxide radicals is of inter-molecular origin, it is natural to attempt extracting information on the translational dynamics from the relaxation dispersion. Nevertheless, the nuclear relaxation dispersion is affected by hyperfine interactions between the electron and nitrogen spins and electron spin relaxation. In addition, one has to take into account that due to non-central positions of the interacting spins, the inter-molecular DD coupling is also influenced by rotational dynamics of the solvent and solute molecules (eccentricity effects).[1]

The outlined issues are illustrated by ^{1}H relaxation dispersion (10kHz – 20 MHz) studies for decalin and glycerol solutions of nitroxide radicals, 4-oxo-TEMPO-d_{16}-^{15}N and 4-oxo-TEMPO-d_{16}-^{14}N. The data are interpreted in terms of a theory which is valid for arbitrary magnetic field as it includes the hyperfine interactions. Special attention is given to the effect of isotope substitution (^{14}N/^{15}N). The role of the rotational dynamics is considered in parallel. The conditions are specified under which the influence of the hyperfine coupling and the electron spin relaxation on PRE in solutions of nitroxide radicals has to be taken into account. It is shown that these effects are of considerable importance (in the range of lower frequencies) even when the translational motion is fast. The role of the rotational dynamics is considerable not only in the range of higher frequencies (like for diamagnetic liquids),[2] but also at low and intermediate frequencies. It is also demonstrated that NMR relaxometry of paramagnetic solution has the potential to probe much faster dynamics than in the case of diamagnetic systems. These findings are of large relevance when dynamical processes are investigated by means of NMR relaxometry applied to solutions of nitroxide-labeled compounds.

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351TH

Broadband Solid-State MAS NMR Spectroscopy of Paramagnetic Lithium Transition Metal Phosphate Materials

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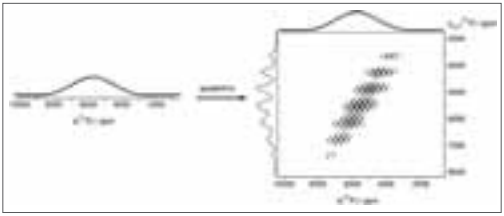
Lithium transition metal phosphate olivine materials are amongst the most promising candidates for a new generation of lithium-ion cathodes. However, a complete atomic-level description of the structure is extremely difficult to obtain by NMR due to the presence of the paramagnetic transition metal ions, which lead to broad overlapping spectra over 100s kHz, which are difficult to excite efficiently. We have developed a new experiment that enables NMR characterization of such complex paramagnetic materials, yielding complete separation of the individual isotropic chemical shifts. The new pulse sequence, which we refer to as 'adiaMATic', makes use of short high-powered adiabatic pulses (SHAPs), which can achieve 100% inversion over a range of isotropic shifts on the order of 1 MHz and with anisotropies >100 kHz. The methodology is demonstrated on a family of lithium metal phosphate compounds $LiFe_xMn_{1-x}PO4$ where the two transition metal ions result in several overlapping sideband patterns in the ^{31}P spectrum over a range of 4000 ppm. The adiaMATic spectra allow us to fully resolve dozens of different sites. From both hybrid functional DFT, and direct fitting of the peaks in the adiaMATic spectra we identify the individual contributions from each TM—O—P spin transfer pathway in the structure.



353TU

Paramagnetic Effects and Dynamics of Loop-Lanthanide-Binding-Tags in Interleukin-1β

Katja Barthelmes, Henry Jonker, Dominic Wagner, Harald Schwalbe

University of Frankfurt, Frankfurt am Main, Germany

Lanthanide-binding-tags (LBTs) are valuable tools for investigation of protein structure, function and dynamics. Their versatile use in NMR spectroscopy, X-ray crystallography and luminescence studies could be demonstrated for several proteins and LBT attachment strategies.[1-3] Our new strategy involves the incorporation of encodable LBTs into loop regions of proteins. We could previously demonstrate the feasibility of this approach using a small library of Interleukin-1β constructs (loop-IL1β), which were designed to bear LBTs of varying length at different loop positions. We were able to measure paramagnetic effects such as residual dipolar couplings (RDCs) or pseudo-contact shifts (PCSs).[4] However, the magnitude of these effects differs with respect to the choice of the loop for LBT- insertion. Intrinsic motions of the protein backbone reduce the size of the measurable parameters. Therefore, we here investigate the paramagnetic effects and dynamics of three different loop positions (denoted L2, R2 and S2). Considerable effects could be measured for Terbium or Thulium induced RDCs and PCSs as well as for Gadolinium induced paramagnetic relaxation enhancement (PRE). In addition, the calculated generalized order parameter S^2 from $\{^1H\}$-^{15}N HetNOE, ^{15}N longitudinal relaxation rates (R_1) and ^{15}N transversal relaxation rates (R_2) will provide insights into the loop-LBT dynamics.

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354WE

Protein - Ligand Interaction in an Artificial Metalloenzyme Monitored by Pseudo Contact Shift ^{19}F-NMR Spectroscopy

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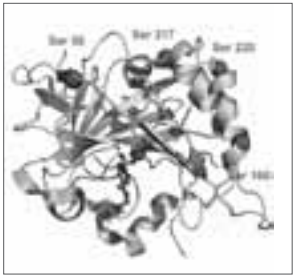

Artificial metalloenzymes based on catalytically active organometallic compounds bound to human carbonic anhydrase type II (hCA-II) have recently been shown to perform stereoselective hydrogenation reactions.[1] For a deeper understanding of the stereoselectivity we are interested in a structural characterisation of the catalytically active protein ligand complex.

As a model system, N-(2,3-difluorobenzyl)-4-sulfamoylbenzamide bound to hCA-II was investigated by PCS NMR spectroscopy. Therefore the thulium derivative of DOTA-M8[2] was site-specifically attached to the 30 kDa protein ligand complex. In order to obtain different labelling sites four different mutants of the 15N labelled protein were prepared by site-directed mutagenesis (S50C_C206S, S166C_C206C S217C_C206S and S220C_C206S). Pseudo contact shifts for the fluorine nuclei of the ligand were determined by simple one-dimensional ^{19}F-NMR experiments. The location of the fluorine atoms could precisely be resolved using only pcs data.

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Acknowledgements: We gratefully acknowledge T.R. Ward for his expertise on hCA-II and R.A. Byrd for providing us with a sample of tetramethylcyclen.

355TH

A Toolbox of Two Stereo-Isomeric DOTA-M8 Chelating Tags with Six Different Lanthanides – Twelve Magnetic Susceptibility Tensors to Choose From!

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Pseudocontact shifts (PCSs) offer a powerful long-range tool for the structural characterization of proteins and their complexes [1]. In order to generate large PCSs, rigid chelate – lanthanide complexes can site-specifically be attached to a cystein residue in the protein. We have synthesized and characterized twelve different lanthanide – DOTA M8 [2] complexes and determined their structure by NMR. The strong PCSs observed in the framework of the ligands of up to 1000 ppm in the ^{1}H NMR were used to calculate the corresponding magnetic susceptibility tensors. Depending on the nature of the ligand and the metal, a large variation of PCSs and tensors was obtained. For selected cases the tag was conjugated to a 30 kDa protein and the PCSs induced in the protein nuclei were compared with the magnetic susceptibility tensors obtained from the ligand alone.

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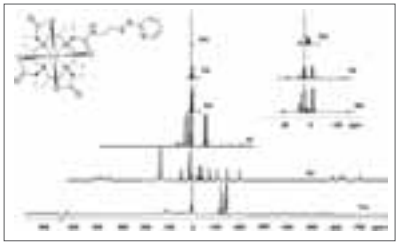

356MO

The Intracellular Conformation of α-synuclein Observed by In-Cell NMR Spectroscopy

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In-cell NMR spectroscopy is a powerful method to directly observe the structure and dynamics of proteins within their native cellular environment. Here we report the use of NMR to study the intracellular conformation of α–synuclein, a small protein strongly implicated in the pathogenesis of Parkinson's disease and dementia with Lewy bodies. We describe simple new spectroscopic methods to directly and rapidly verify the intracellular localisation of observed resonances, and to quantify the extent of leakage, and we report the development of deconvolution methods to reduce inhomogeneous line broadening within cellular samples, so allowing the accurate measurement of backbone chemical shifts within crowded in-cell NMR spectra. These were used to evaluate secondary structure populations within the cytosol, and we find no significant differences when compared to the protein in bulk solution, indicating that the protein remains in a disordered state within the cell.

357TU

Characterization of Intrinsic Disorder in Measles Virus Nucleocapsids in Situ

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[1]Institut de Biologie Structurale, Grenoble, France, [2]Unit for Virus Host Cell Interactions, Grenoble, France, [3]AFMB-CNRS, Marseille, France

The genome of measles virus is encapsidated by multiple copies of the nucleoprotein (N), forming helical nucleocapsids of molecular mass approaching 50 Megadalton. The intrinsically disordered C-terminal domain of N (Ntail) is essential for transcription and replication of the virus via an interaction with the phosphoprotein P of the viral polymerase complex. Here, we characterize the conformational behaviour of Ntail in its isolated and P bound forms using chemical shifts and residual dipolar couplings showing that the molecular recognition element (MoRE) of Ntail undergoes alpha-helical folding upon binding to P. The pre-recognition state of the Ntail interaction site samples a dynamic equilibrium of specific, N-capped helical conformers arguing for conformational selection in the complex formation.

We also report the first in situ structural characterization of Ntail in the context of entire N-RNA nucleocapsids. Using a combination of solution NMR spectroscopy, small angle scattering and electron microscopy, we demonstrate that Ntail is highly flexible in the intact nucleocapsids. We present a model for which the first 50 disordered amino acids of Ntail are conformationally restricted as the chain escapes from the inside to the outside of the nucleocapsid. We show that the MoRE of Ntail exchanges on and off the nucleocapsid surface providing a mechanism by which it can catch the viral polymerase complex and transfer it to the RNA binding site at the surface of the nucleocapsid.

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358WE

Conformational Plasticity of Alpha-Synuclein in Live Mammalian Cells

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Intracellular aggregates of the intrinsically disordered human protein alpha-synuclein (AS) are pathological hallmarks of Parkinson's disease (PD) (1). Until recently, most structural *in vitro* studies on AS have been performed on isolated protein samples, under conditions that differ substantially from the crowded *in vivo* environments of intact cells (2). Here, we present high-resolution in-cell NMR data on the structural and dynamic properties of AS in five different mammalian cell lines that also include dopaminergic neurons of the Substantia nigra. Employing a novel protocol for the efficient delivery of isotope labelled protein samples into live mammalian cells, we are able to produce in-cell NMR samples of highly reproducible quality that even permit residue-resolved relaxation measurements. By directly comparing these in-cell NMR results with AS data from different *in vitro* environments mimicking intracellular viscosity and macromolecular crowding, we are in the process of delineating physical and biological contributions to AS's different *in vivo* behaviours.

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359TH

Protein "Stickiness" Limits Feasibility for In-Cell NMR Spectroscopy

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The potential wealth of in situ knowledge attainable through in-cell NMR spectroscopy is compromised by a major limitation: many globular proteins do not yield in-cell spectra (*1*, *2*). When over-expressed in *E. coli*, ^{15}N-labelled ΔTat-GB1 cannot be observed through in-cell NMR spectroscopy while GB1 yields a high quality spectrum(*1*)(Fig. 1).

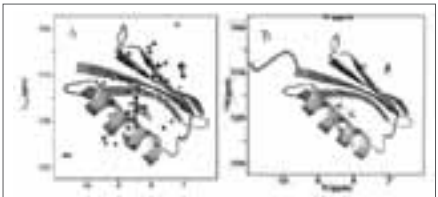

Fig. 1 Structures of (A) the biologically inert GB1 and (B) the sticky fusion protein ΔTat-GB1 with the resulting ^{15}N-^{1}H HSQC spectra superposed.

The rotational correlation time (RTC) of a protein is the most important determinant for its feasibility to in-cell NMR yet the contribution to this factor of macromolecular crowding, viscosity and macromolecular interactions is only beginning to emerge(*3*). We performed size exclusion chromatography (SEC) on cell lysates containing over-expressed GB1 and ΔTat-GB1 to investigate the extent of their interactions in the *E. coli* cytosol(*1*). A distinct correlation was observed between protein interaction propensities and their viability for in-cell NMR. "Sticky" proteins elute from an SEC column with a high apparent molecular weight and cannot be studied by in-cell NMR. Biologically inert proteins qualify as model systems for in-cell studies and elute from a column with volumes related to the molecular weight of the pure protein. This suggests that (i) protein complexation is significant in its contribution to in-cell RTC's and (ii) the lack of an in-cell spectrum for a protein can still provide physicochemical clues.

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360MO

Structure Propensities of the Partially Unstructured bHLHZipper-Domain of v-Myc

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The C-terminal domain of the oncogenic transcription factor Myc is one of the most interesting fragments of the basic-helix-loop-helix-zipper (bHLHZip) protein family. A deregulation of Myc via either gene amplification or chromosomal translocation or several other mechanisms leads to many severe carcinosis like Burkitt lymphoma and multiple myeloma. In fact, the oncogene myc is a highly potent transforming gene and capable to transform various cell lines. Its oncogenic activity initialized by deregulated expression leads to a shift of the equilibrium in the Myc/Max/Mad network towards Myc/Max complexes. The Myc/Max heterodimerization is the prerequisite of the transcriptional functionality of Myc.

Primarily, we are focusing on the apo-state of the C-terminal domain of v-Myc, the retroviral homolog of human c-Myc. With multi-dimensional NMR neither a fully structured nor an unstructured characteristic of v-Myc could be seen. The bHLHZip domain of v-Myc does not exist as a random coil but exhibits partially pre-structured α-helical regions in its apo-state.
We were able to show that even the flexibility of so-called natively, in this case partially, unstructured proteins still can bear a propensity to transiently form a preferred structure in its flexible “unstructured” regions. Just recently, paramagnetic resonance enhancement in addition to multi-dimensional NMR showed very interesting and exciting evidence concerning a propensity of Myc for a particular transient pre-structure involving its bHLH motif. Next to be analyzed is the question if this structure propensity can be initialized and stabilized with fragments of its main binding partner Max.

361TU

Spin Temperature in a Multi-Spin System: Simulations of Thermal Mixing DNP

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Weizmann Institute of Science, Rehovot, Israel

The interpretation of results from NMR experiments on large coupled spin systems requires in many cases some understanding of their quantum spin dynamics. For example, to describe the nuclear signal enhancement during the Dynamic Nuclear Polarization (DNP) experiments on solid samples, one can use the quantum mechanical spin density operator formalism including relaxation [1], or the thermodynamic model [2]. The quantum approach limits the size of the system that can be explored due to the exponential growth of the calculation size and time with the number of spins. The thermodynamic model assumes that the collective state of many coupled spins can be described using spin temperature/s, and describes the dynamics of the system as an equilibration process of these temperatures. Three main mechanisms have been used to describe the solid state DNP enhancement: The Solid-Effect (SE), the Cross-Effect (CE) and Thermal Mixing (TM). Quantum mechanics is used to explain the basics of the SE and CE mechanisms, and thermodynamics for TM.

In this presentation we consider solid state DNP and demonstrate the creation of spin temperatures, while simulating the behavior of a coupled spin system using the quantum description. This is done on a small spin systems containing nuclei coupled to unpaired electrons, using parameters which can give rise to the TM and SE mechanisms. This study provides some insight into the creation and equilibration of well defined spin temperatures that appear during the polarization of nuclei.

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362WE

New Hyperpolarized Glucose Derivatives for ^{13}C-Magnetic Resonance Imaging

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[1]Bracco Imaging SpA, Colleretto Giacosa (TO), Italy, [2]University of Torino, Torino, Italy

The extraordinary enhancement of the Nuclear Magnetic Resonance signal obtained from Para-Hydrogen Induced Polarization has been exploited in the investigation of hydrogenation mechanisms and, more recently, in the development of hyperpolarized contrast agents for Magnetic Resonance Imaging (MRI). In particular the high signal/noise ratio that can be achieved on heteronuclei such as ^{13}C or ^{15}N allows to obtain molecules that can be traced *in vivo*. In fact the complete absence of those signals in biological tissues leads to images in which the background signal derives uniquely from instrumental noise. Furthermore, due to long T_1 values that can be reached on these nuclei, hyperpolarization can be maintained for a time long enough to allow the acquisition of images in *in vivo* conditions.

We present here the synthesis and parahydrogenation experiments of a series of novel substrates, with the aim of obtaining an in-depth understanding of the potential of these species as ^{13}C hyperpolarized contrast agents. Special attention is focused on bio-compatible, water soluble parahydrogenated products. In particular, we have synthesized and tested for para-hydrogenation a set of molecules in which glucose is bound to an hydrogenable synthon (butynoic acid), in order to select suitable candidates for an in vivo MRI method focused on the assessment of glucose cellular uptake.

363TH

Parahydrogen-Induced Polarization in Gas-Phase Hydrogenations over Immobilized Ir Complexes

Vladimir Zhivonitko, Ivan Skovpin, Igor Koptyug

International Tomography Center SB RAS, Novosibirsk, Russia

The NMR sensitivity problem is especially crucial in studies involving gases because of much lower spin concentration as compared to liquids. Heterogeneous hydrogenations using parahydrogen provide an efficient approach of producing hyperpolarized gases for the sensitivity boosting. This technique can be utilized as an alternative to optical pumping of noble gases like ^{129}Xe, which applications scope grows exceedingly nowadays. Moreover, recent research activities showed that under particular experimental conditions the sensitivity boost given by parahydrogen-induced polarization (PHIP) provided even greater S/N ratios than that in ^{129}Xe experiments.1 In addition, experimental implementation of the gas-phase PHIP experiments is relatively easy, which was utilized successfully in ^{1}H NMR imaging experiments,[2,3] including those with the use of remote-detection NMR.[1]

On the other hand, an efficient catalyst is required for the production of hyperpolarized gases using PHIP. Immobilized Rh catalysts were involved in the previous studies.[1-3] In this work, we investigate several silica immobilized Ir complexes in terms of production of hyperpolarized propane and propene gases in gas-phase hydrogenations of propene and propyne, respectively. Both ALTADENA and PASADENA PHIP experiment types were performed. It was shown that propyne hydrogenation over the immobilized Ir complexes provide a two orders of magnitude signal enhancement in ^{1}H NMR spectra, whilst propene hydrogenation provide much lower enhancements. In addition, ^{31}P solid-state NMR was used for the catalyst characterization and investigation of the structure of catalytic sites.

This work was supported by grants SB RAS (##57, 60, 61 and 122), 11.G34.31.0045, RFBR (11-03-93995-CSIC_a and 11-03-00248-a), program of support of leading scientific schools NSh-2429.2012.3.

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364MO

Online Monitoring of Intelligent Polymers Using Hyperpolarized Xenon

Stefan Gloeggler[1], Markus Raue[1], Johannes Colell[1], Pierre Tuerschmann[1], Bernhard Bluemich[1], Stephan Appelt[2]

[1]RWTH Aachen University, Aachen, Germany, [2]Research Center Juelich, Juelich, Germany

Poly(N-isopropylacrylamide) (PNiPAAm) [1,2] can be crosslinked with N,N'-methylene-bis-acrylamide (MBAm) to form a hydrogel, that can absorb large amounts of liquid compared to its own weight. An interesting property of the crosslinked PNiPAAm is that it undergoes a lower critical solution temperature (LCST) phase transition at 32°C in water, meaning that previously absorbed solvent is released due to a collapse of the hydrogel network. Because of this property, crosslinked PNiPAAm has attracted particular interest in nano science as a drug delivery system [3].

We will demonstrate the possibility of differentiating between PNiPAAm hydrogels with different concentrations of crosslinker and different swelling degrees in single scan experiments using hyperpolarized xenon and a newly developed low field NMR system that operates at 14 mT (166 kHz ^{129}Xe-frequency). An outstanding observation can be made if solvent is added to the hydrogel: The subsequent absorption of solvent can be monitored on line by changes in chemical shift in the xenon spectrum.

This development shows great potential for investigating and monitoring hydrogel nanoparticles or hydrogel coated nanoparticles that are used as drug delivery systems and to non-invasively obtain information about its state, for example, if the hydrogels are loaded with drug molecules.

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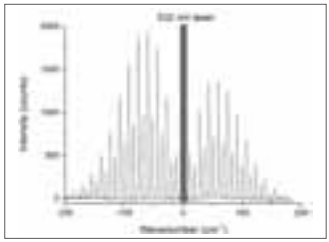
365TU

In situ Raman Spectroscopy to Determine N_2 Gas Temperatures in Spin-Exchange Optical Pumping Cells for Use in Hyperpolarised Noble Gas NMR/MRI

Hayley Newton[1], Nicholas Whiting[1], Michael J. Barlow[1], Peter Morris[1], Laura Walkup[2], Boyd M. Goodson[2]

[1]Sir Peter Mansfield Magnetic Resonance Centre; University of Nottingham, Nottingham, UK, [2]Department of Chemistry & Biochemistry; Southern Illinois University, Carbondale, IL, USA

Hyperpolarised (HP) ^{129}Xe is capable of being used for diagnostic lung MRI, due to high image contrast in the gas phase and spectroscopic information in dissolved phase studies. Spin-exchange optical pumping (SEOP) is typically used to produce HP gases; the process involves transferring angular momentum from resonant circularly polarised photons to the electronic spins of an alkali metal vapour and subsequently the nuclei of the noble gas. Nitrogen is often loaded into the OP cell to both pressure broaden the alkali metal absorption line and to collisionally de-excite the alkali metal atoms. Previous studies (*Walter, et. al., Phys. Rev. Lett. 86; 2001*) using *in situ* Raman spectroscopy to directly measure N_2 rovibrational temperatures (T_{N2}) during SEOP found that T_{N2} could be elevated by hundreds of degrees compared to the cell surface when using only ~15 W of light from broadband laser diode arrays. These studies also used ^{4}He as the dominant buffer gas species, which has a relatively high thermal conductivity to dissipate heat to the cell walls. In this work, we attain Raman signals of N_2 gas during SEOP using frequency-narrowed lasers with an order of magnitude higher power. We exclude 4He from our gas mixtures and have greater N_2 partial pressures than have previously been characterised. *In situ* Raman spectroscopy was collected using an orthogonal excitation/detection method, with a 532 nm excitation laser and a two-stage Raman spectrometer for detection; the resulting spectra can be analysed to determine T_{N2} with a spatial resolution of <1 mm^3. We will show the temperature of the gas mixes under various conditions: Xe and N_2 partial pressures, cell surface temperature, laser power, and spectral offset, with corresponding in situ low field NMR signals and transmitted laser spectra to show how these values vary with optical pumping conditions. Measurement of the OP cell surface temperature is often a poor indicator of T_{N2}—necessitating the *in situ* Raman measurements explored here. This will allow SEOP apparatuses to be more fully characterised, leading to advances in HP ^{129}Xe production.



366WE

SABRE: A New Hyperpolarisation Technique to Enhance NMR Sensitivity

Lyrelle Lloyd[1], Ryan E. Mewis[1], Simon B. Duckett[1], Gary G. R. Green[2], Mike A. Bernstein[3], Steven Coombes[3], Richard Lewis[3], Chris J. Sleigh[3], Joost A. B. Lohman[4], David Kilgour[4]

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Nuclear Magnetic Resonance (NMR) is an inherently insensitive technique because the level of interaction between a proton and the magnetic field is low. This results in a small population distribution across the energy levels it probes resulting in only 1 in every 32,000 protons being observed at room temperature in a 9.4 T field. We have employed parahydrogen which exists solely in the αβ-βα spin configuration, to artificially increase this population difference.

Our new SABRE (Signal Amplification By Reversible Exchange) technique has proven that this non-Boltzmann distribution of spin states can be imposed on a substrate through a metal dihydride catalyst of the form $[Ir(NHC)(substrate)_3(H)_2]^+$, (where NHC = *N*-heterocyclic carbene), without incorporation of parahydrogen into the substrate itself. Here, both parahydrogen and the substrate reversibly exchange with the metal centre, leading to the generation of hyperpolarised free substrate which is ready for detection. To date, work carried out by the our group exploiting this technique has shown it to yield significant enhancements with ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P nuclei.

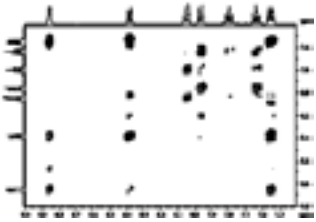

Figure 1. An OPSYdq-COSY 2D NMR spectrum of quinoline where the initial polarisation transfer step was undertaken at 50 G.

The development of a new apparatus has provided the means to interrogate these materials using 2D NMR sequences. To date, we have succeeded in rapidly obtaining COSY, HMBC and HMQC data at low concentration. When the OPSY coherence filtering sequence is introduced into the COSY sequence, as demonstrated in **Figure 1**, such measurements can be completed in protio solvents.

This poster will illustrate how SABRE can be used to characterise a range of molecules by NMR spectroscopy at low concentration.

367TH

Moving High Sensitivity MRI Towards Clinical Applications

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Parahydrogen has been used to sensitise NMR and MRI measurements by hydrogenation for a number of years [1]. Recently, it has been successfully used to hyperpolarise substrates without changing their chemical composition via a process called SABRE (Signal Amplification By Reversible Exchange). This process can be catalysed by an iridium metal complex, **figure 1**.

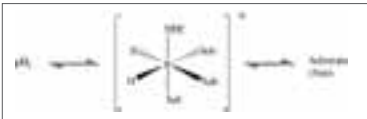

Figure 1: Schematic representation of the SABRE method using an iridium catalyst and substrate when parahydrogen is added.

Before SABRE can be used to make in-vivo measurements a number of challenges must be overcome to make this chemical process suitable for clinical applications. This poster illustrates progress towards this objective via;

(i) The successful immobilisation of the catalyst.
(ii) The optimisation of the hyperpolarisation level and measurements at physiological conditions.
(iii) The demonstration that the 1H enhanced signal can be recorded in an ex-vivo experiment.
(iv) The production of a long lived signal through the polarisation of pyrazine in the presence of heart tissue.

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368MO

Method of Quantifying the Relative Enhancements Originating from the Solid Effect and the Cross Effect for Solid State Dynamic Nuclear Polarization

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Proton and carbon-13 Dynamic Nuclear Polarization (DNP) experiments were conducted on static samples on a 3.4T homebuilt hybrid pulsed-EPR-NMR spectrometer. During these DNP experiments NMR signals are enhanced with the help of microwave (MW) irradiation on or close to the EPR spectrum of the stable free radicals in the sample, transferring polarization from the free electrons to the nuclei. In the solid state a distinction is made between three DNP enhancement mechanisms: The Solid Effect (SE), the Cross Effect (CE) and Thermal Mixing (TM).

In an effort to describe the development of the nuclear polarization in samples, where the SE-DNP and the CE-DNP processes are simultaneously responsible for the enhancement, we present here an analytical method to distinguish between the two processes. Individual SE-DNP and CE-DNP spectral lineshapes are generated by using theoretical simulations on a small electron-nuclear spin system combined with the EPR lineshape of the radical in the sample. Comparing these DNP lineshapes with the experimental DNP spectra it is possible to quantify the relative enhancements originating from the SE and the CE mechanisms of different samples.

In this poster we explain the method of analysis and its restrictions. Applications are shown in the case of 1H-DNP and 13C-DNP experiments on samples containing stable radicals or biradicals such as TEMPOL, trityl and TOTAPOL in the temperature range 6 – 50 K.

369TU

Comparison of Liquid State DNP Enhancements of Solvents at High Magnetic Field (9.4 T)

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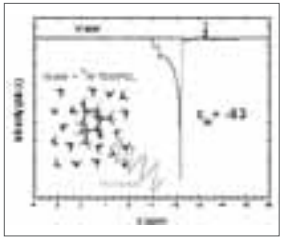

Improving sensitivity is a key issue in NMR spectroscopy. One way of tackling this issue is hyperpolarization by Dynamic Nuclear Polarization (DNP). In DNP, hyperpolarization of nuclei is achieved by microwave irradiation of the unpaired electron spin of radicals transferring their larger Boltzmann polarization to the nuclei. It has been shown, that by this method also high enhancements can be obtained at high magnetic fields in the solid state[1,2] and, more recently, in the liquid state.[3] Here, we present DNP experiments performed at a magnetic field of 9.4 T (corresponding to 260 GHz EPR and 400 MHz proton NMR frequency)[4] on four different solvents: water, DMSO, acetone and toluene, using ^{14}N-TEMPOL as polarizing agent. We observed ^{1}H-NMR enhancements as high as -80, -20, -30 and -25 for the different solvents, respectively. The liquid state Overhauser enhancement is defined as a product of leakage, saturation and coupling factor and the ratio of electron and nuclear gyromagnetic ratio[5]. All these factors can be independently determined by NMR relaxation measurements, the paramagnetic NMR line shift as a function of MW excitation power and NMR dispersion measurements[6] and will be compared with the experimental achieved DNP enhancements.

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370WE

New Developments for Using Hyperpolarized ^{129}Xe and SPIONs as Contrast Agents: 1) "Scaling Up" High-[Xe] SEOP for an 'Open-Source' Clinical-Scale Xe Polarizer; 2) Towards Physiological pH-Sensing with Dendron-Functionalized SPIONs

Panayiotis Nikolaou[1], Aaron Coffey[1], Laura Walkup[2], Brogan Gust[2], Nicholas Whiting[3], Hayley Newton3, Iga Muradyan[4], Mikayel Dabaghyan[4], Kaili Ranta[2], Gregory Moroz[5], Matthew Rosen[6], Samuel Patz[4], Michael J. Barlow[3], Eduard Chekmenev[2], Shaoyi Xu[2], Ayse Yilmaz[2], Ping He[2], Max Gemeinhardt[2], Yong Gao[2], Boyd M. Goodson[2]

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Our groups have been working separately on the development of hyperpolarized 129Xe and dendron-functionalized SPIONs (superparamagnetic iron oxide nanoparticles) as MRI contrast agents, respectively involving: (1) the fundamental study of spin-exchange optical pumping (SEOP) and development of an 'open-source' clinical-scale xenon polarizer; and (2) the synthesis and characterization of a novel class of dendron-functionalized SPIONs capable of sensing pH variations in physiologically relevant regimes.

In support of (1), here we present preliminary results from our first-generation "open-source" xenon polarizer, dubbed "XENA" (XEnon pumpiNg Automated)—recently installed at Harvard. XENA was created by "scaling up" SEOP results obtained from fundamental studies of SEOP under conditions of high-[Xe] and resonant laser fluxes—and ongoing fundamental studies will be summarized. Unlike other polarizers, XENA runs on xenon-rich gas mixtures and is capable of operation in either single-batch or stopped-flow modes and subsequent transfer to Tedlar bags without having to freeze or accumulate the Xe over time; additionally, the simplified, open-source design makes for ready implementation in other laboratories. In-cell PXe values of >70% at >700 torr (in a 500 cc cell) have been observed; apparent T1's in Tedlar bags at high and low fields of ~5 h and ~38 min, respectively, have been measured.

In support of (2), we are interested in how SPIONs may be designed with novel surface functionalities to facilitate the creation of MR contrast agents capable of sensing changes in local environments. Local reductions (or variations) in tissue pH have been associated with several pathological conditions including many cancers. Recently we have synthesized a new class of dendron-SPIONs with surface functionalities (comprising of branch-shaped molecules called dendrons) designed to provide peak pH sensitivity in the range of pH~6-8. We are also studying the MR responses of such SPIONs with improved mathematical models. Improved understanding of how surface functionalization determines pH sensitivity should allow for tuning of the SPION surface chemistry to exhibit highly sensitive MR responses to pH variation in physiologically relevant regimes and generate a new class of SPIONs capable of MR mapping of pH in vivo.

371TH

Improved Polarizing Agents and Sample Preparation for DNP Surface Enhanced NMR Spectroscopy

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Our research group has recently demonstrated that dynamic nuclear polarization (DNP) enhanced solid-state NMR spectroscopy is a sensitive tool to probe the surface of a variety of materials – such as functionalized (and mesostructured) silica, alumina or metal-organic frameworks.[1-4] In this approach, the material is impregnated by a radical-containing solution, then the sample is spun at the magic-angle at 100 K under microwave irradiation.

Here we present some of our recent findings concerning the influence of the nature of the polarizing mixture (both the solvent[5] and the radical) and of the type of functionalization of the material on the DNP enhancements (ε_H). Biradicals have been demonstrated to be the most efficient polarizing agents at high magnetic fields. Their efficiency as a polarizing agent depends on many parameters, such as the relative orientation of the electron g tensors or the strength of the dipolar coupling. However, in addition to these well known parameters, we demonstrate that the electron longitudinal relaxation time (T_{1e}) is a key factor for obtaining high ε. A new biradical that possesses longer electron relaxation times (T_{1e} and T_{2e}) and yields high enhancements at 9.4 T (ε_H ~100) is presented.[6] We will also demonstrate that the nuclear relaxation properties and spin density of the material also strongly influence ε. For instance, when dealing with metal-functionalized materials, it is necessary to passivate the surface with inert functional groups. The effect of the surface concentration and NMR relaxation properties of these groups on the DNP enhancements will be discussed, casting light on the mechanism of polarization transfer at the surface.

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373TU

Dissolution-DNP at 6.7 T: Unprecedented Polarization P(^{13}C) > 75% in 20 Min

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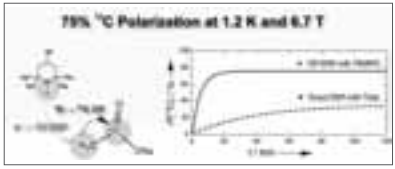
The polarization of ^{13}C nuclei can be enhanced by 'dissolution-DNP'.[1] Typically, ^{13}C nuclei are polarized directly to P(13C) = 35 % by saturating the ESR transitions of Trityl radicals at 94 GHz at T = 1.2 K and B0 = 3.35 T. An alternative approach [2] consists in polarizing protons followed by cross polarization from ^{1}H to ^{13}C at low temperature, preferably using widely available nitroxide radicals such as TEMPO, which can lead to P(^{1}H→^{13}C) = 25 % in one-fiftieth of the time. A further substantial improvement can be achieved with TEMPO by doubling the microwave frequency to 188 GHz and the magnetic field to B0 = 6.7 T. Direct ^{13}C polarization leads to P(^{13}C) = 45% and P(^{1}H) = 95%, the latter building up with a characteristic time constant τDNP(^{1}H) < 10 min. The dominant mechanism is thermal mixing. Cross polarization leads to P(^{1}H→^{13}C) > 75%, 130'000 times more than the Boltzmann polarization at T = 300 K and B0 = 6.7 T. Some technical challenges of high frequency DNP and low temperature cross polarization will be discussed.



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374WE

Quantitative Metabolic Imaging Methods for Hyperpolarised 13C

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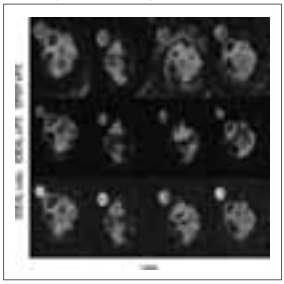
Introduction: Metabolic imaging with hyperpolarised [1-^{13}C]pyruvate enables the unique detection of cellular metabolism minimally invasive. Images of pyruvate and its downstream metabolites are typically given in arbitrary units due effects impairing absolute quantification such as liquid-state polarisation, T1 relaxation, perfusion of the region-of-interest, dilution and RF coil sensitivities. In this work we outline three different approaches to a more quantitative metabolic imaging and compare these different methods.

Methods: Four rats bearing subcutaneous MAT BIII tumours were imaged with two different acquisition methods: (1) IDEAL Spiral CSI; (2) saturation-recovery with spectral-spatial excitation and spiral imaging. Both acquisitions were reconstructed to time-resolved metabolic images of pyruvate, lactate and alanine by gridding reconstruction and additionally for (1) by chemical-shift modelling to the present chemical shifts. The IDEAL Spiral CSI data was quantified by two methods: (A) turnover mapping from pyruvate to lactate based on frequency domain kinetic two-side exchange modelling; (B) ratio of the summed lactate to pyruvate data. The relaxation rate can be neglected in the saturation-recovery approach and therefore the metabolic turnover maps can be extracted by the ratio of lactate to pyruvate images scaled with flip angle and metabolite repetition time. Using the formalism of frequency domain modelling, we generalised this approach to take into consideration the whole set of time-resolved metabolic data, leading to a ratio of summed lactate over pyruvate ratio.



Results: It is possible with both acquisition methods to generate reliable turnover maps, yielding quantitatively similar turnover rates (figure on the left: top=k_{PX} with saturation-recovery, middle =k_{PX} with IDEAL, bottom=ratio with IDEAL). The turnover is highly elevated in the tumour region as compared to the surrounding tissue (guts and muscle). The contrast is comparable to the summed ratio, however with the advantage of being more quantitative with units in sec^{-1}.

375TH

LANTHANIDES AS LOW TEMPERATURE RELAXATION SWITCHES

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We report a series of longitudinal relaxation studies designed to evaluate the effectiveness of certain lanthanide metal ions as relaxation switches. The aim of the work is to enable the 'brute force' polarisation of material containing nuclear spin species such as ^{13}C. This technique has been hindered by the extremely long relaxation times found at very high ratios of magnetic field to temperature. It is also desirable to have reasonably long survival time for the hyperpolarisation on returning to room temperature. We believe that lanthanides such as dysprosium and holmium may provide a practical solution to the problem because at room temperature they are regarded as shift reagents with a very short correlation time, whereas at low temperatures, the correlation time becomes comparable to the Larmor period which leads to a reduction in T1. We performed a series of proton T1 measurements on samples containing lanthanides including dysprosium and holmium. Typically 1 mM solutions of the lanthanide chloride or DTPA chelate were made in a water/ glycerol solvent. The proton T1 times were measured at 4-200 K at 2T and below 4 K at a range of fields. The T1 relaxation rates are linearly dependent on the lanthanide concentration, as expected. As shown in Fig.1, the T1 values exhibit a minimum at around 20K allowing correlation times to be found. Interestingly we find a significant dependence on the pH value of the solution, indicating that a low pH is advantageous. Below 4 K we are able to achieve absolute proton polarisations of about 2% but we find that T1 varies approximately as $(B/T)^n$ where n~3-4 and therefore tends to become very long below 0.5 K for fields of a few tesla and above. The high proton polarisation can be transferred to other spin species such as ^{13}C and ^{31}P by thermal mixing (*Gadian DG, Panesar KS, Perez Linde AJ, Horsewill AJ, Köckenberger W, and Owers-Bradley JR (2012). Phys. Chem. Chem. Phys. 14: 5397-5402*).

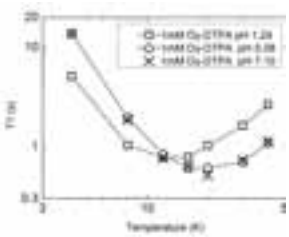

Fig. 1. [1]H T1 vs T. Lines are guides.

376MO

DISSOLUTION DNP AND QUADRUPOLAR NUCLEI: POLARIZATION OF NITROGEN-14

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In solution DNP experiments, enhanced nuclear polarization is generated at temperatures close to 1 K in a solid sample containing a stable radical (electron source) acting as polarizing agent and the molecule of interest in a glassing solvent. When the nuclear polarization has built up, the sample is rapidly dissolved and shuttled to a conventional NMR instrument for observation [1]. Dissolution DNP has been applied in recent years to a variety of nuclei, however much of the research has been focused on spin-1/2. As far as our knowledge goes, the only quadrupolar nucleus studied by dissolution DNP is 6Li [2]. This nucleus has a very small quadrupolar moment (-0.0808 fm2) and it behaves like a spin-1/2 nuclei in the context of DNP. Therefore, it is clear that further efforts have to be done towards the study of quadrupolar nuclei by DNP which are a majority in the periodic table. In the current work we present, for the first time, the study by direct hyperpolarization of 14N compounds (tetramethylammonium chloride (TMA) and choline) by dissolution DNP, showing the feasibility of DNP studies in nuclei with strong quadrupolar moment (2.044 fm2) and low gyromagnetic ratio[3].

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377TU

Novel Polarizing Agents for High-Field Dynamic Nuclear Polarization

Björn Corzilius[1], Albert A. Smith[1], Olesya Haze[2], Matthew K. Kiesewetter[2], Claudio Luchinat[3], Ivano Bertini[3], Timothy M. Swager[2], Robert G. Griffin[1]

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In various biochemical applications dynamic nuclear polarization (DNP) has been used to enhance nuclear polarization in MAS NMR by factors of up to ~200. This signal enhancement allows NMR experiments which would not be feasible otherwise. To date, biological DNP applications require electron spins to be added to the sample in the form of exogenous radicals. This electron spins provide a large spin polarization subsequently transferred to nuclei. The development of improved organic mono- and biradicals for DNP is of crucial importance. Furthermore, endogenous electron spins in the form of transition metals (e.g., Mn(II)-centers) in biomolecules (*e.g.*, metalloproteins) and materials open the possibility of site-specific DNP in a native environment, therefore minimizing impact on sample structure (*e.g.*, protein folding). The investigation of this site-specific polarization could yield important in¬formation for structure determination.

We introduce two organic polarizing agents that are soluble in glycerol/water for cross-effect (CE) and solid-effect (SE). A stable and highly water-soluble derivative of BDPA exceeds enhancements obtained with trityl radical (OX063) and retains the typical but unique electron spin properties of its precursor (i.e., narrow EPR linewidth and long relaxation times). Recently, we have developed a water-soluble, rigid biradical for CE DNP NMR which has outperformed TOTAPOL. We discuss the impact of structure, solubility and electronic coupling on the DNP performance.

High-spin metal and rare earth (Mn(II) and Gd(III)) complexes will also be discussed as novel sources of polarization for SE DNP. Besides yielding significant ^{1}H enhancements, the narrow EPR linewidth of these complexes also allows efficient direct polarization of ^{13}C by the SE which is not feasible with conventional polarizing agents. We will show direct 13C enhancement factors of $\varepsilon > 100$ observed with Gd-DOTA in fully protonated samples. Additionally, by effectively increasing the microwave field strength in a microwave resonator, ^{1}H enhancements of $\varepsilon > 100$ are observed under static NMR conditions at 80 K, and > 150 at 20°K, with sensitivity increases up to 240-fold.

378WE

Optimization of Decay Times for DNP-Enhanced Multidimensional Solid-State NMR: Respecting Spin-Lock and Spin-Echo Periods

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Thanks to instrumental and theoretical development carried out at MIT [1] and notably the access to high-power / high-frequency microwave sources, high-field Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) currently appears as a promising solution to enhance nuclear magnetization in many different types of systems.

In high-field MAS-DNP experiments, systems of interest are usually dissolved, impregnated or suspended in glassy matrices doped with polarizing agents (TEMPO, TOTAPOL, etc.) and measured at low temperature (down to ~100 K). The polarizing agent concentration (typically 1 to tens of mM) is adjusted in order to maximize the DNP enhancement factor measured with and without microwave irradiation.

Nevertheless, many challenges still need to be addressed in order to fully benefit from the 1D enhancement factor of nuclear polarization (typically measured using ^{13}C or ^{29}Si CPMAS experiments). In this work, we discuss the influence of electron concentration and sample temperature on DNP polarization transfer efficiency, nuclear T_{1n} and electron T1e spin-lattice relaxation times, apparent versus refocused transverse decay times [2], spin-locking decay times, etc. All the DNP/SSNMR measurements performed at ~10 Tesla are complemented by high field EPR measurements performed at the same magnetic field.

We notably highlight that, contrary to apparent line-widths, refocused transverse decay times and spin-locking decay times are strongly dependent on sample temperature and electron concentration. This is of major importance for optimal sensitivity enhancement of multidimensional experiments that employ refocused evolution (Refocused Inadequate, SAR-COSY, etc.) and/or spin-locking blocks. Applications of these studies will be demonstrated on different samples ranging from small molecules to larger sytems, including a 20 kDa protein (YajG) and a bacterial cell wall.

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379TH

CHARACTERIZATION OF A NEW HIGH PRESSURE HYPERPOLARIZATION GENERATOR: TECHNICAL SETUP AND APPLICATIONS

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Hyper-polarized (HP) ^{129}Xe NMR methods are uniquely suited to study porosity, surface conditions and hiereachial order of particulate, mesoscopically structured materials. Interfacial interactions and processes near the surface make the elucidation of the specific properties of such materials crucial for the development of functionalized materials. Recent advances in surface enhancement techniques using HP ^{129}Xe have inspired us to construct a new hyper-polarizer at the University of Bayreuth to help transform solid-state NMR spectroscopy, which usually is a bulk method, into a surface sensitive technique [1,2]. Unlike most other high pressure systems, our apparatus employs a 20cm long pre-saturation cell separate from the optical pumping region, allowing for external control of the Rb density. The vertical arrangement and unique design of the pumping cell ensures full adsorption of the laser light. Our cell is novel in that it possesses two optical faces with two laser beams centered and aligned along each cell body (120W total available power). The oven is completely closed, resulting in 5°C temperature difference between the inner/outer surfaces of the glass body. The apparatus allows for a variety of gas delivery techniques: direct expansion (i.e. batch mode) and flow-through mode (recirculation through the sample region, and controlled flow-through MFCs to MAS without recirculation), and is portable.

Here, we will discuss the performace quality of the new apparatus and demonstrate its utility to investigate the inter-connectivity of co-existing adsoprtion sites within MIL-53 using ^{129}Xe 2D EXSY continuous flow NMR. MIL-53 (Al) is metal organic framework tethered by di-carboxylate linkers, forming diamond shaped channels in the absence of guest molecules. The "breathing" phenomenon observed in this framework refers to a reversible structural transition wherein the open, large pore (LP) conformation collapses into a more narrow structure (NP) with gas adsorption—the NP form in turn reopens to the LP structure with increased pressure [4, 5]. Results indicate intimate connectivity between the LP and NP structures and sheds light on the collapsing mechanism.

380MO

HOW TO SIMULTANEOUSLY ESTIMATE T_1 AND THE SAMPLING FLIP ANGLE FOR HYPERPOLARIZED SPINS

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To monitor the magnetization decay of hyperpolarized nuclear spins and estimate T_1, a sequence of read pulses of small flip α is applied. With the exception of the initial inversion-pulse (not needed for hyperpolarized spins), the pulse sequence is similar to that of Look and Locker [1]. Each radio-frequency pulse removes part of the magnetization thus increasing the rate of return of the magnetization to equilibrium. This apparent T_1 denoted T_1* can be expressed as:

$$\frac{1}{T_1^*} = \frac{1}{T_1} - \frac{\ln[\cos(\alpha)]}{\tau}$$

where τ is the delay between the magnetization-sampling pulses.

When running hyperpolarization experiments, it is not always possible to optimally adjust all parameter values such as the flip angle α; instead an approximate estimate is made.

Estimating both T_1 and α using linear sampling of the magnetization does not remove the correlation between both parameters and only T_1*can be uniquely estimated. Thus we propose the use of spectral acquisition based on a geometric sequence of times. This non-uniform sampling of the magnetization removes the correlation between T_1 and α and allows the simultaneous estimation of each parameter value.

We experimentally demonstrated this approach by using hyperpolarized ^{13}C-urea. While the linear sampling scheme gave an estimate of T_1* = 37.0 s, the non-uniform sampling gave T_1 = 44.2 s and α = 4.1o. This was in agreement with both T_1 and the pulse angle estimates obtained from the same sample after the loss of the hyperpolarised state.

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381TU

Application of Nuclear Singlet Order in Hyperpolarised Metabolic NMR

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Hyperpolarised NMR is a valuable technique for grading tumours and detecting treatment response through real-time imaging of bio-chemical reactions in vivo. Currently, however, the range of detectable reactions is limited by fast T_1-dependent decay of the nuclear hyperpolarisation. Use of coupled spin-1/2 nuclei may improve this situation, since these may decay more slowly than T_1 when the polarisation is trapped in the nuclear singlet (spin-0) state [1-3]. Singlet order escapes many of the processes that result in signal loss, thereby preserving spin order over longer timescales, and potentially allowing detection of slower metabolic processes. Singlet order may also preserve hyperpolarization during transport to sites of interest in vivo, such as tumours, or allow more handling time of the sample before injection.

In this presentation, we report that ^{13}C singlet order of the metabolite [1,2- C_2] pyruvate is longer-lived than the longitudinal T1, at low field, when dissolved in human blood. We show also that singlet order also survives metabolic reactions in vivo, as long as the chemical bond between the two carbons is preserved. Using 1D-MRS in a mouse model, we have observed the reaction product singlet-[1,2- C_2] lactate. Potential applications are discussed.

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382WE

Preparation of Highly Polarised Nuclear Spin Systems Using Brute-Force and Low-Field Thermal Mixing

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Several strategies have been developed for generating highly polarised nuclear spin systems, including dynamic nuclear polarisation, optical pumping, and methods exploiting parahydrogen. We report here on investigations using an alternative strategy based on a 'brute-force' approach. The central notion of 'brute-force' NMR is that as the temperature is reduced and the field is increased, the equilibrium nuclear polarisation will increase, according to the Boltzmann distribution. The main problem is that it may take an extremely long time for the nuclear polarisation to approach thermal equilibrium at low temperatures and high fields, since nuclear relaxation becomes very slow. Cross-polarisation techniques can alleviate this problem.

Experiments were carried out using (i) a rapid field-cycling spectrometer that operates at temperatures down to 4.2K and delivers magnetic field switches of 10 Ts^{-1}, and (ii) a millikelvin spectrometer that operates at any chosen field up to 15T, and at temperatures as low as 10mK. Field cycling on this system can be carried out at rates of 1T min^{-1}. Experiments were carried out on [1-^{13}C] sodium acetate, either in the form of a powder, or dissolved in a 50/50 water/glycerol solution. In some experiments, sodium phosphate was also added to the water/glycerol solution.

We show that low-field thermal mixing can be used to transfer polarisation from the relatively rapidly relaxing 1H reservoir to more slowly relaxing ^{13}C and ^{31}P nuclei. The effects are particularly dramatic for the 31P nuclei, which at 2T and 4.2K showed a 75-fold enhancement in their effective rate of approach to equilibrium, and an even greater (150-fold) enhancement in the presence of a relaxation agent. The mixing step is also very effective in terms of the amount of polarisation transferred – 70-90% of the maximum theoretical value in the experiments reported here. These findings (1) have important implications for brute-force polarisation. We also show that the ^1H reservoir can be tapped repeatedly through a number of consecutive thermal mixing steps; this could provide additional sensitivity enhancement in solid-state NMR.

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383TH

ACHIEVING HIGH SPATIAL RESOLUTION AND HIGH SNR IN LOW-FIELD MRI OF HYPERPOLARISED GASES WITH SLOW LOW ANGLE SHOT IMAGING

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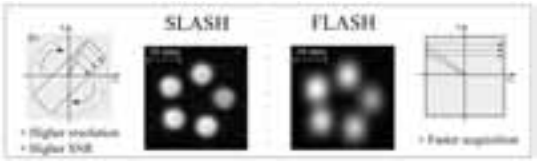
MRI of hyperpolarised gases is usually performed with fast data acquisition to achieve high spatial resolutions despite rapid diffusion-induced signal attenuation. We describe a k-space sampling scheme suitable for slow low angle shot (SLASH) acquisition and yielding an increased SNR. It consists of a series of anisotropic partial acquisitions with a reduced resolution in the read direction, which alleviates signal attenuation and still provides a high isotropic resolution. The advantages of SLASH imaging over conventional FLASH imaging are evaluated analytically, using numerical lattice calculations, and experimentally in phantoms cells filled with hyperpolarised ^3He-N_2 gas mixtures. Low-field MRI is performed (here at 2.7 mT), a necessary condition to obtain long enough T_2^* values in lungs for slow acquisition. The SLASH scheme is less sensitive to the artefacts caused by concomitant gradients. Finally, with a double-echo acquisition scheme, SLASH allows measuring maps of apparent diffusion coefficients for an extended range of time and length scales.



Double-cross and Cartesian k-space sampling patterns used for slow (left) and fast (right) low-angle shot data acquisition, and examples of corresponding axial projection images of a phantom cell filled with hyperpolarised gas. In-plane resolution, SNR, and total imaging time are 1x1mm², 60, and 1.5s for SLASH (1.8x2.5mm², 75, and 0.12s for FLASH).

384MO

NEW MULTI-DIMENSIONAL SINGLE-SHOT NMR STRATEGIES FOR DISSOLUTION DNP

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While dissolution-DNP is capable of routinely delivering a sensitivity enhancement of approximately 4 orders of magnitude, there are still several challenges associated with exploiting this enhancement. Perhaps the foremost challenge faced in dissolution-DNP is to maximize the amount of information obtained in a single scan, as this is all that can be provided by the meta-stable hyperpolarized state. Here we present a methodology for acquiring mult-dimensional data in a single scan, and show how it can be generally applied to achieve higher dimensionality in other situations.

The technique presented here termed HyerSPASM (Donovan et. al.) borrows from two previous developments termed SWAT (Hurd et. al.) and SPEED (Kupce et. al.). The *HyperSPASM* sequence is shown in figure 1. We will show how the *HyperSPASM* methodology allows one to introduce an extra dimension by means of calculated chemical shift offsets. This methodology is not restricted to 2 dimensions and can be applied in a conventional 2D experiment to calculate chemical shifts in a third dimension. Furthermore this methodology can be combined with Ultra-Fast spatial encoding, thereby giving a 3 dimensional correlation experiment in a single scan that is accessible to dissolution DNP.

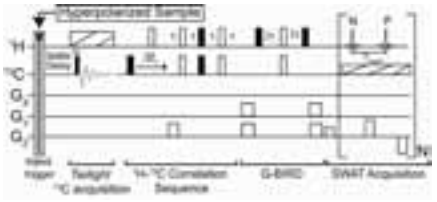

Figure 1. HyperSPASM pulse sequence. Immediately following injection a carbon-13 spectrum is acquired using a low flip-angle pulse. Following carbon-13 acquisition single quantum ^1H-^{13}C coherence is created that has evolved for a time Δt_1 and been encoded by a gradient. A G-BIRD pulse sequence element is used to suppress any carbon-12 bonded magnetization, and then data is acquired using alternating gradients to reverse the sign of the coherence order between each data point.

385TU

Dynamic Nuclear Polarization With Quadrupolar Nuclei: New Effects

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We have explored direct dynamic nuclear polarization of the most naturally abundant isotope of nitrogen, 14N in quasi spherically symmetrical environments to minimize the effect of quadrupolar relaxation.

In contrast to the well know behaviour of spin ½ nuclei studied so far, we found that polarization can be effectively transferred irradiating at four different microwave frequencies (two at either side of the allowed EPR transition), using trityl radicals as the source of unpaired electrons. Spectra were measured at room temperature after dissolution and transfer of the sample to a standard NMR instrument

Complex polarization build up curves were observed for each irradiation frequency.

The polarization profiles of matched 15N samples show the standard behaviour, showing that the observed effects are related to the presence of the quadrupolar isotope.

Under optimal polarization conditions natural abundance choline could be easily detected in a single scan

Similarly, multiple excitation frequencies as well as non exponential build up curves could be measured directly in a frozen 13C enriched sample of tetramethyl ammonium nitrate, with natural abundance nitrogen.

Our measurement show the feasibility of observing natural abundance choline, instead of its 15N labelled form. Choline is a well know cancer marker.

In addition, our data strongly suggest new DNP phenomena that had not been previously observed. Their possible origin and implications will be discussed.

386WE

Evaluation of a Shuttle DNP Spectrometer by Calculating the Coupling and Global Enhancement Factors of L-Tryptophan

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A liquid state shuttle dynamic nuclear polarization (DNP) spectrometer is presented, featuring several technical modifications that increase stability and improve reproducibility. For the protons of L-tryptophan, the signal enhancement and the DNP spin properties, such as relaxation, were measured and compared with each other. The calculated coupling factors suggest that the proton accessibility for the polarizer molecule has an important influence on the DNP enhancement. In general, short proton spin longitudinal relaxation times without radical reduce the detectable enhancement by decreasing the leakage factor and increasing the relaxation losses during the course of the sample transfer. The usage of a global enhancement factor gives a more complete overview of the capabilities for the described experimental setup. Global enhancements of up to 4.2 for L-tryptophan protons are found compared to pure Boltzmann enhancements of up to 2.4.

387TH

Quantification of Sensitivity Enhancements from Surface Enhanced DNP Solid-state NMR and Applications to Metal Organic Frameworks

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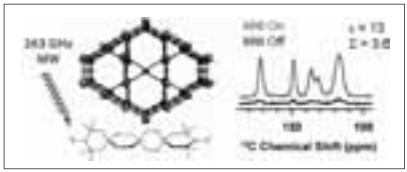
Dynamic nuclear polarization (DNP) has attracted much interest as a method to increase the sensitivity of solid-state NMR experiments.[1] Recently our research group has described the application of DNP enhanced solid-state NMR spectroscopy for the characterization of the surface of inorganic mesoporous and nano-particulate materials.[2,3] This approach yields ^{1}H DNP enhancements (εH) on the order of 20 to 40, however the sensitivity enhancement available from this approach was not been quantified. Here, we investigate the *sensitivity enhancements* provided by low temperature (~ 100 K) DNP solid-state NMR experiments, as compared to standard room temperature experiments, for mesoporous silica materials.4 The integrated intensity of DNP enhanced ^{29}Si CP/MAS NMR spectra were measured as a function of biradical concentration. It was observed that paramagnetic effects induced by the biradical (i.e., PRE) reduce the magnitude of the surface NMR signals by a factor of 30-70% (depending upon radical concentration), and that sample preparations which provide the largest ε do not necessarily provide the largest absolute sensitivity enhancements (Σ). We find that absolute sensitivity enhancements are on the order of 10 to 40 (including thermal Boltzmann and relaxation effects). We also demonstrate the utility of DNP for the characterization of metal organic framework (MOF) materials.5 DNP enables the rapid acquisition of 1D ^{13}C and ^{15}N CP/MAS spectra and 2D ^{1}H-^{13}C HETCOR spectra at natural isotopic abundances.



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388MO

Liquid State DNP Probes with Fabry-Perot Resonator at 9.2 Tesla

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Recent achieved DNP enhancements in liquids at high magnetic fields [1-4] have initiated strong interest in possible applications of this method to biomolecular research. However, due to the excessive sample heating by the microwave electrical field component in liquid solutions, only very small sample volumes have been used so far, dictated by dimensions of fundamental mode microwave resonators. For instance, the helical double resonance structure, used for our first demonstrations of the applicability of Overhauser DNP to aqueous solutions at 9.2 T [5], restricted the aqueous sample to a volume of only 3 nl. Together with a poor spectral resolution and low RF filling factor this resulted in small overall signal amplitude, hampering sensitive observation of biomolecules. Here we present new double resonance structures for liquid-state DNP by combination of a Fabry-Pérot resonator for the microwave excitation at 260 GHz and a stripline/coplanar line resonance structure for NMR detection at 400 MHz [6]. These new double resonance structures offer an increase in aqueous sample volume (>100 nl) with respect to the helical probe and exhibit improved NMR sensitivity and linewidth.

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389TU

Para-Hydrogen Induced Polarization of Small Peptides at High Magnetic Field

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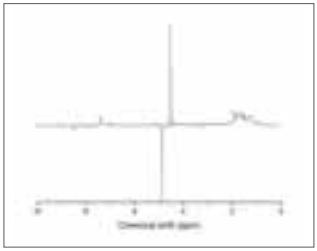
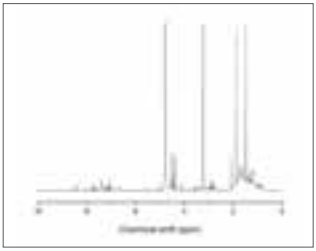
The issue of sensitivity in NMR experiments provides a continuous challenge and methods for signal enhancement are being developed. Here we are using the SABRE (Signal Amplification by Reversible-Exchange) method of para-hydrogen induced polarization (1) which consists in hyperpolarizing a substrate via scalar coupling in a transition metal complex (Crabtree catalyst) between the parahydrogen and the substrate. We have tested various small molecules as substrates. As an example, experiments were performed under PASADENA (Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment) (2) conditions on a Lys-Trp-Lys tripeptide in methanol solvent and at high magnetic field. Hyperpolarization was observed on the aromatic rings of tryptophane.





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390WE

Liquid State NMR Hyperpolarization Induced by the Haupt-Effect

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The inherently low sensitivity of NMR experiments is still a limiting factor in solving complex problems by NMR spectroscopy. Due to this fact there exist several methods to achieve an increase in signal intensity by hyperpolarization, i.e. to enlarge the difference in population far away from Boltzmann distribution at thermal equilibrium. One possible approach to achieve dynamic polarization was discovered by Haupt in 1972 [1]. He applied a sudden change in temperature from 8°K to 30°K and observed a 100-fold signal enhancement for the ^{1}H-resonances 4-methylpyridine (in the solid state). A fundamental condition for substances showing this so-called Haupt-effect is the presence of symmetrical molecular groups with a low rotational barrier, e.g. methyl groups, referred to as quantum rotors [2]. We could show that a modification of this approach can be used to obtain large ^{1}H and ^{13}C NMR hyperpolarization in the liquid state yielding an enhancement factor of 90 in the case of 4-methylpyridine. The proton coupled carbon signal of the methyl group yields a remarkable up and down multiplet pattern [3]. Using our approach, we have been able to observe a similar signal pattern for toluene, dibromomesitylene and several acetates of lithium, sodium or barium. The respective hyperpolarization factors correlate with the tunneling frequencies of the methyl group rotation.

Our aim is to identify more substances showing the Haupt-effect and to transfer magnetization from hyperpolarized spins to heteronuclei or other analytes dissolved in that matrix [4].

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391TH

Long-Lived States Originating from Para-Hydrogen Stored for Minutes in High Magnetic Fields

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The problem of low sensitivity in NMR can be overcome by hyperpolarization techniques. Yet, this approach is restricted to relatively short time scales depending on the nuclear T_1 relaxation times, in the range of seconds. This makes long-lived singlet states very useful in hyperpolarization studies, where Para Hydrogen Induced Polarization (PHIP) is particularly attractive because of the para-H_2 singlet symmetry. Most PHIP experiments, however, are performed on asymmetric molecules in order to directly convert the initial singlet state to an NMR observable triplet state. We demonstrate that in Cs-symmetric molecules a long-lived singlet state created by PHIP can be stored for several minutes on protons in high magnetic fields. Subsequently, it is converted into high non-thermal magnetization by controlled singlet-triplet conversion via level anti-crossing and observed.

392MO

DNP/ss-NMR Probe for Membrane Protein Study

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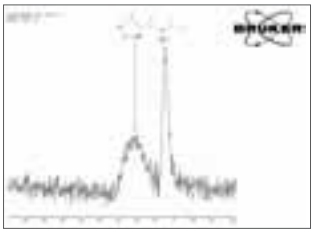

NMR is widely used for determining molecular structures with an angstrom precision. In this project we intend to use dynamic nuclear polarization DNP as a hyperpolarization method which can enhance the NMR signal by orders of magnitude [1]. This is due to the transfer of the large Boltzmann polarization of paramagnetic species to the nuclei of interest by microwave irradiation of the sample. It has been demonstrated that DNP/solid-state NMR is feasible also for oriented membranes [2]. This poster reports about our work in progress with the aim to build and use a static DNP/ NMR probe specially designed for the investigation of polypeptides reconstituted in oriented membranes at low temperature. It is intended to use ^{31}P ss-NMR spectroscopy to monitor phospholipid membrane alignment and ^{15}N ss-NMR spectra to determine polypeptide orientation in the membrane [3]. In this poster, we present technical challenges to overcome to reach optimal B1 field homogeneity in the active region of the NMR coil as well as work in progress on oriented samples. The 3D model of the NMR coil configuration of the probe is shown figure 1. The field distribution inside the coil has been simulated with the chosen configuration improving the ^{15}N channel sensitivity. In addition, initial one dimensional ^{15}N ss-NMR cross polarization spectra have been acquired at room temperature (model peptide and histidine). A first standard experiment has been realized on histidine at low temperature (i.e. 130 K, figure 2).

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393TU

Multiple Dipolar Echoes in Hyperpolarized ^{129}Xe and ^{3}He Solutions

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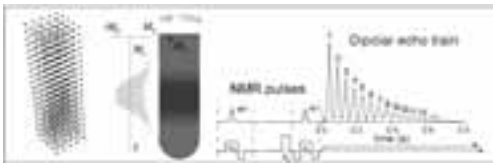
We report results of multiple dipolar echo measurements in hyperpolarized liquids using optically pumped spin-1/2 noble gas atoms: either ^{129}Xe dissolved in cyclohexane or ^{3}He dissolved in superfluid ^{4}He. Long echo trains have been obtained in the presence of applied gradients with a 90°-τ-90° NMR pulse sequence (using slice-selective flipping pulses for the ^{129}Xe experiments). A mean field description is valid for explaining the echoes observed in these liquids, even for spin temperatures as low as 10mK for ^{129}Xe or 10μK for ^{3}He. The echoes originate from the distant dipolar fields within the samples. Systematic numerical lattice simulations have been used to assess the effects of slice selection and of finite sample size in addition to those of atomic diffusion. They account for the observed echo widths and amplitudes much better than previously published models which disregard finite size effects although they appear to be of key importance. This opens the way to using multiple dipolar echoes for the determination of the absolute magnetization in hyperpolarized liquids without external signal calibration.

Numerical lattice simulations (left, with up to 2x10^{6} coupled magnetic moments) are used to compute the time evolution of magnetization maps in hyperpolarized liquids due to rf and gradient pulses and to distant dipolar couplings. With a 90°-τ-90° pulse sequence (center), multiple dipolar echoes are numerically obtained. They quantitatively match the experimentally observed echo trains (right).



394WE

Optimizing Cross Polarization for Dissolution Dynamic Nuclear Polarization

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In dissolution Dynamic Nuclear Polarization (DNP) experiments the build up of the polarization of low-γ spins is typically slow and in the order of minutes to hours. Therefore, methods to accelerate the polarization transfer while retaining a high steady-state polarization value are important since they can help speed up the experimental throughput significantly. This has led to the combination of DNP with cross-polarization (CP) [1]. At liquid-helium temperatures CP can be used most efficiently in systems undergoing thermal mixing where a common DNP-enhanced spin temperature is reached for all participating nuclei.

The knowledge of the underlying spin dynamics is beneficial. In this work, we apply a spin-thermodynamic model to the system of interest by describing the participating spin species ^{13}C and ^{1}H and the electron non-Zeeman (NZ) system as thermal baths. Numerical solution and optimization of the model parameters allows predictions of the system upon changes of specific parameters. In this manner, it correctly anticipates the increase of the final DNP enhancement upon sample deuteration by according reduction of the thermal capacity of the ^{1}H spin bath.

The combination of CP with dissolution DNP experiments is usually hampered by the restriction of the radio-frequency (rf) circuit due to non-ideal coil geometry and enhanced arcing in the helium atmosphere. We show an optimized CP sequence that includes the usage of adiabatic half-passage pulses as well as multiple contact times to circumvent the limitations. The sequence yields CP efficiency of up to a factor 3.5 and allows ^{13}C

polarization levels at 4.2K of >10% and >6% for 100 kHz and 20 kHz rf-field strengths, respectively, with a build up time of 18 s on protonated [1,4-^{13}C]fumarate in a fully deuterated solvent.

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395TH

Quantitative Analysis of High Field Liquid State DNP at 3.4 T

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We presented a double resonance structure for high field DNP at 95 GHz and 144 MHz. A miniaturized radiofrequency coil is integrated within a single-mode non-radiative dielectric resonator. With this probe it is possible to perform double resonance experiments on nanoliter samples in which enhancement factors of up to -160 are observed. By combining temperature dependent NMR relaxation, EPR, and DNP experiments, we have shown that the Overhauser model is still valid at 3.4 T [1].

Previously, NMR signals were obtained by using a single loop coil, in combination with a sample plug with a size matching the length of the microwave cavity. The sample was surrounded with a fluorized liquid. In this way, the enhanced NMR signal results solely from the microwave cavity and the microwave irradiation over the length of the sample is homogeneous. At the moment a μ-RF coil is used to obtain localised NMR detection. Two different types of μ-RF coils were tested. The first is a coil is sputtered on a 200 μm square capillary by using shadow mask lithography. The second is a solenoid coil made by winding a 100 μm copper wire around the sample capillary. Depending on the size of the coils, the sensitive volume is approximately 5-10 nl. Both coils have a higher sensitivity as compared to the single loop detection.

The in-situ NMR detection results in a number of improvements. Firstly, a quantitative enhancement is obtained since only the enhanced part of the sample is being measured. Secondly, the NMR resolution is improved significantly. Thirdly, the length of the sample is not limited to the size of the cavity which is advantageous for microfluidic sample handling, e.g.sample shuttling. A downside is the decreased quality factor of the microwave resonator, which limits the number of windings of the solenoid coil.

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396MO

Overhauser DNP at 3.4 Tesla with Frémy's salt

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Dynamic Nuclear Polarization (DNP) based on the Overhauser mechanism constitutes a valuable tool to enhance the sensitivity of Nuclear Magnetic Resonance (NMR) experiments in the liquid state and in recent years, detailed mechanistic studies have provided insight into the specific parameters needed to achieve optimal enhancements of the NMR signal. Particularly, in view of applications in high-resolution NMR, the field dependence of liquid state DNP is a major topic of current research. These measurements are usually aggravated by increasing heating effects at higher microwave frequencies.

In this study, using Frémy's salt dissolved in water as model system, NMR signal enhancement of the bulk water protons has been recorded as a function of the irradiation time and the polarizer concentration at 3.4 T. Maximum enhancements of -50 were achieved, as compared to -170 at 0.34 T, which illustrates the strong field dependence of the DNP effect. To compare our results at 3.4 T with recent reports in the literature, we have evaluated the kinetics of the DNP build-up which is given by the observable nuclear relaxation rate. The build-up rates are consistent with the T1n of the observed water protons at room temperature (for 9 GHz/0.34 T) and at about 50 ± 10 °C (for 94 GHz/3.4 T).

Moreover the use of Frémy's salt allowed the determination of the saturation factors at 94 GHz by PELDOR experiments. These studies confirm that the enhancements observed at 3.4 T are well consistent with the Overhauser mechanism and are subject only to moderate microwave heating, while higher enhancements can only be obtained at higher temperatures.

397TU

PROBING THE MAGNETIC STATES OF NICOTINAMIDE USING SIGNAL AMPLIFICATION BY REVERSIBLE EXCHANGE (SABRE)

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SABRE (Signal Amplification By Reversible Exchange) is a newly emerging methodology for transferring polarization from parahydrogen without its incorporation into the analyte. As a result of polarization transfer, the analyte is hyperpolarised, thus entailing an increased magnetic response which translates to increased signal intensity after the employment of 1D and 2D NMR methods.

Nicotinamide has been interrogated using 1D and 2D methods in conjunction with the use of a polarizer. Initially the effect on the ^{1}H polarisation levels due to the length of the parahydrogen bubbling time and the delay prior to and after transfer to a NMR spectrometer were probed. Optimised values were then used to investigate the magnetic states generated after polarization transfer at low magnetic field using the OPSY (Only *Para*hydrogen SpectroscopY) sequence. The application of different quantum filters enabled the magnetic states produced to be selectively observed, thus informing the observer of the different types of spin state present. Zero, single, double and triple quantum states were all identified. Modification of the OPSY sequence in which the $\varpi/2$ pulses were made selective enabled the magnetic states to be probed further in terms of each spin pair located around the aromatic ring system of nicotinamide (see Figure 1).

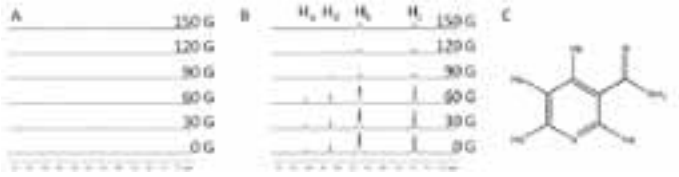

Figure 1- Application of a modified OPSY sequence in which the first $\varpi/2$ pulse is replaced by two simultaneously applied selective pulses after polarisation transfer at the field indicated. A) Protons H_a and H_d selectively pulsed; B) Protons H_c and H_b selectively pulsed; C) Chemical structure of Nicotinamide.

398WE

NOVEL PROBE FOR LIQUID STATE DNP AT 9.3 T

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Dynamic nuclear polarization (DNP) is an experimental technique where the high electron spin polarization is transferred to the nuclear spins by partial or full saturation of the ESR transition (e.g. [1-5]). DNP is an experimental technique that is particularly challenging when applied to aqueous samples due to the high absorption of electric fields at microwave frequencies. In this poster we present a novel DNP probe that allows running quantitative experiments on liquid samples and control the sample temperature. For a field of 9.3 T we present first experimental DNP data.

The essential part of our DNP probe is a temperature stabilized microfluidic chip that combines a high-Q semiconfocal Fabry-Perot microwave resonator with a planar multiply tuned NMR coil. Two different geometries of such a microfluidic chip were realized with the goal to (i) homogeneously pump the electrons in the entire sample volume, and (ii) to evaluate an optimized sample geometry to obtain a spectral resolution that is comparable to that typical in high resolution NMR.

In the presented experiments we put special emphasis on the quantification of DNP enhancements as function of temperature.

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399TH

TIME-SCALING OF XE DIFFUSION IN NANOTUBULAR STRUCTURES BY PULSED FIELD GRADIENT NMR IN COMBINATION WITH HYPERPOLARIZED XE-129 NMR

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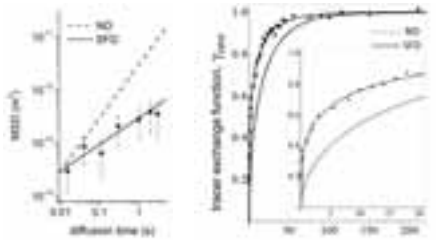
Single-file diffusion (SFD) occurs in 1D channels under conditions when sorbate molecules cannot pass one another. This fascinating phenomenon is not only of fundamental interest, it has implications for catalysis, separations, and nanofluidics. Despite the large number of computational/theoretical studies, only a few experimental reports of molecular SFD have appeared in the literature. One of the most interesting, yet unverified predictions of the simple random-walk model is a cross-over of the time scaling of the MSD from that of SFD to normal (Fickian) diffusion at sufficiently long observation times, depending on channel length and occupancy. Here, the results of Xe-129 NMR studies of diffusion in several types of self-assembled nanotubular materials, including dipeptides, gallium molecular wheels, and bis-urea macrocycles, will be presented. The channels formed from L-alanyl-L-valine (AV) have an internal diameter of 0.51 nm, which is just slightly greater than the 0.45 nm diameter of the Xe atom, thereby fulfilling the geometrical criterion for SFD. Xe-129 PFG NMR was performed using large (up to 30 T/m) gradients and high (17.6 T) magnetic field. Continuous-flow hyperpolarized Xe-129 selective saturation recovery experiments were also performed on the same AV sample under identical conditions. Both methods yielded distinct signatures of SFD. However, these results are incongruous to the simple random-walk model which predicts Fickian diffusion time-scaling at these observation times. Implications for the diffusion mechanism will be discussed.



A B

A. Time dependent mean-squared displacements of Xe in AV nanotubes probed by PFG NMR at 25 °C. B. Continuous-flow hyperpolarized Xe-NMR tracer exchange data along analytical modeling, with no fitted parameters, where the parameter T_1=36 s has been measured in a separate saturation-recovery experiment with thermally polarized Xe in long channels.

400MO

STRUCTURAL CHARACTERIZATION OF GUANINE RICH SEQUENCE FROM N-MYC ONCOGENE

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Guanine rich DNA and RNA can in the presence of cations form G-quadruplexes. There has been accumulating evidence that these four stranded structures comprising stacked G-quartets could play important roles in vital cell mechanisms such as regulation of transcription, translation and recombination. The apparent biological importance of G-quadruplexes supported by their stabilization being accompanied by altered gene regulation classifies these uncanonical structures as potential therapeutic targets. Furthermore, due to their structural variability and high temperature stability G-quadruplexes are considered as potential building blocks for nanodevices.

In our study we used multidimensional heteronuclear solution-state NMR in order to obtain insights into structural features of guanine rich DNA oligonucleotide originating from intron of N-myc oncogene. We showed that G-quadruplexes were formed in the presence of NH_4^+, K^+ and Na^+ ions. In K^+ ions containing solution unexpected equilibrium was observed between monomeric and dimeric G-quadruplexes, which we characterized in the terms of DNA oligonucleotide and K^+ ion concentrations. The monomeric and dimeric forms are an interesting example of G-quadruplex polymorphism providing new insights into the impact of sequence details of loop regions on G-quadruplex folding. We calculated NMR-based structures of both of the G-quadruplex forms in K^+ ions containing solution. The monomeric form represents a missing element in structures of parallel G-quadruplexes comprising three G-quartets as it exhibits three flexible single nucleotide loops. Furthermore, its structural properties and high temperature stability suggest possibility of specific biological role of G-quadruplex formation within intron of N-myc oncogene. The dimeric G-quadruplex formed in the presence of K^+ ions exhibits interesting structural feature of two single nucleotide links between central G-quartets, which enable consecutive stacking of six G-quartets in the core of the structure.

401TU

Programming the Self-Assembly of DNA G-Quadruplexes

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DNA is usually double stranded with Watson-Crick base-pairing, a major groove, and a minor groove. It can also fold into four-stranded architectures, with pseudo-planar base-pair alignments of four bases. These architectures are known as quadruplexes and have four grooves. A large number of topologies are accessible to these associations of four guanosine segments (1). This architectural diversity is of biological relevance (2). Quadruplexes are associated with the telomeres, gene regulation, and other biological functions. Four-stranded DNA has a few advantages over its double stranded cousin. It is mechanically more robust, has greater resistance to temperature denaturation and enzymatic degradation. However, quadruplex self-assembly is principally regulated by cation-dipole, solvophobic, and base stacking interactions, as well as base-composition of the DNA sequence including the availability of guanine-rich segments (1, 3). These and other variables make it difficult to programme the self-assembly of DNA G-quadruplexes. Thus far, precious little has been achieved regarding its programmability (1, 3, 4, 5).

With the aim of establishing principles for DNA G-quadruplex programmability we have been investigating structural determinants through design and control of self-assembly. In the course of this work we are determining solution structures of a few novel topologies, and we have developed methods for their rapid assessment in solution (4, 5). In this report we will demonstrate control of folding of a few new topologies. An understanding of this interplay is the basis for formalizing our understanding of principles for folding of these architectures in the biological context, and for the design of technological materials based on quadruplex DNA.

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402WE

Structure of a Bent DNA Studied by a Combination of NMR and EPR Spectroscopy

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Long-range distance constraints in the nm-range obtained from Pulsed Electron-Electron Double Resonance (PELDOR) spectroscopy[1] have been used to predict the global structure[2] and the conformational flexibility[3] of a DNA molecule with two double-stranded stems and a bulge in the center. To determine the angle and the orientation between the two stems a rigid cytidin-analogue nitroxide spin label (Ç) has been incorporated pairwise at different positions into the DNA molecule.[4] Orientation selective PELDOR experiments performed at X- and Q-band frequencies allowed determination of the mutual orientation and the distances between this two spin labels[5] The PELDOR data predicts a larger flexibility in the bulge region of the DNA compared to structures derived from NMR constraints[6] alone. Combining both, NMR and PELDOR constraints, permits us to obtain a more detailed picture of the conformational flexibility of the DNA molecule.

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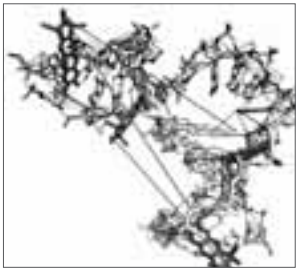

403TH

Structural Investigation of Inosine-Edited RNA Duplex and Its Interaction with RNA-Induced Silencing Complex Component p100

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The protein p100 (also known as SND1 or Tudor-SN), a component of the RNA-induced silencing complex, is involved in binding and cleaving inosine-edited RNA duplexes thereby excluding them from downstream events in RNA interference. This function is performed by p100's two tandem pairs of staphylococcal nuclease (SN) domains that were shown to be implicated in edited RNA binding and cleavage.

However many key aspects of this interaction remain unclear. What is the structural basis for the discrimination between edited and unedited RNA sequences? Does the p100 possess RNA binding capability alone or also have RNAse activity? If so, which structural aspects modulate this activity? To address these questions, we set out to solve the three-dimensional structure of the inosine-edited RNA substrate of p100 by NMR spectroscopy as well as study the structural changes that occur in the p100 protein upon RNA binding.

NMR studies of the inosine-edited RNA (12 kDa), show that the presence of inosine produces a destabilizing effect on the RNA duplex structure. Non-standard sugar puckers are observed for the residues in the inosine-containing motif, an indication of a deviation of this region from standard A-form geometry. In addition, the central inosine residue (I10) exhibits multiple conformations.

Multidimensional NMR experiments were used to conduct a backbone assignment of the p100 39 kDa construct containing SN domains 3 and 4 up to 85%. There is an indication that an interaction between these two domains takes place, which contributes to the overall dual domain fold. Protein constructs containing single residue mutations in the purported RNA binding site however, maintain the overall conformation present in the wild type p100.

To further investigate the p100/edited RNA duplex binding, this interaction will be studied by titrations, measurements of relaxation and residual dipolar couplings, as well as by electrophoretic mobility shift assays and isothermal titration calorimetry.

404MO

NMR Structural Studies on Covalent Pyrrolobenzodiazepine-DNA Adducts

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Pyrrolobenzodiazepines (PBDs) are well-known antitumor antibiotics and bind through the formation of a covalent linkage to the 2-amino group of a guanine base in the duplex minor groove. We here report on the use of [3-^{15}N] labeled thymidines specifically incorporated into oligonucleotides to serve as NMR probes for an NMR structural characterization of covalent PBD-DNA adducts. The specifically labeled DNA duplex allows for NMR editing techniques, simplifying the spectra and signal assignments around the covalent drug binding site and gives structural information within the local environment of the isotope label. As an additional benefit, the better resolved ^{15}N resonances can give independent information on any structural changes in their immediate environment.

In the present study, two different oligonucleotide duplexes were employed for complex formation with a dimeric PBD drug possessing DNA cross-linking ability: a self-complementary duplex with guanines on opposite strands, enabling an interstrand cross-link by the drug; and a non-self-complementary duplex with two guanines located on the same strand, thus only allowing for an intrastrand drug-mediated link. In both cases the established aminal bond formed in the covalent adducts shows an S configuration at PBD C11. Different orientations of the PBD aromatic A-ring with respect to the covalently modified guanine as observed in the non-symmetric complex is shown to result in characteristic changes of PBD H11 and H11a proton chemical shifts. Based on a compilation of available NMR data on various PBD complexes, these differences may be used as valuable probes for the simple identification of PBD orientational preferences in DNA-PBD adducts.

In general, a simple and straightforward NMR analysis of covalent PBD adducts in terms of their binding modes and stereochemistries may benefit from the introduction of specific 15N labels in the nucleic acids and the observed characteristic chemical shift changes of individual drug protons, especially for complexes that exhibit severe signal overlap.

405TU

NMR Spectroscopic Characterization of Coexisting DNA-Drug Complexes in Slow Exchange

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Several members of the indoloquinoline family of drugs exhibit antifungal, antibacterial, antiinflammatory and antitumor activities. An aminoalkyl-derivativized phenyl-substituted indolo[3,2-b]quinoline has previously been shown to specifically bind triplex DNA and to constitute a potential antitumor drug. Whereas its binding to DNA was initially investigated by optical methods suggesting an intercalative mode of binding, we now analyzed preferential binding sites on triplexes by NMR spectroscopic methods.

Due to a reversible binding at different sites with slow exchange of free triple-helical DNA and several complexes of different population, strongly overlapped signals prevent a more detailed analysis of the spectrum. The specific incorporation of [3-^{15}N]-labeled thymidines within the triplex allows to unambiguously determine the number of coexisting complexes by heteronuclear ^{1}H-^{15}N experiments. After the assignment of the imino proton signals in the free DNA, corresponding resonances in the complexes could be identified by exchange crosspeaks in an EASY-ROESY experiment. To increase exchange rate dependent ROE crosspeak intensities, the temperature can be varied to reach the slow intermediate exchange regime. In this way, even coexisting complexes of low population can be identified. Within a complex the imino signals could be assigned by intramolecular NOE contacts for major species.

Due to an electrostatic repulsion expected between the positively charged drug and protonated cytosines within the third strand, neighboring TAT-triads and the duplex-triplex junction were originally proposed as potential intercalation sites. These could be confirmed through chemical shift footprints, indicating a significant preference for the duplex-triplex junction. Three low-populated complexes could be found with drug binding sites at a TAT tract within the triplex stem and two higher populated species bind the drug at the junction. The major complex was further structurally characterized by intermolecular NOE contacts found between drug and DNA protons.

406WE

Solution Structure of Duplex DNA with an Embedded Fluorescence Probe

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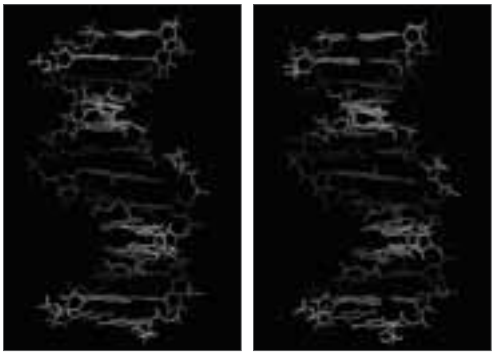
The THz vibrational activity of a biopolymer can be measured locally with an embedded molecular probe. For this purpose, the new polarity probe 2-Hydroxy-7-Carboxyfluorene was linked into a 13mer DNA duplex opposite an abasic site. The NMR solution structure shows that the fluorene molecule occupies the position of a base pair but can flip around the long axis.



407TH

Investigating the Melting Process of the Cyanobacterial Hsp17 RNA Thermometer at Base Pair Resolution

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The acclimation to varying environmental stimuli poses a significant challenge to microorganisms. Changes in temperature for example can lead to heat stress resulting in misfolding of proteins. In order to adapt to these conditions, bacteria have evolved a set of tools to sense and react appropriately upon temperature changes. Several classes of cis-regulatory RNA thermometers are able to trap the Shine-Dalgarno sequence in a helix and thus allow a rapid response in gene expression through their specific melting behaviour. Previously, we characterized the melting process of the Salmonella fourU RNA thermometer using a combination of Circular Dichroism and Imino-exchange NMR spectroscopy [1]. Here, we take the same approach to investigate the Hsp17 RNA-thermometer, which is found in Cyanobacteria and constitutes a novel class of RNA-thermometers [2]. We examine the thermodynamics of the melting process at base pair resolution and compare them to the Salmonella fourU RNA thermometer to identify common mechanism for tailored response to temperature.

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408MO

NMR Study on Anomalous Phase Transition of 1,3-Dimethyl- imidazolium bis(pentafluoroethylsulfonyl)amide

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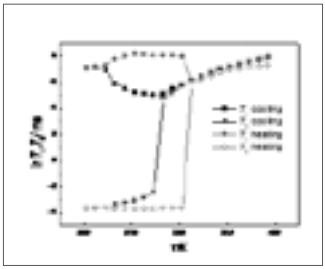
The ^{1}H-T_1, T_2 values of 1,3-dimethylimidazolium bis(trifluoromethylsulphonyl) amide: [C_1mim]NTf_2 and 1,3-dimethylimidazolium bis(pentafluoroethylsulphonyl) amide: [C_1mim]BETA were measured as a function of temperature in the wide range from 203 to 403 K. The ^{1}H-T_1, T_2 values of [C_1mim]NTf_2 changed discontinuously at 283 K in the cooling process by crystallization. The melting point of the sample is 303 K. The ^{1}H-T_1,T_2 values of [C_1mim]BETA discontinuously changed at 273 K and 223 K in the cooling process. However, only the ^{1}H-T_2 values of [C_1mim]BETA changed discontinuously though the ^{1}H-T_1 values changed continuously with lowering temperature at 273 K. Only the ^{1}H-T_1 values discontinuously changed at 223 K. The discontinuous change of the T_2 at 273 K values may be caused by glass transition. The discontinuous change of T1 at 223 K is not crystallization. The crystallization occurred at around 213 K in the heating process after cooling down to 203 K. The melting point of [C_1mim]NPf_2 is 303 K.



409TU

NMR STUDY ON PHASE TRANSITIONS AND MOLECULAR DYNAMICS OF 1-ALKYL- 3-METHYLIMIDAZOLIUM BROMIDE

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In previous work, we investigated the phase transition of ionic liquids 1-alkyl-3-methylimidazolium bromide [$C_n$mim]Br with n=2, 3 and 4 using the ^{1}H-T^1,T^2 values measured as a function of temperature in the wide temperature range [1]). In this time, we try to measure the ^{1}H-T_1,T_2 values of [C_5mim]Br and [C_6mim]Br as a function of temperature in the wide temperature range from 173 K to 403 K. The ^{1}H-T_1,T_2 values of [C_5mim]Br continuously changed in the cooling process. In the heating process, however, [C_5mim]Br discontinuously changed at about 253 K by crystallization. The crystallization speed is very slow like that of [C_4mim]Br. The melting point was 343 K. However, [C_6mim]Br did not crystallized in the both cooling and heating process. The behaviors are the same as that of [C_3mim]Br. Moreover, we measured the ^{13}C spectra and the ^{13}C-T_1,T_2 values as a function of temperature for both sample with lowering temperature in order to obtain information of the molecular dynamics in the liquid state. We will demonstrate the phase transition behavior and the molecular dynamics depending on the alkyl chain length.

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410WE

NMR STUDY ON SLOW PHASE TRANSITION OF IONIC LIQUID INCLUDING 1-BUTYL-3-METHYL- IMIDAZOLIUM CATION

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^{1}H-T_1, T_2 of ionic liquids 1-butyl-3-methylimidazolium bromide:[C_4mim]Br, 1-butyl-3-methylimidazolium hexa- fluorrophosphate:[C_4mim]PF_6 and1-butyl-3-methylimidazolium bis(trifluoromethylsulphonyl)amide: [C_4mim]NTf_2 were measured as a function of temperature in the range from 173 to 403 K. There was no discontinuous change of ^{1}H-T_1, T_2 for all samples in the cooling process. In the heating process, however, the^{1}H-T_1, T_2 values of [C_4mim]Br and [C_4mim]PF_6 changed discontinuously at 273 and at 233 K respectively by crystallization. The crystallization speed extremely slows in both samples. We need more than one hour for crystallization of approximately 0.5ml sample. [C_4mim]NTf_2 also crystallized at 213 K in the heating process and the crystallization speed also very slow. However, the ^{1}H-T_1 values of [C_4mim]NTf_2 did not change discontinuously. We will demonstrate and discuss detail of the anomalous phenomenon.

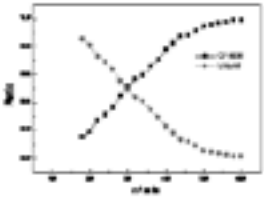

Fig. Crystallization speed of [C4mim]Br

411TH

How to Measure Transverse Relaxation of Protons?

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Measurements of transverse relaxation rates $R_2 = 1/T_2$ in proton spin-systems are challenging due to modulations arising from homonuclear J-couplings. In this contribution, we compare different techniques to circumvent this problem.

Suppression of echo modulations in the time domain is the analogue of decoupling multiplets in the frequency domain. For the purpose of decoupling, it is irrelevant if the homonuclear *J*-couplings are *refocused* or *locked*, as long as the splitting falls inside the line-width. But this difference becomes crucial when R_2's are measured. The relaxation rates of in-phase and anti-phase coherences are generally not identical. While a spin-lock can measure the decay of a pure in-phase coherence, a refocusing sequence measures a mixture of in- and anti-phase decay. The duration of the delays between the echoes determine the in-/anti-phase ratio.

Transverse relaxation rates of different proton spin-systems were investigated by:

- Spin-lock techniques to measure R1ρ
- Carr-Purcell-Meiboom-Gill (CPMG) echo trains with moderate radio-frequency pulses[1]
- Perfect Echo to refocus homonuclear *J*-couplings in an AX-system[2,3]
- "Multiple Quantum-Filtered" sequence[4]

Assets and drawbacks of the different approaches will be discussed.

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412MO

Dynamics and Function of the KIX Domain

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Protein dynamic processes play an important role for the regulation of biological function, since they provide the conformational plasticity that is required for interaction with different binding partners and for exposing functionally important amino acid residues to the environment, thereby rendering them accessible to activation.

In this study we investigate the dynamics and the function of the KIX domain of CREB binding protein (CBP) by combining nuclear magnetic resonance (NMR) spectroscopic structural and dynamic studies with other biophysical techniques. CBP is a co-activator protein that participates in transcriptional regulation by forming a direct link between transcription factors and the basal transcriptional machinery. The KIX domain of CBP plays a key role in the regulation of gene transcription because it mediates cooperativity between transcription factors by simultaneous binding through two interaction sites. Methylation of KIX disables the binding of biological targets, representing a novel aspect of the regulation mechanism of gene transcription.

We focus our research on NMR relaxation experiments, such as CPMG relaxation dispersion experiments or T1 and T2 relaxation, to gain insights in local unfolding and dynamics of the KIX domain. We examine a possible functional role of a recently described folding intermediate of the KIX domain in its methylation mechanism. Local unfolding of the KIX domain could render the side-chain of an arginine residue susceptible to methylation.

Acknowledgement: Supported by the Austrian Science Fund (P22735 to MT)

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413TU

T_1-Relaxation in Scalar Coupled Multi-Spin Systems at Variable Magnetic Field

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Investigations of spin-lattice relaxation, especially, of its magnetic field dependence – relaxation dispersion (NMRD) – provide valuable information on the characteristic times of molecular mobility. Features in NMRD are due to the following three reasons: (i) noise spectral density changes with the field; (ii) spin-operators causing relaxation change with the field; (iii) eigen-states of the spin system change with the field. As (i) and (ii) have already been investigated in detail we studied theoretically the mechanism (iii) in the situation of coupled multi-spin systems.

We assumed that the spins are coupled by scalar interactions and found the following new peculiarities in NMRD curves. First, individual spins, which have strongly different T_1 times at high magnetic field, relax with a common longitudinal relaxation time at low fields. Second, at certain fields the NMRD curves exhibit sharp features, such as peaks or dips. We explain these new features by the strong coupling between the spins so that the eigen-states of the spin system are collective states of spins, which then no longer relax individually. The peaks and dips in the NMRD curves are explained as effects of nuclear spin level anti-crossings. In addition, coherent contributions to T_1-relaxation have been found and analyzed. The theoretical results are in very good agreement with our experimental results for coupled protons in several amino acids and nucleotides.

Our study demonstrates that for liquid systems scalar couplings must be taken into account for understanding and simulating their relaxation dispersion curves as a prerequisite for their use in differentiating between relaxation mechanisms and determining the parameters of molecular mobility.

Financial support by RFBR (Projects No. 11-03-00296) and the Program P-220 of the Russian Government (grant No. 11.G34.31.0045) is acknowledged.

414WE

Peptide Self-Assembly Studied by Anisotropic Rotational Diffusion Derived from ^{13}C NMR Relaxation

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The $^{13}C^{\alpha}$ relaxation rate constants, R_1 and R_2, are known to be very sensitive to the degree of anisotropy of the molecular object and to the orientation of the CH bond vector. Here, this property is exploited in a novel method to investigate the organization of a supramolecular assembly[1]. The self-assembling system on which this method will be applied is the pore forming cyclic lipodepsipeptide pseudodesmin A[2], which assembles into supramolecular structures of indefinite size in non-polar organic solvents. Based on the monomer conformation of this small peptide building block and diffusion data, a model was previously proposed for the pseudodesmin A self-assembly[2].

By confronting R_2/R_1 ratios with the CH bond vectors within the known monomer conformation, the orientation of the monomers within the supramolecular assemblies can be assessed. The rotational diffusion coefficients of the assemblies can be obtained from the data, leading to their average dimensions. In the case of pseudodesmin A, it is demonstrated that the length of the cylinder-like structures increases with concentration, while the diameter remains constant. In addition, the orientation of the monomer molecules vs. the direction of growth reveals the surface area where the intermolecular contact takes place, which for pseudodesmin A is in agreement with an end-to-end helix stacking, validating the proposed model. We demonstrate that this method can be used for the study of the self-assembly of small molecules into anisotropic supramolecular structures[1], provided they contain sufficient distinguishable CH (or NH) groups that sample various orientations. The main advantages of this technique are that it provides structural information about the orientation of the monomer molecule within the assembly and that it can be applied in the solution state.

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415TH

Radiation Damping Effects in Small Flip Angle and Spin Noise NMR Spectra

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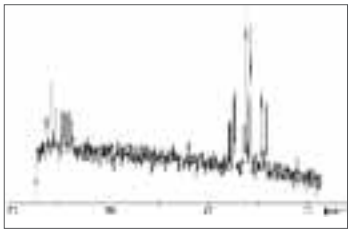
If radiation damping affects one of two or more overlapping NMR lines, *all* line shapes are no longer Lorentzian - independent of the flip angle and even in excitation-free noise spectra. In small flip angle pulse spectra narrow negative signal distortions within broad radiation damped signals at the positions of weak resonances, resemble "hole-burnt" spectra due to cross-precession [1]. In spin noise spectra narrow positive peaks on top of broad "dips" at the same positions allowing sensitive detection of small satellite peaks.

Only if radiation damping, which often dominates noise spectra as absorbed circuit noise (ACN) [2], is rigorously excluded, can pure nuclear spin noise be observed.

This can be attained

(1) by inhomogeneous B0 fields such as in spin noise imaging (SNI) experiments [3],
(2) for intrinsically broad lines such as in static solids [4] or paramagnetic solutions [5], or
(3) at low spin densities and/or with low gyro¬magnetic ratio nuclei.



The latter case is demonstrated by the, to our knowledge, first ^{13}C-spin noise spectra ever recorded, exemplified by the spectrum shown in the Figure: a 176 MHz ^{1}H-coupled ^{13}C spin noise power spectrum of ^{13}C-enriched glycerol.

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416MO

Dynamic Tunnelling Polarisation: A Quantum Rotor Analogue of DNP and the Solid Effect

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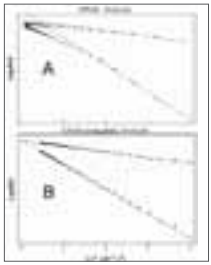
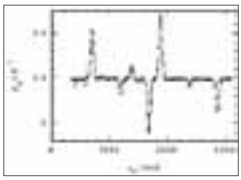


We report a quantum rotor analogue of Dynamic Nuclear Polarisation and the nuclear solid effect. [1] Comparing with conventional DNP experiments, in general terms the rotational angular momentum associated with the quantum tunnelling states of CH_3 rotors replaces the role of electron spin angular momentum.

The rotation of the methyl rotor CH_3 is characterised by quantum tunnelling with frequency ν_t twhere the exchange splitting $h\nu_t$ separates A and E nuclear spin-symmetry species. We shall describe experiments where the populations of the CH_3 tunnelling states are manipulated in a customised manner by rf irradiation of weakly allowed sideband transitions within the manifold of tunnelling magnetic levels. We show how substantial positive and negative polarisations of the A and E states are achieved using a specially designed double resonance field-cycling NMR pulse sequence. As part of this procedure, level crossings between the tunnelling and Zeeman systems are used to measure the tunnelling polarisations. In a further adaptation, the lifetimes of the A and E states are measured and investigated as a function of magnetic field. Spectra reveal evidence for mechanical cogwheel-like coupling between CH_3 rotors. The contribution these new experiments make to our understanding of quantum rotors will be discussed including the outlook for possible new techniques for nuclear spin polarisation.



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417TU

On the Self-Diffusion Measurement by the Modulated Gradient Spin Echo, a Legacy of Sir Paul T. Callaghan

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An exceptional NMR tool is the method, in which the spectral density of the translational velocity autocorrelation[1] is "probed" by a sampling function given by the frequency spectrum of the effective magnetic field gradient waveform. Sir Paul T. Callaghan termed it as "the modulated gradient spin echo" (MGSE)[2]. The frequency range of MGSE variant with the pulsed gradients is limited to below 1 kHz due the self-induction of gradient coils. Here, we consider the MGSE technique with the fixed gradient and with the CPMG train of π–rf pulses to modulate the effective gradient[3]. Much faster switching rate of the rf-pulses increases the high frequency limit of method for about two orders of degree and enables studies of the molecular dynamics in porous materials[4], and the motions in fluidized granular systems[5]. However, the simultaneous application of rf-pulses and gradient fields leads to the resonance offset artefacts as shown in Fig. A. We have exploited a way of their reduction by the analysis with the expansion of spin evolution operator into the Magnus series. This gives that a proper phase cycling of rf-pulses removes the offset terms. We confirmed it also by the experiment (Fig. B). The method enables precise measurements of the velocity autocorrelation of liquids in various systems.

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418WE

Exceeding the Limit of Dynamics Studies on Proteins Using NMR Spectroscopy

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Internal motions of proteins are tightly related to their particular function. These motions occur in a broad range of timescales from picoseconds to seconds and slower. Relaxation dispersion is uniquely sensitive to the kinetics and amplitude of motions that occur within the μs-ms timescale that manifest themselves within the residual line-width of peaks. Within relaxation dispersion, rotating-frame transverse relaxation ($R_1\rho$) experiments function by modulating the strength of spin-lock pulses to effectively quench this residual line-width. However, the strength of the applied spin-lock pulse is directly related to the fastest kinetics that can be observed, and due to hardware limitations, motions faster than 40 μs could not be detected up to now. Yet, motions between the globular rotational correlation time (4 ns) and the 40 μs, deemed the supra-τc range, have recently been proposed to play a role in molecular recognition.[1] So far, it could only be accessed by lowering the temperature, thus slowing the kinetics down such that they were within the detectable range of $R_1\rho$ experiments.[2] Therefore, we have set out to exceed our view into this time window by generating large spin-lock pulses that could be used to observe faster kinetics. Using a cryogenically cooled NMR probe, a tool commonly found in many NMR laboratories, we have been able to increase our resolution into motions that could occur as fast as 25 μs, 10 μs, and 4 μs for ^{15}N, ^{13}C, and 1H nuclei, respectively. We demonstrate that large spin-lock pulses can be applied without damaging the integrity of the probe nor heating of the sample, and validate their use for $R_1\rho$ experiments. In addition, sensitivity to smaller amplitude motions is observed, and the detection of motion within the sidechains of ubiquitin with kinetics that are within the supra–τc range is presented but slower than predicted from MD simulations on BPTI.[3] This technique allows for the observation of faster motions in proteins and the accurate characterization of parameters that define the dynamic process.

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419TH

Thermal Coefficients of Methyl Groups Within Ubiquitin

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Protein folding and molecular recognition are physiological processes that are intricately linked to their dynamic signature and reflected in their thermal coefficients. Additionally, the local conformational entropy is directly related to the degrees of freedom, which each residue possesses within its conformational space. Therefore, the temperature dependence of the local conformational entropy may provide insight into understanding how local dynamics may affect the stability of proteins. Here, we analyze the temperature dependence of internal methyl group dynamics derived from the cross-correlated relaxation between dipolar couplings of two CH bonds within ubiquitin (Sabo et al Prot. Sci. 2012, 21, 562). Spanning a temperature range from 275 K to 308 K, internal methyl group dynamics tend to increase with increasing temperature, which translates to a general increase in local conformational entropy. With this data measured over multiple temperatures, the thermal coefficient of the methyl group order parameter, the characteristic thermal coefficient, and the local heat capacity were obtained. By analyzing the distribution of methyl group thermal coefficients within ubiquitin, we found that the N-terminal region has relatively high thermostability. These results indicate that methyl groups contribute quite appreciably to the total heat capacity of ubiquitin through the regulation of local conformational entropy. We have also investigated the influence of denaturant using guanidinium chloride on these thermal coefficients. From these measurements, we found that two methyl groups, L8δ1 and L73δ1 appear to be early reporters of ubiquitin unfolding. Taken together, a per residue gauge of local protein thermodynamics, as well as local responses to the presence of denaturants, offers a powerful complement to the already well-established methods for determining global thermodynamic parameters in proteins.

420MO

Measuring the Kinetics of Conformational Sampling during Binding with NMR Spectroscopy

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Molecular recognition is typically described as either following a conformational selection (CS) or induced-fit (IF) mechanism. In the CS model, a protein samples a subset of structural configurations which mimic structures from the bound form. An important facet of this is that the sampling of different conformations limits the on-rate for binding. In the IF model, a free conformation is altered to a bound conformation induced upon binding and the on-rate is governed by the rate of this conformational change. For ubiquitin, a protein that forms many different interactions with various proteins, large heterogeneous conformations are sampled in the free form on a timescale of about 2-38 μs at room temperature.1,2 Here, we evaluate the role of this sampling event during the molecular recognition process using ubiquitin complexed with the UBA domain of Dsk2p (Dsk2)3 by utilizing Nuclear Magnetic Resonance (NMR) spectroscopy. For this purpose, concentration dependent CPMG relaxation dispersion, which is sensitive to lowly populated intermediates, has been used for evaluating the efficacy of CS and IF.4 In brief, data fit with a simple two-state model are in contradiction with HSQC-based titration results and indicate that the binding scheme is more sophisticated. Techniques on how to fit the data to higher order models and results from expanded kinetic schemes will be presented as well as a comparison of both CS and IF binding models for the ubiquitin and Dsk2 interaction. This approach can provide pivotal information regarding molecular recognition mechanisms.

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421TU

Solid State ^{19}F-NMR Relaxometry to Monitor the Local Mobility of Membrane-Bound Peptides

Stephan Grage[1], Oleg Babii[1], Ronald Lochte[1], Sergii Afonin[1], Parvesh Wadhwani[1], Pavel Mykhailiuk[2], Igor Komarov[2], Anne Ulrich[1]

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Membrane-active peptides can have a range of different structures (α-helical, β-sheeted, disordered), with diverse functions such as antimicrobial, cell-penetrating, fusogenic, or cytotoxic. Many of these amphiphilic peptides are cationic, but they interact with lipid bilayers in different ways. They can adopt distinct alignment and oligomerisation states in the membrane, and they can undergo reversible self-assembly or irreversible aggregation. While the secondary structure and orientation in the membrane has been studied intensively, the focus of this study is to monitor the local mobility of some representative peptides along their primary amino acid sequence. This way we expect to get insight into the degree of folding and flexibility of the peptides in the different insertion or oligomeric states.

We used solid-state ^{19}F NMR relaxation as a highly sensitive tool to characterize the local mobility of the α-helical antimicrobial PGLa as a first case study. The intrinsically high ^{19}F sensitivity was increased further by exploiting the rotational diffusion of the studied peptide in macroscopically oriented lipid bilayers that are placed at the magic angle in the magnetic field. To obtain information of the backbone mobility (rather than side chain flexibility), we employed a special artificial amino acid linking a CF_3-group directly to the backbone. This approach enabled us to scan a large number of amino acid positions along the PGLa sequence, and to obtain a detailed mobility profile of this membrane bound peptide.

422WE

Direct Observation of Correlated Protein Motions Detected by NMR Spectroscopy

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Protein function is inextricably linked to protein dynamics. In the case of molecular recognition, the ground state conformational ensemble of ubiquitin possesses all conformers captured to date in crystal structures of ubiquitin complexes (Lange, Science, 320, 1471). These findings support the concept of conformational selection, specifically that amplitudes of motion or dynamics resulting from conformational inter-conversion on the μsec time-scale are limiting the on-rates for complex formation (Ban, Angew. Chem. Int. Ed., 50, 11437). For ubiquitin, a majority of these conformational dynamics are concentrated in a single collective mode described by a pincer-like motion. To alleviate the entropic costs associated with sampling conformers along the pincer-like trajectory, the ubiquitin conformational ensemble predicts a significant amount of correlated motions (Fenwick, J. Am. Chem. Soc., 133, 10336). Fifteen years ago, experimentalists were challenged to develop methodology for detecting correlated protein motions (Dill and Chan, Nat. Struct. Biol., 4, 10). Until now, the direct observation of long-range correlated motion has been hindered by the lack of experimental methodology. A technique suitable for the characterization of such dynamic processes is NMR spectroscopy. Here, we present a novel method to detect long-range correlated motions using cross-correlated relaxation (CCR) between pairs of backbone CαHα bonds located on adjacent β-strands of the third immunoglobulin-binding domain of the streptococcal protein G (GB3) (Rief, Science, 276, 1230). We report CCR rates for 4 pairs of CαHα bonds, Q2-K19, K4-T17, V6-E15, and Y45-F52. The CCR rates indicate that all 4 CαHα pairs engage in correlated motions (Pelupessy, J. Biomol. NMR, 25, 265). In order to complement our experimental results, we generated a conformational ensemble of GB3 utilizing previously measured residual dipolar couplings (Yao, J. Phys. Chem. B, 112, 6045), which was cross-validated with backbone NH-NH and NH-CαHα CCR rates (Vögeli and Yao, J. Am. Chem. Soc.,131, 3668) and hydrogen bond scalar couplings. We show that our measured CCR rates are predicted by this GB3 ensemble. Taken together, we provide the first experimental evidence for long-range correlated motions. These findings demonstrate that the synchronized β-sheet motions reduce the degrees of freedom and contribute to the stability of the protein ensemble.

423TH

Application of Field-Cycling NMR Relaxometry to the Investigation of Magnetic Order in Nanoparticle Suspensions

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In this presentation the application of 1H field-cycling NMR relaxometry, in the 10E3 – 10E8 Hz range, to study aqueous and non-aqueous magnetic nanoparticle suspensions will be described.[1] These studies are of interest, as the physicochemical properties of solvated nanostructures are critical to their performance in biomedical applications, and in particular as contrast agents for MRI. These developments have been facilitated by the development of a workable theory for solvent relaxation due to superparamagnetic nanoparticles, by Robert Muller and his colleagues at Mons-Hainault. [2] Our research demonstrates that for magnetic nanoparticle and nanocomposite suspensions, 1H NMR relaxation time measurements of the solvent provide insight into the extent of solvation, the surface and bulk magnetisation, and the magnetic order.[3-10] This arises because the diffusing solvent molecules interact dynamically with the nanocomposite surface. The information obtained on the structures in situ is complementary to that available from dynamic light scattering, magnetometry and electron microscopy. The implications of the findings for developing other NMR probes of both nanostructure and confined dynamics will be discussed.

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424MO

Overhauser Dynamic Nuclear Polarization for the Quantitative Study of Hydration Dynamics

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Overhauser dynamic nuclear polarization (ODNP) offers a unique insight into the colorful variation of hydration dynamics at the surfaces, interfaces, and interiors of proteins, lipid vesicles, and other biological macromolecules with sub-nanoscale specificity. It uniquely allows measurements on "dirty" samples, which may be opaque, extremely viscous, or contain particularly large, immobile macromolecules. ODNP analyzes the fast translational motion of water molecules with high selectivity, thus allowing a unique insight into the layer of hydration water that surrounds macromolecules in aqueous solution, and which plays a hotly debated role in the interactions between macromolecules and macromolecular interfaces.

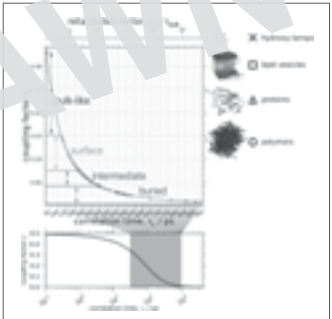

Figure 1: One can map out the timescale of the translational dynamics of a variety of samples by measuring their ODNP coupling factor, ξ

Here, a new experimental approach and data analysis model demonstrate a previously unparalleled level of accuracy. We will show how this novel approach grants access to the quantitative analysis of samples with spin label concentrations as low as 10 μM, as opposed to the previous practical limit of 500 μM. We show how, previously, subtle effects of very slight microwave heating could lead to overall shifts in the quantitative values of hydration dynamics measured by DNP. We present a correction for these effects that allows us to extract ODNP data that routinely demonstrates maximum saturation in a standard cylindrical cavity. Thus, this new approach and analysis provide a now standardized tool for measuring the translational mobility of the hydration water with quantitative accuracy.

NOT FOR DRAWN

426WE

The Water Dynamics of Superparamagnetic Iron Oxide-Loaded Vesicles

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Superparamagnetic iron oxide (SPIO) nanoparticles have been introduced as contrast agents for magnetic resonance imaging (MRI) in clinic. Recently, SPIO has been also used for tracking cells. Bowen *et al*. shows that the MR relaxation of water molecules have different behaviors in SPIO solution and SPIO-loaded cells.

In this study, we used water-in-oil-in-water (W/O/W) double emulsions to mimic the cellular environments. We compared the MR relaxation between SPIO-loaded vesicles and SPIO solution. The detailed contribution of water in each compartment has been studied. The results display that water relaxation in SPIO-loaded vesicles has different behavior from that in SPIO solution. Both samples indicate a strong iron concentration dependent T_2^* relaxation. However, T_2 relaxation of water in SPIO-loaded vesicles is almost independent of iron concentration while that of SPIO solution still have similar trend as T_2^* relaxation. It may result from different relaxation mechanisms, e.g. SMR and MAR. However, the present theories can not completely interpret the iron concentration dependent T_2 and T_2^* relaxation when SPIO in confinement due to the restricted water diffusion. It might have other source to contribute the perturbed field in the vesicles. When vesicles distance is close enough, the local field in a vesicle may be perturbed by the SPIO in nearby vesicles and shows the anomalous concentration dependency of T_2^* relaxation. The results can be applied to the analogous SPIO-loaded cell system.

427TH

Are {^{1}H}-^{15}N NOE Data Indispensable in the Determination of Protein Backbone Dynamics?

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Since the first successfully applied ^{15}N relaxation method to backbone amide nitrogen nuclei for local mobility determination in proteins has been frequently studied by means of this approach. Owing to the complexity of motions in proteins analysis of experimental ^{15}N relaxation data has been almost exclusively carried out using model-free approach rather than analytical model of motion. Soon it was found that model-free approach sometimes poorly described experimental data, especially {^{1}H}-^{15}N NOEs which were determined together with longitudinal, R_1, and transverse, R_2, relaxation rates of ^{15}N nuclei. The crucial weakness of {^{1}H}-^{15}N NOE experiment relays on inherently low sensitivity as compared to relaxation rate measurements. Moreover, amide hydrogens in proteins exchange with solvent requiring very careful approach to the solvent signal suppression in order to avoid magnetization transfer and other side effects.

A time saving, alternative approach relying on the substitution of NOE data with relaxation rates determined at additional magnetic field strength(s) is faster and retaining or exceeding a number of initial data. Provided there is an access to different magnetic field NMR spectrometers one can to carry out R_1 and R_2 experiments at several magnetic fields instead of a single NOE measurement.

Concluding, ^{15}N relaxation rates in proteins determined at multiple magnetic field strengths are superior to the data including nuclear Overhauser effects for two reasons. First, total experimental time required for collecting data becomes significantly shorter. Second, interpretation of internal motions in the frame of genuine model-free approach rather than the extended one often turns out to be satisfactory.

428MO

Shielding and Indirect Spin-Spin Coupling Tensors in the Presence of a Heavy Atom – an Experimental and Theoretical Study

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Magnetic shielding and indirect spin-spin coupling phenomena are tensorial properties and both their isotropic and anisotropic parts do affect NMR spectra. There are numerous examples of determination of Δσ values either in oriented phases (solids or liquid crystal solvents) or even in isotropic solutions via relaxation effects. On the other hand, observation of ΔJ effects and determination of their values are not a trivial task, as they are always heavily masked by the dipolar coupling between the same pair of nuclei. The interaction tensors mentioned above can nowadays be theoretically calculated using commercial quantum chemistry programs, although the reliability of such methods in the case of ΔJ in systems involving heavy nuclei has hardly ever been tested experimentally.

In this communication we report the results of the experimental and theoretical investigations of bis(phenylethynyl)mercury (I) labeled with ^{13}C at positions neighbouring Hg. The theoretical calculations of molecular geometry and values of NMR parameters for I have been performed by ZORA/DFT method, including the relativistic scalar and spin-orbit coupling contributions, using PBE0 functional and TZP (or jcpl) basis set. The experimental values of isotropic parameters have been measured by the standard ^{13}C and ^{199}Hg NMR spectra. The shielding anisotropies for atoms from the central part of I have been determined using the method based on the interpretation of the magnetic relaxation data measured for liquid solution, within the rotational diffusion theory and assuming symmetrical top reorientation model. The anisotropies of ^{13}C-^{199}Hg and ^{13}C-^{13}C spin-spin couplings have been determined exploiting the temperature-dependent ^{13}C NMR spectra of I in the ZLI1167 liquid-crystal phase. We have found out that for compound I a very good reproduction of the experimental values of both isotropic and anisotropic NMR parameters has been achieved by theoretical calculations.

Taking into account the above results we can conclude that a sufficiently advanced theoretical approach should provide reliable predictions of the values of NMR parameters also in the cases involving heavy nuclei.

429TU

The MRI Contrast Enhancements of Superparamagnetic Iron Oxide-Loaded Cells by Phase Nutation Imaging

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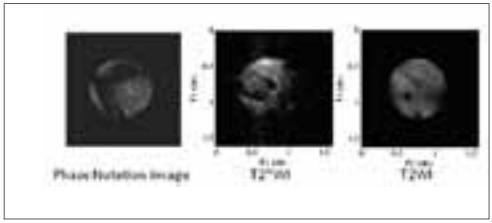
In recent years, Magnetic Resonance Imaging (MRI) has been wildly used in physiology because of its power to obtain image of the human body noninvasively. To get the better image quality, the superparamagnetic iron-oxide contrast agents play a crucial role in MRI contrast enhancements.

In our previous study, we found that the difference between T_2 and T_2* and the signal attenuation due to water diffusion in in vitro environments are varied a lot when contrast agent presents. Resuming these discoveries, we design the new imaging pulse sequences that is sensitive to the off-resonance effect in the sample which is suit for the characteristic of SPIO-labeled cells.

In this study, we used water-in-oil-in-water (W/O/W) double emulsions to mimic the cellular environments for phantom test and also took *in vitro* imaging of SPIO-labeled cells. The new design pulse sequence shows a good contrast between SPIO-labeled cell and normal tissue. Comparing with the image by post-procesing of spin-echo (T2WI) and gradient-echo (T2*WI) imaging, the phase nutation images of SPIO-labeled regime (highlighted by the red circle) show a positive contrast.



430WE

Carbon-13 NMR Relaxation Study of the Dynamics of *Escherichia coli* O91 O-Antigen Polysaccharide

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The dynamic of the O-antigen part of the lipopolysaccharide from the enterohemorrhagic Escherichia coli O91 has been studied by means of carbon-13 NMR relaxations. Previous study [1] shows that the O-antigen consists of pentasaccharide repeating units being linked through a secondary carbon of the pyranoin sugar rings. The material used in this study was grown on specifically labeled D-[6-^{13}C]Glc which lead to site specific ^{13}C labeling at the C_6 positions (three hydroxymethyl groups and one methyl group). Carbon-13 relaxation data including relaxation times T1 and T2 and heteronuclear Overhauser enhancement, were acquired at two magnetic fields (16.4 and 21.1 T). Based on the observations made about dynamics of differently labeled O-antigen polysaccharide [2], highly intricate internal dynamics was expected, both in amplitude and timescales. Relaxation data were analyzed using Lipari-Szabo and two site-jump model, revealing internal dynamics of the sugar residues ranging from tenths picoseconds to nanosecond timescales.

[1] Kjellberg, A.; Weintraub, A.; Widmalm, G.; Biochem. **1999**, 38, 12205-12211
[2] Lycknert, K.; Widmalm, G.; Biomacromolecules, **2004**, 5, 1015-1020

431TH

Diverse Mobility of D_2O Molecules in Faujasite Cages: A Deuteron NMR Investigation

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Study of dynamic behavior of water molecules in zeolites is a part of investigations aiming to elucidate also catalytic properties at molecular level. Here we apply deuteron NMR methods to follow D_2O dynamics in faujasites in a wide range of temperature. Synthetic zeolites NaY with Si/Al = 2.4, 1.8 and NaX (1.3) were used. Amounts of D_2O were equal 100%, 200%, 300% and 500% respective to the number of Na+ cations in the unit cell. Thus in terms molecules per unit cell 100% refers to 56, 68 and 86 molecules in NaY(2.4), NaY(1.8) and NaX(1.3), respectively. In a detailed microscopic model one expects features related to interactions: electrostatic water-sodium cation, hydrogen bond water-framework oxygen and water-water bonding, and their dependence on the loading and Si/Al ratio. These interactions provide local potential value and symmetry, which may be deduced from observed mobility by means of deuteron NMR spectroscopy.

Activation energy obtained from relaxation rates measured above 200K decreases with decreasing Si/Al and loading. There are two components of different width observed in the spectra above 200K for all samples considered. Their width at room temperature increases with decreasing Si/Al ratio. Their relative weights change with temperature. Contribution A_n of the narrow line decays according to the common dependence $A_n \sim \exp(-aT)$, where $a = 0.04K^{-1}$. The narrow line can be attributed to water molecules in a chain on the cage surface, with O-D performing tetrahedral jumps. Such jumps average the quadrupole interaction most effectively. Other molecules perform translational-rotational jumps. All motions slow down on decreasing temperature and at temperature about 200K all molecules become localized as indicated by extreme broadening of deuteron NMR spectra. Deuteron NMR spectra provide a direct evidence for the symmetry of deuteron mobility. Three shapes are present here. Pake doublets, with peak separation $3/4C_Q$, are observed for rigid deuterons. Twofold exchange of deuterons in D_2O leads to a pagoda shape. Gaussian spectra with decreasing width represent chaotic reorientations leading to isotropic reorientations at high temperature. All these components are found in the spectra with contributions depending on Si/Al ratio and loading. Pake doublets dominate at low temperature in all cases. Twofold exchange enters on increasing temperature.

432MO

Insight into Interdomain Dynamics by Using Paramagnetic NMR Data

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Inter-domain motions within multi-domain proteins play a crucial role in many biologically important processes involving protein-protein or protein-DNA recognition. The overall dynamics of multi-domain proteins is constituted by time dependent changes in both the internal conformations of individual domains and relative orientations of these domains with respect to each other. The paramagnetic NMR-based constraints pseudocontact shifts (PCS) and residual dipolar couplings (RDC) contain precious long-range information to detect intra- and inter-domain dynamics. Here, we report a new strategy to describe the translational and rotational motions of protein domains using a combination of NMR and Molecular Dynamic (MD) simulation. As a model system we used a complex of calmodulin (CaM) with the IQ-recognition motif from the voltage-gated calcium channel $Ca_v1.2$ (IQ), which adopts two different interdomain orientations in the crystal structure solved by X-ray crystallography1. Using the N60D-CaM mutant [2,3] we collected a large number of PCSs and RDCs for six different paramagnetic lanthanide ions spanning the whole range of magnetic susceptibility tensors, in terms of magnitude and anisotropy. Our results verify the co-existence of the X-ray conformers in solution, but also indicate that their presence is not sufficient to fully explain the experimental NMR data. Interestingly, the addition of MD models to X-ray structures provided a significant improvement in the evaluation, indicating a larger basin of conformational space sampled by CaM-IQ complex. Our ensemble approach represents an alternative method to assess the possible conformations experienced by a protein displaying conformational heterogeneity. The results are discussed in the light of other strategies of using paramagnetic data for multidomain proteins.

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2. Bertini I, Del Bianco C, Gelis I, Katsaros N, Luchinat C, Parigi G, Peana M, Provenzani A, Zoroddu MA, *Proc. Natl. Acad. Sci. USA* **2004**, 101, 6841-6846
3. Bertini I, Kursula P, Luchinat C, Parigi G, Vahokoski J, Wilmanns M, Yuan J, *J.Am. Chem.Soc.* **2009**, 131, 5134-5144

433TU

Water Spin Magnetization Exchange NMR Study Using New Mathematical Methodology

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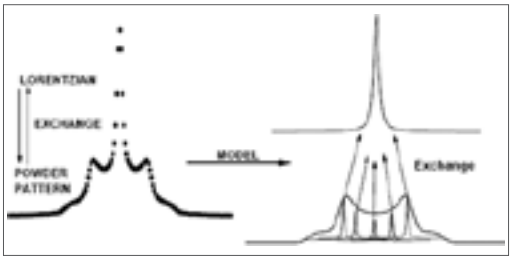
Water behaviour on the pore surface of nano-silica MCM-41, at a hydration level corresponding to one water molecule per OH group, is studied using ^{2}H NMR spectra in the temperature range 213 K to 313 K. In an earlier studies [J. Hassan, E. Reardon, H. Peemoeller, Microporous Mesoporous Materials, 122 (2009) 121-127 and J. Hassan, Physica B 407 (2012) 179–183] it was shown that at this hydration level, deuterons of water at single OH sites exhibit a Lorentzian line shape and deuterons of water at hydrogen-bonded OH sites exhibit a powder pattern. Here it is shown that magnetization exchange occurs between these two deuteron spin groups. This exchange cannot be described using the common, two-site exchange model, involving two Lorentzians. We successfully apply a multi-Lorentzian exchange model, developed by Woessner [D. E. Woessner, Mol. Phys. 34, 4, (1977) 899-920] to study effects of motion on the shape of powder pattern spectra. For this low hydration sample the rate of magnetization exchange out of the hydration site, where the water deuterons exhibit a Lorentzian line in the ^{2}H spectra, is 1.3 ms^{-1} and the activation energy for the exchange is found to be 3.4±0.1 kcal/mole.



434WE

Regulation and Dynamics of the Plasmodium falciparum Cell Invasion Motor: Insights from NMR

Christopher Douse[1], Ernesto Cota[1], Ed Tate[1], Pete Simpson[1], Tony Holder[2]

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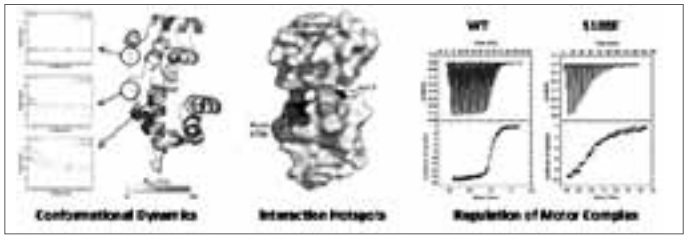
A key event in the complex life cycle of *Plasmodium* spp., the protozoan parasites that cause malaria, is the invasion of erythrocytes by blood stages known as merozoites. The motive force required for this process is provided by a conserved actomyosin motor consisting of an unusual myosin (MyoA) that is part of a multi-protein assembly making up the biomolecular invasion machinery. One of these proteins is Myosin Tail Interacting Protein (MTIP), which links the motor to the inner membrane of the merozoite.

The MTIP/MyoA complex can be reconstituted *in vitro* using peptides mimicking the C-terminal tail of MyoA, and since inhibition of the interaction in vivo should stall invasion and disrupt the parasitic life cycle, it has been identified as a target for the development of novel antimalarials and chemical genetic tools.

In this talk I will describe the application of NMR spectroscopy and other biophysical techniques in studying the MyoA binding domain of MTIP from *Plasmodium falciparum*. In particular, I will show how these experiments have informed inhibitor development and enabled us to extract structure-function relationships concerning the regulation and dynamics of this pathologically relevant system.



435TH

Tailored Real-Time Scaling of Heteronuclear Couplings

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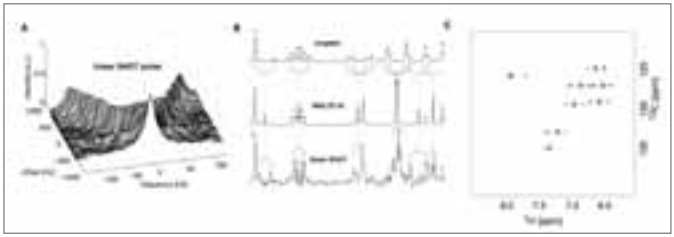
Technische Universität München, Department of Chemistry, Garching, Germany

Heteronuclear couplings are an extremely valuable source of molecular information, encoded in the multiplet splittings of an NMR spectrum. Radiofrequency irradiation on one coupled nuclear spin allows to modify the coupling strength, scaling down the multiplet splittings in the spectrum observed at the resonance frequency of the other nuclear spin. Those decoupling pulse sequences are oftentimes used to collapse a multiplet into a singlet and can therefore simplify NMR spectra signifi-cantly. Continuous-wave decoupling has an intrinsic non-linear off-resonance dependence of the scaling of the effective *J*-coupling. Using optimal control pulse optimization, we show that virtually arbitrary off-resonance scaling of the *J*-coupling strengths, covering the full range between complete coupling (line-splitting equal to *J*) to complete decoupling (no line-splitting), can be achieved. Linear scaling for off-resonance decoupling can be achieved (see Fig. A) The new class of tailored decoupling pulses is named SHOT (Scaling of Heteronuclear couplings by Optimal Tracking). In an experiment we show that a 1D SHOT {1H}-13C experiment yields comparable information to a 2D HSQC and can give full assignment of all coupled spins (see Fig. B,C). Using SHOT pulses with linear J-scaling, we present an alternative, robust, easy-to-implement, and fast method of encoding chemical shift information indirectly through off-resonance decoupling.



436MO

Solvent-Free High Field Dynamic Nuclear Polarization of Mesoporous Silica Impregnated or Functionalized with Nitroxide Radicals

Olivier Lafon, Herve Vezin, Aany Sofia Lilly Thankamony, Fabien Aussenac, Melanie Rosay, Xingyu Lu, Julien Trebosc, Jean-Paul Amoureux

UCCS, Univ. Lille Nord de France, Lille, France

High-field Dynamic Nuclear Polarization (DNP) is a promising technique to enhance the NMR signals of solids through a transfer of magnetization from unpaired electrons to nuclei. However, the extension of this technique to inorganic materials requires the incorporation of paramagnetic agents in the materials.

Here we compare two methods to introduce nitroxide radicals into the materials: (i) the post-synthesis impregnation with radical containing solution[1], (ii) the functionalization of silica surface with nitroxide radicals[2] (see Fig. a). Direct and indirect ^{29}Si DNP via ^{1}H were tested for these two incorporation protocols. The functionalization procedure permitted to demonstrate the feasibility of solvent-free DNP for porous solids. Furthermore, the absence of frozen solvent within the pores results in fast polarization build-up, which will be useful for investigation of direct DNP below 100 K.

[1] O. Lafon *et al. Angew. Chem. Int.* Ed. 2011, 50, 8367-8370
[2] A. S. Lilly Thankamony *et al. Appl. Magn. Reson.* Submitted

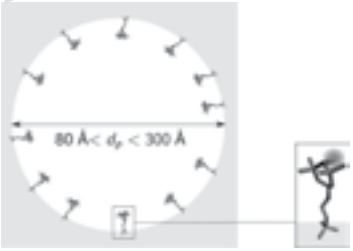


Fig. Schematic structure of mesoporous silica functionalized with TEMPO.

437TU

Development and First Results of a New High Resolution Low Field NMR Spectrometer

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In this contribution we present the development and first results of a newly developed, low field NMR spectrometer allowing for the measurement of high resolution spectra (0.7 ppm) at 1 mT- 19mT (41kHz- 800 kHz ^{1}H-frequency). The core of the spectrometer consists of a high homogeneity B_0-electromagnet with four shims and six ultra-stable current sources. The probehead of the spectrometer is equipped with modular resonators using a universal rf-excitation coil for all modules, allowing for high rf-pulse homogeneity and best sensitivity with individually optimized pickup coils. The highly rf-shielded field cycling probehead is applicable for measurements with all spin ½ nuclei and nuclei with spin > ½ with low quadrupole moments (e.g. ^{6}Li).

Until now high resolution proton spectra have been recorded in a single scan with thermally polarized samples (B_0 = 10 mT). Upon application of hyper- and prepolarization methods (2T Halbach magnet, PHIP) ^{1}H and naturally abundant ^{13}C and ^{29}Si homo- and heteronuclear *J*-coupled spectra could be measured in a single scan. Furthermore hyperpolarized ^{129}Xe spectra could be recorded in real time allowing for the measurement of chemical processes in real time.

NOT DRAWN

438WE

NMR Spectroscopic Analysis of Diesel Fuel Oxidation Stability

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Diesel fuel properties are dependent primarily on the composition of diesel fuels, which consist of hundreds of individual components belonging to aromatic, paraffinic, olefinic and polar hydrocarbon classes. Oxidation degradation is the most important limiting factor for diesel fuel stability which determines the amount of insoluble sediments formed during oxidation.

NMR spectroscopy is one of the most powerful methods in identification and structural analysis of complex mixtures. It has already been used for the analysis of oil, oil derivatives and products. In complex mixtures such as mineral fuels, high resolution ^{1}H and ^{13}C NMR spectroscopy can provide wealth of information on average structural parameters and quantitative distribution of hydrogens and carbons.

A combination of ^{1}H and inverse gated ^{13}C NMR spectroscopy together with a comprehensive two dimensional gas chromatography (GCxGC) and statistical analysis has proven useful to analyze diesel fuels prior and after oxidation and to determine its oxidation stability. Classes of hydrocarbons present before and after oxidation have been identified and quantified. A decrease in aromatic iso- and n-paraffinic hydrocarbons and an increase in the total saturated hydrocarbons have been observed after oxidation. It has been shown that integrated peak intensities of certain functional groups in ^{1}H NMR spectra correlates well with oxidation stability.

439TH

New ^{129}Xe Chemical Shift Ranges in Novel HXeY Compounds

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A new group of metastable noble gas compounds of the common HNgY structural formula, where Y is an electronegative fragment, was discovered in 1995 by Pettersson et al. [1]. Recently, a methodology for accurate theoretical predictions of NMR parameters in Xe molecules was presented in a calibration study [2] on the prototypic HXeCCH [3] compound.

In here, it is shown that ^{129}Xe NMR chemical shift in the recently prepared matrix isolated xenon compounds, HXeY (Y=H, F, Cl, Br, I, CN, NC, CCH, CCCCH, CCCN, CCXeH, OXeH, OH, SH) as well as ClXeCN and ClXeNC, appear in new, so far unexplored 129Xe chemical shift ranges. State of the art theoretical calculations predict ^{129}Xe NMR chemical shifts of ca. 500 1000 ppm (wrt. Xe gas) for HXeY species and ca. 1100 1600 ppm for ClXeCN and ClXeNC that fall between the so far characterized Xe molecules and atomic Xe guest-host systems with chemically non-bonded Xe atom [4].

While the relativistic effects only slightly modulate the ^{129}Xe chemical shift, the spin orbit induced shielding effects on the 1H chemical shifts of the H1 atom directly bonded to the Xe centre largely overwhelm the nonrelativistic deshielding effects leading to an overall negative 1H chemical shift in the range between 5 to 35 ppm (wrt. CH_4).

Thus, the relativistic effects induced by the heavy Xe atom appear considerably more important for the chemical shift of the neighbouring, light hydrogen atom than that of the Xe nucleus itself. The predicted NMR parameters facilitate for an unambiguous experimental identification of these novel compounds.

[1] M. Pettersson, J. Lundell, and M. Räsänen, J. Chem. Phys. **102**, 6423 (1995).
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[3] L. Khriachtchev, H. Tanskanen, J. Lundell, M. Pettersson, H. Kiljunen, and M. Räsänen, J. Am. Chem. Soc. **125**, 4696 (2003).
[4] M. Gerken and G. J. Schrobilgen, Coordination Chemistry Reviews, **197**, 335 (2000).

440MO

Analysis of Glucose Metabolism of Free Swimming Farmed Seabass in ^{2}H-Enriched Seawater by ^{2}H NMR Analysis of Plasma Glucose Positional ^{2}H-Enrichments

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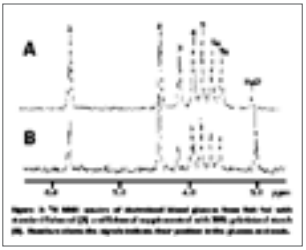
[1]Center for Functional Ecology, Faculty of Science & Technology University of Coimbra, Coimbra, Portugal, [2]Center for Neurosciences & Cell Biology, University of Coimbra, Coimbra, Portugal, [3]Department of Life Sciences, Faculty of Science & Technology, University of Coimbra, Coimbra, Portugal

The sparing of feed protein conversion to glucose is a key economic and environmental objective for aquaculture. However, study of fish glucose metabolism by traditional tracer methods is challenging because of difficulties in tracer delivery via intravenous infusion. We developed a novel approach based on the analysis of plasma glucose ^{2}H-enrichment from ^{2}H-enriched seawater. In this setting, constant body water ^{2}H-enrichment is rapidly attained and subsequent ^{2}H-enrichment of blood glucose informs the sources of glucose appearance. Positional enrichment of plasma glucose is fully resolved by ^{2}H NMR analysis following derivatization (Figure 1). These data inform the fraction of blood glucose derived from protein (gluconeogenesis) *vs.* non-gluconeogenic sources, such as dietary carbohydrate and glycerol. The ^{2}H NMR spectra of Figure 1 are quite distinct from each other and reflect significant differences in plasma glucose sourcing for fish fed on standard fishmeal that is naturally low in carbohydrate (3% of total calories) compared with fishmeal supplemented with gelatinized starch (30% of total calories). The changes in positional ^{2}H-enrichment distributions reflect a reduced gluconeogenic contribution to plasma glucose appearance and a sparing of protein conversion to glucose. In conclusion, ^{2}H NMR analysis of metabolite enrichments from ^{2}H-enriched water is a powerful and versatile method for the study of nutrition and metabolism in fish and other species.



441TU

The Impact of Oxidized Phospholipids on Lipid Membrane Organization and Membrane-Mediated Apoptotic Regulation by Apoptotic Members of the Bcl-2 Protein Family

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The unique organization of lipid membranes together with their inherent protein constituents enables them to act simultaneously as a cellular barrier but also as a control interface regulating the cellular communication with its extracellular surrounding. In general, eukaryotic cells contain a wide range of different membrane systems, which mainly belong to the various organelles and exert therefore often very specific functions. While for a long time lipids have been seen as merely structural membrane units with proteins doing the actual work, this view has changed in recent years where it was shown that lipids are also directly involved in numerous physiological processes. However, how membranes change under intracellular oxidative conditions, which e.g. trigger mitochondria-mediated programmed cell death, is still a mystery. Nevertheless, the oxidation products of lipids – mainly via oxidation of their unsaturated fatty acid chains – generate lipids with molecular properties very different from normal membrane lipids. These alteration can cause e.g. truncated fatty acid chains which bear polar or charged carboxyl group at the chain ends; properties which will dramatically alterate membrane organization and their biological function. To obtain insight into the role of oxidized lipids on mitochondrial membrane structure and subsequent consequences for the regulative interplay of the mitochondrial apoptosis controlling Bcl-2 protein family with these membranes under oxidative stress conditions, we used a combined DSC and solid state NMR approach. By incorporating defined oxidized lipids such as PazePC and PoxnoPC, with both having truncated sn-2 chains and a carboxyl or carbonyl group at the hydrophobic chain terminal position, we observed drastic changes on the phase-behaviour and dynamics of DMPC-based model membranes. There we could reveal a OxPl-poor and OxPl-rich domain which both could coexist as two different types of lamellar structures as visible by 31P MAS NMR. Wideline 2H NMR also revealed dramatic changes in the membrane hydration pattern. By using mitochondrial outer membrane mimicking lipid mixtures (PE,PC, Cardiolipin), the presence of PazePC also improved the association and incorporation of the apoptotic Bax protein; an observation which is in agreement with the oxidized lipid induced onset of apoptosis as observed in vivo.

442WE

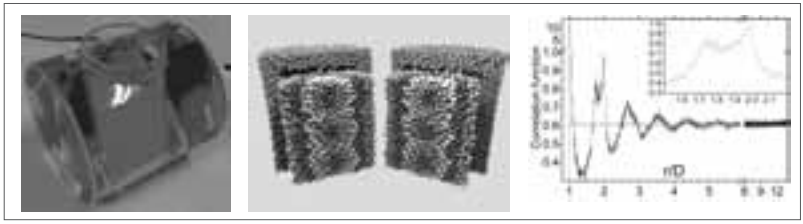
A New MRI Reconstruction Technique Based on Real Space Hough Transform for Granular Samples

Riccardo Balzan[1], Alessandro Sellerio[2], Arthur Magill[1], Daniele Mari[2], Arnaud Comment[1], Gerard Gremaud[2]

[1]Institute of Physics of Biological Systems, EPFL, Lausanne, Switzerland, [2]Groupe de Spectroscopie Mécanique, EPFL, Lausanne, Switzerland

A new 3D reconstruction technique developed for analyzing granular samples composed of as much as $3\text{-}10^4$ spheres will be presented. It was applied to study the structural geometry of samples composed of 3mm diameter plastic beads immersed in water. The MRI acquisitions were performed on a 7T Siemens clinical scanner using a single proton channel birdcage volume coil and a standard gradient echo sequence (TR = 50ms, TE = 3.79ms, total acquisition time about 5h). We obtained 3D images of volumes up to 500mL with an isotropic resolution of 150µm.

Unlike the previously proposed methods based on Fourier transformation [1], our technique makes use of a real-space Hough transform. The algorithm we propose contains optimized features to obtain a very high reconstruction precision and it was possible to determine the position of every single bead with a precision higher than 10µm, similar to the dispersion of the spheres diameter.



The unprecedented precision in the reconstruction allowed us studying the details of the structural behavior of sphere packing. In particular, we were able to discover and highlight a relation between the short and long range properties of granular materials and observe the presence of crystallization.

The proposed technique could lead to important breakthroughs both in experimental and theoretical approaches to the field of granular materials as well as to improvements in industrial manipulations involving granular raw materials (e.g. coal, sand, powders).

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443TH

Organochlorine Pesticides Residues in Soil and Vegetables in Different Growing Systems

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In this paper are presented the research results obtained in 2011, regarding the assessment of some organochlorine pesticide residues (20 active substances) from 80 soils samples and 25 vegetables samples (tomatoes, cucumber, peppers, eggplant) from different growing systems (ecological and conventional).

Determination of the organochlorine pesticide residues in soil and vegetables samples were performed by Gas chromatograph (GC Shimadzu, model 2100), equipped with an electron capture detector.

In soil samples harvest from conventional farm on Roman Farm and Tg.Frumos Farm were detected Endosulfan I (*range 0.002 – 0.015mg/kg*); Endrin aldehide (*range 0.004 – 0.01 mg/kg*) and Endosulfan sulfate (*0.001 mg/kg*).

In vegetable samples from conventional farm, the content of organochlorine pesticide residues in some samples analysed were none detectable. In others samples, tomatoes, papper were detected *heptachlor epoxid residues* (range 0.001 – 0.006 mg/kg); *endosulfan I residues* (range 0.001 – 0.003 mg/kg) and *endrin aldehide residues*, but in admissible limits (< 0.01 mg/kg).In all samples analysed the organochlorine pesticide residues were included in admissible limits (Regulation (EC) 396/2005).

444MO

Investigation of the Bioactive Conformation of 26RFa, an Orexigenic Neuropeptide

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26RFa, a RFamide neuropeptide, is the ligand of the G-protein-coupled-receptor GPR103 and the system 26RFa/GPR103 is involved in feeding behaviour. Analysis of the 26RFa precursor reveals that it may generate several additional RFa-peptides including an N-terminally extended form, 43RFa, which binds and activates GPR103 similarly to 26RFa.

Using molecular modeling under NMR restraints, we showed that, in DPC micelles - a membrane mimetic medium - both peptides adopt the same conformation, i.e. an alpha-helix $^{P4\text{-}K19/P21\text{-}K35}$ followed by a β-turn $^{F22\text{-}R25/F39\text{-}R42}$ suggesting that this helix-linker-turn motif is essential for the biological activity.

SAR studies on truncated fragments of 26RFa [1] indicated that, the deletion of the first nine amino acids does not markedly alter the biological activity (EC_{50}: 26RFa: = 10,4nM; $26RFa_{10\text{-}26}$ = 37,5nM) while the suppression of additional residues (EC_{50} : $26RFa_{13\text{-}26}$ = 95,3nM; $26RFa_{16\text{-}26}$ =237nM; $26RF_{a20\text{-}26}$ =739 nM) provokes more important effects.

In order to explain these differences, we investigated the solution conformation of $26RFa_{11\text{-}26}$ and $26RFa_{13\text{-}26}$ in DPC micelles. $26RFa_{11\text{-}26}$ encompasses a nascent helix and a β-turn $^{F22\text{-}R25}$ while $26RFa_{13\text{-}26}$ only possesses the C-terminal turn structure F22-R25. The absence or destabilization of the helical structure in 26RFa N-terminal truncated fragments could thus explain the decrease of the biological activity.

To gain more insight into the importance of a stabilized helix on the biological activity, we are currently synthesizing, using the Hydrogen Bond Surrogate method [2], analogues of $26RFa_{11\text{-}26}$ and $26RFa_{13\text{-}26}$ in which the main hydrogen bond (11-15 or 13-17) is replaced by a covalent link. Structural studies and biological analyses will allow us to further elucidate the bioactive conformation of 26RFa.

Acknowledgments: Part of this work is supported by ERDF funding through the IS:CE-Chem project and Interreg IV A France-(Channel)-England programme. The authors also thank the Crunch network for its financial support.

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445TU

15N NMR Spectroscopic Studies on the Tautomerism of Substituted 2-Aminothiazolines

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2-aminothiazolines can exist in two tautomeric species (Fig. 1) and lack of regioselectivicty. Several methods and techniques have been used to evaluate tautomeric process. In our case ^{15}N NMR spectroscopy was used due to tautomeric process involves two nitrogen atoms.[2] HMBC $^{1}H\text{-}^{15}N$ spectra were acquired at room temperature for several substituted 2-aminothiazolines. It was observed from HMBC contour plot correlation between five membered ring CH2 groups with sp^2 nitrogen around 250 ppm, while aliphatic hydrogen correlates with sp^3 nitrogen around 90 ppm, suggesting that amino form is the major or the unique tautomer present in solution. These results were corroborate by theoretical calculations at the M06-2X/cc-pVDZ level, which provides amino tautomer as more stable (~1 kcal mol^{-1}) than imino one (Fig. 1).

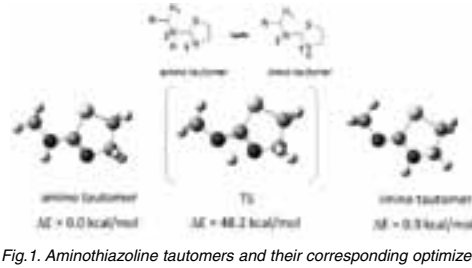

Fig.1. Aminothiazoline tautomers and their corresponding optimized geometries and transition state.

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Authors are grateful to FAPESP and CNPq for financial support.

446WE

Suppressing Background Signals via the Electronic Mixing-Mediated Annihilation (EMMA) Method

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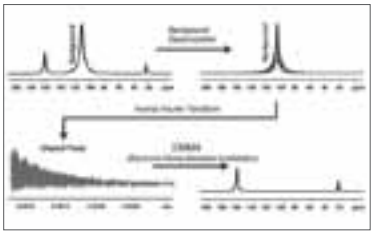
A simple procedure to effectively suppress background signals arising from various components (e.g. probe heads, stators, rotors, inserts, tubes) in NMR is presented. Similarly to the ERETIC™ method, which uses an electronic signal as an internal standard for quantification, the proposed scheme is based on an electronically generated time-dependent signal that is injected into the receiver coil of the NMR probe head during signal acquisition. More specifically, the line shape, width and frequency of this electronic signal are determined by deconvoluting the background signal in the frequency domain. This deconvoluted signal is then converted into a time-dependent function through inverse Fourier Transform, which is used to generate the shaped pulse that is fed into the receiver coil during the acquisition of the Free Induction Decay. The power of the shaped pulse is adjusted to match the intensity of the background signal, and its phase is shifted by 180° with respect to the receiver reference phase. We are presenting here some experimental results obtained using the so-called Electronic Mixing-Mediated Annihilation (EMMA) methodology.



447TH

Towards Cysteine Mediated Protein Self-Assembly

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Chemical modifications of proteins can be used to evaluate the contribution of non-native surface features to self-assembly[1].

Here, we show a mutant of the copper protein plastocyanin with a cysteine residue exposed to the solvent at the "hydrophobic patch". Compounds with different functional groups were used to modify this surface-exposed cysteine in order to study the effect on self-assembled structures of plastocyanin[2].

The ^{1}H-^{15}N backbone chemical shift perturbation map revealed intense perturbations for the residues in the β-sheet which precedes the cysteine. Current work is focused on NMR studies of protein-protein assembly induced by surface functionalization.

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448MO

Dynamics in Lipids and Peptides Studied by Oriented Solid-State NMR Spectroscopy and MD Simulations

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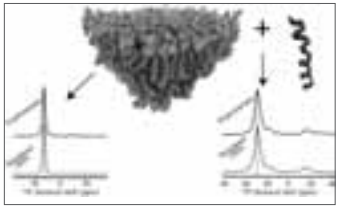

The dynamics of biological molecules are of utmost importance for the functions conducted in living organisms. Using membrane mimics composed of lipids as orientation media it is possible to align polypeptides, and study either a labelled polypeptide in the membrane or natural abundance ^{31}P and ^{13}C of the lipids. We use a new model to reproduce Solid-State NMR spectra of natural abundance ^{31}P in lipids based on MD simulations. This allows us to include the effect of time averaging on the simulated ^{31}P NMR spectra, thereby closer mimicking the conditions during a real NMR experiment.

Using Oriented Solid-State NMR spectroscopy we can access orientational information about the membrane-interacting antimicrobial peptide Alamethicin. This is done by measuring one or more of the following properties: ^{15}N chemical shift, ^{1}H-^{15}N dipole-dipole coupling and the ^{2}H quadrupole coupling. The resulting orientational constraints are used as input for calculations of the peptide orientation in the membrane and dynamics of single residues in this isolated environment.

449TU

1H NMR Metabolomics Analysis of Exhaled Breath Condensate, Serum and Urine Allow to Differentiate Sleep Apnea from Obstructive Lung Disease

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by persistent and progressive airflow limitation and by an enhanced chronic inflammatory response in the airways and in the lung to noxious particles or gases. Chronic inflammation causes structural changes and narrowing of the small airways. COPD is the fourth leading cause of mortality worldwide and results in an increasing economic and social burden. Obstructive Sleep Apnea (OSA) is a sleep disorder characterized by recurrent interruptions of breathing during sleep due to relaxation of the throat muscles; the soft tissue in the rear throat collapses and closes, resulting in blocked airways, with resultant hypoxemia. OSA is estimated to affect 3,5% of males and 1,5% of females aged over 40 years.

In this studies we used NMR spectroscopy to analyse the exhaled breath, serum and urine. The obtained data were evaluated by advance statistical analysis supported by supervised chemometric PLS-DA and OPLS-DA tools. Our studies have shown that the applied methodology allows to discriminate patients with COPD, sleep apnea and control group. Numerous of low molecular compound has been identified and such fingerprinting gave insight into disturbed by disease metabolic pathways. Investigation of all these biofluids may gave insight into the understanding of lung diseases and particularly of COPD.

450WE

Heterogeneity of Homopolymer and Heteropolymer Microgels by NMR

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The Flory temperature-induced volume transition theory for microgels was extended to account for a crosslinker density distribution reflected in high resolution transverse relaxation nuclear magnetic resonance experiments. Using this newly developed theory we have characterized quantitatively homo (PVCL) and copolymer (PVCL:PNIPAAm/PNIPMAAm) microgel systems in the bimodal crosslink density heterogeneous approximation.

Proton transverse magnetization relaxation NMR proved directly the existence of a bimodal heterogeneous morphology of the microgel particles. The ratio of the crosslink density in core and corona could be evaluated from the transverse relaxation times T_2 of each decay components. The ratio of the transverse relaxation times of core and corona was related to the Flory transition theory parameter, the number of subchains, through the scale invariant theory of polymer networks.

By fitting the dynamic light scattering temperature transition data with the extended Flory transition theory state equation, we obtain quantitative information about the microgel particle: the volume polymer fractions in the deswollen state, the number of subchains for the core and corona, and the ratio between the radius in the core and the hydrodynamic radius. From a number of good fit sets of parameters we choose the most probable morphological parameters from the microscopic and thermodynamic constraints imposed by ^{1}H transverse relaxation NMR and Flory equation of state in the approximation of a homogeneous morphology.

As an outlook to this work, the theoretical and experimental principles of Flory transition theory and NMR transverse relaxation are being used to characterize core-shell microgels composed of a homopolymer core and a homopolymer shell, and having two transition temperatures.

451TH

Cooperative Mercury Motion in the Ionic Conductor Cu2HgI4 *

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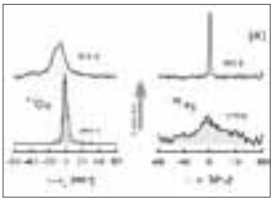
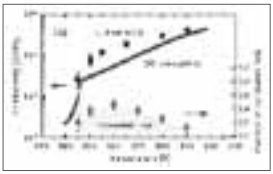


In glass-forming materials particles increasingly move together as the glass transition is approached. Such cooperativity is also found in other arrested systems and seems to be intimately connected to the slow dynamics. Here we report on the observation of large-scale dynamic correlations in a distinctly non-glassy system – the conductive phase of the ionic conductor Cu_2HgI_4 [1]. Using carefully designed nuclear magnetic resonance experiments we prove that mercury ions are the main contributors to conduction (establishing Cu_2HgI_4 as the first known mercury conductor), and show that mercury diffusion is anomalous. Nonlinear conductivity measurements, used as a probe for dynamical heterogeinity, reveal a characteristic correlation timescale $\tau_{corr.}$ The same timescale is confirmed by stimulated echo NMR measurements, where the fraction of ions trapped after τ_{corr} can be directly measured. To explain the cooperativity we propose a simple model with two essential ingredients – disorder and existence of two kinds of particles, slow (copper) and fast (mercury). We compare the results with recent studies of arrested and glass-forming materials [2, 3], thus establishing an unexpected connection between seemingly different fields.



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452MO

Static State ^{13}C NMR Study of Rubbers Deformed During MAS

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We investigated the effect of deformation of natural rubbers (NR), polyisobutylene (PIB) and cis-1,4-polybutadiene (cis-1,4 PB) caused by high centrifugal pressure of fast magic-angle spinning (MAS) on ^{13}C NMR spectra. Solid state ^{13}C MAS NMR spectra with high-power ^{1}H dipolar decoupling (DD) of rubbers show liquid-like very narrow signals. However, static state ^{13}C DD NMR spectra of rubbers after MAS showed broadened and apparently anisotropic peaks (top-left of Fig.1), although the static ^{13}C NMR spectra before MAS exhibited an isotropic and relatively narrow peak. Static state ^{13}C DD NMR spectra of the maximum elongated NR and cis-1,4 PB in a rotor became the apparently doublet. For PIB, the observed spectra were anisotropic and overlapped a doublet peak. Static ^{13}C DD NMR spectra of a strip cut from the deformed NR, cis-1,4 PB and PIB showed an angular dependence against the static magnetic field. The angular dependent ^{13}C NMR spectra of the maximum elongated rubbers showed similar tendency against the static magnetic field. However, for the restricted extension rubbers, the angular dependent ^{13}C NMR spectra of PIB differed from those of the NR and cis-1,4 PB (right of Fig.1). Magnetic susceptibility measurement was employed to elucidate the chemical shift change. The magnetic susceptibility of the strip cut from the maximum elongated (rolled) NR and cis-1,4 PB were different from those from the restricted elongation NR and cis-1,4 PB, although no difference was observed for PIB. The difference in the angular dependence of ^{13}C DD NMR spectra was attributed to the difference in magnetic susceptibility caused by π electron interaction with the static magnetic field.

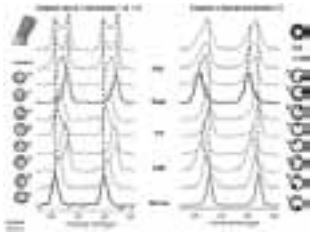

Fig. 1. Angular dependent ^{13}C NMR of NR (left: maximum elongated NR, right: dough-nut shaped NR).

453TU

Conjugated Polymer Microstructure Probed by Triplet State ODMR Spectroscopy

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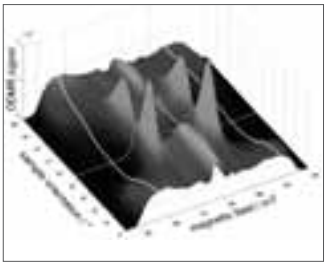
The formation of triplet excitons is considered as a factor strongly influencing the performance of organic optoelectronic and photovoltaic (OPV) devices. In OPV, studying triplets in conjugated polymers is quite challenging, as the commonly used highly performing D-A systems do not exhibit phosphorescence. Moreover, the morphology of their solid-state thin films is very complex, as the amorphous and crystalline domains coexist making the analysis of optical data ambiguous. In this contribution, we demonstrate angular-resolved optically detected magnetic resonance (ODMR) studies of triplet excitons in thin films of several novel low band-gap polymers used in highly performing solar cells. We found complex triplet patterns similar to the one shown below, which were strongly dependent on the relative orientation of the polymer film to the static magnetic field. The experimental data was simulated with *easyspin*, and the ZFS parameters D and E of triplets as well as their orientation were deduced. The studied conjugated polymers are aligned either in a so-called “edge-on“ or “face-on” configuration, i.e. the polymer side chains being perpendicular or parallel to the substrate, respectively being in agreement with the X-ray analysis. The observed dependence of the ODMR signal implies that the orientation of chromophores, on which triplet excitons are formed, is not limited to the substrate surface, but sustains across the ~100 nm thick sample. The main contribution to ODMR apparently stems from triplets formed in crystalline domains rather than in amorphous regions. In conclusion, the ODMR provides a tool for linking the microstructure information with molecular excited states in materials relevant for polymer electronics and OPV.



454WE

EXPLOITING UNUSUAL $^{1}J(^{1}H\text{-}^{14}N)$ AND $^{1}J(^{2}H\text{-}^{14}N)$ SCALAR COUPLINGS IN HR-MAS NMR SPECTRA TO CHARACTERIZE THE CHEMICAL MORPHOLOGY OF DOUBLE NETWORK HYDROGELS

Pavletta Shestakova[1], Rudolph Willem[2], Elena Vassileva[3]

[1]Bulgarian Academy of Sciences - NMR Laboratory - Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria, [2]Vrije Universiteit Brussel - High Resolution NMR Centre - Department of Materials and Chemistry, Brussels, Belgium, [3]Sofia University - Faculty of Chemistry, Sofia, Bulgaria

Hydrogels are materials with extremely high swelling capacity by water, and with promising potential to applications in biomedical, agricultural, pharmaceutical and soil engineering. They have, in general, poor mechanical performances, as they can be teared easily. By contrast, Double Network (DN) hydrogels display remarkably high resistance to mechanical stress and unusually high micro-hardness. The DN hydrogel synthesized photochemically or thermally from the individual PAMPS hydrogel (PAMPS = poly(2-acrylamido-2-methyl-1-propanesulfonic acid) swollen up with a solution of a AAm (PAAm = poly(acrylamide), in the presence of MBAA (N,N'-methylene bis(acrylamide) as a covalent cross-linker is a good example of such a DN hydrogel. High resolution magic angle spinning (hr-MAS) NMR data (, water suppressed as well as diffusion filtered ^{1}H, ^{2}H hr-MAS spectra, 2D ROESY, HSQC and ^{13}C spectra) in H_2O and D_2O reveal the existence of a pool of PAMPS amide protons or deuterons displaying respectively 1:1:1 triplet like ^{1}H and ^{2}H resonance patterns characterized as the rarely observed but nevertheless well-known $^{1}J(^{1}H\text{-}^{14}N)$ and $^{1}J(^{2}H\text{-}^{14}N)$ scalar couplings [1]. Monitoring by ^{1}H hr-MAS NMR the influence of increasing AAm concentration on the morphology and chemical structure of the DN hydrogel reveals a concerted amplitude decrease of residual olefinic resonances from the MBAA cross-linker and amplitude increase of the triplet splittings, unambiguously evidencing a cooperative mechanism in the DN generation. The latter is correlated to a parallel strengthening of mechanical stress resistance and micro-hardness of the PAMPS/PAAm DN.

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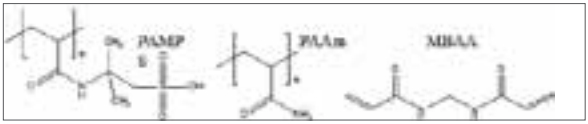

455TH

DYNAMIC DEUTERIUM MAS NMR OF MOLECULES GRAFTED AT THE INNER SURFACE OF MESOPOROUS MATERIALS

Jayanthi Sundaresan, Veronica Frydman, Shimon Vega

Weizmann Institute of Science, Rechovot, Israel

Dynamics of molecules on the surface has been an area of interest due to its extended applicability in various fields. The work presented here is an attempt to look at the water induced local mobility of small organic molecules covalently attached to the inner walls of mesoporous materials using solid state NMR. Towards this study we have mainly employed deuterium magic angle spinning (MAS) NMR because of the sensitivity of the deuterium quadrupole spectra to dynamics. A dynamic deuterium MAS NMR study was carried out on a molecule with a deuterated methyl (CD_3) group at the free terminus that was grafted to MCM-41 which enabled efficient monitoring of their local mobility. Deuterium sideband spectra were recorded under high and low hydration levels Spectral changes were observed indicating dynamics offered by hydration of the mesoporous surface. Observation shows that a part of the grafted molecule remains static irrespective of the hydration state of the sample, while the rest show spectral changes induced by single water molecules.

To further substantiate the experimental results, Molecular Dynamic (MD) simulation has been carried out mimicking the grafted molecule on a mesoporous template. Analysis of the MD simulation provided a description of the molecular mobility resulting in the averaging process of the quadrupolar tensor components. The results corroborate the earlier observed two site jump model describing the dynamic molecules. Similar studies were extended to molecules with deuterated methylene grafted to mesoporous materials. Analysis of the experimental observations followed by MD simulations could again provide a dynamic model for the local mobility of these molecules.

456MO

Combined Solid-State NMR and DFT Investigation of the Effects on Local Structure of Boron Incorporation in Non-crystalline Layered Silicates

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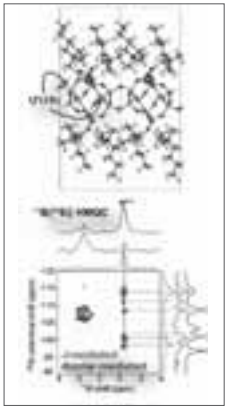
[1]CEMHTI CNRS-UPR3079, Université d'Orléans, Orléans, France, [2]Department of Chemical Engineering, UCSB, Santa Barbara, USA

In recent years, much exciting research has focused on the incorporation of heteroatoms into porous materials for catalysis, ion exchange, and gas separation. Controlling the heteroatom distributions and locations in the silicate materials is a major challenge, since their incorporation deteriorates the molecular order by generating compositional and geometric local disorder, which limits the effectiveness of diffraction-based characterization techniques. Boron atoms were successfully incorporated in non-crystalline layered silicate materials with short-range molecular order. A combination of solid-state NMR experiments and first principle calculations was used to explore the effects on the local structure of silicon to boron substitution within the layered silicate frameworks. They reveal profoundly different incorporation behaviours for two strongly-related materials, which contrast with their otherwise highly similar morphologies and compositions. We demonstrate for the first time a preferential incorporation of boron in single crystallographic tetrahedral site for one material but not for the other. The nature of the incorporation site and the resulting structural modifications, which are also profoundly different in the two materials, are described on the basis of $^{11}B\{^{29}Si\}$ NMR correlation experiments probing B-Si spatial proximities and B-O-Si connectivities, and calculations of ^{11}B chemical shifts at the density functional level of theory (DFT). This opens new routes to understand and control heteroatom locations in three-dimensional zeolite catalysts obtained by co-condensation of these and other heteroatom-containing layered silicate materials.



457TU

Mesoscopic Dynamics and the Mechanism of Gelatin Sol-Gel Transition

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We present an extensive analysis of polypeptide dynamics in gelatin water solutions at the micron scale, revealing an emergent length scale and slow cooperative diffusion. Combining pulsed field gradient NMR diffusometry and low frequency conductivity spectroscopy[1] we are able to closely track the motion of polypeptide chains during the transition from sol to gel, resolving two distinct diffusion regimes.

At short timescales we observe an effective diffusion coefficient which is shown to correspond to single chain diffusion. This effective diffusion survives down to a characteristic, temperature-independent length, playing the role of a pore size. Simultaneous measurements of diffusion and conductivity also directly provide the number of non-bound chains in the solution, enabling us to follow chain aggregation and gel network formation. A concentration master curve is found for the aggregation process, with scaling exponents different from microscopic polarimetry measurements[2]. This is explained by taking additional, emergent hydrophobic interactions between chains into account.

At longer times (~1 s) both NMR and conductivity measurements detect an additional diffusion process, related to cooperative motion of chains and similar to dynamics observed in glasses[3] and coloidal gels[4]. Direct measurements of the four-point correlation function using PFG-NMR show a peak at the characteristic timescale of the slow process, confirming its cooperative nature. The slow process abruptly transforms from elastic to dissipative at the sol-gel transition.

Our results prove that the gelation process of gelatin is significantly more complex than simple percolation[2,5] or phase separation6 models indicate, with emergent interactions playing an important role.

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459TH

STRUCTURAL AND DYNAMICAL PROPERTIES OF INDOMETHACIN MOLECULES EMBEDDED WITHIN THE MESOPORES OF SILICATES AND METAL-ORGANIC FRAMEWORK MATERIALS: A SOLID-STATE NMR VIEW

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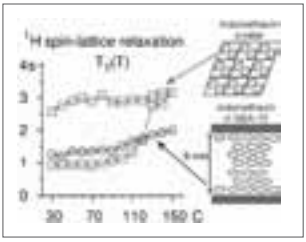
The structural properties of the mesoscopically confined drug, and drug-drug and drug-matrix interactions were investigated in model drug-delivery systems prepared from MCM-41 and SBA-15 mesoporous silicate matrices, and Cr-MIL-101, Fe-MIL-101, and Al-MIL-101 metal-organic frameworks, loaded with different amounts of indomethacin molecules. ^{1}H MAS and ^{1}H-^{13}C CPMAS NMR spectra indicated that only when the concentration of indomethacin within the mesopores becomes sufficiently high, hydrogen bonds between the drug molecules become abundant. Nitrogen sorption analysis and comparison of ^{1}H spin-lattice relaxation times in progressively loaded mesoporous matrices suggested that at low loading concentrations indomethacin forms layers on the walls of the mesopores, and that at moderate or high loading concentrations rigid nanoparticles that extend throughout the entire mesopore cross-section are formed. Variable-temperature ^{1}H spin-lattice relaxation measurements showed that the mesoscopically confined indomethacin is significantly different from the bulk amorphous indomethacin. It does not become rubbery and it exhibits a solid-solid transition that is similar to the phase transition of the crystalline indomethacin solvate. The obtained results are important for the understanding of the release kinetics and for the estimation of the total amount of the released drug from the drug-delivery system.



460MO

STRUCTURAL BIOLOGICAL STUDIES OF PLANT CELL-WALL: RESONANCE ASSIGNMENT OF ^{13}C LABELED LIGNOCELLULOSE BY SOLUTION AND SOLID-STATE NMR

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Lignocellulose, the major component of plant cell wall, is one of the most abundant and available biomass as non-food materials. However, effective uses of lignocellulose are hampered by the its structural complexity, consisting of cellulose microfibrils embedded in a matrix of hemicelluloses with lignin. Therefore, establishing effective methods for structural analysis and characterization for the lignocellulose are expected. Previous studies of lignocellulose structural analysis using NMR required isolation into each compornent. In recent study, an analytical method of lignocellulose without isolation was developed using ^{13}C-HSQC with DMSO-type solvent system. However, it was difficult to assign signals of lignocellulose unambiguously, especially those of polysaccharides which have similar chemical structures, due to significant signal overlapp on the spectrum.

In this study, we analyzed structure of lignocellulose without isolation into each component using high resolution multidimensional NMR spectroscopy. For the signal assignment of lignocellulose in complicated spectra, we employed homo- and heteronuclear correlation spectroscopy such as ^{13}C-HSQC-TOCSY, ^{13}C-HSQC-NOESY, ^{13}C-INADEQUATE, 3D-HCCH-COSY for ^{13}C labelled lignocellulose dissolved in DMSO-pyridine mixture solvent to achieve ^{1}H-^{13}C and ^{13}C-^{13}C correlations, and subsequently signals were assigned with nuclei of lignocellulose from ^{1}H-^{13}C and ^{13}C-^{13}C correlations. In addition, we attempted to analyze structure of lignocellulose using solid-state NMR.

In solution state analysis, 16 structural units (89 signals) were assigned. 3D-HCCH-COSY, which is traditional 3D pulse sequence for aliphatic carbon resonance assignment using transferring ^{13}C-^{13}C magnetization after INEPT transfer, are the powerful method for signal assignment of lignocelluloses, because it made overlapped signals spread. In solid-state NMR, crystalline and amorphous cellulose signals were assigned by ^{13}C-INADEQUATE and ^{13}C-DARR (Dipolar Assisted Rotational Resonance): conventional ^{13}C-^{13}C correlation pulse sequences in solid-state NMR. For analysis of lignin and hemicelluloses which were mobile and less detectable components, we employed NQS-DARR combined NQS dipolar dephasing pulse for non-quaternary suppression with DARR dipolar recoupling experiments for achieving structurally neighbored ^{13}C-^{13}C correlations.

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Host-Guest Interactions of Charged Dendronized Polymers Analyzed with DEER

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We present data from double electron-electron resonance (DEER) spectroscopy characterizing the interaction between peripherally charged dendronized polymers (denpols) and different guest-molecules.

Denpols are macromolecules that carry a dendron side-group on every repetition unit of their backbone. Due to the enormous sterical crowding of the side-groups denpols can be approximated as very stiff cylindrically shaped molecules.[1]

Exploiting self-assembly of paramagnetic probe molecules (Fremy's salt) on the cylindrical surface of the denpols and detecting the distance distribution between the probes, we are able to determine the size and the shape of the cylindrical denpols in solution. These results show that the radii of the cross-sections of denpols grow with increasing generation of the dendron side-groups and that the radii (generation 4: approx. 2 nm) are fully independent of the environment of the denpols. This extraordinary shape-persistence makes charged denpols excellent candidates for fitting into the concept of molecular objects (persistent shape, defined envelope, molecular precision).[2]

Further, the potentially enormous interior of the denpols gives rise to the possibility to not only assemble molecules on the denpols' surface, but also incorporate guest-molecules inside them. Applying CW EPR we found that up to 2.2 spin-labeled fatty acids can be incorporated into a generation 4 dendron side-group and can be released by varying the pH or the ionic-strength of the aqueous environment.

Yet, the arrangement of guest-molecules in dendritic structures is a complex issue. We therefore propose a DEER-technique that features isotopically labeled nitroxides, to determine the approximate distance of the spin-labels of the fatty acid guest-molecules inside the denpols from ^{15}N-labeled Fremy's salt assembled on the surface of the denpols.

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462WE

Monitoring the Activation Process of the Giant Pore MIL-100(Al) by Solid State NMR

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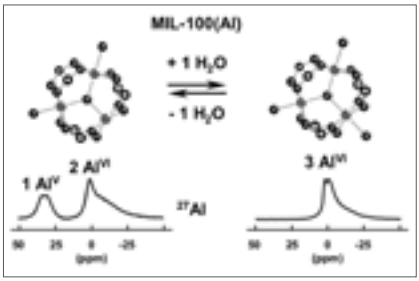
The ability of a reversible removal of coordinated water in the mesoporous MIL-100(Al) upon dehydration/rehydration process has been investigated using solid-state NMR techniques. Double resonance techniques such as ^{1}H{^{27}Al} TRAPDOR and ^{27}Al{^{1}H} HETCOR were used in order to probe proximity between species containing protons and the inorganic framework. On the other hand, ^{1}H-^{1}H correlation experiments using the DQ-BABA and RFDR sequences were employed to investigate the interaction between the different kinds of protonic species including those of the organic framework. The compound shows a remarkable thermal stability up to 370 °C, with small structural alterations. Only one water molecule per Al3 trimer, the main inorganic building unit, was found to leave the trimer producing only one coordinatively unsaturated site (cus) at 350 °C. Compared to the chromium isotype MIL-100(Cr), the difference of generated number of cus site per trimer, explains at the same time the different origin of the stability of the aluminum trimer versus chromium, and the different chemistry that it will induce between both MIL-100(Al) and MIL-100(Cr). The as-synthesized compound contains an important amount of extra-framework 1,3,5-benzenetricaboxilic acid, $(H_3btc)_{EF}$, encapsulated into the large pores and most of it could be removed upon DMF/water activation. However, 0.3-0.5 molecules of $(H_3btc)_{EF}$ per Al_3 trimer were found strongly interacting with the framework and their complete removal was difficult to achieve.



463TH

Advanced Solid-State NMR Study of Anhydrous and Hydrated Ordinary Portland Cement

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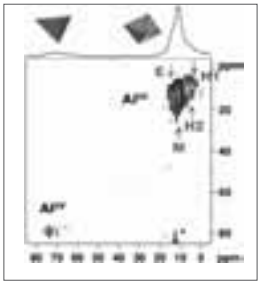
A suite of multinuclear multidimensional solid-state NMR experiments has been employed to investigate the hydration features of ordinary Portland cement (OPC) pastes. Both anhydrous and hydrated OPC pastes were examined, paying particular attention to the structural modification of hydrated pastes as a function of the hydration degree. ^{1}H MAS NMR was used to follow the nature and dynamics of water in OPC paste as well as the formation of various hydroxyl species. ^{29}Si MAS NMR has been extensively used to characterize the silicate species and their network formation mechanism upon hydration. ^{27}Al MAS and MQMAS NMR experiments revealed the formation of different calcium aluminate hydrate species after cement hydration. The molecular nature of calcium silicate hydrates and substituted calcium aluminate hydrates in the hydration products were determined and quantified. Moreover, the evidences of chemical and structural modifications of various phases in pastes submitted to accelerated carbonation have also been investigated in order to clarify the degradative behavior of exposed cement pastes.



^{27}Al MAS and MQMAS NMR spectra of hydrated OPC paste cured for 28 days

464MO

Selective Suppression of Molecular Signals in NMR Spectra by Specific Matrix Addition

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The purpose of the work is to test the selectivity of Molecularly Imprimented Polymers (MIPs) for the adsorption of a target molecule during the NMR experiment. The net result on the NMR spectrum of an effective capture of the target by the polymer would be the disappearance of its signal. The experiment is demonstrated here for Bisphenol A (BPA), which is a dangerous food contaminant originating from plastic containers. Commercial chromatographic phases have been thus developed to detect this molecule with a very high selectivity. The signal suppression experiment is depicted in the Figure of BPA, for a mixture containing Biphenyl, Dibenzyl and Aniline. BPA has peaks (arrows) in the aliphatic and aromatic region of the ^{1}H NMR spectrum. More specifically, it presents an almost perfect overlap with one of the resonances of aniline, at about 7.1ppm. Removal of the BPA signal by any irradiation scheme will be impossible without strong perturbation of the aniline peaks. Conversely the spectra in Figure (Panel B) shows as the addition of the specifically capturing MIP achieve this goal without introducing significant deviations for the rest of the signals. This method has thus the potential of finding a broad range of applications, provided the adapted materials are made available.

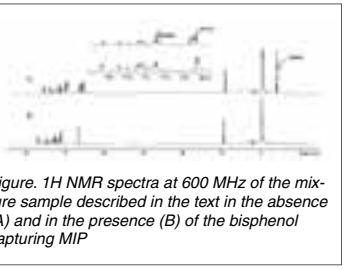

Figure. 1H NMR spectra at 600 MHz of the mixture sample described in the text in the absence (A) and in the presence (B) of the bisphenol capturing MIP

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465TU

High Resolution Calcium-43 NMR Studies of Calcite to Aragonite Phase Transformation

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How calcium carbonate forms calcite and aragonite in mollusk shell is a long-standing enigma. It has been known that the formation of calcium carbonate in mollusk shell starts at the prismatic calcite layer, whereas the nacre layer appears subsequently in the inner side. To shed more light on this biomineralization process, we employ solid-state ^{43}Ca NMR to study the in-vitro phase transformation of calcite to aragonite. Using the gas diffusion approach, we obtained the phase of Mg-calcite initially and the system subsequently transformed to aragonite as the reaction time proceeded. We find that the ^{43}Ca NMR parameters of Mg-calcite, which is a very important biomineral phase, are very similar to those of pure calcite. Under the high-resolution condition provided by magic-angle spinning at 4 kHz, we can monitor the variation of the ^{43}Ca NMR parameters of the aragonite signals for the samples obtained at different reaction times, viz. 2 hours, 24 hours, and 9 days. In particular, the C_Q and η_Q data of the aragonite phase have significant variation between the 2-h and the 24-h samples, whereas the ^{43}Ca NMR parameters of the 24-h and 9-d samples are very similar. In addition, the ^{43}Ca T_1 parameters were determined to increase monotonically with the reaction time. We conclude that the initial precipitation of Mg-calcite is a kinetic-driven event. In the presence of significant amount of Mg^{2+} ions, the system will transform to aragonite, where the ions in the lattice can have considerable motional dynamics. As a result, the corresponding C_Q value is reduced and the spin-lattice relaxation becomes relatively more efficient for the aragonite site of the 2-h sample. As the crystallinity of aragonite improves further, the NMR parameters for the aragonite phase of the 24-h sample become very similar to those obtained for pure aragonite (9-d sample). To our knowledge, this is the first illustration of how ^{43}Ca NMR spectroscopy can be exploited to monitor the biomineralization process of calcium carbonate.

466WE

Detection of Phase Biaxiality in Liquid Crystals by Xenon NMR

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The existence of biaxial nematic liquid crystals (LCs) was predicted already in the 1970's. In recent years scientists' interest has focused synthesizing compounds that could form thermotropic biaxial nematic phases as, due to symmetry properties different from conventional uniaxial LCs, these new materials are expected to lead to improvements in the properties of the LC displays as well as to new applications. There are several experimental techniques, such as ^{2}H NMR and X-ray diffraction, which can be used to confirm the phase structure of liquid crystals. Nevertheless, all of these methods have been criticized whether they give unambiguous prove of the phase biaxiality in nematics.

NMR of dissolved xenon gas has over the years proven to be an extremely sensitive tool to study the properties of all kinds of materials. In this work we show that ^{131}Xe second order quadrupole shift (SOQS) observable in NMR spectra of xenon dissolved in liquid crystals provides a method to unambiguously distinguish between uniaxial and biaxial liquid crystal phases [1]. Besides revealing the biaxiality, the ^{131}Xe SOQS offers a novel method to determine the tilt angle in smectic C phases. As an example, the ^{131}Xe SOQS in a ferroelectric liquid crystal is reported.

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467TH

Towards Understanding NMR Shift Distributions in Doped and Nanoscale Semiconductors

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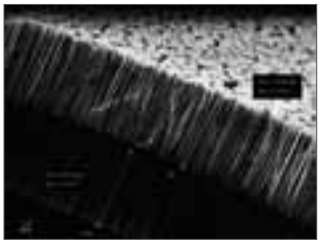
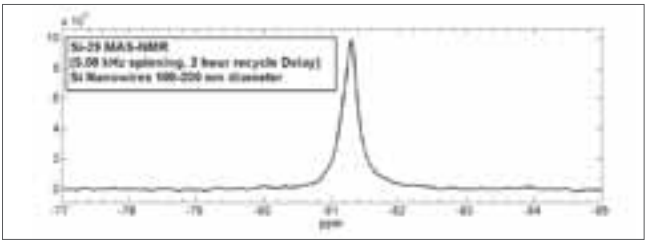
NMR spectra of semiconductors either intentionally doped or of submicron size often are inhomogeneously broadened by distributions of either Knight shifts or chemical shifts. Understanding the structural and electronic factors responsible for such distributions, and being able to distinguish between the two types of shift interactions, is at a primitive stage. Both silicon and (hexagonal) gallium nitride (h-GaN) are important systems for better understanding the nature of NMR shift distributions.

The ^{71}Ga and ^{14}N MAS-NMR spectra of h-GaN doped n-type with Ge exhibit large distributions of Knight shifts that have been previously interpreted in terms of inhomogeneous doping. Variable-temperature results indicate a highly metallic semiconductor.

The ^{29}Si MAS-NMR spectra of silicon *uniformly* doped n-type with Sb surprisingly show a large distribution of Knight shifts that can be studied using a SPARTAN hole-burning sequence. A marked temperature dependence points to the effects of disorder in this barely-metallic semiconductor, and suggests that ^{29}Si MAS-NMR should be a powerful probe of the microscopic nature of the Mott metal-insulator transition.

Chemical shift distributions in ^{29}Si MAS-NMR of a variety of nanosilicon materials can be up to three orders of magnitude larger than the starting material (crystalline silicon). A highly regular silicon nanowire sample (see SEM below) yields the narrowest chemical shift distribution seen for any nanoscale semiconductor (see spectrum below). *The results clearly show that large inhomogeneous linewidths are not an intrinsic feature of semiconductors in nanoscale form.*





468MO

Solid-state NMR Characterization of Metal-Organic Frameworks at Ultrahigh Magnetic Field

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Metal-organic frameworks (MOFs) are a new generation of porous materials with many potential applications. They are hybrid inorganic-organic solids prepared via self-assembly of metal cations with organic linkers to form three dimensional porous networks with novel topologies. Although the structures of many MOFs can be determined by single crystal X-ray diffraction, a significant number of MOF structures have to be refined from more limited powder XRD data due to the lack of suitable single crystals. In such cases an unambiguous structure solution requires additional information from complementary techniques such as solid-state NMR (SSNMR). However, many important MOFs contain metal ions whose NMR active isotopes (such as ^{67}Zn, ^{91}Zr, ^{25}Mg) are quadrupolar and unreceptive. As a result, SSNMR has not been used to characterize these low-gamma nuclei.

In this talk, we present natural abundance solid-state ^{67}Zn and ^{25}Mg NMR spectra of several representative Zn- and Mg-containing MOFs acquired at ultrahigh magnetic field of 21.1 T. The results indicate that ^{67}Zn and ^{25}Mg MAS, 3QMAS and static spectra are highly sensitive to the metal local environment. In combination with theoretical calculations, the analysis of ^{67}Zn and ^{25}Mg spectra yields valuable insights into the meal local structure as well as host-guest interaction. The work demonstrates that ^{67}Zn and ^{25}Mg solid-state NMR at very high field is a useful tool which can now be added to the arsenal of the techniques for characterization of MOFs.

469TU

NMR Studies on Polymer Composites

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Polymer materials are usually composites from polymer and an inorganic fillers. The interaction between the polymer defines the materials properties (thermal, electric and mechanical) and the long-term performance. The modification of the filler for enhanced compatibility is followed by solid-state NMR. In particular 27Al spectra of layered double hydroxides provide insight into the structure modification in the filler during the modification steps and the incorporation into the polymer. The interaction of the polymer with the filler is evident from shortening proton T2 and T1rho. Shorter relaxation times are indicative of the interaction between filler and polymer resulting in reduced polymer mobility. Both relaxation times in the polymers exhibit at least two components, which are evaluated by inverse Laplace transform. The long components associated with more mobile polymer chains are most affected. Data are compared to rheo-NMR results from the melt. Upon shear the unfilled polymer exhibits an initially unexpected prolongation of T2, which is explained by loss of chain entanglements as result of shear. In the filled polymer the filler interaction is more significant, no signatures of disentanglement has been observed. While mechanical stress on elastomers results in reversible changes, time-dependent effects are observed, in semi-crystalline polymers, consisting of rigid crystallites interconnected by rather flexible amorphous regions, time-dependent effects are observed. The reason is creep of polymer chains through the crystallites under mechanical load. This has been investigated by in-situ NMR studies under uniaxial stress. Drastic changes are observed in both T2 and T1rho. Both have been showing a time dependence from the start of the mechanical load. The NMR results are correlated to the simultaneously measured stress-strain curves. Notably changes in the NMR parameters are observed on a timescale significantly longer than the timescale of mechanical stress relaxation, which has been monitored simulateously in the same apparatus.

470WE

Advances in *In Situ* NMR and MRI Studies of Energy Materials

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Ex situ studies of batteries are limited by self-relaxation of the electrode materials before a measurement can be obtained. The application of advanced magnetic resonance techniques, such as MRI and complex NMR experiments, *in situ* has the potential to monitor dynamics and visually monitor changes in functioning electrochemical systems in real time. Here we present *in situ* NMR and MRI of symmetric Li metal batteries and carbon based supercapacitors.

Although, Li metal is the lightest and most electropositive anode material, giving the greatest capacity and voltage for battery, commercial anode materials are most commonly made of carbon, due to safety issues associated with dendritic lithium (Li formation. Previous studies utilized bulk magnetic susceptibility and skin depth effects to quantitatively study Li microstructure growth using *in situ* NMR [1]. Utilizing both *in situ* ^{7}Li NMR and MRI we have further investigated the electrochemical conditions under which Li microstructures form in LIBs [2].

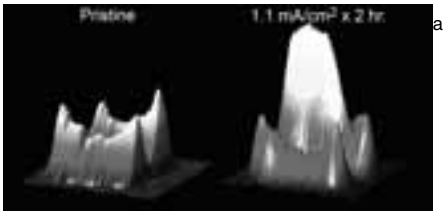

Figure 1. In situ 2D ^{7}Li MRI images of a symmetric Li metal battery before (pristine) and after charging.

Supercapacitors function based on the separation of the cations and anions of the electrolyte to the negative and positive charged electrodes, respectively. The application of *in situ* ^{11}B NMR to study the liquid electrolyte provides insight into the mechanism of supercapacitors, by monitoring changes in the BF_4^- environment in carbon electrodes with a tetraethylammonium tetrafluoroborate ($TEABF_4$) electrolyte [3].

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471TH

NMR Study of Thermosensitive Homopolymers and Copolymers in Aqueous Solutions

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It is well known that thermosensitive polymers show in aqueous solution during heating a phase separation which is a macroscopic manifestation of a coil-globule transition followed by aggregation and formation of so-called mesoglobules. Their thermosensitivity makes these polymers interesting for miscellaneous biomedical and technological applications, e.g., as drug release polymers.

Formation of globular structures results in a marked line broadening in NMR spectra. The fraction of units with significantly reduced mobility (phase-separated fraction) can be determined from integrated intensities in high-resolution ^{1}H NMR spectra. The strong dependence of the transition temperatures on polymer concentration was revealed in this way for poly(N-vinylcaprolactam) (PVCL) solutions in D_2O while for poly(*N*-isopropylmethacrylamide) (PIPMAm) solutions the transition interval is independent of polymer concentration. NMR spectroscopy also enabled us to determine thermodynamic parameters ΔH and ΔS characterizing the phase transition. Comparison with ΔH values obtained by DSC confirmed that the cooperative unit is the whole macromolecule. The existence of water bound in mesoglobules and different character of the water released from mesoglobules with time were shown for PVCL and PIPMAm solutions by ^{1}H T_2 relaxation times of HDO.

Combination of NMR and DSC was also used to investigate phase separation in D_2O solutions of P(IPMAm/AAm) random copolymers. From the NMR results it follows that in respective mesoglobules there are domains where both hydrophilic AAm sequences (units) and surrounding IPMAm sequences are hydrated and therefore mobile. Both phase- separated fraction determined by NMR and ΔH determined by DSC decrease faster than the content of thermosensitive IPMAm units in the copolymer. Comparison of ΔH values determined by NMR (using van't Hoff plots) and DSC shows that the size of the cooperative units undergoing the transition as a whole decreases with increasing AAm content in the copolymer.

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472MO

Characterization of the Phase Transformation of Calcite to Aragonite

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Calcium carbonate is one of the most commonly found biominerals in organisms. The mechanism of how Nature selects different phases of calcium carbonate in the nacreous and prismatic layers of mollusk shell remains an outstanding mystery. Previous studies show that magnesium ions may play a role in inducing the phase transformation of calcite to aragonite. To shed more light on the biomineralization process, we have developed an in-vitro model system to study the phase transformation of calcite to aragonite, which is based on the gas diffusion approach in the presence of magnesium ions. The samples collected at different reaction times have been characterized by a number of physical techniques, with particular emphasis on solid-state 43Ca NMR. We found that the initial sample is mainly constituted of Mg-calcite, where the incorporation of magnesium ions in calcite has been confirmed by X-ray powder diffraction. As the reaction time proceeds, the formation of aragonite takes place, which is accompanied by the slow dissolution of the Mg-calcite. The transition from calcite to aragonite in our system might be caused by the increase in the Mg/Ca ratio of our solution as calcium carbonate continuously precipitates. As indicated by the SEM images, the crystallinity of aragonite improves with the reaction time. By measurements on isotopically enriched samples, the 43Ca chemical shift anisotropy and quadrupole parameters at the calcium sites of aragonite were extracted, from which we show that 43Ca NMR is a very sensitive spectroscopic technique to follow the phase transformation of calcium carbonate.

473TU

NMR Investigations of Acid Doped Polybenzimidazole, an Electrolyte Membrane for Fuel Cells

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Fuel cells (FCs) are devices that convert the chemical energy of their reagents into electrical energy without resorting to a thermal Carnot cycle. Polymer electrolyte membrane FCs (PEMFCs) have raised considerable interest for their high energy conversion efficiency, high power density, ease of assembly, silent operation, and good environmental compatibility. Fuel cells consist mainly of an anode, a cathode and a membrane acting as electrolyte and which allows charges to selectively move between anode and cathode. Acid doped polybenzimidazole (PBI) polymers have become a very promising system for use as membranes in high temperature PEMFCs.

The conductivity in FCs is well known to be directly related to the mobility of the ions through the membrane, which might involve a multi-step proton hopping process (Grotthuss mechanism) or the bulk mobility of a proton carrier.

We report investigations of poly(2,5-benzimidazole) (AB-PBI) doped with phosphoric acid (PA) with different methods of NMR [1]. We have characterized the molecular interactions between the polymer matrix and the acid dopant. Heteronuclear correlation experiments were used to study hydrogen bonding characteristics. We have further studied the dynamics and order in the system at different doping levels and temperatures using 2H-NMR and 1H-Double-quantum spectroscopy. This information is of fundamental importance in order to elucidate the role of phosphoric acid in the conductivity mechanism of polybenzimidazole [2, 3].

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474WE

Full Differentiation and Assignment of Boron Species in the Electrolytes $Li_2B_6O_9F_2$ and $Li_2B_3O_4F_3$ by Solid-State ^{11}B-NMR Spectroscopy

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A new class of potential ionic conductors, namely crystalline lithium fluorooxoborates, has been synthesized in our group. The rationale behind the design was to provide a migration path along which the ions experience a flat electrostatic potential profile. In oxoanionic matrices, oxygen atoms in terminal positions are notorious for trapping cations. This situation can be alleviated by adding fluorine to boron in trigonal planar coordination, resulting in four-fold boron coordination, with a formal negative charge located on the boron atom. Thus, in fluorooxoborates, the negative effective charges at the terminal oxygen or fluorine atoms are reduced, enhancing lithium ion mobility.

Three new fluorooxoborates were discovered and characterised by X-ray diffraction, namely LiB_6O_9F [1], $Li_2B_6O_9F_2$ [2], and $Li_2B_3O_4F_3$ [3]. The bulk ionic conductivity of LiB_6O_9F is 6.6 x 10^{-9} S.cm^{-1} at 673 K [1], classifying it as a solid electrolyte, but is much lower at room temperature. To improve conductivity, the compounds $Li_2B_6O_9F_2$ and $Li_2B_3O_4F_3$ with higher stoichiometric lithium content were synthesized. Both $Li_2B_6O_9F_2$ and $Li_2B_3O_4F_3$ have also been extensively studied by solid-state NMR spectroscopy. In a first step, the complex ^{11}B-MAS-NMR spectra exhibited by the fluorooxoborates were deconvoluted using the information gained from 3QMAS spectra. The sub-spectra were assigned to the boron species in the crystal structure by evaluation of chemical shift and quadrupolar interaction, which allows clear differentiation of boron atoms in tetrahedral and trigonal coordination. Efficient ^{19}F spin decoupling techniques [4] were used to determine the proximity of boron atoms to fluorine by analyzing the response of the ^{11}B spectrum to application of decoupling, allowing full assignment of all components of the complex MAS spectra [5].

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475TH

Microstructure of Pluronic F127 Alginate Composite Hydrogel Probed by NMR and Rheology

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A composite hydrogel formed by Pluronic F127 and alginate in water, crosslinked by divalent ions, was devised to address the in situ sustained delivery of innovative antiproliferative agents [1], employed to prevent coronary restenosis. Here we report a study of the composite gel with Ca^{2+} as cross linking agent, as well as of the parent systems, namely the Pluronic F127 water thermogel and the alginate-Ca^{2+} water, ionotropic gel. They were extensively investigated through NMR, by measuring water transverse relaxation,T_2, by CPMG, and diffusion, by PGSE NMR, at low field (0.47 T), and the diffusion of the probe molecule theophylline at high-field (11.74 T) by PGSTE NMR (one-shot sequence [2]). All measurements were carried out at body temperature, 37 °C. Diffusion both of water and of theophylline in alginate-Ca^{2+} hydrogel is slightly hindered compared to pure water, while in the Pluronic one it is much slower. Nonetheless, echo decays are exponential with diffusion coefficients independent of diffusion time, since both materials are quite homogeneous. Conversely, the diffusion in the composite gel is anomalous, the echo decays deviating from exponential trends. Thus, either a stretched exponential or a fractional order analysis model [3] has to be employed to rationalize the experimental trends. The parameters returned by the best fit procedures indicate high structural complexity (porosity and tortuosity). The fractional space parameter, μ,[3] increases with diffusion time, indicating that diffusion decreases at longer times. These observations support the hypothesis of the interaction of the PEO head-groups of Pluronic with the biopolymer, decreasing their hydration, opening water channels in the Pluronic composite gel, and, at the same time, forming barriers that hamper long range diffusion, thus succeeding in a sustained release of embedded drugs. The mechanical spectra, along with T_2 values of water, suggest that the mesh size increases from the pure polysaccharide to the composite gel, while the gel strength reduces.

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476MO

Study of the Structural, Phase and Dynamic Properties of an Anion-Exchange Membrane for Polymeric Fuel Cells by Means of Solid State NMR Techniques

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Polymeric materials are playing a very important role in the development of the fuel cell technology thanks to their low cost, easy processability and high flexibility.[1] In particular, a great interest is now focused on the polymeric anion exchange membranes (AEM) since they provide several advantages with respect to the proton exchange membranes widely studied in the past, such as the use of cheaper catalysts, a faster kinetics at the electrodes and a decrease of the fuel cross-over.[2]

Since the performances of these membranes in the final fuel cells are strongly dependent on their structural and dynamic properties on a nanometric and sub-nanometric scale, the study of these properties is very important to devise and prepare optimized and improved systems. In this field solid state NMR (SSNMR) is one of the most promising techniques,[3] even if its use is still limited.[4]

In this work the structural, phase and dynamic properties of an anion exchange membrane, in which the unit responsible for the ionic conduction, 1,4-diazabicyclo[2,2,2]octane (DABCO) containing at least one quaternary ammonium site, is grafted to a LDPE film through a 1-chloro-4-vinyl-benzene (VBC) moyety, have been investigated and characterized by means of a multi-nuclear and multi-technique SSNMR approach. ^{13}C CP/MAS selective, ^{1}H MAS, and ^{1}H-^{13}C HETCOR experiments allowed detailed information on the phase and dynamic properties of LDPE, DABCO and VBC to be obtained, and the comparative study of the systems obtained at three different steps of the preparation process was very useful to understand the morphological changes, concerning in particular LDPE, that occurred during the preparation of the membrane. In particular, the presence of quite rigid amorphous and crystalline PE domains, whose dynamic behaviour in the range of kHz significantly changes (as shown by temperature-variable CP/MAS experiments) during the preparation process, has been observed. Moreover T_1 and $T_{1\rho}$ measurements allowed to get insights into the degree of homogeneity of the polymeric film, as well as into the DABCO distribution in PE amorphous and crystalline domains.

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477TU

^{1}H AND ^{31}P DIFFUSION NMR - CHARACTERIZATION OF PLATINUM(II) DINUCLEAR COMPLEX CONJUGATED TO POLYPHOSPHOESTERS

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Drug conjugation to a macromolecular carrier is a promising strategy especially in the field of cancer therapy. It has been demonstrated that long-circulating polymeric carriers can preferentially and effectively accumulate in solid tumors – a phenomenon known as the "Enhanced Permeability and Retention (EPR) effect". Dinuclear platinum complexes with polyamine linkers constitute a class of compounds displaying novel antitumor and DNA-binding properties. These compounds are of particular interest because they show high activity in vitro and in vivo against tumor cell lines that are resistant to cisplatin.

In the present investigation spermidine-bridged dinuclear platinum(II) complex prodrug forms were obtained via conjugation to poly(oxyethylene H-phosphonate)s. These are polymers with repeating PEG building block linked by phosphoester groups. Two precursor polymers were used derived from polyethylene glycols with molecular weight 200 g/mol and 600 g/mol (PEG200 and PEG600).

^{1}H and ^{31}P spectra in combination with diffusion ordered NMR spectroscopy (DOSY) were applied to prove the conjugation reaction between the polymer carrier and the bioactive agent as well as to characterize the obtained new structures.

The results from the DOSY spectra show that the new polymeric conjugates form particles with average hydrodynamic radii from 5 nm to 18 nm depending on the polymer used.

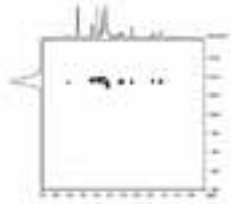

Figure. 1H DOSY spectrum of Pt(II) complex conjugated to PEG600 based polyphosphoester.

Acknowledgement: The support by the NSF of Bulgaria (Grants: DO 02-198/2008 and DRNF 02/13/ 2009) is highly acknowledged.

478WE

DIFFUSION NMR CHARACTERIZATION OF DRUG DELIVERY SYSTEM BASED ON CORE-SHELL TYPE STAR COPOLYMER

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The present communication reports the NMR characterization of a new core-shell type star copolymer whose interior presents hyperbranched polystyrene bearing arms of poly(acrylic acid). This macromolecular architecture possesses key features for application as cisplatin carrier. Changes of star size and mobility upon drug complexation were followed by conventional and diffusion ordered NMR spectroscopy (DOSY). The signal broadening observed in the ^{1}H NMR spectra evidenced the reduced chain mobility as result of drug immobilization at the coordination sites of the polymer. The DOSY spectra indicate star contraction at the initial stage of cisplatin loading. Further increase of the drug amount resulted in appearance of larger particles due to cisplatin induced crosslinking.

To avoid crosslinking upon loading the star macromolecules were PEGylated applying cisplatin as reversible linker. The DOSY spectra confirm the PEGylation of the particles and their higher stability in an aqueous solution as compared to the non-PEGylated system. The formation of a PEG shell reduced the star coupling and only one type of loaded particles was observed. The PEG shell was released under physiological conditions within 24 h as monitored by changes in the DOSY spectra.

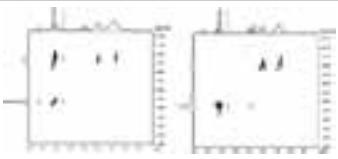

Figure. DOSY spectra of: PEGylated star macromolecules (left); After release of the PEG shell (right).

Acknowledgement: The support by the NSF of Bulgaria (Grants: DO 02-198/2008, DO 02-166/2008 and DRNF 02/13/ 2009) is highly acknowledged.

479TH

SOLID-STATE NMR STUDY ON THE BINDING SITES OF ACETONE MOLECULES IN FUNCTIONALIZED MIL-53 MOFS

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Metal-organic frameworks (MOFs) offer a huge potential for different applications like drug delivery, gas storage and sensor devices. All these applications rely on the interaction between the framework and the incorporated guest molecules. To obtain a better understanding of such interactions, we performed a study on a series of functionalized MIL-53 MOFs. Beside MIL-53, MIL-53-NH_2 and MIL-53-NHCHO, with anchor groups providing different hydrogen bond donor acceptor pattern, were investigated. Acetone was chosen as guest molecule because of its ability to act solely as hydrogen bond acceptor. At first we determined the spatial arrangement of the binding site offered to the guest molecule by static proton-driven spin diffusion exchange experiments. For the study of the host-guest interactions we used high-resolution ^{1}H solid-state NMR techniques at high magnetic fields, taking advantage of DUMBO decoupling schemes. The assignment of the resonances was done using high-resolution 2D HETCOR spectra (^{1}H-^{13}C, ^{1}H-^{27}Al and ^{1}H-^{14}N). The binding sites of the acetone molecules with respect to the framework were unraveled using ^{1}H-^{1}H-spindiffusion build up curves. These analyses were supported by DFT calculations which were used to determine and evaluate structure solutions for the arrangement of the functional groups as well as to build models for different binding scenarios of the acetone molecules, which showed that the carbonyl groups are bound to the functional groups while the methyl groups are close to the aromatic H's of the MOFs.

480MO

MEASUREMENTS AND THEORETICAL PREDICTION OF WATER SELF-DIFFUSION IN MACROPOROUS MEDIA

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We present a comparison of the results of PFG NMR experiment with the results of original predictive model of self-diffusion in macroporous media. The pore structures of the materials made of fused Al_2O_3 (corundum) and a ceramic binder were obtained by applying a method of stochastic reconstruction [1,2]. The method was based on simulated annealing constrained by a set of microstructural descriptors: the two-point probability function for the void phase and the lineal-path functions for the void and solid phases. These descriptors were derived from a large number of back-scattered electron images of planar cuts through the medium, which was impregnated by using epoxy resin. An initial random configuration of voxels was gradually transformed into a correlated (pore) structure in order to get a close match between the calculated and experimental microstructural descriptors. Subsequently, simulations of the Brownian motion in pores of stochastic replicas resulted in effective diffusion coefficients and tortuosities. The corresponding experimental data were obtained by means of PFG-NMR stimulated echo pulse sequence in a broad diffusion time range 5–2000 ms. The obtained apparent self-diffusion coefficients were analyzed within the Padé approximation [3].

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481TU

Poly (ethylene glycol) as an Alignment Medium

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Next to J-coupling, chemical shifts and NOEs, anisotropic parameters like residual dipolar couplings (RDCs) are a useful tool for determining complex molecular structures. For obtaining RDCs it is necessary to partially orient the molecules via a so-called alignment medium. In such partially aligned samples dipolar couplings do not fully average to zero, and can be observed as residual contributions.

During the recent years a number of gel-based alignment media like polydimethylsiloxane (PDMS), polyacrylonitrile (PAN), poly(methylmethacrylate) (PMMA), or polystyrene (PS) have been developed. All these alignment media are suitable for a relatively small range of NMR solvents.

In contrast to these alignment media, poly (ethylene oxide) (PEO) forms gels with a large variety of solvents ranging from apolar over polar organic solvents to water, including mixtures of corresponding solvents. Furthermore, PEO is applicable for a wide range of molecules like sugars, peptides, and other small molecules.

482WE

Exploring Zero Time-to-Echo Imaging of Glass Fibre Materials at 9.4T: How Relaxation Times and Frequency Shifts May Serve as an Indicator of Structural Defect

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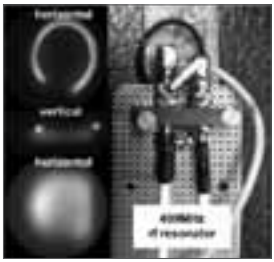

Glass fibre materials, used in the manufacture of wind turbine blades, can suffer unpredictable, post-manufacturing, in-situ structural failure. The economic cost of remediation of such blade failures is very significant, both on land and offshore. We suggest using zero-time-to-echo (ZTE) magnetic resonance imaging (MRI) [1] as a method for characterising glass fibre materials. This work explores the efficacy of using zero time to echo (ZTE) magnetic resonance imaging (MRI) to detect the rapidly decaying ^{1}H NMR signal from a representative sample (4mm thickness, 25mm width, 100mm length). A 400MHz surface resonator was developed made of a 20mm diameter loop formed with 1.5mm thick silver wire and designed with variable tuning and matching in order to investigate the ^{1}H-MRI signal at 9.4T. Following 3D ZTE parameters were set-up: 250kHz/FoV bandwidth, (40x40x40)mm^3 FoV, TR/TE=4000/4μs, TA=1min29sec, 5.7° flip angle, 5μs pulse length, (200x200x200)μm^3 nominal voxel size. ^{1}H images of the blade material were acquired demonstrating that ZTE is a suitable technique for acquiring image data from glass fibre materials. Very short T_2* (<20μs) of the material led to stronger blurring artefacts for the blade material compared to heat shrink used for insulating the silver wire of the detector. Work is on-going in studying the relaxation time parameters and chemical frequency shifts for materials with and without structural weaknesses in order to improve the predictive power of the technique. In conclusion, ZTE-MRI can provide useful insight about the mechanical properties of glass fibre materials.

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483TH

Atropisomerization of Para Di Substituted Propyl Bridged Biphenyl Cyclophanes

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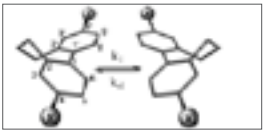
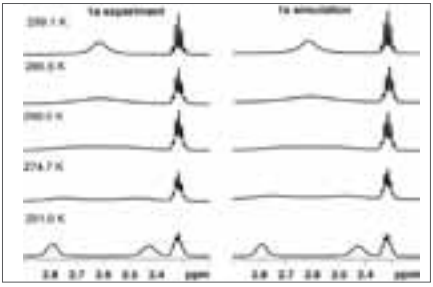
The interaction of matter with light and especially the possibility to manipulate light by the design of matter is one of the most exciting topics in science. Such structurally engineered matter are second-order non-linear optic materials. The atropisomerization process in 4 and 4` electron donor and acceptor substituted 2, 2` propyl bridged axial chiral biphenyl cyclophanes was studied. Free activation energies ΔG‡(T) of the rotation around the central biphenyl bond were calculated from ^{1}H-NMR variable temperature measurements. Correlations of ΔG‡(T) with Hammett parameters showed a linear relationship. To gain deeper insights full lineshape analysis was performed and the resulting enthalpic and entropic contributions of the free activation energy correlated with the Hammett parameter. Strong correlations to the Resonance parameter show that the influence of the substituents is mainly affecting the ϖ-system. DFT calculations delivered comparable free activation energies ΔG‡(T) and the mechanistic studies showed a planar transition state.

a: X = NO2
b: X = CN
c: X = NC
g: X = F
h: X = H
i: X = pip, NO_2



d: X = Sac
e: X = Br
f: X = Cl
j: X = OMe
k: X = NH_2
l: X = pip



484MO

Characterization of Macroporous Polymeric Supports by Relaxation Measurements

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The aim of most of the research in the field of ion exchange is to enhance functional properties such as selectivity and capacity, which have direct impact on the applicability of the resins (1). These supports can also be employed as anion exchangers or in affinity chromatography after attaching a ligand (2). In this way, matrix derivatives with alkyl amines can be used in affinity chromatography for the purification of amine oxidases or other proteins with affinity for the amino group, for instance, bilirubin. In this work we study the influence of changes in the content of ethylene glycol dimethacrylate (EGDMA) percentage and stirring speed on the formation of the macroporous network. It has previously been shown that the polymer mesh changes radically with the percentage of EGDMA upon absorption of bovine serum albumin (BSA), which was used as a representative large biological substrate (3). In this work we use 1H-CPMG sequences to obtain information on the distribution of transverse relaxation times of polar and non-polar solvents contained in the matrices. We observe a narrow distribution of relaxation times for the networks with non-polar solvents, while for polar solvents, the distribution is expanded and it is possible to distinguish three different behaviors which represent the swelling process for such networks.

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CONICET, FONCYT, SECYT-UNC, MPIP-MPG

485TU

Natural Abundance 13C NMR Study of the Effects of Thermochemical Treatments on the Lignocellulosic Structure of Wheat Straw

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The effects of thermochemical treatments (aquathermolysis and pyrolysis) on the lignocellulosic structure and composition of wheat straw were studied with ^{13}C solid state CPMAS NMR spectroscopy and proton T1ρ relaxation measurements. Results show that aquathermolysis removes hemicellulose, but stabilizes cellulose and lignin. Pyrolysis of untreated wheat straw degrades lignin and removes (hemi)cellulose completely. In contrast, pyrolysis of aquathermolysed wheat straw leaves traces of cellulose in the char. In addition, although lignin methoxy peaks remain clearly visible in the spectra of both pyrolised samples, they are much more intense in the pyrolised aquathermolysed wheat straw. This is most likely due to the removal of catalytically active (potassium) salts by the aquathermolysis, although recondensation due to the aquathermolysis treatment can also play a role. Furthermore, it was also found that both chars contain aliphatic chains, which were attributed to the presence of cutin or cutin-like materials, a macromolecule that covers the aerial surface of plants, not soluble in water and stable under the pyrolysis conditions applied.

486WE

Changes in Process of Water Releasing from the Polymer Globules in Presence of Additives

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According to the results from the NMR, FTIR and quantum mechanics calculation we can conclude that partially hydrophobic additives, namely methyl-ethyl ketone (MEK), methyl-isopropyl ketone (MIPK), and methyl-t-butyl ketone (MTBK) and tert-butyl methyl ether (tBME) seem to be attached to the polymer (poly N-isopropylmethacrylamide PIPMAAm, poly vynil methyl ether PVME) chains even before phase separation. This effect leads to the decreasing of the LCST with increasing of additive concentration. This interaction as well affects the solubility of the additive in water. Interaction of the additive with polymer leads to the increasing the amount of additive what can be solved in water. This polymer-additive interaction forms through the water molecules what leads to the decreasing of the melting point of the investigated systems.

Both ketone- and ether-based additives change the way of globules formation by pushing the water molecules out the globular structures, what leads to the formation of core inside the globular structure formed by polymer segments only. Shell of the globule is more porous and consists of three components: polymer segments, additive ad water molecules. Layer of additive/water molecule complexes attached to the surface of polymer globules protect the individual globules from interaction. This effect was observed by optical microscope and certified by the PFG NMR self-diffusion measurements and chemical exchange NMR experiments. This model can be confirmed by the cooling experiments where reorganization of the polymer globules is observed in two steps. First one corresponds to the re-solving process of the porous shell (~1-2K less then LCST), second step characterize the reorganization process in more rigid core part (require the lower temperature, 5-7K less then LCST). In such a structure no effect of water releasing observed, only releasing of a small part of an additive is taking place.

Acknowledgement
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487TH

EFFECT OF GLASSY MODES ON SPIN-LATTICE RELAXATION OF TEMPO RADICAL IN ETHANOL

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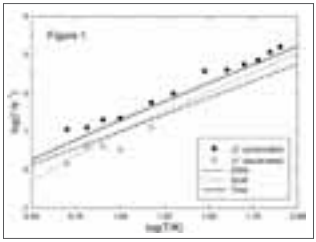

Spin-lattice relaxation (SLR) of TEMPO radical in the glassy (G) and crystalline (C) ethanol was measured by X-band EPR technique between 5 and 80 K. The SLR rates $\Gamma=1/T_1$ are higher in G-ethanol than in C-ethanol for both protonated and deuterated samples, while the excess SLR rate in G-ethanol $\Delta\Gamma=\Gamma G-\Gamma C$ is much higher for the protonated sample (Fig. 1). The results indicate that $\Delta\Gamma$ is produced by extra modes existing in glassy matrix, which are coupled to the electron spin of radical by the electron-nuclear dipolar (END) interaction. Using the soft-potential model for glassy modes and assuming END interaction between the electron spin and matrix protons, the expressions for SLR rates due to various mechanisms of glassy modes was derived. The SLR rates were evaluated for protonated G-ethanol from reported experimental data. The results indicate two effective mechanisms: thermally activated relaxation of double-well systems (DWS) and phonon-induced relaxation of quasi-harmonic local modes (QLM). The SLR rate induced by these mechanisms correlates well with the experimental $\Delta\Gamma$ in both the order of magnitude and the temperature dependence (Fig. 1).

488MO

TRACING THE TRANSIENT CONFORMATIONAL SIGNAL IN BACTERIAL PHOTOTAXIS USING SDSL-EPR SPECTROSCOPY

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In microbial photo- and chemotaxis a two-component signaling cascade mediates a regulated response of the flagellar motor to environmental conditions. Upon activation, photo- and chemoreceptors transfer a signal across the plasma membrane to activate the histidine kinase CheA. Successive regulation of the CheY-phosphorylation level controls the flagellar motor.

In Natronomonas pharaonis a sensory rhodopsin II – transducer complex (SRII/HtrII) mediates negative phototaxis.[1] As the initial signal, a light-induced outward movement of receptor helix F leads to a conformational change of transducer helix TM2, which in turn propagates the signal to the adjacent HAMP domain.[1,2]

For the HAMP domain, a widely abundant signaling module, several mechanisms were suggested[3], all comprising two distinct conformational states. These can be observed by two-component cw-EPR spectra at ambient temperatures existing in a thermodynamic equilibrium which can be driven by salt-, temperature- and pH-changes.[4]

To trace the conformational signal and it's propagation throughout the elongated transducer, we applied cw- and pulse-EPR spectroscopy in conjunction with nitroxide spin labeling. We follow transient changes by time-resolved cw-EPR spectroscopy and compare the resulting spectral changes to difference spectra corresponding to the above shifts in the thermodynamic equilibrium. The light-driven conformational changes are in agreement with a shift towards a more compact state of the HAMP domain.

Following this signal beyond the HAMP domain requires a mechanism compatible with the formation of trimers of SRII/HtrII dimers which activate CheA. An activation scheme within the framework of hexagonal arrays formed by the trimers of SRII/HtrII will be the key step to understanding the enormous cooperativity leading to signal amplification in networks formed by clusters of interacting receptors.

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489TU

The Influence of Annealing Temperature on the Properties of $Sn_{1-x}Mn_xO_2$ Powders as Seen by EPR

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Recently, an intense research activity is being pursued on diluted magnetic semiconductors (DMSs) since the predicted room temperature ferromagnetism in these materials could be useful in spintronics [1].

Tin oxide (SnO_2) doped with Mn ions is one of these DMSs where the presence of high-temperature ferromagnetism was reported [2]. It was shown that there is a strong dependence of their magnetic properties on both the sintering temperature and doping content. It is believed that oxygen vacancies and substitutional incorporation are important to produce ferromagnetism in semiconductor oxide doped with transition metal ions.

The present paper reports detailed electron paramagnetic resonance investigations (EPR) of the samples in order to investigate the influence of annealing temperature on the properties of $Sn_{1-x}Mn_xO_2$ powders.

X-band and Q-band electron paramagnetic resonance (EPR) studies of Mn^{2+} ions in $Sn_{1-x}Mn_xO_2$ powders with x = 0.5%, 1%, annealed at different temperatures is reported. These samples are interesting to investigate as Mn doping produce ferromagnetism in SnO_2, making a promising ferromagnetic semiconductor at room temperature.

490WE

EPR Characterization of Micro and Nanostructured $Zn_{1-x}Fe_xO$ Powders

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ZnO is an attractive system for quite a wide variety practical applications, being a chemically stable oxide semiconductor. It has been shown that Fe doping produces ferromagnetic semiconductor at room temperature [1]. This material, therefore, has the potential for use in spintronic devices such as spin transistors, spin light emiting diodes, very high density nonvolatile semiconductor memory and optical emitters. It is believed that oxygen vacancies and substitutional incorporation are important to produce ferromagnetism in semiconductor oxide doped with transition metal ions.

The present paper reports detailed electron paramagnetic resonance investigations (EPR) of the samples in order to investigate how Fe ions are incorporated into the ZnO lattice and their interaction with environment.

X-band electron paramagnetic resonance (EPR) studies of Fe^{3+} ions in $Zn_{1-x}Fe_xO$ powders with X = 1%, 3%, 5% is reported. These samples are interesting to investigate as Fe doping produce ferromagnetism in ZnO, making a promising ferromagnetic semiconductor at room temperature.

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491TH

Combining Gd^{3+} Chelates and Nitroxide Radicals for DEER Based Nanometer Range Distance Measurements

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The methodology for the double electron-electron resonance (DEER)-based distance measurements with Gd^{3+} paramagnetic centers has been developing extensively over the last decade. Combining the Gd^{3+} centers and nitroxide radicals [1] opens up a possibility to selectively measure different distances in a single sample. The concept of such measurements has been recently proposed by us [2]. The first proof of principle applications of such methodology have been recently performed [3,4]. The use of Gd^{3+} complexes at all detection frequencies requires a detailed analysis of the dipolar interaction and spin dynamics of the Gd^{3+} ions and Gd^{3+}-nitroxide spin pairs. This is especially important for commonly performed X- and Q-band experiments (10 and 35 GHz detection frequency), where the strength of sero field splitting term in the spin Hamiltonian of Gd^{3+} centers can become comparable to the strength of the electron Zeeman interaction.

We present the performance of the DEER experiment in Gd^{3+}-nitroxide pairs on several model systems, report the results of applying the selective distance measurement strategy in a study of bifunctional Au-nanoparticles [4] and present detailed analysis of dipolar frequencies excited in high-spin Gd^{3+} centers [4]. Additionally we discuss possible mechanisms, contributing to the reduction of the DEER echo amplitude in the Gd^{3+}-nitroxide DEER experiment. Such a reduction of detected spin echo has significant influence on the sensitivity of Gd^{3+}-based distance measurements and is thus of high importance for the optimization of the technique. The work is supported by SNF Grants 20020_132255/1 and 200021_121579.

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493TU

Probing Protein-Cofactor Interactions in Blue-Light Photoreceptors

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Three different classes of flavin-containing blue-light photoreceptors, namely LOV domains, BLUF domains and cryptochromes, are known to date. Although these proteins share the same flavin chromophore, their primary photoreactions differ significantly. Electron-transfer reactions are supposed to be involved an all three classes of blue-light photoreceptors, therefore electron paramagnetic resonance (EPR) methods in all flavours are particularly suited to identify and characterize (short-lived) paramagnetic intermediates (radicals, radical pairs and triplet states) even in *in vivo* systems.

In this contribution, we describe how fine-tuning of the surroundings of LOV domains can be utilized to gain information on the flavins micro-environment and to exploit the influence of these protein-cofactor interactions on the protein's reactivity. Moreover, we were able to identify key amino acids for efficient electron transfer in cryptochromes (and photolyases), and we could demonstrate that the kinetics of this photo-induced electron transfer reaction is magnetically sensitive, which argues that cryptochrome is fit for purpose as a magnetoreceptor.

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494WE

Electrically Detected Magnetic Resonance under Pulsed Illumination: From the Measurement of Recombination Times to Hyperpolarisation in ENDOR

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Electrically detected magnetic resonance (EDMR) is a highly versatile method to study defects and recombination centres in semiconductor nanostructures. Using pulsed microwave excitation, coherent control, electron spin readout as well as decoherence measurements in device structures have been demonstrated [1,2]. By additionally pulsing the illumination, we have recently extended the toolbox of pulsed EDMR to allow selective depopulation of spin states, the direct measurements of the singlet and triplet recombination times and the very sensitive electrical detection of ENDOR, allowing for example to study both the NMR of the neutral as well as the positively charged state of Phosphorus donors in Silicon and the investigation of the nuclear decoherence [3,4].

This contribution will address low-field EDMR, where due to the hyperfine interaction combined electron spin-nuclear spin transition are excited and where we can systematically measure the recombination and decoherence times of the dominant spin-dependent transition at Si/SiO2 interfaces as a function of the mixing angle of the spin states and of the magnetic field applied using pulsed EDMR. In addition, we present experiments addressing hyperpolarisation in ED-ENDOR, where in the same microscopic system non-thermal nuclear polarisations exceeding 50 % can generated for the 31P nucleus, and discuss the origin of this hyperpolarisation as well as possible applications.

This work is funded by DFG through SFB 631 “Solid-State Quantum Information Processing” and through the JST-DFG Joint Programme on Nanoelectronics.

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496MO

Surface Electrostatics of Lipid Bilayers by EPR of pH-Sensitive Spin-Labelled Lipid

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Many essential biophysical processes such as protein membrane insertion, membrane fusion, as well as local curvature are governed by lipid bilayer electrostatic potential. Currently, the arsenal of analytical methods for assessing electrostatic properties of the lipid bilayers is limited to a few techniques and molecular probes. We present a new spin-probe EPR approach for assessing electrostatic surface potentials of lipid bilayers that is based on recently synthesized phospholipid-based EPR probe (IMTSL-PTE) containing reversibly ionizable (protonatable) nitroxide tag attached to the lipids' polar head group. EPR spectra of the probe directly report on its ionization state and, therefore, electrostatic potential through changes in both magnetic parameters and nitroxide motion regime. Further, in IMTSL-PTE the nitroxide moiety is directly tethered to the lipid polar head defining location of the measured potential with respect to the lipid bilayer interface. Finally, lipoid nature of the probe makes it an integral part of lipid bilayers. Comparison of experimental surface potentials measured by EPR of IMTSL-PTE showed a remarkable (<±2%) agreement with the Gouy-Chapman theory for anionic lipid bilayers in the fluid phase such POPG at 17 ˚C and DMPG at 48 ˚C. Agreement with the theory is becoming worse for several bilayer systems such as DMPG vesicles in gel phase (17 ˚C), fluid phase bilayers formed from mixtures of DMPC and DMPG, and DMPG vesicles at various electrolyte concentrations. Possible reasons for such deviations as well as the proper choice of an electrostatically neutral interface have been discussed. The application of the new EPR method is further illustrated by studies of a series of small unilamellar vesicles of defined curvature (from 30 to 100 nm in diameter) as well as well as bio-nano interfaces such as formed in substrate-supported lipid nanotubes. We expect that the new method would be broadly applicable for studying interfacial electrostatic phenomena in more comprehensive models of cellular membranes that, for example, include sterols and integral proteins as well as bio-nano interfaces we are studying with spin-labelling EPR. Supported by US BES DOE contract DE-FG02-02ER15354 to AIS.

497TU

pH Sensitive EPR Labels to Probe Local Dielectric Gradients in Protein-Membrane Systems

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Nitroxide spin-labeling in combination with EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been a substantial interest in utilizing EPR to characterize local effects of polarity and hydrogen bonding in these systems. Here we report on employing an arsenal of advanced spin-labeling EPR methods to profile heterogeneous dielectric and hydrogen bonding environment along the α-helical chain of an alanine-rich WALP peptide that is anchored in a lipid bilayer in a transmembrane orientation. A series of WALP single cysteine mutants was labeled with a pH-sensitive nitroxide IMSTL (S-(1-oxyl-2,2,3,5,5-pentamethylimidazolidin-4-ylmethyl) ester) that is similar in molecular volume to phenylalanine. The protonation state of this nitroxide could be directly observed by EPR allowing us to follow proton gradient across the membrane in the vicinity of the WALP α-helix, and, thus, to reconstruct the gradient in the effective dielectric constant across the membrane on membrane-protein interface. Q-band DEER experiments with symmetric double-labeled WALPs were employed to derive positions of nitroxides upon protonation. This system provided another estimate of the local dielectric constant. Local polarity was also evaluated from characteristic changes in EPR spectra that were enhanced by the use of perdeuterated and ^{15}N-substituted nitroxides and high field EPR at 130 GHz (D-band). Formation of hydrogen bonds between the nitroxides and membrane-penetrating water molecules was observed directly in HYSCORE X-band experiments. Such measurements allowed us to derive experimental profiles of heterogeneous dielectric and hydrogen bonding environment along a typical transmembrane α-helix.

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498WE

Gd^{3+}-nitroxide DEER on Site-Specific Orthogonally-Labelled T4-Lysozyme; Performance of Gd^{3+}-DOTA and Gd^{3+}-DTPA Complexes for Nanometer Range Distance Measurements.

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Double Electron Electron Resonance (DEER) in nitroxide-nitroxide spin pairs is broadly employed to obtain structural information in biomolecules. Recently, Gd^{3+}-nitroxide DEER has been reported exhibiting promising performance.[1] This approach requires attachment of two different spin labels to the sample under investigation. Such an orthogonal labelling can be performed by using an unnatural amino acid (K1) bearing a nitroxide side chain in combination with an engineered cysteine.[2] The sulphur-specific maleimido moiety can then be exploited to covalently link a maleimido Gd^{3+}-complex derivative to cysteine. We used two different T4-lysozyme mutants possessing a cysteine in position 109 and the K1 label at position 68 and 131 respectively. We labelled the 109C using maleimido-DOTA or maleimido-DTPA derivatives enclosing Gd^{3+}, to obtain four site-specific orthogonally-labelled T4-lysozyme mutants. We find distance distributions in all four labelled mutants in agreement with previous results obtained from nitroxide-nitroxide DEER in the 109R1-68K1 and 109R1-131K1 T4-lysozymes.[2] The optimum conditions for sensitivity are discussed and the performance of Gd^{3+}-DOTA and -DTPA in the DEER experiment at X and Q band is compared. For example, Gd^{3+}-DOTA manifests a stronger DEER echo reduction and a narrower spectrum compared with the Gd^{3+}-DTPA complex. At X band, the width of Gd3+ spectrum allows to tune the detection frequency in a broad range resulting in a strong reduction of the nuclear modulation artefacts. The orthogonal labelling approach in combination with X- and Q- band Gd^{3+}-nitroxide DEER successfully enables extraction of nanometer-range distance information for proteins having 30-40 μmolar spin concentrations. These concentrations reach the sensitivity limit for X band and are well above the sensitivity limit for Q band Gd^{3+}-nitroxide DEER.

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499TH

HIGH FIELD ELDOR-DETECTED NMR STUDIES OF THE Ca^{2+}- AND Sr^{2+}-CONTAINING OXYGEN EVOLVING COMPLEXES IN PHOTOSYSTEM II: WATER BINDING AND WATER SPLITTING IN PHOTOSYNTHESIS

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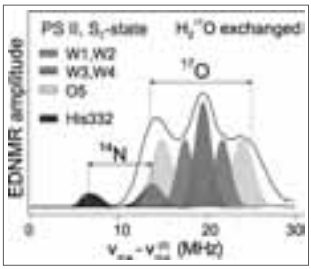

High field ELDOR-detected NMR (EDNMR) is demonstrated as a sensitive technique for the detection of electron-nuclear interactions of spectroscopically 'difficult' nuclei such as ^{17}O and ^{14}N ligands of paramagnetic metal complexes. This method allows the detection of hyperfine and nuclear quadrupole couplings and is of superior sensitivity and of wider applicability as the more standard ENDOR and ESEEM (HYSCORE) techniques. We have applied EDNMR at W-band (94 GHz) to detect the interaction of the water oxidizing Mn_4O_5Ca cluster in the S_2 state (Seff = ½) in photosystem (PS) II with magnetic nuclei of amino acids and attached exchangeable water (-derived) ligands. In samples incubated with $H_2{}^{17}O$, couplings of three different types of ^{17}O-containing ligands were detected (W1-4, O5; see figure). They were assigned based on a structural model derived from X-ray crystallography,[1] which has been refined using density functional theory[2] and comparison to model complexes. Comparison of the native Ca^{2+} to the Sr^{2+} containing clusters and the use of the substrate analogue NH_3 confirmed these assignments. The data further refine the reaction pathway for O-O bond formation supporting an oxo/oxyl coupling mechanism in the S_4 state of the cycle.[3]

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500MO

ON THE ROOM TEMPERATURE FERROMAGNETISM IN IRON DOPED NANO-TiO_2 ANATASE PHASE

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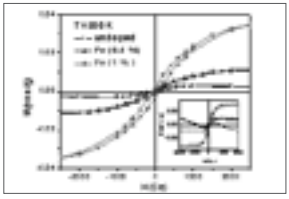
Since several years diluted magnetic semiconductor oxides [1 and references therein], with simultaneous control of charge and spin, have attracted intensive interest for the potential applications in spintronics. Among various such oxides, TiO_2 has been suggested as being a good material which combines magnetic and transport properties. As electronic structure calculations indicate that different 3d-dopant ions (Co, Fe, Mn etc.) may be ferromagnetic in TiO_2 [2], numerous investigations were performed. In spite of this effort the data are often contradictory on the existence or not of the ferromagnetic ordering. Therefore, in this contribution, a study of local structure and magnetic properties of iron-doped (0-4% at.) hydrothermally-grown nano-TiO_2 anatase phase was performed with EPR, Mössbauer, and magnetic measurements, as well by XRD and TEM analyses. Different Fe^{3+} ions positions, and oxygen defects, with various g and ΔH parameters were evidenced in X-and Q-band EPR spectra of as-prepared and post annealed TiO_2 samples. Significantly, in all samples unusual temperature behaviour of the double integral EPR intensity is observed. This feature, associated with the presence of the 2+, 3+, and 4+ iron ionization states in Mössbauer spectra, with relative high hyperfine interactions [3], together with the *M* versus *H* data measurements at 300 K (see the figure below), confirm the existence of a clear ferromagnetic ordering at room temperature.



One suggests a bound magnetic polaron (BMP) mechanism for magnetic ordering in Fe-nano-TiO2.

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501TU

RELAXATION ENHANCEMENT BASED DISTANCE MEASUREMENTS ON ORTHOGONALLY LABELLED T4-LYSOZYME

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The relaxation enhancement of a nitroxide spin-probe, induced by a fast relaxing paramagnetic lanthanide center can be used as an alternative to DEER/PELDOR experiments to measure distances in macromolecules[1]. In the present work we apply the longitudinal relaxation enhancement approach to determine distances in spin labelled T4-lysozyme.

The sample preparation requires attaching two different spin labels to the protein[2]. This orthogonal spin labelling uses an exposed cysteine for attaching a Ln^{3+}-complex at position 109C and an unnatural amino acid (K1) exhibiting a nitroxide radical at position 68K1 or 131K1. As complexing agents we used maleimido-DOTA or maleimido-DTPA, loaded with Dy^{3+} as paramagnetic species and Lu^{3+} as diamagnetic reference. We have measured and analyzed four different T4-lysozyme samples 109C/68K1 and 109C/131K1 labelled with DOTA and DTPA.

We present relaxation enhancement data for all T4-lysozyme samples and compare the extracted distances to Gd^{3+}- nitroxide DEER data. We discuss the shape of the relaxation enhancement curve, maximum relaxation enhancement temperature, and overall performance for two different types of lanthanide labels (DTPA- and DOTA-based). Advanced methods for data evaluation and adjusted measurements for dealing with partially labelled samples are demonstrated.

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502WE

INFLUENCE PHOSPHOLIPIDS COMPOSITIONS ON THE HYDROPHOBICITY OF MEMBRANES

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The lipid bilayer represents the fundamental permeability barrier to the passage of polar molecules into and out of a cell due to its high hydrophobicity. Incorporation of saturating amounts of cholesterol into lipid membranes ensures the rectangular shape of the hydrophobicity profile across the membranes [1]. However in one of our results [2] we discovered a similar hydrophobicity profiles in membranes without cholesterol. The membranes of fiber-cell eye-lenses are main object our investigation as a part of exploring mechanism of protect lens from cataract disease.

For preparing of membranes with similar phospholipid composition as real membranes of fiber-cell eye-lenses we used next phospholipids: phosphocholine (POPC), phosphoethanolamine (POPE), phosphoserine (POPS) and sphingomyelin (SM) phospholipids. The profiles of hydrophobicity across these model bilayer membranes the similar different animal species but without cholesterol content showed high effect of hydrophobic barrier. This effect seems like hydrophobic barrier if it were cholesterol content but not rectangular shape [1]. We supposed that this effect may be a result of influence of phospholipid compositions or influence may be one of these phospholipids. The aim of this work was to investigate influence of the phospholipid membrane composition on its hydrophobic barrier using electron paramagnetic resonance (EPR) spin-labeling methods.

We investigated a successively influence of each lipid for a hydrophobicity of membranes. The conventional electron paramagnetic resonance (CW EPR) spectroscopy and using phospholipid analog spin labels which can easy incorporate to phospholipid membranes can provide unique information about structure of membranes such as hydrophobicity order parameter, oxygen transport and other parameters. The hydrophobicity of membranes in this method is defined from parameters of hyperfine splitting of EPR spectra.

POPE is dominant in creating a high hydrophobic barrier at the center of membranes. Effect of other phospholipids on membrane hydrophobicity is as follow: SM<PC<<PS=PE.

Membranes containing POPE are slightly more ordered.

The major contribution to the hydrophobicity of membranes made of phospholipid mixtures comes from the phospholipid with higher hydrophobicity in single component membranes.

High hydrophobic barriers across membranes in which cholesterol content is very low can be formed by the presence of PE.

Polar heads are responsible for creating the hydrophobic barrier in the hydrocarbon membrane center.

Our investigations show also that the evaluation of membrane hydrophobicity in frozen samples is more sensitive and informative than the evaluation in fluid-phase membranes at high temperature.

This work was supported by grants EY015526, EB002052, and EB001980 from the National Institutes of Health.

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503TH

ELECTRON SPIN-LATTICE AND SPIN-SPIN RELAXATION TIMES OF ATOMIC HYDROGEN: CAN H@POSS RIVAL ENDOHEDRAL FULLERENES AS QUBIT EMBODIMENTS?

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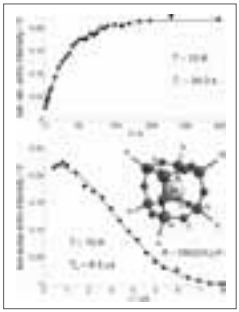

Paramagnetic atoms encaged in molecular cages like endohedral fullerenes (e.g. $N@C_{60}$) are promising components of spin-based quantum computing because they can be precisely placed into large arrays by chemical engineering. Trapped atomic hydrogen is even more attractive due to its simpler electronic 1s state and the exceptionally large hyperfine coupling of 1420.406 MHz. Whilst C_{60} cannot stably host atomic hydrogen, it has been found that polyhedral octa-silsesquioxanes (POSS) are ideal cages for this purpose. Crucial properties for quantum computing like the spin-lattice T_1 and spin-spin T_M relaxation times depend strongly on the type of the peripheral organic substituents. Recently [1] we showed that the room-temperature phase memory time T_M=13.9 μs for the system with R=$OSi(CH_3)_2H$ is the longest observed so far for this kind of cages. Moreover, this study proved that the spin-spin relaxation (representing the coherence time scale) is determined by nuclear spin diffusion and at low temperatures it is strongly enhanced by dynamic processes like rotation of the methyl groups. Here we investigate for the first time the system with R=OCH_3 which provides the following potential advantages: 1) it has a high group electronegativity, similar to that of $OSi(CH_3)_2H$, ensuring small delocalization of electron spin density over the cage, 2) it possesses less magnetic nuclei, and 3) it provides an easy way to prepare the deuterated species with R=OCD_3. The results are compared to T_M=160 μs of $^{15}N@C_{60}$ [2] which is the longest electron spin coherence time of any molecular radical.

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504MO

A CONFORMATIONALLY UNAMBIGUOUS ISOINDOLINE-DERIVED EPR PROBE FOR DISTANCE MEASUREMENTS IN NUCLEIC ACIDS

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EPR spectroscopy has been increasingly used to investigate the organizational and dynamic properties of nucleic acids, using persistent nitroxide spin probes. Spin labels that are conjugated to biopolymers with a tether that has some flexibility decrease the accuracy of EPR-based distance measurements between labels. In contrast, rigid spin labels, like **Ç (Figure 1)** do not move independently of the biopolymer to which they are attached. The rigidity of **Ç** makes is advantageous for the determination of accurate distances and orientations of structural elements in nucleic acids. However, for simple distance measurements one needs to deconvolute the orientational effect of **Ç**.

We report here synthesis of a conformationally unambiguous isoindoline-derived nitroxide spin label (^{Im}T, **Figure 1**). Although rotation around the single bond between the benzimidazole to the base is possible, such rotation would be around an axis that goes through the N-O bond of the nitroxide. Therefore, rotation around the single bond should only cause a minor displacement of the nitroxide relative to the nucleic acid, making this a promising probe for determination of accurate distances within oligonucleotides.

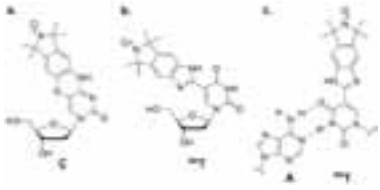

Figure 1. *Spin-labeled nucleosides **Ç** (a), ^{Im}T (b) and base pairing of the latter with **A** (c).*

505TU

PARAMAGNETIC DEFECTS IN HYDROTHERMAL-GROWN ANATASE TiO_2 NANOPARTICLES

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Since the discovery of its photocatalytic properties by Fujishima [1], nanosized TiO2 has been extensively studied [2] and used in a variety of industries including coatings, cosmetics, pollution control, solar energy conversion, and as potential material for spintronics. Its electronic properties can be controlled by doping, the creation of defects having a profound effect on the properties of the surface, either by separating the charge carriers or by trapping them. Electron paramagnetic resonance (EPR) spectroscopy, due to its high sensitivity, can put in evidence paramagnetic defects on as grown or UV irradiated samples [3, 4].

We report in this contribution the EPR investigations, at different temperatures in X- and Q-band, on as-prepared and annealed hydrothermal-grown TiO_2 nanosize particles. Pure and doped (Fe, Co) anatase samples, thermal treated in controlled atmosphere, evidence different paramagnetic defects, identified by their g_{eff} values. Both electron and hole active centers are observed in samples treated in partially reduced oxygen atmosphere. Dependence on the dopant nature and content, local structure, and TiO_2 particles size is discussed. Relevance to the magnetic properties is mentioned too. In the case of iron doping, polarization of impurity ion spins via electrons delocalization of paramagnetic defects supports a polaronic model for the observed magnetic properties [5 and references therein].

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506WE

Q-BAND ELECTRON PARAMAGNETIC RESONANCE STUDIES OF THE Mn_4CaO_5 CLUSTER OF PHOTOSYSTEM II IN THE S_3 STATE.

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The water splitting process in higher plants, cyanobacteria and algae is catalyzed by the Oxygen Evolving Complex (OEC) of Photosystem II (PSII). During its catalytic cycle OEC undergoes four one-electron oxidation transitions, $S_0 – S_1$, …,$S_3 – (S_4)S_0$ (S-state transitions) driven by the photo-excitation of the chlorophyll species P_{680}. There are two major steps on each S-state transition; the oxidation of an appropriately positioned tyrosine, tyr Z, by P_{680}+ and the subsequent oxidation of the central catalytic core, the Mn_4CaO_5 cluster, by oxidized Tyr Z.

Electron Paramagnetic Resonance (EPR) spectroscopy has been valuable in the characterization of the OEC. One important conclusion from these studies is that the spin of the Mn_4CaO_5 cluster alternates between half integer and integer values during the S-state transitions. This shows that the electrons during the S-state transitions are extracted from Mn itself or its immediate vicinity. The most extensively studied oxidation states of the Mn_4CaO_5 cluster by EPR are those with half integer spin, S_0 and S_2, because half integer spin systems are more accessible by conventional EPR than integer spin systems.

EPR studies of the integer spin S-states are rather scarce. It was not until just before the turn of the century that rather weak but characteristic low-field EPR signals in perpendicular and parallel mode X band (9.5 GHz) attributable to the critical S_3 state were observed. Early studies analyzed the spectra and suggested that they arise from a low-lying state of an exchange-coupled system with S = 1. However, an unambiguous interpretation of the EPR signals of this kind requires a multi-frequency approach.

Subsequent experiments at Q-band frequencies indicated that the spin associated with the ground state of S_3 is S = 3 rather than S = 1. This conclusion was supported in turn by detailed simulations of the X-band EPR spectra.

The X-band EPR signals from the S_3 state depend on the treatment of the PSII preparations. In the present work we extend our preliminary Q-band EPR experiments and study also the effect of such treatments on the Q-band EPR spectra from this critical S-state.

507TH

Pulse EPR on the [FeS] Clusters of the Membrane-Bound Hydrogenase from Ralstonia eutropha

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Understanding the exceptional tolerance of certain [NiFe] hydrogenases towards oxygen is of high importance given their potential future application as catalysts for H_2 production. [NiFe] hydrogenases mainly consist of a large subunit with the [NiFe] active site and a small one containing [FeS] clusters as part of the electron-transfer chain. While standard hydrogenases are inactivated by traces of O_2 there are examples sustaining high activity even under ambient oxygen levels. Since many redox states of the named cofactors are paramagnetic, EPR spectroscopy is well-suited to characterise them and their coordination. Here we focus on the oxygen-tolerant membrane-bound hydrogenase from *Ralstonia eutropha*. The enzyme exhibits in its (resting) Ni_r-B state a magnetic coupling between the [NiFe] centre and two paramagnetic species, a distinct feature not found in standard hydrogenases. Previous research revealed high similarity of the [NiFe] site in both standard and O_2-tolerant hydrogenases [1], leaving the [FeS] region as probable origin of the O_2 tolerance.

Recent crystal structure analyses showed a new kind of iron-sulfur cluster most likely involved in that tolerance [2,3]. This [4Fe3S] cluster with an unusual binding motif presumably acts as electron acceptor during H_2 oxidation but as donor when the protein is under attack of O_2. We present pulsed EPR studies on the proximal iron-sulfur cluster of MBH possibly being the origin of the remarkable O_2 tolerance. Two distinct interactions with nearby nitrogens are found: A strong one attributable to a Fe-N backbone coordination, and a weaker one assignable to a histidine.

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508MO

Characterization of Paramagnetic Species in Seeds by EPR

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Paramagnetic species were characterized in the seeds of radish wild, rice, mustard, wheat, oats, sorghum, sunflower, soybean, cotton, beans, maize and barley by EPR. Some iron complexes such as goethite, hematite, magnetite and ferrihydrite, normally present in the soil, were also investigated by EPR, since their signals can, a priori, be present in EPR spectra of seeds. The EPR experiments were performed at X-band microwave frequency (9.5 GHz) on the JEOL spectrometer (JES-PE-3X) at room temperature, 77K, 30K and in a temperature range from 132 to 385 K, with the purpose of studying the thermal behavior of the species. A g marker of $MgO:Mn^{2+}$ (g=1.981 in the fourth line of the spectrum) was maintained in the cavity of the spectrometer, so that the date were obtained simultaneously with the samples spectra. In the EPR spectra of the seeds, we detected the same complex of Fe^{3+} found in goethite, with g = 2.0 in all the investigated seeds. In addition, free radicals have also been detected with g = 2.004, on all seeds, and with g = 2.013 only in sorghum seeds at room temperature. The sunflower seeds showed the highest signal intensity of the free radical with g = 2.004. During the temperature variation from 132 to 273 K, significant change was not observed in the spectra, whereas, when the seeds and goethite were submitted to the temperature of 77 K, their EPR spectra changed significantly, but similarly, appearing lines of EPR at g = 6.0 and g = 3.7, which are due to rhombic symmetry and higher symmetry than rhombic symmetry, respectively. When the samples of seeds and goethite were submitted at temperature of 30 K, again the spectra changed significantly and showed similar aspect to that of which had been found at room temperature, but with higher intensity, in addition, the spectra of the seeds also showed traces of Mn^{2+}, and other paramagnetic species normally present in soil samples*. Thus, these results confirm that the seeds showed structures of Fe^{3+} similar to goethite.

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509TU

Applications of Adiabatic and Fast Passage Ultra-Wideband Pulses in Electron Paramagnetic Resonance Spectroscopy

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Only a fraction of all electron spins can typically be excited in pulsed electron paramagnetic resonance (EPR) spectroscopy due to the technically constrained excitation bandwidth of rectangular pulses. In many EPR experiments the measurement sensitivity is thus related to the excitation bandwidth. We apply adiabatic and fast passage ultra-wideband (UWB) pulses to achieve inversion over several hundreds of MHz.

Technically, frequency-swept pulses are generated by a 12 GS/s arbitrary waveform generator and upconverted to X band frequencies, therefore providing UWB excitation pulses that can have maximum bandwidths larger than 3 GHz. This pulsed UWB source is utilized as an incoherent channel in an ordinary pulsed EPR spectrometer with an overcoupled split ring resonator. A severe excitation bandwidth limitation for such UWB excitation being the resonator, we discuss experimental methodologies and simulation techniques to account for the resonator profile. Herein, complications in simulating the electron spin response to such UWB excitation are addressed by using a set of precomputed spin propagators in Hilbert or Liouville space.

Aided by these procedures, we demonstrate optimized inversion recovery and double electron electron resonance (DEER) experiments. First, virtually complete inversion of the nitroxide spectrum with an adiabatic pulse of 128 ns length is achieved. Consequently, spectral diffusion between inverted and non-inverted spins is largely suppressed and the observation bandwidth can be increased to increase measurement sensitivity. Second, DEER is performed on a terypridine-based copper(II) complex with a nitroxide-copper distance of 2.5 nm. As previously demonstrated on this complex when pumping copper spins and observing nitroxide spins, the modulation depth is severely limited by the excitation bandwidth of the pump pulse. By using fast passage UWB pulses with a maximum length of 64 ns, we achieve a significant enhancement of the modulation depth.

Based on these experimental results and forthcoming innovations, the advantages of UWB pulses for several application fields are summarized.

510WE

Conformational Transitions of the *E. coli* Vitamin B_{12} Importer in Micelles and Liposomes Revealed by Double Electron Electron Resonance

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The type II ABC (ATP-binding cassette) importer BtuCD-F mediates the uptake of vitamin B_{12} in *E. coli.* Unlike for the type I importers, the details of the substrate transport are not well understood for BtuCD-F. Compared to liposome-reconstituted samples, detergent-solubilized BtuCD-F exhibits a higher basal ATP-ase activity and the release of the substrate is ATP-independent. To elucidate the structural origin of the environment-dependent functional differences and to characterize the 'alternate-access' of the translocation channel, we used DEER (Double Electron-Electron Resonance) on spin-labelled mutants in the core TM5 and TM10 helices. In detergent, the interspin distance distributions reveal a more open translocation channel with enhanced backbone dynamics which we propose to be the reason for the higher ATP-ase activity and the ATP-independent trans-release of the substrate. Combined with our previous results (1), we confirm that unlike in ABC exporters and type I importers, upon ATP binding the translocation channel in BtuCD-F adopts an inward-facing conformation by an independent movement of the core TM5 and TM10 helices. In the ADP-state, the channel is restored to an occluded apo-like conformation in agreement with the crystal structure. The EPR data suggests that the vitamin B_{12} can enter in the translocation channel and be translocated only in the ATP state (2).

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511TH

Local Glass Transition Temperature Around Spin Labels in Proteins Determined by CW Power Saturation EPR

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For structural studies by pulsed EPR methods, spin-labeled water soluble or membrane proteins are routinely exposed to cryogenic temperatures. For example, DEER measurements are usually performed at 50 K and the samples are shock frozen either directly in liquid nitrogen or in a cooling bath (e.g. ethanol/dry ice) before insertion in the pre-cooled cavity. Freeze-quenching is also used in some applications. Recently, a comparison between different freezing methods showed that the mean interspin distance in T4-lysozyme was unaffected [1]. Although the DEER experiment is done at 50 K, equilibration of the conformational ensemble occurs to a good approximation at the glass transition temperature (T_g) during shock-freezing of the sample. Targeting the most commonly used conditions at which DEER experiments are performed, the MD simulations of the rotamers used in MMM to model the conformations of the spin labels in proteins were performed at 175 K [2], which is an estimate of the glass transition temperature of the protein hydration water. To refine the rotamer simulation approach, we address the possibility to extract experimentally the local glass transition temperature of spin labeled sites in proteins in different water-glycerol mixtures by continuous wave power saturation measurements at variable temperatures, a method recently applied to extract the T_g values of spin-labeled polymers [3,4]. The results obtained with two spin-labeled water soluble proteins, namely T4-lysozyme and the maltose binding protein MBP are presented and compared to the data obtained with free nitroxide probes in a water-glycerol mixture both in the presence or absence of proteins in the matrix. The method is unique because it allows to compare T_g values at different sites in proteins and can be extended to membrane proteins in micellar or lipid environments.

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512MO

Suppression of Multiple-Spin Correlations Manifesting in Ghost Distances in Double Electron-Electron Resonance

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Distance distributions of a pair of dipolar coupled spins are routinely measured in the range of 1.5 - 6.0 nm with the double electron-electron (DEER) experiment. However, if spin-labeled oligomers are studied, the presence of more than two spins in a sample within the sensitivity range of the DEER experiment is not a rare event. More than two dipolar coupled spins manifest in an increased total modulation depth and in sum and difference frequency contributions in the form factor [1]. This leads to line broadening in frequency domain and eventually to additional peaks appearing in the distance distribution, which do not correspond to the real interspin distances of the system and are hence referred to as ghost distances.

We present an approach to largely suppress ghost distances in order to obtain true interspin distances by manipulating the experimentally obtained form factor during data analysis. In contrast to a previously developed approach, which demands a series of very high signal-to-noise ratio experimental traces at variable attenuation of the pump pulse, we present a procedure that only requires a single DEER trace and hence reduces experimental cost enormously, which should enable the application to biological systems rather than only model samples.

The approach is validated on simulated test cases and applied to rigid synthetic model samples with different spin geometries. The suppression of ghost distances with the presented approach works best for symmetric geometries and rigid molecules for up to four-spin systems. The distance distributions obtained by such suppression are consistent with distributions that were previously obtained with two alternative approaches and agree with the expectations for the true interspin distance distributions in these compounds.

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513TU

Tetrameric Supramolecular Assembly of a Cu(II) Complex Containing Schiff Base and Its Catecholase Activity Investigated by Electron Paramagnetic Resonance

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Multicopper enzymes containing more than one copper ion in their active sites are widely utilized in nature for transporting copper ions, transferring electrons, activating dioxygen molecules, and catalyzing many other reactions. One of the ways to understand the nature of the enzymes is the construction and characterization of model compounds. Recent development of metal-assisted self-assembly has made enormous success in mimicking the multicopper enzymes. In this study, we have synthesized a Schiff-base ligand containing two N-, O-donor groups linked by a spacer. By controlling the length and rigidity of the spacer, we could successfully build a tetrameric supramolecular Cu(II) complex (Cu_4L_4 = tetrakis((N,N'-bis(salicylaldimine)-1,2-ethylenediamine)Cu(II)). Single crystal X-ray crystallography of Cu_4L_4 revealed that the copper sites are non-coupled, However, the complexes showed unexpectedly-high catecholase activity when Cu4L4 was treated with 3,5-di-tertbutylcatechol (3,5-DTBC) in the presence of air at basic condition, Electron paramagnetic resonance (EPR) measurements were performed during the time course of the oxidation of 3,5-DTBC by Cu_4L_4. Two kinds of EPR spectra, one with g = [2.050 2.050 2.263] and A(Cu) = [83 83 565] MHz, and the other with g = [2.050 2.050 2.242] and A(Cu) = [75 75 575] MHz, were observed. In this poster, we discuss the changes of the EPR spectra during the oxidation of 3,5-DTBC and compare those with previously reported EPR of binuclear copper enzymes including catecholase to search a possible mechanism of catecholase activity of Cu_4L_4.

514WE

EPR Investigation of Super Reduced State of [FeFe] Hydrogenase from Chlamydomonas reinhardtii

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Hydrogenases catalyze the heterolytic splitting and formation of H_2 and are of high interest in biotechnology, aiming at the generation and conversion of H_2 as renewable energy carrier. The [FeFe] hydrogenases of marine algae are highly active in H_2 production. The active site of this enzyme is highly conserved and contains the H-cluster consisting of a "classical" $[4Fe4S]_H$ cluster coupled via a protein cysteine side group to a unique $[2Fe]_H$ sub-cluster containing CN and CO ligands as well as a dithiol bridging ligand. Currently, two EPR active states have been described, H_{ox} (active oxidized) and H_{ox}-CO (CO inhibited form) having an electronic ground state of S=1/2. In these states the binuclear sub-cluster is in a mixed valence $[FeIFeII]_H$ configuration and exchanged coupled to one of the high spin iron atoms in the diamagnetic $[4Fe4S]^{2+}$ cluster[1,2]. Our combined EPR and FTIR study of the [FeFe] hydrogenase from *Chlamydomonas reinhardtii* (*Cr*) enabled us to identify a third paramagnetic state of the H-cluster, named H_{sred} (active super-reduced). This super-reduced state can be generated by treatment with its natural product/substrate H_2 or by chemical reduction with sodium dithionite. It is suggested that this newly identified state plays an important role in the catalytic mechanism of all [FeFe] hydrogenases. Its g-parameters are consistent with those of a reduced [4Fe-4S] cluster, suggesting a $[4Fe4S]_{H+}$ coupled to the $[FeIFeI]_H$ sub-cluster[3]. HYSCORE and ENDOR studies suggest that similar to the H_{ox} and H_{ox}-CO states spin exchange occurs between the [4Fe4S] and [2Fe] sub-clusters in H_{sred}.

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515TH

Investigation of Influences of Ceramides on Phospholipid Membrane Dynamics by Solid-State NMR and MD Simulations

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Ceramides are lipid molecules that have sphingosine and acyl chain in their structure. They occur mainly in eukaryotic cell membranes. It has been known for a long time that ceramides are very important molecules for many different cell processes, such as apoptosis, cell growth and signaling. Although, the role of ceramide on the mechanisms of such processes is not known yet, there are studies, which reveals some links between the function of ceramide and its effects on membrane dynamics. Therefore, we aimed to investigate the effects of different ceramides on the dynamics of phospholipid membranes by using solid-state NMR techniques in combination with molecular dynamics simulations.

In this study, pure C16-Cer and DMPC liposomes and DMPC – C16-Cer (20% C16-Cer) mixture were examined. Temperature dependent ^{1}H-, 14N-, 31P static and MAS NMR, DIPSHIFT spectra were recorded. These allowed to investigate membrane phase behavior, domain formations, changes in electrostatic surface potential, orientation of lipid head groups and order parameters of both of the chains of DMPC and ceramide. We have combined these data with MD simulations to obtain more detailed information at molecular level. Therefore, the combined usage of solid-state NMR methods with theoretical calculations helps to investigate the effects of ceramides on membrane dynamics.

516MO

Pathway for the Transmission of $^{TS}J_{FH}$ Coupling in 2-fluorobenzaldehyde

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Understanding NMR scalar couplings mainly transmitted through-space are of paramount importance for applying NMR parameters for interpreting molecular electronic structures. However, mechanisms involved in their transmission are not always clear, e.g. recently[1] it was shown that in 2-fluorophenol $^{TS}J_{FH}$ is not transmitted through a hydrogen bond, as previously accepted. Instead, that $^{TS}J_{FH}$ coupling was shown to be mainly transmitted by exchange interactions taking place where F and OH electronic clouds overlap. Here it is studied theoretically [at the SOPPA(CCSD) level] and experimentally that $^{TS}J_{FHa}$ coupling for the 2-fluorobenzaldehyde (Fig. 1) is transmitted more effectively, $^{TS}J_{FHa}$ = -2.6 Hz, when H_a and F are in the trans arrangement. For the cis form $^{TS}J_{FHa}$ coupling is around 0.5 Hz.

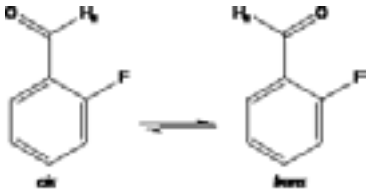

Fig.1. For 2-fluorobenzaldehyde the optimized geometry (isolated molecule) yields cis as preferential in 2.2 kcal mol^{-1} respect to the trans conformation and it is observed that $^{TS}J_{FHa}$ = -2.6 Hz is transmitted mainly due to the F---O close contact.

For the *trans* form, there is steric interaction between $LP_2(F)$ and $LP_2(O)$, which increases the $LP_2(F)$ s character to 0.32 %, allowing the FC interaction to be transmitted from $LP_2(F)$ to $LP_2(O)$; the latter undergoes a strong $LP_2(O)\rightarrow\sigma^*_{C\text{-}Ha}$ interaction transferring the FC information from $LP_2(O)$ to the formyl proton (H_a). For the *cis* form the $LP_2(F)$ % s character is about 15 times smaller than that for the trans form. This FC coupling pathway is confirmed resorting to the FCCP-CMOs.[2] This calls for some caution when intending to use $^{TS}J_{XY}$ to gauge the proximity between the X, Y coupling nuclei.

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FAPESP, CNPq

517TU

Conformational Preferences of Indole-Based Receptors and Their Interactions with Anions

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The conformational preferences of indole-based anion receptors and their complexes with different anions have been studied by a combination of heteronuclear NMR spectroscopy and quantum mechanical calculations. In the first group of receptors, a single indole scaffold has been functionalized with an amide group at C2 and a variety of amide, urea and thiourea functional groups at C7 [1-3]. NOE experiments showed that anti–anti conformation across C2–C2α and C7–N7α bonds is favored in an acetone-d6 solution in the absence of anions. Upon anion binding to receptors, *syn–syn* conformation becomes predominant. The second group of receptors exhibited an extra indole group, which resulted in diindolyl(thio)ureas [4]. NOE enhancements showed that the anti–anti conformer along the C7–N7α bonds is preferred in DMSO-d6 solution in the absence of anions. Anion-induced ^{1}H and ^{15}N chemical shift changes suggested weak binding of chloride anions and negligible conformational changes. Strong deshielding of the ureido protons and moderate deshielding of the indole NH has been observed upon the addition of acetate, benzoate, bicarbonate and dihydrogen phosphate, which indicates that the predominant hydrogen bond interactions occurred at urea donor groups. Binding of oxoanions caused remarkable conformational changes along the C7–N7α bonds and the *syn–syn* conformer was preferred for anion–receptor complexes. The conformational changes in functionalized indoles and diindolyl(thio)ureas upon anion binding are in good agreement with the energy preferences established by *ab initio* calculations.

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519TH

Peculiarities of NMR Spectra of Adamantyl-Substituted Dihydroxynaphthalenes

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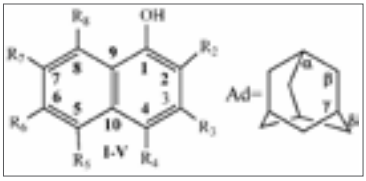
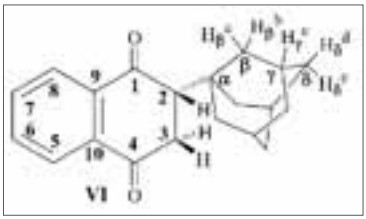
We synthesized adamantyl derivatives of different dihydroxynaphthalenes and while determining of their structures with NMR spectroscopy some peculiarities were found.





For compounds **I** (R_2=Ad; R_6=OH; R_3=R_4=R_5=R_7=R_8=H); **II** (R_3=R_7=Ad; R_6=OH; R_3=R_4=R_5=R_7=R_8=H) and **III** (R_3=R_6=Ad; R_7=OH; R_3=R_4=R_5=R_7=R_8=H) containing adamantyl group in ortho-position to hydroxyl groups of 1,6- and 1,7-dihydroxynaphthalenes low-field shifting from six protons of Hβ and three nuclear of carbon Cβ was observed.

For compounds **IV** (R_3=Ad; R_6=OH; R_2=R_4=R_5=R_7=R_8=H) and V (R_3=Ad; R_7=OH; R_2=R_4=R_5=R_6=R_8=H) containing adamantyl group in metha-position to hydroxyl groups of 1,6- and 1,7- dihydroxynaphthalenes strong-field shifting from six protons of Hβ and three nuclear of carbon Cβ was observed.

In adamantyl fragment of ^{1}H NMR spectra of compound **VI** two multiple signals from six protons of Hβ and two multiple signals from six protons of Hδ were observed. This can be explained that adamantyl group is added to chiral carbon C_2, and by this very reason protons $H\beta^a$ and $H\beta^b$ are decoupled with ^{2}J=12.3 Hz.

520MO

The Influence of Electronic Modifications on Dynamics and Reactivity of Bis-NHC-Complexes as Observed by NMR Spectroscopy

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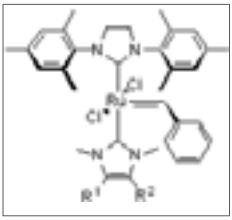

The development of efficient precatalysts for various applications in olefin metathesis is one of the most active research fields of current organometallic chemistry. A promising class of systems is the class of Bis-NHC-complexes **1**, which can show high yields for ring-closing metathesis [1].

To understand the dynamics and reactivity of these systems, we investigated the rotational barriers, corresponding to bond strengths, of the two Ru-NHC-bonds of four Bis-NHC-complexes with NMR spectroscopy.

Analogous to previous studies by our group [2], we used exchange spectroscopy at different temperatures to quantify the rotational barriers. We first identified the temperature range of interest using the EASY-ROESY experiment [3]. With the help of the PANIC-approach [4], we were able to extract the values of the rotational barriers from 1D PFGSE NOE spectra [5,6] at various temperatures.

It was possible to link these rotational barriers to the reactivity of the complexes. The presentation will show the results of this study [7].

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521TU

The Nuclear Quadrupole Resonance - Based Screening of Medicines

Georgia Kyriakidou[1], Iain Poplett[1], Michael Rowe[1], John Smith[1], Kaspar Althoefer[1], Jamie Barras[1], Shota Katsura[2], Hideo Itozaki[2]

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Medicines counterfeiting is a growing problem worldwide, and techniques for screening medicines to tell real from fake are receiving increasing attention.One such technique is Nuclear Quadrupole Resonance (NQR). The observed frequencies are characteristic of the nucleus and its chemical environment. When applied to medicines screening the measurement can be arranged such that signals are seen only from the active pharmaceutical ingredient (API) and not from any other materials present. The technique is quantitative and can estimate the API present to within 2%. Signals are only seen from solids so suspensions can be examined without interference from the medium. The key nucleus in medicines screening is ^{14}N, present in over 80% of medicines. Other quadrupolar nuclei are also found in medicines, such as ^{23}Na, ^{35}Cl, $^{79,81}Br$ and ^{127}I.

In this presentation we report progress in a project sponsored by the Wellcome Trust to develop a portable prototype to detect NQR signals from the API in medicines in tablets or capsules from bottles, blister packs and cartons containing them – a unique feature of the method. ^{14}N NQR signals from sealed packets of, for example, paracetamol at 2.564 MHz can be obtained in only a few seconds with no interference from the packaging material. NQR signals are usually inhomogeneously broadened, due to effects such as the presence of impurities or crystal defects, giving information on the likely origin of the medicine and its method of manufacture. We have shown that we can differentiate between real and fake medicines at a quantitative level and detect NQR signals from medicines including antimalarials (metakelfin, SP), antibiotics (ampicillin, amoxicillin), antihypertensives (furosemide), slimming pills (orlistat), antibacterials (sulfadoxine and sulfapyridine) and more.

As blister packs come in many configurations, medicines' screening would be aided by the use of variable-pitch coils to create a more homogeneous RF field across the whole length and breadth of the coil. We show the results of measurements with one such variable-pitch coil that demonstrate that it is much less sensitive to the position of the sample within the coil that a comparable fixed-pitch coil, increasing the utility of the technique in this application.

522WE

Accurate NOE-Derived Interproton Distances - Fact of Fiction?

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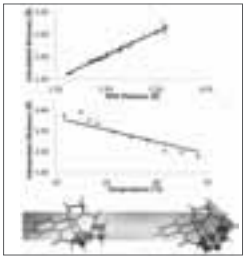
University of Bristol, Bristol, UK

Establishing an Initial Level of Accuracy
Accurate interproton distance determination in solution using NOE data is an area of significant interest and complexity. We present a simple method to derive accurate interproton distances from within rigid and flexible systems using NOE data. Strychnine is used as a rigid model system to test the validity of this method. A comparison of the 1D NOE-derived distances and the computed structure of strychnine[1] (right) gives an average error of only 2.3% (0.07Å).[2]

Low Level Conformer Identification
A second low-level conformer of strychnine is identified using NOE data and confirmed computationally, showing the potential of accurate NOE measurements to determine minute contributions to structure ensembles in solution.[3]

Modelling Populations in Flexible Compounds
NOE data is further applied to the small flexible molecule, 4-propylaniline, to confirm and predict the relative populations of the multiple possible conformers.[4] It is suggested that with the highly accurate interproton distances determined using this method, there is less need for reliance on the large numbers of loose restraints, such as scalar couplings, typically used in the dynamical analysis of flexible molecules.



Monitoring Changes in Conformer Populations with Temperature
NOE-distance relationships are also shown to be sufficiently accurate to monitor very small changes in conformer populations in solution (<0.5%/10 °C) – in response to temperature – in good agreement with Boltzmann-predictions, illustrating the effectiveness of accurate NOE-distance measurements in obtaining high quality dynamical, as well as structural, information for small molecules.

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523TH

Solid State Solubility of Miconazole in Poly[(ethylene glycol)-g-vinyl alcohol] Using Hot-Melt Extrusion

Victor Litvinov[1], Sandra Guns[2], Peter Adriaensens[3], Boudewijn Scholtens[4], Mai Quaedflieg[4], Robert Carleer[3], Guy Van den Mooter[2]
[1]DSM Resolve, Geleen, The Netherlands, [2]University of Leuven, Leuven, Belgium, [3]University of Hasselt, Diepenbeek, Belgium, [4]DSM Xplore, Geleen, The Netherlands

The use of hot-melt extrusion for preparing homogeneous API-excipient mixtures is studied for miconazole-PEG-*g*-PVA [poly(ethylene glycol) – poly(vinyl alcohol) graft copolymer] solid dispersions with a 5 cc table-top, twin-screw co-rotating micro-compounder (DSM Xplore). Phase behavior of PEG-*g*-PVA, miscibility of miconazole in PEG-*g*-PVA and the partitioning of miconazole between PEG and PVA amorphous phases are characterized using a combination of temperature modulated DSC, XRPD, and solid-state ^{1}H and ^{13}C NMR methods. Phase composition (PVA crystallinity) is not largely affected by hot-melt extrusion and the presence of the drug. Miconazole preferably resides in the PEG amorphous phase and its molecules are well dispersed in the PEG-*g*-PVA matrix using hot-melt extrusion mixing. Miconazole forms amorphous nano-clusters whose average size equals approximately 1.6 nm, indicating solid solution formation (molecular level dispersion) of the drug in the polymer. The study also shows that hot-melt extrusion can be a very efficient method for preparing pharmaceutical formulations.

524MO

Study of Near-Symmetric Cyclodextrins by Compressed Sensing 2D NMR

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Modified cyclodextrins are very attractive compounds which can act as chemosensors, artificial enzymes, drug carriers, molecular reactors etc.

Here, we demonstrate that compressed sensing 2D NMR spectra [1] allow for the full spectral assignment of near-symmetric β-cyclodextrin derivatives (one sugar unit C6-mono- modified), which were designed to be receptors of the anticancer drug doxorubicin [2]. The complete assignment of ^{1}H and ^{13}C chemical shifts of asymmetrically functionalised cyclodextrins is a challenging task, and cannot be simply done by means of conventional experiments, owing to the peaks overlap caused by the similarity of seven sugar units.

The CS approach to reconstructing 2D NMR spectra from the NUS data ensures experimental time saving and the resolution improvement, what is here highly desired for unambiguous assignment. The IRLS reconstruction [3] of HSQC spectrum from 5.12% of the data (512 pts out of 10000, 1 hour of exp. time) delivered highly resolved 2D HSQC spectra. Moreover, reconstructed 2D HSQC-TOCSY spectra yield information about the correlations within one sugar unit and 2D HSQC-NOESY technique allows the sequential assignment of the glucosidic units (in both cases: 10,24% of the data, measurement time ca. 5 hours).

The method of CS makes it possible to obtain the set of properly resolved 2D NMR spectra required for the full assignment recorded in overnight experimental time, and can be further recommended for other oligosaccharides and other complex organic molecules.

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525TU

INPHARMA: Paving the Way Towards Structure-Based Drug Design for Proteins with Poorly Known Structure and Membrane Receptors

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EMBL, Heidelberg, Germany

Success in structure-based drug design relies on the knowledge of the structure of the receptor of interest in complex with a library of selected fragment-ligands. When crystal structures cannot be obtained, the generation of complex models through *in silico* docking represents a viable alternative. Unfortunately, the accuracy of the scoring of docked poses is severely limited by the quality of the target structure and by the inherent approximations of the docking algorithms.

The scoring of docked poses can be substantially improved by the inclusion of experimental data, derived for example in NMR experiments. In this perspective, our laboratory developed the INPHARMA methodology. INPHARMA-NOEs generate from the protein-mediated magnetization transfer occurring between two ligands that bind competitively to the target receptor (1). The measured INPHARMA-NOEs are compared to the ones computed by means of the full-relaxation-matrix approach on combinations of ligands docking poses. Using INPHARMA it was possible to correctly identify the binding mode of two fragment-ligands interacting with the hamster protein kinase A (2).

The methodology robustness was put to test using target structures of different quality (3). As a result of this analysis, INPHARMA proved to be very reliable for target structures of good quality, while its performance decreases with the quality of the protein structure.

Here I will show how INPHARMA can be successfully used to rescore docking poses that have been generated with protein structures of poor quality. The approach is based on extending the experimental set by measuring INPHARMA on several pairs of ligands and by intersecting the results (multiplexing). Furthermore, I will show how this approach can be applied to membrane proteins, which are challenging targets for both modelling and experimental approaches.

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526WE

NMR Chemical Shifts to Characterize Binding Ag^+ to Quinalphos

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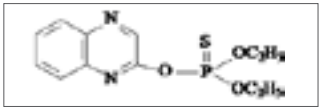
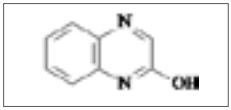
Hydrolysis of the organophosphorothioate ester, quinalphos (**Q**, O,O-diethyl O-quinoxaline-2-yl phosphorothioate) was studied in the absence and presence of Ag^+ at 25°C and pH 4.0, 7.0 and 10.0 using HPLC with UV detector. The hydrolysis products were 2-hydroxyquinoxaline (**HQ**) and O,O-diethyl phosphorothioic acid (**PA**). The kinetic data show clearly that Ag^+ facilitated the hydrolysis of **Q**; *viz* the catalytic rate increased with increasing the pH.

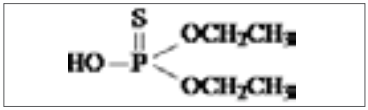
In the second part of this study, we employed NMR technique to investigate how Ag^+ coordinates the substrate (**Q**) in ways that accelerate hydrolysis. ^1H, ^{13}C, and ^{31}P NMR data show that Ag+ has the ability to bind to both sites S and N atoms. This leads to the hypothesis of that this metal ion (Ag^+) may form a six membered ring with Q and that this complex facilitates nucleophilic attack, enhancing hydrolysis through S_N2 (P) process.



(Q)



(HQ)



(PA)

527TH

Crosslinked Helically Chiral Poly-(γ-benzyl-L-glutamate) as Enantiodifferentiating Alignment Medium

Tobias Montag, Christina M. Thiele

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The development of new alignment media – for the structure determination of small molecules by RDCs[1] – is of great interest. For the alignment of organic compounds mainly liquid crystals like homopolypetides[2] or polymer gels like crosslinked poly(acrylonitrile)[3] are used. Polymer gels have several advantages.[4] Unfortunately most organic solvent based gels are achiral.[5] Homopolypeptide based media do have the benefit of a helically chiral structure, which allows enantiodiscrimination.[2, 6]

Therefore we study the syntheses of polymer gels based on crosslinked helically chiral poly-(γ-benzyl-L-glutamate) (PBLG)[7] which swell in $CDCl_3$. We show the reliable measurement of RDCs with the help of (+)-/(-)-isopinocampheol and (+)-/(-)-camphorsultam and observed enantiodifferentiation for the two enantiomers of both analytes.

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528MO

New Microemulsions and Applications Bring NMR Chromatography into the Analytical Chemistry Mainstream

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NMR spectroscopy is an excellent tool for structural analysis of pure compound NMR spectroscopy is an excellent tool for structural analysis of pure compounds. However, for mixtures it performs poorly because of overlapping signals. Diffusion ordered NMR spectroscopy (DOSY) can be used to separate the spectra of compounds with widely differing molecular weights, but the separation is usually insufficient. Recently developed NMR 'chromatographic' methods increase the diffusion separation.

Using nanostructured dispersed media, such as microemulsions, yields high-resolution spectra. The phase diagram of water/SDS/butan-1-ol/cyclohexane and its perdeuterated form is investigated and its oil-in-water (O/W), water-in-oil (W/O) and bicontinuous regions are defined by NMR diffusion and conductivity measurement. The diffusion behavior of analytes in O/W, W/O and bicontinuous microemulsions, both fluorinated and deuterated, serving as NMR chromatography 'solvents' are analyzed and compared. They seem to reflect the analytes' hydrophilicity and lipophilicity. A model is developed for prediction and control of the diffusion separation, allowing one to make an intelligent choice between the several NMR chromatography solvents that have been developed. Mixtures of up to 11 compounds have been separated by this new method.

NMR spectroscopy has been applied to the separation of pharmaceuticals, vitamins, fragrances and sugars and is expected to have many more applications.

529TU

Interactions between Cucurbiturils and Gases in Aqueous Solution Observed by NMR

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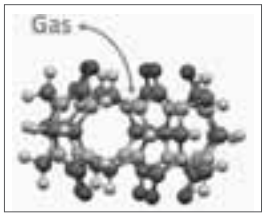

There is a growing interest for the development of molecular containers dedicated to gas trapping in water. (1) Among them, cucurbiturils are particularly rigid and able to trap a wide variety of gases. Here we present NMR experimental results on interaction of noble gases and small alkanes with cucurbiturils. An emphasis has first been placed done on the xenon@cucurbit[5]uril system in water. (2) It shows a spontaneous but very slow binding at room temperature, due to the small diameter of the cucurbituril portals. A large dependence with temperature of the in/out exchange kinetics is also observed. Theoretical calculations are in agreement with NMR data, allowing a better interpretation of the interactions with noble gases in aqueous solution. Intermolecular interactions between cucurbiturils have also been deduced from observations. Then the study was extended to lighter noble gases and light alkanes in interaction with new cucurbit[5]uril derivatives. We show that for the smallest gases the binding is entropically driven. We also observe a large dependence on kinetic and thermodynamic binding as a function of the cage-molecules substituents. This may have impact on selective encapsulation of gases of economical or ecological interest.

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530WE

Recent Developments in NMR Spectroscopy- Applications in Natural Product Chemistry

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Natural products are highly evolved, specific, and effective gene products. Their diverse structural and stereochemical characteristics make them valuable templates for exploring novel molecular diversity. Globally, there is a revival of interest in the use of natural products for the treatment of various ailments. This is mainly due to increased awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of currently available synthetic medicines, reported cases of adverse side-effects of modern medicines and perceived gentleness of natural medicines. Nuclear magnetic resonance spectroscopy has been extensively employed to not only solve complex structures of natural products, but also used to understand their interactions with the biological targets as a drug discovery tool. The development in hardware and introduction of new technologies in NMR has changed the paradigm in drug discovery to a greater extent.

During our studies on bioactive natural products in last three decades, we have isolated a large number of heterocyclic compounds with novel chemical structures and interesting biological activities. This includes steroidal lactones (withanolides), indole, diterpenoid, isoquinoline and steroidal alkaloids, flavonoids, coumarins, and other classes of compounds. A large number of these compounds were systematically screened for *in vitro* and *in vivo* biological activities by employing high-throughput mechanism based bioassay screening and bioactive constituents were further analyzed by using STD NMR spectroscopy. Several bioactive natural products were also subjected to biotransformation and large libraries of novel structures were obtained. As a result, several new classes of potent enzyme inhibitors, antioxidant, immunomodulating, antiglycating and antiparasitic compounds were identified, some of which are in different phases of development.

During this presentation, the effective use of NMR spectroscopy in solving natural product specific problems, such as stereochemical assignments, very limited quantities, complex mixture analysis, as well as epitope mapping in biological targets will be presented, along with an overview of emerging technologies and advent of hardware to solve many issues which are specific to natural products.

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Epitop Mapping of Novel Urease Inhibitors by NMR Spectroscopy

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Research in the field of enzyme inhibition has enormous potential to introduce new drug candidates against different clinical conditions. Urease catalyzes the hydrolysis of urea to ammonia and carbamate. The net effect of the reaction is the localized increased in pH. Urease activity plays an important role in the pathogenesis of gastric and peptic ulcers, urolithiasis, hepatic encephalopathy, and hepatic coma. Keeping in view the therapeutic importance of ureases, we have selected them as potential targets to discover new lead molecules for the treatment of gastric ulcers, and other urease associated pathologies.

The NMR spectroscopy is a powerful techniques to study the protein-ligand interaction. It has high sensitivity to detect weak interactions. This has become a method of choice to screen the library of compounds with enzymes and other biologically important proteins.

In our laboratory, over 2,000 natural and synthetic compounds were systematically screened against the urease enzyme by using high-throughput mechanism-based assays. As a result several new classes of potent urease inhibitors have been discovered. We have extensively employed NMR spectroscopic methods to understand the ligand-recptor interactions, particularly for epitope mapping of any of potent urease inhibitors. Saturation Transfer Difference NMR (STD) and Transferred Nuclear Overhauser Effect NMR (TrNOE) techniques were used to obtain insight into the interactions of inhibitors and enzyme at atomic resolution. The results of such studies were found to be consistent to many of the in silico and modeling studies conducted in our laboratory. This led to the discovery of high affinity ligands against urease enzyme. The results of these studies will be presented as an example to the use of NMR in the discovery of new and high affinity inhibitors.

WITHDRAWN

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Configurational and Conformational Studies of Marine Natural Products

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The configurational and conformational assignment of natural products is essential to understand their biological activity on a molecular level and to allow their procurement through total synthesis. Methods such as X-ray crystallography require crystalline products, and chemical synthesis is usually very time consuming and not always definitive. The structural elucidation of amorphous molecules with several unknown stereogenic centers would benefit greatly from a method that could simultaneously analyze all configurations. The fc-rDG/DDD method (floating chirality restrained DG/DDD) is a combination of distance geometry (DG) and distance-bounds driven dynamics (DDD) calculations using interproton distances and floating chirality in order to determine the relative configuration of small molecules (including low molecular-weight natural products). The application of the fc-rDG/DDD method to several dimeric pyrrole-imidazole alkaloids will be discussed. These molecules have eight contiguous stereogenic centers, necessitating a method which allows a simultaneous determination of all unknown centers [1]. This investigation will include the application to axinellamine A and 3,7-*epi* massadine chloride using interproton distances derived from ROESY spectra [2]. Another example is an intermediate in the total synthesis of palau'amine, 20-deoxymacropalau'amine azide [3] which will be the main focus of this contribution. This compound has a very interesting structure with a 9-membered ring. Besides the configurational assignment of the five stereogenic centers, a detailed conformational analysis was carried out [4]. This is of special importance because macro-palau'amine is the direct precursor of palau'amine in its total synthesis. The final step in this synthesis is transannular ring closure of macro-palau'amine to palau'amine.

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533TU

Application of DOSY NMR Spectroscopy to Study Molecular Self-Assembly in Solution

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In the present work we deal with the application of Diffusion Ordered NMR SpectroscopY (DOSY) to determine thermodynamic parameters associated with molecular self-assembly in solution. The set of molecules known to aggregate in neutral solution with equilibrium constants K ranging from 10 M^{-1} up to 60000 M^{-1} have been investigated by means of ^{1}H DOSY and ^{1}H chemical shift titration at 600 MHz. The set of self-association models, either dependent or not on the hydrodynamic shape of aggregates, have been applied in order to evaluate the *K* values, viz. dimer, indefinite association (EK model), cylinder, ellipsoid and sphere, each of which includes or excludes the intrinsic attenuation of *K* on aggregate growth. As a reference we used *K* values derived from a ^{1}H chemical shift dataset using the EK model, the most widely used approach to study molecular assembly by means of NMR. It was found that none of the tested models was able to reproduce the *K* values derived from chemical shift measurements for the whole series of studied molecules. An alternative approach based on the empirical dependence of diffusion coefficient of an aggregate as a function of the number of molecules in it was suggested and showed the most satisfactory agreement with ^{1}H chemical shift data. The results of computations over a large set of molecules were also analysed in terms of answering the question as to whether the aggregation proceeds beyond the dimer stage. It was found that the DOSY-based approach may potentially be able to solve the problem of distinguishing dimer from EK models, a problem commonly encountered when dealing with molecular self-assembly.

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Reporter Ligand Screening for Inhibitors of 2OG Oxygenases

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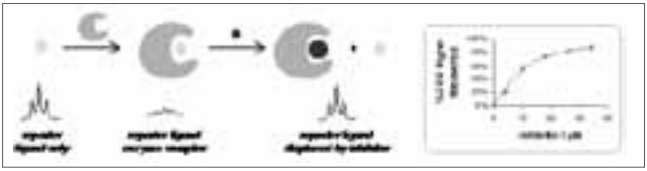
2-Oxoglutarate (2OG) dependent oxygenases are ubiquitous in plants, micro-organisms and animals. In humans, they are involved in a diverse range of important biological roles, including but not limited to oxygen sensing, fatty acid metabolism and epigenetic regulation. Many 2OG oxygenases are current inhibition targets for diseases such as cancer, ischemia and anaemia. To date, most small molecule-based oxygenase inhibitors are designed to bind to the iron(II) in the enzyme active site and act as competitors for the enzyme co-substrate 2OG. Various biophysical methods have been applied to the study of inhibitor binding to 2OG oxygenases, and one the most widely used screening tools is non-denaturing electrospray ionisation mass spectrometry. However, as non-covalent protein-ligand complexes may not always survive the transition from solution phase to gas phase, the search for an alternative solution-based screening technique is still desirable.

The NMR reporter screening method is a useful technique for the site-specific detection of both high- and low-affinity ligands. All 2OG oxygenases utilise 2OG as co-substrate, therefore making 2OG a good candidate as reporter ligand for generic binding assays. Here, using unlabelled and ^{13}C-labelled 2OG as reporter ligand, combined with CPMG-edited ^{1}H and 1D ^{1}H-^{13}C HSQC experiments for spectral editing, we describe the applicability of 2OG displacement for inhibitor screening and quantification (K_i measurement) by NMR.



535TH

Solid-State NMR - a Tool for Investigating the Molecular Architecture of New Pharmaceutical Forms

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The study of different types of solid pharmaceutical forms (polymorphs, salts, hydrates, solvates or co-crystals) is on the critical path of the drug development process, since they tend to have different physical and chemical properties, such as solubility and bio-availability which are essential for the drug development. The structural investigation of new pharmaceutical solid forms leads to the elucidation of the crystal packing modes and the types of intra- and inter-molecular interactions [1].

The present work is focused on the structural characterization of Ethoxzolamide and a series of new compounds based on Ciprofloxacine HCl [2]. The practical innovative approach proposed here is the combination of complementarily structure-elucidation techniques, with the main focus on solid-state NMR and molecular modelling.

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536MO

1D AND 2D NMR STUDIES ON A NONAGGREGATED ZINC PHTHALOCYANINE AS A CONSTITUTIONAL ISOMER MIXTURE

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One of the base methods for phthalocyanine synthesis is condensation of substituted phthalonitrile derivatives. However, condensation of phthalonitrile derivatives with at least three different substituent in all cases leads to constitutional isomers mixture[1]. The four possible isomers are those with molecular symmetries D_{2h}:C_{4h}:C_{2v}:C_s in ratio of 1:1:2:4.Up to now, the successful separation of these four isomers with common column chromatography or by recrystallization has not been reported in the literature. The isolation of limited number of isomers separately was achieved for only specific phthalocyanines using specially designed HPLC columns[2]. NMR spectra of these isomer mixtures show broad signals when compared to phthalonitrile precursor. Identification of regioisomers by NMR spectroscopy is difficult in mixtures because of slight differences in chemical shifts and aggregation behaviour of phthalocyanine macrocycle. Here we present ^{1}H and ^{13}C NMR studies of a hexadecasubstituted zinc phthalocyanine (ZnPc) complex which shows low aggregation and, accordingly, narrow NMR signals. We expect that ZnPc was obtained as a statistical mixture of four regioisomers (figure1) owing to the various possible positions of the phenoxysulfonic acid and chloro side-groups relative to one another.

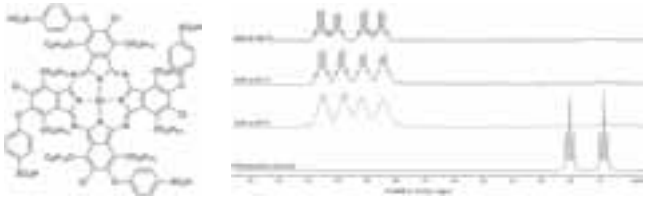

Figure 1 : Structure of ZnPc (only C_{4h} isomer shown) and ^{1}H NMR of -O-CH_2- region

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537TU

LOW TEMPERATURE NMR MEASUREMENTS FOR THE CHARACTERIZATION OF MOLECULAR DYNAMICS IN CRYSTALLINE SOLIDS: STUDY OF SODIUM IBUPROFEN AT CRYOGENIC TEMPERATURES

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The study of molecular motions occurring in solid systems is of particular interest, not only for the intrinsic value of such a deep knowledge of a chemical system, but also because molecular dynamics can be connected with fundamental properties, such as stability of solid phases, solid–solid phase transitions, intra- and intermolecular interactions, and chemical reactivity.

Sodium Ibuprofen represents an interesting case study for a thorough dynamic investigation. Molecular fragments in its dihydrated form are characterized by inter-conformational motions in the kHz and MHz regime even below room temperature, as found from a previous solid-state NMR (SSNMR) study [1]. Low-temperature SSNMR may give a crucial contribution to the quantitative determination of motional parameters such as activation energies and correlation times. However, performing MAS NMR experiments at temperatures below 100 K is challenging. The experiments here reported have been carried out using the custom-built NMR cryo-equipment at University of Southampton. In particular, the experiments done at temperatures ranging from 300 to 140 K have been performed in a system where a standard Varian VT stack was modified such as that both bearing and drive gas flows are cooled by thermal contact with the exhaust gas of the cooling flow [2]. On the other hand, for temperatures below 140 K a cryogenic NMR equipment that uses supercritical helium to reach temperature as low as 1.8 K in static conditions and 13 K under 15 kHz MAS has been used [3]. The simultaneous analysis of ^{13}C and ^{1}H relaxation time measurements, as well as ^{13}C CP-MAS and 2D PASS spectra, performed in the temperature range between 230 and 40 K, along with previously acquired higher temperature data, led to a comprehensive and quantitative characterization of all interconformational motions occurring in crystalline Sodium Ibuprofen.

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538WE

STRUCTURE AND ANTITHROMBIN III BINDING OF HEPARIN-LIKE OLIGOSACCHARIDES: NMR AND THEORETICAL STUDY

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Heparin, isolated from natural sources, is a polysaccharide with blood anticoagulant properties. Binding to antithrombin III accelerates its inhibitory activity against thrombin and factor Xa in the blood-coagulation cascade.[1] The shortest oligosaccharide chain of the heparin sequence having an antithrombotic effect was identified in the 1980s.[2] Subsequently, several research groups synthesized heparin-like derivatives displaying better pharmacological properties than heparin.

During our research we studied the structure of heparin-like oligosaccharides,[1] prepared by the Research Group for Carbohydrates at the University of Debrecen, and their interaction with antithrombin III.

Unambiguous $^{1}H/^{13}C$ assignments of the studied carbohydrate derivatives were accomplished on the basis of standard 2D NMR spectra. Proton-proton scalar coupling constants were determined from phase sensitive TOCSY spectra and internuclear proton-proton distances were estimated from ROESY spectra. These data provide valuable information about the three-dimensional structure of the free oligosaccharides.

The interaction of oligosaccharides with AT-III was assessed by ^{1}H and STD NMR spectra. The hydrogen atoms of carbohydrates in close proximity of the protein binding site were assigned with the use of STD NMR technique.[3] The internuclear proton-proton distances of the oligosaccharides bound to AT-III were determined from transferred NOESY experiments.[3] On the basis of these data molecular modeling has been initiated.

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539TH

$^{n}J(^{77}Se,^{1}H)$-BASED CONFORMATIONAL AND STEREOCHEMICAL ANALYSES OF SELENOSUGARS: NMR AND THEORETICAL STUDY

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Se-glycosides are important starting materials in synthetic carbohydrate chemistry and are useful in studies of biological interactions as well. It was expected that $^{n}J(^{77}Se,^{1}H)$ spin-spin couplings would be instrumental to gain insight into the conformations around the C-Se bond in a series of derivatives with the following structures:

R = H, Me, Ar; R' = H, Ac

Experimental values of $^{n}J(^{77}Se,^{1}H)$ were determined by 1D and/or 2D ^{77}Se-^{1}H CPMG-HSQMBC[1] pulse schemes or by their selectively ^{1}H-decoupled variants. The CPMG cycle was applied at reduced power level to avoid undesired sample heating for the temperature sensitive Se nucleus. Theoretical calculations were carried out at the Second-Order Polarization Propagator Approach (SOPPA) level[2] taking into account all four non-relativistic coupling contributions to the total coupling constant, as implemented in the DALTON program code.[3] All calculations of spin-spin couplings were performed using equilibrium geometries of the true-minimum conformers localized at the MP2/6-31G** level.

It is very encouraging that the population-averaged values of the calculated geminal, $^{2}J(^{77}Se,^{1}H)$, and vicinal, $^{3}J(^{77}Se,^{1}H)$, couplings are in a good agreement with experiment indicating the adequacy of theoretical level of the approach. More importantly, the *two*-bond ^{77}Se-^{1}H coupling constants, as opposed to $^{3}J(^{77}Se,^{1}H)$, turned out to be very sensitive to the torsion angle around the C-Se bond. These findings are of importance for the conformational analyses of Se-glycosides and related molecules.

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540MO

NMR Studies of Nanocrystalline Calcium Hydroxyapatites Prepared by Dry Milling

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Introduction: Stoichiometric calcium hydroxyapatite with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is a reference compound of apatite minerals. Biological and synthetic apatites are usually nonstoichiometric because of ionic substitutions. Apatites are mineral components of teeth and bones. In medicine, they are commonly used as constituents of bioceramics and dental implants. The crystal surface of synthetic and biological apatites is covered with a structured water layer [1]. It serves as an interface between mineral and proteinaceous compartments of hard tissues. Hydroxyapatite contains intracrystalline structural OH groups. The minerals of bone, enamel, dentin and cementum contain ca. 21%, 73%, 18% and 18% of the structural OH groups, respectively, normally present in the stoichiometric hydroxyapatite [2,3]. It has been assumed that the content of the OH groups is dependent on the crystal size [4,5].

Material and methods: Samples of synthetic hydroxyapatite were ground, without any solvent added, in a ball mill. The resulting apatites were found nanocrystalline with the crystal dimensions in the 20 – 100 nm range (TEM), the size being dependent on the grinding time. The samples were then analyzed using solid state ^{1}H and ^{31}P NMR.

Results and conclusions: The study showed that the grinding process severely affected both the amount of water feasible to be adsorbed on the crystal surface and the content of the structural OH groups located in the crystal lattice. The former value increased and the latter decreased on grinding. It has been found that the content of the structural OH groups in calcium hydroxyapatite decreases with the reduction of the crystal dimensions.

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541TU

Towards Chiral Discrimination Using RDCs

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The determination of the molecular configuration is an important task within the structure elucidation of pharmaceutical compounds. To determine the absolute configuration of a stereocenter, X-ray crystallography or VCD spectroscopy are the most common and most powerful tools. In the field of NMR, the use of chiral shift reagents, such as Mosher's ester, Eu-complexes or Pirkle's alcohol, contributed to the instruments for configuration determination. In the last decade, chiral liquid crystals have been introduced and exploited for the investigation of stereocenters[1-2].

In the current study, we describe the application of PELG and PBLG liquid crystals together with ^{13}C detected NMR experiments towards the discrimination of testosterone from 17-epitestosterone using the cross-fitting method[3]. A surprising effect was observed due to the degeneracy of orientations in the aligned state and the molecular symmetry during the analysis of 5a-dihydrotestosterone and 5b-dihydrotestosterone. In the end, an attempt was made to distinguish between 19-nortestosterone and ent-19-nortestosterone as has been described for (R)- and (S)-iboprufen[4].

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542WE

NMR Structural Investigation of Drug Loaded Poly(butylcyanoacrylate) Nanoparticles

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The main challenge in the design of nanoparticles (NPs) as drug delivery systems is to obtain a higher therapeutic effect with minimal toxicity. The efficacy of drug loaded NPs is affected by the physicochemical properties of the drug and nano-formulation and the interplay of these factors with the transport, binding and metabolism of the drug in the body.

Here we present results from NMR-based structural investigation of drug loaded poly(butylcyanoacrylate) NPs (PBCN). ^{1}H and ^{19}F NMR was applied to study the structure and composition of PBCN loaded with daunorubicin (DAU), 5-fluorouracil (5FU) or mixtures of both, considering their potential as nanomedicines for cancer therapy. Diffusion ordered NMR spectroscopy (^{1}H and ^{19}F DOSY) combined with spin-lattice relaxation time (T1) and nuclear Overhauser effect (NOE) studies provided valuable structural information. The combination of these data allowed the description of the mechanisms of drug incorporation into the polymer matrix, drug – polymer interactions and therefore the overall structure of drug-loaded NPs studied.The results confirm the coexistence of different types of drug inclusion into PBCN depending on the drug properties and method of drug loaded NPs preparation. Three forms of DAU inclusion were defined: (i) association by H-bonds and/or dipole-charge interactions; (ii) physical entrapment; and (iii) adsorption on the surface of NPs. The study of 5FU loaded PBCN allowed determining the role of 5FU as an initiator of the anionic polymerization process by its nucleophilic centers (N-1 and N-3). The existence of physically entrapped and covalently bonded to the polymer chains 5FU was defined.

The results based on the NMR analysis indicate that the chemical composition and overall structure of drug loaded PBCN can be tuned by choosing a correct drug loading strategy. Due to the presence of the different modes of drugs inclusion, the PBCN are suitable for sustained and controlled delivery/release of DAU and 5FU.

This work has been carried out with the financial support of NSF/Bulgaria (DO 02-168/2008); FCT/ Portugal, contracts PTDC/SAU-FAR/111414/2009 and REDE/1517/RMN/2005 (with funds from POCI 2010 (FEDER) and FCT).

543TH

Determining the Relative Stereochemistry of Biologically Active Poly-Hydroxylated Pyrrolizidinones and Pyrrolizidines by NMR Spectroscopy

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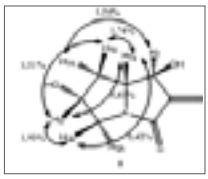
α-Glicosidase inhibitors are important class of compounds that can be used for the treatment of several diseases. Natural and synthetic poly-hydroxylated pyrrolizidinones and pyrrolizidines are good candidates to be used as α-glycosidase inhibitors. Recently, we have developed a new, simple and direct approach for the synthesis of poly-functionalized pyrrolizidinones and pyrrolizidines, based on a highly stereoselective group-directed Morita-Baylis-Hillman (MBH) reaction (Scheme 1).[1]

Scheme 1. Stereoselective group-directed Morita-Baylis-Hillman reaction

The stereoselectivity attained in this MBH reaction was rationalized through the data collected in NMR studies (Figure 1).



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544MO

New Type Inhibitor Studies of Carbonic Anhydrase Using NMR and Molecular Modelling

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Carbonic anhydrases (CA) are proteins that are well-suited to serve as models in many types of studies in biophysics, bioanalysis, the physical-organic chemistry of inhibitor design, and medical chemistry. In vivo, these enzymes catalyze the hydration of CO_2 and dehydration of bicarbonate. There are 16 different isoforms of CA, which are expressed in different cell types or compartments and a few of them are associated with disease development, e.g. CA IX is associated with cancer development and CA II is involved in glaucoma. These isoforms are promising drug targets, but it is important that the obtained inhibitors are very selective.

We expressed and purified from *E. coli* a ^{2}H, ^{13}C and ^{15}N labeled mutant of CA II, which incorporates the active site mutations A65S and N67Q and is designed to mimic CA IX. We were able to assign 243 amino acid residues out of 245 (not including 15 prolines). Recently at our institute a new class of potentially selective CA IX inhibitors – sulfocoumarines – was discovered. We used 1D (^{1}H, STD, WaterLOGSY) and 2D (^{15}N-^{1}H HSQC) NMR experiments to study the interactions of these compounds with CA. Based on protein backbone amide chemical shift perturbation data, we modeled the 3D structures of the protein-inhibitor complex using molecular docking (OPLS 2005 force field). Chemical shift changes of the inhibitor suggested that sulfocoumarines bind in an open form and the sulfate group interacts with the zinc ion. The ligand additionally forms a hydrogen bond between a hydroxyl group and a threonine located in the active site. We also observed that the backbone amide signals of some amino acid residues disappeared from the HSQC spectra during the titrations, indicating a possible conformational change upon ligand binding.

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545TU

Fragment-Based Discovery of Novel Plasmepsin II Inhibitors by NMR Screening

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Malaria, one of the major re-emerging parasitic diseases, is caused by protozoal parasites belonging to the genus plasmodia. To date, resistance has emerged towards all classes of antimalarial drugs except artemisinins, although artemisinin-resistant parasites are starting to appear in some regions. Hence, drugs with novel mechanisms of action are desperately needed to combat the disease. However, large pharma companies are not urging to invest in the development of new antimalarial drugs due to low purchasing power of the potential consumers.

We chose plasmepsin II, an aspartic protease essential in ensuring *Plasmodium falciparum* life cycle, as target for development of new antimalarial drugs. We used NMR spectroscopy to screen a library of 1000 fragment-like compounds against plasmepsin II. Three different NMR experiments were used (T1ρ, WaterLOGSY and STD) to detect protein-ligand binding. In each of these experiments changes of different parameters for ligands in bound and free state are observed. The combined use of such essentially different experiments decreases the possibility of false positives. To probe that the binding is specific to the plasmepsin II active site all hits were tested in competition experiments with a known inhibitor.

We show that our approach is successful in identifying micro-molar fragment hits against plasmepsin II. The binders were confirmed using surface plasmon resonance and enzymatic bioassay. The binding pose was determined by molecular docking to guide further hit optimization. In parallel we are also working on the NMR assignment of plasmepsin II that will facilitate the hit optimization process. We show that we were able to express the protein using ^{2}H, ^{13}C and ^{15}N labelling, and to record heteronuclear triple resonance NMR data.

Acknowledgments: We thank EU European Regional Development Fund (through project 2DP/2.1.1.1.0/10/APIA/VIAA/074) and the EC 7th FP Project Bio-NMR (number: 261863) for financial support and for access to the 1 GHz NMR spectrometer at the Center for High-Field NMR in Lyon, France.

546WE

Protein Camouflage in Cytochrome c-calixarene Complexes

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Molecules that target the cationic side chains of lysine and arginine have potential as generic protein surface binders.[1] Lysine recognition, in particular, is important given the prevalent roles of histones (bearing modified lysines) in the regulation of gene expression.[2] Here, the complex formed between the lysine-rich cytochrome c (cyt c) and p-sulfonatocalix[4]arene (sclx4) is reported.[3]

An NMR titration (Figure 1A) of ^{15}N-labelled cyt c with $sclx_4$ suggests that the ligand is sampling at least two binding regions on the protein surface, with K_d values in the 0.6-1.8 mM range. A crystal structure of the complex, involving two molecules of cyt c and three molecules of $sclx_4$, was determined to 1.4 Å (Figure 1B). The features of the binding sites, all of which involve one or more Lys side chains, will be presented. The potential of sclx4 to generate protein assemblies and to camouflage protein surfaces will also be discussed.

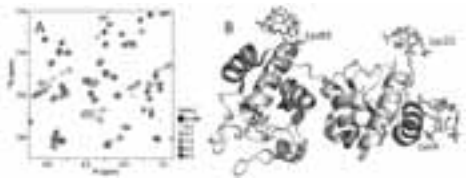

Figure 1: Spectral region from overlaid ^{1}H-^{15}N HSQC spectra of cyt c in the presence of increasing concentrations of sclx4 (A). Crystal structure of cyt c-$sclx_4$ complex (B). Cyt c is represented as a cartoon. Lysine side chains, $sclx_4$, and heme groups are represented as sticks.

Key references:
(1) Renner et al. Journal of the American Chemical Society 2005, 128, 620
(2) Li et al. Nature 2006, 442, 7098
(3) McGovern et al. Nature Chemistry 2012, In Press

547TH

NMR Assignment of the 30 kDa Fucose-Binding Lectin from Ralstonia solanacearum

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Ralstonia solanacearum is a worldwide distributed plant pathogen, responsible for lethal wilting in a wide range of agriculultural crops. Lectins are a family of proteins, known for their carbohydrate-binding activity.

RSL (30 kDa trimer) is a fucose-binding lectin,[1] which is involved in the attachment of *Ralstonia* to cell walls in plant host. In the present study, NMR experiments were carried out to assign the ^{1}H,^{15}N HSQC spectrum of RSL. This task is essential as it provides a useful "fingerprint" for future studies.

A set of 2D and 3D NMR spectra has been acquired in order to perform the assignments for both RSL-mannose and RSL-fucose complexes. In addition, NMR titrations with mannose and fucose have been performed, to attribute chemical shifts to amino acids in the active sites.

This is the first report of the spectrum which describes the RSL backbone amide (N-H). The results will aid the identification of inhibitors of RSL that function by blocking the sugar-binding site. Such inhibitors could be further developed to prevent pathogen binding in crops.

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548MO

"Tautomeric Switching" in Anion Receptors Promotes Remarkable Binding Selectivity

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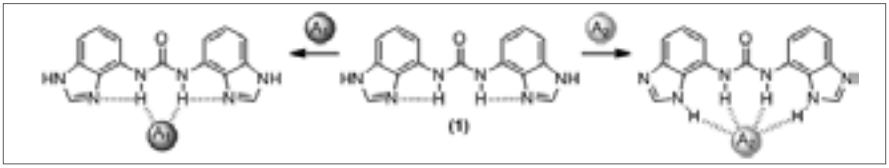
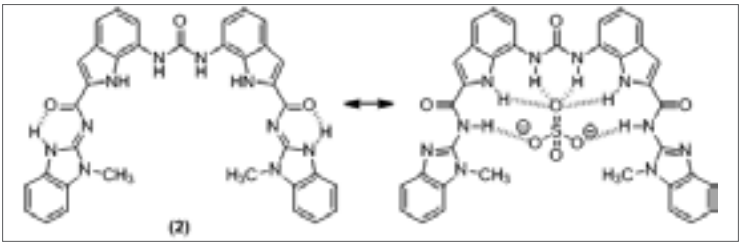
Dibenzoimidazoleurea anion receptors (**1**) have been shown to change their anion-binding motif according to the basicity of the anion via a "tautomeric switch" process and in the process exhibit remarkable binding selectivity:[1]



A similar phenomenon has been observed in a series of pseudo-guanidinium receptors (2) using a combination of multidimensional NMR experiments and NMR titration studies.



1. P. A. Gale, J. R. Hiscock, N. Lalaoui, M. E. Light, N. J. Wells, M. Wenzel, *Org. Biomol. Chem.* **2012**, Advance Article. **DOI**: 10.1039/C1OB06800H

549TU

Structural Characterization with NMR of Derivatized Dextrans as Cancer Diagnostic Agents

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Linear dextrans of MW 10-70 kDa are among the nanoparticle systems currently explored for cancer diagnosis and therapy being inexpensive, stable, easily derivatizable, and of low pharmacological activity and toxicity. The hydrophilic surface of dextran allows for long circulation times and effective transport to the tumor site while properly attached targeting molecules, which specifically bind to surface epitopes or receptors at the tumor site, ensure tumor localization.

In the present work, two dextran (MW approx. 34 kDa) derivatives have been designed and synthesized having as base the incorporation of reactive cysteine moieties on the dextran backbone. The first derivative carries mannose moieties for targeting the mannose receptors of sentinel lymph nodes, the first site of tumor metastasis. The second derivative carries a cysteinyl-analogue of bombesin, a 14-peptide used as tumor marker for small cell lung carcinoma, gastric cancer, and neuroblastoma.

The final products and all intermediates were characterized with NMR employing an array of ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments, carried out on a Bruker AVANCE 500 MHz spectrometer. For bombesin, the sequential assignment strategy was used to identify the amino acid spin systems and fully assign the ^{1}H resonances. NMR is the only means of characterization of dextran nanoparticles that cannot be crystallized for X-ray analysis, and, in addition, are not amenable to MS analysis due to their polymeric nature that generates in each synthetic step mixtures of dextrans differing in the degree of substitution.

For application of the dextran derivatives in cancer radiodiagnosis, complexation of the remaining free cysteine moieties with technetium-99m, the most commonly used radioisotope in clinical practice, was effected. The structural characterization of the complexed dextran derivatives was achieved with NMR through the employment of rhenium, a non-radioactive analogue of techentium-99m. Upon complexation, the cysteine moiety gives broad peaks (typical for cysteine complexes) and characteristic ^{1}H and ^{13}C shifts, that allow the identification and quantification of the chelated cysteine moieties present on dextran.

550WE

NMR Characterization of S-Nitroso-β-cyclodextrin Derivatives

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NCSR "Demokritos", Aghia Paraskevi, Attiki, Greece

Cyclodextrins (CDs) are cyclic oligosaccharides able to capture in their molecular cavity a variety of small organic molecules, thus several CDs have been approved for medical applications as drugs carriers. Selective modification of the CD hydroxyls provides functional derivatives that may merge the action arising from the fuctionalisation with the cavity binding capacity. In the present work, mono-6-S-nitrosothiol-β-cyclodextrin (bmSNO) and per-6-S-nitrosothiol-β-cyclodextrin (bpSNO) were prepared, designed to combine light-triggered and regulated release of nitric oxide (NO) with drug-carrying capacity by complexation with a chemotherapeutic agent in the CD cavity.

RSNO compounds are typically unstable due to facile NO release (e.g. under mild heating), thus they can only be characterized spectroscopically. Although UV-Vis spectra of RSNOs display two characteristic bands, the structure can only be described with NMR spectroscopy. The SNO-CDs were prepared in situ from an acidified solution of the thiols, bmSH and bpSH, and $Na^{15}NO_2$ in DMSO solution. The ^{13}C NMR spectra showed clear signal shifts due to the –SH to –SNO transformation (from ~26 ppm to ~35.3 ppm). A one pulse as well as inverse gated decoupling sequences provided ^{15}N NMR signals of comparable intensity indicating no ^{1}H-^{15}N 3J coupling. Both bmSNO and bpSNO appeared as broad peaks at ~ 800 ppm, close to the frequency of the $S^{15}NO$ resonance of a reference compound, S-nitroso-glutathione ($GS^{15}NO$) at 804 ppm. The broadening of the $GS^{15}NO$ resonance (185 Hz), compared with that of residual nitrite, is attributed to *syn*- to anti-conformation exchange of $–S^{15}N=O$. This could also account for the severe broadening of the $S^{15}NO$ signals of the SNO-CDs, in which additional conformations can be expected *via* possible rotations about the C6-SNO bond as well as cavity inclusion of the lipophilic SNO group(s). Indeed, analyzing the *J*-coupling patterns of the precursor, mono-SH-βCD, using limiting *J* values derived from the Haasnoot-Altona equation, the corresponding populations of the lowest energy conformations about the C5-C6 bond, gauche-gauche (*gg*) and gauche-trans, were found 33% and 67%, respectively, suggesting that the –SH group is rotated mostly toward the cavity. Finally the chemotherapeutic tamoxifen enters the CD cavity without disturbing the SNO integrity, as was shown by 2D ROESY spectra.

551TH

NMR Spectroscopic and Molecular Interaction Studies of Incretin Peptide - GIP

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Diabetes and its related disorders are a major threat to the global community. In this regard, incretin hormones play an important role for secreting insulin for the beta-cell. One of the hormones, glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that stimulates insulin secretion by interacting with a G-protein coupled receptor located in pancreatic β-cell. Due to its glucose lowering and insulinotropic properties, GIP is considered as a potential target for treating type 2 diabetes. In our laboratory, we identified the solution structures of GIP in various solution conditions including membrane mimicking media using NMR spectroscopy and computational modelling techniques. In order to exploit the potential of GIP for diabetes therapy, we focus our research on understanding the hormone-receptor interactions. In this work using NMR based docking approach we have determined the likely docking position of the hormone with its receptor binding region and revealed a likely interaction of GIP amino acid side chains with specific residues on the extra cellular domain from the GIP receptor. These results provide a basic understanding of the interaction mechanism of GIP with its receptor that can be useful for studying the development of peptide or non-peptide drugs for treating type 2 diabetes and other related disorders.

Reference

Venneti K, Hewage C, **Regulatory Peptides**, submitted

552MO

Carbon-13 NMR Studies of Protease Inhibitor Complexes

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Catalysis by proteases involves formation or breakdown of a tetrahedral intermediate [1]. In the serine [1,3,5,6] and cysteine proteases [2] an enzyme nucleophile reacts with the peptide carbonyl carbon to form a tetrahedral intermediate while with the aspartyl [4] and metalloproteases [7]water reacts with the peptide carbonyl to form the tetrahedral intermediate. Inhibitors that mimic these tetrahedral intermediates are expected to be potent protease inhibitors.

We will show how we have used ^{13}C NMR to show how the glyoxal group (RCOCHO) [1,2,3,4,5,6,7] and carboxyl group (COOH) [8] interacts with different classes of proteases.

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[4] Cosgrove, S., Rogers, L., Hewage, C.M. and Malthouse, J.P.G., *Biochemistry,* 46,11205-15, (**2007**).
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[7] Howe, N., Ceruso, M., Spink E. and Malthouse, J.P.G., *Biochimica et Biophysica Acta*, 1814, 1394-1403, (**2011**).
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Acknowledgements
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553TU

Heteronuclear Double Quantum Correlation Experiments Involving Protons for the Study of Partially Ordered and Rigid Systems

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The tremendous success of solution state NMR in deciphering structures of chemical and biological molecules have been largely due experiments that could map proton – proton proximities both through scalar and dipolar couplings. Similar approaches to study molecular structures in solids have been hampered mainly by the presence of very large proton-proton dipolar couplings. However, for the case of partially ordered and fully rigid systems the methodology is undergoing evolution. This is due to the requirement that the homonuclear dipolar couplings need to be eliminated while retaining chemical shifts and the heteronuclear couplings. In the case of protons, the presence of very strong proton-proton interactions requires special techniques and developments in this regard have been continuing. Multiple quantum correlation spectroscopy in the case of rigid and semi rigid systems can provide useful proximity information, as the coherences can be generated between dipolar coupled spin systems. Here, we present our efforts in utilizing proton double quantum and carbon single quantum correlation experiments for the case of static oriented liquid crystal samples and rigid biological samples. Correlations based on both scalar and dipolar couplings are being explored.

Such experiments have the following advantages, viz., (a) provide more definite assignments; (b) uncertainties in proton chemical shifts can be reduced by making redundant information available due to possibilities of several DQ cross peaks present for the same carbon; (c) provide chemical shifts of protons, bonded to some other atoms such as the amide protons; (d) enable ^{13}C-^{13}C correlation. The application of this technique to 5CB liquid crystal and Glutathione tri peptide (GSH) will be presented.

554WE

New Insights into the Spatial Proximities between Spin-1/2 and Quadrupolar Nuclei using D-HMQC Technique Incorporating Different Recoupling Sequences

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UCCS, Univ. Lille Nord de France, Lille, France

We show that valuable and unreported information on the spatial proximities between spin-1/2 nuclei, such as ^{31}P, ^{13}C or ^{1}H, and quadrupolar nuclei, such as ^{27}Al, ^{11}B or ^{51}V, can be obtained using *D*-HMQC.[1] This method alleviates the main limitations of the CP-HETCOR technique: it is robust to rf-field inhomogeneity, MAS frequency fluctuations, spread in resonance frequencies and in quadrupolar coupling constants. Moreover, it does not require complicated and time-consuming optimization procedures, and is efficient at high magnetic field. These advantages permitted (i) to probe ^{31}P-^{27}Al proximities in P_2O_5-doped alumino-silicateglass, (ii) to record hetero-nuclear correlation 2D spectrum showing proximities between ^{31}P nuclei and both tri- and tetragonal ^{11}B sites and (iii) to record the first ^{31}P-^{51}V correlation 2D spectrum.

Furthermore, we show that the choice of the heteronuclear recoupling sequence depends on the magnitude of homonuclear dipolar interactions between the spin-1/2 nuclei.[2] SFAM-1 must be employed for weak homonuclear dipolar interactions, whereas SR 4^2_1 recoupling has to be used for *D*-HMQC involving ^{1}H or ^{19}F.

1 G. Tricot *et al Phys. Chem. Chem. Phys.* 2011, 13, 16786

2 X. Lu *et al J. Chem. Phys.* submitted

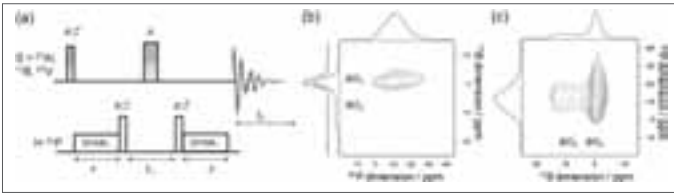

Fig. (a) D-HMQC sequence, Observation of ^{11}B-^{31}P proximities in borophosphate glass using (b) CP-HETCOR and (c) D-HMQC.

555TH

Static 2D Method for Disentangling Quadrupolar Coupling from Chemical Shifts:Application to Sodium NMR in Saturated Rocks

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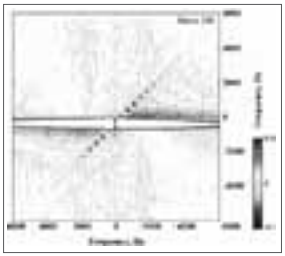
The quadrupolar coupling (f_Q) of Na^+ can be used as a probe for the electric field gradient around the ions in solution or in different media. Since the formation rocks in oil reservoirs are occasionally saturated with highly concentrated NaCl brine, studying the dynamics of Na^+ using NMR offers the opportunity to assess the petrophysical properties of the rock. However, conventional multiple-quantum filtered experiments give spectra with poor signal-to-noise ratios, possibly due to a distribution of quadrupolar couplings as well as chemical shifts, making it impossible to extract coupling information. In this presentation, we show a method based on the quadrupolar jump-and-return (QJR) sequence to demonstrate the existence of a distribution of quadrupolar couplings as well as the chemical shift (or susceptibility) distribution of the ^{23}Na brine signals in rock.

The QJR sequence selectively detects quadrupolar nuclei with quadrupolar coupling. [1] When the sequence is applied in 2D fashion to examine 10% NaCl brine saturated rock samples, each produces unique spectrum. The 2D QJR spectrum of Berea sandstone (Figure), for example exhibits diagonal peaks, which contain information regarding the quadrupolar coupling that is usually unattainable from double quantum filtered experiments. On the other hand, no diagonal peaks are observed in the 2D QJR spectrum from Indiana limestone, which is consistent with the assumption that sodium is repelled from the surface due to the positively charged pore surface.



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556MO

THIRD-SPIN ASSISTED HETERONUCLEAR POLARIZATION TRANSFER BY SECOND-ORDER RESONANT RECOUPLING

Vipin Agarwal[1], Ingo Scholz[1], Mariana Sardo[2], Anja Böckmann[3], Matthias Ernst[1], Beat Meier[1]

[1]ETH Zurich, Laboratory of Physical Chemistry, Wolfgang-Pauli Strasse, 8093 Zurich, Switzerland, [2]a CICECO – Chemistry Department, , University of Aveiro, 3810-193 Aveiro, Portugal, [3]IBCP, UMR 5086 CNRS/Université de Lyon 1, 7 Passage du Vercors, 69367 Lyon, France

Long-range distance restraints are paramount for obtaining the correct fold and structure of biomolecules by solid-state NMR. Polarization transfer based on second-order recoupling sequence play an important role in obtaining long-range distance restraints in biomolecules. Despite the smaller magnitude of couplings resulting from second-order cross-terms at higher MAS frequency, sequences based on second-order cross terms remain popular because they are less sensitive to dipolar truncation which is commonly observed in application of first-order recoupling sequences to uniformly labeled biomolecules. Several second-order recoupling sequences between homo/heteronuclear dipolar couplings have been reported in the literature. e.g. PDSD[1], DARR[2], the CHHC/NHHC experiments, PAR[3], PAIN-CP[4], MIRROR[5] and RESORT[6]. These sequences can be classified as non-resonant or resonant sequences based on the requirement to match a rf-field amplitude or a modulation frequency to the MAS frequency.

The PAR (homonuclear) and PAIN-CP (heteronuclear) sequences exploit the cross terms between two heteronuclear dipolar couplings for magnetization transfer and this mechanism was termed as third-spin assisted recoupling (TSAR) where the third passive spin is usually a proton. The PAIN transfer is always accompanied by a PAR transfer and gives, therefore, rise to additional relay peaks in a two-dimensional heteronuclear N-C correlation spectra.

We propose a new heteronuclear second-order recoupling sequence based on the principles of RESORT. The sequence dubbed heteronuclear-RESORT promotes heteronuclear polarization transfer while at the same time suppressing homonuclear polarization transfer. The heteronuclear-RESORT experiment has been analytically analyzed in the framework of quadri-modal Floquet theory and the effective Hamiltonian at different resonance conditions will be discussed. Through simulations and experiments, a comparison of the heteronuclear polarization-transfer efficiency with the homonuclear polarization transfer at different resonance conditions will be shown. As a practical application, data on U-[^{13}C,^{15}N] Ubiquitin and a mixed sample of [U-^{15}N]/[U-^{13}C] Crh samples will be presented.

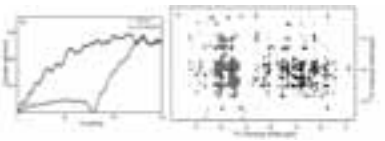

Figure1: a) Simulation depicting transfer efficiency of RESORT and Het-RESORT as a function of ^{1}H r.f field at a particular resonance condition. B) Overlay of PAIN (black) and Het-RESORT spectra for U-[^{13}C,^{15}N] Ubiquitin

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557TU

MEASUREMENT OF ^{27}AL-^{13}C DISTANCES USING OVERCOUPLED RESONATORS AND S-RESPDOR EXPERIMENT

Frédérique Pourpoint[1], Julien Trébosc[1], Régis Gauvin[1], Qiang Wang[2], Olivier Lafon[1], Feng Deng[2], Amoureux Jean-Paul[1]

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Following a lack of inquiry concerning the proximities between nuclei exhibiting close Larmor frequencies, intend is to develop a new solid state NMR method to reach this structural information between nuclei, such as ^{27}Al and ^{13}C, which differ by only 3.6% in Larmor frequencies. Over-coupled resonators are used allowing the tuning and matching of the X channel of the double resonance ^{1}HX probe at the ^{27}Al and ^{13}C Larmor frequencies. This device permits the successive irradiation of ^{27}Al and ^{13}C isotopes in a pulse sequence and we demonstrate ^{27}Al-^{13}C J-HMQC, D-HMQC and S-RESPDOR experiments. The J-HMQC provides information on 27Al-13C connectivities, the D-HMQC allows probing the ^{27}Al-^{13}C proximities qualitatively and the ^{13}C-^{27}Al S-RESPDOR yields accurate Al-C distances. We show that in ^{13}C natural abundance, this approach allows the measurement of Al-C distances of 216 pm for the covalent bond in lithium tetra-alkyl-aluminate, which is commonly used as a co-catalyst in olefin polymerization processes, and ranging from 274 to 381 pm for the three carbons in aluminum lactate. These methods are expected to be useful for the characterization of supported heterogeneous catalysts, which lack long-range order. It will help to bring further understanding in the nature of the Al-C bond in terms of distances and hence, of reactivity.

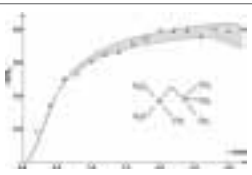

Figure: ^{13}C-^{27}Al S-RESPDOR fraction curve of Li[Al(CH$_3$)$_3$(CH$_2$Si(CH$_3$)$_3$)]

1 F. Pourpoint et al. Phys. Chem. Chem. Phys. submitted

558WE

LOCAL STRUCTURES OF RARE EARTH (RE=LA, Y, LU, SC) ALUMINOSILICATE GLASSES EXPLORED BY MULTINUCLEAR SOLID-STATE NMR

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Thanks to their high glass transition temperatures and beneficial mechanical and optical properties coupled with an excellent corrosion resistance, rare earth (RE) aluminosilicate (AS) glasses of the ternary RE_2O_3—Al_2O_3—SiO_2 systems constitute host materials in a multitude of optical devices, and are suggested for radioactive waste confinement. Many physical properties of RE AS glasses, such as their micro-hardness and Youngs modulus enhance for increasing RE^{3+} cation field strength, CFS=z/r^2, where z and r denotes the charge and radius of the cation, respectively.

Here we compare the network structures of RE_2O_3—Al_2O_3—SiO_2 (RE = La, Y, Lu, Sc) glasses, as explored primarily by ^{27}Al and ^{29}Si magic-angle-spinning (MAS) NMR. We elucidate the dependence of the local structural features of RE AS glasses on variations of their RE/Al/Si cation composition, as well as the RE^{3+} CFS, where the latter increases along the series $La^{3+}<Y^{3+}<Lu^{3+}<Sc^{3+}$.

Narrowed ^{29}Si resonances reveal an AS network ordering for an increase in either the RE or Al content of the glass. Al mainly assumes tetrahedral coordination, but significant fractions of AlO_5 and AlO_6 polyhedra are present in all structures. The relative populations of higher Al coordinations grow for increasing RE^{3+} CFS, where the AlO5 fractions typically constitutes <10% in the La—Al—Si—O glasses, but >30% in their Sc-based counterparts. The local ^{29}Si and ^{27}Al environments, as reflected by chemical shifts and quadrupolar couplings, are overall similar in all samples associated with a constant n_{RE}/n_{Si} molar ratio, except for unexpectedly shielded ^{29}Si NMR signals observed from the Sc–Al–Si–O glasses. Further, ^{45}Sc isotropic chemical shifts derived from MAS spectra acquired at different magnetic fields and from triple-quantum (3QMAS) NMR experiments are typical for ScO_6 environments.

559TH

ENHANCED TWO-DIMENSIONAL CORRELATION SPECTROSCOPY OF QUADRUPOLAR NUCLEI USING DYNAMIC NUCLEAR POLARIZATION; METHODS AND APPLICATIONS

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Porous materials are a cornerstone of the chemical industry since they can be employed in heterogeneous catalysis, separation processes, sensors, photonics, biomaterials etc. However, their rational design is currently limited by the lack of applicable atomic-level characterisation methods. NMR spectroscopy presents itself as a technique that is able to provide detailed information on the atomic-level structure and dynamics of these often disordered systems. Nevertheless, the intrinsic insensitivity of NMR prevents the examination of interfaces; these regions being crucial to the properties of porous materials.

The presented work shows how dynamic nuclear polarization (DNP), in combination with state-of-the-art solid-state NMR methodologies, can be used to overcome this limitation. In this study the gain in sensitivity using DNP in the analysis of a specimen of mesoporous alumina significantly reduced required experimental times by a factor of approximately 400. This allowed the fast acquisition of data that were previously deemed unrealistic. Interface-selective, two-dimensional homonuclear dipolar correlation spectra were recorded using the $BR2^1_2$ recoupling sequence on the quadrupolar nucleus, aluminium-27, in only 4 hours. These spectra elucidated the connections between surface aluminium sites, in particular the catalytically important aluminium-V site. The importance of heteronuclear decoupling during this method is illustrated along with an investigation into the nature of protons close to the interfacial aluminium sites. Also detailed is an evaluation into the determination of the proximity of each aluminium site to the surface by means of a comparison between intensity, T_2* and T_2' measurements and a critique of the use of cross-polarisation build-up curves for analyses in similar systems.

560MO

Thermodynamic Isotope Effects in the ^{2}H NMR Spectra of Partially Deuterated Amino Acids

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Full and partial deuteration of methyl ($-CD_3$) and ammonium ($-ND_3+$) is widely used in both solid- and liquid state NMR to study dynamics and reduce proton linewidths. Implicit in such studies are the assumptions that deuteration is non-perturbative, and that anisotropic NMR interactions are averaged by rapid rotation or hopping between all three sites of the $-CD_3$ or $-ND_3+$ group. Neither assumption is fully valid.

We selectively deuterium-labelled three crystalline amino acids on the ammonium group with deuterium percentages from 10% to ~ 99%. In all three cases, the ^{1}H decoupled deuterium powder spectra show a non-zero asymmetry parameter η because the three deuteration sites have different hydrogen bond strengths, and the ammonium group lacks perfect threefold rotational symmetry. Both factors lead to imperfect three-fold averaging, which ideally should give an axially symmetric spectrum reduced in width by a factor of 3 from that of a static deuteron. Significantly, however, we find that the measured value of η itself depends of the level of deuteration. In all three amino-acids, 10% deuterated samples, in which the predominant deuterium-containing species is $-NDH_2+$, show substantially larger values of η than fully deuterated samples. In L-alanine, for example, η increases from 0.18 to 0.23 between $-ND_3+$ and NDH_2+. At higher levels of deuteration, we can observe distinct singularities in a single spectrum for the three deuterated isotopomers.

The most plausible explanation for this phenomenon is a thermodynamic isotope effect. Because of the uncertainty principle, deuterons are sterically substantially smaller than protons, and they also have lower zero-point energies. They tend to partition into weak hydrogen bonds (which have large zero-point energies, as well as larger ^{2}H quadrupole coupling constants) and out of strong hydrogen bonds. For this reason, the deuteron in an $-NDH_2+$ group will spend less than 1/3 of its time in the strongest hydrogen bond with the smallest C_Q, and more than 1/3 in the weakest hydrogen bond, with the largest CQ. This results in a substantial perturbation of the motionally averaged spectrum from that of the

$-ND_3+$ group, in which each deuteron must by symmetry spend exactly 1/3 of its time in each of the three sites. Full thermochemical quantum mechanical calculations reproduce these isotope effects in a reasonably quantitative fashion.

562WE

Natural Abundance ^{43}Ca Solid State NMR in Minerals and Materials of Biological Origin

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Calcium is one of the most abundant elements in the nature with a prominent significance to the life. The only NMR active isotope ^{43}Ca is notoriously difficult to study due to its low natural abundance (0.135%) and small magnetogyric ratio. Even with very high magnetic fields available today, natural abundance ^{43}Ca Solid State (SS) NMR requires substantial efforts and remains largely under-explored. In this work natural abundance ^{43}Ca SS NMR at 21 T was employed to obtain information about calcium environment in a variety of minerals and materials of biological origin. The spectra of biogenic materials were compared to the spectra of calcium compounds with known structure that commonly considered as models for calcium environment in biological material. We demonstrate that the ^{43}Ca SS NMR is capable to distinguish Ca in a different chemical environment of biological materials of diverse origin. A variety of common mineral such as sulfates, silicates and phosphates have been also explored. The spectral assignments were assisted by the first principles calculations of the chemical shift and quadrupolar tensors of studied nuclei. The calculations were performed using the Gauge Included Projector Augmented Wave (GIPAW) approach[1] with periodic boundary conditions (CASTEP[2]). In some cases an almost quantitative agreement was observed between the experimental and calculated parameters, and the calculations were of major assistance in interpreting the experimental data. The potential and the utility of ^{43}Ca SS NMR in studies of biological materials and minerals will be discussed.

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563TH

Deuterium MAS NMR Studies of Dynamics

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The spin I = 1 deuterium (^{2}H) nucleus has been one of the most widely exploited quadrupolar nuclei in solid-state NMR studies of molecular order and dynamics. The ^{2}H quadrupolar interaction, which generally ranges from 150 to 250 kHz, dominates the lineshapes and is very sensitive to molecular motion over a wide kinetic window. ^{2}H MAS NMR leads to an increase in both sensitivity and resolution and hence allows the site-specific study of dynamic processes.

In this work, we use ^{2}H MAS NMR to monitor the dynamics of the labile deuterons of α-oxalic acid dihydrate and L-histidine monohydrate hydrochloride, which were obtained by recrystallization in heavy water. For these two samples, we measured the ^{2}H Zeeman spin-lattice (T_{1Z}) and quadrupolar (T_{1Q}) spin-lattice relaxation times under MAS at different temperatures for all ^{2}H sites. At low temperatures, the envelope of the spinning sideband manifold forms a powder-pattern-like lineshape. Direct experimental evidence of dynamics can then be obtained by comparing the anisotropy of T_{1Z} and T_{1Q}. We also recorded a series of single-quantum (SQ) and double-quantum (DQ) two-dimensional ^{2}H MAS spectra that can reveal processes occurring on a slower, microsecond timescale.

Different T_{1Z} values were obtained for the various non-degenerate ^{2}H sites in each of the two compounds, suggesting the absence of MAS-induced spin diffusion. We experienced difficulties related to the measurement of ^{2}H T_{1Q} under MAS using broadband Jeener-Broekaert experiments and these will be discussed. Differences between the ^{2}H SQ and DQ linewidths clearly indicate intermediate-timescale motions for D_2O in α-oxalic acid dihydrate and ND_3+ in L-histidine monohydrate hydrochloride. The motions responsible for the line broadening are believed to be 180° flips around the C_2 axis of D_2O molecules and 3-site 120° jumps for ND_3+ groups. The temperature dependence of the ND_3+ SQ linewidths and T_{1Z} obey a simple Arrhenius-type relation that gives access to the activation energy of the dynamic process.

564MO

Characterization of the ^{1}H Double Quantum Fourier and Laplace Spectra of Aged Natural Rubber Samples

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In the last years the nuclear magnetic resonance (NMR) spectroscopy and relaxometry has been used effectively in the analysis of aged natural rubber (NR) samples. The DQ build-up curves for a series of cross-linked natural rubber samples [1] naturally aged during six years is presented and characterized. These curves of ages NR samples presents two peaks which can't be describes by classical functions. The effect of one year of aging in natural conditions can be observed by the apparition of a new component in the DQ build-up curve as a small shoulder shifted to larger time while the six years aging present a larger effect mostly on samples with low values of cross-link density. A successful approach in the analysis of multiple component DQ curves is via DQ Fourier and Laplace spectra. A dedicated program written in C++ was used to obtain the DQ Fourier spectra using a correction term. This can be described by the distributions of the residual dipolar coupling constants characteristic to the distributions of the end-to-end vector associated to the natural rubber polymer network. The deconvolution of DQ Fourier spectra with a sum of four Gaussian distributions are presented and characterized for the entire series of aged natural rubber samples. The DQ Fourier spectra obtained from the DQ build-up curves treated in the approximation of spin -½ pair and the dependence of the correction effective relaxation time values function of cross-link density it's shown for the entire series of aged cross-linked NR samples. These natural rubber DQ build-up curves were analysed also with inverse Laplace transform and the corresponding Laplace spectra are obtained. The differences between the Fourier and Laplace spectra consist mainly in the spectral resolution. The centres of four Gaussinan distributions obtained via bout methods are, in general, consistent.

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565TU

Morphology of Filled EPDM Rubber by Laplace Distribution of ^{1}H NMR Spin-Diffusion Decays

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The ^{1}H NMR spin-diffusion with a DQ filter experiments were recorded for a series of filled EPDM samples function of eight filler types (based on carbon-black, silica and calcium carbonate) and filler content. The EPDM polymer chains can be found i) as segments attached to the filler clusters, the bound rubber or ii) as mobile segments with one or two ends connected to filler aggregates and/or agglomerates. The morphological components of filled EPDM samples are discriminated using the ^{1}H NMR spin-diffusion decays which are analyzed, for the first time using the Laplace inversion procedure [1], and not by Fourier transform and deconvolution of spectra. The morphological components are clearly resolved in the Laplace distribution by their specific relaxation times. The inverse Laplace procedure applied to the ^{1}H NMR spin-diffusion decay curves of filled EPDM sample requires the use of a complex kernel [2] as a sum of Abragamian and exponential functions. The Abragamian peaks are associated with the bounded EPDM rubber to the filler clusters and the exponential peaks are associated to the mobile EPDM polymer chain segments. In order to extract the bound and mobile spin-diffusion coefficients D_b and D_m and length of the EPDM polymer chains segments l_b and l_m, the dependences of the Abragamian and exponential peaks integral areas function of the spin-diffusion time were fitted with a theoretical 1D spin-diffusion equation. With the obtained values for D_b, D_m and l_b the theoretical 1D spin-diffusion equation was used to produce, for the first time, a numerical kernel of a new Laplace-like inversion procedure. This kernel is characterized by a series of mobile EPDM polymer chain segments length l_m values, and was used to extract the distribution of lm. Finally, we observe the multi-modal distributions of the mobile EPDM polymer chain segments length lm, characteristic to each filler type and content.

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566WE

Calculations of Solid-State NMR Parameters of Isocytosine and Sesquiterpene Lactones

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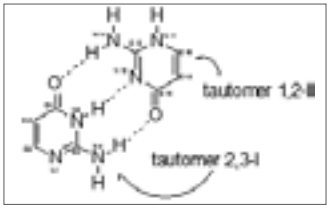
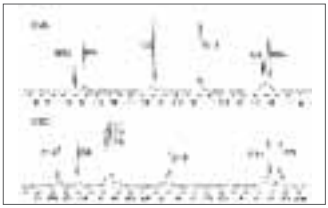
Isocytosine crystallizes as a 1:1 ratio of two tautomers in a manner similar to that of the guanine and cytosine pairs in DNA. The experimental solid-state NMR chemical shifts of crystalline neutral ^{15}N labeled isocytosine were compared with those calculated by three different methods: (1) calculations on isolated molecules, (2) calculations on isocytosine clusters of various sizes, and (3) infinite crystal calculations, that is, the gauge including projector-augmented wave (GIPAW1) method. The data obtained with the GIPAW method were in best agreement with the experimental data.2

The GIPAW method was also used for calculations of solid-state ^{13}C chemical shifts of a series of sesquiterpene lactones. Two polymorphs of helenalin and aromaticin were studied. In the asymmetric unit cells of geigerinin and badkhysin, two geometrically different molecules are present. The experimental differences in ^{13}C chemical shifts between the polymorphs and between the geometrically different molecules were reproduced very well with the GIPAW calculations.[3]





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567TH

Magic Angle Spinning NMR Study of Interaction of N-terminal Sequence of Dermorphin (Tyr-D-Ala-Phe-Gly) with Phospholipids.

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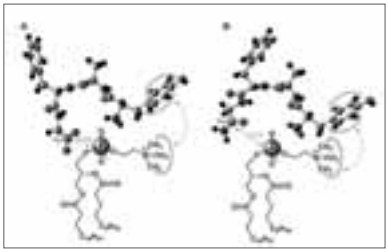
Peptides are important class of natural products responsible for number of vital processes eg metabolism regulations, signal transductions, inhibition the grows of microorganisms etc. Two modifications of Tyr-D-Ala-Phe-Gly tetrapeptide, with different C-terminal groups (Tyr-D-Ala-Phe-Gly-OH (**A**) and Tyr-D-Ala-Phe-Gly-NH2 (**B**)) containing in the sequence, message domain (Tyr-D-Ala-Phe) of important opioid peptides, dermorphin and delthorphins I, II were investigated employing various NMR sequences. Peptides were searched in the solid phase as crystalline powder and as a sample embedded into membrane constructed using DMPC:DMPG phospholipids in the sub-gel Lc and liquid crystalline Lα phases. ^{1}H MAS, ^{13}C MAS, ^{13}C CP/MAS, ^{13}C-^{13}C DARR, ^{13}C-^{31}P REDOR, ^{13}C-^{13}C INADEQUATE MAS and ^{1}H-^{1}H RFDR MAS NOESY experiments were employed.

Our study clearly proved that functional group bonded to terminal carbonyl residue have great impact on alignment and presumably on conformation of pharmacophore. It is known that natural opioid peptides are in most cases ended with amide (B). Thus, information showing that geometry of message domain (A, B) is influenced by the chemistry of C-end can be useful in the design of chemically modified opioids.



568MO

CP-MAS NMR Discrimination of Pure Isomorphic Manidipine α/β and of Their Mixtures

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The two pure isomorphs of manidipine α and β have been studied by CP-MAS NMR to discover remarkable differences as the fingerprints in the solid state NMR spectra. Different regions of the CP-MAS NMR spectra, where the α and β forms remarkably differed, were chosen and compared, and even small amounts (up to 5%) of one form into the other manidipine form were observed. The corresponding mixtures of manidipine α and β at different ratios (50-50%, 5-95%, 95-5%, 85-15%, 15%-85%, 65-35%, 35-65% (w/w)) have been also investigated to try to detect and quantify the amount of one pure form into the other. A new quantitative analysis strategy for polymorphs mixtures has been proposed. The NMR data acquired on the polymorph mixture were directly fitted by experimental NMR data obtained from the individual polymorphic forms. To avoid mathematical complications of fitting using frequency-domain NMR spectra, the time-domain NMR signal was used instead. The proposed mathematical procedure provides reliable quantitative results for mixture analysis of pharmaceutical polymorphs.

569TU

TOPOLOGICAL DETERMINATION OF THE ANIMICROBIAL PEPTIDE HYLASEPTIN P2 IN MEMBRANE-MIMICKING ENVIRONMENT THROUGH SOLUTION AND SOLID-STATE NMR

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The Hylaseptin P2 (HSP2) is an antimicrobial peptide present on *Hypsiboas punctatus* anurans, mainly found at the Amazon rainforest. It has considerable activity against pathogens such as bacteria (both *Gram*-positive and *Gram*-negative) and fungi. Like most of the antimicrobial peptides, it is believed that its mechanism of action is guided by its affinity with the bacterial membrane, involving steps of interaction with the membrane surface followed by the lysis of the cell. The full pathway of the mechanism proposed in the literature is not yet completely unveiled, hence the need to perform different studies to elucidate it better.

In this work, conjoint use of solution and solid-state NMR studies results provided important information regarding the topological features of HSP2 when in membrane-like environments. The solution NMR experiments were performed with a sample containing 40% of TFE and rendered geometric information regarding the tertiary structure of HSP2. The final set of calculated structural models consisted of highly-helical linear chains, with stark amphipatic character.

The solid-state NMR experiments were performed with a sample containing the peptide with selectively placed isotopic labelings (^{15}N label on Leu-16 and C^2H_3 label on A-10) reconstituted on mechanically-oriented POPC bilayers over stacked thin glass plates. The solid-state NMR results provided information on the orientation of HSP2 when interacting with phospholipid bilayers. While the chemical shift gave mostly information on tilt angle of the helical chain, the deuterium quadrupolar splitting yielded data regarding mostly rotational pitch angles of the helix.

Adding up these two results together, it was possible to better describe the topology responsible for the activity of HSP2 on pathogenic microbes, further relating its tertiary structure with the most probable orientation of the molecule during the interaction with bacterial membranes and thus aiding the elucidation of the complete antimicrobial activity mechanism.
ACKNOWLEDGMENT CAPES, CNPq, FAPEMIG

570WE

INTERMOLECULAR STACKING BEHAVIOR DEPENDENT ON SIDE CHAIN STRUCTURE OF A,B-ALTERNATING POLY(ARYLENEVINYLENE) COPOLYMERS

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Molecular packing behavior was investigated in a regioregular poly(arylenevinylene) copolymers semiconductor (S258 and S191B) which has the different side chain structure. First of all, UV analysis was studied and we analyed S191B has the lamellar structure. And also 1H VT and DSC analysis indicated to local mobility has dependent upon the chain size. Second, from 1H VT and DQ BABA (Double Quantum NMR spectroscopy using Back-to-Back pulse sequences) experiments, we conclude that the polymer containing oxygen in the side chains have more rigid and tight interlayer structures than those without oxygen. Using REPT-HDOR (Recoupled Polarization Transfer-Hetero nuclear dipolar-order rotor encoding) experiments, we can measure dipolar coupling (D_{CH}) between C-H bonds for individual CHn groups and extract information about their local mobility.

In this paper, we proved that the new polyarylene(vinylene) copolymers derivatives (S191B and S258) with a small change in polymer back-bone resulted in the change of the molecular packing and the hole mobility[1-7]

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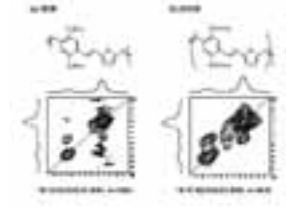

Fig 4. (a) 1H-1H Double-Quantum BABA NMR spectrum of S258 (850MHz , Spinning rate 25kz) (b) 1H-1H Double-Quantum BABA NMR spectrum of 191B (850MHz NMR, Spinning rate 25kz)

571TH

^{1}H Double-Quantum Correlations at Ultra-Fast MAS and Long Excitation Times

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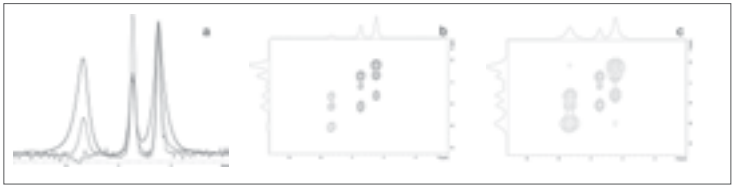
Advances in the ssNMR instrumentation, in particular the extension to spinning frequencies of up to 80 kHz, the so-called ultra-fast MAS regime, has stimulated the development of new or improved methods under the modified experimental conditions. In this context, we report here preliminary results of a study aimed at understanding the behaviour of ^{1}H DQ coherences generated at ultra-fast MAS by the simplest two-pulse sequence ($\varpi/2$-$n\tau_R$-ϖ-$n\tau_R$- $\varpi/2$), of which DQ-excitation mechanism relies upon the *dephasing part* of the time propagator under MAS, as defined in [1]. Interesting features have been observed indeed compared with conventional spinning frequencies, and with sequences (such as BABA) which exploit the *rotor-modulated* part of the MAS time-propagator for DQ-excitation. As an example, the gain in resolution at long DQ-excitation times is briefly illustrated in the figures below for the particular case of L-alanine: (a) DQ filtered spectra recorded with the two-pulse sequence at ν_R = 65 kHz and τ_{ex} ~ 0.18 (blue), 1.84 (red), and 3.68 ms (green); (b) 2D DQ spectrum recorded at τ_{ex} = 1.84 ms; (c) 2D DQ spectrum recorded using BABA at τ_{ex} = 0.03 ms. Qualitatively, this can be explained by the fact that both, the excitation and evolution of the DQ-correlations are governed by an effective Hamiltonian that can be essentially reduced to three-spin interaction terms under ultra-fast MAS.



[1] C. Filip, S. Hafner, I. Schnell, D.E. Demco, H.W. Spiess, *J. Chem. Phys.* **110** (1999) 423

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Understanding the Heteronuclear Decoupling Parameter Space

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Advances in spectrometer technology, such as rising limits of achievable spinning speed, B0 field, and nutation frequency, have created new challenges for heteronuclear decoupling in the solid state. For instance, it is often harder to find good decoupling parameters at increasing MAS rates, and higher B0 fields tend to adversely affect the observed decoherence times. To better understand these issues, a systematic analysis of the factors determining decoupling performance is being carried out in collaboration with a consortium of research groups[1], combining experimental observations with efficient multi-spin simulations under MAS and time-dependent RF[2].

The detailed evolution of the parameter-space with increasing MAS, B0, and nutation rate has been systematically measured for the most widely applied decoupling sequences: CW, TPPM, XiX, and SPINAL-64; with dephasing time under spin-echo, representing the homogeneous limit in resolution, as the primary metric of decoupling performance. Dramatic changes in the peak dephasing times and ease of optimisation for the different sequences have been observed as a function of these parameters. Also, identical experiments on different molecular environments, CH2 of glycine and CH of alanine, have revealed an intricate dependence of peak decoupling on both neighbouring and remote couplings.

Ab-initio simulations of decoherence times have provided additional insights into the effect of pulse transients and RF inhomogeneity on decoupling performance, as well as reinforcing the theoretical understanding of how the experimental results depend on the other parameters.

[1] community.dur.ac.uk/nmr.solids/decoupling/

[2] Zorin, V. E.; Ernst, M.; Brown, S. P. & Hodgkinson, P. Insights into homonuclear decoupling from efficient numerical simulation: Techniques and examples. *J. Magn. Reson.*, **2008**, 192, 183

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100+ kHz MAS NMR

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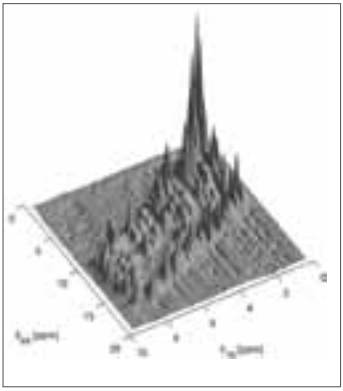

We report initial 1,2 and 3D results with a new, micro-rotor generation of the MAS unit. One of the obvious high speed application categories is related to suppression of homogeneous interactions and improvement of resolution. For the case of adenine methanesulfonate salt there are three hydrogen bonding environments. These protons can be seen clearly with 90 kHz spinning, however at 60 kHz resolution is poorer resulting in less information about the hydrogen bonding environments. As a rule, higher rotation rates come at the cost of reduced sample volume (in our case 0.6-1 uL) which may critically affect sensitivity and may even compromise feasibility of experiments in higher dimensions. However, this is not always the case. Depending on asymptotic value of the linewidth, intensities may grow almost linearly with MAS rate thus compensate for volume loss of S/N. We observe this phenomenon in rigid proton systems spinning beyond 100 kHz (ANPNA peptide 1Q-2Q in figure).

One has to realize, that in this case even a 200 KHz rf field is just a factor of 2 more than mechanical spinning rate and it may be difficult to accommodate multiple "ideal" pulses in less than 10 us rotation period. A general improvement in MQ excitation for *rf* fields up to 410 kHz was observed.

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Radio-Frequency Driven Dipolar Recoupling Experiments at Ultra-Slow MAS

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Slow MAS correlation experiments, which provide direct information on relative molecular orientations, are derived from static 2D CSA correlation techniques. The advantages of the slow MAS experiment over its static counterpart is the possibility to observe samples having multiple sites and to obtain better signal-to-noise ratio while retaining the CSA information in the spinning sideband pattern. Most of the commonly used homonuclear dipolar recoupling sequences are designed to work best at moderate or fast MAS. In many cases rotor-synchronized pulses are used and thus the rf-fields strength decreases with slower sample spinning, leading to a reduced excitation bandwidth. Sites with very different isotropic chemical shift and / or large CSA would therefore no longer be correctly excited. To overcome this problem we employed either composite pulses or adiabatic sweeps such as WURST within classical double-quantum (e.g. $C7_2^1$) or zero-quantum (e.g. fpRFDR or $SR6_6^2$) recoupling sequences. The relative performance of these different sequences will be demonstrated. Additionally, since the rotor period becomes very long at slow MAS, the t1 evolution time can no longer be rotor synchronized. We, thus, employed a time reversal scheme to obtain purely absorptive 2D spectra.

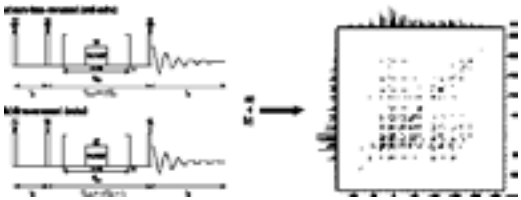

Figure: Pulse sequences and 2D ^{31}P-^{31}P wurst-fpRFDR spectrum of MgP_4O_{11} (four ^{31}P sites) at a MAS of 3kHz and with a mixing time of ~16ms

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CEDARLANE

ROTOTEC-SPINTEC

Sunday 1st July		Time	Monday 2nd July		Tuesday 3rd July		Wednesday 4th July		Thursday 5th July	
10.00 - 16.30	Registration	Chair	Emsley		Pons		Meier		Bodenhausen	
		08.30-09.15	Nicholson		Levitt		Lesage		Schwalbe	
		09.15-10.00	Oschkinat		Van Doorslaer		Blackledge		Brindle	
		10.00-10.45	Coffee		Coffee		Coffee		Coffee	
			Theatre L	Theatre M	Theatre L	Theatre M	Theatre L	Theatre M	Theatre L	Theatre M
		Chair	Brennan/ Crowley	Madhu	Mok	Böckmann	Grey	Navon	Blümich	Jerschow
		10.45-11.20	Griffin	Ernst	Atreya	Duer	Jaroniec	Cohen	Walls	Huster
14.00 - 16.15	**Tutorial Lectures** **Brennan Grandinetti Zilm**	11.20-11.45	Ala-Korpela	Nishiyama	Warren	Wang	Bode	Dumez	Bajaj	Müller
		11.45-12.10	Selenko	Thiele	Kalbitzer	Demers	Maly	Jerschow	Demas	Akbey
		12.10-12.45	Pielak	Nielsen	Morris	Ravotti	Carlier	Nicolay	Appelt	Rienstra
		12.45-13.45	Lunch		Lunch		Lunch		Lunch	
		13.45-15.45	Poster Session (MO) and Tea		Poster Session (TU) and Tea		Poster Session (WE) and Tea		Poster Session (TH) and Tea	
			Theatre L	Theatre M	Theatre L	Theatre M	Theatre L	Theatre M	Theatre L	Theatre M
		Chair	Malthouse	Goldfarb	Williamson	Dolinšek	Griesinger	Köcken-berger	Bax	Van Doorslaer
16.30 - 19.15	**Opening & Prize Session Debelouchina Emsley Tycko**	15.45-16.20	Sattler	Kay	Güntert	Arčon	Hosur	Kuzma	Opella	Blank
		16.20-16.45	Overduin	Roberts	Balbach	Cottrell	Chill	Thurber	Petzold	Jeschke
		16.45-17.10	Tate	Biskup	Giraudeau	Giesecke	Frueh	Hilty	Schnell	Wedge
		17.10-17.45	Feigon	Bennati	Nilges	Deschamps	Wand	Duckett	Neudecker	Dyakonov
		Chair	Karlsson		Delepierre		Jeschke		Papavassiliou (Theatre L)	
		17.55-18.40	Wagner		Vendruscolo		Wrachtrup		Prisner	
19.15	Reception	18.45	Bruker Hospitality Suites		Agilent Hospitality Suites		Free Evening		19.45 Dinner	