EENC 2005

Magnetic Resonance for the Future

Veldhoven, The Netherlands, 3-8 July 2005

Programme and Abstract Book

Programme Schedule

Sunday, 3 July 2005

12.00 - 17.30 21.00 - 23.00	Registration
15.00 - 16.30	Workshops: Rooms 65 (1), 63 (2), 80-81 (3), 82-83 (4), 64 (5) 1) Average Hamiltonian Theory; 2) Multivariate analysis of NMR data; 3) Structure generation and Validation 4) Alignment and Orientation; 5) Computation of NMR parameters
17.00 - 19.45	Mixer and Buffet Dinner
20.00 - 21.00	Opening of the Conference Opening Lecture: R. Kaptein
12.00 - 17.00 21.00 - 24.00	Mounting of posters Mounting of posters; Hospitality Suites

Monday, 4 July 2005

07.00 - 08.30	Breakfast	
08.30 - 10.30	Plenary lectures: Brabant Hall Beat Meier, Nico Tjandra and Heinz-Jürgen Steinhoff,	
10.30 - 11.00	Break	
11.00 - 12.40	Brabant Hall Dynamics: Liquids 1	Auditorium Solids 1
12.45 - 14.00	Lunch	
14.00 - 16.00	Brabant Hall Bio-NMR: Liquids 2	Auditorium Methods: Solids 2
16.00 - 17.30	Workshops 1-5	
17.00 - 18.00	Vendor presentations, Brabant Hall and Auditorium	
16.00 - 18.00	Poster Session with Refreshments	
18.00 - 18.50	Keynote lecture: Brabant Hall Alex Pines	
19.00 - 20.30	Dinner	
16.30 - 01.00	Varian evening, Hospitality Suites	

Tuesday, 5 July 2005

07.00 - 08.30	Breakfast	
08.30 - 10.00	Presentation of awards: Brabant Hall Ampère prize: Malcolm Levitt E.R. Andrew prize: Christian Hilty Russell Varian prize: To be announced	
10.00 - 10.30	Break	
10.30 - 12.40	Brabant Hall Fast NMR: Liquids 3	Auditorium Emerging methods, EPR 1
12.45 - 14.00	Lunch	
14.00 - 16.10	Brabant Hall Biomolecular Interactions: Liquids 4	Auditorium Methods: Solids 3
16.00 - 17.00	Guided poster tours	
17.00 - 18.00	Vendor presentations, Brabant Hall and Auditorium	
16.00 - 18.00	Poster Session with Refreshments	
18.30 - 20.00	Dinner	
20.00 - 01.00	Bruker evening, Hospitality Suites	

Wednesday, 6 July 2005

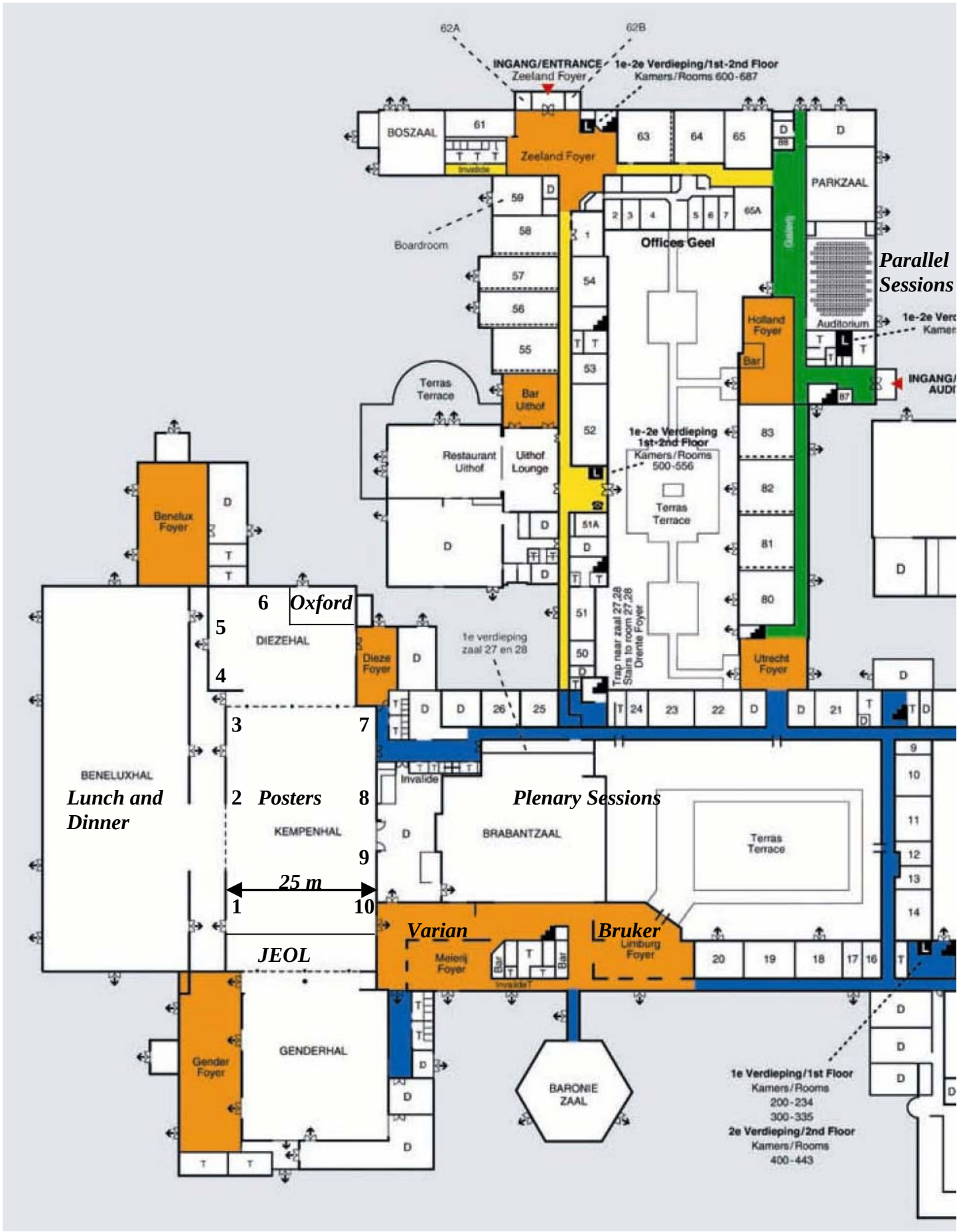
07.00 - 08.30	Breakfast	
08.30 - 10.30	Plenary lectures: Brabant Hall John Sidles , Bob Griffin and Lucio Frydman	
10.30 - 11.00	Break	
11.00 - 12.40	Brabant Hall BioNMR: Solids 4	Auditorium EPR 2
12.45 - 14.00	Lunch	
14.00 - 16.00	Brabant Hall Dynamics: Liquids 5	Auditorium MRI
16.15 - 17.00	Auditorium The future of NMR in Structural Genomics	
16.00 - 17.00	Guided poster tours	
17.00 - 18.00	Vendor presentations, Brabant Hall and Auditorium	
16.00 - 18.00	Poster Session with Refreshments	
18.00 - 18.50	Keynote lecture: Brabant Hall Ad Bax	
19.00 - 20.30	Dinner	
20.30 - 01.00	JEOL evening, Hospitality Suites	

Thursday, 7 July 2005

07.00 - 08.30	Breakfast	
08.30 - 10.30	Plenary lectures: Brabant Hall Hartmut Oschkinat, Jeremy Nicholson and Clare Grey	
10.30 - 11.00	Break	
11.00 - 12.40	Brabant Hall Screening: Liquids 6	Auditorium Diffusion
12.45 - 14.00	Lunch	
14.00 - 15.50	Brabant Hall Computing	Auditorium Bio-NMR: Liquids 7
15.50 - 16.20	Break	
16.20 - 18.00	Brabant Hall Protein folding: Liquids 8	Auditorium Materials: Solids 5
18.10 - 18.20	Closure	
18.20 - 19.30	Take down of posters	
19.30 - ???	Farewell dinner in Caribbean Style	

Friday, 8 July 2005

07.00 - 08.30	Breakfast
08.30 - 12.00	Departure
11.30 - 15.00	Opportunity to visit Dutch NMR Centers



Room 20 (Blue Zone): Meeting Room
Room 21 (Blue Zone): Meeting Room
Room 22 (Blue Zone): Doty Scientific
Room 24 (Blue Zone): Stelar s.r.l.
Room 25 (Blue Zone): RS2D
Room 26 (Blue Zone): Rototec-Spintec

Room 23 (Blue Zone): Conference Office

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Magnetic Resonance for the Future

Veldhoven, The Netherlands, 3-8 July 2005

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EUROMAR was organized with extensive logistics support from:

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Section Biophysical Organic Chemistry/SS NMR, Leiden Institute of Chemistry, Leiden Universiy
HF-NMR facility, Institute for Molecules and Materials, Radboud University, Nijmegen
NH Koningshof

INTRODUCTION

The European Magnetic Resonance Community is moving towards an integration of Magnetic Resonance Conferences in Europe. In this the Ampère, EENC and UK NMR-DG Committees are working together for joining their meetings into one common annual series. This EUROMAR conference takes the place of EENC 2005 and represents a way point in this move. It is organised under the auspices of the EENC International Organising Committee with support from the Ampère and UK NMR-DG Committees. EUROMAR is organised by the "Stichting Chemische Congressen I", which is affiliated to the Royal Netherlands Chemical Society and the Netherlands NMR Discussion Group.

The three conferences all have a long history. The following gives a partial view of this for the EENC and AMPÈRE meetings.

EENC	***Congress Ampère***	***UK NMRDG***
Euromar, 2006, York	Euromar, 2006, York	Euromar, 2006, York
18th EENC 2005/EUROMAR (Joint EENC + Ampere + UK-NMRDG (2005 Veldhoven)	Colloque AMPÈRE (2005 Veldhoven)	(Biannual Series)
17th EENC 2004 Lille (Joint EENC + Ampere)	32nd Congress AMPÈRE (2004 Lille)	
16th EENC 2002 Prague	31st Congress AMPÈRE, 2002, Poznan	
15th EENC 2000 Leipzig	30th Congress AMPÈRE, 2000, Lisbon	
14th EENC 1998 Bled	29th AMPÈRE-13th ISMAR, 1998, Berlin	

Earlier EENC meetings took place in Paris (1996), Oulu (1994), Lisbon (1992), Veldhoven (1990), Bad Aussee (1988), Spa (1986), Altavilla Milicia (1984), Super Nendaz (1982), Königstein (1980), Grenoble (1979), Elsinore (1977), Enschede (1975) and Canterbury (1974)

The Commission of the European Communities has recognized the importance of this move and has provided substantial support for this EUROMAR meeting through its Marie Curie large conferences support programme. Through this programme some 50 young scientist could be fully supported for attending the conference.

The importance of integrating the three conference series has been recognized too by the large vendors of NMR equipment. Their generous support has enabled the organizers to set up the EUROMAR grant fund, through which almost one hundred scientists could obtain a grant that compensated at least half their accommodation and registration costs.

The reduced registration costs for students were also made possible by the generous support of several vendors of NMR related equipment, Netherlands companies and Netherlands scientific organizations.

GENERAL INFORMATION

Registration Badges

The registration badges have to be visibly worn at all conference activities. Please wear them throughout the duration of the conference. Any registration gives access to the poster sessions and to the price award session on Tuesday morning. The refreshments provided during the breaks and the social activities are part of all registration packages, as well as the surcharge for attending the conference dinner on Thursday. All registrations entitle access to the Benelux Hall for the meals. The shuttle services to and from the Eindhoven railway station are included in the registration. The following categories of badges are distinguished:

White badges:
Participants with a regular, student or grant registration are entitled to take part in all scientific and other activities offered during the conference. Thus the participant may attend the lectures, take part in the guided poster tours, workshops and meet the speaker sessions. The participant is allowed to present a poster, provided the scientific committee has accepted the poster(s).

Badges with a black bar:
Delegates with this badge have registered as vendor delegates. A vendor registration only applies to those participants that take part in vendor activities during the conference. This registration does not give access to the normal lecture programme, the workshops, the guided poster tours and so on. It does not allow for the presentation of a poster in the poster sessions. However, the vendor registration gives access to the vendor presentations on Tuesday and Wednesday.

Badges with a blue bar:
This registration applies to accompanying persons. It does not give access to the normal lecture programme, including the vendor presentations on Tuesday and Wednesday, the workshops, the guided poster tours and so on. It does not allow for presenting a poster in the poster sessions.

Badges with a green bar:
These badges apply to the lecturers. The badge gives the same rights as the white badges.

Badges with a yellow bar:
These badges identify participants that are part of the conference organization. It applies to the stewards that provide assistance for ensuring a smooth progress of the conference, to the National Scientific Committee and to the Local Organizing Committee.

Location of Sessions

All plenary lectures take place in the “Brabantzaal” of the “NH Koningshof”. Parallel lectures take place in the “Brabantzaal” and the “Auditorium”. The posters are continuously on display in the “Kempenhal” from Monday morning through Thursday afternoon. The workshops take place in rooms 80-83 and 63-65.

Special sessions

Meet the speaker sessions will be organised on Wednesday and Thursday morning.

Vendor presentations will take place on Tuesday and Wednesday during the poster sessions from 17.00 to 18.00 in the Brabant Hall and the Auditorium. The programme for these presentations will be announced on flyers.

A discussion moderated by Lucia Banci will be held in the Auditorium on July 6 from 16.15-17.00 on the role of NMR in Structural Genomics. This discussion is important for generating advice to the European Commission on the contents of its Life Sciences and Research Infrastructures programmes in the 7th Framework Programme. The discussion takes place in the context of an EU Specific Support Action.

Poster categories

Except for poster abstracts that arrived after the print deadline of 17 June 2005, all poster abstracts are found in the poster-abstract book, sorted by category:

BL	Biomolecular NMR in liquids	EP	EPR
BS	Biomolecular NMR in the solid state	IM	Imaging and in-vivo NMR
CA	Computation/Calculation	ML	Methods development for liquid NMR
MI	Miscellaneous	MS	Methods development for SS NMR
MA	Materials		

Poster sessions

There are three formal poster sessions, on Monday, Tuesday and Wednesday afternoon from 16.00 - 18.00. Authors of odd-numbered posters are asked to present their posters on Tuesday afternoon; authors of even-numbered posters may present their poster on Wednesday afternoon. The Monday session is has no formal rules for the presence of the authors of posters.

Guided poster tours

Guided poster tours will be arranged on Tuesday and Wednesday, during the poster sessions. Registration lists for these tours will be available at the conference office.

Poster set-up and removal

Poster set-up for all posters is possible from 12.00 PM on Sunday, July 3. The poster boards allow for poster size A0+ (portrait orientation, maximum 90*120 cm^2). The boards are numbered by category. The number corresponds to the number in your abstract book. Your poster can be located easiest through the author index. In mounting your poster, please keep in mind the poster category. Posters are displayed either on poster boards or on the walls of the Hall. For fixing the posters on the poster boards thumbtacks or pins may be used. Posters that are to be displayed on the walls have to be fixed by painters' masking tape and not with thumbtacks or pins. This tape is easily removed from the walls and from your poster, without damaging either. The fixing materials are available from the stewards and from the conference office. The posters have to be removed before 19.00 PM on Thursday, July 7, 2005. Authors who leave their posters on the poster boards after 19.00 PM do so on their own risk.

Refreshments, Coffee and Tea

Refreshments, coffee and tea will be served during the breaks, poster sessions and after dinner in the "Kempenhal" and, for those delegates attending the parallel sessions in the "Auditorium", in the "Galerij" and "Holland Foyer".

Meals

Breakfast will be served in the restaurant "Binnenhof" from 7.00 AM. Lunches and dinners (which are all buffet style) will be served in the "Benelux Hall". Dinners generally include one free drink (wine, beer, soft drink). Additional drinks are charged to you personally. At the Conference dinner and Caribbean evening free drinks (within reason) are provided. Lunch will be served daily from 12.45PM. Dinner will be served from 19.00 PM (Conference dinner starts at 19.30 PM).

Reception and mixers

The mixers and the conference reception will take place in the "Kempenhal" or outside the "Limburg Foyer" (weather permitting).

Conference Office

The Conference Office is in Room 23, Blue Zone. Office hours:
12.00 PM - 17.00 PM Sunday
08.30 AM - 18.00 PM Monday
11.00 AM - 18.00 PM Tuesday, Wednesday and Thursday

Internet

The NH Koningshof has an Internet Corner with four PC's. In addition there is a WIFI network in the NH Koningshof. Cards for using this network can be bought at the hotel reception desk. The costs are € 9.00 for two hours service and € 15.00 for 24 hours service. In addition to these possibilities we will provide an Ethernet hub in room 80 (Green Zone) from Tuesday morning till Thursday evening. You can attach your PC to this hub with your own cable (Cat. 5, standard RJ45 plug).

Messages

Letters to both resident and non-resident participants should be addressed to the individual:

NH Koningshof
c/o Conference office EENC 2005 / EUROMAR
Locht 117 - P.O. Box 140 - NL-5500 AC Veldhoven - The Netherlands
Phone: +31-40-2537475; Telefax: +31-40-2545515; E-mail: secr@euromar.org

Messages may be exchanged at the Conference Office and a board will carry the names of those for whom mail and messages have been received. A message board for announcing job opportunities will be located in the "Kempenhal".

Speakers

Please arrive 15 minutes before your session, to familiarize yourself with the audiovisual system and to set up your presentation. Stewards are available to assist you. Room 21, Blue Zone is set up for previewing your slides, and to give the finishing touch to your presentation.

Vendor activities

The vendor activities are at the following locations:

- "Limburg Foyer": Bruker Biospin
- "Meierij Foyer": Varian
- "Kempenhal": JEOL and table top displays from Campro-Scientific, CIL, EURISO-TOP, NewEra/CortecNet, NMRtec, Ritmeester, Umetrics, VenusCorp and Wiley
- "Diezehal": Oxford Instruments
- Room 22 (Blue Zone): Doty Scientific
- Room 24 (Blue Zone): STELAR
- Room 25 (Blue Zone): RS2D
- Room 26 (Blue Zone): Rototec-Spintec

Hospitality activities of the vendors may take place from 12.30 PM - 01.00 AM each day during the Conference at the vendors discretion.

Leisure opportunities

The "NH Koningshof" is located in a very nice environment. There are several walking routes marked. Bicycles may be rented through the "NH Koningshof" reception desk. The "Dommelpoort" sports and recreation center offers a wide range of active and passive recreation.

Accompanying Persons Programme

In view of the small number of accompanying persons, there will be no regular Accompanying Persons Programme. At the Conference Office we have available brochures from the Eindhoven Tourist Office on several possibilities. Attractive opportunities are:

- A visit to the Van Abbemuseum
- A city walk through Eindhoven with help of a brochure
- A visit to the "Heuvel Galerie" with shopping guide and discount coupons
- A visit to the DAF Museum

It is very attractive too to pay a visit to ‘s-Hertogenbosch (25 minutes by train) and Maastricht (1 hour by train). Both cities have nice old town centers, a large variety of museums and churches and attractive mid town shopping and leisure centers.

Transportation to Eindhoven is convenient with public transport. A time schedule is available at the Conference Office.

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Meeting Rooms

Meeting rooms are available for private meetings and for special presentations. These rooms may be booked through the Conference Office.

Bar Service

The “Abdijbar” will be open from 16.00 PM. The “Porticato” and “Dommelpoort” bar services are available throughout the day. Please obtain consumption tokens (offering a discount on regular prices) from the hotel reception desk, as cash payment for bar services will not be accepted. These tokens are accepted too for any additional drinks you might want during dinner. The “NH Koningshof” will refund unused tokens.

Departure

Please note that the hotel rooms have to be vacated by 10.00AM on the day of departure.

On Friday 8 July a bus service will be available for transport between the “NH Koningshof” and the Eindhoven Railway Station. Buses will depart at 9.30, 10.30 and 11.30 AM. Please sign up timely for this service

Post-conference visits

There is an opportunity to visit the NMR laboratories of the Technical University Eindhoven, Leiden University, Radboud University Nijmegen, the Wageningen University and Research Center and Utrecht University from 11.30 AM. Travel to the laboratories is not organised. All laboratories are easily reached by public transport. Time schedules and travel instructions are available at the Conference Office.

The organising committee for EUROMAR/EENC 2005 gratefully acknowledges the support of the following companies and organizations

Budget guarantees and financial support

www.kncv.nl

www.bijvoet-center.nl

Stichting Magnetische Resonantie NMR-DG

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www.bruker.de

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www.ritmeesterbv.nl

www.wiley.com

www.elsevier.com

www.infrae.com

www.NMRtec.com

www.spectrastableisotopes.com

www.cortecnet.com

www.iconisotopes.com

www.wangnmr.com

www.rs2d.com

www.newera-spectro.com

www.campro-scientific.com

www.umetrics.com

www.rototec-spintec.com

www.isotope.com

www.dotynmr.com

www.oxford-instruments.com

www.eurisotop.fr

www.stelar.it

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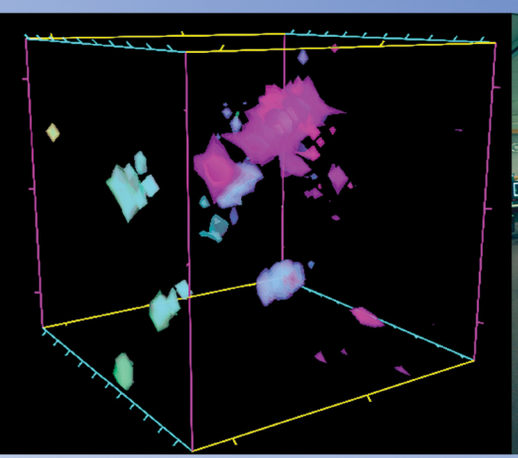
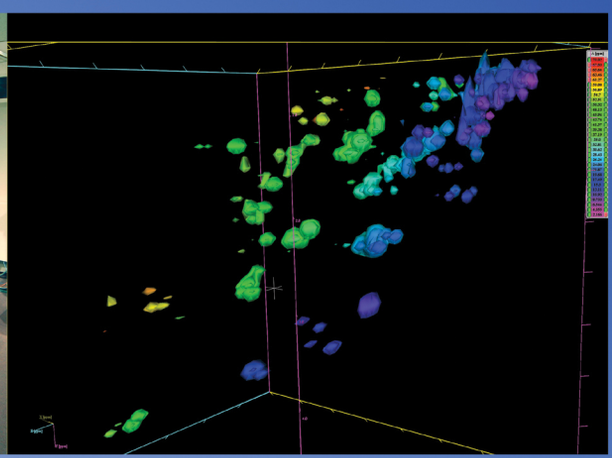
JEOL

Welcomes you to the Euromar meeting 2005





Come and see the NMR and software capabilities from JEOL at the poster room.
You are also invited to the JEOL party on Wednesday evening from 7pm on the JEOL booth

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TOP 5 HIGHLY-CITED ARTICLES OVER LAST 3 YEARS:

- M, Bak, J.T. Rasmussen, N.Chr. Nielsen, **"SIMPSON: A general simulation program for solid-state NMR spectroscopy"**, J. Magn. Reson. 147 (2000) 296-330
- I. Schnell, H.W. Spiess, **"High-resolution 1H NMR spectroscopy in the solid state: Very fast sample rotation and multiple-quantum coherences",** J. Magn. Reson. 151 (2001) 153-227
- C.D. Schwieters, J.J. Kuszewski, N. Tjandra, G.M. Clore, **"The Xplor-NIH NMR molecular structure determination package",** J. Magn. Reson. 160 (2003) 65-73
- S. Skare, M. Hedehus, M.E. Moseley, T. Li, "**Condition number as a measure of noise performance of diffusion tensor data acquisition schemes with MRI"**, J. Magn. Reson. 147 (2000) 340-352
- K. Saalwachter, R. Graf, H.W. Spiess, "**Recoupled polarization-transfer methods for solid-state 1H-13C heteronuclear correlation in the limit of fast MAS"**, J. Magn. Reson. 148 (2001) 398-418

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Infrae is a software services company focusing on document, asset, and content management for enterprises. Its customer base spans both the private and public sectors throughout Europe. Based in Rotterdam, the Netherlands, the company has operated successfully since early 2001.

Approach

Infrae builds software on request and provides integration, customization, and support services.

The company takes a synergy-seeking approach to software development: instead of building only client-specific applications, Infrae develops generic solutions that are useful for multiple parties. If projects can turn into open source products, other users will have a vested interest in future development and maintenance of the software, resulting in sharply reduced IT expenditures.

Clients therefore participate in a network of excellence, sharing expenses and trading knowledge. Infrae is customer-driven and collaboration-powered.

Products

Infrae's products are strategically designed for longevity and interoperation in technical ecosystems. Content is made future-proof by compliance with established standards.

Silva CMS

Silva is an enterprise-class CMS for managing content for the web, print, and other media, built on the advanced Zope application server. Content is stored in clean and future-proof XML, independent of layout and presentation. Features include a multi-version workflow system, integral WYSIWYG editor (Kupu), content reuse in multiple publications, sophisticated access management, import/export facilities, XSLT rendering, fine-grained templating, and hi-res image storage and manipulation.

Railroad Repository

Railroad is a standards based repository for binary files such as digital media, along with their metadata. Railroad can be seamlessly integrated into CMS's, leveraging the open WebDAV protocol and simple HTTP messaging. Multiple clients (CMS's and DAM systems) can access the resources in the repository at the same time, allowing large data files and their metadata to be centrally managed, shared, and served efficiently.

Kupu Editor

Kupu is a browser-based editor for Mozilla, Firefox, and Internet Explorer. Written in Javascript, Kupu can be easily integrated into a variety of CMS's, and is currently used in Apache Lenya, Silva, Plone, Twiki, Magnolia, OLAT, and the Union CMS. Kupu has become an OSCOM (Open Source Content Management association) project.

Services

Software Development

Infrae offers expertise in content management, web authoring, digital asset management, user interface design, and infrastructure. Infrae people build a close relationship with their counterparts in client organizations, and apply a proven methodology for software design and project management. Infrae can accommodate almost any need within the diverse IT environments of its customers.

Integration

Infrae has long experience integrating applications into existing, complex technical surroundings. Collaboration with customer staff results in top quality infrastructure.

Support

The user friendliness of Infrae's products can be traced to one aspect: the developers talk – and listen – to users. Infrae people view support as a learning process. It's a continuous feedback loop that keeps the ecology clean and healthy.

Customization

Many projects customize an existing product or act as middleware to interoperate with other systems. The same exacting standards are applied to these solutions.

Documentation

Infrae's working procedures ensure that documentation is produced as part of the process, ready to deliver at any stage of development.

Training

Infrae offers training for its own products, and also provides general training in foundational technologies such as the Python programming language.

References

Erasmus University Rotterdam, The Netherlands

ETH Zürich (The Swiss Federal Institute of Technology), Switzerland

Wirtschaftsuniversität Wien (Vienna University of Economics and Business Administration), Austria

The University of Luton, UK

University College London, UK

Institute of Netherlands History, The Netherlands

Bijvoet Center for Biomolecular Research, The Netherlands

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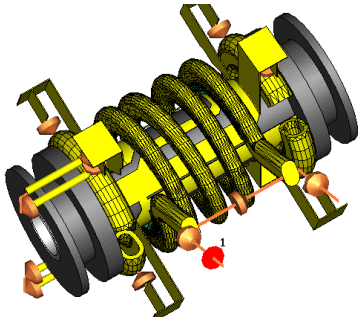
Doty MAS *Two-coil* Method: ^{1}H Cross Coil, X/Y Solenoid Coil

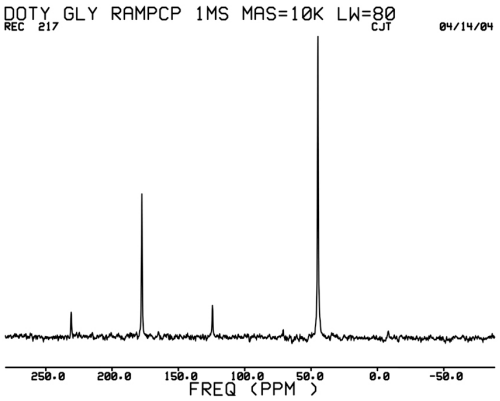


What is a cross coil ?
The cross coil is a "saddle-type" coil that has an inner one-turn loop in parallel with a segmented turn on each side.

Why use two coils ?
Using two orthogonal coils simplifies tuning and permits higher RF fields in larger samples and very high B_0.

How does this help ? The low inductance cross coil contributes much less to sample heating during decoupling. Compared to a solenoid, it's a factor of 10-25 times less !!

^{13}C CP/MAS on Glycine, H/C/N tuning @750 MHz



S/N ≈250:1, 70 µg sample, CP@50 kHz for 1 ms; 110 kHz TPPM decoupling, LB = 80 Hz, nt = 4, ***Spectrum*** - **MIT**

Fastest Spinning for the Sample Size

Maximize S/N with larger sample volume, fast spinning rotors.

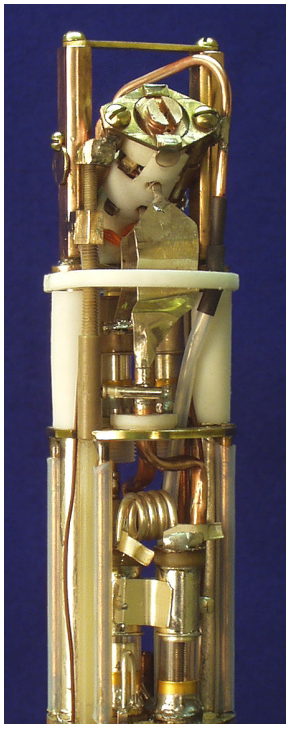
Doty 7 mm, 5 mm, & 4 mm rotors

Recent advancements in our turbine technology have provided improved axial stability and thus improved resolution.

25 kHz MAS, 4 mm rotors, for wide or narrow bore magnets.

18 kHz MAS, 5 mm rotors, for wide or narrow bore magnets.

12 kHz MAS, 7 mm rotors, for wide bore magnets only.



A Doty H/X/Y XC4 NB Probe

Triple-resonance, Double-broadband Tuning

H-F/X/Y in wide or narrow bore magnets.

H/X/Y/lock wide bore, H/X/lock narrow bore.

H/F/X wide bore or narrow bore.

Maximize versatility with X and Y channels that are multinuclear, even in probes where ^{1}H resolution is optimized – to 0.003 ppm FWHM.

Doty Scientific, Inc. 700 Clemson Rd. Columbia, SC 29229 USA Web: www.dotynmr.com
Phone: (803) 788-6497 Fax: (803) 736-5495 Email: sales@dotynmr.com

Programme of the EUROMAR/EENC 2005 meeting

3-8 July 2005, Veldhoven, the Netherlands

Sunday, 3 July 2005, Afternoon

12.00-17.30 Registration and room allocation (Entrance Hall of the "NH Koningshof"). Registration will continue from 21.00-23.00 at the same location and in the following days will take place in the Conference Office (room 23, Blue Zone)

12.00-17.00 and 21.00-24.00 Mounting of posters

15.00-16.30 Workshops 1-5

Workshop 1: Room 65 (Yellow Zone)
Workshop 2: Room 63 (Yellow Zone)
Workshop 3: Room 80-81 (Green Zone)
Workshop 4: Room 82-83 (Green Zone)
Workshop 5: Room 64 (Yellow Zone)

17.00-19.45 Mixer and Buffet Dinner

20.00-21.00 Brabant Hall **Plenary Session**
Chair **Rolf Boelens**

20.00-20.10 *Opening of EUROMAR/EENC 2005*

20.10-21.00 Rob Kaptein *Protein-DNA Interaction: Insight from the lac Repressor System*

Hospitality Suites

Monday, 4 July 2005, Morning

08.30-10.30	**Brabant Hall**	**Plenary Session**
	Chair	**Daniella Goldfarb**
08.30-09.10	Beat Meier	*Solid-state NMR under fast MAS: techniques and application to amyloids*
09.10-09.50	Nico Tjandra	*Chemical shift anisotropy in solution NMR structure determination and relaxation analysis*
09.50-10.30	Heinz-Jürgen Steinhoff	*The structure and conformational dynamics of membrane proteins as revealed by site directed spin labelling and multi-frequency EPR spectroscopy*

Break

11.00-12.40	**Brabant Hall**	**Parallel Session: Dynamics, Liquids 1**
	Chair	**Geoffrey Bodenhausen**
11.00-11.30	Hashim Al Hashimi	*Resolving hierarchal motional modes in RNA by NMR*
11.30-12.00	Markus Zweckstetter	*Residual structure and slow dynamics in alpha-synuclein*
12.00-12.20	Karine Loth	*Structural Information through Cross-Correlated Relaxation in 6PGL*
12.20-12.40	Sivanandam Veeramuthu	*Evaluation of cross-correlation effects between H_α-C_α dipolar and H_α-Curie-spin interactions in paramagnetic proteins*

11.00-12.40	**Auditorium**	**Parallel Session: Solids 1**
	Chair	**Huub de Groot**
11.00-11.30	Dieter Suter	*Scalability of quantum computation: contributions from magnetic resonance*
11.30-12.00	Jonathan Jones	*NMR quantum computing with para-hydrogen*
12.00-12.20	Kan Nian Hu	*Cross Effect Dynamic Nuclear Polarization Using Biradicals*
12.20-12.40	Christian Degen	*Microscale Localized Spectroscopy by Mechanical Detection of NMR.*

Lunch

Monday, 4 July 2005, Afternoon

14.00-16.00	**Brabant Hall**	**Parallel Session: Bio-NMR, Liquids 2**
	Chair	**Miquel Pons**
14.00-14.30	Torleif Härd	*Molecular recognition between engineered protein domains*
14.30-15.00	Jesús Jimenez-Barbero	*Conformational and structural features of carbohydrate-protein interactions. Insights from STD, tr-NOE, and related NMR experiments*
15.00-15.20	Klaartje Houben	*NMR studies of the dynamics and metal exchange properties of two C4C4 RING domain*
15.20-15.40	Isabella Felli	*Towards complete assignment of proteins through protonless NMR*
15.40-16.00	Bin Wu	*Folding Rules for Branched DNA Prediction of alignment of nucleic acids in aligned media*

14.00-16.00	**Auditorium**	**Parallel Session: Methods, Solids 2**
	Chair	**Arno Kentgens**
14.00-14.30	Niels Nielsen	*The use of optimal control theory for systematic design of solid-state NMR experiments with improved performance*
14.30-15.00	Ago Samoson	*Advances in sample spinning experiments*
15.00-15.20	Melinda Duer	*Solid-state NMR: a tool for probing the protein-mineral interface and other structures in bone*
15.20-15.40	Bénédicte Elena	*^{1}H High-Resolution Solid-State Nuclear Magnetic Resonance for Structure Determination in Powdered Solids*
15.40-16.00	Jan van Bentum	*Developments in Solid-State NMR micro-spectroscopy*

16.00-17.30	Workshops 1-5	Workshop 1: Room 65 (Yellow Zone) Workshop 2: Room 63 (Yellow Zone) Workshop 3: Room 80-81 (Green Zone) Workshop 4: Room 82-83 (Green Zone) Workshop 5: Room 64 (Yellow Zone)
16.00-18.00	Poster Session	Kempen Hall
18.00-18.50	**Brabant Hall**	**Plenary Session**
	Chair	**Rolf Boelens**
18.00-18.50	Alex Pines	*Molecular-Target Magnetic Resonance from Microfluidics to Human Imaging*
19.00-2030	*Dinner*	

Varian evening. A festive event on the occasion of awarding the Russell-Varian prize on Tuesday to a person that made significant contributions to NMR with a single paper.

Tuesday, 5 July 2005, Morning

08.30-10.25	**Brabant Hall**	**Plenary Session**
	Chair	**Chairmen Prize Committees**
08.30-09.15	Ampère Prize	
	Malcolm H. Levitt	*Principles and Applications of Symmetry-Based Recoupling in Solid-State NMR*
09.15-09.40	Andrew Prize	
	Christian Hilty	*NMR Studies of the Membrane Protein OmpX in DHPC Micelles*
09.40-10.25	Russell-Varian Prize	

Break

10.50-12.40	**Brabant Hall**	**Parallel Session: Fast NMR, Liquids 3**
	Chair	**Lucio Frydman**
10.50-11.20	Philippe Pelupessy	*Adiabatic Pulses in Single Scan Multi-Dimensional NMR Spectroscopy*
11.20-11.50	Bernhard Brutscher	*Very fast 2D NMR spectroscopy for real-time investigation of dynamic events in proteins*
11.50-12.10	Ji Won Yoon	*Reconstruction of Multidimensional NMR Spectra*
12.10-12.30	Tammo Diercks	*Novel Flip-Back Schemes and Queued Spectroscopy: New Methods for Recovery of Lost Magnetisation*
12.30-12.50	Rosa Tamara Branca	*A new method for ultrafast high resolution iZQC spectroscopy in vivo*

10.50-12.50	**Auditorium**	**Parallel Session: Emerging methods, EPR 1**
	Chair	**Jan Schmidt**
10.50-11.20	Jack Freed	*New Frontiers in ESR Technology: ESR Microscopy and Millimeter Wave Two-Dimensional ESR Spectroscopy*
11.20-11.50	Brian Hoffman	*Structure, Binding Modes, and Electron Inventory of Nitrogenase Enzymatic Intermediates by Multinuclear ENDOR Spectroscopy*
11.50-12.10	Malte Drescher	*ESR-Imaging*
12.10-12.30	Tomasz Czechowski	*2D imaging of two types radicals by the CW-EPR method*
12.30-12.50	Maria Fittipaldi	*Investigation of the Nuclear Interactions with the S = 5/2 Electron Spin of the Fe(III) in Aquometmyoglobin by HYSCORE Spectroscopy*

Lunch

Tuesday, 5 July 2005, Afternoon

14.00-16.00	**Auditorium**	**Parallel Session: Methods, Solids 3**
	Chair	**Rien de Bie**
14.00-14.30	Leo van Wüllen	*Solid-state NMR methods for the characterization of the structure and dynamics of amorphous solids*
14.30-15.00	Dimitris Sakellariou	*Approaches to Solid State NMR of (Radioactive) materials*
15.00-15.20	Jair Freitas	*Coexistence of FM and AFM in Cu-doped manganites detected by NMR and magnetic measurements*
15.20-15.40	Marica Cutajar	*Use of ^{2}H double-quantum MAS NMR for studying molecular motion in solids*
15.40-16.00	Pedro Aguiar	*Solid-State NMR Spectroscopy of Paramagnetic Transition-Metal Cyanides*

14.00-16.00	**Brabant Hall**	**Parallel Session: Biomolecular Interactions, Liquids 4**
	Chair	**Mike Williamson**
14.00-14.30	Michael Sattler	*NMR studies of structure, dynamics and RNA recognition in the regulation of gene expression*
14.30-15.00	Jacob Anglister	*Mechanism of HIV-1 co-receptor selectivity probed by NMR of antibody-peptide complexes*
15.00-15.20	Remco Sprangers	*NMR and biochemical studies of the 305kDa ClpP protease establish dynamic side pores for product release*
15.20-15.40	Zhara Shajani	*Motion in the Binding Site for Human U1A Protein: A Solution and Solid-State NMR Study of RNA dynamics*
15.40-16.00	Ravi Pratap Barnwal	*Insight into methyl-dynamics of dynamically different motifs of dsRBD of PKR to understand hydrophobic core packing*

16.00-18.00	Poster Session	Kempen Hall
19.00-20.30	*Dinner*	

Bruker evening. A memorable evening will be organised with Alsatian and specific Dutch accents for food and drinks.

Wednesday, 6 July 2005, Morning

08.30-10.30	**Brabant Hall**	**Plenary Session**
	Chair	**Beat Meier**
08.30-09.10	John Sidles	*The MRFM Program for Achieving Single Nuclear Spin Detection*
09.10-09.50	Bob Griffin	*Dynamic Nuclear Polarization at high magnetic fields: why two electrons are better than one*
09.50-10.30	Lucio Frydman	*Principles and Progress of Spatially Encoded NMR and MRI*

Break

11.00-12.40	**Brabant Hall**	**Parallel Session: Bio-NMR, Solids 4**
	Chair	**Huub de Groot**
11.00-11.30	Marc Baldus	*Studying protein structure and dynamics by solid-state NMR methods*
11.30-12.00	Clemens Glaubitz	*Solid-state NMR approaches to proton pumping and drug efflux systems*
12.00-12.20	Joerg Matysik	*Up to factor 5000 above Boltzmann equilibrium: photo-CIDNP MAS NMR on photosynthetic RCs*
12.20-12.40	Antoinette Killian	*Influence of the lipid environment on the orientation of protein transmembrane segments as determined by solid state ^{2}H NMR*

11.00-12.40	**Auditorium**	**Parallel Session: EPR 2**
	Chair	**Hans-Wolfgang Spiess**
11.00-11.30	Edgar Groenen	*High-frequency pulsed ENDOR and its application to copper proteins*
11.30-12.00	Robert Bittl	*Protein-Cofactor and Cofactor-Substrate Interactions Studied by High-Field EPR*
12.00-12.20	Sushil Misra	*Virtues of high-field//high-frequency EPR: Advantages of multi-frequency approach in EPR*
12.20-12.40	Jorge Caldeira	*Protein microcrystals oriented in a liquid crystalline polymer medium: an EPR spectroscopy study*

Lunch

Wednesday, 6 July 2005, Afternoon

14.00-16.00	**Auditorium**	**Parallel Session: MRI**
	Chair	**Henk van As**
14.00-14.30	Robert Müller	*Magnetic Resonance Molecular Imaging, the Challenges*
14.30-15.00	Peter van Zijl	*Studying and exploiting the properties of mobile proteins in situ*
15.00-15.30	Mathias Hoehn	*Observing cell migration in the brain: in vivo MR imaging*
15.30-16.00	Armin Purea	*Cellular and molecular magnetic resonance microscopy at 17.6 T*

14.00-16.00	**Brabant Hall**	**Parallel Session: Dynamics, Liquids 5**
	Chair	**Christian Griesinger**
14.00-14.30	Martin Blackledge	*Application of Residual Dipolar Couplings to the Study of Flexibility in Folded and Unfolded Proteins*
14.30-15.00	Rafael Brüschweiler	*New Aspects of Covariance NMR*
15.00-15.30	Stephan Grzesiek	*Polypeptide folding observed by high-resolution NMR methods*
15.30-16.00	Erik Zuiderweg	*Allosterics and dynamics*

16.00-18.00	Poster Session	Kempen Hall

18.00-18.50	**Brabant Hall**	**Plenary Session**
	Chair	**Rolf Boelens**
18.00-18.50	Ad Bax	*Weak alignment provides new opportunities*

19.00-20.30 *Dinner*

JEOL evening. Hospitality with Japanese accents.

Thursday, 7 July 2005, Morning

08.30-10.30	**Brabant Hall**	**Plenary Session**
	Chair	**Rob Kaptein**
08.30-09.10	Hartmut Oschkinat	*Protein Structure Determination by MAS Solid-State NMR*
09.10-09.50	Jeremy Nicholson	*NMR Spectroscopy and Understanding the Global Systems Biology of Disease Processes*
09.50-10.30	Clare Grey	*Probing Local Structure and Ionic Motion in Disordered Systems: Applications to Fuel Cell Materials, Batteries and Catalysis*

Break

11.00-12.40	**Brabant Hall**	**Parallel Session: Screening, Liquids 6**
	Chair	**John van Duynhoven**
11.00-11.30	Bernd Meyer	*STD-NMR to characterize ligands to membrane bound receptors including G-protein coupled receptors (GPCRs)*
11.30-12.00	Gregg Siegal	*Target Immobilized NMR Screening, new capabilities for fragment-based lead discovery*
12.00-12.20	Gareth Morris	*New Techniques in DOSY*
12.20-12.40	Rosa Fayos	*STD based methods to study protein-protein interactions*

11.00-12.40	**Auditorium**	**Parallel Session: Diffusion**
	Chair	**Pieter Magusin**
11.00-11.30	Ioan Ardelean	*NMR investigations of vapour contribution to the effective diffusion in partially saturated porous media*
11.30-12.00	Henk van As	*A 3D Tree MRMicroscope: from porous grains to intact trees*
12.00-12.20	André Pampel	*Pulsed Field Gradient NMR in Combination with Magic Angle Spinning - New Possibilities for Studying Diffusion and Making NMR Microscopy of Heterogeneous Materials*
12.20-12.40	Janez Stepisnik	*Velocity autocorrelation function of fluidized granular system measured by the novel spin echo technique*

Lunch

Thursday, 7 July 2005, Afternoon

14.00-15.50	**Auditorium**	**Parallel Session: Computing**
	Chair	**Vladimir Sklenar**
14.00-14.30	Alexandre Bonvin	*Data-driven docking for the study of biomolecular complexes*
14.30-15.00	Michele Vendruscolo	*Structure determination of non-native protein conformations using NMR-derived restraints*
15.00-15.30	Torsten Herrmann	*Automated NOESY analysis for side-chain resonance assignment and collection of conformational restraints*
15.30-15.50	Norbert Mueller	*DFT Calculation of CSA Tensors in Model Peptides. Dependence on secondary structure*

14.00-15.50	**Brabant Hall**	**Parallel Session: Bio-NMR, Liquids 7**
	Chair	**Sybren Wijmenga**
14.00-14.30	Lucia Banci	*NMR studies on partially unfolded proteins*
14.30-14.50	Anastasia Zhuravleva	*The role of internal motions in protein function: a backbone and side-chain multiple-field NMR relaxation studies of two globular proteins azurin and barnase*
14.50-15.10	Fabien Ferrage	*Measurement of intra-residue dipolar cross-relaxation rates between C_α and CO nuclei in proteins*
15.10-15.30	Hugo van Ingen	*CEESY: a new method to measure Chemical Exchange to Excited States by NMR spectroscopy*
15.30-15.50	Sabine Ruth Quadt	*NMR Structural Studies of the Interferon α Signalling Com*plex

Break

16.20-18.00	**Brabant Hall**	**Parallel Session: Protein Folding, Liquids 8**
	Chair	**Nico van Nuland**
16.20-16.50	Harald Schwalbe	*NMR of Protein Folding and Aggregation*
16.50-17.20	Peter Hore	*^{19}F CIDNP of peptides and proteins*
17.20-17.40	Carine van Heijenoort	*NMR multidomain proteins stability and folding analysis : conformational exchange, temperature and pH stability variation in human annexin 1 first domain*
17.40-18.00	Boris Fuertig	*NMR real time studies of RNA folding*

16.20-18.00	**Auditorium**	**Parallel Session: Materials, Solids 5**
	Chair	**Bernhard Blümich**
16.20-16.50	Laurie D. Hall	*Characterisation of Spatially Heterogeneous Soft Solids by MRI*
16.50-17.20	Kay Saalwächter	*Chain Ordering, Slow Motions, and Heterogeneities in Elastomers: New Insights by Low-Field NMR*
17.20-17.40	Laurent Delevoye	*Characterisation of a devitrified glass through homo- and heteronuclear correlation experiments*
17.40-18.00	Anton Buzlukov	*Hydrogen in Ti_3Al intermetallic compound: a proton NMR study*

19.30-???? **Conference Dinner and Caribbean evening**

Workshops

All workshops will take place in rooms 80-83 and rooms 63-65. The workshops will be held on Sunday from 15.00 – 16.30 PM and Monday from 16.00 – 17.30 PM. A certificate for having attended a workshop will be available on Tuesday at the Conference Office. The organisers are indebted to the staff of the workshops and especially appreciate the complete sponsoring of workshop 2 “Multivariate analysis of NMR data” by Umetrics, Sweden.

Workshop 1. Average Hamiltonian Theory (Room 65)

Workshop Staff

Dr. Thomas Vosegaard, University of Aarhus, Danmark (Monday)
Dr. A. Brinkmann, Radboud Universiteit, Nijmegen, The Netherlands (Sunday)

Workshop Content

The concept of the average Hamiltonian will be introduced and its applications will be illustrated. A minimal knowledge of elementary quantum mechanics (including spin angular momentum, Zeeman eigenstates, propagators, density operator) is assumed and a set of notes setting forth some mathematical foundations for the subject will be distributed. Opportunities for discussion and the solving of exercises will be provided.

Initial illustrations will emphasize the 1st order average Hamiltonian and its importance for the design of experiments: Examples will include truncation of internal Hamiltonians (dipolar, etc.) in a high external field, interaction frame of the radio-frequency field, composite pulses,
homonuclear decoupling and dipolar recoupling.

Higher order corrections will be described, and their importance will be illustrated. Examples will be given by hands on computation with the simulation program SIMPSON.

Participants

Bankmann, Dennis
Biron, Zohar
Branca, Rosa Tamara
Czechowski, Tomasz
Diercks, Tammo
Duma, Luminita
Fagiolo, Gianlorenzo
Ferrage, Fabien
Filipp, Fabian Volker
Frey, Mike
Gili, Tommaso
Hautle, Patrick
Houben, Klaartje
Kaiser, Christoph
Kehlet, Cindie
Knijn, Paul
Kobzar, Kyryl
Kumar, Ashutosh
Luy, Burkhard
Mauri, Michele
Nikkhou Aski, Sahar
Ogloblichev, Vasiliy
Orr, Robin
Purea, Armin
Quadt, Sabine Ruth
Shajani, Zahra
Silakov, Alexey

Workshop 2. Multivariate analysis of NMR data (Room 63)

Workshop Staff

Dr. M. Dyrby, Umetrics, Sweden

Workshop Content

The aim of the workshop is to demonstrate how multivariate methods can be used to analyse and visualise NMR data. Two basic techniques Principal Components Analysis (PCA) and Partial Least Squares (PLS) will be demonstrated and it will be shown how these can be used to solve different problems.

The problems can be divided into three main groups:

1. Overview of data,
2. Classification of data answering the question "Is a new sample of type A or type B",
3. Calibration using NMR as a fast and non-invasive technique to substitute traditional wet chemistry methods.

Data and problems will come from metabonomics as well as other biological studies but also include the analysis of polymer data. The workshop will start with a short theory part followed by a demonstration and will end with the possibility for attendees to do hands-on exercises from supplied training examples.

Participants

Andersen, Nikolas
Andronesi, Ovidiu Cristian
Berti, Francesco
Bloemberg, Tom
Caytan, Elsa
Clayden, Nigel John
Dancea, Felician
De Vries, Koen
Duval, Franck
Eijkelenboom, Astrid
Elena, Bénédicte
Kloks, Cathelijne
Koskela, Harri
Linssen, Harrie-Aj
Morris, Gareth
Ní Bhriain, Nollaig
Orekhov, Vladislav
Orza, Ramona
Pikkemaat, Jeroen
Soininen, Pasi
Tomaselli, Simona
Van De Molengraaf, Roland
Van Tilborg, Paul
Voelkel, Ruediger
Weglarz, Wladyslaw
Yoon, Ji Won
Zubenko, Dmitry

Workshop 3. Structure Generation and Validation (Room 80-81)

Workshop Staff

Dr. G. Vuister, Radboud Universiteit, Nijmegen, The Netherlands
Dr. C. Spronk, Radboud Universiteit, Nijmegen, The Netherlands
Dr. T. Herrmann, ETH, Zürich, Switzerland

Workshop Content

The development of reliable, smart, well-documented and foremost useful computational tools for structure calculation and structure validation has become increasingly important for NMR-based structural projects. This workshop is aimed at giving you answers to the following two questions:

1. How can I speed-up the process of structure calculation by automation?
2. How can I verify that my results are correct?

The workshop will introduce you to RADAR, a program for automated structure calculation, recently developed at the ETH. The program allows for rapid structure generation from the experimental data directly, and thus greatly alleviates the cumbersome and time-consuming data analysis steps.

Unfortunately, no program can tell you if your results are correct. There are, however, tools that can assist you in finding unlikely elements in your data and structure ensembles and there are tools to improve your results. We will provide you with an overview how to validate your data and resulting NMR ensembles, and improve your results.

Of course, we plan on a 'hands-on' experience, which will give you the opportunity to get a first impression of what is around and how your research could benefit from using the tools. For the already more experienced participants, we hope on a lively discussion with you, which will guide the future directions of our efforts.

Participants

AB, Eiso
Alaña, Iñigo
Allan, Martin
Ampt, Kirsten
Apponyi, Margit
Bettendorff, Pascal
Darwish, Tamim
De Jong, Rob
Deporte, Sylvaine
Etzkorn, Manuel
Farrant, Duncan
Ganapathy, Swapna
Heikkinen, Outi
Joli, Flore
Kurashima-Ito, Kaori
Lelli, Moreno
Loth, Karine
Neuhaus, David
Nielbo, Steen
Niemitz, Matthias
Oekonomopulos, Raymond
Papadopoulos, Evangelos
Petrova, Svetlana
Pirnat, Janez
Plavec, Janez
Pukala, Tara
Samoson, Ago
Schallus, Thomas
Schnegg, Alexander
Schnur, Einat
Schwedhelm, Kai
Sowerby, Andrew
Stevens, Timothy
Tripsianes, Konstantinos
Van Der Werf, Ramon
Veeramuthu Natarajan, Sivanandam
Vlach, Jiri
Vogtherr, Martin

Workshop 4. Alignment and orientation (Room 82-83)

Workshop Staff

Dr. G. Parigi, CERM, Florence, Italy
Dr. M. Zweckstetter, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Workshop Content

Anisotropic interactions, such as residual dipolar couplings (RDCs), can be observed in solution when a molecule is aligned with the magnetic field, either as a result of its own magnetic susceptibility anisotropy, or caused by an anisotropic environment such as an oriented liquid crystalline phase or an anisotropically compressed gel. The structural information provided by these quantities can either be used for further refinement of structures determined by conventional methods, or for determining a structure directly. They can also be used for structure validation, for determination of relative orientations of molecular fragments or domains, or for studying dynamic effects. Analysis of the relation between a molecule's 3D shape and its alignment tensor values can yield important insights into all of these processes. In this workshop the following topics will be covered:

1. Theory of RDCs;
2. Molecular alignment;
3. Determination of a molecular alignment tensor including RDC distribution analysis, back-calculation of alignment tensors, charge/shape-prediction of alignment tensors, evaluation of alignment tensor accuracy and quality measures;
4. Liquid crystal theory.
5. Usage of PALES (http://www.mpibpc.gwdg.de/abteilungen/030/zweckstetter) for efficient analysis of RDCs.

In the second part of the workshop the usage of paramagnetism-based restraints will be discussed. The presence of a paramagnetic metal ion in the protein structure provides restraints for solution structure determination arising from the orientation dependence of the electron magnetic moment and of the energy of a magnetically anisotropic molecule. Both effects are related to the anisotropy of the metal magnetic susceptibility. As a consequence:

1. The dipole-dipole coupling of protein nuclei with the electron magnetic moment does not average to zero upon rotation, and pseudocontact shifts arise
2. The dipole-dipole coupling between coupled protein nuclei does not average zero upon rotation, and self-orientation residual dipolar couplings arise.

Participants

Alho, Nanna
Angulo, Jesus
Bentrop, Detlef
Bernado, Pau
Canales, Angeles
Claridge, Tim
Díaz-Quintana, Antonio
Douglass, Bradley
Edlich, Christian
Ferrer-Anglada, Núria
Fiala, Radovan
Flinders, Jeremy
Grage, Stephan
Hrabal, Richard
Johar, Zeena
Kloks, Cathelijne
Koenig, Bernd
Lancelin, Jean-Marc
Mari, Silvia
Marsden, Brian
Maruyoshi, Keisuke
Matsumori, Nobuaki
Musco, Giovanna
Nabuurs, Sanne
Najda, Andzelika
Nieto Mesa, Pedro Manuel
Noguera, Valerie
Odeyale, Kayode
Rademacher, Christoph
Rohonczy, János
Sanath, Chinthaka
Saunavaara, Jani
Schmidt, Claudia
Stevens, Kristof
Tallavaara, Pekka
Thibaudeau, Christophe
Thompson, Gary
Underhaug, Jarl
Van Heijenoort, Carine
Vyushkova, Maria
Wiesner, Silke
Wu, Bin
Zuckerstaetter, Gerhard

Workshop 5. Computation of NMR parameters (Room 64)

Workshop Staff

Prof. Dr. V. Sklenar, Masaryk University, Brno, Czech Republic
Dr. E. van Lenthe, SCM, Amsterdam, The Netherlands
Prof. Dr. Martin Kaupp, Universität Würzburg, Würzburg, Germany

Workshop Content

Theoretical background of NMR parameters has tremendously benefited from recent advances in quantum chemical theory, namely in gradually maturing quantitative treatments based on *ab initio* and density functional methods (DFT). Due to the improvement in computational techniques and the incredible increase in speed of computers in the last decades, the calculation of NMR and EPR parameters can now be routinely performed using several software packages. The first part of this workshop will give theoretical background on calculations of NMR chemical shifts, spin-spin coupling constants, and nuclear quadrupole coupling constants. The following topics will be covered: some basics of *ab initio* and density functional theory, diamagnetic and paramagnetic terms, the Fermi-contact, orbital, and spin dipolar terms, the gauge problem (GIAO, IGLO), relativistic effects (in particular the important spin-orbit chemical shifts), inclusion of environment and molecular dynamics, accuracy, interpretation of the results. Applications of DFT to biomolecules will be summarized and demonstrated on studies of protein and nucleic acids constituents. Possibilities and application examples using ADF and ReSpect program packages will be reviewed, including several applications to systems with heavy atoms and discussion of relativistic effects.

Participants

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How Regulatory Proteins Interact with DNA: The Lac Repressor System

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An overview will be given of our recent work on lac repressor DNA interactions. The aim is to provide a detailed picture of protein-DNA recognition in terms of structure, dynamics, kinetics and (in the future) thermodynamics.

Using a covalently linked dimeric lac headpiece the structure of the complex with the O1 lac operator was solved. The large assymetry in the mode of binding to left and right sides of the operator shows that the headpiece has a considerable plasticity.

The first step in finding the regulatory target site is binding to non-specific DNA. The complex of the dimeric lac headpiece with non-cognate DNA showed very different structural and dynamic features compared to the specific complexes. Interactions are only electrostatic in nature (including H-bonds with the DNA backbone). The complex is highly dynamic in the μs to ms time range.

Finally from the measurement of H/D exchange rates of amide protons a detailed view could be obtained of the molecular processes occurring in protein-DNA association and dissociation.

Solid-state NMR under fast MAS: techniques and application to amyloids

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Fast, small diameter MAS rotors have opened new opportunities and have posed new challanges for solid-state NMR. Progress in this respect will be reviewed and discussed in this contribution. In particular, we will emphasis recent developments in decoupling and recoupling in the fast spinning regime. MAS rotation frequencies up to 70 kHz are now possible and allow for new experiments. Solid-state NMR applications to a de novo designed amyloid fibril (17meric peptide) and to the prion protein HET-s (from the fungus Podospora anserina) will be presented. The prion fibrils, in particular, show quite narrow resonance lines in the ^{13}C and ^{15}N spectra (down to .25 ppm) and an almost complete assignment of all visible resonances is available. The solid-state NMR chemical shifts indicate the position of four beta sheets in the primary sequence and correlate well with H/D exchange data and with the infectivity of the prion protein.

Chemical shift anisotropy in solution NMR structure determination and relaxation analysis

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Advances in computation and ab initio methodologies have made the theoretical calculation of chemical shielding of complicated systems feasible. This in turn allows one to isolate the various geometrical contributions to the shielding tensor. The results can be partially validated through experimental data. This can provide a starting point to evaluate how we use the chemical shift information in structure determination and relaxation analysis. We have illustrated this with the amide proton CSA tensor. We isolated contributions from dihedral angles, hydrogen bond angle and hydrogen bond distance. This leads to a better understanding of how amide proton shift depends on these variables and the development of its use in structure refinement. Furthermore in the process of implementing the residual CSA in structure refinement we learned a great deal on how carbonyl CSA tensor depends on the local backbone geometry. This prompted us to try to characterize its dynamics. One aspect is to differentiate the dynamical behavior of the carbonyl CSA tensor relative to the amide N-H dipolar, and the other is how one can use the geometrical dependence of the carbonyl CSA relaxation as structural restraints. All of these studies show that there are still a lot to be gained by better understanding what governs chemical shift tensors

The structure and conformational dynamics of membrane proteins as revealed by site directed spin labeling and multi-frequency EPR spectroscopy

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Recent developments including pulse techniques and multi-frequency approaches make the combination of electron paramagnetic resonance (EPR) spectroscopy, site-directed spin labeling (SDSL), and molecular dynamic simulations an attractive approach for studying the structure and conformational changes of membrane proteins and membrane protein complexes. Analysis of the spin label side chain mobility, its solvent accessibility, the polarity (1) of the spin label micro-environment and distances between spin label side chains provide information for restraint modeling of protein domains or protein - protein interaction sites with spatial resolution at the level of the backbone fold. Structural changes can be followed with sub-millisecond time resolution. The talk focuses on the application of this method on two membrane proteins: the Na^+/proline transporter PutP of *Escherichia coli* (2), and the halobacterial phototaxis receptor sensory rhodopsin (pSRII) in complex with the receptor-specific transducer protein (pHtrII) (3). Light activation of pSRII induces a signal which is transferred across the plasma membrane by pHtrII that binds tightly to the photoreceptor. Inter-spin distances determined from pairs of interacting nitroxide spin labels introduced into the pSRII-pHtrII complex lead to a unique structural model of the dimeric complex. Time resolved detection of inter-spin distance changes after light activation reveals conformational changes of pSRII and uncovers the mechanism of the signal transfer from pSRII to the associated transducer pHtrII. Doubly spin labeled variants of the Na^+/proline transporter PutP reconstituted in proteoliposomes were studied by means of four-pulse double electron-electron resonance (DEER) techniques. Inter-spin distance changes observed between loops 2 and 6 upon Na^+ binding suggest ligand induced structural alterations of PutP which might be essential for the transport mechanism.

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Resolving Hierarchal Motional Modes in RNA by NMR

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By applying a domain-elongation strategy, we are able to resolve three motional modes in ribonucleic acids (RNA) using ^{15}N spin relaxation methods which otherwise evade detection owing to violations in the so-called NMR "decoupling approximation". In addition to Brownian rotational diffusion and bond vector librations, we observe collective dynamics of helical domains in two distinct bulge-containing RNAs from HIV-1: the transactivation response element (TAR) and dimerization initiation site (DIS). These collective motions occur across exceptionally short linkers (<4 nt) and provide a molecular basis for functionally important RNA structural transitions. Extended model free analysis of the relaxation data leads to determination of an overall diffusion tensor which is in excellent agreement with structure-based hydrodynamic calculations. The amplitude (S^2 = 0.83-0.87) and timescale (30-40 ps) of the individual N-H librational motions are in agreement with previous values reported for Watson-Crick residues. In contrast, the collective motions have diffusion limited timescales (~1.6 ns) and larger amplitudes (S^2 = 0.73-0.77) which exhibit a linear temperature dependence over the 283 K - 313 K temperature range. Remarkably, the collective motions are predicted to converge to maximum order at temperatures near the protein glass transition temperature (~180-220 K). The collective motions in TAR can be arrested by recognition of ligands bearing multiple cationic groups, indicating that they originate in part from lack of electrostatic stabilization at the bulge linker. The elongation strategy promises to overcome a key barrier that currently precludes the discovery and characterization of fundamental motional modes in RNA by NMR

Residual structure and slow dynamics in alpha-synuclein

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In Parkinson's disease, intra-cytoplasmic neuronal inclusions (Lewy bodies) containing aggregates of the protein a-synuclein are deposited in the pigmented nuclei of the brainstem. a-synuclein, however, is highly soluble in its native, healthy state with no ordered structure detectable by Far-UV CD or FTIR. Therefore, it is unknown what allows the soluble, unstructured protein to remain harmless, why point mutations, ligand binding or changes in solution conditions can cause protein aggregation and what kind of structural rearrangements are involved in these processes.

Employing paramagnetic relaxation enhancement, NMR dipolar couplings and 15N relaxation time measurements we show that monomeric a-synuclein assumes conformations that are stabilized by long-range interactions and act to inhibit oligomerization and aggregation. Residual dipolar coupling indicate that the autoinhibitory conformations arise on a timescale between nano- and microseconds corresponding to the time scale of secondary structure formation during protein folding. Residual dipolar couplings further allow identification of domains displaying concerted motion. In addition, RDCs report on a hydrophobic cluster involving the C-terminus of a-synuclein and the NAC region, which is essential for aggregation. Polyamine binding and/or temperature increase, conditions that induce aggregation *in vitro*, release the autoinhibitory structure, leading to a completely unfolded conformation that associates readily.

Two genetic mutations in a-synuclein, A30P and A53T, have been identified in autosomal-dominantly inherited early onset PD. An increased tendency to form intermediate oligomeric neurotoxic species is a property shared by both mutants, though no particular structural feature has been yet identified when compared to the wild type protein. We show that paramagnetic relaxation enhancement and in particular RDCs are a very powerful tool to investigate the structural transitions induced by these mutations. Our results explain several innate features of both A30P and A53T a-synuclein genetic mutants regarding the increased neurotoxicity observed in Parkinson's disease.

Structural Information through Cross-Correlated Relaxation in 6PGL

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The parasitic protozoa *Trypanosoma brucei* is transmitted to humans through the bite of the Tse-Tse fly, causing sleeping sickness in sub-Saharan Africa. Because the parasites tend to acquire increasing resistance to available drugs, there is a crucial need to identify new targets in order to develop new therapies against this highly disabling disease. The Pentose Phosphate Pathway (PPP) plays a crucial role in the parasites. A better characterization of the associated enzymes is necessary to evaluate this pathway as a potential drug target. In particular, the role and mechanism of action of a particular enzyme, 6-phosphogluconolactonase (6PGL), needs to be better understood.

6PGL is a 266 amino-acid protein, with a molecular weight of 30 kDa. Only a limited number of structures of proteins of this size have been solved by NMR, because the spectra of such proteins usually suffer from poor sensitivity and large line-widths that are due to rapid proton relaxation. The spectral assignment of proteins over 25 kDa can only be achieved using ^{2}H, ^{15}N, ^{13}C triply labeled proteins. However, the determination of the 3D structure, which relies on estimates of distances between protons through the nuclear Overhauser effect, still remains a serious challenge even for per-deuterated proteins. NOE spectra of such samples contain only signals between exchangeable protons, which provide too few distance constraints to reconstruct the structure.

We propose a new strategy, based on recent NH^{N}-$C^{\alpha}H^{\alpha}$ dipole-dipole cross correlation experiments, for the determination of Ψ torsion angles in 6PGL. One of the practical problems encountered in this study is that the relationship between the Ψ-angles and the experimental cross correlation rates is not unique. Indeed, because of the periodic dependence of the relaxation rates on Ψ, up to four values of Ψ_{exp}, are compatible with a given relaxation rate, so that the Ψ-angles are underdetermined. We therefore used the TALOS software as a companion tool. Our method consists in confronting Ψ_{pred} angles predicted by TALOS with the different Ψ_{exp} angles that are compatible with experimentally measured relaxation rates. If one of the angles Ψ_{exp} matches the value Ψ_{pred} estimated by TALOS, this Ψ_{exp} is retained as the "true" value. The angle Ψ_{exp} can then be used as a structural constraint, in contrast to Ψ_{pred} which is based on statistical analysis.

Evaluation of cross-correlation effects between Hα-Cα dipolar and Hα-Curie-spin interactions in paramagnetic proteins

V.N.Sivanandam

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Long-range geometry restraints can improve the quality of biomolecular structures determined by NMR. In recent years, the angular dependence of cross-correlated relaxation arising from the interference of different relaxation mechanisms has been exploited frequently [1, 2] to derive long-range orientation restraints. In metal containing paramagnetic proteins, the electron-nuclear interactions can provide distance and angle restraints. In particular, Curie-spin relaxation (CSR) [3] delivers angular restraints via cross-correlation effects with dipole-dipole(DD) interactions. Previously the structural dependence of $^{1}H_{N}$-^{15}N CSRxDD cross-correlated relaxation in paramagnetic proteins has been demonstrated [4, 5]. Here, we report on the use of $^{1}H\alpha$-$^{13}C\alpha$ CSRxDD cross-correlation effects to derive additional angular restraints for the $^{1}H\alpha$-$^{13}C\alpha$ bond vectors with respect to the paramagnetic center. Quantitative measurements of these effects were achieved by the application of a spin state selective HACACO- αβ differential relaxation experiment. The approach was tested by application to recombinant doubly labeled paramagnetic high-spin (fluoro,aquo) and low-spin (cyano) met-myoglobin complexes. Preliminary results obtained from differential relaxation rates of the $^{13}C\alpha$ coupled Hα doublet components show good agreement to the rates predicted from the crystal structure.

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Scalability of quantum computation: Contributions from magnetic resonance

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Quantum information processing holds enormous potential for some problems that cannot be solved efficiently on classical computers. The most successful experimental demonstrations of quantum computing so far were based on liquid-state nuclear magnetic resonance (NMR), where Shor's algorithm was implemented on a 7-qubit quantum computer [1]. While these demonstration experiments have shown that it is in principle possible to implement quantum algorithms on suitable physical systems, really useful implementations will have to operate on at least dozens of qubits to tackle problems that cannot be solved by classical computers. So far, no viable solutions have been found for increasing the number of qubits in liquid-state NMR systems into a range where the resulting processors are more powerful than conventional electronic computers. For this purpose, solid-state systems may have some advantages.

Decoherence, the decay of quantum information, ranges among the biggest obstacles to large scale quantum computation. So far, the discussion of decoherence times in different physical systems was based on a comparison of T_1 and T_2, i.e. the relaxation of individual, uncorrelated qubits. However, quantum information processing requires the creation of highly correlated states involving all the qubits in the quantum register. It is expected that these correlated states will decay much more rapidly than the individual spins: in the extreme case of macroscopic bodies, the quantum correlations disappear too rapidly to be observable [2].

For a quantitative analysis of the dependence of the relaxation times on the number of correlated qubits (spins), me measured the decay rate of cluster states of spins [3]. While the data show a clear increase of the decoherence rate with the size of the system, the increase appears to be slower than some theoretical predictions. Decoupling techniques can be used to reduce the decoherence rates of clusters of all sizes. If confirmed in other systems, this finding improves the prospects for large-scale quantum computation.

Apart from long enough decoherence times, quantum computers must satisfy several other requirements, such as the possibility to initialize the system into an appropriate state and perform logical operations on selected individual and pairs of qubits. We are working on these issues in a number of systems based on spins in molecules and semiconductors.

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Monday 4 July, morning Solids 1

NMR quantum computing with para-hydrogen

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The practical implementation of quantum algorithms has so far been dominated by liquid state NMR techniques. The many algorithms successfully implemented on liquid state NMR systems include, most notably, Deutsch's algorithm, Grover's quantum search, and Shor's factoring algorithm. This unrivalled success can be attributed to the high degree of coherent control over small spin systems and also to the long decoherence times in comparison with the timescales of the computation. However, one of the problems haunting the prospects of liquid state NMR as a practically scalable and "truly quantum" implementation, was that the states encountered in *almost all* of the previous implementations were highly mixed.

The highly mixed states used in NMR raised two major concerns. First, there was the issue of initializing the spins into a well-defined state. It was shown that one could circumvent this problem by preparing pseudopure states, which behave *effectively* (up to a certain scaling factor) as pure states. This approach worked well for small spin systems, but cannot be extended to larger systems, because of the exponential loss in signal with increase in the number of spins. Secondly, the highly mixed states encountered in conventional liquid state NMR are separable. As entanglement could not exist in these systems, some authors suggested that NMR quantum computing could perhaps be explained using classical models. With separable states, even the quantum nature of NMR quantum computing was under question!

Motivated by these problems, we have demonstrated the preparation of an almost pure two-spin state, which lies above the entanglement threshold, for the two hydride ^{1}H nuclei in the organometallic compound $Ru(H)_2(CO)_2$(dppe), where dppe indicates 1,2-bis(diphenylphosphino)ethane. This was achieved using laser induced addition of pure para-hydrogen to $Ru(CO)_3$(dppe). We have also demonstrated implementations of the Deutsch and Grover algorithms on this and a related spin system, demonstrating that coherent manipulations of spins in our pure spin systems can also be carried out.

Although developed for quantum computing these ideas may also find applications in more conventional NMR studies involving *para*-hydrogen. By using *para*-hydrogen prepared at 20K, rather than the usual 77K, we increase the signal strength by a factor of three. Using fully coherent addition and a spin-selective excitation sequence increases the signal strength by a further factor of two. Similar ideas have been previously described by Glaser and co-workers, but the spin state purity achieved (around 10%) lies below the entanglement threshold.

Cross Effect Dynamic Nuclear Polarization Using Biradicals

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Dynamic Nuclear Polarization (DNP) is a process that transfers the greater spin polarization of electrons to nuclei and therefore enhances the sensitivity of NMR spectroscopy. With stable paramagnets in insulating solids, both theories and experiments indicated that the cross effect (CE) mechanism is more efficient for DNP at high magnetic fields. CE occurs with two dipolar coupled electron spins and is differentiated from the solid effect (SE) with one electron spin and from the thermal mixing (TM) with multiple electron spins. A theory based on quantum mechanics of spin interactions was developed to understand SE, CE and TM. Specifically, CE requires that the EPR frequency separation matches the nuclear Larmor frequency, i.e. $w_{e1} - w_{e2} \sim w_n$ and that the microwave frequency is close to one of the EPR frequencies, i.e. $w_M \sim w_{e1}$ or w_{e2}. Nonetheless, a proper $e^- - e^-$ dipolar interaction is indispensable in the CE mechanism, and its optimization can be accomplished by tuning the molecular tether between two TEMPO moieties in a *biradical* (BTnE). The BT2E, *bis*-TEMPO tethered by 2 polyethylene glycol chains, was found to yield the maximal proton DNP enhancement of 200 (at 90 K, 5 T), an improvement by a factor of about 4 compared to monomeric TEMPO. While the matched frequencies in the powder EPR lineshape of TEMPO is determined by a random distribution, it can be optimized by using two different radicals, which may be tethered to form a new *biradical* that is utilized to transfer more electron spin polarization to nuclei.Solid-State NMR Spectroscopy of Paramagnetic Transition-Metal Cyanides

Microscale Localized Spectroscopy by Mechanical Detection of NMR.

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The "Magnetic Resonance Force Microscope" (MRFM) combines (nuclear) magnetic resonance with atomic force microscopy (AFM) to perform magnetic resonance imaging on the micro- and nanoscale. A MRFM employs extremely force sensitive AFM cantilevers to measure the tiny attractive (or repulsive) ~aN to ~fN forces between the sample's nuclear magnetization and a small nearby ferromagnetic particle. This mechanical method of detection improves the poor sensitivity of inductively detected NMR - as used in any commercial spectrometer - and has lead to the observation of a single electron spin and to sub-surface nanometer scale imaging in three dimensions.

The combination of the MRFM with high-resolution magnetic resonance spectroscopy is, however, hindered by the large field gradient associated with the ferromagnet.Our strategy to circumvent this problem is to introduce suitable rf-irradiation schemes to study the local spin interactions selectively. This allows to obtain spatially resolved spectroscopic information and chemical contrast while benefiting from the exquisite sensitivity and imaging capacity of the MRFM. In this contribution first examples of such experiments as part of a systematic approach will be presented.

Molecular recognition between engineered protein domains

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We study the structural and thermodynamic basis for protein-protein recognition and binding using engineered affibodies as model systems. Affibodies are small binding proteins selected by phage display from combinatorial protein libraries in which the 58 amino-acid residue three-helix Z domain is used as a scaffold. A large number of binders to different targets have been identified in this way and binding affinities are in some cases strong with dissociation constants as low as 10 pM. Our objective is to use high-precision structures determined by NMR in combination with studies of dynamics and in particular studies of protein folding and binding thermodynamics to understand the recognition process. I will in this lecture discuss the binding of the Z_{SPA-1} affibody that was selected as a binder to the (originating) Z domain, and the structure of the Z_{Taq}:Z_{ZTaq} complex - an 'anti-idiotypic' affibody pair. The studies illustrate how seemingly simple structural topologies may conceal a bewildering complexity that arises when coupled folding/binding and induced fit operate simultaneously in molecular recognition, but that, on the positive side, several of the structural and thermodynamic characteristics of protein-protein interactions can be rationalized and perhaps also exploited in protein engineering.

Published work includes Wahlberg *et al.* (2003) *Proc. Natl. Acad. Sci. USA* **100**, 3185-3190, and Dincbas-Renqvist *et al.* (2004) *J. Am. Chem. Soc.* **126**, 11220-11230.

Conformational and structural features of carbohydrate-protein interactions. Insights from STD, tr-NOE, and related NMR experiments

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This presentation is focused on the application of NMR methods[1] to the study of molecular recognition proceses between carbohydrates and proteins.[2] Particular attention will be paid to the use of TRNOE, STD, RDC, DOSY,[3] and heteronuclear relaxation methods to detect the association proceses and to find out the bioactive conformation of sugars and glycomimetics at the recognition site of the corresponding receptors. As Lectins and encimes, both wild type and mutants, have been used as receptors with the final aim to know and to evaluate the relative importance of polar (hydrogen bonding, electrostatic interactions) and apolar (van der Waals, CH-π) forces[4] in the molecular recognition process. As examples, structural and conformational details of chitooligosaccharide recognition by hevein domains will be shown.[5] Additional examples will be presented that include the binding of GM1 glycomimetics by cholera toxin,[6] and between heparin analogues and the acidic fibroblast growth factor.[7] I have to emphasise that all these projects have been performed with the collaboration of a number of people at Madrid and in collaboration with several international groups.

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NMR studies of the dynamics and metal exchange properties of two C4C4 RING domains

Klaartje Houben

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Zinc fingers are small structured proteins that require the coordination of zinc for a stable tertiary fold. Together with FYVE and PHD, the RING domain forms a distinct class of zinc-binding domains, where two zinc ions are ligated in a cross-braced manner, with the first and second pairs of ligands coordinating one zinc ion, while the second and fourth pairs ligate the other zinc ion. We studied the dynamcis and metal exchange of the C4C4 RING domains of CNOT4 and the p44 subunit of TFIIH. We found that Zn^{2+}-Cd^{2+} exchange is different between the two metal-binding sites in the C4C4 RING domains and also differs dignificantly between the two proteins. In order to understand the origins of these distinct exchange rates, we have studied the backbone dynamics of both domains in the presence of zinc and of cadmium by NMR spectroscopy. The differential stability of the two metal-binding sites in the RING domains, as reflected by the different metal exchange rates, can be explained by a combination of accessibility and an electrostatic ion interaction model. The higher metal exchange rate in the p44 RING domain correlated with an increased backbone flexibility as compared to CNOT4. This difference in dynamics may be related to the distinct types of protein-protein interactions that the two C4C4 RING domains are involved in.

Towards complete assignment of proteins through protonless NMR

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The complete heteronculear sequence specific assignment of proteins is feasible through ^{13}C direct detection 3D experiments thanks to the improvements in probe technology and to the development of new NMR experiments. The set of NMR experiments we present is based on direct detection of ^{13}CO and ^{13}Ca through spin state selective methods to remove the large one bond ^{13}C-^{13}C scalar coupling constants in the direct acquisition dimension (IPAP for ^{13}CO and DIPAP for ^{13}Ca). The experiments presented here allow us to identify spin systems (CACO-IPAP, CBCACO-IPAP, CCCO-IPAP, Ca-TOCSY-DIPAP) and to link them in a sequence specific manner (CON-IPAP, CBCACON-IPAP, CANCO-IPAP). This "protonless NMR" strategy is demonstrated on monomeric and dimeric Cu,Zn superoxide dismutase [1-3]. It offers alternative solutions for demanding situations (paramagnetic and/or large molecules) and it can be useful in general in conjunction with conventional experiments. The set of experiments developed now allows us to perform protein sequence specific assignment of backbone and side chains.

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Folding Rules for Branched DNA Prediction of alignment of nucleic acids in aligned media

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On the basis of high-resolution structural data we have recently shown[1,2] that two folding rules can completely account for the stacking behavior of all reported DNA three-way junctions containing two unpaired residues at the junction (3HS2). These two rules can now be used to predict the stacking preference of 3HS2 molecules of any sequence. Following this procedure we designed three novel sequences that were predicted to fold into a preferred A/C-stacked conformation. NMR data indeed show that all three new 3H molecules fold into a stable A/C-stacked conformation. Residual dipolar couplings (RDC) were crucial to derive the first high-resolution DNA 3H structures. To further improve the usage of RDCs in DNAs and RNAs and specifically branched DNAs we developed a fast analytical method for alignment prediction. We demonstrate for nucleic acids in aligning medium (i.e. Pf1 phage) that the molecular alignment tensor **A** can be predicted reliably and accurately, via a simple and fast calculation based on the radius of gyration tensor spanned out by the phosphate charges. The 26 published RDC-derived DNA/RNA structures provided sufficient statistics to validate the proposed prediction approach. We also show theoretically that pure electrostatic alignment leads to a quadratic dependence on size, while steric alignment is linearly dependent on size. Moreover, our analytic expression predicts that under usual conditions of salt strengths the alignment essentially independent of salt strength in good agreement with published experimental observations.

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The use of optimal control theory for systematic design of solid-state NMR experiments with improved performance

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The aim of obtaining information about structure and dynamics for increasingly complicated molecular systems has been a driving force for the development of more and more advanced NMR experiments with improved characteristics in terms of information content, sensitivity, and spectral resolution. This general statement certainly applies to solid-state NMR spectroscopy where the instrumental capability to tailor the complicated nuclear spin Hamiltonian to a manageable form has led to highly advanced re- and decoupling NMR experiments. These experiments have primarily been designed using combinations of intuition, analytical description, and numerical calculations, for example based on average Hamiltonian theory. In this presentation, we demonstrate that optimal control theory – analytically and numerically – is a very powerful vehicle for designing NMR experiments with improved performance in terms of sensitivity, selectivity, and robustness towards instrumental imperfections. These aspects – along with an introduction to our optimization procedure - are demonstrated by the presentation of various new improved methods for homo- and heteronuclear dipolar recoupling in spin-1/2 systems and excitation of multiple-quantum coherences for quadrupolar nuclei. The methods will be described and demonstrated analytically, numerically, and experimentally.

Advances in sample spinning experiments

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We report first DOR measurements at 900 MHz and MAS at 40 KHz and 20K. Dynamics of new generation MAS rotors is demonstrated by comparative rotation sweep and PDSD in peptides.

Solid-state NMR: A tool for probing the protein-mineral interface and other structures in bone

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Bone is a highly complex composite material consisting of a protein matrix (predominantly collagen) with calcium phosphate crystals deposited in it. The nature of the calcium phosphate phase in bone has been the subject of considerable discussion in the literature for many years. Of key importance is the structure of the surface of the crystals as it is the surface, which interacts with the surrounding matrix and binds the whole material together. As for any crystal, the surface is disordered and not easily amenable to study by conventional diffraction techniques. Solid-state NMR however is an ideal method for studying both this surface structure and the interface between the surface and protein matrix.

This work will describe the combination of ^{1}H-^{31}P 2D HETCOR, ^{1}H-^{31}P REDOR and other experiments, which have allowed us to characterize the surface of the mineral phase in bone in some detail. Subsequent ^{13}C-^{31}P REDOR experiments have both identified one of the protein groups responsible for binding to the crystal surfaces (a glutamate carboxylate group) and allowed determination of the distance of this group from the crystal surface. This is the first direct experimental evidence of such an interaction in bone and supports a long-standing hypothesis that glutamate coordination to calcium ions in the mineral phase is a primary mechanism for the mineral – protein binding.

The structure of the protein matrix in bone is very important to the material properties of bone. In particular, this matrix is thought to be responsible for dissipating stresses, which might otherwise damage the delicate mineral crystals. Accordingly, we have used solid-state NMR to assess the structure of collagen in bone. The primary method we have used here is measurement of ^{13}C chemical shift anisotropies/ asymmetries via a new method developed in Cambridge which allows the scaling of the effective sample spinning rate, and so measurement of chemical shift anisotropies via spinning sideband patterns whilst magic-angle spinning at high spinning rates. First principles electronic structure calculations enable us to calculate the expected chemical shift parameters for a variety of trial structures. Comparison with the experimental parameters then allows us to determine the range of structures which can exist in bone collagen.

Other experiments which are being developed in to determine structural parameters for the collagen in bone which utilize ^{14}N (which occurs as every third nucleus in the protein backbone) will also be discussed.

Proton High-Resolution Solid-State NMR for Structure Determination in Powdered Solids

Bénédicte Elena

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Proton resolution is largely determined in rotating powders by the quality of homonuclear dipolar decoupling sequences. We show how significantly improved performance in decoupling is provided by application of the recently introduced direct spectral optimization procedure (dubbed eDUMBO). Using the resulting sequences, proton line-width less than 0.2 ppm (after correction for the decoupling scaling factor) can be obtained at 700 MHz for crystalline powdered solids, using standard equipment and moderate magic-angle spinning frequencies.

Efficient proton homonuclear decoupling is critical to the observation of $^{1}J_{CH}$ couplings, and for the efficiency of through-bond correlation experiments in solids. We demonstrate the feasibility of ^{1}H-^{13}C INEPT through-bond polarization transfer to rigid crystalline solids. The use of efficient proton homonuclear dipolar decoupling in combination with direct spectral optimization procedure provides minimization of transverse decoherence, and leads to efficient ^{1}H-^{13}C INEPT transfer for uniformly ^{13}C-labeled, as well as natural abundance, crystalline organic compounds. Application of this technique to 2D heteronuclear correlation spectroscopy provides, for a carbon-13 enriched sample of L-Isoleucine, an enhancement factor up to three in sensitivity in comparison to standard MAS-J-HMQC experiments.

Furthermore, the increased performance in proton homonuclear dipolar decoupling is essential for the acquisition of highly resolved proton spectra in the solid-state, and their applications. We present high resolution ^{1}H-^{1}H correlation experiment, such as high resolution 2D double–quantum single–quantum CRAMPS correlation experiments, and ^{1}H-^{1}H spin diffusion recorded with high resolution in both indirect (t_1) and direct (t_2) dimension of acquisition, using windowed-DUMBO-1 decoupling. We show how those experiments can provide extremely valuable structural information for small powdered compounds at natural abundance. Notably we have recorded a series of directly detected ^{1}H-^{1}H spin diffusion experiments that can be quantitatively exploited to measure the dipolar correlations between sites. The investigation of ^{1}H-^{1}H spin diffusion build-up curves using a rate matrix analysis approach shows that high resolution magic-angle spinning NMR of protons provides a method to probe crystalline arrangements. The comparison between experimental ^{1}H data and simulation is shown to depend strongly on the parameters of the crystal structure, for example on the unit cell parameters or the orientation of the molecule in the unit cell, and those parameters are experimentally determined for a model organic compound, the dipeptide β-L-Aspartyl-L-Alanine

Monday 4 July, afternoon Methods, Solids 2

1 GHz NMR and beyond

Jan van Bentum

Jan van Os, Arno Kentgens (Radboud University Nijmegen), Claude Berthier, Mladen Horvatic (CNRS Grenoble), Juergen Haase (IFW Dresden) and Ago Samoson (ICPB Tallinn).

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This year, a new EC collaboration project has started to explore the possibilities for very high field NMR.

The main goal of this project is to develop the methodology for performing NMR experiments in resistive high-field magnets up to 40 T and in pulsed fields above 60 T. The project unites all high-field NMR initiatives in Europe, and is associated with high magnetic field facilities that provide free access for all users. Medium to high resolution NMR spectroscopy at 1.25 GHz (30 T) will be developed for chemical and biological research. A versatile multi-frequency low temperature spectrometer for fields up to 40 T will be established for the study of physical problems for example with field-induced phase transitions. Finally, a unique pulsed field NMR facility will be established to explore material properties of for example high T_c superconductors at fields above 60 T. The project is coordinated by the Solid State NMR group at the Radboud University in Nijmegen. Partners are the high magnetic field laboratory in Grenoble, the pulsed field facility in Dresden and the National Institute of Chemical Physics and Biophysics in Tallinn. In the poster we will outline the methods that are used to achieve the required resolution in very inhomogeneous and relatively unstable magnets. This includes novel ways of ferromagnetic shimming, the use of efficient microcoils and new very high speed MAS probes. Preliminary results include the first 1 ppm spectra obtained at 1277 MHz in the new high field installation in , the first 2 GHz spectra obtained in pulsed fields and examples of very low temperature NMR analysis of quantum spin phase transitions at magnetic fields above 25 Tesla.

Recent developments in microtesla, ex-situ and remote NMR and MRI

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Novel methodologies of NMR and MRI from molecular to macroscopic scales will be described. Microtesla magnetic resonance with SQUID detectors has extended from *in vitro* solution spectroscopy to *in vivo* human imaging. We have further extended the observation of high-resolution NMR and MRI in inhomogeneous fields using "shim pulses" in inhomogeneous fields and nonlinear gradients. First results have been obtained using one sided systems, thereby enhancing the promise of an approach to scanning *ex-situ* detection of magnetic resonance which would make it possible to obtain high-resolution information about objects or subjects that are immobile or otherwise inaccessible to traditional methods of NMR and MRI. The combination of optical and magnetic resonance using laser-polarized atoms and enhanced *remote* detection using SQUIDs and lasers for reconstruction of images and spectra allows the exploration of species identification, distribution and flow, in solution, in porous materials and in microfluidic channels. Furthermore, *functionalized* molecular biosensors using hyperpolarized agents with continuous bubbling/dissolution and flow have been used to detect target molecules and protein conformational changes upon substrate binding. The NMR biosensor methodology is being combined with *remote* detection for enhanced sensitivity in the examination of ultralow concentration species in solution. Such approaches also open the possibility of magnetic resonance for multiplexed molecular assaying, with applications in physics, chemistry, materials science, and biomedicine.

Collaborating with Jean-Pierre Dutasta and Thierry Brotin in Lyon, Yiqiao Song, Pabitra Sen et al at Schlumberger-Doll Research, and the groups of Bernhard Bluemich in Aachen, and John Clarke, Dmitry Budker, Jeffrey Reimer and David Wemmer at Berkeley

Principles and Applications of Symmetry-Based Recoupling in Solid-State NMR

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Around 30 years ago, two major classes of solid-state NMR methods were developed for improving resolution, sensitivity and spectral information content. Magic-angle spinning averaged out anisotropic interactions by rotating the molecular environment in space. Multiple-pulse sequences averaged out interactions by rotating the nuclear spins using applied fields. Although several steps were made in the direction of combining magic-angle spinning with multiple-pulse sequences, the complicated nature of combined spin and space rotations inhibited a rapid development in this area.

Our research group has used symmetry theorems to simplify the problem of combined spin and space manipulations. If the pulse sequence obeys certain symmetry relationships, its first-order behaviour is governed by simple selection rules whose consequences may be predicted diagrammatically. It is now rather straightforward to construct recoupling and decoupling pulse sequences with a wide range of different properties, simply by choosing sets of integers called symmetry numbers.

I will give an overview of how this is done, and show some of the applications of these symmetry-based pulse sequences to biological solid-state NMR and to materials science.

NMR Studies of the Membrane Protein OmpX in DHPC Micelles

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NMR has become well established for determining the structure and dynamics of biological macromolecules. It is often the only applicable method when working with integral membrane proteins, which constitute up to 30% of a typical genome. However, the study of these proteins in solution is intrinsically challenging due to the need for a lipophilic phase for solubilization, giving rise to large particle sizes. Here, NMR methods allowing to work with such systems were explored, using as a model the outer membrane protein X (OmpX) from *Escherichia coli*. Based on backbone and partial side-chain assignments, the solution structure of the β-barrel protein OmpX in dihexanoyl phosphatidylcholine (DHPC) micelles was determined. Thereby, the side-chain assignments were obtained with newly developed TROSY-based NMR experiments, which were applied together with specific isotope labelling techniques. Based on the NMR structure, detailed information about the solvation of the protein by its surrounding detergent micelle was gained using intermolecular nuclear Overhauser enhancement, which identified contacts of the protein with lipid molecules. This was complemented by determining the effect of paramagnetic relaxation agents with different solubility properties on the protein resonances. The thus obtained model of the detergent micelle showed that it closely mimics the lipid bilayer into which the protein is embedded in its natural environment. Therefore, it seems plausible that the NMR structure obtained in solution faithfully represents the protein in its functional form, and that for example information on protein dynamics can be extrapolated to a proposed structure-function relationship.

Adiabatic Pulses in Single Scan Multi-Dimensional NMR Spectroscopy

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In 2002 Frydman and coworkers (PNAS, vol. 99, pp. 15858-15862, 2002) introduced a method to acquire multidimensional spectra in only one scan. This was achieved by obtaining a non-uniform evolution over the sample in the indirect dimension, mimicking a gradient with an amplitude proportional to the chemical shift. This purpose was achieved by a train of discrete frequency shifted selective pulses in presence of a gradient. There are several problems that arise with this scheme: artifacts due to the discrete scheme, phase-distortions and losses due to the non-complete excitation of the polarization over the full sample-volume. Moreover, for nuclei with a low gyro-magnetic ratio it is impossible to achieve a decent resolution due to the limited frequency differences that a gradient induces in the sample. In this presentation it will be shown that the use of adiabatic pulses during the excitation of the indirect dimension can overcome these difficulties. In addition, some strategies to improve the quality of the spectra and the sensitivity of the method will be discussed.

Very fast two-dimensional NMR spectroscopy for real-time investigation of dynamic events in proteins on the time scale of seconds

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Ultra-fast multidimensional NMR provides a new tool for site-resolved kinetic NMR experiments, e.g. real-time studies of protein folding, conformational changes related to biomolecular function, or the measurement of fast hydrogen-deuterium exchange rates characterizing the solvent accessibility of different parts of the protein. Ultra-fast acquisition schemes are also important for high-throughput NMR studies, such as the systematic evaluation of sample conditions for structural studies by either NMR or X-ray crystallography, and library-based ligand screening. Conventional multidimensional NMR offers the spectral resolution required to resolve the many resonance peaks of macromolecular systems, but it requires long measurement times that are exponentially increasing with the dimensionality of the experiment. Several fast acquisition techniques (projection NMR, Hadamard NMR, single-scan NMR, …) have been developed recently that can speed up NMR data acquisition by several orders of magnitude. Especially, "single scan NMR", introduced by L. Frydman and co-workers [(1)], allows the recording of any multidimensional spectrum within a single repetition of the experiment. Here we will present an alternative technique, SOFAST-HMQC [(2)] that allows recording of two-dimensional protein spectra within a few seconds. The experiment is based on standard data sampling in the indirect dimension, and it is very easy to implement. The performance of the new experiment in terms of sensitivity and resolution will be demonstrated for various proteins at millimolar concentration. Real time measurements of fast hydrogen-deuterium exchange rates in proteins illustrate the potential of SOFAST-HMQC for site-resolved kinetic NMR studies.

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Reconstruction of Multidimensional NMR Spectra

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Reconstruction of an image from a set of projections is a well-established science, successfully exploited in X-ray tomography and magnetic resonance imaging(MRI). We have adapted this principle to generate multidimensional NMR spectra, with the crucial difference that these spectra are made up of discrete features. sparsely distributed, rather like the stars in the night sky. Consequently a reliable reconstruction can be made from a small number of projections. This reduces the duration of the measurements by orders of magnitude, of crucial important for structural investigations of biomolecules such as proteins. We explore several practical schemes for reconstruction -- back-projection, iterative least-squares fitting, the lower-value algorithm, and Bayesian methods. The results are compared in terms of their reliability, sensitivity and speed of computation.

Novel Flip-Back Schemes and Queued Spectroscopy: New Methods for Recovery of Lost Magnetisation

Tammo Diercks

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The majority of NMR experiments only converts a part of all 1H polarisation into observable coherence. Recovery of the unused polarisation could obviously make NMR considerably more economical when used in one of two possible ways:

1. indirectly and unspecifically to cool the proton lattice and, thus, accelerate reequilibration of the selected proton subset as in LTROSY[1].
2. directly and specifically, as we propose here, in an immediately (i.e., without reequilibration delay) appended second NMR experiment; such queued experiments on different types of proton polarisation are largely uncorrelated and may therefore differ broadly (in contrast to strictly correlated, specific concatenation schemes recovering lost coherence, such as CoCONOESY[2] or the sensitivity enhancement3 scheme).

For both applicat ions, we present two novel schemes to recover unused polarisation in different types of heteronuclear correlation experiments, based on initial orthogonal separation of observed and lost magnetisations:

1. qTROSY (queued TROSY) recovers anti-TROSY polarisation in TROSY experiments
2. efb (extended flip-back) preserves proton magnetisation not bound to a specific heteronucleus in HSQC-type experiments

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A new method for ultrafast high resolution iZQC spectroscopy in vivo

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Localized detection of metabolites is useful for detecting and diagnosing diseases. In many organs, susceptibility differences between tissues and or interfaces, especially air/tissue interfaces, create strong local fields which broaden the resonance frequency lines from each proton species. It then becomes difficult to shim the magnet to create an acceptable linewidth. The broadening can be improved through choosing very small voxels, at a great sacrifice to sensitivity and linewidth.

Intermolecular zero quantum coherences (iZQC) are intrinsically not sensitive to large scale inhomogeneities, and can give sharp lines along the indirect dimension despite variations in the magnetic field[1-4]. Our work on cold-blooded animals at low temperatures has increased resolution by an order of magnitude over a direct acquisition.

Though in vitro ZQC spectroscopy gives narrow lines, in vivo for warm blood animals[5] the t1 noise inherent to the long experimental times of two dimensional spectroscopy decreases the resolution. In principle, ultrafast two-dimensional methods such as those developed by Frydman et al.[6,7] can overcome this problem. However, their approach (time offset excitations of many different narrow slices) will not work for our application, since the molecules involved in an iZQC scan inevitably suffer some chemical shift mis-registration, and their coupling would therefore be eliminated. Recently, we have developed a new pulse sequence that enables the acquisition of 2D ZQC localized spectra within a single data acquisition scan. We have also used the same approach to speed up other standard 2D-spectroscopy pulse sequences such as standard COSY or intramolecular double quantum filtered spectroscopy. Preliminary work in vitro, with a simultaneous acquisition of up to 31 indirect-dimension points per sequence, will be presented.

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New Frontiers in ESR Technology: ESR Microscopy and Millimeter Wave Two-Dimensional ESR Spectroscopy

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High resolution (ESR) imaging of microscopic samples is an emerging field of modern ESR technology. The goal is to obtain images of small biological specimens or samples for materials science applications, with a spatial resolution of about 1 micron in a few minutes of acquisition. NMR microscopy is a well developed technique but is limited, due to Signal-to-Noise ratio and proton diffusion, to a practical resolution of ca. [10 μm] [3]. ESR has potential virtues for microscopic magnetic resonance imaging. For example, the signal per spin is much higher than in NMR, diffusion does not limit the resolution in the short time scales (T_2's ~ μs) of the ESR measurements, ESR micro-resonators detect with a high quality factor (Q) of ~ 1000 and the ESR line-shape is more sensitive to dynamic effects, leading to richer information. Since most samples do not contain stable free radicals, paramagnetic species must be added in a manner similar to that of adding contrast agents in NMR or dyes in optics. We have developed a 3D ESR microscope operating in CW mode, at 9 GHz [1] which utilizes a miniature imaging probe consisting of resonator plus gradient coils. This system is capable of obtaining 3D images of 64x64x64 voxels with a resolution of 10x10x30 μm at 60 minutes of acquisition. While it is a strong technique, it suffers from some drawbacks, e.g. it requires longer image acquisition times, it cannot obtain directly image contrasts such as T_1,T_2 and diffusion, and the time resolution of the CW is low. To overcome these limitations we have recently developed a pulsed 9/16 GHz ESR microscope [2] using short, but intense, pulsed magnetic field gradients. It is capable of obtaining 3D images of 256x256x50 voxels with a resolution of 3.5x7x11μm in 20 minutes of acquisition time. These performance criteria may be considered as the current state-of-the-art for magnetic resonance microscopy that can be readily applied to wide variety of biological and materials science applications. ESR microscopy at higher frequencies is expected to enable approaching 1 micron resolution.

High-field/high-frequency (HFHF) ESR offers improved sensitivity and resolution compared to ESR at conventional fields and frequencies. However, most HFHF ESR spectrometers suffer from limited millimeter-wave power, thereby requiring long mm-wave pulses. This precludes their use when relaxation times are short, e.g. in fluid samples. Low mm-wave power is also a major factor limiting the achievable spectral coverage and thereby the multiplex advantage of Fourier transform ESR (FTESR) experiments. High-power pulses are needed to perform two-dimensional (2D) FTESR experiments, which can unravel the dynamics of a spin system in great detail, making it an excellent tool for studying spin and molecular dynamics. We describe a high-power, high-bandwidth, pulsed ESR spectrometer operating at 95 GHz.[3] One of the principal design goals was the ability to investigate dynamic processes in aqueous samples at physiological temperatures with the intent to study biological systems. In experiments on aqueous samples at room temperature, we achieved 200-300 MHz spectral coverage at a sensitivity of $1.1 \times 10^{10}\sqrt{s}$ spins and a dead time of less than 50 ns. 2D-electron-electron double resonance experiments on aqueous and membrane samples of spin probes and spin labeled proteins illustrate the practical application of such a spectrometer.

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Tuesday 5 July, morning Emerging methods, EPR 1

Structure, Binding Modes, and Electron Inventory of Nitrogenase Enzymatic Intermediates by Multinuclear Q-band CW and Pulsed ENDOR Spectroscopy

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Nitrogenase catalyzes the reduction of N_2 to NH_3 on the most complicated metalloenzyme active site, the molybdenum-iron cofactor (FeMo-cofactor; FeMo-co; [Mo, Fe_7, S_9 -homocitrate]). This talk will describe the results of a collaboration between our group and those of Dennis Dean, Lance Seefeldt, and Brian Hales, in which we use EPR and Q-band CW and pulsed electron-nuclear double resonance (ENDOR) spectroscopies to characterize intermediates trapped during substrate reduction by nitrogenase. We have now: *(i)* trapped an intermediate that form during the reduction of alkynes and have used novel *quantitative* ^{1,2}H ENDOR measurements (Stochastic, Field-modulated; Mims pulsed), plus ^{13}C ENDOR measurements, to show that it contains a novel bio-organometallic structure: a complex of the alkene product bound as a ferracyle to Fe6 of FeMo-co; *(ii)* characterized by ^{1,2}H ENDOR an intermediate formed during proton reduction which most likely containing two hydrides bound to the cofactor; *(iii)* trapped three intermediates in which N_2 and/or its reduced forms are bound to the cofactor, and initiated their ^{14,15}N and ^{1,2}H ENDOR characterization; *(iv)* used ^{57}Fe ENDOR results to arrive at the first complete 'electron inventory' of a reduction intermediate, determining the the number of electrons, *n*, delivered to the molybdenum-iron protein and its partition into *m* electrons residing on the FeMo-cofactor and *s* delivered to the substrate ($n = m + s$); *(v)* correlated for the first time a kinetic intermediate with a well-defined chemical state of the enzyme; *(vi)* made advances in characterizing the 'thing in the middle' of the cofactor, a first row interstitial atom recently shown to be part of the cofactor. This developing story will be presented as dictated by time and emerging results.

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ESR-Imaging

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We present here our recent results on ESR-imaging via temperature dependent pulsed X-Band ESR and the backprojection reconstruction method focussing on quasi one-dimensional organic conductors, where even pulsed conduction electron spin resonance is possible.

We manipulated the defect concentration in a $(fluoranthene)_2PF_6$-crystal by spatially modulated proton irradiation and used a spatially resolved ESR-analysis to quantify the resulting local defect concentration, spin diffusion coefficient or electron spin concentration. It could be proven that spin diffusion coefficient and Peierls transition can be tailored via the radiation induced defect concentration and monitored via spatially resolved ESR.

Since flow measurement is an established tool in NMR-imaging, the possibility of performing ESR experiments on conduction electrons allows in principle to determine the electron spin drift velocity for given electrical current. We present an appropriate pulsed gradient and current ESR detection scheme and the first successful experiment of detecting the phase shift in the ESR-signal induced by an applied electrical current. The ohmic correlation is demonstrated in a range of more than 0.5 ampere and a maximum drift velocity of 18 m/s was found in the metallic phase of a radical cation salt.

2D imaging of two types radicals by the CW-EPR method

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The CW-EPR method of image reconstruction is based on the sample rotation in a magnetic field of a constant gradient (50G/cm). In order to obtain a projection (radical density distribution) along a given direction the EPR spectra should be recorded with and without the gradient. At the next step the two spectra are subjected to deconvolution, which gives the distribution of the radical density. Having performed this operation for e.g. 36 different angles we get the information necessary for reconstruction of the radical distribution [1]. The problem becomes more complex when there are at least two types of radicals in the sample, then the procedure of deconvolution does not give satisfactory results. We have proposed a solution of getting projections for each radical, based on iterative procedures. The images of density distribution obtained for each radical obtained by our procedure have proved that the method of deconvolution in combination with iterative fitting provides correct results. The test was performed on a sample of polymer PPS Br 111 with glass fibres and minerals. The results indicated a homogeneous distribution of radicals in the sample volume. The images obtained were in agreement with the real shape of the sample.

Another technique of imaging of hyperfine interactions concentration distribution by the electron nuclear double resonance imaging (CW-ENDOR) we would like to propose is based on a step-by-step change of the magnetic field intensity. The procedure was tested on a sample of a monocrystal of succinic acid.

Acknowledgment

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Investigation of the Nuclear Interactions with the S = 5/2 Electron Spin of the Fe(III) in Aquometmyoglobin by HYSCORE Spectroscopy

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We have investigated the possibility of using HYSCORE (hyperfine sublevel correlation) spectroscopy to study the electronic distribution of systems with an electron spin larger than ½, such as high-spin Fe(III) centers present in some heme proteins. We studied frozen solutions of aquometmyoglobin from equine skeletal muscle by HYSCORE spectroscopy. In this protein, the iron-containing heme group is bound to the protein via a proximal histidine. Since the distal ligand is a water molecule, the Fe(III) is in a high-spin state (S = 5/2). A previous ENDOR (electron nuclear double resonance) study on single crystals of aquometmyoglobin revealed hyperfine and quadrupole tensors of the heme and histidine nitrogens[1]. Since this protein has been already so well characterized, we have chosen it as a model system for our study.

We have used variants of the HYSCORE experiments in order to detect the nitrogen signals over a wide range of magnetic field and therefore of selective orientations. Scholes *et al.* used an effective spin Hamiltonian to simulate the ENDOR spectra. We followed the same approach to simulate the HYSCORE spectra. The features in the HYSCORE spectra are reproduced by the simulations using the hyperfine and quadrupole tensors of the heme and the histidine nitrogen coordinated to the Fe(III) as obtained from the ENDOR study. This shows the possibility to extract hyperfine and quadrupole tensors from the HYSCORE spectra of high-spin systems.

Moreover, the HYSCORE spectra reveal the signals of the remote nitrogen of the hystidine as well as those of the protons interacting with the Fe(III). In order to investigate the proton interactions, HYSCORE experiments have been performed on aquometmyoglobin in deuterated water.

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Solid State NMR methods for the characterization of the structure and dynamics in amorphous solids

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Continuing interest in these materials relies on the fact that the macroscopic physical and chemical properties can be adjusted – via a variation of the composition and synthesis route – in wide ranges. For a controlled optimization of key properties however, a detailed understanding of the macroscopic physical and chemical properties in terms of microscopic structural information constitutes a prerequisite.

In the first part of the presentation it is shown that using advanced solid state NMR strategies including MAS, MQMAS and various double resonance techniques it is possible to elucidate the structure of amorphous solids on short and intermediate length scales. Presented examples include nitridic ceramics and borosilicate glasses and show how to correlate composition, structure and function of these materials.

Different from crystalline compounds, however, an amorphous solid is not fully described by its composition. An important additional parameter is the thermal history of the material which affects the structure (network organization, phase separation) and hence function (macroscopic properties) of the amorphous solid. Equilibration during the melt to glass transition is impeded by rather slow kinetics; hence the structure of the amorphous solid at ambient temperatures depends not only on the equilibria present in the melt but also on their temperature dependence and the employed cooling rates.

This highlights the importance to correlate the structure in the amorphous solid at ambient temperature to the structural features present in the melt and to the equilibria governing the microstructure in the melt. This entails the necessity to perform NMR experiments at temperatures near and above the glass transition temperature. Methods to perform MAS NMR experiments at temperatures in the range of 673 K to 1173 K will be presented and preliminary results on glasses and glass melts will be given.

Approaches to Solid State NMR of (Radioactive) materials

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Various types of materials are under investigation as potential nuclear waste storage matrices. Some of them are crystalline (hollandites, phosphates, zircolonites) and some are amorphous borosilicate glasses. In both cases solid-state NMR is a well suited technique for their structural characterization. Studies of materials containing radioactive samples have been limited because of safety issues. The samples have to be properly confined resulting in a dramatic reduction of sensitivity as well as of the spinning rate that can be used. We are investigating slow spinning NMR techniques, as an alternative for high resolution NMR of amorphous and crystalline natural abundance materials. We propose the use of ultra slow NMR techniques and the use of microcoils and we show preliminary experimental results that allow to record highly sensitive spectra under safe spinning conditions.

Coexistence of FM and AFM in Cu-doped manganites detected by NMR and magnetic measurements

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Manganites have attracted great interest from scientific community since the 1950's, due to the complex and intriguing interaction between their structural, magnetic, and electric transport properties. The most notorious example is the effect of colossal magnetoresistance, responsible for the renewed interest in these materials in the last years. The properties of manganites with composition $R_{1-x}A_xMnO_3$ (R=rare earth, A=divalent metal) are controlled by the coexistence of Mn^{3+} and Mn^{4+} ions, created by the substitution of A^{2+} for R^{3+} ions. An interesting way to better understand the intrinsic properties of manganites is by doping the Mn site, usually with other transition-metal elements. This work describes the study of $La_{0.86}Sr_{0.14}Mn_{1-y}Cu_yO_{3+d}$ compounds, with y=0, 0.05, 0.10, 0.15, and 0.20. Both ^{139}La and ^{55}Mn zero field NMR measurements were used to investigate the local magnetic configuration in the samples, whereas X-ray diffractometry, scanning electron microscopy, and magnetization measurements were employed for additional structural and magnetic characterization. The Cu for Mn substitution did not show strong influence in the crystalline structure of the compounds, which preserved rhombohedral symmetry in the doping-range investigated. However, the volume of the unit cell decreased and an increase in the rhombohedral distortion was observed for doped samples with y = 0.15 and 0.20. Scanning electron microscopy images revealed homogeneous microstructure in all samples, besides a trend for smaller grains and larger porosity with increasing Cu content. The magnetic properties were found to be quite sensitive to the Cu doping. Magnetization vs. temperature measurements revealed paramagnetic to ferromagnetic transitions in all samples, with lower Curie temperatures and broader transitions for samples with higher Cu contents. A drop in the magnetization of the Cu-doped samples was also found at temperatures below the Curie temperature. These observations were interpreted in terms of the coexistence of ferro and antiferromagnetic phases at low temperatures, where the existence of the antiferromagnetic phase is favored by the presence of Cu ions in the Mn sub-lattice. Zero-field ^{139}La NMR measurements reinforced this idea, since the spectral shape remained essentially the same, whereas the bulk magnetization was drastically reduced in doped samples. The detected ^{139}La NMR signal was associated with ferromagnetic regions present in all samples. ^{55}Mn NMR spectra in Cu-doped samples indicated a reduction in the average hyperfine field along with a broadening of the resonance line, caused by the disorder introduced by the Cu doping. However, all lines detected by zero-field ^{55}Mn NMR measurements were again typical of ferromagnetic metallic regions. The results indicate coexistence of different magnetic phases in the studied manganites, although crystal structure has not presented significant changes.

Use of 2H double-quantum MAS NMR for studying molecular motion in solids

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We describe the use of a two-dimensional ^{2}H (I = 1) double-quantum MAS NMR experiment.[1-3] The experiment presented here correlates single- and double-quantum coherences to provide information about molecular motion in solids. Spin I = 1 double-quantum coherences are less sensitive to motional broadening under MAS than single-quantum coherences in certain motional regimes, as they are not subject to quadrupolar broadening to a first-order approximation. Hence, by comparing single- and double-quantum linewidths it is possible to obtain information about motional rates and mechanisms. (This phenomenon is similar to the difference in linewidths recently observed for half-integer quadrupolar nuclei in multiple-quantum (MQ)MAS and satellite-transition (ST)MAS spectra when motion is present[4]).

We present results from a variety of deuterated solids, including oxalic acid dihydrate, $(CO_2D)_2.2D_2O$. In this latter system, the water molecules undergo rapid rotation about their DOD bisector and, as a result, the double-quantum MAS linewidth of this peak is significantly narrower than the single-quantum linewidth. In contrast, this effect is not observed for the OD deuterons.

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Solid-State NMR Spectroscopy of Paramagnetic Transition-Metal Cyanides

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Over the past decade there has been a resurgence in metal-cyanide chemistry, in part due to the utility of bridging cyanides for constructing organometallic frameworks and materials possessing useful electronic and opto-electronic properties. Solid-state NMR spectroscopy has been successfully applied in the structural investigation of diamagnetic metal-cyanides, however, the electron-nucleus interactions in corresponding paramagnetic solids have hindered both the acquisition and the interpretation of spectra. Fast magic-angle spinning (MAS) has recently been shown to allow the acquisition of spectra of spin-1/2 nuclides in paramagnetic materials. We apply standard MAS techniques to a series of transition-metal cyanides containing stoichiometric paramagnetic cations. Variable-temperature experiments are used to gauge the contribution of Fermi and/or pseudocontact contributions to the observed shifts. In favourable cases, reliable shielding tensors can be determined from this analysis. These results will be discussed in the context of the available structural information to provide a basis for the characterization of paramagnetic metal-cyanide solids by MAS NMR.

NMR studies of molecular recognition and dynamicsin the regulation of gene expression

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We use NMR to characterize the structural basis for RNA recognition in splicing and RNA interference. Recently, we have also combined quantum chemical calculations, molecular dynamics and NMR to characterize local anisotropic dynamics of the peptide backbone in proteins.

Gene silencing mediated by RNA interference depends on short interfering RNAs (siRNAs) and micro RNAs (miRNAs). SiRNAs exhibit unique features, namely a defined size of 19-21 base pairs with characteristic 2-nucleotide single-stranded 3' overhangs and 5' monophosphates. We have determined the structural basis for the recognition of the characteristic 2-nucleotide single-stranded 3' overhangs of siRNAs by the Ago2 PAZ domain. These results and work of others reveal unique and novel ways of sequence-independent recognition of the characteristic features of siRNAs and miRNAs in the RNAi pathway.

Splicing of nuclear pre-mRNA, i.e. the removal of non-coding, intervening intron sequences, is a key step in eukaryotic gene expression and contributes to gene regulation and protein diversity by joining of alternative exons. Early spliceosome assembly involves protein-protein and protein-RNA interactions for cooperative intron RNA recognition. We have developed strategies for studying larger protein-RNA complexes using residual dipolar couplings based on the available structures of smaller fragments. Examples of complexes involved in spliceosome assembly will be discussed.

Mechanism of HIV-1 Co-receptor selectivity probed by NMR of antibody-peptide complexes.

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The third variable region (V3) of the HIV-1 envelope glycoprotein gp120 is involved in gp120 binding to the chemokine receptors CCR5 and CXCR4 which serve as co-receptors in HIV-1 infection. The sequence of V3 determines whether the virus binds to CCR5 and infects predominantly macrophages (“R5 virus”) or to CXCR4 and the virus infects mostly T-cells (“X4 virus”). To gain insight into the structure of the V3 loop, not provided by the crystallographic structure of gp120-core, we have studied the structure of V3 peptides in complex with V3-specific antibodies that were either elicited against native gp120 or isolated from an HIV-1 infected donor. We assume that flexible V3 peptides, upon binding to these antibodies, fold into the native V3 conformation by an induced fit mechanism. The NMR structures of two highly divergent V3 peptides in complex with the two HIV-1 neutralizing antibodies 0.5β and 447-52D revealed that V3 forms a β-hairpin and that the N-terminal strand of this hairpin is more dominant than the C-terminal strand in its interactions with virus-neutralizing antibodies. Two alternative conformations were found for the N-terminal strand of V3. These conformations differ by 180° in the orientation of the side chains creating two surfaces with differing topology. On the basis of these findings, we proposed a model for HIV-1 selectivity suggesting that the formation of these two different surfaces by otherwise very similar V3 sequences accounts for the difference in specificity (receptor selectivity) between R5 and X4 viruses

NMR and biochemical studies of the 305kDa ClpP protease establish dynamic side pores for product release

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We have used a combination of NMR techniques and biochemical experiments to study the 305kDa E-coli ClpP protease[1]. This protease is a representative member of the of family cylindrical self-compartmentalizing degradation machines, that also includes the 20S proteasome. The high molecular weight challenges the possibility to obtain NMR data of good quality. We show that the newly developed methyl TROSY technique[2] yields high quality correlation maps of the ILE-δ_1 groups at temperatures as low as 0.5ºC (estimated molecular tumbling tme 450ns). To obtain assignments of these methyl groups we used a mutational approach where ILE residues of interest were replaced with closely related aminoacids. Interestingly, we found that a region connecting the 2 ClpP heptameric rings is highly dynamic and adopts two distinct conformations at low temperatures. An increase in temperature shows a clear increase in the exchange rate between those two states. Using magnetization transfer and methyl TROSY relaxation dispersion experiments we were able to obtain data about the rates and populations of the exchange process between 0.5 and 40ºC, which allowed us to extract thermodynamic properties. A restriction of the observed motional freedom using a disulphide cysteine link resulted in a protease that was not able to release peptides, linking the observed dynamical process with the biologically relevant process of substrate release. Our data shows the potential of methyl TROSY techniques to study molecular complexes of extremely high molecular weight.

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Motion in the Binding Site for Human U1A Protein: A NMR Study of RNA dynamics

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The widespread importance of induced fit in RNA recognition makes it imperative that RNA motional properties are characterized in a systematic, quantitative way. Until now, however, very few studies have been dedicated to the systematic characterization of the RNA motional properties that accompany protein recognition. We are using both solution and solid-state NMR to determine the motional range and amplitudes. Solution NMR techniques are particularly useful in providing a global view of the molecule whereas solid-state NMR is sensitive to local ordering and can provide site-specific information. One can use the former to determine motion in all sites simultaneously and locate residues of particular interest. Such sites can then be selectively labeled and probed with solid state NMR to determine trajectories of motion. Together, both solution and solids state NMR cover a large motional timescale ~10 orders of magnitude; however to date, they have not been applied collectively to look at a single particular system. We propose to study the U1A protein-RNA complex as it provides some of the best-studied examples of the role of RNA motional changes upon protein binding. Here, we report both the ^{13}C NMR relaxation studies of base and sugar dynamics for the RNA target of the human U1A protein in both its free and protein-bound form, and preliminary solid-state results investigating the dynamics of the C43 residue using static deuterium lineshape data. For the free RNA, we have analyzed fast (ns-ps) and intermediate (µs-ms) motions of a construct of 3'UTR U1A mRNA by measuring ^{13}C T_1, $T_{1\rho}$ and heteronuclear NOEs for both sugar and base nuclei, as well as the power dependence of $T_{1\rho}$. The measurements indicate a broad range of dynamic heterogeneity in both the bases and sugars. In the binding region, the bases U40, U41, G42 and C43 all exhibit significant motion on the ns-ps timescale and residues A24, G42 and A44 exhibit conformational exchange. The analysis of these measurement paints a much clearer picture of the type of motions experienced by this RNA in the absence of the protein than was provided by the analysis of the structure based solely on NOEs and scalar couplings. The results lead to a model where the internal loop region to which U1A binds `breathes' on a µs- ms time scale at the hinge connecting the internal loop to the double helical regions. Superimposed on this motion, the residues at the very tip of the loop and lacking strong stacking interactions undergo fast (ns-ps) motions. The majority of these motions were found to be quenched upon binding to the U1A protein.

Insight into methyl-dynamics of dynamically different motifs of dsRBD of PKR to understand hydrophobic core packing

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Double stranded RNA binding domains (dsRBD) regulate distinct cellular functions and the fate of an RNA molecule in the cell. This highly homogeneous domains present in multiple copies in a number of species, exhibiting individual functional specificity. Number of NMR and X-ray crystallographic structural studies reveal that several such domains take common α-β-β-α tertiary fold. However the functional specificities of these domains could be due to the dynamic variations of the individual amino acids, as has been shown earlier in the case of backbone dynamics of ^{15}N-^{1}H of dsRNA binding motifs (dsRBMs) of human PKR. In order to further investigate if the differences in dynamics of the two dsRBMs are restricted to only backbone or if the side-chain motions are also different to the extent of influencing their packing of the two hydrophobic cores, we have been investigating the methyl group dynamics using ^{13}C relaxation experiments. The result of these investigations shows that it has a large implementation for disease-protein's activity and will be discussed in this paper.

Programs for Achieving Single Nuclear Spin Detection

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This lecture discusses the technical feasibility of comprehensive molecular imaging, and what such a capability might imply. Care has been taken to make this material accessible to non-specialists, yet the discussion is reasonably rigorous.

The following questions are addressed:

(1) What is a reasonable technical path to single-nuclear-spin detection?

(2) What are appropriate performance metrics and technical milestones?

(3) When might this technology reasonably be ready?

(4) What tasks could this technology accomplish?

(5) What new resources might this technology create?

The year 2004 was a breakthrough year for magnetic resonance force microscopy (MRFM): the detection of a single electron spin by Dan Rugar's IBM group culminated a twelve-year effort during which MRFM's signal-to-noise ratio has improved by 140 dB.

This equates to a doubling of MRFM sensitivity every 3.1 months for twelve years; a rate of progress unequalled by any sensor technology in history. The natural technical path to further improvements is by continued development and testing of smaller, colder, more quiet MRFM devices.

A natural performance metric is the single-spin channel capacity, defined as the bits-per-second of imaging information received from each spin. A natural milestone is 0.1 bits-per-second, which is a threshold rate at which single-spin detection becomes feasible.

When might this technology reasonably be ready? Atomic-resolution imaging will become feasible in ~2010, if present progress is sustained. Sustaining this progress will require three coordinated efforts: (1) fabricating the next generation of MRFM devices: smaller, colder, and quieter, (2) testing these devices in realistic imaging environments, and (3) synthesizing reliable engineering principles from emerging nanoscale physics.

To the extent that maturing MRFM technologies can approach the quantum limits to channel capacity, tabletop-scale devices will observe hundreds of atomic coordinates per-second. A cluster of several hundred such devices, deployed like the clusters of sequencers in the Human Genome Project, would observe ~ 10^12 coordinates per year.

Archiving, interpreting, and sharing this rich flow of structural data would comprise the largest scientific project in history. The unifying context of the project would amplify the value of a broad spectrum of atomic-scale imaging projects relating materials science, nanoscale electronics, quantum physics, biology, and medicine.

The concluding part of the talk will focus on mission-critical technical issues. For the coming year, we take these issues to be: (1) the need for large-scale quantum spin simulations, (2) measurements of thermal dielectric noise in polymer and biological samples, and (3) advanced image reconstruction algorithms.

High Frequency Dynamic Nuclear Polarization: Why Two Electrons Are Better Than One

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Over the last few years we have developed two gyrotron microwave sources that operate at frequencies of 140 GHz (λ= 2.14 mm) and 250 GHz (λ= 1.20 mm), and a third that is under development at ~460 GHz (λ= 0.65 mm) that permit DNP enhanced NMR (DNP/NMR) experiments in magnetic fields of 5-16.4 T (^{1}H NMR frequencies of 211, 380, and 700 MHz, respectively). We review the instrumentation (low temperature MAS probes, microwave transmission lines, and gyrotron sources) used for these experiments. In addition, we discuss two mechanisms that are currently used for DNP experiments in insulating solids at high fields – the solid effect and thermal mixing/cross effect -- and the paramagnetic polarizing agents appropriate for each. These include a new class of biradicals that enable increased enhancements at reduced concentrations of the paramagnetic center. Finally, we discuss applications of DNP/NMR that illustrate its utility in enhancing signal-to-noise in MAS NMR spectra of a variety of biological systems including membrane proteins and amyloid fibrils whose structures are of considerable scientific interest. In particular we review results that illustrate enhancements that are routinely available and range from 20—200 depending on experimental variables such as temperature, magnetic field, microwave B_1, polarizing agent, etc. In addition, we describe low temperature MAS experiments where we detect phase transitions in the 100-200 K range that may be connected with the so called “protein glass transition.”

Principles and Progress in Spatially Encoded NMR and MRI

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The present contribution will discuss some of the ongoing progress in our laboratory in connection to single-scan 2D NMR spectroscopy. While the bases of the methodology remain unchanged, a number of new strategies that should improve the sensitivity and overall performance of ultrafast 2D NMR have been devised. A number of spin excitation alternatives capable of affording the spatial encoding of the spin interactions that underlies ultrafast 2D NMR will be presented, and their relative merits compared. A variety of spin acquisition strategies involving the use of the interlaced Fourier transformation, as well as acquisition alternatives based on monitoring the two-dimensional data in the absence of any field gradient oscillations, will also be presented. In addition to these various spin excitation and data acquisition schemes, we shall also discuss some extensions of the spatial encoding principle towards the single-scan acquisition of 2D MRI images. Time permitting, the possibility to use these concepts in order to compensate various field inhomogeneities which arise in magnetic resonance spectroscopy and imaging, will also be presented.

Studying molecular structure and dynamics by high-resolution solid-state NMR

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Solid-state NMR represents a spectroscopic technique to relate spin-spin and spin-electron interactions to molecular conformation in an insoluble and non-crystalline environment. We discuss recent methodological progress to construct 3D molecular structures and monitor dynamics using a single NMR sample[1]. Such techniques are useful for the study of ligand-binding to G-protein coupled receptors[2] and ion channels[3] and offer new means to investigate large membrane proteins and protein complexes at atomic resolution.

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Solid-state NMR Concepts to study bacterial efflux pumps

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Membrane bound efflux pumps with broad substrate specificity provide an important mechanism for multidrug resistance. They are found in all major transporter families and use energy provided by ATP hydrolysis or proton motif force for substrate translocation. Unsolved problems include the mechanism of energy coupling to drug translocation, drug recognition, and characterisation of the catalytic cycle as well as resolving their 3D structure. Here, we report a systematic solid-state NMR approach towards the understanding of ABC and secondary transporters.

^{1}H-HR-MAS NMR has been used to screen a large number of antibiotics with respect to their location probability within the membrane. Our data support the “hydrophobic vacuum cleaner” model for protein mediated drug removal from the membrane.

The molecular dynamic of these proteins has been approached by residue specific ^{2}H labelling (Ala-d3) of two different transport proteins (LmrA, Hsmr).

Long range distance constraints are required for studying conformational re-organisations during the transport cycle or for defining the oligomeric state of the transport systems under investigation. ^{19}F-^{19}F dipolar couplings provide an useful approach to obtain long-range information with suitable sensitivity. Single and double cysteine substitution mutants of TBsmr, EmrE and LmrA have been expressed and ^{19}F-labeled through the attachment of a trifluoroacetone group by a disulfide linkage. First ^{19}F-MAS-NMR dipolar recoupling experiments on these transporters will be presented.

Finally, prospects for uniform ^{13}C/^{15}N labelling and multi-dimensional MAS-NMR experiments on these systems will be discussed.

Photo-CIDNP MAS NMR on photosynthetic reaction centres: What makes MAS NMR at factor 5000 above Boltzmann possible?

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Due to the light-induced electron transfer, photosynthetic reaction centres (RCs) produce photochemically induced dynamic nuclear polarisation (photo-CIDNP). This effect allows the detection of NMR signals from the photochemically active region of RCs with an enhancement of factor up to 5000. The origin of photo-CIDNP in frozen RCs has recently been re-evaluated [1]. One target of our investigations are the conditions allowing to observe such dramatic increase of NMR sensitivity. Understanding these conditions would allow new classes of MAS NMR experiments. The second target are the functional principles of photosynthetic electron pumping. Will be our understanding allow to construct better artificial systems? Obviously, photo-CIDNP MAS NMR is an excellent method to probe the electronic structure of such natural electron pumps. The special pair in RCs of Rhodobacter spheroides can be probed in great detail. Our analysis provides clear evidence for differences the two cofactors of the special pair are different in the electronic ground state. Whereas one BChl shows chemical shifts similar to the accessory BCls, there appears to be one special BChl in the special pair [2]. Since entire photosynthetic units show virtually an identical photo-CIDNP pattern as isolated RCs, we exclude an effect of the antenna on the electronic ground state of the RC [3]. The photo-CIDNP pattern observed in photosystem II indicates a strong asymmetry of the electron spin density distribution on P680 with a maximum on pyrrole ring III. That observation is explained by a local electric field which increases the redox potential without changing the HOMO-LUMO gap [4]. These findings of photosystem II contrast photo-CIDNP data obtained from photosystem I [5]. New data will be discussed.

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Wednesday 6 July, morning Bio-NMR, Solids 4

Inlfuence of the lipid environment on the orientation of protein transmembrane segments as determined by solid state ^{2}H NMR

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The structural and functional properties of membrane proteins are sensitive to interactions with lipids in the host membrane. The aim of the research presented here is to determine how the lipid environment can influence membrane protein structure and organization by affecting their transmembrane segments. By employing designed model peptides, that mimick transmembrane parts of proteins, and incorporating them into well-defined synthetic lipid bilayers of varying composition, we can analyze in detail the influence of lipids on structural properties of transmembrane protein segments. In particular, we used solid state ^{2}H NMR to investigate the influence of hydrophobic mismatch and anchoring interactions with the lipid/water interface on helix tilt, direction of tilt, and structure of the peptide backbone, employing peptides with deuterated alanines at specific positions along the helix axis. The results show that hydrophobic mismatch and interfacial anchoring both are important parameters that determine extent and direction of tilt of protein transmembrane segments in lipid bilayers.

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High-frequency pulsed ENDOR and its application to copper proteins.

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During the last decade a few groups, among those the Leiden group, have pioneered the potential of pulsed Electron Nuclear Double Resonance (ENDOR) spectroscopy beyond X-band microwave frequencies. The high EPR frequency implies an enhanced spectral resolution and absolute detection sensitivity, and the possibility to study paramagnetic centers not accessible at low frequencies. These advantages *ipso facto* apply to high-frequency ENDOR as well, which in addition provides

- an excellent nuclear-Zeeman resolution, facilitating or even enabling the analysis of ENDOR spectra and the assignment of ENDOR lines to different nuclei,
- accessibility to nuclei with small magnetic moments, and
- a nuclear sensitivity orders of magnitude better than for NMR, which is essential when working with tiny and/or valuable samples as is often the case in biology and nano- material science.

To illustrate these features, examples from our research will be discussed. These will include semiconductors, nanoparticles and in particular copper centers in proteins.

Protein-Cofactor and Cofactor-Substrate Interactions Studied by High-Field EPR

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The spectral resolution of the components of the *g* matrix at high-field/high-frequency EPR has been shown to allow for a detailed characterization of the cofactor binding situation in proteins. The particular sensitivity towards hydrogen bonds has been extensively utilized to study the quinone acceptors in photosynthetic reaction centers. Here we apply this approach to analyze substrate binding to a protein-bound cofactor. Pyrroloquinoline quinone (PQQ) is one of several quinone cofactors that is utilized in a class of dehydrogenases known as quinoproteins. We have used continuous-wave EPR at 94 GHz (W-band) to study substrate binding in ethanol dehydrogenase (QEDH) from *Pseudomonas aeruginosa*, taking advantage of the fact that the enzyme is isolated with a substantial proportion of the PQQ cofactor in the paramagnetic semiquinone form. In the substrate-free enzyme, the principal values of the g-tensor, obtained by spectral simulation are: g_x = 2.00585(2), g_y = 2.00518(2), and g_z = 2.00212(2), giving g_{iso} = 2.00438(2). All three principal values of the g-tensor decrease when ethanol is bound to the protein: g_x = 2.00574(2), g_y = 2.00511(2), and g_z = 2.00207(2), giving g_{iso} = 2.00431(2). The results represent the first direct evidence for the tight binding of an alcohol to a PQQ-dependent alcohol dehydrogenase and show that ethanol also binds to the enzyme even when the PQQ cofactor is in the semiquinone form. The decrease in *g* is consistent with an increase in polarity in the immediate vicinity of the PQQ cofactor and probably reflects a changed geometry of the PQQ-Ca^{2+} complex when ethanol binds.

Virtues of high field/high-frequency EPR: Advantages of multi-frequency approach in EPR

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Multi-frequency EPR is a preferred technique today as it enables one to obtain a more complete picture of the various processes affecting a paramagnetic ion in a given environment. It enables one to distinguish between the processes which depend on the external magnetic field and those which are independent of it. In assessing merit of using a particular EPR frequency, the factors which mask features of EPR spectra, notably linewidth and anisotropy, should also be taken into account. The effects due to second-order terms in perturbation in the energies of the levels become important when the Zeeman and non-Zeeman interactions are of the same order of magnitude. The advantages of high-frequency EPR are that it enhances the field-dependent interactions, while suppressing the second-order effects, in particular, the anisotropy. In addition, it enables observation of EPR spectrum when there exist large zero-field splittings (ZFS), which might not at all be observed, or at best results in a rather complicated angular variation of EPR line positions at low frequencies. As for influence of motion on EPR spectrum, the molecular motion does not completely average out at higher frequency due to faster time scale and thus can be better understood at higher frequencies. Further, the spectral shape is strongly influenced by the relative values of the ZFS and hyperfine (HF) interactions, and is easier to analyze at higher frequencies being simpler. This is because at lower frequencies, ZFS can lead to very broad lines in powders due to anisotropy. Nevertheless, low-frequency EPR is preferred to study field-independent interactions in greater detail, in particular the HF interaction, which is masked at higher frequencies when there exist g-anisotropy and g-strain. As well, aqueous-solution spectra of Cu^{2+} complexes are better resolved at lower frequencies, leading to determination of correlation times. Then there are frequency-dependent effects, e.g. the thermally active direct process of spin lattice relaxation, which can only be studied by multi-frequency approach. On the other hand, local-mode and Raman processes are frequency independent. Samples of different sizes and properties are optimally studied at different frequencies, since different sample sizes are required due to varying size of resonant cavity with frequency. Further, large and/or lossy samples are better studied at lower frequencies and vice versa. All these considerations suggest that multi-frequency approach is necessary to obtain a more complete and unambiguous picture. It includes study of Zeeman (g-value) and HF contributions, differentiating spectra of multiple species from each other, determination of number of nitrogens bound to copper, study of paramagnetic ions with spins greaterer than one-half, distance measurements based on dipolar coupling, study of non-Kramers (even-spin) ions, and measurement of rotational correlations. This presentation will discusses all these multi-frequency features in detail with the help of illustrative examples.

Protein microcrystals oriented in a liquid crystalline polymer medium: an EPR spectroscopy study

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A material with oriented molecules can reveal information valuable for computacional and spectroscopic studies of metalloproteins. Correlation between molecular structure and spectroscopic data can be established using single crystals, oriented membrane proteins, paramagnetic molecules linked to DNA fibers and weakly oriented proteins by bicelles, and alligned crystals under intense magnetic fields. Here we report a novel composite solid material with dispersed and partially aligned metalloprotein microcrystals. The thin solid films were obtained with protein microcrystals in a host liquid crystalline polymer medium, with the alignment tuned by a shear flow. A strong orientation effect of the metalloprotein was observed by EPR spectroscopy and optical microscopy. A mathematical model for EPR spectral simulations revealed the orientation distribution function of protein molecules and the orientation of the *g*-tensor within the molecular frame. This new approch provides a general tool for correlating spectroscopic data, with a protein or model compounds structure.

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Magnetic Resonance Molecular Imaging, the Challenges

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Introduced in the radiological practice some 20 years ago, MRI has been quickly recognized as one of the most efficient medical diagnostic techniques. Its power is linked to a very high (subillimetric) spatial resolution and a broad range of natural contrast. In spite of it, contrast agents have been developed to increase the efficacy of the method. The mechanisms through which they modify the contrast is based on the magnetic interactions between the water protons and the magnetic reporter which is the active part of the system. This *contrastophore* can be a paramagnetic ion like gadolinium, complexed with an organic ligand, or a superparamagnetic nanocrystal.

While the contrast agents of the first generation were non specific and had to be administered at relatively high doses (ca 100 micromoles per kg of body weight), large efforts are nowadays devoted to produce specific contrast agents which will bring MRI and its high resolution to the field of molecular imaging. This step requires, as in any other molecular imaging method, the search for appropriate vectors but also a tremendous improvement of the efficiency of the magnetic reporters to which they are linked.

Studying and exploiting the properties of mobile proteins in situ

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Despite the abundance of proteins and peptides inside many cell types, these molecules do not provide intense signals in proton magnetic resonance (MR) spectra, and little has been studied about them *in vivo*. In particular, there have been no magnetic resonance imaging (MRI) methods to spatially assess mobile proteins and their properties *in vivo*. Yet, various lesions may induce changes in the amount of such proteins and peptides, and in the exchange properties of their amide protons. One way to study the signals of such proteins and peptides in the brain and in tumor cells is through the use of water exchange spectroscopy (1-3). However, one problem in assessing such compounds is their low concentration, and the use of such spectroscopy is not trivial in the clinical setting. In order to increase detection sensitivity and practicality, it would be useful if these proteins could be detected via the water resonance, especially for imaging purposes. Because there is exchange between amide protons of intracellular mobile proteins and peptides at 8.3ppm and water protons at 4.7ppm, this is possible by using the chemical exchange saturation transfer (CEST) signal enhancement approach of Balaban et al. (4,5). When applied in tissue on amide protons of cellular proteins/peptides, this approach has been called “amide proton transfer” or “APT” imaging (6,7).

We will demonstrate two applications of APT imaging that are promising. In the first, the pH dependence of the exchange rate of the amide protons with tissue water is used to generate a pH image (6). This can be applied to study ischemia and has the potential to differentiate between ischemic penumbra regions that will go on to infarction (i.e. due to impaired oxygen metabolism) from those that may not. Secondly, some tumors have a high protein/peptide content. One example of that is the 9L glioma in rat brain. When comparing APT imaging of such tumors with standard approaches such as T1, T2, and diffusion imaging, it was found that the APT image provided a well-define tumor outline (7).

In this lecture we will outline the principles of this approach and illustrate them with examples.

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Observing cell migration in the brain: in vivo MR imaging

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Recent developments in high-resolution MR imaging as well as specific cell labeling strategies have been reported in experimental neurology (1-3). With these methodological approaches, the non-invasive observation of the migrational activity of stem cells implanted into the rat brain after stroke induction has become possible for the first time (1). Migration across the corpus callosum from the contralateral hemisphere (site of implantation) to the target zone, the ischemic border zone, can be followed. Such migrational dynamics is in principle observable for stem cells in the context of various cererbal diseases, thus leading to new information on the mechanisms of interaction between implanted cells and host tissue, and on the potential of such labeled cells for their regenerative capacity. From the in vivo data, dynamic parameters such as migration speed of the implanted cells may also be estimated. A rather new approach is presently analyzing the possibility to transfer and extend the methodology to a selective labeling of *endogenous* stem cells, at least for those stem cells being generated in the SVZ (4).

Examples applying different labeling strategies and cell types in conjuction with different (patho-)physiological conditions will be discussed. A new strategy combining molecular biological methods and contrast agent chemistry with the molecular MR imaging tool will be presented (4,5). The latest developments of "smart contrast agents" and of "smart cells" approaches will be presented showing the potential of molecular MRI for the future registration not only of the location of labeled cells but also of their functional fate.

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Cellular and molecular magnetic resonance microscopy at 17.6 T

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The availability of high magnetic field strengths combined with strong imaging gradients and dedicated radiofrequency (rf) coils allows for NMR microscopy on a cellular level, ranging from the detection of specifically labeled cells for *in vivo* applications to the study of individual single cells. Here, we present an overview of our latest efforts in this field.

Lately, stem cell transplantation has become a very promising therapeutic approach for neurological diseases. Further knowledge of the quantification and visualization of cell migration paths is necessary to understand the underlying mechanisms. With cell-internalized VSOP (very small superparamagnetic iron oxide particles) as an appropriate contrast agent, stem cells can be tracked *in vivo* in the rat brain at a spatial resolution of 98 µm isotropic, which is well above the cell size. Unambiguous identification by signal change was possible for 100 labeled cells [1].

By reducing the size of the rf coil and thus improving the coil sensitivity, a spatial resolution on the order of 10 µm isotropic can be achieved as well. Although this resolution is sufficient to depict the intracellular morphology of large cells, it is still inferior to classical light microscopy. However, NMR imaging offers a wide range of contrast mechanisms and functional parameters, such as diffusion. Our focus on single cell imaging lies in the quantification of intracellular relaxation times and diffusion and their change upon chemical fixation by formaldehyde. *Xenopus laevis* oocytes are used as a single cell model. Relaxation times upon fixation show a slight reduction in T1 and a strong reduction in T2. A reduction of the apparent diffusion coefficient (ADC) is also observable. Evidence for protein cross-linking due to fixation is stronger in the cytoplasm than in the nucleus.

Furthermore, the small sample volume to usable experimental volume ratio can be optimized by increasing the number of probe coils and samples used, thus increasing the experiment efficiency. Especially on high-field systems with limited access time, a higher sample throughput is desirable. A four-channel probehead consisting of four individual solenoid coils suited for cellular imaging of *Xenopus laevis* oocytes was designed, allowing simultaneous acquisition from four samples. 3D T1-mapping of fixed oocytes was carried out to demonstrate practical use [2].

Lately, we have focused on performing molecular imaging on *Xenopus laevis* embryos. Antibodies coupled to superparamagnetic dextrane-coated particles serve as a specific probe detectable by NMR microscopy. First *in vitro* experiments have shown contrast improvement over non-labeled specimen and specific signal depletion corresponding to results obtained by fluorescence microscopy. Additionally, the above mentioned instrumentation technique allows screening of multiple specimens simultaneously, e.g. different stages and controls, without sacrifice in resolution.

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Application of Residual Dipolar Couplings to the Study of Flexibility in Folded and Unfolded Proteins

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Molecular dynamics, manifest in backbone and side-chain mobilities, play a crucial role in protein stability and function. While local backbone fluctuations on the ps to ns time scale have been the subject of detailed characterization, slower motions, in the sub-micro to second range, remain poorly understood. These time scales are however of particular interest because functionally important biological processes are expected to occur in this time range. Residual dipolar couplings (RDCs) report on averages over longer time-scales (up to hundreds of milliseconds) and therefore encode key information for understanding slower protein motions over a very broad time range. In this study we have assembled an extensive data set, comprising up to 27 dipolar coupling interactions that sample 6 different directions throughout the peptide plane, to uniquely define the nature and amplitude of protein backbone motion in an Immunoglobulin binding domain of streptococcal protein G (henceforth termed protein G). This analysis reveals a heterogeneous distribution of slow dynamics in the nanosecond to second time range. An alternating pattern of dynamics along the peptide sequence is found to form a long-range network across the _eta-strands, reminiscent of a standing wave. Importantly the extracted motional modes significantly improve the prediction of experimentally measured scalar couplings across hydrogen bonds throughout the molecule. This analysis shows the motion to be correlated across, and propagated via inter-strand hydrogen bonds.

Natively unfolded regions are predicted to be present in a significant fraction of proteins encoded in the genome. Although characterisation of the conformational behaviour of unfolded proteins is crucial to understanding their function, classical structural biology is inadapted to these systems due to the inherent conformational heterogeneity of the disordered chain. In the second part of this talk we will present the structure and dynamics of the natively disordered region of protein X, the nucleocapsid-binding domain of Sendai virus phosphoprotein, using RDCs and small-angle scattering. Experimental RDCs are used to test the validity of different models of conformational sampling of the 57 amino acid unfolded domain using an original Monte-Carlo-based sampling technique. We demonstrate that RDCs in both unfolded and folded domains are accurately reproduced using a remarkably simple model for the flexible region, based only on amino-acid-specific backbone dihedral angle propensity and sidechain volume exclusion. Small angle X-ray and neutron scattering data simulated from this ensemble are also in close agreement with experimental results, confirming the average size and shape of the conformational ensemble.

This analysis reveals that the behaviour of this natively unfolded chain is determined essentially by local, amino-acid specific sampling properties and carries significant consequences for our understanding of the unstructured state.

New Aspects of Covariance NMR

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Covariance NMR spectroscopy is demonstrated for 2D NMR data collected using the hypercomplex and TPPI methods. Absorption mode 2D spectra are obtained by application of the square-root operation to the covariance matrix calculated from sets of 1D spectra. The resulting spectra closely resemble the 2D FT spectra, except that they are fully symmetric with the spectral resolution along both dimensions determined by the favorable resolution achievable along the detection dimension. A highly efficient method is presented for the calculation of the square root of the covariance spectrum by applying a singular value decomposition (SVD) directly to the mixed time-frequency domain data matrix. Applications will be shown for 2D NOESY and COSY data sets and computational benchmarks will be provided for data matrix dimensions typically encountered in practice. The SVD implementation makes covariance NMR amenable to routine applications.

Covariance NMR, which was originally designed for homonuclear experiments, can be generalized to heteronuclear spectra. As a result, one can obtain correlations between spins of the same nuclear species without requiring its direct detection. Experimental demonstrations are shown for a ^{13}C-edited TOCSY experiment of the uniformly ^{13}C-labeled decapeptide antamanide. Furthermore, the applicability of covariance NMR to double-quantum experiments is demonstrated

Backbone dynamics from $^{13}C^{\alpha}$ Relaxation in perdeuterated proteins and polypeptide folding observed by RDCs

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We have determined $^{13}C^{\alpha}$ R_1, R_2 and $R_{1\rho}$ relaxation rates in perdeuterated U-^{15}N/15% random-^{13}C-labelled protein G at various temperatures and 600 and 800 MHz proton frequency. Strong differences between $R_{1\rho}$ and R_2 on the order of several Hertz are found throughout the entire backbone. We interpret these differences as a chemical exchange contribution (R_{ex}) to the overall transverse relaxation rate R_2. The exchange contributions increase strongly at higher temperatures, but are clearly detectable more than 30 K below the thermal denaturation temperature of protein G.

As a second topic, we will discuss insights that can be obtained on polypeptide folding from RDCs. We have recently characterized the disintegration of T4 fibritin foldon, a trimeric β-hairpin propeller, into its constituent monomeric parts and followed the thermal unfolding of the β-hairpin monomer by chemical shifts and residual dipolar couplings. Near-turn residues show particular thermal stability in RDC values and hence in local order parameters. At high temperatures, the RDCs converge to non-zero average values consistent with predictions from random chain polymer models. Residue-specific deviations above the unfolding transition reveal the persistence of residual order around prolines, large hydrophobic residues and at the β-turn. We have extended these measurements to other model peptides. The results show that preferences for local backbone conformations can be detected according to the amino acid type.

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Allosterics and Dynamics

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Despite the availability of crystal structures of many different ligand states of Hsp70 chaperone proteins, the allosteric mechanism could not be delineated. Our recent NMR studies on the Hsp70 chaperones reveal that dynamic rearrangements of subdomains in 60 kDa chaperone are most likely the basis of the allosteric mechanism [1,2]. These crucial low-energetic, slow time scale rearrangements were completely quenched in the crystal lattices.

At the pico-second time scale, peptide-backbone motions are more extensive than indicated by the NH-order parameters, and contribute to the entropy of the induced-fit of protein ligand binding as demonstrated by our CO-Ca cross-correlated relaxation studies of calmodulin / peptide binding[3]. We will discuss new approaches to extract more dynamic parameters from our cross-correlated CO relaxation studies.

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Weak alignment offers new opportunities in NMR structure determination

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Biomolecular structure determination by NMR to date has relied almost exclusively on local parameters, such as NOEs and J couplings. In contrast, dipolar couplings measured in weakly aligned macromolecules provide information on the orientation of individual internuclear vectors relative to the molecular alignment tensor. To date, this information has been used primarily for structure refinement as well as structure validation. Alternatively, intrinsically it is possible to build a model that satisfies all dipolar restraints. However, when using only one-bond dipolar couplings this latter approach requires a very complete set of dipolar couplings.

Instead, a more robust hybrid approach will be described that searches the PDB for all fragments compatible with a given set of dipolar couplings, and these fragments (after individual refinement) are assembled into a full structure using regular simulated annealing protocols. The procedure benefits greatly from having a modest number of d_{NN} NOE connectivities, and from the use of a potential of mean force, describing the empirical energy associated with hydrogen bonding, where partners are identified in a fully automated manner. Application to the protein gS crystallin shows remarkably close correspondence to X-ray structures solved previously for other members of the crystallin family, for its two globular domains.

Structure determination of proteins by magic-angle spinning solid-state NMR

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Structure determination of membrane proteins and amyloids by magic-angle spinning solid-state NMR requires a robust concept that still works in cases of high spectral complexity and non-ideal line width. In this context, we have applied different different techniques to prepare membrane protein samples, various labelling strategies and spectroscopical approaches to achieve suitable MAS NMR spectra and to be able to assign them. This is currently applied to the 281-residue membrane protein OmpG, of which we obtained first assignments of individual amino acids.

The dependency of cross peak amplitudes on the length of the mixing period in proton driven spin diffusion spectra will be analyzed. A closer look at the accuracy of these distance measurements by PDSD spectroscopy showed the necessity for using generous bounds for distance restraints. This is taken care of in automated structure calculations by ARIA. The procedure we have chosen takes into account isotope labelling pattern as used in solid-state NMR and was applied to the α-spectrin SH3 domain. Furthermore, a concept for detecting protons and hydrogen bonds in salt bridges will be demonstrated with the α-spectrin SH3 domain as an example.

NMR Spectroscopy and Understanding the Global Systems Biology of Disease Processes.

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Global systems biology concerns the statistical integration of genetic, metabolic and environmental data to describe the effects of disease processes at several levels of biomolecular organization. High frequency NMR is a well-established tool for characterizing and quantifying a wide range of metabolites in biological fluids and tissues and exploring the biochemical consequences of drug-induced toxicity and human disease (1-3). Metabolic profiles and NMR spectra are changed characteristically in different toxicity or disease conditions according to the exact site and mechanism of the lesion. ^{1}H NMR is particularly useful as it allows rapid non-destructive analysis of small quantities of biofluids using robotic flow-injection systems. This can be coupled to cryo-flow probe technology to give excellent sensitivity or the ability to analyse less receptive nuclei without isotopic enrichment. The use of pattern recognition (PR) allows interrogation of MR data and can give direct diagnostic information and aid the detection of novel biomarkers of disease. We have also developed novel statistical correlation methods (STOCSY) that can be used to create pseudo 2 dimensional spectra from 1D data sets obtained from high throughput screening (4). Expert system approaches can also be implemented to give direct diagnostic outputs on toxicity type based on spectroscopic input data. Such diagnostics can be extremely sensitive for the detection of low level damage in a variety of organ systems and is potentially a powerful new adjunct to conventional procedures for toxicity and disease assessment and can help explain environment-gene interactions that give rise to idiosyncratic toxicity of drugs in man. In particular novel methods of metabolic screening using probabilistic modelling approaches (10) have been developed that will enable rapid toxicological and clinical assessments based on MRS of biofluids that are now suitable for wider diagnostic implementation.

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Probing Local Structure and Motion in Disordered Materials: Battery Materials and Ionic Conductors

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Detailed information can be obtained from the NMR spectra of paramagnetic materials, by exploiting the hyperfine interaction, the measure of unpaired spin density transferred from the paramagnets to the nuclei under observation (typically ^{6}Li/^{7}Li). This interaction can be exploited to obtain detailed structural information from electrode materials used in Li-ion batteries. Results from two different systems will be discussed, Li_7MnN_4 and the solid solution xLi_2MnO_3-$yLi(NiMn)_{0.5}O_2$. Li_7MnN_4 adopts an anti-fluorite structure, the Li^+ and Mn^{5+} ordering on the tetrahedral sites, due to the large coulombic repulsions of the Mn^{5+} ions. This ordering creates five different sites for Li^+, which could be individually resolved in the ^{6}Li MAS NMR spectrum of this sample. The Li resonances were assigned to the different crystallographic sites, by considering the multiplicities of the sites and the numbers of Mn5+ ions in the 1st, 2nd and 3rd coordination shells surrounding the Li ions. Mobility between the different sites could be detected by following the changes of the spectrum as a function of temperature, or by performing a two-dimensional magnetization exchange experiment. The differences in mobility were rationalized by considering the jump pathways for Li^+ ions in the solid. On oxidation of these materials, the NMR spectra collapse and a single resonance is observed. This is indicative of rapid motion of the Li+ ions in the lattice. The shift of the resonance indicates that the Li^+ is preferentially removed from the Li1 and Li5 sites in the lattice – sites that contain Mn^{5+} ions in the 1st cation coordination shell.

The second half of this talk will focus on the use of ^{17}O methods to study local structure and motion in the perovskite Sr- and Mg-doped lanthanum gallate and molecular sieves. The results for the perovskites will be compared with mobility in aurivilius-type phases such as Nb^{5+}-doped Bi_2WO_6. Finally, the application of a series of ^{1}H/^{17}O double resonance (CP, TRAPDOR, HETCOR, REDOR) NMR experiments to locate the Bronsted acid site resonance in an acidic zeolite will be described.

STD-NMR to characterize ligands to membrane bound receptors including G-protein coupled receptors (GPCRs)

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The use of STD NMR for assaying the interactions between ligands and cellular transmembrane receptors is described. STD NMR can be used to analyze the binding of small molecules to large membrane integrated receptors in living cells using a newly developed variant of the STD protocol called STDD (Saturation transfer double difference) spectroscopy.

The STDD technique has also been used to analyze the binding of peptides and glycopeptide to the CCR5 chemokine recptor. The receptor was obtained from cells overexpressing the protein, a seven helix trans membrane protein, by collecting membrane fragments of the cells by fractional centrifugation. Subsequent extrusion yields liposomes with the membrane fragments fused without solubilization of the protein. The STDD spectra can be used to assign a binding epitope and to arrive at a binding model for the interaction between the glycopeptide and the 7TM receptor CCR5. This interaction ist he crusial Stepp for the HIV to enter a human macrophage.

TINS: Fragment Based Drug Discovery for (Nearly) All Targets

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Fragment based drug discovery methods are gaining increased use because they generate “lead like” compounds that are highly amenable to modification without violating Lipinski’s “Rules of Five”. Fragment based methods have successfully generated high affinity lead compounds for targets that have been particularly difficult to attack using other approaches, such as protein-DNA complexes. However, until now, fragment based methods were only applicable to proteins that were available in large quantities and that were soluble or could be solubilized.

We have developed a method we call TINS, for target immobilized NMR screening, that is applicable to targets that are not possible to obtain in large quantities and/or are insoluble such as integral membrane proteins. In TINS, the target is immobilized on solid support. The mixture of compounds to be tested for binding (10 at a time so far) is pumped over the support in a stop-flow setup and binding is detected by simple 1D 1H NMR spectroscopy of the ligands. Binding to the immobilized target results in a simple reduction in peak amplitude, which is conveniently detected by comparison with a control sample. The ligand mixture can then be washed away and a new mixture applied. NMR analysis of the ligands alleviates the necessity for isotopically labeled target, of which only 3-4 mg are required for a typically sized molecule (50 kDa). We have demonstrated that a single sample of target can be used to screen a 10,000 compound library, easily detecting binding as weak as 2.5 mM KD. Due to the sensitivity to weak binding TINS holds great promise in bringing fragment based methods to very large targets such as enzymes, nucleic acids and insoluble, hard to produce membrane proteins.

We have developed a dual-chamber, flow-injection probe in collaboration with Bruker, to support a high-throughput TINS screening station. The TINS principle, NMR experiments designed to simultaneously acquire data on both samples and the function of the new probe will all be rerported on.

New Techniques in DOSY

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Information about the sizes of molecules present in a liquid sample can be obtained from self-diffusion coefficients. In diffusion-ordered spectroscopy (DOSY), diffusion coefficients are determined by incrementing the amplitudes of pulse field gradients in, for example, a stimulated echo sequence, and then fitting the resultant decays of signals to the appropriate theoretical expression. The data are typically presented in 2D plot with a spectral dimension and a diffusion dimension; in the latter the lineshape is a Gaussian centred on the apparent diffusion coefficient and with a width determined by the standard error estimated in the fitting process.

A variety of effects conspire to make this less straightforward than it sounds. Where signals overlap it is difficult or impossible to extract reliable diffusion information for individual components; many instrumental effects can complicate the analysis; and in concentrated samples the nuclear magnetization itself perturbs the measurements.

Frequently, overlap is present in the normal spectrum, rendering it impossible to achieve the high resolution in the diffusion dimension that is obtainable where the decay can be fitted to a monoexponential curve. In cases of overlap a natural extension of the basic DOSY experiment is to add diffusion encoding to a 2D experiment, to form a 3D DOSY experiment, where spectral overlap is much less likely. Such pulse sequences have hitherto been created almost exclusively by concatenating a diffusion encoding sequence with an existing 2D experiment. This is often a practical approach, but in some cases the experiment can be greatly improved by incorporating the diffusion encoding internally in the existing 2D experiment, in a type of pulse sequence christened IDOSY, where I stands for internal. This scheme lends itself particularly easily to experiments that are displayed in absolute value mode, such as COSY or 2DJ spectroscopy. The main advantages of IDOSY experiments are that the minimum experimental time is substantially reduced – up to 32-fold compared with previous 3D-DOSY sequences – and that using a spin-echo instead of a stimulated echo gives an approximate 2-fold increase in signal-to-noise ratio.

Convection is highly detrimental to diffusion measurements, and is commonly encountered for samples in low-viscosity solvents (such as chloroform) away from ambient temperature. To achieve convection compensation in a stimulated echo experiment, two stimulated echoes are needed, throwing away 75% of the signal. In IDOSY sequences, convection compensation can be achieved with little or no loss in signal-to-noise ratio.

Improved pulse sequences for diffusion encoding in HMQC, COSY and 2DJ experiments, HMQC-IDOSY (1), 2DJ-IDOSY (2) and COSY-IDOSY (3) will be presented; these are faster and more sensitive than existing methods, and allow easy compensation for convection.

Radiation damping can complicate measurements on concentrated samples very significantly, even where special hardware is used to switch the probe Q (4). Diffusion measurements, however, can be carried out even on pure H_2O samples using a very simple modification of existing techniques to to limit the net magnetization used (5).

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Detection of Plant Metabolon formation in Polyamine Biosynthesis by NMR Relaxation Methods

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Polyamines are considered critical regulators of cell growth, differentiation and cell death [1]. The biosynthesis of the polyamines spermidine and spermine takes place from putrescine by two sequential steps catalyzed by the enzymes Spermidine Synthase (SPDS) and Spermine Synthase (SPMS) respectively [2]. Macromolecular complexes of enzymes that effect consecutive metabolic transformations, known as metabolons, provide an effective channeling mechanism between the active sites of the two enzymes. Recent studies performed by Tiburcio and coworkers using a yeast two-hybrid system [3] suggest the formation of a metabolon involving SPDS and SPMS.

Direct detection of protein-protein interactions by NMR is hindered by the need to use concentrations well above the physiological range. This requirement is even more stringent if one wishes to use relaxation effects which are the most sensitive indicators of macromolecular aggregation [4]. Previous attempts to solve this problem have made use of line width measurements in one dimensional spectra [5] or fusion proteins incorporating a reporter domain that is probed through fast exchange with a small molecule spy (SMS) [6].

In this communication we report our ongoing work on the characterization of in vitro metabolon formation through Saturation Transfer Difference (STD) [7] studies of specific small molecule reversible inhibitors acting as SMS for the different enzymes involved in the complex.

Macromolecular association leads to an increase in the correlation time that is sensed by the transient complex involving specific SMSs. Amplification of the signal takes place by fast exchange between bound and free SMS and accumulation of saturated free SMSs due to their long relaxation times. This allows the use of protein concentrations that approach the physiological ones.

Ultimately, the method aims to study complex interactions in multiprotein systems at physiological concentrations, by defining matching pairs of protein-SMS to identify at low which of the possible complexes are actually formed near physiological concentrations.

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NMR investigation of the vapor contribution to diffusion in porous media partially filled with liquids

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If a macroscopic sample contains a two-phase system formed by a liquid in thermal equilibrium with its vapor, the acquired NMR signals normally are entirely dominated by the magnetization of the liquid phase. The reason is that the density of the vapor phase at room temperature is three orders of magnitude less than that of the same species in the liquid phase. Consequently the contribution to the signal from the vapor phase can be neglected. However, in some cases, diffusion measurements of liquids partially filling porous media indicated an enhanced self-diffusion coefficient relative to the bulk phase [1-4]. The reason for such observations is the molecular exchange process between the two phases: liquid and saturated vapor. Molecular exchange means that the solvent molecules are intermittently subject to diffusion features in either phase. Translational displacements in the vapor phase are much faster than in the liquid phase and contribute to the enhancement of the effective diffusion coefficient.

In our report the contribution of the vapor phase to molecular diffusion in silica glasses with nanometer (Vycor: pore diameter 4 nm) and micrometer (Vitrapor#5: pore diameter 1 µm; Vitrapor#4: pore diameter 10 µm; Vitrapor#C: pore diameter 50 µm) pores partially filled with cyclohexane (non-polar) or water (polar) was investigated with the aid of field-gradient NMR diffusometry. For the same liquid species and for the same diffusion time a transition from "fast" to "slow" exchange was observed by increasing the pore dimension. It can be concluded that the pore size plays a crucial role for the relevance of molecular exchange limits relative to the experimental diffusion/exchange time. A general two-phase exchange model has been developed in order to explain the experimental results [4]. The model is based on a two-region approximation as suggested for gas diffusion in zeolites [5].

The vapor phase effect on diffusion as a function of the filling degree was clearly demonstrated in this way. Due to the molecular exchange, the values of the effective diffusion coefficient in the case of cyclohexane in VitraPor#5 (1 µm pores), exceed the bulk value by a factor of ten for low filling factors. Moreover, for diffusion in the vapor phase, two mechanisms could be distinguished, namely ordinary ("Einstein") and Knudsen diffusion with (possibly different) tortuosity effects for a given pore space structure. Finally, the mean residence time of the solvent molecules in the liquid phase could be evaluated from fits of the theory to the experimental echo attenuation data.

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A 3 Tree MRMicroscope: from porous grains to intact trees

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Magnetic Resonance Imaging (MRI) and Magnetic Resonance Microscopy (MRM) techniques are widely used for a large number of applications. Among them slow transport processes (e.g. in intact plants and trees) and (semi-) solid state imaging at high spatial resolution (e.g. starch in rice kernel and its gelation during cooking or the solid matrix in baked bread and its mobility as a function of moisture content) are not straightforward. These applications strongly depend on the hardware: a combination of rf coil dimension, maximum gradient strength and switching time, dimension of the bore and climate control within the bore of the magnet. We recently have realised a 3 T MRI/MRM system (Bruker and Magnex) that combines hardware to do all these jobs. The 70 cm bore vertical magnet can be used in combination with three different gradient systems: a 50 cm inner diameter gradient system, gradient strength up to 30 mT/m, a 15 cm (10 cm rf coil) gradient system up to 280 mT/m and an openable 7 cm (4 cm rf coil) gradient system up to 1 T/m. The central bore can be connected to a remote climate control unit.

The system has successfully been applied to study phloem and xylem flow in trees (up to 2.5 m length) and plants, to realise SPI semi-solid images (TE from 26 µs and longer), and to study the hydrodynamics in porous media like packed beds and reactors.

Pulsed Field Gradient NMR in Combination with Magic Angle Spinning - New Possibilities for Studying Diffusion and Making NMR Microscopy of Heterogeneous Materials

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The **combination of MAS NMR spectroscopy** with **Pulsed Field Gradient (PFG) NMR** opens novel possibilities for **investigating diffusion process**es and also for **NMR microscopy of heterogeneous, non-liquid samples**. The advantages are based on the effect of line narrowing and concern (i) a prolongation of the intervals during which the magnetic field gradients may be applied and a corresponding enhancement in the sensitivity towards small molecular displacements as well as higher spatial resolution, and (ii) an enhanced resolution in the chemical shift scale. MAS PFG NMR facilitates investigations of materials that were hardly accessible by conventional PFG NMR-based techniques so far. In this contribution, recent progress and achievements in this field is demonstrated using a few examples.

1. A remarkable **increase in gradient strength available** is achieved by using a narrow-bore solid-state MAS probe inserted in a triple-axis micro-imaging device. Strong pulsed field gradients up to **2.6 T/m** along the magic angle can be created with extreme high accuracy and stability. The micro-imaging device does not influence the performance of the probe and is not inducing forces on the sample. It is shown that PFG NMR-experiments using stimulated echo sequences can be performed on this system with great accuracy.
2. MAS PFG NMR is demonstrated to have remarkable advantages in comparison with conventional PFG NMR, if applied to **diffusion measurements in beds of nanoporous particles**, notably zeolites. Those investigations mainly profit from the elimination of the disturbing influence of the inhomogeneity of the sample susceptibility. This is illustrated by measuring the diffusion coefficients of organic molecules adsorbed in silicalite-1. The strong gradients in combination with high spectral resolution allow the investigation of mixtures of molecules containing very fast and very slow diffusing molecules. This is demonstrated measuring the diffusion coefficients of water, benzene, ethene, and ethane simultaneously sorbed in NaX zeolite.
3. The described system can be used to create gradients that are following the sample rotation. Under such conditions, as it was shown earlier, **NMR imaging of rotating sample**s becomes possible. The aforementioned equipment opens novel possibilities with respect to spectral resolution and achievable spatial resolution. The accuracy of the triggered MAS images is proofed by a phantom. Spatially resolved spectroscopy was performed using a semi-solid sample and resulted in a resolution never observed before.

The new possibilities for studying diffusion in membranes, catalysts, drug delivery samples, and spatially resolved NMR of biopsy samples are outlined.

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Velocity autocorrelation function of fluidized granular system measured by the novel spin echo technique

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NMR gradient spin echo is a tool that yields not only macroscopic but also microscopic dynamic variables because the spin echo attenuation is directly related to the displacement or the velocity autocorrelation function of spin bearing particles[1,2], which is the key quantities from which the underlying nature of the dynamical processes can be revealed. However, this spin echo potential has been only partially exploited particularly, when applied to the study of granular flows[3].

The modulated gradient spin echo method involves a repetitive train of radiofrequency pulses with interspersed magnetic field gradient pulses or a gradient waveform that periodically modulate the phase of tracking spins. The resulting spin-echo attenuation is proportional to the value of displacement power spectrum or velocity autocorrelation power spectrum at the modulation frequency, depending on the applied sequence. The frequency range of measurement is determined by the rate of spin phase modulation, which is between a few Hz to about 100 kHz at the present state of art. The method is a low frequency complement to the non-elastic neutron scattering method with range above GHz frequencies.

A variant of the MGSE sequence is the CPMG echo train applied to spins in the constant magnetic field gradient, G. It creates the spectrum of the effective gradient with the dominant sampling peak at the frequency of modulation, ω_m. By changing the modulation frequency, the peak can be adjusted in position in order to samplethe frequency dependence of the displacement autocorrelation power spectrum, $I_z(\omega)$. After N modulation cycles (t=NT), the spin echo attenuation is

$$\beta(t, \omega_m) = 8 \gamma^2 G^2 I_z(\omega_m) t /\pi^2 .$$

Here we report about the first application of this method to obtain the displacement autocorrelation spectrum of air-fluidized granular beads. The granular system consists of pharmaceutical oil-filled, hard plastic spherical beads with the restitution coefficient of 0.85 filled in the cylindrical container. The measurement gives the displacement autocorrelation spectrum, which provides the velocity autocorrelation function of bead motion. An oscillatory decay of function with the long negative tail agrees with the simulation of hard-sphere fluids of Alder and Wainwright[4], who found the exponential decay of velocity autocorrelation function only for very low densities of particles. At higher densities the exponential form breaks down and the velocity autocorrelation exhibits a negative tail. The results of our measurements are similar to the measurement by the positron emission tracking as reported in Ref.[5] where the lobe of spectrum was interpreted as a restriction to bead motion by walls of the experimental cell and not as the result of bead caging within the space of adjacent colliding beads, as follows from the analysis of our measurements.The measurements enable to define the characteristic cage size, the mean collision frequency of beads and the diffusion like constant denoting the beads motion between the cages.

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Data-driven docking for the study of biomolecular complexes.

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With the presently available amount of genetic information, a lot of attention focuses on systems biology and in particular on biomolecular interactions. Considering the huge number of such interactions, and their often weak and transient nature, conventional experimental methods such as X-ray crystallography and NMR spectroscopy will not be sufficient to gain structural insight into those. A wealth of biochemical and/or biophysical data can however easily be obtained for biomolecular complexes. Combining these data with docking, the process of modeling the 3D structure of a complex from its known constituents, should provide valuable structural information and complement the classical structural methods.

We have developed for this purpose a data-driven docking approach called HADDOCK (High Ambiguity Driven protein–protein DOCKing) (http://www.nmr.chem.uu.nl:/haddock). HADDOCK distinguishes itself from ab-initio docking methods in the fact that it encodes information from identified or predicted protein interfaces in ambiguous interaction restraints (AIRs) to drive the docking process. The AIRs are defined as ambiguous distance restraints between any identified residue at the interface of one protein ("active residues") and all identified ("active") plus surface neighbor ("passive") residues on the other protein and vice versa. The AIRs only enforce that the identified, "active" residues make contacts with active or passive residues of the partner protein but do not define the relative orientation of the molecules. Flexibility is accounted for in different ways during the docking which allows to model (small) conformational changes taking place during complex formation.

In my talk I will discuss the various sources of data that can be used to map interactions and illustrate their use in HADDOCK with examples from our laboratory together with results from our participation to the blind docking experiment CAPRI (Critical Assessment of PRedicted Interactions) (http://capri.ebi.ac.uk).

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Structure determination of non-native protein conformations using NMR-derived restraints

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In recent years increasingly detailed information about the structures and dynamics of protein molecules has been obtained by innovative applications of NMR techniques and of theoretical methods, in particular molecular dynamics simulations. I will discuss how such approaches can be combined by incorporating a wide range of different types of experimental data as restraints in computer simulations to provide unprecedented detail about the ensembles of structures that describe proteins in a wide variety of states from the native structure to highly unfolded species. This strategy has provided, in particular, new insights into the mechanism by which proteins are able to fold into their native states, or by which they fail to do so and give rise to harmful aggregates that are associated with a wide range of debilitating human diseases.

Automated NOESY analysis for side-chain resonance assignment and collection of conformational restraints

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The presentation will focus on recent developments in the field of automatic NOESY analysis. In this context, a novel algorithm for automated side-chain resonance assignment as well as an improved algorithm for automated NOESY peak picking and NOE assignment will be introduced.

- Automated side-chain resonance assignment

A novel algorithm for automated resonance assignment that operates directly on the raw NMR data is presented (ASCAN: automated side-chain assignment from NOESY spectra). Minimal input requirements for ASCAN are sequence-specific assignments of at least one proton/heavy atom pair and one additional heavy atom per residue, heteronuclear-resolved [1H,1H]-NOESY and possibly heteronuclear-resolved [1H,1H]-TOCSY spectra. In its standard application, ASCAN starts with the complete backbone sequence-specific assignments (including HN/N/Cα/Cβ) and uses them first to guide the peak picking of NMR spectra and then to exploit the covalent polypeptide structure for identifying new side-chain resonance assignments.

ASCAN was validated with experimental data sets of the three proteins PBPA (1-128), TM1442 and FimD (25-125), which consist of single polypeptide chains with 128, 110 and 100 residues, respectively. Proof of principle for ASCAN was established by comparison of the resulting side-chain resonance assignment with those obtained by interactive procedures. For each of the three proteins, more than 80% of the side chain protons and heavy atoms were assigned by ASCAN with a correctness rate above 90%. To assess the potential of ASCAN in the context of fully automated protein 3D structure determination, two structure calculations were performed for each of the three test proteins with the software ATNOS/CANDID (1, 2) and one of the common structure calculation algorithms using automated and interactively generated side-chain resonance assignment. In all three cases the two structure bundles coincide closely in terms of precision and accuracy, confirming the reliability of the ASCAN procedure.

Overall ASCAN automates a labor intensive step of NMR data analysis requiring a minimal set of NMR spectra as input.

- Automated NOESY peak picking and NOE assignment

An improved implementation of two previously developed algorithms for automated NMR data analysis, ATNOS (1) for identification of NOE signals in 2D and 3D heteronuclear-resolved [1H,1H]-NOESY spectra based on chemical shift lists and the covalent polypeptide structure, and CANDID (2) for automated NOE assignment, is presented. In the new algorithm, a parallelized and fully probabilistic analysis of the NMR spectra will replace the serial use of ATNOS and CANDID, and will thus allow a maximal combined assessment of all properties for a given signal. After establishing an initial, tentative network of mutually supportive NOEs, the quality of all potential signals is re-evaluated using the embedding into this initial NOE network. This iterative procedure of quality assessment based on network-anchoring for both peak picking and NOE assignment allows for robust artefact discrimination, and increases furthermore the number of high-quality signals available in the first cycle of a structure calculation.

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DFT Calculation of CSA Tensors in Model Peptides. Dependence on secondary structure

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Density Functional Theory (DFT) calculations were used to asses the dependence of backbone-atom chemical shielding anisotropy (CSA) tensors on backbone conformation. The molecular models used in the simulation of protein secondary structure elements and CSA calculations were a capped alanyl-alanine di-peptide and a capped Ala_8 octa-peptide.

The *Gaussian98* program package with the DFT (B3LYP) method was used for geometry optimizations and NMR parameter calculations (GIAO) with the 6-311G(d,p) basis set for the former and 6-311++G(3df,3pd) for the latter.

For each backbone atom of the dipeptide the CSA tensor was analyzed by itself and, additionally, in the context of cross-correlated relaxation with selected dipolar interactions. This was to allow comparison to both solid state and liquid state NMR experiments. The octa-peptide model served to account for the hydrogen bonding influences in alpha helices.

The results obtained on backbone ^{1}H, ^{13}C and ^{15}N CSA tensors showing dependence on protein secondary structure are visualized by way of Ramachandran-type diagrams for the dipeptide model. Comparisons of predicted CSAxDD cross-correlation rates using symmetric and fully asymmetric CSA tensors are shown. The relative influences of hydrogen bonding and conformation on CSA tensors in an α-helical secondary structure are compared.

Acknowledgement

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NMR Studies of partially unfolded proteins

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The knowledge of the genome of a continuously increasing number of organisms has open the challenge of characterizing their products. Structural properties are one of the key features of proteins needed to define and describe their function in the physiological process. During the post-genomic era, the perspective and approach to structural characterization has drastically changed; characterization of a large number of proteins is needed to have a wide coverage of the genome products.

With more and more proteins being produced and characterized, it starts to become evident that a relatively large number of proteins exist in vivo in a partially unfolded or molten globule state. This property allows for a facile interaction with their partners, but also makes them more prone to protein aggregation. This state can be therefore particularly toxic as it is found that several pathologies are related to the formation of protein aggregates.

In the talk I will present some examples of proteins characterized by local unstructured segments and by local enhanced conformational disorder, which can contribute to protein aggregation. I will show how NMR can provide exclusive information on their properties. The results will be mainly focused on FALS-related superoxide dismutase mutants, as well as on a protein with an unknown function which may be evolutionary related to superoxide dismutase.

The role of internal motions in protein function: a backbone and side-chain multiple-field NMR relaxation studies of two globular proteins azurin and barnase

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The NMR relaxation allows studying local protein dynamics on the different time-scales, subnanosecond-nanosecond and microsecond-millisecond. Application of the NMR methods for real protein systems has provided the compelling correlation between local motions and protein function. Dynamic properties of two globular proteins, Pseudomonas aeruginosa azurin and Bacillus amyloliquefaciens barnase, were explored using extensive NMR relaxation measurements at different magnetic fields (500-900 MHz). Following a careful model-free analysis of 15N relaxation measurements at two temperatures and four magnetic fields, several regions with different dynamic regimes are found for small cupredoxin azurin. Detection of increased mobility in the nanosecond range for residues from hydrophobic surface patch, including surface-exposed copper-ligand His117, are consistent with a gated electron transfer process according to the “dynamic docking” model. The residues along intramolecular electron transfer pathway show high rigidity that is remarkably conserved when increasing temperature. Additionally three different conformational exchange processes were observed in the millisecond time-scale. The two of them found near the Cu-site may indicate an additional gated electron transfer mechanism at slower time-scale.

We used H2 relaxation measurements at three magnetic fields for characterization of side-chain methyl dynamics in guanyl-specific ribonuclease barnase and its complex with natural intracellular inhibitor, barstar. Effect on methyl mobility as well as on chemical shifts upon binding was found mainly on the interdomain surfaces. It was suggested that binding with barstar shifts the conformation equilibrium between several co-existed states with slightly different domain orientation. Those results provide new insight in mechanisms that propagate signal across entire protein

Measurement of intra-residue dipolar cross-relaxation rates between Cα and CO nuclei in proteins

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Nitrogen-15 relaxation has been successfully used as the main NMR probe of protein backbone dynamics over the past fifteen years[1]. Several α ^{13}C and carbonyl ^{13}C relaxation experiments have been developed over the same period and some recent studies[2] suggest that the dynamics of the ^{13}Cα-^{13}CO vector reveal motions not sampled by the amide ^{15}N-^{1}H vector.

One of the methods to study the dynamics of the ^{13}Cα-^{13}CO vector is the measurement of the dipolar cross-relaxation rate between the α ^{13}C and the carbonyl ^{13}C nuclei. Two different methods have been suggested to characterize this rate. In one method, the measurement of a steady-state nuclear Overhauser effect[3] (nOe) suffers from systematic errors from the selective saturation profile and inter-residue nOe's. In the alternate scheme developed to measure transient effects[4] this latter problem is effect, as well as the problem of normalizing the transferred polarization.

We present a new experiment based on an HN(CO)CA scheme using symmetrical reconversion[5] to normalize the polarization transferred. The observation of the two-spin orders involving the amide ^{15}N of the next residue isolates the intra-residue cross-relaxation pathway. Application and analysis with comparison to ^{15}N dynamics data using [U-^{13}C,^{15}N,^{2}H] human ubiquitin are presented.

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CEESY: a new method to measure Chemical Exchange to Excited States by NMR spectroscopY

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Abstract We present a new method to detect 'invisible' excited protein states that exchange with an observable ground state. Our CEESY (Chemical Exchange to Excited States) experiment provides an immediate identification of exchanging residues by exploiting any nucleus as a probe for chemical exchange.

Background. Biomolecules often exist in a ground state that is interconverting with an excited state. This excited state can be of functional significance, but is difficult to study since it is only marginally populated. However, the presence of this 'invisible' excited state can be detected indirectly by NMR since both relaxation rates and peak positions are affected by chemical exchange. The magnitude of this effect depends on both kinetic (exchange rates), thermodynamic (relative populations) and structural parameters (size and sign of chemical shift difference between the two states.

Method. The CEESY method relies on the differential effect of exchange on the Larmor-frequencies of SQ and MQ coherences. Kay and coworkers have exploited this effect in their H(S/M)QC-method in which the sign of the chemical shift difference between ground and excited state is determined from the small difference in peak position between a HSQC and HMQC spectrum[1].

The approach taken in our method is to translate this Larmor-frequency difference directly into a peak intensity, allowing an easy qualification of exchanging residues and determination of the sign of the chemical shift difference. It offers the unique possibility to use any nucleus as a probe for chemical exchange to excited states and provides reliable results even in crowded regions of the spectrum.

Results. The method is applied to the ^{15}N backbone nuclei of the 105-residue PAH2 domain. Out of 72 non-overlapping residues, 33 residues give a distinct negative peak, indicating a smaller 15N Larmor frequency for the excited state (i.e. a negative chemical shift difference). Conversely, positive peaks were found for 10 residues.

The sign of the chemical shift difference was confirmed using the H(S/M)QC method. Previously acquired ^{15}N SQ relaxation dispersion measurements could reveal only 26 out of the 43 exchanging, illustrating the high sensitivity of CEESY to exchange.

Conclusion & outlook. The CEESY method outlined here offers the opportunity to recognize exchanging residues at first glance to determine the sign of their chemical shift difference. The results found with our method match those of the H(S/M)QC approach. Application of CEESY to other backbone nuclei is currently in progress. A full analysis of the dispersion data combined with the sign information should result in detailed information about the nature of the excited state and the exchange process.

Note. CEESY should be pronounced as "Kay-sy" and "Cees-sy"

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NMR Structural Studies of the Interferon α Signaling Complex

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Type I interferons (IFNs) are a family of homologous helical cytokines which exhibit pleiotropic effects on a wide variety of cell types including antiviral activity, antibacterial, antiprozoal, immunomodulatory and cell growth regulatory functions.

Consequently, IFNs are the human proteins most widely used in the treatment of several diseases (e.g. several kinds of cancer, hepatitis C, MS), however severe side effects hamper the treatment. All type I IFNs bind to a cell surface receptor consisting of two subunits, IFNAR1 and IFNAR2, associating upon binding of interferon.

Here we describe our ongoing studies on the structure and dynamics of the 44 kDa complex of IFNα2 with the extracellular domain of IFNAR2 (R2-EC) and of the ternary 95 kDa complex between IFNα2, R2-EC and IFNAR1 (R1-EC) by multidimensional NMR techniques. These two complexes are challenging projects for NMR studies and require the use of all available state of the art techniques.

The structure of free R2-EC was previously solved in our lab. Backbone assignment of IFNα2 in the IFNα2/R2-EC complex was achieved using a set of TROSY experiments including HSQC, HNCO, HNCA, HNCOCAB, HNCB, HNCACO and ^{15}N NOESY. The secondary structure was predicted using the programs PsiCSI and TALOS. The binding sites for R2-EC and for R1-EC on IFNα2 were studied using TROSY HSQC, intermolecular NOEs and saturation transfer experiments. The results of these experiments suggest conformational changes in the IFNα2/R2-EC binding side upon formation of the ternary complex.

This structural information provides important insight into interferon signaling processes and may allow improvement in the development of therapeutically used IFNs and IFN- like molecules

NMR of Protein Folding and Aggregation

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The detection of an increasing number of protein misfolding diseases with polypeptide chains forming fibrils under specific conditions, and the observation that some of these proteins lack a persistent globular fold in their monomeric form, has renewed the interest in the investigation of non-native, often unfolded states of proteins. NMR has been vital for the characterization of conformation and dynamics in these states.At Euromar, we will present the following new experiments in this context:

1. Photo-CIDNP experiments to probe accessibility of aromatic clusters to dyes. With new experiments, all tryptophan indole side NH can be assigned in non-native states of proteins. Mutations known to disrupt hydrophobic clusters modulate the Photo-CIDNP intensities of these side chains (Schloerb et al., submitted).

2. Heteronuclear relaxation data are sensitive parameters for the detection of dynamic restrictions of polypeptide chains. We will present data for various non-native states proteins and discuss possible models for their interpretation (Collins et al., Wirmer et al.).

3. We have applied the above experiments to the investigation of the human prion proteins. Interesting deviations from random-coil behavior can be identified along the polypeptide chain. We have identified conditions under which those states form fibers very rapidly.

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^{19}F CIDNP of peptides and proteins

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The NMR spectra of samples in which free radical reactions are proceeding often display strongly perturbed intensities for the reaction products. This phenomenon, first observed in 1967, was initially and erroneously attributed to a dynamic nuclear polarization effect and named CIDNP (Chemically Induced Dynamic Nuclear Polarization). Two years later Closs and Kaptein independently arrived at the correct explanation, the Radical Pair Mechanism, in which nuclear polarization arises from coherent interconversion of electronic singlet and triplet states of pairs of spin-correlated radicals. Driven principally by nuclear hyperfine interactions, this process causes the probability of radical recombination to depend on the nuclear spin configuration of the radicals; hence the polarization.

For 30 years CIDNP has been used to investigate free radical reaction mechanisms, and to identify and characterise radicals too short-lived to be detected directly by NMR and, in some cases, by EPR. In 1978 Kaptein showed that CIDNP could also be used as a surface probe for bio-macromolecules, in particular proteins. By adding to the protein solution a small quantity of a photosensitiser, usually a flavin, and irradiating the sample inside the NMR probe with a laser, one can generate nuclear polarization in the side-chains of histidine, tryptophan and tyrosine residues provided they are physically accessible to the photosensitiser. The technique has been used to study the changes in side-chain exposure brought about by protein interactions with ligands, nucleic acids, lipids, and other proteins, and to compare related protein structures.

We report recent work on photo-CIDNP of ^{19}F-substituted tyrosine residues in peptides and proteins which exploit the large polarization and chemical shift dispersion of the fluorine nuclei. Exploratory experiments on 3-fluorotyrosine show CIDNP buildup rates, defined as the initial rate of growth of z-magnetization relative to the magnetization at equilibrium, of 34 s^{-1} for the ^{19}F, compared to just 5 s^{-1} for the ^{1}H at position 3 in tyrosine itself (*conditions*: 4 mM 3-F-Tyr, 0.2 mM flavin mononucleotide photosensitiser, 5 W argon ion laser power, 600 MHz ^{1}H frequency).

The mechanism of ^{19}F magnetization pumping in fluorinated amino acids and model compounds has been studied using a microsecond time-resolved photo-CIDNP technique aided by *ab initio* calculation of hyperfine and g-tensors in the intermediate radicals. The distinctive feature of the ^{19}F nucleus in these radicals is a large (up to 20 mT) hyperfine coupling anisotropy, which causes the ^{19}F relaxation in the radicals to occur on the same timescale as certain stages of the radical pair mechanism, resulting in a dependence of the sign of the CIDNP enhancement on the rotational correlation time of the radical.

Applications to ^{19}F-labelled green fluorescent protein and the Trp-cage protein will be discussed.

NMR multidomain proteins stability and folding analysis: conformational exchange, temperature and pH stability variation in human annexin 1 first domain.

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Annexins constitute a family of cytosolic, water soluble proteins, which bind negatively charged phospholipids in a calcium-dependent manner. They share a commune quaternary structure, composed of a planar cyclic arrangement of four domains (Huber et al., 1992; Concha et al., 1993). These four domains have very similar 3D structures, which consist of five alpha-helical segments wound into a right-handed compact superhelix, but show a low identity in primary sequence. Annexins are thus excellent models for studying the folding mechanisms of multidomain proteins, and also for the analysis of the relationship between sequence, structure and stability of domains.

When produced and purified isolated, human annexin 1 four domains don't behave the same way: domain 1 has been proven to be an autonomous folding unit (Gao et al., 1999), folded in a highly similar way to that when together with the other three domains. Domain 2 is largely unstructured (Ochsenbein et al., 2001), and the other two domains can't be isolated in water solution.

Previous relaxation studies in our laboratory had revealed presence of conformational exchange in the first domain, but didn't allow the determination of physical exchange parameters. The study we present here is based on a CPMG analysis of conformational exchange. We have realized experiments at 500Mhz, 600Mhz and 800Mhz, and at multiple temperatures, to study the exchange variations with thermal energy, thereby allowing the calculation of activation energies associated with movements. We have also sampled different pH conditions, combined with circular dichroïsm experiments, to study domain 1 stability with pH variations.

Our results show that conformational exchange in human annexin 1 first domain is localized, both in secondary and tertiary structure. Exchange constants calculated at different field values show that results are meaningful, and exchange variations with temperature allowed us to determine a global activation energy. Finally, exchange variations with pH show that exchange is most effective, relaxation-wise, for pH 4-4.5. The results will be discussed in the context of the isolated domain 1 and of the entire annexin.

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NMR real time studies of RNA folding

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The catalytic and regulatory functions of RNAs, such as ribozymes, riboswitches and the spliceosome, depend strongly on tertiary structure reorganisation and secondary structure refolding.

New techniques have been developed in our group that allow investigation of RNA folding processes by NMR in real time. Equilibrium as well as non equilibrium processes can be monitored. Application of this technique to several RNA systems (such as bistable RNA sequences and riboswitches) will be shown characterizing secondary structure refolding as well as of tertiary conformational switching events for the first time.

Characterisation of Spatially Heterogeneous Soft Solids by MRI

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Many important materials in everyday life can be defined as being "soft-solid" ….. for example, most living tissues. The fact that many of those are water-rich makes them ideal systems for Magnetic Resonance Imaging (MRI), and the ever increasingly important roles for MRI in diagnostic radiology is certainly the most spectacular example of the power of MRI to distinguish the different components in spatially heterogeneous systems. Although at present relatively little use is made of MRI to characterise their internal structural features this presentation will use examples from two classes of soft solids to illustrate the current status and some future potential of this area.

First, attention will be drawn to the substantial progress which has already been made to use MRI to quantitate the composition of human articular cartilage, and thereby to characterise the at present unknown aetiology of osteoarthritis. Specifically, this has involved the development of MRI scan protocols which are quantitative for the relaxation parameters (T_1, T_2, M_{sat}/Mo) of water, and sufficiently rapid that they can be used to study clinical patients. Work in Cambridge has concentrated on finger and knee joints, and both will be discussed. Those studies have resulted in the NIH establishing the $US 40 million "Osteoarthritis Initiative" to study damaged knees.

Second, mention will be made of equivalent measurements to characterise a wide range of foodstuffs. Industrially important applications not only include measurements of natural ripening but modern methods of food preparation. The latter include on-line measurements of the novel cooking régimes now associated with "molecular-gastronomy". (N.B. i, A typical meal in a molecular gastronomy restaurant will cost £140 per person and will comprise 15 courses, each bite-sized; ii, the use of MRI to measure 140,000 temperatures in 52 seconds constitutes the world's most powerful (and expensive) thermometer.)

Chain Ordering, Slow Motions, and Heterogeneities in Elastomers: New Insights by Low-Field NMR

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We have recently developed a new approach to ^{1}H multiple-quantum solid-state NMR, which yields qualitatively new information on chain ordering, dynamics, and heterogeneities in polymer networks. The technique can be implemented on simple low-field NMR spectrometers without compromise in data quality [1], and has been applied in investigations of filler effects in elastomers and the swelling process of model networks. In the latter case, substantial chain-order heterogeneities were found to develop on swelling, thus challenging established models [2]. These findings are in agreement with computer simulations of the NMR response of simple network models, thus proving the generality of the phenomenon.

We further report our recent results on details of the chain dynamics in diene rubbers, where our findings are in substantial contrast to accepted model predictions. It is shown that the commonly used Andersen-Weiss approach in conjunction with an exponential decay of inter-cross-link orientation correlations is not applicable. Traditional Hahn echo relaxometry was found to be subject to potentially serious artifacts, in particular when applied to filled elastomers. Finally, we elaborate on new insights into the effect of clay nanofillers on natural rubber matrices and the correlation of NMR results with the macroscopic mechanical and swelling behavior.

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Characterisation of a devitrified glass through homo- and heteronuclear correlation experiments

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Phosphate based glasses have several applications. They can be used as sealing glasses for electronical applications (vanadophosphate), as biomaterials (calcium phosphate) or as encapsulating phase for radioactive waste (iron or aluminium phosphate). In all these applications, devitrification plays an important role and a good understanding of the crystallisation behaviour is therefore necessary. In such a case, both thermal range in which crystallisation occurs and the nature of the formed compounds must be determined. Devitrification often leads to the formation of a large number of compounds with a low cristallinity. Thus, NMR spectroscopy reveals to be a very useful technique, complementary to XRD, especially in the case of remaining amorphous parts which reduce the efficiency of X-ray diffraction techniques.

We have studied a sodium aluminophosphate glass which has been proposed as a host matrix for treated radioactive waste. Devitrification occurs at 612°C and leads to the formation a several compounds.

The local environment of this devitrified glass has been studied by multinuclear NMR spectroscopy. ^{31}P network connectivity has been investigated "through space" and "through bond" using the following pulse sequences: RFDR (determination of the number of phases), INADEQUATE and double quanta (BABA) techniques (connectivities). High-resolution spectra of quadrupolar nuclei (^{27}Al and ^{23}Na) have been recorded using MQ-MAS and ST-MAS techniques. Finally, the Dipolar and J coupling MQ-HETCOR sequences have been applied to determine ^{31}P/^{27}Al and ^{31}P / ^{23}Na connectivities. The efficiency of the latter experiments has been improved by using the recent development of SPAM techniques. We will present NMR data obtained at different magnetic fields, including triple resonance spectra acquired at very high field (18.8T).

This work confirms the presence of several phosphate compounds and brings new information about the nature of the network (phosphate / aluminophosphate). Moreover, the NMR caracterisation of new phases gives the possibility to expand the chemical shift database of sodium aluminophosphates.

Hydrogen in Ti_3Al intermetallic compound: a proton NMR study

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The Ti_3Al–H system has attracted recent attention because of the wide possibilities of its practical applications, particularly as a hydrogen storage material. This compound can absorb large amounts of hydrogen (up to a content H/M ~ 1.45), and owing to its light molecular mass it corresponds to one of the highest hydrogen weight concentration (~ 3.3 wt. %) for all the studied intermetallic–hydrogen systems. With increasing H content, the lattice structure undergo several transitions and change from the hexagonal structure $D0_{19}$–Ni_3Sn (at H/M ~ 0) through the tetragonal $L6_0$–$CuTi_3$ (at H/M ~ 0.5) to the face centered cubic structure $L1_2$–Cu_3Au (H/M ~ 1) [1]. This suggests a study of the effects of the lattice structure changes on the behavior of H-atoms. Our interest to the Ti_3Al–H system is based also on the fact that the structure $D0_{19}$ is related to the hcp structure of Sc, Y and Lu where the jump motion of dissolved hydrogen is characterized by two frequency scales: the slower jump process being responsible for the long-range diffusion and the very fast localized H motion (see, for example, [2]). The coexistence of two types of H motion has been attributed to the structure of the hydrogen sublattice which consists of pairs of tetrahedral interstitial sites separated by about 1 □. This suggests the possibility of the fast localized H jumps within such pairs. Hence, the existence of similar features in the case of Ti_3AlH_x system (at least, at low H concentrations) could be expected.

To obtain information on the hydrogen diffusion parameters, we have performed the measurements of the proton spin-lattice relaxation rates, R_1, for the samples $Ti_3AlH_{0.31}$ and $Ti_3AlH_{0.51}$ (with the Ni_3Sn-type structure), $Ti_3AlH_{1.0}$ and $Ti_3AlH_{2.0}$ (with the $CuTi_3$-type dominant phase), and $Ti_3AlH_{4.32}$ (with the Cu_3Au-type dominant phase). The measurements were performed on a modernized Bruker SXP pulse NMR spectrometer at the resonance frequencies $\omega_0/2\pi$= 90, 23.8 and 14.6 MHz using the saturation-recovery method.

In the range $80 < T < 430$ K the temperature dependences of R_1 for $Ti_3AlH_{0.31}$ and $Ti_3AlH_{0.51}$ are characterized by a monotonous increase of R_1 values with no signs of frequency dependence. The behavior of R_1 shows significant deviations from the linear law which is related to the electronic (Korringa) term, $R_{1e} = C_eT$ (where C_e is believed to be proportional to the square of the density of electron states at the Fermi level, $N^2(E_F)$), and which usually dominates in M–H systems with slow hydrogen diffusion. These deviations can be attributed to the temperature dependence of C_e which is expected for compounds with the Fermi level lying close to a peak in the density of electron states [3]. The shape of the $R_1(T)$ dependence for $Ti_3AlH_{1.0}$ is qualitatively similar to those for Ti_3AlH_x with $x = 0.31$ and 0.51; however the deviations from the linear behavior for $Ti_3AlH_{1.0}$ are stronger.

For samples $Ti_3AlH_{2.0}$ and $Ti_3AlH_{4.32}$ a distinct frequency dependence of the proton relaxation rate appears at $T >$ 320 K (the values of R_1 increase with decreasing ω_0). Such a dependence is typical for the low-temperature slope of the relaxation rate peak which is related to the modulation of dipole-dipole interaction due to hydrogen motion and which is often observed for M–H systems with fast H diffusion. The motional contribution to the relaxation rate, R_{1d}, reaches its maximum value at a temperature at which the characteristic frequency of H jumps, τ_d^{-1}, is approximately equal to the resonance frequency, ω_0. The maximum of R_{1d} has not been found up to 550 K. Thus, our results indicate that the hydrogen mobility in Ti_3AlH_x increases with increasing H content but remains to be rather slow.

The most interesting results have been obtained at low temperatures ($T < 80$ K). For all the samples the proton spin-lattice relaxation rates exhibit distinct frequency-dependent peaks (near 15 K at 90 MHz). The nature of these low-temperature R_1 peaks is not clear at present. The appearance of such peaks may be caused by H motion, so that our results may indicate the coexistence of several H jump processes with strongly differing characteristic frequencies. In this case the motion responsible for the appearance of these peaks must be very fast (the rough estimate yields $\tau_d^{-1} \sim 10^7$ s^{-1} at 10 K, and this is one of the highest values for all known M–H systems). The other mechanism which can lead to similar behavior of R_1 is the relaxation due to paramagnetic impurities (R_{1p}). In particular, qualitatively similar $R_1(T)$ dependences were observed for hydrides of some rare earth metals (for example, for LaH_x [4]) and were attributed to the paramagnetic impurity ions of Gd^{3+} and Ce^{3+}. The position of $(R_{1p})^{max}$ is determined by the condition $\omega_0\tau_i = 1$, where τ_i is the spin-lattice relaxation time of a paramagnetic impurity ion. It should be noted, however, that the low content of Gd and Ce in La and other rare earth elements is a typical situation even for nominally "pure" metals, but this is unlikely for Ti and Al. In order to clarify the situation, additional investigations (including the magnetic susceptibility and ^{27}Al NMR measurements) are required.

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Biomolecular Evidence for Calcium-Dependent Interactions between Lewis^X Oligosaccharides by NMR in Weakly Oriented Media

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Weak carbohydrate-carbohydrate interactions are believed to be responsible for the first step of cell adhesion. One of the structures involved in this mechanism is the Lewis^X (Le^X) trisaccharide. Two Le^X moieties can interact to form a homotypic complex in the presence of divalent cations such as calcium. We designed a protocol to characterize these low affinity saccharide-saccharide interactions in weakly aligned dilute liquid crystalline solutions, using a mixture of pentaethylene glycol monododecyl ether with *n*-hexanol and bicelles.

Residual ^{13}C-^{1}H dipolar couplings in Le^X oligosaccharides in natural ^{13}C isotopic abundance indicate partial alignment of the saccharides. The degree of alignment, and hence the residual dipolar couplings, are affected by the presence of calcium ions, thus indicating the existence of a Le^X-Ca^{2+}-Le^X complex. It is an intriguing aspect of our observations that unambiguous evidence about the formation of this complex can be obtained *without knowledge of its structure*.

Structure determination of Helix 7 from the Vph1 membrane domain of V-ATPase by high-field NMR

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This work is part of a major project concerning the characterization and protein-drug interactions of the transmembrane protein complex V-ATPase (Vacuolar ATPase).

For the structural analysis of the protein complex 2D high-field NMR spectroscopy is applied. The protein complex was subdivided into a range of peptides for simplicity of analysis. This study presents the results obtained for the 4.2 kDa (37 residues) Helix 7 peptide present in the proton translocation channel of the Vph1 membrane domain of yeast V-ATPase. The chemically synthesized peptide was dissolved in d_6-DMSO and ^{13}C-^{1}H-HSQC, ^{13}C-^{1}H-HMBC, ^{1}H-^{1}H-TOCSY, and ^{1}H-^{1}H-NOESY spectra were collected. The assignment was accomplished by using the HMBC, HSQC, and TOCSY spectra. The NOE contacts were used to determine distance constrains for the structural calculations. For the unassigned amino acid, molecular dynamics calculations were performed and the best fit was used for structure prediction.

Signal Enhancement in Saturation Transfer Experiments: Binding Epitopes of Myelin-Associated-Glycoprotein Inhibitors

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Numerous biomolecular ligand-receptor interactions have been investigated by the saturation transfer difference (STD) experiment (1). Information provided from these studies has permitted the high-throughput acquisition of structure activity relationships and greatly enhanced role of NMR in drug design. Herein, methods are described allowing a considerable gain in sensitivity with respect numerous published schemes reported by various research groups and pharmaceutical companies.

The second part of this contribution will describe the interactions responsible for ligands binding to the myelin-associated-glycoprotein (MAG). Following injury to the adult CNS, regeneration is inhibited by a signal transduction pathway initiated by three proteins, Nogo-A, Omgp and MAG, interacting with the Nogo receptor. It has recently been shown that blocking the binding site of MAG diminishes the inhibition of regeneration (2). Herein we provide an interpretation of the binding of related ligands through the synergistic use of STD NMR, surface plasmon resonance experiments and molecular modeling.

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Cytochrome c6 binds to both Photosystem I and Cytochrome f using the same interaction area, as determined by NMR

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In many photosynthetic organisms, the transfer of electrons between the membrane complexes Cytochrome b_6f and Photosystem I (PSI) is mediated by soluble Cytochrome c_6 (Cc_6). This process constitutes an excellent case study of transient interactions in electron transport chains. Here, we studied the physiological interactions between Cc_6 and PSI - at its both monomeric (340 kDa) and trimeric (1064kDa) states - by following the specific line-width broadening effects on Cc_6 TROSY spectra upon titration of Cc6 with β-dodecyl-maltoside-solubilised PSI particles. In this case, the binding equilibrium corresponds to an intermediate-slow exchange regime. On the other hand, we have analysed the physiological interaction between Cc6 and the soluble domain of Cytochrome f by Chemical Shift Perturbation Mapping and HADDOCK approach. Despite the differences in their binding dynamics, both complexes involve the same interaction area in Cc_6, which consists of a hydrophobic site and an adjacent electrostatic patch surrounding the heme cleft. Binding of Cc_6 to PSI also affects the backbone of some internal residues, a fact that is attributed to small binding-induced conformational changes inside the heme protein.

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Mapping the interaction surface of a membrane protein: Unveiling the conformational switch of phospholamban in calcium pump regulation

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We have used magnetic resonance to map the interaction surface of an integral membrane protein for its regulatory target, an integral membrane enzyme. Phospholamban (PLN) regulates cardiac contractility via its modulation of sarco(endo)plasmic reticulum calcium ATPase (SERCA) activity. Impairment of this regulatory process causes heart failure. To map the molecular details of the PLN/SERCA interaction, we have functionally reconstituted SERCA with labeled PLN in dodecylphosphocholine micelles for high-resolution NMR spectroscopy and in both micelles and lipid bilayers for EPR spectroscopy. Differential perturbations in NMR linewidths and chemical shifts, measured as a function of position in the PLN sequence, provide a vivid picture of extensive SERCA contacts in both cytoplasmic and transmembrane domains of PLN and provide structural insight into previously reported functional mutagenesis data. NMR and EPR data show clear and complementary evidence for a dynamic (μs-to-ms) equilibrium between two conformational states in the cytoplasmic domain of PLN. These results support the hypothesis that SERCA attracts the cytoplasmic domain of PLN away from the lipid surface, shifting the preexisting equilibrium of PLN conformers toward a structure that is poised to interact with the regulatory target. EPR shows that this conformational switch behaves similarly in micelles and lipid membranes. Based on structural and dynamics data, we propose a model in which PLN undergoes allosteric activation upon encountering SERCA.

The high-resolution structure of the complex between SPC-SH3 and the proline-rich peptide P41: understanding binding affinity by comparison with ABL-SH3

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SH3 domains are small protein modules of 60-85 amino acids that bind to short *proline-rich* sequences with moderate-to-low affinity and specificity. Interactions with SH3 domains play a crucial role in regulation of many cellular processes (some are related to cancer and AIDS) and have thus been interesting targets in drug design.

The decapeptide APSYSPPPPP (p41) binds with high affinity to Abl-SH3, while it has low affinity for Spc-SH3. The binding affinity, however, is increased 3-fold upon mutation of the arginine at position 21 to a alanine residue (R21A Spc-SH3).

Here we present the high-resolution structure of the complex between R21A Spc-SH3 and p41 derived from NMR data using a combination of CANDID, ARIA and HADDOCK. The structural differences between the protein in its free and bound form are very small and limited to the binding interface, which consists of a conserved hydrophobic surface flanked by two non-conserved loops. The structure of the R21A Spc-SH3:p41 complex resembles that of the Abl-SH3:p41 complex that was solved previously by X-ray crystallography. The difference in affinity between Spc-SH3 and Abl-SH3 can be rationalized by the different interactions that are involved in the binding to p41. In addition, the 3-fold lower binding affinity of the wild-type (WT) Spc-SH3 domain compared to the R21A mutant can be understood in terms of steric hindrance caused by the longer side chain of the arginine residue in WT Spc-SH3. The mutation has no other major consequence, as the overall structures of the WT and R21A mutant are almost identical.

Chemically pumped ^{19}F NMR studies of fluorine-labelled Green Fluorescent Protein and Trp-cage protein.

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^{19}F labeled Green Fluorescent Protein and Trp-cage protein have been prepared and studied with ^{19}F NMR and chemically induced dynamic nuclear polarization (CIDNP) techniques. The ^{19}F nucleus was introduced in the form of 3-fluorotyrosine, using either biosynthetic preparation (GFP) or solid state synthesis (Trp-cage). For the latter procedure a chemical synthesis of the (N)-FMOC-(O)-tBu-3-fluoro-L-tyrosine precursor has been developed.

^{19}F NMR signals of all ten fluorotyrosines in GFP have been assigned using a combination of site-directed mutagenesis, NMR and photo-CIDNP techniques. An interesting feature of 3-fluorotyrosinated proteins is the existence of fluorotyrosine ring-flip isomers. Introduction of fluorine into the phenyl moiety breaks its symmetry and in general two ^{19}F signals per fluorotyrosine residue are expected, because the chemical environment around the two possible fluorine positions will in general be different. The ring-flip isomers provide a probe for the protein dynamics and side chain packing density. Coalescence of the two signals during unfolding means that the amino acid packing around the phenyl ring has become loose enough to allow ring flips, and the relative intensity of the two signals gives a probe of the energy difference between the two rotamers.

Depending on the rotational correlation time of the tyrosine residue in question, the amplitude of the photo-CIDNP magnetization for solvent-exposed residues varies from -20 to +40 times the Boltzmann equilibrium value. The sign and amplitude of the ^{19}F CIDNP enhancement in 3-fluorotyrosine is determined by nuclear paramagnetic relaxation rates in the intermediate radicals. It provides a sensitive real-time probe of the local motional correlation time, allowing extraction of this parameter even from heavily broadened spectra of partially denatured states.

The mechanism of ^{19}F magnetization pumping in fluorinated amino acids and model compounds has been studied using microsecond time-resolved photo-CIDNP technique aided by *ab initio* calculation of hyperfine and g tensors in the intermediate radicals. The distinctive feature of the ^{19}F nucleus in these radicals a is large (up to 20 mT) hyperfine anisotropy, which makes ^{19}F paramagnetic relaxation extremely fast and short-circuits certain stages of the radical pair mechanism, resulting in the observed correlation time dependence of the sign of the CIDNP enhancement.

Circumventing NMR probe modification in time-resolved photo-CIDNP hardware.

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Chemically induced dynamic nuclear polarization (CIDNP) is a non-Boltzmann nuclear spin level population produced in chemical reactions that proceed through radical pair intermediates. The theory of CIDNP is well developed and allows extraction of radical reaction rate constants, hyperfine couplings, g factors and nuclear paramagnetic relaxation rates in the intermediate radicals. The primary application of this technique, specifically of its photo-induced variant (photo-CIDNP), has been to investigating radical reaction mechanisms and relatively recently to protein structure investigation.

Two primary kinds of photo-CIDNP hardware are continuous-wave (CW) and time-resolved (TR) setups, of which only the latter may be used to extract magnetic and kinetic parameters of the intermediate radicals. While assembling a fibre-optical CW setup is relatively straightforward, the more informative TR equipment used to be a technical challenge, requiring a manual probe modification in order to bring high-intensity pulsed laser light into the narrow magnet bore. However, with today's complex multinuclear, multiple-gradient and cryogenic probes such a modification is quickly ceasing to be an option for a mainstream physical chemist.

This poster describes the technical and software detail of the TR-CIDNP setup that we had recently assembled at Oxford, based on the frequency-tripled Nd:YAG laser and a shared Varian INOVA 600 NMR spectrometer. We describe a cheap and efficient light routing system that avoids probe modification and a number of TR-CIDNP pulse sequences optimized for use with Nd:YAG laser running at a constant pulse repetition rate. A pulse and flash sequence is suggested which adds up time-resolved photo-CIDNP signals from successive laser flashes not in the acquisition computer, but in the spin system itself, resulting in a substantial improvement in S/N over the traditional signal averaging technique.

Uniform illumination of optically dense NMR samples

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A variety of photochemical NMR experiments require illumination of the sample inside the NMR probe, including but not limited to: photo-CIDNP (Chemically Induced Dynamic Nuclear Polarization), metal ion release from photolabile cage compounds, photochemical kinetics and studies of photoactive proteins.

One of the biggest technical challenges faced in such experiments is to deliver light efficiently into the active region of the NMR sample. Ideally, the entire sample volume should be uniformly illuminated to maximise sensitivity and to avoid concentration and temperature gradients, which might distort the observed kinetics. Such problems are likely to be most severe for optically dense samples. It is highly desirable that uniform illumination is achieved without extensive probe modifications, which may compromise the NMR performance, and in a manner that allows facile transfer from one spectrometer to another.

We demonstrate a simple, inexpensive method for in situ laser illumination of NMR samples using a stepwise tapered optical fibre to deliver light uniformly along the axis of a 5 mm NMR tube. The optical path length of the incident light inside the sample is about 3 mm, allowing efficient illumination of optically dense samples. The degradation in spectral resolution and the reduction in filling factor are both minimal. Probe modifications are not required.

Photo-CIDNP of Amino Acids and Proteins in the presence of paramagnetic lanthanide(III) ions

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Photo-Chemically Induced Dynamic Nuclear Polarisation (photo-CIDNP) is a powerful technique for recording NMR spectra of amino acids and proteins with a large sensitivity enhancement and a high spectral resolution [1,2]. The method employs the reaction of a photosensitizer, usually flavin mononucleotide (FMN), in its first photoexcited triplet state with the aromatic residues of the amino acids tryptophan (Trp), tyrosine (Tyr) or histidine (His) to form a spin-correlated radical triplet pair leading to enhanced absorptive and emissive NMR signals of individual side-chain nuclei in the intact protein. CIDNP occurs only when the side-chains of these amino acids are physically accessible to the photoexcited flavin, thereby permitting the monitoring of changes in protein structure during substrate, inhibitor or cofactor binding, folding or unfolding [3,4].

We have explored the feasibility of applying the photo-CIDNP technique to amino acids and proteins in the presence of paramagnetic lanthanide(III) ions with the aims of improving both spectral resolution and sensitivity. We found that the presence of such ions, however, not only shifts CIDNP signals substantially but also leads to a significant attenuation of the observed polarization. Steady-state CIDNP experiments were performed in the presence of either paramagnetic ytterbium(III) or diamagnetic lanthanum(III) ions to explore the cause of the signal attenuation. The results of these experiments show that in the presence of diamagnetic lanthanide(III) ions no attenuation of polarization signals occurs. It was therefore concluded that acceleration of spin relaxation rather than increased spin-orbit coupling leading to a strong acceleration of intersystem crossing rates in the radical pair is the main reason for the observed attenuation.

Furthermore, we were able to show that the formation of a complex between ytterbium(III) and the photosensitizer (FMN) results in the formation of a stable and insoluble precipitate, hindering the acquisition of CIDNP spectra. The structure and chemical composition of this complex has been determined. Moreover, we show ways to circumvent these problems associated with CIDNP of biologically active molecules in the presence of ytterbium(III) using other flavin derivatives as a photosensitizer.

The concept of combining the advantages of 'steady-state' and 'real-time' protein CIDNP, together with the improved spectral resolution of employing paramagnetic lanthanide(III) ions, is explored using bovine α-lactalbumin (BLA), a 14 kDa protein responsible for the regulation of lactose biosynthesis in the lactating mammary gland. The denatured apo-form of this protein refolds upon the addition of a variety of ions including ytterbium(III) [5,6] and therefore serves as a promising testing ground for showing the advantages of protein CIDNP in the presence of paramagnetic lanthanide(III) ions.

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NMR Studies of the Photochemical Intracomplex Reaction between β-Cyclodextrin and Anthraquinone-2,6-disulfonic Acid Disodium Salt in Water Solution.

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Study of the interactions between short-lived radical and cyclodextrin is concerned to understanding the influence of non-covalent binding of reactants on the radical reaction pathway. These interactions are essential factor in such biological processes as fermentative reactions, drug – receptor binding and others.

Earlier we investigated the monomolecular reaction of dibenzyl ketone photodecay in β-cyclodextrin (CD) water solution. It has been shown that benzyl radical formed in this reaction kept in the CD cavity not less then 10 μs [1].

However not always CD stays indifferent to photoreactants. In the presence of photo-exited quinones CD becomes H-donor [2]. We investigated this processes in detail. The photointeraction between anthraquinone-2,6-disulfonic acid disodium salt (AQDS) and CD were studied in water solution. CIDNP experiment has shown that the main primary product of photoreaction is hemiacetal, which can be formed only by recombination of semiquinone radical with C6-radical of CD. The location of H-donating group is caused by complexation geometry.

The formation of AQDS-CD inclusion complex has established by 1D and 2D NMR methods. Downfield shifts of the AQDS protons, and upfield shifts of the CD protons are obtained in 1D NMR spectra. The 1:1 stoichiometry of complex is estimate by Job's method. The complex formation constant was calculated from the change in the observed chemical shifts of AQDS versus CD concentration (NMR shift titration) and was equal to 800±100 M^{-1}.

CIDNP experiment has also shown that polarization intensity of the forming hemiacetal depends on the CD concentration. After normalization this dependence almost coincides with that of NMR shift titration. This means the concentration of geminate pairs increases in the same manner as that of the complex with increasing CD concentration. Therefore the formation of the product of selective CD oxidation can be unambiguously assigned to the formation of complex between reagents.

Since the 1D NMR spectra fail to give the unambiguous information about the complex structure, we used the rotating frame nuclear Overhauser effect spectroscopy (ROESY) for determining the main structural complex parameters. Intermolecular ROESY cross-peaks indicate that AQDS is localized near the narrow side of the CD cavity. A possible location of AQDS at a given position was verified by molecular modeling using the AM1 method. For the optimized inclusion complex geometry the minimal distances between AQDS and CD protons are varied from 0.23-0.25 nm (for H5 and H3) to 0.36-0.45 nm (H6 and H4) and 0.55-0.61 nm (H1 and H2). Taking into account the sensitivity of ROESY method to distances between protons of guest and host molecules up to 0.45 nm [3] the presented model of inclusion complex is in fair agreement with the NMR data. This structure of the complex also corresponds well to the selective oxidation of CD at C6 position.

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Structure and dynamics of a biologically active human FGF-1 monomer, complexed to a hexasaccharide heparin analogue by NMR

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The fibroblast growth factors (FGFs) constitute a family of more than twenty signalling polypeptides, that have been demonstrated to be involved in a variety of biological processes including cell proliferation, differentiation and angiogenesis. It is well stablish that FGF biological functions are triggered by binding of the polypeptide to heparan sulfate glycosaminoglycans (HS-GAGs) and specific transmembrane tyrosine kinase receptors at the cell surface (FGFR). It has also been proposed that FGF dimerization is a key process in the formation of the FGF:FGFR signalling complex mediated by GAGs either free or bound to the cell surface.

However, using well defined synthetic HS-GAG-type oligosaccharides sequences we have recently described sedimentation-equilibrium based data that strongly suggest that GAG-induced FGF dimerization is not an absolute requirement for biological activity [1]. In particular, it was shown that an hexasaccharide in which all the sulfates groups are oriented on only one side of its helical-like structure generates monomeric species that induce the same mitogenic activity as low molecular weight heparin.

Herein, we described the structure and dynamics of the biologically active monomeric complex between FGF1 and this heparin-like hexasaccharide, as determined by NMR. Structure calculations were performed using DYANA, with 1432 NOE upper distance constraints. At each stage, 200 structures were calculated using 35000 steps of simulated annealing and a final ensemble of 30 structures was selected based on the DYANA target function values. The selected structures were refined by restrained molecular dynamics with the AMBER 5 program. The final structure is fairly well defined, with a average pairwise r.m.s.d. for the protein backbone atoms of 0.99 Å.

Futhermore, in order to study the hexasaccharide conformation in the bound state, we have also adquired the ^{13}C-double half filter NOESY for the complex in D_2O solution. This experiment has allowed us to assign the carbohydrate signals in the complex and to determine the ligand bound conformation. The detected intermolecular NOEs and a chemical shift perturbation analysis also permits to position hexasaccharide in the complex. A 3D picture of the complex can then be proposed on the basis of all experiments.

Finally, we have also used the program AutoDock to study the carbohydrate-protein interaction and the model obtained is in agreement with the experimental intermolecular NOEs.

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Which Liquid Crystalline Solvents Are Relevant for NMR Spectroscopy? Solvent Independent Unambiguous NMR Structures of Oriented Molecules

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In NMR spectroscopy of oriented molecules, when the structural parameters of a given molecule are measured in different solvents there is often a discrepancy in results that significantly exceeds the experimental error of the measurement. Moreover, sometimes structural parameteres also show large deviation from the data obtained with other structural methods, such as X-ray crystallography, electron diffraction, or microwave spectroscopy. This discrepancy has been the major obstacle for NMR spectroscopy to become a structural method as well-established as others mentioned above. One of the main consequences of these discrepancies is an ambiguity of NMR structure, because the solvent dependence of the apparent structure does not allow one to choose the correct undistorted structure from the set of determined ones. As a rule, the largest deviations are observed for small molecules weakly oriented in thermotropic liquid crystals.

On the other hand, last few years residual dipolar couplings (RDC) of weak oriented biomolecules are used for three-dimensional (3D) structure determination of biomolecules by NMR spectroscopy [1]. The good match of determined NMR structure with the X-Ray structure is very strange comparing with the high distortions expected for such weak aligned solutes.

Recently we have shown that the distribution of structural parameters measured in different solvents is not random, and there is a difference in results between thermotropic and lyotropic liquid crystalline (LC) solvents. Particularly, in weakly aligned lyotropic systems there are no considerable deviations of the structural data of model molecules, whereas in thermotropics the apparent deviations were observed to be inversely proportional to the order parameter of solute [2]. This result can be considered as confirmation of the appropriateness of the use of weakly aligned solvents in studies of biomolecular structures using RDCs. Also we found out that nematic thermotropic LC solvents used for NMR spectroscopy can be separated into two groups: one in which large apparent deviations of the measured structural parameters are observed, and the group of other LCs for which the measured structural parameters are solvent independent.

First group mainly consists of LCs with a large positive anisotropy of diamagnetic susceptibility and their mixtures. This result indicates that many of traditionally used nematic LC solvents, such as EBBA, Phase IV, HAB, or mixtures on their bases are not relevant for structural NMR spectroscopy. Another characteristic features of these solvents are their small negative dielectric anisotropy and the positive sign of D^{D}_{CH} of methane, referenced to the director of LC.

The second group contains thermotropic LCs with negative or small positive diamagnetic anisotropy, positive dielectric anisotropy and negative sign of D^{D}_{CH} of methane, used as a reference. This group includes LCs like ZLI 1132, ZLI 1167, ZLI 1695.

The right choice of the LC solvent will ensure unambiguous, relevant and precise NMR structure. This conclusion is based in the study of halomethanes, benzene, furan, thiophene and several other probe molecules in large set of LC solvents [3].

The possible reason of the phenomenon will be discussed. Some evidences for the model will be demonstrated.

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Conformational analysis of oGM3 and Gal-GM3 using residual dipolar coupling and solvated molecular dynamics

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Molecular recognition of both sialic acid and galactose moieties seem to play a key role for GM1 [Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1Cer] recognition by cholera toxin. However, GM1 proper conformation for toxin binding[a] is probably mediated by the interaction between the acetamide group of the GalNAc residue and the sialic acid carboxylic group, although no direct experimental evidence has supported this hypothesis so far.

In order to test this hypothesis, we have investigated the conformational features of smaller models of GM1, namely GM3 [NeuAcα2-3Galβ1-4GlcOH] and Gal-GM3 [Galβ1-4(NeuAcα2-3)Galβ1-4GlcOH] using a combined NMR and MD approach. Molecular dynamics in explicit water over a period of 10 ns for each compound has been performed in order to predict the rotamer populations around the different glycosidic linkages. The simulations predict that the flexibility of the sialic acid residue strongly decreases when passing from trisaccharide 2 to the branched tetrasaccharide 3. From the NMR side, experimental hetero- and homo-nuclear residual dipolar couplings were measured using two different alignment media, PEG and phage. Accurate RDC values were measured by employing MestReC software's tools[b]. In order to get the conformational behavior from RDC values, an order matrix analysis was performed for each pyranose ring[c], by combining the D_{CH} and D_{HH} experimental values and Redcat software[d].

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Selective refocusing 2D NMR experiments combined with Chiral Liquid Crystalline Solvents: A great Tool to simplify Enantiomeric Visualisation

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Considerable efforts are continuously made in the field of the asymmetric synthesis in organic chemistry. Consequently, the development of new and efficient NMR tools allowing the visualization of enantiomers is a great motivation for spectroscopists. Among them, the use of NMR in chiral liquid crystalline solvents has proved to be a method of choice.

One of the most interesting chiral liquid crystals is the chiral nematic phase formed by poly-(g-benzyl-L-glutamate) (PBLG) in solution with various organic co-solvents such as chloroform, dichloromethane, DMF, THF etc. We have shown that we can visualize an enantiomeric discrimination through NMR spectra in this chiral medium. This method was successfully applied to a wide range of chiral compounds including solutes which are chiral by virtue of isotopic substitution and using deuterium, carbon-13, fluorine-19 or Natural Abundance Deuterium NMR (NAD-NMR)**(1-6)**. We present here simple and robust methods for simplifying the analysis of proton or carbon-13 spectra of molecules dissolved in weakly oriented media. The NMR approach investigated is based on the use of proton selective refocusing 2D experiments (SERF 2D) to improve the resolution of proton spectra of solutes and the measurement of their proton-proton dipolar couplings **(7**) . The analytical potential of proton SERF experiments is applied to the case of enantiomers dissolved in chiral polypeptide liquid crystals. In an another part, it is demonstrated that the carbon-proton heteronuclear selective refocusing 2D NMR experiments enable to extract carbon-proton residual dipolar couplings for each enantiomer from a complex or unresolved proton-coupled ^{13}C spectral patterns **(8).** Illustrative examples are analysed and discussed. It is shown that an accurate determination of enantiomeric excess is possible within a short experimental time.

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Ultrafast 2D NMR spectroscopy and stopped-flow methods applied to protein folding studies

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Structural and kinetic information on protein folding processes can be gained from stopped-flow NMR spectroscopy which, when combined with photo-CIDNP (chemically induced dynamic nuclear polarization) yields higher sensitivity and spectral resolution [1,2].

Many folding processes, though, happen on a very short time scale, much faster than can be observed by NMR. Nevertheless, with stopped-flow methods, folding events happening on a time scale of seconds have been observed. As for resolution, it would be preferable to do 2D NMR spectroscopy, but as the time scale of such an experiment is in the order of ten minutes to hours, it is basically impossible to do conventional stopped-flow 2D NMR spectroscopy, except for the very slowest of folding reactions. Furthermore, coupling 2D NMR spectroscopy with photo-CIDNP leads to the problem of photo bleaching, as the dyes used in photo-CIDNP experiments tend to photo-degrade after prolonged irradiation.

Photo-CIDNP signal enhancement can be understood in terms of the radical pair mechanism. The dye molecule, in this case a flavin derivative, is photoexcited with a laser flash and reacts with the sidechains of certain amino acid residues on the surface of the protein, generating CIDNP [3,4].

In 2002, Frydman and co-workers demonstrated a protocol for acquiring a 2D NMR spectrum within a single scan. This technique is based on spatial encoding of spin evolution during t_1 with the aid of a magnetic field gradient and subsequent decoding during t_2 while spins are subjected to a train of gradient echoes, i.e. a EPI (echo planar imaging) read-out scheme. It was shown that coupling photo-CIDNP and ultrafast NMR spectroscopy is advantageous for single scan protein NMR due to the increase of sensitivity. On the other hand, the photo-degradation problem in 2D photo-CIDNP NMR spectroscopy is alleviated, as the ultrafast scheme requires only a small number of scans to obtain an acceptable 2D CIDNP spectrum of a protein [5,6].

Combining the ultrafast technique with stopped-flow methods opens up the possibility of monitoring protein folding processes in two dimensions. In this poster, spectra from ultrafast TOCSY NMR experiments recorded after the rapid injection of ribonuclease A into an unfolding medium will be shown. It will be shown that ultrafast 2D NMR and stopped-flow methods can be combined and can be used to gain insight into protein folding processes. Further work will be discussed and the possibility of other NMR experiments explored.

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Structural studies of antigenic determinants recognized by a protective monoclonal antibody in view of developping a vaccine against shigellosis

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Shigella, an invasive enterobacterium, is the causative agent of human dysentery or shigellosis. It raises a serious health problem, especially in those regions of the Third World where bad sanitary conditions and malnutrition prevail. Development of a vaccine against shigellosis is one of the priorities of the WHO. There are four different species of the bacterial agent causing shigellosis, each of them possessing many serotypes which are defined by the structure of the O antigen (Ag-O), the main target of the immune response protecting against Shigella and that differ mainly by the position of the glucosyl redisue (E).

Interactions between pentasaccharide corresponding to the repeating unit of both Shigella flexneri 5a and 2a and peptides that mimic the pentasaccharide with protective antibodies, IgA and IgG, were investigated by NMR and molecular modeling. First the average structures of the free ligands were established and then their conformations in the bound form were investigated using transferred NOE (TrNOE) experiments. The pentasaccharide structures in the bound form is similar to the average structure in the free form. Similarly, the peptides both free and bound to the antibody adopt a turn conformation. In order to map the epitopes at the molecular level, contacts between ligands and antibodies were evidenced using saturation transfer difference (STD) experiments with selective saturation of the antibody resonances. Finally, NMR derived pentasccharide and peptide models coupled to STD informations were used to generate antibody:pentasaccharide and antibody:peptide complexes. Interactions of pentasaccharides with protective antibodies show that the glucose residue remains in close contact with the antibodies and thus is part of the epitope. The peptide side-chains of residues forming the turn, contact the antibodies residues in the Ab-peptide complexes.

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Characterisation of the structure and dynamics of the unfolded state by NMR and optical spectroscopy

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The folding of proteins has been studied intensively during the past decades and includes the 179-residue *Azotobacter vinelandii* flavodoxin. The topology of flavodoxin is characterised by a five-stranded parallel β-sheet surrounded by α-helices. An intermediate is populated during denaturant-induced equilibrium unfolding of flavodoxin. The intermediate has molten globule-like characteristics, which as demontrated by fluorescence and CD spectroscopy. The population of the intermediate depends on the temperature, at certain denaturant concentrations the population is up to 80% at 35ºC. To further examine flavodoxin folding at residue-level, NMR spectroscopy is used. Since protein folding starts with unfolded polypeptides, flavodoxin, unfolded with guanine hydrochloride, will be structurally and dynamically characterised by heteronuclear NMR spectroscopy. Resonances of the unfolded protein need to be assigned and chemical shift deviations from random coil values can will reveal residual secondary structure. In addition ^{15}N relaxation studies will lead to a better view of the unfolded protein. Once the unfolded state is assigned, the folding of the molten globule-like intermediate of flavodoxin can be followed at the residue-level by NMR spectroscopy.

NMR investigation of binding between dynein light chain to a non-coiled-coil tail domain of myosin-Va

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Class V myosins are involved in short-range intracellular transport along actin filaments. Of the three mammalian myosin-V heavy chain genes, mutations of MYO5A are responsible for the dilute phenotype and Griscelli syndrome type 1 in mice and humans, respectively. A dynein light chain (DLC) has been identified as a tail domain light chain of myosin-Va (myo5a). DLC may function as a cargo-binding and/or regulatory subunit of both motor proteins. Our goal is to identify and characterize the binding site of DLC on myo5a. DLC is a homodimer, build up of 94 residues each, myo5a consists of 15 residues.

Resonance assignments on ^{13}C, ^{15}N labelled DLC are obtained from 3D HNCA, HNCOCA, CCONH experiments. Binding of the myo5a fragment is monitored by ^{1}H-^{15}N HSQC measurements using ^{15}N labelled DLC and unlabelled myo5a. At sub-stoichiometric amounts of peptide two sets of resonances are observed; one set corresponding to the free DLC, another to the complexed form; indicating the species are in slow exchange on the NMR time scale, and making possible the estimation of the K_d constant, cca. 30μM. The detected chemical shift changes indicate that binding occurs on a surface groove on DLC. As DLC acts as a homodimer two molecules of target peptide fit in the two binding channels. Titration experiments indicate that there is no communication between the two channels.

Besides myo5a, testing other small peptides (nNOS, bim) that share very little amino acid sequence homology, it is found that the DLC binding sites are the same, and binding is similar. Binding sites from peptide site are determined by saturation transfer difference (STD) measurements.

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Fast assignment of NMR backbone resonances for proteins of known 3D structures using residual dipolar couplings.

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Structural genomic projects today yield an increasingly high number of proteins structures, mainly obtained by X-Ray diffraction, whose functions remain to be elucidated. NMR can play here a crucial role, through its ability to quickly identify binding sites of ligands on proteins, by measuring the evolution of chemical shifts on fast and sensitive HSQC type experiments (Chemical Shift Mapping). An important NMR limiting step is the assignment of the HSQC spectra, which remains often fastidious, and big efforts are being made to provide to the NMR community automated assignment methods. To be applicable to high throughput procedures, the methods need to be fast and reasonably priced, and thus to use (i) a minimal amount of preferably only 15N-labeled protein, (ii) fast, highly sensitive, and generally practicable experiments.

For proteins whose 3D structures alone are already known, the comparison of back-calculated with experimental Residual Dipolar Couplings (RDCs) values can be a very efficient way to obtain a straightforward assignment of HSQC spectra. It is however in practice not sufficient, and a number of methods have been proposed that use additional information such as HN-HN nOes, chemical shifts, accessibility of amide protons, etc. (Hus 2002, Erdmann 2002, Langmead 2004, Jung, 2004).

Here, we propose a new approach that exploits RDCs to get a fast and automated assignment of interaction sites in proteins of known 3D structures. We also analyze in detail the theoretical and experimental limits of the use of RDCs in this context.

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NMR multidomain proteins stability and folding analysis : conformational exchange, temperature and pH stability variation in human annexin 1 first domain.

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Annexins constitute a family of cytosolic, water soluble proteins, which bind negatively charged phospholipids in a calcium-dependent manner. They share a commune quaternary structure, composed of a planar cyclic arrangement of four domains (Huber et al., 1992; Concha et al., 1993). These four domains have very similar 3D structures, which consist of five alpha-helical segments wound into a right-handed compact superhelix, but show a low identity in primary sequence. Annexins are thus excellent models for studying the folding mechanisms of multidomain proteins, and also for the analysis of the relationship between sequence, structure and stability of domains.

When produced and purified isolated, human annexin 1 four domains don't behave the same way: domain 1 has been proven to be an autonomous folding unit (Gao et al., 1999), folded in a highly similar way to that when together with the other three domains. Domain 2 is largely unstructured (Ochsenbein et al., 2001), and the other two domains can't be isolated in water solution.

Previous relaxation studies in our laboratory had revealed presence of conformational exchange in the first domain, but didn't allow the determination of physical exchange parameters. The study we present here is based on a CPMG analysis of conformational exchange. We have realized experiments at 500Mhz, 600Mhz and 800Mhz, and at multiple temperatures, to study the exchange variations with thermal energy, thereby allowing the calculation of activation energies associated with movements. We have also sampled different pH conditions, combined with circular dichroïsm experiments, to study domain 1 stability with pH variations.

Our results show that conformational exchange in human annexin 1 first domain is localized, both in secondary and tertiary structure. Exchange constants calculated at different field values show that results are meaningful, and exchange variations with temperature allowed us to determine a global activation energy. Finally, exchange variations with pH show that exchange is most effective, relaxation-wise, for pH 4-4.5. The results will be discussed in the context of the isolated domain 1 and of the entire annexin.

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Fast assignment of NMR backbone resonances for proteins of known 3D structures using residual dipolar couplings.

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A Novel Class of Amphibian Peptides: Investigating the Signiferins

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Amphibian skin contains a rich store of biologically active compounds which serve as the animal's principle defence against predation. Our work to date has allowed the identification of more than 150 bioactive peptides from the dermal secretion of some 25 Australian anurans, with activity ranging from antibiotic, antifungal and anticancer properties, to effecting analgesia or smooth muscle stimulation.[1,2] The characterisation of such host-defence peptides has recently been extended to include the genus *Crinia,* in which seven novel peptides were identified in the skin secretion of *C. signifera*.[3] This group comprises a number of short (10 – 17 residue) peptides subsequently named the signiferins, and includes the first ever cystine-bridged cyclic peptide isolated from an Australian amphibian, as well as unusual peptides which begin with two isoleucine residues. Nuclear Magnetic Resonance Spectroscopy and Restrained Molecular Dynamics calculations have been used to investigate the solution structure of the novel cyclic peptide, as well as a second analogue isolated from a closely related species of frog. Details of the bioactivity of the signiferins is presented, and is discussed with reference to the unusual structures of these peptides.

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Rotational Dynamics of Adamantanecarboxylic Acid in Complex with β-cyclodextrin

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The reorientation of 1-Adamantanecarboxylic acid (AdCA) within the β-cyclodextrin cavity is investigated by means of multiple-field ^{13}C NMR relaxation. The dissociation constant describing the complexation equilibrium is determined using translational diffusion measurements for the guest during a titration by the host. The reorientation of bound AdCA at 25^{o}C can be described by either the Lipari Szabo or the axially symmetric rotational diffusion model while at lower temperature, assignment of the "spinning" and "rocking" motion of AdCA inside the cavity to the two local correlation times is proposed.

Structural Characterisation of the pleckstrin protein

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The pleckstrin protein is the major protein kinase C (PKC) substrate in blood platelets. The protein consists of 3 domains: a PH domain at each terminus and a central DEP domain. Phosphorylation of three sites in the linker region between N-PH and DEP domain leads to the functional activation of pleckstrin. Recently, we solved the structure of the C-terminal PH domain and showed that it binds selectively to a lipid second messenger, PtdIns(3,4)P_2. The structures of the other two domains of pleckstrin are also known.

We have now moved on to delineate the overall structure of full-length pleckstrin by NMR and other biophysical as well as biochemical techniques. For this purpose, we use the combination of residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE) induced by spin-labels in order to obtain both orientational and translational information on pleckstrin double-domain constructs. Furthermore, assignments and RDCs of *in vitro* phosphorylated pleckstrin are now available and allow first structural interpretation of pleckstrin's activated state.

NMR structure of HIV-1 co-receptor CCR5 amino terminus

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HIV-1 entry into target cells is mediated by the successive interaction of the viral envelope glycoprotein gp120 with the cellular receptor CD4 followed by binding to a chemokine co-receptor, mainly CCR5 or CXCR4. A tyrosine rich segment at the CCR5 N-terminus is involved in the interaction with both types of CCR5 ligands, i.e. CC-chemokines and the HIV-1 gp120/CD4 complex, and sulfation of 2-4 of the tyrosine residues in this segment is crucial for the binding.

In this study we determined the three dimensional structure of doubly sulfated and unsulfated forms of peptides corresponding to the N-terminus of CCR5 using high resolution 2D NMR spectroscopy.

Our results show that residues within the N-terminus of CCR5 have a tendency to form a helical conformation in both the sulfated and unsulfated peptides. This helical domain is stabilized and extended in the sulfated peptide. Consequently a highly negative surface is formed in the sulfated peptide that may be crucial for interaction with complementary domains on gp120 or CCR5 chemokines. This study offers a new insight to the structure- function relationship of tyrosine sulfation in peptides.

NMR study on the membrane proximal domain of HIV-1 envelope protein

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HIV-1 envelope glycoprotein gp41 is responsible for the fusion of the virus with the host cell. The fusion process, as well as the full structure of gp41, are not completely understood. One of the strongest inhibitors of HIV-1 fusion, which is now used as an anti HIV-1 drug (Fuzeon, also called Enfuvirtide or T-20) is a 36-residue peptide named DP-178, gp41(638-673). In the course of this study we Established an expression system and purification protocol for the 42-residue peptide NN-DP-178-NITN (gp41 (636-677)) to produce a 13C/15N labeled peptide. The labeling enables us to use heteronuclear 2D and 3D NMR methods in order to determine the secondary structure of gp41(636-677). our results suggest that NN-DP-178- NITN peptide is composed of an N-terminal unstructured region and C-terminal helical region, in agreement with the structural results for the 13-residue peptide gp41(659-671) that was already determined in our laboratory. As such, it is very likely that the helical conformation in this region are maintained in gp41 throughout the different tertiary structures of the envelope protein that form during the process of viral fusion.

Structural Analysis of Thiomethylene Bridged 'Oligonucleotide Foldamers' by NMR Spectroscopy

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All the modified oligonucleotides investigated so far maintain a separation between nucleobase and the backbone. This led to the question whether this structural invariance implies a functional differentiation of nucleobase and the backbone which is indeed required for the formation of pairing systems. To answer the above question a new type of oligonucleotide analogues that include the nucleobase in the backbone were introduced [1]. In these analogues (referred to as 'Foldamers'), the 3'-5' phosphodiester linkage between two successive ribose moieties is replaced by a link between the base of one nucleoside unit and the ribosyl moiety of the following unit.

These foldamers aim at exploring in more detail the structural limits of the organizational principle of oligonucleosides and thereby a better understanding of the structural requirements for the formation of stable duplexes. The central question is obviously if the linkers and the geometry they impose will be compatible with hydrogen bond formation and stacking interactions or even favour them, and thus allow the formation of stable duplexes.

The observation that partially protected type of oligonucleotide dimers form stable duplexes in $CDCl_3$ suggests strongly that the dedifferentiation of nucleobase and backbone is not a requisite for base pairing.

The pairing and folding properties of a tetramer (AUAU) with thiomethylene linker were investigated with the help of NMR. The calculations were performed with XPLOR-NIH version 2.0.4 [2] using distance restraints generated from NOESY (mixing time 300msec) with the aid of SPARKY [3]. The analysis of tetramer-duplex ensemble proves the existence of a stable duplex which predominantly has a bent-helical structure and shows normal Watson-Crick pairing of the base pairs.

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Structural comparison of the wt matrix protein from Mason-Pfizer Monkey Virus with its R55F mutant.

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Mason-Pfizer monkey virus (M-PMV) belongs to the genus of betaretroviruses, in which matrix protein (MA) plays an essential role in different stages of retrovirus maturation. MA participates on the assembly of immature viral particles, their transport to the outer cell membrane and budding. Several single- or double-point mutants of MA (e.g. A18V, R55F, T41I/T78I, Y11F/Y28F) are known to cause dramatic changes in the virus life cycle. [1,2] For example, M-PMV capsids are assembled at the plasma membrane instead of in the cytoplasm in case of R55F mutant, which is a behavior typical for the lentiviruses (*e.g.* HIV-1). [1]

We have focused our attention on the study of three-dimensional structures and dynamics of wt MA protein and its R55F mutant by NMR spectroscopy to reveal possible structural changes caused by the mutation. Although a structure of the wt MA has already been published as a C-alpha trace [3], we decided to perform a new study of this protein to obtain a complete 3D structure as a reference for structural comparisons with other MA mutants.

Doubly labeled (^{13}C, ^{15}N) proteins were prepared using recombinant techniques and standard triple resonance NMR experiments were used for the assignment of protein resonances. Based on NMR parameters (NOE interactions, *J*-couplings, chemical shifts) three-dimensional structures of both proteins were calculated using ARIA [4].

The structural fold of matrix proteins from M-PMV consists of four α-helical regions connected by short loops. We have found that the replacement of the originally solvent-exposed arginine for buried phenylalanine is probably the driving force leading to the reoriented single helical domains in R55F. In order to describe the structural changes more precisely, we used the directional information from rotational diffusion anisotropy based on relaxation properties of ^{15}N nuclei. The reorientation of helices resulted in partial hiding of the signal amino acid sequence Pro 43 to Gly 60 (cytoplasmic targeting/retention signal), which was demonstrated to be responsible for transporting of immature viral particles to the site of assembly [5].

Acknowledgement

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Structural Studies of Lac Repressor Headpiece in Complexes with the O2 and O3 Operators.

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The Lactose Operon is a textbook example of gene regulation in prokaryotes. The lac operon has three lac repressor recognition sites in a stretch of 500-bp called O1, O2 and O3. O3 lies within the lacI gene, which is 93 bp upstream of O1, which lies within the promoter. O2 lies 401 bp downstream of O1, within the lacZ gene. All cognitive sequences have a nearly dyad symmetry and are not of equal quality. Full repression can only be obtained by the simultaneous binding of one tetrameric Lac repressor to the strong operator O1 and to either O2 or O3 creating one of two alternative DNA loops.

The structures of the lac repressor headpiece in complex with O1 and non-specific DNA sequence were reported earlier.[1,2]

We report now the solution structures of lac repressor protein bound to its specific operators O2 and O3 together with the results of biochemical experiments. Electrophoretic mobility shift assay showed that O1 and O2 are both high affinity operators while the O3 affinity is comparable to that of weak nonspecific binding. However in circularly permutation assay experiments we observed a bend for all specific operators with O3 even having the largest bending angle.

Structural information combined with the results of biochemical experiments will give insight into the binding mode of lac repressor protein to various DNA sequences, explain affinity differences and lead to further understanding of the functions of the auxiliary operators.

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NMR and molecular modelling studies of an RNA hairpin containing a G-rich hexaloop

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We study a RNA hairpin which corresponds to the mRNA of the PGY1/MDR1 gene encoding the transmembrane P-glycoprotein (P-gp). Over-expression of the P-glycoprotein is responsible of the multi-drug resistance (MDR), an important obstacle in cancer chemotherapy. This hairpin is the target of antisense oligonucleotides that suppress the P-gp function of multidrug resistance [1].

In the present work, the solution conformation of the 5'(GGGAUG)3' loop closed by the G-U mismatch containing stem is studied by NMR and molecular modelling in explicit solvent. Special attention is given on the sugar and the backbone conformations. In particular, the hexaloop intrinsic properties of these two components are investigated. The stem structures obtained by molecular dynamics with and without NMR constraints converge to the same A-type conformation. The wobble G-U mismatch moderately perturbs the overall conformation, despite of sugars in C2'-endo conformations of the sugar and unusual backbones values between the mismatch and the loop. For the hexaloop part of the hairpin, strongly ordered, we can establish the following facts. First, sugar puckers are found in majority in C2'-endo conformations, probably to extend the strand with the help of numerous but limited unusual backbone angles conformations. This seems to be an intrinsic property for hexaloops, nevertheless, further investigations are required to better describe the backbone behaviour. Second, the loop is stabilized by stacking interactions and hydrogen bounds. Thus, on the 5' side, the four purine bases (G7, G8, G9 and A10) are stacked together, while, at the 3' end of the loop, U11 stacks on G12, while remaining far from the stem. This fold is different but coherent with the two other available solution structures of A-rich hexaloops (UGAAAG (2rp2 [2]) and GUAAAA (1bvj [3]).

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Solution structure of the CARD domain of NOD1

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NOD1 is a cytosolic signaling PRR (host pattern-recognition receptor) playing a crucial role in innate immunity. It has been shown to regulate pro-inflammatory pathways by mediating the activation of the nuclear factor NF-κB via its downstream effector the kinase RICK following the recognition of a specific bacterial ligand. RICK is recruited by NOD1 through interaction of their respective CARD domains (Caspase Activating Recruitment Domain).

The precise activation mechanism of NOD1 remains unclear. The goal of this study is to determine the structural basis of this process. The determination of the 3D structure of the CARD domain of NOD1 alone or in complex with its partner together with biochemical studies would help achieve this goal.

The CARD domain of NOD1 has been cloned, expressed and purified. Most backbone and aliphatic and aromatic side-chain resonances have been assigned and NOESY experiments have been performed.

The program ARIA was used for automated assignment of NOEs and NMR structure calculation (using CNS). The RMSD obtained for the backbone atoms is currently 0.5 Å and 0.9 Å for all heavy atoms. The CARD domain of NOD1 is composed of 6 α-helices and its fold is similar to that of the CARD domains of known structure (RAIDD or APAF1).

The study of the complex formed by the CARD domain of NOD1 with the CARD domain of RICK by NMR mapping will help to characterize CARD-CARD interactions.

Mapping the binding surface of RANTES chemokine complexed with CCR5-derived peptides

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RANTES (regulated upon activation, normal T cell expressed and secreted) chemokine was first identified as powerful leukocyte activator relevant in inflammatory disorders. During the last decade, RANTES attracted particular attention because its HIV suppressive activity. This discovery is associated with the identification in 1995 of three beta-chemokines, namely RANTES, MIP-1a and MIP-1b, as potent inhibitors of both human (HIV-1 and -2) and simian (SIV) immunodeficiency viruses replication in vivo. Because HIV virus exploits chemokine receptors as cellular entry gateways, chemokines that physiologically bind such receptors are nowadays becoming prime targets for new therapeutic strategies of AIDS.

NMR studies of the binding of a native and a S66E mutated RANTES to CCR5-derived peptides will be reported. In order to derive the mapping of the major structural determinants of RANTES-CCR5 binding, titration experiments monitored by 1H-15N HSQC spectra have been performed. It allowed us to precisely locate the amino-acid residues involved in chemokine-coreceptor binding and which might be therefore critical for the HIV blocking. The monomer-dimer equilibrium of RANTES chemokine at micromolar concentration and various experimental conditions is currently under study.

In vitro auto-association of poplar Grx and its competition with glutathione in reducing medium studied by NMR

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Glutaredoxins (Grx) are thiol disulfide oxidoreductases present in numerous organisms from bacteria to human [1]. They are essential for the maintaining of the thiol redox status of the cells. Through a CxxC or CxxS active site, Grxs catalyse the reduction of disulfide or glutathione (GSH) mixed disulfide. They are maintained reduced by GSH using electron donated by NADPH via glutathione reductase.

Peroxiredoxins (Prx) [3] belong to the new Grx targets identified in highter plants [2] and pathogenic bacteria. Due to the crucial functions of Prxs in the cells, the study of PrxGrx interaction is a challenging task in which NMR could offer relevent analysis. To understand molecular interactions of GrxPrx system, we have initiated the study of the poplar system by NMR. Sequencespecific assignment of both proteins have been first obtained [4,5], and then their dynamic properties have been characterized. Poplar Prx behaves as a defined homodimer 2x18 kDa [6], and poplar Grx displays relaxation data compatible with a selfassociation equilibrium. We will describe the characteristics of this autoassociation : its dissociation contante, the association interface, and the in vitro dynamic behavior of the reduced Grx. This equilibrium can be displaced using GSH. The binding site of GSH is in part shared with the autoassociation interface.

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Antibiotic recognition and transcriptional regulation by TipA proteins

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The *tipA* gene from *Streptomyces lividans* is expressed as two autonomous in-frame translation products, TipAL and TipAS. TipAS recognizes and covalently binds various thiopeptide antibiotics. The solution structure of free TipAS has been solved by NMR spectroscopy[2]. TipAL contains an additional DNA binding domain and upregulates *tipA* transcription in response to antibiotic binding.

Our aim is to understand how TipAS recognizes and binds structurally different antibiotics, and the mechanism by which antibiotic binding to TipAL leads to increased *tipA* transcription. This knowledge may be helpful in understanding multidrug recognition and resistance in pathogenic bacteria.

We obtained a complex of TipAS with the antibiotic promothiocin A, and studied it by NMR spectroscopy[1]. A series of triple-resonance spectra was recorded, and the protein backbone was assigned. NOE, scalar coupling and residual dipolar coupling data were collected. Secondary chemical shifts and preliminary structure calculations showed that while the TipAS core remained largely unchanged, the previously flexible N-terminus assumed an α-helical conformation upon binding of the antibiotic[2]. We are presently attempting to obtain resonance assignments for the antibiotic in the complex, and a structure of the TipAS*promothiocin A complex.

We also obtained and studied a complex of TipAS with the antibiotic nosiheptide. By comparing it to TipAS*promothiocin A, we are hoping to gain insight into how TipAS accommodates different ligands.

The N-terminal, DNA binding domain of TipAL was produced as an independent construct named TipAN. NMR spectroscopy showed that TipAN formed aggregates at concentrations between 30 and 300 mM, making it an unlikely candidate for high resolution NMR spectroscopy. However, we have obtained diffracting crystals of TipAN in complex with cognate DNA, and we are now attempting to solve its structure by X-ray crystallography.

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Interaction of the muscular LIM protein MLP with α-Actinin2

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MLP is the muscle specific variant of the cysteine rich protein family that has been known for several years to bind α-Actinin. Proteins of this family contain two LIM domains connected by a long glycine rich linker. MLP is transcriptionally upregulated during muscle development, but is also found in adult cardiac myocytes. Apart from α-Actinin MLP was shown to bind β1-Spectrin, MyoD and most recently telethonin. The later interaction is proposed to stabilize the correct anchoring of the elastic muscle filament Titin at the Z-disc and translate mechanical stress sensed by Titin into signaling pathways by a yet unkown mechanism. We have started a functional and structural study of this interaction network in which MLP is involved. We expressed full-length MLP and the isolated LIM domains in E. coli and started NMR based characterization. The LIM domains appear to act as independent units since the 15N-HSQC spectrum of the full-length protein can be reconstituted from spectra of the single domains. Linker residues and the C-terminal part apparently do not contribute to the structured part of MLP. Chemical shift assignment of the second LIM domain has been accomplished and structure calculation is almost completed.Our current data show that both LIM domains interact independtly and specifically to the central rod dimer of α-Actinin2 based on NMR titrations and solid phase binding assays.

Insight into protein-glycans interactions through data-driven flexible docking with HADDOCK.

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Lectins and defensins belong to a family of proteins that interact specifically with carbohydrates. They provide the first defense mechanism of an organism against bacteria, parasites or viruses by binding sugars on their surface. Up to date, little is known about the specific interactions involved in this process. In order to gain insight into these we studied the interaction between several mini-lectins and carbohydrate using NMR information in combination with our data-driven docking approach HADDOCK . Due to the small size and the rather dynamical nature of the interacting molecules, a fully flexible docking protocol was followed.

Results will be presented for several peptides, including retrocyclin , a lectin which can interact with HIV-1, and a human alpha-defensins HNP2, which binds with relatively high affinity the gp120 protein from the HIV virus. We will present docking studies of the interaction of these peptides with various disaccharides including β-Gal(1-4)β-Gal (lactose), β-GlcNAc(1-4)β-GlcNAc (chitobiose) and mannose, which can be found on the surface of HIV-gp120.

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NMR and fluorescence anisotropy studies of the interaction between the bacterial nucleoid associated proteins HHA and H-NS

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Bacterial pathogens are able to sense environmental changes, which distinguish host from external environments, and respond by modifying the expression of, among others, genes that code for virulence factors. The toxin hemolysin, produced by many uropathogenic *Escherichia coli* strains, is one of the virulence factors whose expression is modulated by environmental factors, such as temperature and osmolarity (1).

The nucleoid associated protein H-NS is a negative regulator of transcription of a large number of genes in Gram negative bacteria and is also involved in the thermoosmotic regulation of hemolysin expression (2). H-NS is organized in two independent structural domains: a carboxi-terminal DNA binding domain and an N-terminal domain involved in homo- and heterooligomerization processes. H-NS binds preferentially to intrinsically curved regions of DNA and the degree of oligomerization of H-NS is related to the specific binding to curved DNA (3).

Studies focused on the regulatory features of hemolysin expression led to the identification of the Hha protein (4). Hha does not bind DNA directly but it interacts with H-NS, suggesting that the Hha/H-NS complex is responsible for the repression of the hemolysin gene (5).

Here we report NMR and fluorescence anisotropy studies of the interaction between Hha and H-NS64, a truncated form containing only the N-terminal dimerization domain.

The interaction between Hha and H-NS64 causes important broadening effects in the NMR spectra of Hha that affect residues buried in the hydrophobic core of the protein, indicating that a substantial conformational change in Hha is involved in the interaction between both proteins. Similar broadening effects in Hha were also observed in the absence of H-NS at , suggesting that Hha has intrinsic conformational plasticity (6).

Fluorescence anisotropy has been used to monitor changes in correlation time caused by the interaction between Hha and H-NS64 under different conditions. These results reveal a salt dependence in the stoichiometry of the complex formed. A complex containing one Hha and two H-NS64 molecules is formed in NaCl. At lower salt concentrations (100mM NaCl) a different complex, containing one Hha and one H-NS64 molecule, is formed. Titrations with increasing NaCl concentrations show a sharp transition between the two forms at the physiological concentration of NaCl.

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Understanding inactivation of industrial enzymes: a protein folding problem

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Enzymes are elegant catalysts, many of which work at room temperature in aqueous media with high specificity. They are used in a number of commercial applications. In cleaning, enzymes have proven to be important for the removal of stains or for colour retention. The goal of this study is to understand in details by the use of NMR spectroscopy and complementary biophysical techniques how the folding and stability of an engineered enzyme derived from Pseudomonas mendocina lipase are related to its inactivation in harsh conditions such as detergent, high temperatures and alkaline pH. NMR spectroscopy has proven to be a powerful tool in the field of protein folding in that it enables to monitor structural changes at a residue level. This poster will present different aspects of the structure, stability and inhibition of this enzyme.

Screening of RING-finger domains of ubiquitin E3 ligases for NMR structural studies

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The proteasome proteolytic pathway is a crucial pathway for protein degradation in the cell. A series of enzymes, called E1, E2 and E3, target protein to be degraded via the ubiquitination pathway. The Ring finger domain of E3s (ubiquitin ligase enzyme) interacts specifically with E2s (ubiquitin-conjugating), whereas other domains of E3s specifically bind to substrates. UbcH5 (E2) can recognize a number of E3s, like c-Cbl, MDM2, hRing2. To understand the mechanism of ubiquitin transfer from E2 to the target protein and the specificity in the E2-E3 enzyme cascade, we study the structural similarities and differences between these E2/E3s.

We have cloned the Ring finger motif of 20 E3s which can interact with UbcH5, each with 4 different tags, 6 proteins have been purified and recorded ^{15}N-HSQC to see if we can determine the solution structure by NMR. It turns out that only one E3 Ring was folded. Possibly the low yield in stable Ring domain is related to the fact that the cysteines involved in Zn^{2+} coordination are very active, and can easily be oxidized, which prohibits correct folding. The successive is currently under process.

NMR solution structure of the peptide fragment (1-30), derived from mouse Doppel prion protein, in DHPC membrane mimicking micelles.

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The structure and positioning of a peptide with a sequence derived from the N-terminal part (1-30) of the mouse Doppel prion protein has been investigated in DHPC phospholipid membrane mimicking environment.

mDpl(1-30): MKNRL GTWWV AILCM LLASH LSTVK ARGIK.

CD spectroscopy shows the peptide to be one-third α-helical in DHPC and SDS solvents while it is mostly random coil in water. NMR spectrum assignments and structure calculation was successful in DHPC. The NMR spectra had sufficient quality to obtain a sequence specific assignment and lead to high-resolution structural data.

The experimentally derived structure, together with D2O exchange and NMR diffusion measurements are in good agreement with the hypothesis of a trans membrane α-helical middle part. A computer model was build as a PDB for the DHPC micelle and a cylindrical shaped DHPC conformation with a central pocket for the peptide. The diameter of this theoretical model particle fits well with the diffusion measurement experiments.

The structure is deposited under the code PDB-1Z65.
The chemical shift assignments are deposited under BMRB-6598.

You may see the structure models and a more thorough presentation at:
http://www.biophys.su.se/~evangelo/mDpl/

Stability of multi spin-echo sequences to the initial phase variation

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It is well known that multi spin-echo sequences are very sensitive to length of refocusing RF pulses in the RF pulse train. Any deviation from the ideal 180 degrees flip angle may lead to a complete loss of transversal magnetization at the end of the sequence. As it is practically impossible to design RF coils with perfect RF homogeneity this problem cannot be avoided unless proper phases of RF pulses are used in multi spin-echo sequences. The simple Carr-Purcell sequence [1] $(90_x\text{-}(t\text{-}180_x\text{-}t)^N\text{-AQ})$ where phases of all RF pulses are identical very soon accumulates a large error in magnetization orientation due to imperfect refocusing pulses. This problem is elegantly solved by Carr-Purcell-Meiboom-Gill modification of the sequence [2] where refocusing pulses in the pulse train have their phase shifted for 90 degrees with respect to the phase of the excitation pulse $(90_x\text{-}(t\text{-}180_y\text{-}t)^N\text{-AQ})$. Similar effect may be obtained also by alternating phases of refocusing pulses during refocusing pulse train $(90_x\text{-}(t\text{-}180_x\text{-}2t\text{-}180_x\text{-}t)^{N/2}\text{-AQ})$. However, both the approaches fail when there is another mechanism that additionally alters phase between the excitation and the first refocusing pulse, i.e., when there is an initial phase variation. This problem can be quite easily encountered; for example if the delay between the excitation and the first refocusing pulse is not exactly t or if the phase is altered by an external magnetic filed pulse. Such instability of the magnetization orientation during refocusing RF pulse train makes use of some fast imaging techniques difficult for registration of events that change phase of NMR signal. For example if flow is imaged by phase sensitive methods then velocity is proportional to the phase of the signal. One may then try to design a pulse sequence that would be a combination of a standard phase sensitive flow sequence and a multi spin-echo fast imaging sequence (RARE, U-FLARE) [3]. In the first part of the sequence a phase is accumulated and is proportional to the measured physical quantity (velocity). This may be done by the spin echo sequence with a pair of gradient pulses; one before and one after the refocusing pulse and the second part of the sequence consists of the train of refocusing RF pulses by which signals form several k-space lines are acquired and which starts at the echo of the phase accumulating part of the sequence; i.e., $90_x\text{-}G\text{-}180_y\text{-}G\text{-}t_{AQ/2}\text{-}(180_y\text{-AQ})^N$. The sequence will produce nice images as long as there is no flow in the system or no flow encoding gradients on. At flow and flow encoding gradient on images are largely distorted (transversal magnetization is lost in some parts of the image) due to the additional phase shift accumulated before the refocusing pulse train. The phase shift is largely of external origin and is not caused entirely by static magnetic field inhomogeneity as is the case for phase shifts in the rest of the sequence.

I have studied by computer simulations and also by NMR and MRI experiments effects of the initial phase variation to multi spin-echo sequences at different phase cycling schemes. The goal was to find a phase scheme that would be the least sensitive to any initial phase variation and would be therefore applicable for phase registration by fast multi spin-echo imaging sequences. So far I have not found any such phase cycling scheme that would be better than others, since there always exists an initial phase variation at which the performance of any phase cycling scheme is ideal and the other where it is about equally poor for all schemes. Lost transversal magnetization may be recovered is signals acquired by several different phase cycling schemes are acquired.

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Structural Information of paramagnetic metal sites in metalloproteins extracted from ^{13}C-^{13}C COSY.

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The relative intensity of C^{α}-C' cross peaks in homonuclear ^{13}C-^{13}C COSY spectra depends on the relaxation properties of C^{α} and C' spins which, in the proximity of a paramagnetic center, are related to the metal-to-carbon distance. The quantitative analysis of asymmetry in such spectra has lead, for the Cerium substituted dicalcium protein Calbidin D_{9k}, to the straightforward identification of peaks arising from metal coordinating groups. The monodentate or bidentate metal binding mode of carboxylates was directly identified via NMR.

An NMR study of a three-domain construct of ATP7A with copper(I) and the partner HAH1: the interplay of domains

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ATP7A is a membrane-bound P-type ATPase involved in copper(I) homeostasis in humans. It possesses a long N-terminal tail which protrudes into the cytosol and contains six copper(I)-binding domains, which are individually well folded and capable of binding one copper(I) ion. ATP7A receives copper from a soluble protein, the metallochaperone HAH1 and then transfers it to a multicopper oxidase passing through the trans-Golgi organell. The exact role and interplay of the six soluble domains is still quite unclear, as it has been extensively demonstrated that they are strongly redundant with respect to copper(I) transport *in vivo*. To understand the possible molecular mechanisms of ATP7A and the role of the six soluble domains *in vivo,* a three-domain (fourth to sixth) construct has been investigated by NMR, in absence and in presence of copper(I). In addition, its interaction with copper(I)-HAH1 has been characterized. From these studies it can be proposed that the fourth domain is the preferential site for the initial interaction with the partner. A significant dependence of the overall domain dynamics on the metallation state and on the presence of HAH1 is observed. This dependence could constitute the molecular mechanism to trigger copper(I) translocation and/or ATP7A relocalization.

The various behaviour of this construct will be presented and discussed

Evidence of a Bimodal Binding between Diclofenac-Na and β-Cyclodextrin in Solution

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The complexation between diclofenac sodium and β-cyclodextrin was investigated in solution by 1D and 2D ^{1}H NMR. The continuous variation method was used to establish the stoichiometry. The obtained results indicate that simultaneous inclusion of both rings occur giving rise to two isomeric 1:1 complexes. The view of a bimodal binding between diclofenac sodium and β-cyclodextrin was also supported by ROESY experiments. The association constants for the two 1:1 complexes were calculated by non-linear least square regression analysis, applying an iteration procedure. The geometry of the two 1:1 complexes, according to the obtained NMR data is given.

^{13}C Direct Detection in the Study of the Paramagnetic Metallo-Proteins

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In paramagnetic metalloproteins, the Curie and dipolar relaxations may induce significant line broadening of the NMR resonances. Often, they are responsible for the lack of the ^{1}H-^{1}H and ^{1}H-X cross-peaks close to the metal center in 2D/3D spectra. The effect is particularly severe for large metalloproteins and at high magnetic field, and it hampers the study of the region close to the metal center: often the most important part of the protein for the biological activity.

A possible solution may derive from ^{13}C direct detected experiments, taking advantage of the smaller gyromagnetic ratio of carbon that make the Curie and dipolar contributions about 16 times smaller. The consequent lower sensitivity of the ^{13}C detection is at least in part compensated, in the perspective, using dedicated probes (possibly cryo) and/or tailored sequences.

With this in mind, several ^{13}C-^{13}C correlation experiments (^{13}C-^{13}C COCA MQ,[1] ^{13}C-^{13}C NOESY,[2] ^{13}C-^{13}C COSY QF, etc.) were adapted to the observation of paramagnetic proteins, in order to fill the hole of close-to-metal residues lacked in the ^{1}H-detected spectra.[3] Several experiments were conduced on a paramagnetic sample of human oncomodulin, a calcium binding protein in which calcium is replaced by the paramagnetic lanthanides Tb^{3+} and Gd^{3+}.

Compared with a traditional ^{1}H--^{15}N HSQC where only 48% of the backbone peaks are observed in the Tb-Oncomodulin, using ^{13}C direct detected experiments (^{13}C-^{13}C COCA MQ, ^{13}C-^{13}C NOESY, ^{13}C 1D) more than 90% of the backbone peaks can be assigned. The pseudocontact shifts of the close-to-metal assigned carbon provide precious paramagnetic constrains that were used in the structural refinement of the NMR structure around the metal center.

The extension of these techniques to other strong paramagnetic proteins as Gd-oncomodulin will be also discussed.

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Application of STD NMR to an Arabinofuranoside Antibody Complex and a Virus Recognizing Oligosaccharide Determinants

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A. Among the Actinomyces family the gram-positive Mycobacteria play a significant role, as this genus contains M. leprae and M. tuberculosis. Two major polysaccharides are located on the glycocalyx of Actinomyces. The structures of both polysaccharides, Arabinogalactan (AG) and Lipoarabinomannan (LAM), have been published. The furanosidic rings that make up these polysaccharides are only found in lower organisms such as bacteria, parasites, or fungi. Since these saccharides are responsible for the pathogenicity of the microorganisms, and since they are not known in human biology, they are attractive targets for drug design.

A branched hexasaccharide motif caps the terminal ends of LAM and AG plays a key role in antigenic recognition [1]. Consequently, antibodies specifically directed against this antigen have to recognize these furanose rings. Furthermore, novel trends in vaccination strategies rather employ structurally well defined fragments instead of attenuated pathogenic organisms as vaccines. Therefore, an epitope mapping of this hexasaccharide motif is a first step towards the development of potent vaccines.

In order to achieve this goal, we have performed a complete ^{1}H and ^{13}C NMR assignment for this hexasaccharide, and fragments thereof. STD NMR spectra [2] of the saccharides in the presence of a monoclonal antibody CS35 were then used to perform a group epitope mapping to deliver the binding epitope at atomic resolution.

B. The Hepatitis A Virus (HAV) is a non-enveloped icosahedral-shaped single stranded RNA virus. It belongs to the picornaviral family. One of the first steps during host cell infection is the attachment of the virus to its receptor. It is suggested that the receptor is mucin-type glycoprotein (havcr1) consisting of two domains, an immunoglobulin-like domain and a mucin-like domain. A recent study has demonstrated that virus attachment to the host cell is sialic acid dependent [3]. Here we apply STD NMR experiments to explore the binding of sialic acid containing oligosaccharides to native Hepatitis A viruses.

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NMR investigation of complex formation between antimalarial drugs, chloroquine and dioncophylline C, and their presumed target FPIX

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The principal mode of action of some antimalarial drugs is to inhibit the hemozoin formation from ferriprotoporphyrine IX heme (FPIX). The most commonly used antimalarial drug is chloroquine, but growing resistance of the malaria parasites imposes a major issue. A very promising novel antimalarial compound is dioncophylline C [1], which has been isolated from the tropical liana Triphyophyllum peltatum belonging to the plant family Dioncophyllaceae.

We have determined the structure of chloroquine and dioncophylline C bound to FPIX. Distance restraints were obtained using the influence of the paramagnetic metal centre FeIII, found within FPIX, on the relaxation rate of nearby proton spins. These effects depend significantly on the metal-proton distance. In a series of high field NMR inversion recovery experiments at 9.4, 14.1 and 17.6 T the T1 relaxation times of different protons in chloroquine and dioncophylline C were measured. The effective correlation times of FPIX-drug complexes were calculated by fitting the relaxation times obtained at the three field strengths to the Solomon-Bloembergen equation. Precise FPIX Fe-drug H distances for the solution structure of the complexes formed between FPIX µ-oxo dimer and the antimalarial drugs were derived from the relaxation rates. The distances were used to perform distance restrained MD calculations to determine the lowest-energy structures of the complexes. Both drugs form non-covalent complexes with FPIX that are not stabilized by hydrogen bonds. The complexes are presumably stabilized by pi-pi interactions. These findings correspond to previous investigations by NMR and Raman spectroscopy [2-4].

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Molecular recognition in the peroxisomal import pathway studied by NMR spectroscopy

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We have studied various aspects of molecular recognition events that are involved in the peroxisomal import pathway by using a combination of biophysical and structural biology techniques.

The first example involves the tethering of the receptor-cargo complex towards the peroxisomal membrane, which is mediated by a multimeric membrane anchored protein assembly. A central scaffold for docking of the peroxisomal import complex is the cytosolic SH3 domain of the membrane-bound protein Pex13p. Pex14p binds in a classical PxxP-type mode to the SH3 domain. In yeast an additional alpha helical peptide stretch of Pex5p interacts with the Pex13p SH3 domain independently at a structurally separated site. Mutations within the Pex13p-Pex5p interface specifically impair PTS1-dependent protein import into yeast peroxisomes.

In a second example, a non-specific lipid transfer protein was chosen as a model cargo protein to be imported into the peroxisomal organelle. Its peroxisomal targeting signal type 1 (PTS1) is specifically recognized by the tetratricopeptide repeat (TPR) domain of Pex5p, a cytosolic receptor, which conducts PTS1 proteins towards a docking complex at the peroxisomal membrane. Here we show that the PTS1 of sterol carrier protein 2 (SCP2) displays considerable conformational flexibility to facilitate recognition by the TPR domain of Pex5p. This binding mode allows two different isoforms of SCP2 to interact comparably with Pex5p while the overall fold of SCP2 is not altered. NMR binding experiments using spin-labeled paramagnetic fatty acid derivatives show that the lipid binding function of SCP2 is largely unaffected by the TPR interaction. Chemical shift perturbation identifies physiological fatty acid derivatives to bind into a hydrophobic cavity, formed by alpha helices framing a central beta-sheet. The burying of the lipid ligand is accompanied by a change in the backbone dynamics of SCP2 protein. Intriguingly, two regions comprising non-PTS1 residues, which exhibit reduced backbone flexibility upon lipid binding, also mediate secondary contacts for the recognition of the TPR domain.

Structural studies of the C-terminal domain of the human XPF protein

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Nucleotide excision repair (NER) is a DNA repair mechanism that fixes UV light induced DNA damage and also other endogenously inserted DNA damage.

The ERCC1-XPF heterodimer complex is an important NER protein that plays a crucial role in eukaryotes genome stability. ERCC1-XPF complex is a structure specific endonuclease in eukaryotes that preferentially cleaves the duplex DNA adjacent to the 5' side of the DNA lesion.

In archea the XPF exists as a homo dimer while the mammalian XPF forms an obligatory heterodimer with ERCC1. The human XPF can also form symmetric homodimer as is clear from NMR spectrum. Structural data on these classes of proteins show as an important element a characteristic helix-hairpin-helix (HhH) motif.

We study the dimeric C-terminal domain of human XPF protein. Our structural data on monomeric structures are very similar to the XPF present in the recently solved ERCC1-XPF heterodimer where the two HhH domains form a tight complex.

Deeper insight about the preferential in vivo heterodimer formation rather than homodimer formation by human XPF, structural comparison of XPF in heterodimer and XPF as a homodimer and model for DNA binding domain of XPF will be described.

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NMR and molecular dynamics studies of a DNA interstrand cross-link obtained via a novel furan oxidation strategy

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With the advent of antisense and antigene approach, various cross-linking molecules have been synthesized and successfully applied in medicine. An analogous strategy involves the synthesis of short modified oligonucleotides (ONs) that have the possibility to form a crosslink with a complementary strand. This approach is hampered by the fact that the ON must enter into the cell, and at the same time be inert towards other cellular targets.

Furan is toxic due to the in vivo oxidation catalyzed by cytochrome P-450...[1] Inspired by this toxicity our group in Gent has developed a 2'/2"-furan-modified nucleoside, where the furan is used as a masked functionality. This furan can be oxidized in-situ to the corresponding enal to trigger cross-linking with a complementary ON..[2].

With the help of NMR-spectroscopy and molecular modelling, we are currently studying the position of the cross-link and the nature of the chemical bond that is formed.

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The Global Structure and Dynamics of Human Apolipoprotein CIII in Complex with SDS Micelles by NMR Spectroscopy

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High plasma triglycerides are an independent risk factor for coronary heart disease (CHD). Triglycerides are carried in plasma on very-low-density (VLDL) and low-density (LDL) lipoprotein. Apolipoprotein CIII (apoCIII), a strong risk factor for CHD, impairs the metabolism of both VLDL and LDL, thereby contributing to elevated triglycerides. The solution 3-dimensional structure and dynamics of $^{15}N/^{13}C$ labeled human apolipoprotein CIII (apoCIII) in complex with SDS micelles have been studied by high resolution NMR spectroscopy, based on classical NMR constraints and residual dipolar couplings (RDCs), measured in stretched polyacrylamide gels.To study the dynamics of apoCIII-SDS micelle complex, two sets of $^{1}H-^{15}N$ hetero nuclear relaxation data (T1, T1rho and NOE) have been measured at two magnetic fields (^{1}H frequencies of 600 MHz and 800 MHz). The relaxation data were analyzed using a MATLAB program, called PINATA (Larsson et al., JBNMR 2003). In this way, the correlation time (τ_c) and the order parameter (S) of apoCIII micelle complex, in which independent of the time scale of nano-second (ns) and pico-second (ps) internal motions and conformational exchange, have been determined. The orientation information provided from $^{1}H-^{15}N$ relaxation data also verifies the orientation of the apoCIII-SDS micelle complex derived by classical NMR constraints and RDCs. The 3-dimensional structure and the motional free dynamics data of apoCIII in complex with SDS micelles will be presented.

Interaction of Alzheimer's Aβ peptide with ganglioside micelles

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The amyloid b-peptide Aβ is a major component of plaques in Alzheimer's disease, and formation of senile plaques has been suggested to originate from regions of neuronal membrane rich in gangliosides. Here we demonstrate using NMR on ^{15}N-labelled Aβ(1-40) that the interaction with ganglioside GM1 micelles is localised to the N-terminal region of the peptide, particularly residues His13 to Leu17, which become more helical when bound. The sialic acid residue of the ganglioside headgroup is important for determining the nature of the conformational change. The isolated pentasaccharide headgroup of GM1 is not bound, suggesting the need for a binding surface. These results are discussed in the light of previous findings, and imply a model in which binding to ganglioside-rich membranes causes a transition from random coil to helical in the N-terminal region, leaving the C-terminal region unstructured. The key interaction is with His13, which must undergo the correct conformational change in order to stimulate fibrillogenesis. Formation of mature plaques is suggested to arise from formation of β-sheet structure by the C-terminal tails.

NMR structure, DNA binding, and regulation of R. sphaeroides RegA effector domain

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The RegA protein (also known as PrrA) from *Rhodobacter sphaeroides* forms part of a two-component signalling system that controls expression of genes involved in aerobic and anaerobic metabolism and photosynthesis in response to external oxygen pressure. Here we report the structure of the C-terminal DNA-binding domain, and demonstrate that on treatment with beryllium fluoride the full-length protein dimerises, which enables it to bind to DNA. Chemical shift changes on binding to specific and non-specific DNA sequences show how the protein binds to DNA, and have allowed us to make a model of the complex. Comparison of the non-phosphorylated full-length RegA protein (in which the C-terminal domain is bound to the N-terminal domain) with the isolated C-terminal domain defines the interdomain contact, and demonstrates an allosteric effect on the DNA binding surface.

Structure and function of a bacterial fasciclin I domain

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Fasciclin I domains generally occur in cell surface proteins in a wide range of vertebrates, invertebrates, plants and microorganisms, and where characterized have a role in cell adhesion. Here we report the structure, role and regulation of a simple single-domain extracellular bacterial fasciclin I protein, Flp, in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. The solution structure of the 158-residue protein (including a 21-residue His tag) has been determined by NMR and is typical of fasciclin I domains. Inactivation of the *flp* gene in *R. sphaeroides* resulted in significantly reduced ability to form biofilms, the first clear phenotype identified for any microbial fasciclin I protein. Comparison with homologues, in conjunction with the NMR structure, identified a possible binding site. Reporter studies reveal that *flp* transcription is negatively regulated by the global redox-responsive Prr regulatory pathway in *R. sphaeroides* under all conditions of aerobiosis tested, the first demonstration of regulation of a fasciclin I protein in microbial cells.

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Structural study of the interaction between nucleocapsid from HIV-1 and Primer Binding Site (PBS)

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The Hiv-1 nucleocapsid protein plays an important role in many key steps of the virus such as reverse transcription, integration and encapsidation. These properties point it out as an interesting target for new therapies against the virus. To understand the different mechanisms by which NCp7 acts would put a landmark in the design of protein inhibitors, which would exhibit antiviral activity.

This project focuses on the chaperon activity of NCp7 towards nucleic acids during the second strand transfer within reverse transcription.

The strand transfer takes place thanks to an intramolecular destabilisation of the hairpin structure of the Primer Binding Site (PBS) from the (-)DNA strand, followed by a pairing of this oligonucleotide with its complementary sequence present in (+)DNA. The NCp7 would intervene to favour both mechanisms. In the perspective to inhibit this function, we want to figure out how this action is possible from a structural point of view.

Our aim is to study by NMR the destabilizing activity of the NCp7 toward (-)PBS and (+)PBS sequences. We will also focus on (-)PBS hybridization to its complementary sequence (+)PBS. We already determined the structure of the (-)PBS hairpin and evaluated its fusion temperature. We also determined the structure of the complex made of NCp7(12-55) and ΔP(-)PBS.

This project should enable us to understand the molecular mechanism by which nucleocapsid protein acts on PBS structures to modify their stability. The knowledge of its functioning might be useful to design antiviral molecules intervening on the reverse transcription step.

Studying Paramagnetic Proteins via ^{13}C Direct Detected NMR

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Formerly considered as an enclave for inorganic chemists and metalloprotein biochemists, NMR spectroscopy of paramagnetic systems is now a versatile tool for structural biologists. We are currently exploring perspectives opened in NMR of paramagnetic molecules by the use of ^{13}C direct detection. Thanks also to the recent technological developments, this approach provides a valuable alternative to ^{1}H detection to overcome fast relaxation, due to the smaller magnetic moment of the ^{13}C nucleus and promises to become a general strategy for large molecular weight systems.

We present here a series of ^{13}C detected experiments tailored to paramagnetic molecules. They are based on the optimisation of simple building blocks with respect to relaxation properties of the involved spins. Optimised transfers for Ca-C' and C'(i)-C'(i+1) provide a significant improvement of the percentage of residues that can be identified, with respect to ^{1}H detected experiments. They will also identify the binding mode of carboxylate side chain bound to the metal ion. Cross correlation rates involving Curie spin relaxation of ^{13}C nuclei will also be studied. We will show how direct detection of ^{13}C is not only an alternative strategy for signal assignments but is relevant as a source of additional structural constraints.

Influence of calcium and lithium ions on the dynamic properties of molecules in the structure of human red cells membranes.

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The molecular dynamics heterogeneity in the structure of biological membranes has been studied. Red cells membranes (RCM) were obtained from healthy persons, hemolyzed, washed, dried and suspended in D_2O (39 weight %) in the presence of Ca^{2+} and Li^{+}.

NMR with a pulsed gradient (40 T/m) of magnetic field with the ^{1}H-NMR relaxometer, working frequency 64 MHz, produced by the "Magnetic Resonance" factory (Kazan) has been used. The "stimulated echo" method was used, 310K.

The form of diffusion attenuation of samples was complex and for both diffusion times (t_d = 7 ms, 28 ms) could be represented in the form of the sum of exponential components with different spin populations (P) and the self-diffusion coefficients (D), which were identified as: D_m (10^{-9} m^2/s) - average coefficient of self-diffusion, D_a (10^{-11} m^2/s), P_a – organic components (presumably lipids) of RCM, D_b (10^{-10} m^2/s), P_b – bound water, D_c (10^{-9} m^2/s), P_c – unbound water.

Results (parameter (t_d = 7ms) -> parameter (t_d = 28ms)):

1.RCM: D_m = 1,66 → 1,82; D_a =6,5 → 5,9, P_a =0,11 → 0,05 (P<0,02); D_b,P_b - aren't revealed; D_c =1,86 → 1,95, P_c = 0,89 → 0.95.

2.RCM + Ca^{2+}: D_m =1,73 → 1,76; D_a =4,1 → 5,4, P_a =0,10 → 0,08; D_b =53 → 41,9,

P_b = 0,12 → 0,05 (P<0,01); D_c =2,15 → 1,70 (P<0,01), P_c =0,78 → 0.87 (P<0,05).

3.RCM + Li^{+}: D_m =1,47 → 1,39; D_a =4,5 → 4,1, P_a =0,09 → 0,06; D_b,P_b - aren't revealed; D_c =1,60 → 1,47, P_c = 0,91 → 0.94.

Conclusion:

Dynamics heterogeneity of molecules in structure of RCM could be characterized by a lifetime of molecules in the certain state. Time span during which lipids could be in the structure of associates is about 7 ms. In the presence of Ca^{2+} and Li^{+} lifetime of lipids in associates is increased up to 28 ms at least.

Assignment and dynamics analysis of tryptophan side chains in non-native states of hen egg white lysozyme

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Non-native states of hen egg white lysozyme (HEWL) are not fully unstructured, but contain residual structural elements like hydrophobic clusters. This has been shown by the analysis of ^{15}N transverse relaxation rates of the backbone amide in non-native HEWL. Residues involved in hydrophobic clustering show significantly elevated R_2 relaxation rates. The six tryptophan residues in HEWL are particularly important for the formation of the hydrophobic clusters, which has been shown by mutational studies.

Here we show a new way to assign tryptophan side-chain resonances, in order to analyze the dynamics and the solvent accessibility of these residues in non-native proteins. This information is of significant interest, because the side-chains of tryptophan residues in proteins are important mediators of the hydrophobic clustering and of long-range interactions in non-native states.

The investigated non-native protein is all-Ala-HEWL, a mutant, in which all cysteine residues are replaced by alanines, resulting in a permanently non-native state of hen lysozyme.

The $^{13}C_\gamma$ resonances are well enough resolved to assign these peaks unambiguously to a specific residue, whereas the other aliphatic and aromatic ^{13}C resonances are overlapping in many spectra of non-native proteins. The strategy of assigning the tryptophan side-chain indole $^1H^N/^{15}N_\varepsilon$ resonances is to correlate them and the backbone $^1H^N/^{15}N$ resonances with the $^{13}C_\gamma$ resonances. This is done using two distinct experiments based on the standard HNCACB pulse sequence: The HNCδCγand the HNCαCβCγ experiment: The latter includes an additional transfer from the C_β to the aromatic C_γ. The delay durations in the HNCδCγ experiment are optimized for the transfers in the aromatic tryptophan side chain. Both experiments use soft-WATERGATE water suppression.

The assignment of the tryptophan side chain indole $^1HN/^{15}N_\varepsilon$ resonances allow for the analysis of transversal relaxation rates (R_2) in the tryptophan side chains in addition to the backbone relaxation rates. The pattern of R_2 relaxation rates in the backbone and the side chains is very similar: The rates are higher for the tryptophan residues 62, 63 and 111 than for the others, indicating the involvement of these residues in the most pronounced clusters in non-native HEWL.

Two-dimensional Photo-CIDNP NMR experiments have been used to gather information about the different solvent accessibility of the tryptophan side-chains. In these experiments a single reverse INEPT transfer is used for the ^{15}N-^1H heteronuclear correlation in the tryptophan side-chains.

Time and structural resolved studies on RNA conformational switching

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The catalytic and regulatory functions of RNAs, such as ribozymes, riboswitches and the spliceosome, depend strongly on tertiary structure reorganisation and secondary structure refolding. The underlying dynamical diversity is a fundamental property of RNA and is illustrated by the frequent occurrence of multiple folding pathways, metastable and coexisting, bistable folds. Only a few time-resolved studies of RNA refolding from an unpertubated native state have been reported and the complexity of these systems precluded a detailed structural characterization. Here, we report the investigation of time- and structure-resolved RNA refolding and its application to a detailed kinetical characterisation of a bistable 34mer RNA-sequence adopting two coexisting structures. We revealed that the process of interconversion is cooperative with respect to the temperature of the system. Therefore the process can be trapped kinetically.

NMR real time studies of RNA folding

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The catalytic and regulatory functions of RNAs, such as ribozymes, riboswitches and the spliceosome, depend strongly on tertiary structure reorganisation and secondary structure refolding.

New techniques have been developed in our group that allow investigation of RNA folding processes by NMR in real time. Equilibrium as well as non equilibrium processes can be monitored. Application of this technique to several RNA systems (such as bistable RNA sequences and riboswitches) will be shown characterizing secondary structure refolding as well as of tertiary conformational switching events for the first time.

NMR studies on MAP kinase p38

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MAP kinase p38, a prominent anti-inflammatory drug target, was characterized by NMR spectroscopy. Backbone NMR signals were assigned using uniformly and selectively labeled protein samples, spinlabels and the chemical shift matching procedure.

New ligands for p38 can be found and characterized by NMR methods. We show that high-quality protein-ligand structures can be obtained from a chemical shift perturbation-based protocol called Ligdock or from specific labeling protocols (SOS-NMR).

Large parts of the protein escape detection. This is due to exchange linebroadening arising from motional processes. The motional dynamics was studied in detail for the DFG motif in the activation loop. This motif undergoes motions on the millisecond timescale. These motions can be blocked by ligands that bind to the directly DFG motif, but not by ligands that bind to the ATP-site. This gives insight into the dynamics of the DFG motif and opens a new route to quickly distinguish the binding mode of a ligand.

The role of the hinge peptide versus the intersubunit interface in the swapping of N-terminal ends: the unique case of bovine seminal ribonuclease

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Bovine seminal ribonuclease (BS-RNase), the only dimeric protein among the pancreatic-like ribonucleases, is endowed with some special, non catalytic, activities. The native enzyme is a 70:30 equilibrium mixture of two forms, with (MxM) or without (M=M) exchange (or swapping) of N-terminal 1-20 residues. As a consequence, the hinge region 16-22 has a different tertiary structure in the two forms [1, 2].

By selective reduction and alkylation of the two interchain disulfide bridges, the M=M form of BS-RNase can be converted into a monomeric derivative (mBS) which retains the enzymatic activity, but is devoid of other biological functions. A structural comparison between mBS and RNase A in solution [3] and at solid state [4] shows that significant differences are present in the hinge region 16-22. To investigate the role of the regions which are most affected by the swapping, we have expressed variant proteins by substituting several crucial residues with the corresponding ones of RNase A and compared their structural properties with those of the parent protein.

Both A^{19}-BS-RNase and S^{16}-T^{17}-A^{19}-A^{20}-BS-RNase, in which either Pro 19 or all residues of the hinge sequence have been substituted with the corresponding ones of the pancreatic enzyme, show no differences with respect to the parent protein in the swapping process [5, 6], reaching the same 70:30 equilibrium composition in a comparable time.

The relative insensitivity of the swapping tendency to the substitutions in the hinge region, and in particular to the replacement of P19A, contrasts with the role generally assigned to the proline residues in the hinge loop of swapping proteins [5] and can be rationalized in terms of the unique features of the seminal enzyme, where the swapping process involves an equilibrium between two dimers having a similar and highly constrained quaternary association

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Conformational studies of a reversible alfa to beta transition of Aβ-(1-42) in aqueous media

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The design of molecules able to interact with amyloid peptides to inhibit fibril deposit or membrane pore formation induced by amyloids is of paramount importance in the development of anti-Alzheimer therapies. Current views on the role of the fibrils of amyloid peptides range from regarding them as the cause of Alzheimer pathology to assigning them a protective function. In both cases the study of the conformational properties of Ab-peptides in soluble form constitutes a basic approach for the design of molecules with "anti-amyloid" activity. We have monitored by CD and NMR different conformational states of Aβ-(1-42) in media of different polarity by changing the composition of mixtures of water and hexafluoroisopropanol. The transition of Aβ-(1-42) from a to b conformations occurs when the amount of water is higher than 80%. At high water contents, before the transition point, there is a drastic modification of the peptide folding, characterized by a decrease of the helical character in the C-terminal region, while a significant regularity in the structure is conserved in the N-terminal region. The b conformation can be observed up to 99.8% water (molar), but can be reverted to a-helix simply by adding the appropriate amount of HFIP. The complete conformational path was investigated in detail with Molecular Dynamics simulations in explicit solvent, leading to a precise localization of residues that seed b conformations. The structures obtained might help to find the regions more affected by the environmental conditions also *in vivo*.

NMR structural studies of MIP-1β interaction with peptides derived from the HIV-1 co-receptor CCR5

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CCR5 is a co-receptor for HIV-1 entry into its target cells. The CC chemokine MIP-1beta is one of CCR5 natural ligands, and was shown to inhibit HIV-1 infection. The third variable region (V3 loop) of the gp120 envelope glycoprotein of HIV-1 is involved in CCR5 binding. The V3 loop exhibits structural homology as well as some sequence homology to the 40s loop of MIP-1beta. Therefore, study of the interactions between MIP-1beta and CCR5 is of interest for better understanding of HIV-1 cell entry and drug design. The focus of this study is the characterization of binding of MIP-1beta to CCR5 derived peptides and the structure determination of the formed complexes by NMR. MIP-1beta was expressed in *E. Coli* and successfully bound to a 27-residues long N-terminal CCR5 peptide sulfated at Y10 and Y14. Binding was verified by comparing the NMR spectra of the free and the bound peptides. HOHAHA spectra acquired with a long mixing time were used for this purpose. The binding determinant was mapped using NMR dynamic filtering techniques. Uniformly labeled $^{13}C/^{15}N$ MIP-1beta was successfully expressed using the same system. Sequential assignment of the labeled MIP-1beta will be obtained. ^{15}N-^{1}H HSQC spectra of MIP-1beta titrated with the disulfated CCR5 peptide will be used to map the binding site for CCR5 on MIP-1beta.

Insight into the catalytic activity of HECT domain ubiquitin-protein ligases

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Ubiquitination targets proteins for degradation thereby regulating the abundance of key regulatory proteins in the cell. This reaction is catalyzed by the successive action of an ubiquitin (Ub)-activating (E1), an Ub-conjugating (E2) and an Ub-ligating (E3) enzyme. Members of the Nedd4 family of E3s contain an N-terminal C2 domain of 14kD, multiple WW domains of 4kD each, and a C-terminal catalytic HECT domain of 45kD. Despite recent crystal structures, details of Ub and substrate recognition and consequently the mechanism of HECT domain activity have remained elusive. As a first step towards understanding HECT domain activity we have investigated intra- and intermolecular interactions of the Nedd4 E3 Smurf2 by chemical shift titrations. Our data show that the HECT domain directly recognizes its substrate Smad7 at a site spatially distinct from the E2 binding region thus allowing for a simultaneous interaction of the HECT domain with both the substrate and the Ub-conjugating enzyme. Despite the relatively high molecular weight of the complex we were also able to map the interaction surface between the HECT domain and Ub yielding the first structural insight into the mode of ubiquitin binding by HECT domain-containing E3s. Intramolecular interaction of the N-terminal C2 domain with the HECT domain suggests that the ~1000-residue Nedd4 E3 proteins may adopt a rather compact structure in solution. Whether C2 domain binding provides a means of autoinhibition in HECT domain-containing Ub-ligases is currently under investigation.

Structural basis of Ca^{2+} regulation in the Na^{+}/Ca^{2+} exchanger

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The plasma membrane Na^{+}/Ca^{2+} exchanger (NCX) utilizes the Na^{+} gradient generated by the Na,K-ATPase to rapidly remove Ca^{2+} from animal cells. Binding of intracellular Na^{+} and Ca^{2+} to sites located in the large cytosolic loop of NCX regulates this extrusion.

We report the solution structures of the two regulatory Ca^{2+} binding domains, CBD1 and CBD2 that form an insertion within a membrane-peripheral α-catenin-like domain. Together, the three modules compose the entire cytosolic exchanger loop. CBD1 and CBD2 resemble the overall architecture of cadherin domains, however arrange in an anti-parallel rather than a head-to-tail fashion.

Analyses of the domains in the presence and absence of Ca^{2+} indicate dramatic structural changes in CBD1, but not in CBD2. The alternative splicing site present in CBD2 encodes a large part of the Ca^{2+} binding sites and appears to modulate Na^{+}/Ca^{2+} exchange as required in different tissues. We also present a model of the near complete cytosolic loop, which highlights crucial elements for the regulatory role in response to Ca^{2+} of this part of the NCX molecule.

In addition, CBD1 and CBD2 constitute the first protein structures representing the widely debated Calx-β motif and permit homology modeling of a GPCR of the secretin family as well as a domain in integrin β4.

Binding of aminoacids to proteins and polyelectrolytes

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PFG NMR permits the measurement of the diffusion coefficient and thus the hydrdynamic friction of molecules and complexes in solution. If in addition an electric field is applied the electrophoretic mobility is measured. From the ratio of both the charge of the molecule or complex is determined..

Glutamic acid in solution exhibits a negative electrophoretic mobility, that is increasing by value with pH due to the increasing degree of dissociation, which also leads to a growing solvation shell as reflected in the reduced diffusion coefficent. A similar behaviour is determined if glutamic acid is in solution with a polyelectrolyte (poly diallydimethyl ammonium chloride). The lower electrophoretic mobility compared to the free acid is due to exchange between bound and free acid on the time scale of the experiment. At high pH the acid is fully dissociated and strongly binds to PDADMAC resulting in an electrophoretic mobility close to that of the polycation. A similar effect is seen in the diffusion coefficient, the effect is much more pronounced in the electrophoretic mobility. For more complex systems a novel three-dimensional experiment is demonstrated, that correlates chemical shift, diffusion coefficient and electrophoretic mobility. In the second dimension the electric field strength is incremented while in the third the strength of the magnetic field gradient. FT, States FT and inverse Laplace transform yield a correllation of chemical shift, electrophoretic mobility and diffusion cioefficient.

Effect of Cysteines on the Activity of a Retroviral Protease.

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Mason-Pfizer monkey virus encodes an aspartic protease (M-PMV PR), which is essential for the correct assembly and maturation of the viral particles. Primary sequence of the protease contains two cysteine residues (Cys7, Cys106) that were replaced by alanines in order to stabilize the monomeric form of the 12 kDa protease for structural study by NMR spectroscopy [1]. However, it was demonstrated that the cysteines were not indifferent amino acid residues and they affected maturation of assembled M-PMV capsids by a reversible oxidative modification [2].

Here we present results of structural and biochemical study on the inactive mutants (D26N) of the 12 kDa form of M-PMV PR with either one (C7A, C106A) or both cysteine residues preserved. We demonstrate that replacement of both cysteines affects not only the stability of the protease but also the proteolytic activity of the enzyme. MALDI-TOF measurements of cyanobromide digests of PR_{D26N} proved the existence of an intramonomeric disulphide bridge, which helps to stabilize the monomeric scaffold. Consequently, the chemical equilibrium between monomer and dimer is shifted towards dimer. A comparison of three-dimensional structures of monomers of mutants $PR_{C7A/D26N/C106A}$ and PR_{D26N} revealed minor changes especially on the dimeric interface.

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Solution structure and DNA binding of the zinc-finger domain from DNA ligase IIIα

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DNA ligase IIIα carries out the final step in the base excision repair (BER) and single strand break repair (SSBR) mechanisms of DNA repair. The enzyme recognises and binds single-strand nicks and other damage features in double-stranded DNA, both through the catalytic domain and an N-terminal domain containing a single zinc finger. The latter is homologous to other zinc fingers that recognise damaged DNA, two in the N-terminus of poly(adenosine-ribose)poymerase-1 (PARP-1) and three in the N-terminus of the *A. thalania* nick-sensing DNA 3'-phosphoesterase (AtZDP). Here we present the solution structure of human DNA ligase IIIα, the first structure of a finger from this group. It is related to that of the erythroid transcription factor GATA-1, but has an additional N-terminal β-strand and C-terminal α-helix. Chemical shift mapping using a DNA ligand containing a single-stranded break showed that the DNA-binding surface of the DNA-ligase IIIα zinc finger is substantially different from that of GATA-1, consistent with the fact that the two proteins recognise very different features in the DNA. Likely implications for DNA binding are discussed.

Structure of the Calcium Free Form of the C-Type Lectin-Like Domain of Tetranectin

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Tetranectin is a homotrimeric protein containing a C-type lectin-like domain. This domain (TN3) can bind calcium, but in the absence of calcium, the domain binds a number of kringle-type protein ligands. Two of the calcium-coordinating residues are also critical for binding plasminogen kringle 4 (K4). The structure of the calcium free-form of TN3 (apoTN3) has been determined by NMR. Compared to the structure of the calcium-bound form of TN3 (holoTN3), the core region of secondary structural elements is conserved, while large displacements occur in the loops involved in calcium or K4 binding. A conserved proline, which was found to be in the cis conformation in holoTN3, is in apoTN3 predominantly in the trans conformation. Backbone dynamics indicate that, in apoTN3 especially, two of the three calcium-binding loops and two of the three K4-binding residues exhibit increased flexibility, whereas no such flexibility is observed in holoTN3. In the 20 best nuclear magnetic resonance structures of apoTN3, the residues critical for K4 binding span a large conformational space. Together with the relaxation data, this indicates that the K4-ligand-binding site in apoTN3 is not preformed.

Aspects of Molecular Recognition in Glycopeptide Antibiotics

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For some time, glycopeptide antibiotics have been considered the last line of defense against *Methicillin Resistant Staphylococcus Aaureus* (MRSA). However, vancomycin resistance of Gram-positive bacteria is an increasingly emerging worldwide health problem. The mode of action of glycopeptide antibiotics involves binding of peptidoglycan cell-wall fragments terminating in D-Ala-D-Ala sequence to the carboxylate anion binding pocket of the antibiotic. Dimerization (oligomerization) of the antibiotic enhances the antibacterial effect. Oligomers associated with contiguous back to back and face to face dimers were observed in the crystal structure of vancomycin bound to Nac-D-Ala; the question arises if they persist in aqueous solution which more closely mimics in-vivo conditions. A recent theoretical molecular dynamics calculation suggested that the known cooperativity between antibiotic dimerization and ligand binding can be explained by the non-additivity of entropic costs of dimerization and ligand binding. NMR order parameters S^2 can be derived from ^{15}N NMR auto and cross-relaxation, and they sample a similar fraction of internal timescales (ps-ns range) and can potentially disclose the dynamic aspects of binding. ^{15}N CSA-DD cross-correlated relaxation yielded information on the cis peptide bond in the sequence. Oligomerisation state was determined from global correlation time and DOSY experiments. Possible atropisomers in the aglycones was searched by a new HSQMBC method capable for accurate measurement of heteronuclear long-range scalar couplings.

NMR structure of the 17th domain of Human Filamin A

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Human filamin A (FLNA) is a 24 subdomain protein which binds to actin filaments [1]. It has a role in cytoskeleton and cell signaling. We have solved the solution structure of the 17th domain of human filamin A (FLNA17) using high-resolution NMR spectroscopy. The domain 17 was chosen under investigation since earlier studies have pointed out its role in FLNAs interactions with glycoprotein-Ibα (GP-Ibα) [2]. GP-Ibα is a part of the von Willebrand factor receptor complex which initiates platelet adhesion to injured blood vessel wall [3].

A set of 3D triple-resonance NMR experiments was recorded for sequential backbone and side chain resonance assignment. 3D ^{13}C- and ^{15}N-edited NOESY spectra were recorded to obtain the structural constraints. The assignment of ^{1}H, ^{15}N and ^{13}C resonances was done manually using the program Sparky [4]. The NOE constraint based structure calculations were performed with automatic NOE assignment and structure calculation mode of the program CYANA 2.0 [5].

The rod-like structure of FLNA17 consists of antiparallel b-strands and a short helical segment. The structure closely resembles the immunoglobulin-like fold of F-actin cross-linking gelation factor [6]. The solution structure of free FLNA17 was compared with the structure of FLNA17 complexed with GP-Ibα peptide (the structure of the complex solved by X-ray diffraction). GP-Ibα peptide binds next to the one of the b-strands. However, FLNA17 does not undergo any major structural changes upon interaction with the peptide.

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KChIP4a: Towards a solution structure and a characterization of its interaction with the Kv4.3 N-terminus

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K^+ channel interacting proteins 1-4 (KChIPs) are members of the recoverin/NCS1 family of EF-hand calcium-binding proteins. They interact specifically with the cytoplasmic N-terminus of Kv4 voltage-gated K^+ channels and modulate both channel gating and trafficking. Different from the other KChIPs, KChIP4a removes rapid inactivation of Kv4 channels by virtue of its KIS (K^+ channel inactivation suppressor) domain, an approximately 35 amino acid peptide at the very N-terminus of the protein. At present, neither the molecular mechanism behind the inactivation removal nor the structural properties of the Kv4-KChIP4a interaction are understood.

We use heteronuclear NMR spectroscopy on triple-labeled KChIP4a from a bacterial expression system to characterize its solution structure and its interaction with the Kv4.3 N-terminus. TROSY-based backbone assignment is only suitable for the metal-bound form of the 27.5 kDa protein. NMR data show that KChIP4a can bind both Mg^{2+} and Ca^{2+}, with EF hands 3 and 4 exhibiting a strong preference for Ca^{2+}. EF hand 2 binds Mg^{2+} even in the presence of low concentrations of Ca^{2+}, suggesting that a $1Mg^{2+}/2Ca^{2+}$ state is physiologically relevant. Chemical shift mapping in ^{1}H-^{15}N correlation spectra indicates a moderate to low affinity for the interaction between the N-terminus of Kv4.3 (amino acids 1-40) and KChIP4a.

Characterization of Ligand Binding with NMR

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The saturation transfer difference (STD) NMR technique offers a fast and informative way to explore ligand binding to protein. For a sample, a small amount of protein and large excess of ligand or ligands, binding of which is to be studied, is needed. One of the advantages with NMR is that it does not destroy your sample. In addition, the method is relatively fast to perform; only two 1D spectra are required. Depending on the concentrations used, one can obtain information about the binding during one day or even in one hour. One of the most important features is that the relative intensities of STD spectrum signals tells the binding epitope of the ligand. The aim of the present study was to test the binding of several ligands to PR-10c-His protein and to determine the binding epitope of the ligands in order to explore the role of PR-10 proteins in stress response and plant defence.

Interaction of cations with dimeric G-quadruplexes

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G-rich sequences can adopt multistranded DNA structures called G-quadruplexes that are build of stacked G-quartets. G-quadruplex DNA structures are a subject of considerable interest since their formation has been suggested to play a significant role in variety of important biological processes as well as due to their potential therapeutic applications. Recent studies have provided evidence of G-quadruplex formation *in vivo*. One of the most abundant sites with DNA sequences capable of forming G-quadruplex structures are telomeres, protein-DNA complexes located at the ends of chromosomes. Telomeric DNA consists of multiple copies of repeating units of G-rich DNA like TTTTGGGG in the protozoa *Oxytricha nova*.

DNA oligonucleotide $d(G_3T_4G_4)$ forms a dimeric G-quadruplex in the presence of K^+ ions. NMR structure of $d(G_3T_4G_4)_2$ G-quadruplex exhibits G-quadruplex core consisting of three stacked G-quartets. The two overhanging G3 and G11 residues are located at the opposite sides of the end G-quartets and are not involved in G-quartet formation. $d(G_3T_4G_4)_2$ G-quadruplex represents the first bimolecular G-quadruplex where end G-quartets are spanned by diagonal (T4-T7) as well as edge-type loops (T15-T18). Three of the G-rich strands are parallel while one is antiparallel. The influence of different monovalent cations on the folding of $d(G_3T_4G_4)$ has been studied. Minor differences in key intra- and interquartet NOE intensities in the presence of K^+, Na^+ and NH_4^+ ions are consistent with subtle structural differences while retaining the same folding topology of $d(G_3T_4G_4)_2$ G-quadruplex.

G-quadruplexes exhibit cation binding sites between G-quartets. The use of 2D 1H-15N NMR experiments enabled analyses of $^{15}NH_4^+$ ion movement between the binding sites within the $d(G_4T_4G_4)_2$ G-quadruplex. Na+ ions exhibit a smaller ionic radius that allows them to be coordinated in the plane of G-quartets. The lifetime of the inner ammonium ion within the $d(G_4T_4G_4)_2$ is 270 ms at 298 K. On the other hand, the lifetime of bound Na^+ was found to be 180 μs at 293 K. We studied movement of $^{15}NH_4^+$ ion as a function of increased ionic strength, and in the presence of smaller Na^+ ions which partially occupy binding sites inside $d(G_4T_4G_4)_2$.

NMR Studies of the Structure and Dynamics of a 32-Mer RNA Mimicking the Interaction of a Micro-RNA with its mRNA Target

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Two classes of small single-stranded RNAs (~22 nt), namely short-interfering RNAs (siRNAs) and microRNAs (miRNAs) have been recently identified as key post-transcriptional regulators of gene expression. It has been suggested that only the degree of complementarity between the small RNA and its target determines whether it will induce cleavage of the mRNA or translational repression upon incorporation into the siRISC or miRISC complex, which have been shown to be functionally interchangeable.

Among the several hundreds of known miRNAs, the 21-nucleotide Let-7 has been one of the most extensively studied, owing to its essential role in the regulation of developmental timing, as evidenced in C. Elegans. Hutvágner and Zamore (Science, **2002**, *297*, 2056) have shown that Let-7 can also act as siRNA and cleave its target once it forms a complex with a perfectly complementary RNA, whereas in association with the 3'-UTR of the mRNA Lin-41, it prevents expression of the Lin-41 protein. In the Let-7/Lin-41 complex, only 4 nucleotides of Lin-41 are believed not to be involved in any base-pair with Let-7.

In this work, we will present our NMR studies of the structure and dynamics of a 32-mer RNA construct that has been designed to mimic the Let-7/Lin-41 complex. The uniformly 13C,15N labeled 32-mer RNA has been prepared enzymatically by T7 RNA polymerase transcription. Homonuclear and heteronuclear NMR experiments on this 32-mer hairpin have been performed to ascertain the detailed structure of the asymmetric internal loop and of the adenosine bulge, in particular with respect to potential hydrogen-bonding and stacking interactions. One of our goals was to investigate how the combined presence of an internal loop and bulge, which indeed appears in a broad range of miRNA/mRNAs complexes, affects the structure of the complex.

Hybrid analysis of the conformational dynamics of Z1Z2, a double-Ig segment from the N-terminus of titin

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The experimental determination of the conformational dynamics of biomolecules remains one of the most challenging tasks of modern structural biology. Although a plethora of techniques are currently available to investigate the dynamics or the structure of proteins, they are limited in scope and generally offer only partial glimpses into molecular properties. This is particularly limiting when examining pliant proteins with a high intrinsic flexibility.

In this work, we have examined a two Ig doublet from the N-terminus of titin, termed Z1Z2, essential to human muscle structure and development. We present a combined structure determination of this double domain construct by X-Ray crystallography and NMR where residual dipolar couplings were used to refine the domain orientation of the crystal structure. RDCs present an elegant way of determining domain arrangements but due to the known degeneracy of the RDCs structure calculations of a double domain construct results in four different domain orientations that equally well fit the experimental data. Additional information from a second set of RDCs obtained in a different alignment medium is needed to remove the degeneracy and determine the domain orientation in unambiguous way. The search for appropriate alignment media can, however, be tedious especially since interactions with the medium have to be avoided. Multi-domain constructs that are not entirely rigid may also suffer from interference with the medium that influences their mutual orientation. We used a different approach and compared the results of the RDCs structure refinement with small angle x-ray scattering data that are also obtained under solution conditions. This allowed us to select for one preferred orientation for all three data sets of RDCs and confirm that in solution the domains are markedly more bent than in the crystal.

15 N relaxation data in conjunction with elastic network models allowed us to dissect the conformational and dynamic properties of this fragment. Our results provide a dynamic view of this duet, gained by interpreting crystal structures in the light of data from SAXS and NMR and by MD calculations, all of it which provides static, dynamic, population averaged or sorted information.

Glycyrrhizic acid, a natural inhibitor of the pro-inflammatory activity of High Mobility Group Box 1 protein (HMGB1), functional and structural studies.

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The HMGB1 is a 22 kDa protein with tripartite domain organization, composed of two similar DNA binding domains (HMG box A and B) with an acidic C-terminal tail of 30 residues. HMGB1has a pivotal role as intracellular and extracellular mediator: inside the cell it is a transcription and growth factor, outside it acts as cytokine for lethal systemic inflammation, arthritis and local inflammation (Yang et al. 2004). In particular, recent studies have shown that HMGB1 induces diffuse inflammation in skeletal muscles and is highly expressed in dystrophic muscles (Palumbo et al. 2004). The understanding of the mechanisms underlying its inflammatory activity are therefore of enormous clinical interest and recent research has focused on the identification of effective HMGB1 inhibitors.

We have identified glycyrrhizic acid (GL) as a new modulator of the cytokine activity of HMGB1. GL is a natural triterpene exhibiting a wide range of known pharmacological properties ranging from anti-inflammatory to anti-cancer activities (Baltina 2004). By NMR chemical shift mapping and fluorescence we have shown that GL and its derivative carbenoxolone interact directly with HMGB1 full length with micromolar affinity. The interaction sites are mainly clustered on the first helix of the two HMG boxes, involving highly conserved residues in the HMG family. X-filtered NOESY experiments show that the proton H12 and several methyl groups of GL are in direct contact with aromatic residues of HMGB1. From the functional point of view GL inhibit the HMGB1 induced migratory response and proliferation of endothelial cells and mesoangioblasts. Collectively, these results suggest that GL and derivatives are potential anti-inflammatory drugs which will be also tested as potential therapeutic agents in the treatment of muscular dystrophy. In future we will characterize the complex ligand-HMGB1 at atomic level by the combined use of experimental (NMR) and computational methods (Molecular dynamics, ab-initio calculations, docking methods).

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Characterisation of the Nucleotide-Binding Domain of the Human Mitochondrial ABC Transporter ABCB6 by Hetronuclear Multidimensional NMR and Homology Modelling

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Human ABCB6 (MTABC3) protein is a mitochondrial half-type ATP-binding cassette (ABC) transpoter. Though the function of ABCB6 has not been identified, this protein is proposed to be responsible for the iron homeostasis in mitochondria, as is the case with its Saccharomyces cerevisiae homologue, Atm1p, which transports a precursor of the iron-sulphur (Fe/S) cluster from the mitochondrial matrix to the cytosol.

In order to understand the structural basis for the conformational changes accompanying the substrate transportation cycle, which is controlled by the ADP/ATP-binding and ATP hydrolysis, we have studied the C-terminal nucleotide-binding domain of ABCB6 spanning residues Phe558-Arg842 (ABCB6-C) in both the nucleotide-free and ADP-bound states by heteronuclear multidimensional NMR.

Backbone resonance assignment on the nucleotide-free form of ABCB6-C showed that approximately 30% of non-proline residues, which are mainly distributed around the nucleotide binding loop regions and in the helical domain, were consequently not 'assigned'. This is presumably due to the exchange regime between multiple conformations around these residues at an intermediate rate on the NMR time scale. The localised conformational multiplicity remains in the ADP-bound state with the exception of the regions responsible for the recognition of adenine base and α/β-phosphate groups. We also performed homology modelling of ABCB6-C, which was used to interpret the obtained NMR data including the chemical shift perturbation upon the ADP-binding. These results propose some kind of dynamic cooperativity in conformations between the nucleotide-binding loop regions and the helical domain mediated by the conformational rearrangement of the Q-loop, which connects the RecA-like domain and the helical domain. Since the helical domain is proposed to be responsible for the interaction with the transmembrane domain, this hypothetical cooperativity may explain the mechanism of coupled functions between the nucleotide binding domain and the transmembrane domain in the substrate transportation cycle of ABC transporters.

NMR investigations into the molecular recognition of donor and acceptor substrates of human blood group B galactosyltransferase in solution

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The histo blood group ABO(H) antigens are responsible for failure of mismatched blood transfusions, playing also important roles in cell development, differentiation and oncogenesis. The biosynthesis of blood group antigens is under the control of glycosyltransferases which catalyze the transfer of monosaccharide residues from activated sugars such as uridine-5'-disphospho-a-D-galactose (UDP-Gal) onto acceptor substrates. Human blood group A or B antigens are obtained by the transfer of an α-D-GalNAc, or α-D-Gal residue, respectively, onto the H-type disaccharide α-L-Fuc-(1,2)-β-D-Gal-OR. Employing UDP-GalNAc or UDP-Gal as glycosyl donors the transfer reactions are catalyzed by α1,3 *N*-acetyl-galactosaminyl-transferase (GTA, EC 2.4.1.40), or α1,3 galactosyl-transferase (GTB, EC 2.4.1.37) to yield the A antigen α-L-Fuc-(1,2)-[α-D-GalNAc-(1,3)]-β-D-Gal-OR, or the B antigen α-L-Fuc-(1,2)-[α-D-Gal-(1,3)]-β-D-Gal-OR, respectively. Both enzymes recognize the H-type disaccharide as the acceptor, and the transfer reactions occur with retention of configuration at the anomeric centre of the GalNAc, or Gal unit. Although the A and B antigens only differ in the presence of an *N*-acetyl group instead of a hydroxyl group, the consequences of mismatched blood transfusions are fatal. A recent scientific highlight was the elucidation of the crystal structures of GTA and GTB in complex with UDP and the H-type disaccharide. Yet, many questions regarding the catalytic mechanism remain unsolved.

We have carried out a detailed NMR binding analysis with the retaining blood group B α1,3 galactosyl-transferase, GTB, and its donor and acceptor substrates utilizing STD NMR experiments to infer the ligand binding epitopes at atomic resolution, and transferred NOESY experiments to deduce the bioactive ligand conformations. For the acceptor ligands, STD NMR experiments unambiguously show that the H-type disaccharide acceptor α-L-Fuc-(1,2)-β-D-Gal-octyl, as well as the acceptor substrate analogue α-L-Fuc-(1,2)-β-D-3-deoxy-Gal-O-octyl bind to GTB, even in the absence of donor substrate, or Mg^{2+}. This observation was further substantiated by surface plasmon resonance experiments for the H-type disaccharide yielding a K_D value in the μ-molar range. The bioactive conformation and the binding epitopes of the acceptor ligands are in remarkable accordance with the X-ray data except for the orientation of the hydrophobic aglycon. For the donor ligands, the experiments reveal that the binding epitopes of UDP-Gal and UDP-Glc are almost identical although only UDP-Gal serves as a donor substrate. This result is in sharp contrast to the previously studied inverting b-1,4-galactosyltransferase where pronounced differences were observed for the recognition of UDP-Gal and UDP-Glc. From the STD NMR experiments it is further concluded that the enzyme binds to UDP-Gal and UDP-Glc in the "closed" form. Since there is no crystal structure available yet for the complex between GTB and UDP-Gal, it is proposed a docking model that is in accordance with the experimental STD data, and that explains how the enzyme may distinguish between UDP-Gal and UDP-Glc.

Structural Information through Cross-Correlated Relaxation in 6PGL

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The parasitic protozoa *Trypanosoma brucei* is transmitted to humans through the bite of the Tse-Tse fly, causing sleeping sickness in sub-Saharan Africa. Because the parasites tend to acquire increasing resistance to available drugs, there is a crucial need to identify new targets in order to develop new therapies against this highly disabling disease. The Pentose Phosphate Pathway (PPP) plays a crucial role in the parasites. A better characterization of the associated enzymes is necessary to evaluate this pathway as a potential drug target. In particular, the role and mechanism of action of a particular enzyme, 6-phosphogluconolactonase (6PGL), needs to be better understood.

6PGL is a 266 amino-acid protein, with a molecular weight of 30 kDa. Only a limited number of structures of proteins of this size have been solved by NMR, because the spectra of such proteins usually suffer from poor sensitivity and large line-widths that are due to rapid proton relaxation. The spectral assignment of proteins over 25 kDa can only be achieved using ^{2}H, ^{15}N, ^{13}C triply labeled proteins. However, the determination of the 3D structure, which relies on estimates of distances between protons through the nuclear Overhauser effect, still remains a serious challenge even for per-deuterated proteins. NOE spectra of such samples contain only signals between exchangeable protons, which provide too few distance constraints to reconstruct the structure.

We propose a new strategy, based on recent NH^{N}-$C^{\alpha}H^{\alpha}$ dipole-dipole cross correlation experiments, for the determination of Ψ torsion angles in 6PGL. One of the practical problems encountered in this study is that the relationship between the Ψ-angles and the experimental cross correlation rates is not unique. Indeed, because of the periodic dependence of the relaxation rates on Ψ, up to four values of Ψ_{exp}, are compatible with a given relaxation rate, so that the Ψ-angles are underdetermined. We therefore used the TALOS software as a companion tool. Our method consists in confronting Ψ_{pred} angles predicted by TALOS with the different Ψ_{exp} angles that are compatible with experimentally measured relaxation rates. If one of the angles Ψ_{exp} matches the value Ψ_{pred} estimated by TALOS, this Ψ_{exp} is retained as the "true" value. The angle Ψ_{exp} can then be used as a structural constraint, in contrast to Ψ_{pred} which is based on statistical analysis.

Evaluation of cross-correlation effects between Hα-Cα dipolar and Hα-Curie-spin interactions in paramagnetic proteins

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Long-range geometry restraints can improve the quality of biomolecular structures determined by NMR. In recent years, the angular dependence of cross-correlated relaxation arising from the interference of different relaxation mechanisms has been exploited frequently [1, 2] to derive long-range orientation restraints. In metal containing paramagnetic proteins, the electron-nuclear interactions can provide distance and angle restraints. In particular, Curie-spin relaxation (CSR) [3] delivers angular restraints via cross-correlation effects with dipole-dipole(DD) interactions. Previously the structural dependence of $^{1}H_{N}$-^{15}N CSRxDD cross-correlated relaxation in paramagnetic proteins has been demonstrated [4, 5]. Here, we report on the use of $^{1}H\alpha$-$^{13}C\alpha$ CSRxDD cross-correlation effects to derive additional angular restraints for the $^{1}H\alpha$-$^{13}C\alpha$ bond vectors with respect to the paramagnetic center. Quantitative measurements of these effects were achieved by the application of a spin state selective HACACO- αβ differential relaxation experiment. The approach was tested by application to recombinant doubly labeled paramagnetic high-spin (fluoro,aquo) and low-spin (cyano) met-myoglobin complexes. Preliminary results obtained from differential relaxation rates of the $^{13}C\alpha$ coupled Hα doublet components show good agreement to the rates predicted from the crystal structure.

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NMR studies of the dynamics and metal exchange properties of two C4C4 RING domains

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Zinc fingers are small structured proteins that require the coordination of zinc for a stable tertiary fold. Together with FYVE and PHD, the RING domain forms a distinct class of zinc-binding domains, where two zinc ions are ligated in a cross-braced manner, with the first and second pairs of ligands coordinating one zinc ion, while the second and fourth pairs ligate the other zinc ion. We studied the dynamcis and metal exchange of the C4C4 RING domains of CNOT4 and the p44 subunit of TFIIH. We found that Zn^{2+}-Cd^{2+} exchange is different between the two metal-binding sites in the C4C4 RING domains and also differs dignificantly between the two proteins. In order to understand the origins of these distinct exchange rates, we have studied the backbone dynamics of both domains in the presence of zinc and of cadmium by NMR spectroscopy. The differential stability of the two metal-binding sites in the RING domains, as reflected by the different metal exchange rates, can be explained by a combination of accessibility and an electrostatic ion interaction model. The higher metal exchange rate in the p44 RING domain correlated with an increased backbone flexibility as compared to CNOT4. This difference in dynamics may be related to the distinct types of protein-protein interactions that the two C4C4 RING domains are involved in.

Towards complete assignment of proteins through protonless NMR

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The complete heteronculear sequence specific assignment of proteins is feasible through ^{13}C direct detection 3D experiments thanks to the improvements in probe technology and to the development of new NMR experiments. The set of NMR experiments we present is based on direct detection of ^{13}CO and ^{13}Ca through spin state selective methods to remove the large one bond ^{13}C-^{13}C scalar coupling constants in the direct acquisition dimension (IPAP for ^{13}CO and DIPAP for ^{13}Ca). The experiments presented here allow us to identify spin systems (CACO-IPAP, CBCACO-IPAP, CCCO-IPAP, Ca-TOCSY-DIPAP) and to link them in a sequence specific manner (CON-IPAP, CBCACON-IPAP, CANCO-IPAP). This "protonless NMR" strategy is demonstrated on monomeric and dimeric Cu,Zn superoxide dismutase [1-3]. It offers alternative solutions for demanding situations (paramagnetic and/or large molecules) and it can be useful in general in conjunction with conventional experiments. The set of experiments developed now allows us to perform protein sequence specific assignment of backbone and side chains.

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Folding Rules for Branched DNA Prediction of alignment of nucleic acids in aligned media

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On the basis of high-resolution structural data we have recently shown[1,2] that two folding rules can completely account for the stacking behavior of all reported DNA three-way junctions containing two unpaired residues at the junction (3HS2). These two rules can now be used to predict the stacking preference of 3HS2 molecules of any sequence. Following this procedure we designed three novel sequences that were predicted to fold into a preferred A/C-stacked conformation. NMR data indeed show that all three new 3H molecules fold into a stable A/C-stacked conformation. Residual dipolar couplings (RDC) were crucial to derive the first high-resolution DNA 3H structures. To further improve the usage of RDCs in DNAs and RNAs and specifically branched DNAs we developed a fast analytical method for alignment prediction. We demonstrate for nucleic acids in aligning medium (i.e. Pf1 phage) that the molecular alignment tensor **A** can be predicted reliably and accurately, via a simple and fast calculation based on the radius of gyration tensor spanned out by the phosphate charges. The 26 published RDC-derived DNA/RNA structures provided sufficient statistics to validate the proposed prediction approach. We also show theoretically that pure electrostatic alignment leads to a quadratic dependence on size, while steric alignment is linearly dependent on size. Moreover, our analytic expression predicts that under usual conditions of salt strengths the alignment essentially independent of salt strength in good agreement with published experimental observations.

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Motion in the Binding Site for Human U1A Protein: A NMR Study of RNA dynamics

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The widespread importance of induced fit in RNA recognition makes it imperative that RNA motional properties are characterized in a systematic, quantitative way. Until now, however, very few studies have been dedicated to the systematic characterization of the RNA motional properties that accompany protein recognition. We are using both solution and solid-state NMR to determine the motional range and amplitudes. Solution NMR techniques are particularly useful in providing a global view of the molecule whereas solid-state NMR is sensitive to local ordering and can provide site-specific information. One can use the former to determine motion in all sites simultaneously and locate residues of particular interest. Such sites can then be selectively labeled and probed with solid state NMR to determine trajectories of motion. Together, both solution and solids state NMR cover a large motional timescale ~10 orders of magnitude; however to date, they have not been applied collectively to look at a single particular system. We propose to study the U1A protein-RNA complex as it provides some of the best-studied examples of the role of RNA motional changes upon protein binding. Here, we report both the ^{13}C NMR relaxation studies of base and sugar dynamics for the RNA target of the human U1A protein in both its free and protein-bound form, and preliminary solid-state results investigating the dynamics of the C43 residue using static deuterium lineshape data. For the free RNA, we have analyzed fast (ns-ps) and intermediate (μs-ms) motions of a construct of 3'UTR U1A mRNA by measuring ^{13}C T_1, $T_{1\rho}$ and heteronuclear NOEs for both sugar and base nuclei, as well as the power dependence of $T_{1\rho}$. The measurements indicate a broad range of dynamic heterogeneity in both the bases and sugars. In the binding region, the bases U40, U41, G42 and C43 all exhibit significant motion on the ns-ps timescale and residues A24, G42 and A44 exhibit conformational exchange. The analysis of these measurement paints a much clearer picture of the type of motions experienced by this RNA in the absence of the protein than was provided by the analysis of the structure based solely on NOEs and scalar couplings. The results lead to a model where the internal loop region to which U1A binds `breathes' on a μs- ms time scale at the hinge connecting the internal loop to the double helical regions. Superimposed on this motion, the residues at the very tip of the loop and lacking strong stacking interactions undergo fast (ns-ps) motions. The majority of these motions were found to be quenched upon binding to the U1A protein.

Insight into methyl-dynamics of dynamically different motifs of dsRBD of PKR to understand hydrophobic core packing

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Double stranded RNA binding domains (dsRBD) regulate distinct cellular functions and the fate of an RNA molecule in the cell. This highly homogeneous domains present in multiple copies in a number of species, exhibiting individual functional specificity. Number of NMR and X-ray crystallographic structural studies reveal that several such domains take common α-β-β-β-α tertiary fold. However the functional specificities of these domains could be due to the dynamic variations of the individual amino acids, as has been shown earlier in the case of backbone dynamics of ^{15}N-^{1}H of dsRNA binding motifs (dsRBMs) of human PKR. In order to further investigate if the differences in dynamics of the two dsRBMs are restricted to only backbone or if the side-chain motions are also different to the extent of influencing their packing of the two hydrophobic cores, we have been investigating the methyl group dynamics using ^{13}C relaxation experiments. The result of these investigations shows that it has a large implementation for disease-protein's activity and will be discussed in this paper.

The role of internal motions in protein function: a backbone and side-chain multiple-field NMR relaxation studies of two globular proteins azurin and barnase

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The NMR relaxation allows studying local protein dynamics on the different time-scales, subnanosecond-nanosecond and microsecond-millisecond. Application of the NMR methods for real protein systems has provided the compelling correlation between local motions and protein function. Dynamic properties of two globular proteins, Pseudomonas aeruginosa azurin and Bacillus amyloliquefaciens barnase, were explored using extensive NMR relaxation measurements at different magnetic fields (500-900 MHz). Following a careful model-free analysis of 15N relaxation measurements at two temperatures and four magnetic fields, several regions with different dynamic regimes are found for small cupredoxin azurin. Detection of increased mobility in the nanosecond range for residues from hydrophobic surface patch, including surface-exposed copper-ligand His117, are consistent with a gated electron transfer process according to the "dynamic docking" model. The residues along intramolecular electron transfer pathway show high rigidity that is remarkably conserved when increasing temperature. Additionally three different conformational exchange processes were observed in the millisecond time-scale. The two of them found near the Cu-site may indicate an additional gated electron transfer mechanism at slower time-scale.

We used H2 relaxation measurements at three magnetic fields for characterization of side-chain methyl dynamics in guanyl-specific ribonuclease barnase and its complex with natural intracellular inhibitor, barstar. Effect on methyl mobility as well as on chemical shifts upon binding was found mainly on the interdomain surfaces. It was suggested that binding with barstar shifts the conformation equilibrium between several co-existed states with slightly different domain orientation. Those results provide new insight in mechanisms that propagate signal across entire protein.

Measurement of intra-residue dipolar cross-relaxation rates between Cα and CO nuclei in proteins

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Nitrogen-15 relaxation has been successfully used as the main NMR probe of protein backbone dynamics over the past fifteen years[1]. Several α ^{13}C and carbonyl ^{13}C relaxation experiments have been developed over the same period and some recent studies[2] suggest that the dynamics of the ^{13}Cα-^{13}CO vector reveal motions not sampled by the amide ^{15}N-^{1}H vector.

One of the methods to study the dynamics of the ^{13}Cα-^{13}CO vector is the measurement of the dipolar cross-relaxation rate between the α ^{13}C and the carbonyl ^{13}C nuclei. Two different methods have been suggested to characterize this rate. In one method, the measurement of a steady-state nuclear Overhauser effect[3] (nOe) suffers from systematic errors from the selective saturation profile and inter-residue nOe's. In the alternate scheme developed to measure transient effects[4] this latter problem is effect, as well as the problem of normalizing the transferred polarization.

We present a new experiment based on an HN(CO)CA scheme using symmetrical reconversion[5] to normalize the polarization transferred. The observation of the two-spin orders involving the amide ^{15}N of the next residue isolates the intra-residue cross-relaxation pathway. Application and analysis with comparison to ^{15}N dynamics data using [U-^{13}C, ^{15}N, ^{2}H] human ubiquitin are presented.

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CEESY: a new method to measure Chemical Exchange to Excited States by NMR spectroscopY

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We present a new method to detect 'invisible' excited protein states that exchange with an observable ground state. Our CEESY (Chemical Exchange to Excited States) experiment provides an immediate identification of exchanging residues by exploiting any nucleus as a probe for chemical exchange.

Background. Biomolecules often exist in a ground state that is interconverting with an excited state. This excited state can be of functional significance, but is difficult to study since it is only marginally populated. However, the presence of this 'invisible' excited state can be detected indirectly by NMR since both relaxation rates and peak positions are affected by chemical exchange. The magnitude of this effect depends on both kinetic (exchange rates), thermodynamic (relative populations) and structural parameters (size and sign of chemical shift difference between the two states).

Method. The CEESY method relies on the differential effect of exchange on the Larmor-frequencies of SQ and MQ coherences. Kay and coworkers have exploited this effect in their H(S/M)QC-method in which the sign of the chemical shift difference between ground and excited state is determined from the small difference in peak position between a HSQC and HMQC spectrum[1].

The approach taken in our method is to translate this Larmor-frequency difference directly into a peak intensity, allowing an easy qualification of exchanging residues and determination of the sign of the chemical shift difference. It offers the unique possibility to use any nucleus as a probe for chemical exchange to excited states and provides reliable results even in crowded regions of the spectrum.

Results. The method is applied to the ^{15}N backbone nuclei of the 105-residue PAH2 domain. Out of 72 non-overlapping residues, 33 residues give a distinct negative peak, indicating a smaller 15N Larmor frequency for the excited state (i.e. a negative chemical shift difference). Conversely, positive peaks were found for 10 residues.

The sign of the chemical shift difference was confirmed using the H(S/M)QC method. Previously acquired ^{15}N SQ relaxation dispersion measurements could reveal only 26 out of the 43 exchanging, illustrating the high sensitivity of CEESY to exchange.

Conclusion & outlook. The CEESY method outlined here offers the opportunity to recognize exchanging residues at first glance to determine the sign of their chemical shift difference. The results found with our method match those of the H(S/M)QC approach. Application of CEESY to other backbone nuclei is currently in progress. A full analysis of the dispersion data combined with the sign information should result in detailed information about the nature of the excited state and the exchange process.

Note. CEESY should be pronounced as "Kay-sy" and "Cees-sy"

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NMR Structural Studies of the Interferon α Signaling Complex

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Type I interferons (IFNs) are a family of homologous helical cytokines which exhibit pleiotropic effects on a wide variety of cell types including antiviral activity, antibacterial, antiprozoal, immunomodulatory and cell growth regulatory functions.

Consequently, IFNs are the human proteins most widely used in the treatment of several diseases (e.g. several kinds of cancer, hepatitis C, MS), however severe side effects hamper the treatment. All type I IFNs bind to a cell surface receptor consisting of two subunits, IFNAR1 and IFNAR2, associating upon binding of interferon.

Here we describe our ongoing studies on the structure and dynamics of the 44 kDa complex of IFNα2 with the extracellular domain of IFNAR2 (R2-EC) and of the ternary 95 kDa complex between IFNα2, R2-EC and IFNAR1 (R1-EC) by multidimensional NMR techniques. These two complexes are challenging projects for NMR studies and require the use of all available state of the art techniques.

The structure of free R2-EC was previously solved in our lab. Backbone assignment of IFNα2 in the IFNα2/R2-EC complex was achieved using a set of TROSY experiments including HSQC, HNCO, HNCA, HNCOCAB, HNCB, HNCACO and ^{15}N NOESY. The secondary structure was predicted using the programs PsiCSI and TALOS. The binding sites for R2-EC and for R1-EC on IFNα2 were studied using TROSY HSQC, intermolecular NOEs and saturation transfer experiments. The results of these experiments suggest conformational changes in the IFNα2/R2-EC binding side upon formation of the ternary complex.

This structural information provides important insight into interferon signaling processes and may allow improvement in the development of therapeutically used IFNs and IFN- like molecules

Structural studies of the human nucleotide excision repair complex ERCC1-XPF

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NER is a general repair system for removing virtually all types of lesions from DNA and is the sole repair mechanism for eliminating bulky base adducts[1]. In short its function is accomplished by the sequential assembly of repair factors, each of them playing a key role in the following temporally distinct events: (i) recognition of the damaged DNA; (ii) incision of a 24-32 nt containing the injury; (iii) DNA synthesis to fill the gap; and (iv) ligation of the nick[2]. ERCC1-XPF is a structure specific endonuclease and carries out the incision 5 prime to the lesion. Plenty of in vivo and in vitro studies indicate the instability and functional inability of the individual subunits in the absence of their partner and only the formation of a tight complex, involving amino acid stretches in the carboxyl terminus of both, regains functionality. Thats exactly the epicentre of our project: the last stretches of both proteins known to be crucial for complex formation[3].

Here we present our biochemical results that reveal different stability features in solution for the sequences under investigation. We also analyze in atomic details the mode of the heterodimeric interaction based on the high-resolution structure we obtained using standard NMR methods. Finally comparative structural data highlight the profound similarity of the double helix-hairpin-helix motifs, present in ERCC1 side, as potential DNA recognition elements. We believe that the particular NMR structure provides a great insight for the family of endonuclases that utilize homo- or hetero- interactions in order to perform their action.

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Solid State NMR of the natural ligand bound to its G-protein coupled receptor Bradykinin 2

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Membrane proteins perform a vast amount of important functions in cell membranes. In most genomes, 30% of the genes are responsible for encoding membrane proteins. In spite of their abundance and obvious importance, only 5% of the structures found in the PDB database belong to membrane proteins.

Solid state NMR methods are ideally suited for structural studies of proteins which are reconstituted into membranes.

The neuropeptide Bradykinin (BK) is found in human body fluids, and posseses important pharmacological and physiological properties (increase of vascular permeability, contraction of smooth muscles). This neuropeptide activates Bradykinin 2, a 45kD, 7 transmembrane G-protein coupled receptor which plays a role in the central nervous system, where it is involved in pain stimuli and for the cardinal symptoms of inflammation.

Bradykinin amino acid sequence:

- Pro - Pro Arg - Gly - Phe - Ser - Pro - Phe – Arg

Here, we present the first solid state NMR measurements on Bradykinin, taken in lyophilized form, in an hydrophobic environment and, most notably, bound to its natural G-protein coupled receptor Bradykinin 2.

2D solid state NMR single quantum/double quantum spectra proved helpful in the assignment of the BK signals in its solid form. Spectral assignments and studies of the BK conformation in aqueous solution were carried out with the help of complementary high resolution NMR measurements and agree well with the solid state NMR results.

Interestingly, the solid state NMR on the ligand bound to its receptor (40 µg ligand) becomes feasible only at low temperatures (180 K). Most notably, the signals of the aromatic residues gained in intensity at lower temperatures.

Heteronuclear (^{1}H-^{13}C) MAS NMR resolves the electronic structure of coordinated histidines in bacterial photosynthetic reaction centre

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High resolution solid-state NMR provides clear access to the local spatial and electronic structure around amino acid side chains in large membrane protein complexes. In the present study, different MAS NMR techniques are applied in order to explore the electronic and protonic structure of the histidines co-ordinating to Mg^{2+} in intrinsic membrane bound reaction centres of photosynthetic bacteria. The 2D homonuclear (^{13}C-^{13}C) dipolar correlation MAS NMR spectrum of [$^{13}C_6$,$^{15}N_3$]-histidine labeled bacterial RC shows four separate correlation networks corresponding to at least four different types of histidines. By using two-dimesional heteronuclear (^{1}H-^{13}C) dipolar correlation spectroscopy with phase-modulated Lee-Goldburg homonuclear ^{1}H decoupling applied during t_1 period, a clear and unambiguous assignment of the protons and carbons of histidine interacting with the magnesium of BChl a molecule is obtained. Due to significant ring current effects, the 2D heteronuclear (^{1}H-^{13}C) spectrum shows a clear separation between histidines in close proximity to the BChl cofactors and the other histidines in the reaction centres. These axial hisdines appeared at two different positions in the 2D heteronuclear (^{1}H-^{13}C) spectrum suggesting that histidine coordinating to BChl in the reaction centres have two different types of electronic environment. The intensity of the ^{1}H-^{13}C cross peaks of ε and δ of histidines indicate that out of four axial histidines one histidine has significantly different electron structure than the other three histidines. These results might indicate that this special histidine may be ligating to the special pair in the active branch of bacterial reaction centre.

Solid-state NMR applications of the anomeric effect in monosaccharides

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Carbohydrate plays an essential role in many biological functions including wound healing, embryonic development, blood clotting, cell proliferation and immune response. Recently, it has been reported that a-galactosylceramide (a-GalCer) bound to ceramide in the a-configuration showed significant immuno-stimulatory and anti-tumor activity, and T-cells exhibit carbohydrate-specific recognition for a synthetic glycopeptide that carries a naturally occurring O-b-linked N-acetyl-glucosamine (GlcNAc). In order to address the anomeric effect, we have appealed to high-resolution solid-state NMR spectroscopy. Six monosaccharides: glucose (Glc), mannose (Man), galactose (Gal), galactosamine hydrochloride (GalN), glucosamine hydrochloride (GlcN) and N-acetyl-glucosamine (GlcNAc) were chosen in the NMR analysis, with either ^{13}C or ^{13}C/^{15}N isotope labeling. The ^{13}C spectra reveal two well-distinct resonances readily assigned to anomeric a- and b-form. In all the cases, all tensor values of the a-anomers shifted downfield compared to those of b anomers. And the magic angle spinning sideband were used to extract the chemical shift anisotropy (CSA) tensor elements. It was found that the tensors for the a form are more anisotropic and axial symmetrical. And a high degree of correlation was shown when the ratio of the axial asymmetry of the 13C chemical shfit tensors of the two anomeric forms was plotted in a semilogarithmic plot against the relative population of the two anomers. By applying the REDOR spectroscopy to doubly isotope labeled sugar, we were able to discern whether or not there were any difference in the sugar ring conformation between the anomers. In fact, identical two-bond distance were deduced for both of the a- and b-forms in GlcNAc and GlcN, suggesting that the two anomeric have essentially identical sugar ring scaffolds in these sugars. In light of these REDOR distance measurements and the strong correlation observed between axial asymmetry parameters of the 13C chemical shift tensors and the relative population between the two anomeric forms, we concluded that the anomeric effect arises principally from interaction of the electron charge clouds between the C-1-O-5 and the C-1-O-1 bonds in these monosccharides.

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The Oligomeric Structure of Vaccinia Viral Fusion Protein A27L is Essential for Binding to Heparin and Heparan Sulfates on Cell Surface

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Vaccinia virus is a member of the Poxviridae family, the largest known animal viruses. The soluble domain of the self-assembly vaccinia virus envelope protein A27L, sA27L-aa, consists of a flexible extended coil at the N-terminus and a rigid hydrophobic coiled-coil region at the C-terminus. A basic strip of 12 residues in the N-terminus is responsible for binding of the virus to the cell surface heparan sulfates. Although the C-terminus is believed to mediate self-assembly, its biological role remains unclear until recently. An in vitro bioassay showed that peptides comprising the 12-residue basic region alone do not bind heparin, suggesting that the C-terminal coiled-coil region might serve an indispensable role in biological function. In order to explore structural and functional relationships of the self-assembly vaccinia virus envelope protein, we have performed site-specific mutagenesis in an attempt to specifically disrupt the hydrophobic interaction of the coiled-coil. Based on a previously developed model[1] three single mutants, L47A, L51A and L54A, and one triple mutant, L47,51,54A were expressed and purified from Escherchia coil. The physical properties of these mutants were carefully examined by gel filtration, CD, and NMR spectroscopy, and their biological activities were assessed by an in vitro SPR bioassay and three in vivo bioassays: binding to cells, blocking virus infection and blocking cell fusion. We have shown that the L47A mutant, which is similar to the parental sA27L-aa in forming a hexamer, is biologically active. L51A and L54A mutants form tetramers and are less active. Notably, in the triple mutant, the self-assembly hydrophobic core structure is uncoiled; as a consequence, the tetrameric structure is biologically inactive. Thus, we conclude that the leucine residues, in particular Leu51 and Leu54, sustain the hydrophobic core structure, and is essential for the biological function of vaccinia virus envelope protein A27L binding to cell surface heparan sulfates. Based on these results, we have developed a molecular model to account for the molecular integrity of the multivalent interactions associated with the self-assembly vaccinia virus envelope protein A27L[2].

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Solid State NMR Studies of Changes in Peptide Structure and Dynamics at Reduced Temperatures

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Proteins have been shown to exhibit a motional and conformational transition at low temperatures, ascribed to changes in the dynamics of the amino acid side chains and/or the solvent molecules. This so-called protein glass transition is generally observed in proteins between 100 and 200K. Previous NMR experiments in this range of temperatures have demonstrated that the change in dynamics of the side chains of specific residues, as the temperature is reduced, can complicate or even prevent the acquisition of high-quality NMR spectra.

We present a detailed examination of a motional transition observed in the model peptide and chemotaxin formyl-Met-Leu-Phe (fMLF), which has been the subject of several previous solid state MAS experiments at ambient temperatures. Solid state MAS NMR measurements of the crystalline peptide at temperatures as low as 90K indicate a transition between 200 and 100K. The transition is clearly visible in 2D ^{13}C-^{13}C and ^{13}C-^{15}N correlation experiments at 90K, 175K, and 298K. Single forms are seen below 90K and above 250K, but a mixture of forms is seen at intermediate temperatures. Structural studies were performed at those three temperatures to determine the conformational changes as a function of temperature, featuring TEDOR and R^2-width measurements.

Our results suggest that the transition is correlated to a slowing down of the motion of the methyl groups in the leucine side chain and in the flipping of the phenyl ring side chain of the phenylalanine. This is perhaps to be expected on principles, but notably, these localized dynamics affect the chemical shifts throughout the molecule.

Torsion angle refinement by measuring through space correlation in MAS Solid-state NMR

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Conformation-dependent chemical shifts are, perhaps, the most direct parameters for studying protein backbone conformation under Magic Angle Spinning. In addition, structural information can be obtained by correlating anisotropic chemical shielding and dipolar interactions (see, for example, Ref.[1] for recent reviews). These experiments, however, suffer from spectral overlap and hence can be of limited use for applications in (membrane) proteins.

We show how 2D solid-state MAS-NMR spectroscopy can be used to obtain restraints for the torsional angles Φ,Ψ and X using (^{15}N-^{15}N) spin-diffusion[2] and NHHC[3] correlation experiments. Using a uniformly labelled sample of microcrystalline ubiquitin, we elucidate how variations in experimental conditions affect the structural interpretation. The resulting structural information was used to refine the torsional angles predicted from the TALOS package[4]. In total 20 additional backbone torsional angle restraints could be obtained, which lead to a significant improvement of the RMSD in a model 3D structure calculation[5]. Hence, this approach represents a general routine in structure refinement of proteins with solid-state NMR.

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Structure and dynamics of a membrane-protein complex as seen by MAS NMR

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MAS-based solid-state NMR has recently made progress in studying molecular structure and dynamics of uniformly labelled polypeptides[1]. For applications in larger (possibly membrane-embedded) systems, spectral dispersion and spectroscopic sensitivity are of critical relevance.

We report on recent progress to characterize molecular structure and dynamics in a membrane complex consisting of the photoreceptor sensory rhodopsin II and its cognate transducer (SRII/HtrII)[2,3]. Starting from (^{15}N,^{13}C) and (^{13}C,^{13}C) correlation techniques to obtain sequential resonance assignments[4], we investigate the relationship between molecular structure and dynamics of (^{13}C,^{15}N) labeled (SRII/HtrII)-proteoliposomes in reference to the published crystal structure[3]. Our studies provide the basis to investigate how membrane proximal signalling events are triggered by extracellular and/or membrane associated ligand binding in the ground and active states of SRII/HtrII.

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Exploration of the cofactor state in phytochrome by MAS NMR

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The plant photoreceptor phytochrome (ca. 120 kDa) induces upon light activation a wide variety of physiological and morphological processes, which control the photomorphogenetic development of plants.[1] The chromoprotein phytochrome contains a covalently attached chromophore, phytochromobilin, an open-chain tetrapyrrole that undergoes photoisomerization upon light excitation.[2] Phytochrome exists in two stable forms – the physiological inactive Pr form, which is formed in the dark (absorption maximum: 667 nm) and the photomorphogenetically active Pfr form (absorption maximum: 730 nm).[3]

Magic-angle spinning (MAS) NMR is a powerful method for the investigation of such chromophore-protein systems. Using selective isotope labeling, the functionally relevant partial structure can be elucidated at the atomic precision. To this end, the uniformly ^{15}N-labeled cofactor of the Cph1 phytochrome has been used to record the MAS-NMR spectra of both Pr and Pfr forms. The ^{15}N spectra of both Pr and Pfr/Pr mixture show signals with chemical shifts between 130 and 160 ppm. Such signals can be attributed to protonated nitrogen. This provides clear evidence that all nitrogen atoms are protonated in both Pr and Pfr forms. These results are in agreement with those found previously by liquid NMR.[4] The differences between both spectra will be discussed.

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Photochemically induced dynamic nuclear polarization in photosystem I observed by MAS NMR

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Photochemically induced dynamic nuclear polarisation (photo-CIDNP) has been observed in photosystem I by 13C and 15N magic angle spinning solid-state NMR under continuous illumination with white light. Recently obtained results from uniformly labeled 15N reaction centers show four emissive signals indicating that the radical cation is localized on a single chlorophyll cofactor. An almost complete set of chemical shifts of the aromatic ring carbons of a single Chl a molecule has been obtained [1]. Calculations suggest that appearance of 13C photo-CIDNP signals is from the donor rather than the acceptor. All the light induced 13C NMR signals are emissive indicating the possibility of a predominance of the three-spin mixing mechanism (TSM) over the differential decay (DD) mechanism. The intensity of the photo-CIDNP signals of PSI is very strong compared to other reaction centers. A significant chemical shift difference compared to P680 of photosystem II (PSII) is observed on the carbon C-14. In contrast to PSII, the C13-l resonance appears in the spectrum at the same position as expected from model Chl a molecule. The narrow linewidth provides evidence for a rather rigid, ordered as well as structurally and electrostatically stable donor site without structural heterogeneities. The electron spin density distribution in P700+, which is related to the 13C and 15N photo-CIDNP intensities, will be discussed.

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Combination of MAOSS and photo-CIDNP MAS NMR: Studying electron transfer in membrane proteins

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The membrane bound multiprotein complex of photosystem II (PSII) is a light-driven electron pump and a central player in photosynthesis. The reaction centre of PSII contains following cofactors: 6 chlorophyll *a* molecules, 1 or 2 *β*-carotene molecules, and 2 pheophytin *a* molecules. Despite the process of photosynthesis has been studied for centuries, the electronic structure of P680, the primary electron donor of PSII is not yet understood. Furthermore, the origin of the highest redox potential known in living nature of ~1.2V of $P680^+$ remains still unanswered. Photo-CIDNP (photochemically induced dynamic nuclear spin polarization), observed by magic angle spinning NMR upon illumination, an excellent method has been found to study the electronic structure of the photochemical machinery of reaction centres at the atomic level [1,2]. In this study we combine this method with MAOSS (magic angle oriented sample spinning) in order to obtain orientational information of the nuclear polarised cofactors in PSII. As a consequence the cofactor carrying the cation radical $P680^+$ can be identified. Therefore, the combination of two novel methods allows to study the electronic structure at both, the atomic as well as at the molecular level. Concepts describing the high redox-potential of P680 will be discussed.

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Nanosecond photo-CIDNP in a bacterial reaction centre

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Photo-chemically induced dynamic nuclear polarisation (photo-CIDNP) can be observed by MAS NMR in frozen photosynthetic Reaction Centres (RCs). Therefore, Photo-CIDNP MAS NMR is a powerful technique to probe the working principles of the photochemical machinery of photosynthetic membrane proteins. Nuclear polarisation at a factor up to about 5000 has been detected under continuous illumination in the RC of *Rhodobacter Sphaeroides*. Such enormous polarisation will allow new classes of MAS NMR experiments. Continuous illumination photo-CIDNP in the solid state was observed in uniformly ^{15}N-labelled bacterial RCs of *Rb. Sphaeroides* R-26 [1], in bacterial RCs with both natural and selectively labelled ^{13}C isotope abundance [2,3], in the D1D2 complex of the RC of photosystem II [4] and in the selectively ^{13}C labelled photosystem I of plants [5].

Two mechanisms producing photo-CIDNP from the polarised radical pair are currently under discussion [6]. In the three-step mechanism of coherent Three Spin Mixing (TSM), nuclear polarisation occurs when the nuclei are coupled by the anisotropic part of the hf interaction to the unpaired electrons and the two electron spins in the pair are coupled [7]. In the Differential Decay (DD) mechanism, the nuclear spin sorting is driven by a combination of anisotropic hf interaction and different kinetics of the radical pair decay for singlet and triplet pairs. Until now, mechanistic studies were limited by using continuous illumination. Fast laser experiments will overcome this limit. For the first time, data of a single photocycle of the natural abundance RC of of *Rb. Sphaeroides* R-26 illuminated by 10 nanoseconds flash pulse laser will be presented and the dynamics of the mechanisms producing photo-CIDNP will be discussed.

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The Magnetic Field Effect of photo-CIDNP in reaction centers of Rhodobacter Sphaeroides

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Frozen reaction centers of the purple bacterium Rhodobacter sphaeroides show enormous enhancement of NMR intensities in ^{13}C MAS NMR experiments. This effect is due to photochemically induced dynamic polarization (photo-CIDNP). Photo-CIDNP increases NMR intensities due to the polarization transfer from the polarized radical pair to nuclei, which place the nuclear spin states out of their Boltzmann equilibrium. Currently, there are two mechanisms under discussion. These are electron-electron-nuclear three-spin mixing (TSM) mechanism and the Differential Decay (DD) mechanism. Quantification of the contributions and the interplay between TSM and DD mechanisms is necessary for full understanding of this enhancement mechanism. The field-dependence of photo-CIDNP gains particular relevance in this study. For this purpose, photo-CIDNP MAS NMR experiments were measured at three different field strengths of 17.6, 9.4 and 4.7 T. The largest enhancement is observed at a field strength of 4.7 T. ^{13}C signals out of the photochemically active region are enhanced in the photo-CIDNP MAS NMR spectrum at a field strength of 4.7 T by a factor of about 5000. At field strengths of 9.4 and 17.6 T, the enhancement factor are 500 and 30 respectively. The overall shape of the enhancement spectrum does not depend on the field strength. The enormous enhancement achieved at a field strength of 4.7 T provides a method to overcome the intrinsic problem of sensitivity in solid state NMR.

High Resolution Magic Angle Spinning ^{1}H NMR Spectroscopy of Human Liver Transplants

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This work presents the first application of high resolution magic-angle-spinning (MAS) ^{1}H NMR spectroscopy to human liver biopsy samples, allowing a determination of their metabolic profiles before removal from donors, during cold perfusion and after implantation into recipients. The assignment of peaks observed in the ^{1}H MAS NMR spectra was aided by the use of 2-dimensional *J*-resolved, TOCSY and ^{1}H-^{13}C HMQC spectra. The spectra were dominated by resonances from triglycerides, phospholipids and glycogen and from a variety of small molecules including glycerophosphocholine (GPC), glucose, lactate, creatine, acetate, amino acids, and nucleoside-related compounds such as uridine and adenosine. In agreement with histological data obtained on the same biopsies, two of the six livers were found to contain high amounts of triglycerides by NMR spectroscopy, which also indicated that these tissues contained a higher degree of unsaturated lipids and a lower proportion of phospholipids and low molecular weight compounds. Additionally, proton T_1 and T_2 relaxation times indicated two populations of lipids, a higher mobility triglyceride fraction and a lower mobility phospholipid fraction, the proportions of which changed according to the degree of fat content. GPC was found to decrease from the pre-transplant to the post-transplant biopsy of all livers except for one of the livers with a histologically confirmed high lipid content and might represent a biomarker of liver function post-transplantation. NMR signals produced by the liver preservation solution were clearly detected in the cold perfusion stage biopsies of all livers but remained in the post transplant spectra of only the two livers with a high lipid content and were prominent mainly in the graft that later developed primary graft dysfunction. This study has shown biochemical differences between livers used for transplants that can be related to the degree and type of lipid composition which in turn might relate to donor organ quality.

3D Structure and Dynamics of Phospholamban in lipid bilayers revealed by multi-dimensional MAS solid-state NMR

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Phospholamban (PLN) is a 52-residue membrane protein from the sarcoplasmic reticulum (SR) membrane of heart muscle cells and interacts with the sarco-endoplasmic Ca-ATPase (SERCA). Super-inhibitory forms of PLN are believed to be responsible for heart failure in genetic forms of the disease. Hence, understanding the PLN-SERCA interaction may provide new routes for the treatment of heart failure (*1*).

According to solution-state NMR studies, done in organic solvents or micelles (*2-4*), the monomeric mutant (AFA-PLN) comprises an α-helical cytoplasmic domain and a single trans-membrane helix that are connected by a semi-flexible hinge region. On the other hand, mutagenesis studies and recent EPR (*5*) and FRET (*6*) measurements imply that protein dynamics could play an important role in the functional interaction of PLN with the Ca-ATPase.

We have designed a novel set of multi-dimensional MAS solid-state NMR experiments that permit probing the structure and dynamics of uniformly [^{13}C,^{15}N] labeled AFA-PLN in lipid bilayers, under more relevant biological conditions. Results obtained on AFA-PLN, in the absence and presence of the SERCA1 isoform of Ca^{2+}-ATPase in lipid bilayers, are shown.

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Dimerization of magainin family antimicrobial peptides in lipid bilayers studied by ^{2}H, ^{19}F, and ^{15}N solid-state NMR

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The a-helical antimicrobial peptides PGLa and magainin 2 from the skin of the African claw frog are two members of the magainin peptide family [1]. In order to get insight into peptide interactions in lipid membranes, especially dimerization, these two peptides and the PGLa analog K3 have been studied in the membrane-associated state. We used ^{19}F, ^{2}H and ^{15}N NMR on specifically D_3-Ala, ^{15}N-Gly and CF_3-Phg labeled peptides. at molar ratios between 1:200 and in lipid bilayers composed of DMPC and DMPG. PGLa is found to lie flat on the membrane surface at low concentration, but at higher concentration it inserts obliquely [2,3]. At higher concentrations, especially with DMPG lipids present, the tilt angle increases. This it probably due to dimerization, as substitutions with D_3-Ala at different positions indicated a possible dimerization interface. PGLa and magainin 2 peptides have previously been reported to form heterodimers [4]. These peptides were mixed in a 1:1 ratio and the orientation of labeled PGLa was studied. Likewise, the K3 peptide, a synthetic peptide with an amino acid sequence similar to PGLa, has been shown to form dimers in DPPC/DPPG bilayers [5]. The orientation of these dimers has now been studied using ^{15}N and ^{2}H NMR. Significant differences between PGLa and K3 were observed with regard to the dynamics resulting from the extent of molecular self-association.

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Solid State NMR Experiments on Nano-Crystalline U-^{13}C/^{15}N-Diacylglycerol Kinase

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The integral membrane protein Diacylglycerol Kinase (DGK) from *E. coli* plays an important role in lipid metabolism as it phosphorylates the second messenger diacylglycerol to yield phosphatic acid. It can be purified in very large quantities (~10 mg/L of culture), thus it makes a tempting target for advanced biophysical techniques such as NMR or X-ray crystallography: 3D crystals can be grown, but they diffract very poorly while liquid state NMR experiments are complicated due to the large size of the protein/detergent micelle complex.

Here, we present solid state NMR (SSNMR) experiments conducted on DGK. SSNMR has the advantage over the other high resolution structural techniques in that it can be equally well applied to protein reconstituted into a lipid bilayer, 2D and 3D crystals. Furthermore, even poorly diffracting 3D crystals may still yield excellent line shapes when subjected to solid-state NMR studies.

Along with the large amounts of protein that can be easily obtained, the protein can be reconstituted into single component lipid bilayers at concentrations required for SSNMR experiments whilst retaining full enzyme activity. Furthermore, 3D crystals improve the line shapes as compared to the reconstituted protein.

Here, we show ^{13}C single-quantum/double quantum as well as ^{13}C-^{13}C and ^{15}N-^{15}N correlation experiments using POST-C7, SPECIFIC CP and proton-driven spin diffusion on fully ^{13}C/^{15}N labelled samples.

These studies not only represent the first SSNMR experiments to be carried out on DGK, but also the first time 3D crystals of a membrane protein have been studied with SSNMR. These studies show that our samples produce line shapes that make it possible to consider solid-state NMR as a tool to obtain detailed information on tertiary structures of membrane proteins and nondiffracting crystals.

Solid-state NMR investigations on ion-channel structure of amphotericin B in membrane

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Amphotericin B (AmB) is known to form ion-channel in biological membranes together with sterol and phospholipid, and exert biological activities such as antifungus and hemolysis. To reveal the ion-channel structure, we prepared ^{13}C- or ^{19}F-labeled AmB and sterols with chemical and biosynthetic methods, and performed REDOR experiments in membrane environments. The REDOR experiments of ^{13}C-labeled AmB in dimyristoylphosphatidylcholine (DMPC) membrane showed the prominent dephasing effects between the phosphate group of DMPC and both terminal carbons of AmB, implying that the both terminal parts of AmB reside close to the DMPC bilayer surface. These findings demonstrate that AmB can span across the DMPC membrane with a single-length. Measurements of ^{13}C-^{19}F REDOR between ^{13}C and ^{19}F-AmB molecules have been also performed, and the results will be presented.

Structural studies on modified Amyloid Fibrils

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Amyloid fibrils are a class of protein aggregates associated with a wide range of pathological and debilitating diseases such as Alzheimer's, Parkinson's and Huntington's diseases, hvCJD and BSE. They share strong medium resolution structural characteristics and are thought to be based on the packing of extended beta sheets. Forming fibrils from modified starting sequences, results in amyloid fibrils with modified structural and chemical properties. Recent work has concentrated on a sequence that spontaneously forms amyloid fibrils with covalently attached cytochrome b562 molecules. Resonances from the displayed protein have sufficient conformational flexibility to allow solution state NMR studies. Diffusion studies and porphyrin binding experiments, combined with other biophysical data have allowed insights to be drawn on how amyloid forming sequences assemble into fibrils.

DFT Calculation of CSA Tensors in Model Peptides. Dependence on secondary structure

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Density Functional Theory (DFT) calculations were used to asses the dependence of backbone-atom chemical shielding anisotropy (CSA) tensors on backbone conformation. The molecular models used in the simulation of protein secondary structure elements and CSA calculations were a capped alanyl-alanine di-peptide and a capped Ala_8 octa-peptide.

The *Gaussian98* program package with the DFT (B3LYP) method was used for geometry optimizations and NMR parameter calculations (GIAO) with the 6-311G(d,p) basis set for the former and 6-311++G(3df,3pd) for the latter.

For each backbone atom of the dipeptide the CSA tensor was analyzed by itself and, additionally, in the context of cross-correlated relaxation with selected dipolar interactions. This was to allow comparison to both solid state and liquid state NMR experiments. The octa-peptide model served to account for the hydrogen bonding influences in alpha helices.

The results obtained on backbone ^{1}H, ^{13}C and ^{15}N CSA tensors showing dependence on protein secondary structure are visualized by way of Ramachandran-type diagrams for the dipeptide model. Comparisons of predicted CSAxDD cross-correlation rates using symmetric and fully asymmetric CSA tensors are shown. The relative influences of hydrogen bonding and conformation on CSA tensors in an α-helical secondary structure are compared.

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Automated macromolecular assignment from CCPN: CLOUDS and beyond.

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The CLOUDS protocol is a method of automatically assigning protein NMR spectra. It uses a spatial distribution of hydrogen atoms, generated from NOE distance constraints between anonymous resonances. The collaborative computer project for NMR has incorporated CLOUDS into its CcpNmr software suite to create the CcpNmr Clouds application, thus demonstrating the incorporation of an existing method into the accessible and functionally rich CCPN programming platform. Integration is achieved by providing data exchange interfaces and support programs. New supporting software includes a spin system typing module, which searches for the global optimum mapping of spin system to residue type, and CloudThreader, which automatically fits a protein sequence into a cloud of hydrogen atoms. Also, CcpNmr Clouds is being extended for use with large protein subjects. This involves generating hydrogen clouds from high dimensionality experiments, using homologue structures to assist in assignment and working with selectively deuterated samples.

Ab initio calculation of ^{13}C, ^{19}F chemical shifts and ^{13}C-^{19}F J-coupling constants in a number of substituted fluorobenzenes.

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This poster reports vacuum *ab initio* calculations of ^{19}F, ^{13}C chemical shifts (GIAO HF/6-311G(2d,2p) (^{19}F), CSGT DFT B3LYP/cc-pVDZ(^{13}C) and ^{13}C-^{19}F scalar coupling constants (GIAO DFT B3LYP/aug-cc-pVTZ-J, SOPPA MP2/cc-pVTZ-J, SOPPA CCSD/cc-pVTZ-J), for a number of substituted fluorobenzenes, C_6H_4FX (X=H, F, CH_3, OH, NH_2) using DFT B3LYP 6-311G(2d,2p) geometry.

It was found that the method combination suggested by Tormena and da Silva (CSGT DFT B3LYP/cc-pVDZ(^{13}C)) [1] very accurately reproduces ^{13}C chemical shifts of the compounds under consideration, while GIAO HF [2] does surprisingly well at ^{19}F chemical shifts.

In the direct ^{19}F-^{13}C scalar coupling constants the dominant contribution comes from the Fermi contact term, with other terms (spin-orbit and spin-dipole) contributing less than 10%. B3LYP/aug-cc-pVTZ-J combination, which is known to be very accurate for computing JCH, was found to overestimate FC term and consequently geminate ^{13}C-^{19}F J-coupling constants by as much as 75 Hz. Similar deviations were also found with B3LYP/EPR-III and B3LYP/cc-pCVDZ combinations. Very accurate results were obtained using SOPPA sheme of calculations, with the RMS deviation being around 12 Hz. A full performance analysis of different ab initio methods is given on the poster.

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Projects with the CCPN data model.

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Data management and data exchange are becoming increasingly important in handling NMR projects and are essential for structural genomics and easy deposition of the data. To address this problem the Collaborative Computing Project for the NMR community (CCPN) has developed an abstract 'data model' that can handle and store information common to all projects. This data model covers macromolecular NMR and molecular structure, and we are collaborating to extend it to (biochemical) experiment description and X-ray crystallography. All programs that conform to the CCPN data model can exchange data with other programs without conversion problems or data loss. The current release includes a data model description, Python and Java libraries for interacting with the data, I/O code and schemas for storage in XML and relational databases, a data editor and extensive built-in data consistency checking, all available as open source code

(see http://www.ccpn.ac.uk/ and http://www.sourceforge.net/projects/ccpn/)[1].

To show that the data model approach works, a set of applications (the CcpNmr software suite) has been developed on the basis of the data standard and the extensive subroutine libraries that come with it[2]. The CcpNmr FormatConverter allows formats from common NMR programs to be imported into and exported from the data model, so that at any point in the pipeline another package can be used and the resulting data stored in the data model. CcpNmr Analysis (see poster at this conference) is an entirely new analysis and interactive display program, inspired by Ansig and Sparky. Other integrated software includes ARIA version 2.0 (Institut Pasteur), QUEEN (University of Nijmegen) and CLOUDS program (Carnegie Mellon University) (see poster at this conference).

The architecture of the data model ensures a high level of integrity of the information that is stored, and because of this it is particularly suited to handle large-scale data. The FRED database at the BioMagResBank with 'cleaned' restraints lists for NMR PDB entries (http://www.bmrb.wisc.edu/) and the resulting RECOORD database[3] of over 500 recalculated structures from the PDB (http://www.ebi.ac.uk/msd/recoord/) are based on a step where the restraints and the PDB coordinates are read into the data model and made consistent with each other. The procedures developed in these projects are now being automated to ensure a constant update of the FRED and RECOORD databases.

In a related development at the Macromolecular Structure Database (MSD), this data, and all available NMR data from the BioMagResBank, is entered into an NMR information database that is directly connected to the PDB structures. This will result in services where the original restraints can be directly visualized online together with the coordinates. It will also allow more thorough queries of the relationship between the NMR data and the resulting coordinates.

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Reliability of density functional theory (DFT) in the determination of isotropic hyperfine coupling constants.

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The reliability of density functional theory (DFT) in the determination of the isotropic hyperfine coupling constant (hfcc) of the ground electronic states of organic and inorganic radicals is examined. Predictions using the B3LYP/TZVP// B3LYP/6-31G*, computational protocol are made and compared to experimental values. The set of 150 radicals here studied, were selected using a wide range of criteria. The systems studied are neutral, cationic, anionic, doublet, triplet, quartet, s-, p-, localized, and conjugated radicals, containing ^{1}H, ^{9}Be, ^{11}B, ^{13}C, ^{14}N, ^{17}O, ^{19}F, ^{23}Na, ^{25}Mg, ^{27}Al, ^{29}Si, ^{31}P, ^{33}S and ^{35}Cl nuclei. A large number of hfccs of the above nuclei are compared with their corresponding experimental ones.

After carrying out a regression analysis, we conclude that DFT predictions on the hfccs of the first three rows nuclei are reliable for B3LYP/TZVP by using an optimized geometry with B3LYP/6-31G* combination. By comparison with other much more computational demanding schemes, namely B3LYP/cc-pVTZ and B3LYP/cc-pVQZ, we conclude that the B3LYP functional in conjunction with the TZVP basis set is the most useful computational protocol for the assignment of experimental hfccs, not only for nuclei of first and second rows, but also for those of the third row.

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DFT study of substituent effects on NMR chemical shifts in ^{13}C NMR spectra of trans-azobenzenes

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^{13}C chemical shifts have been determined both experimentally and theoretically (by DFT calculations) for a series of para-monosubstituted trans-azobenzenes (p-X-C6H4NNC6H5) (X=-F, -Cl, -Br, -I, -CH3, -CH3O, -CN, -NH2 and -(CH3)2N). Geometries were optimized by DFT wusing the B3LYP hybrid functional and the 6-311++G** basis set. Nuclear shieldings were computed with DFT using GIAO1 method and 6-311++G** basis set. Among the compunds studied, p-bromo and p-iodoazobezene are singled out as possessing heavy atoms (Br, I) and the chemical shifts were also calculated at the DFT/cc-pVTZ level of the theory with the B3LYP hybrid functional. All calculation were carried out with the Gaussian-98 package. A good correlation is observed between experimental and calculated data. Long-range substituent effects on 13C chemical shifts in monosubstituted trans-azobenzenes are discussed. 1. T. Helgaker, M. Jaszunski, K. Ruud, *Chem. Rev.* 1999, **99**, 293-352.

Excitation Pulse Design in the Presence of Transverse Relaxation

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Introduction

The problem of designing exact excitation pulses in magnetic resonance imaging can be successfully solved either by the use of a sequence of equally spaced hard pulses computed with layer-stripping techniques such as the Shinnar Le Roux (SLR) Algorithm [1], or by the use of soft-pulses obtained with the inverse scattering technique [2]. These methods though neglect any relaxation. Hence in applications where high selectivity is required and transverse relaxation (T_2) is not negligible (e.g. solid state NMR or high-field high-resolution MRI), both the previously mentioned design techniques fail to produce the desired magnetization profile.
We propose a modified SLR algorithm to take into account transverse relaxation for the design of pulse sequences producing a longitudinal magnetization (M_z) profile that compensates T_2-effects in a sense to be described.

Modified approach to spin excitation in presence of fast transverse relaxation

The effect of transverse relaxation cannot be neglected when T_2 is comparable to (or shorter than) the duration of the excitation pulse. The effect of T_2 relaxation on a slice-selective hard pulse sequence computed with the SLR algorithm is to destroy the desired transverse magnetization (M_{xy}) profile and to broaden the M_z-profile.

This loss (due to fast spin-spin interaction) of M_{xy} suggests a possible new way to produce a desired magnetization profile. Instead of designing an excitation pulse that would produce a given M_{xy}-response, it is better to compute a pulse sequence (see next section) resulting in a desired M_z-profile. A non selective 90 degree hard pulse is applied immediately after this pulse sequence and a spin-echo reads the the excited profile.

This differs from the method allowing for T_2 described in reference [3], where the desired response is specified as the stereographic projection ($M_{xy}/(1+M_z)$). The method we describe here is more suitable with fast T_2, as it results in magnetization being stored on the M_z-axis in a specified manner.

Modified SLR algorithm

The modified SLR algorithm can be expressed in terms of the following points:

1. Choose a M_z-profile as a function of ω (i.e. frequency offset)
2. Define as the desired final M_z-profile a truncated Fourier Series to order N of the profile in point 1 (N must be sufficiently large to reproduce it with a good approximation)
3. Find the compatible M_{xy}-profile in absence of relaxation ($M_z^2(\omega)+|M_{xy}(\omega)|^2=1$)
4. Find the sequence of hard pulses that generates it via the SLR algorithm (the result is a sequence of angles (θ_n,φ_n), θ_n is the amplitude of the n-th hard pulse and φ_n is its phase)
5. Fix the ratio $R=\tau/T_2$ between the time interval τ between the SLR hard pulses and T_2
6. Start from initial magnetization $M_0=(0,0,1)$ and at each time step n, find the angles ($\theta_n(R)$, $\varphi_n(R)$) that minimize the L2-distance between the T_2-renormalized M_z-profile computed with the SLR angles and the M_z-profile evolved with T_2 relaxation and the ($\theta_n(R)$,$\varphi_n(R)$) hard pulse.

Results and conclusions

A 59-hard pulses slice selective pulse sequence of duration 6.8ms has been computed with the modified SLR algorithm, to select a slice of 4KHz, assuming T_2=7ms and an available bandwidth of 50 KHz (given by the gradient strength and the sample size). The M_z-profile obtained with it showed a slice with the desired excited frequency range, in contrast with the standard SLR algorithm that provided a broader profile (50% broader at the edges of the slice).

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Nuclear spin diffusion in space disordered subsystem

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Modern state of studies on fundamental problem of delocalization of localized excitations with dipole transition rates is presented. The studied processes can be visualized on the example of depolarization of one β-active nucleus (β-nucleus) ^{8}Li in LiF single crystal containing stable impurity nuclei ^{6}Li with controllable concentration c<<1 [1]. It is described with high accuracy by the master equation [2]

$dp_{i0}/dt=\Sigma_j(w_{ji}p_{i0}-w_{ij}p_{j0}),\ \ p_{i0}(t=0)=\delta_{i0}.$ (1)

Here p_{i0} is polarization (along external static field H_0) of the i-th nucleus from the ^{8}Li-^{6}Li subsystem, if initially the polarization was localized on nucleus ^{8}Li (its number is i=0). Eq. (1) presents main relaxation channel for nuclei ^{8}Li at room temperature and $200G<H_0\tilde{<}3000G$, that is determined by unique coincidence of g-factors: $(g_I-g_S)/g_S=0.0057$, where I and S are spins of ^{8}Li and ^{6}Li. The process is directly measurable because angular distribution of the β-emission relative to β-nucleus polarization is $W(\theta)=1+ap_{00}\cos\theta$, where nuclear constant a is near 0.1. The observable is $P_{00}(t)=<p_{00}(t)>_c$ - the β-nuclei polarization averaged on random distribution of ^{6}Li spins in the crystal sites. If $H_0\tilde{=}$ 200 G, then transfer rate $w_{ij}=w_0r_0^6(1-3\cos^2{}_{\theta ij})^2/r_{ij}^6$ for i no equal to 0, and $w_{0j}=\xi w_{j0}$ with $\xi=I(I+1)/[S(S+1)]=3$. The system gives an unique precise measurable example of spin diffusion in disordered media. Similar problems with dipole transition rates w_{ij} proportional to r_{ij}^{-6} are of importance in many optical studies as well [3]. Existing theoretical results were incorporated into the relation $P_{00}(t)=F(t)$ [2] with

$F(t)=Q(t)+(1-Q(t))(\xi/x^{3/2})(1+\varphi/x^{1/2}),\ \ x=\mu\beta(t+\tau),$ (2)

$Q(t)=<\exp(-\Sigma_j w_{j0}t)>_c=\exp(-(\beta t)^{1/2}).$

Here small concentration limit is assumed, $\beta=(256/243)(r_0^6/\Omega^2)\pi^3c^2w_0$ is Forster parameter, and Ω is prime cell volume. The value $\mu\beta\tau=5.11$ gives interpolation between exact results of the expansion of P_{00} in terms of c^m (in powers of $(\beta t)^{m/2}$ in reality) and expansion in terms of $(\beta t)^{-m/2}$, produced by the approximative or numerical treatment of the long time asymptotics (where dipole long ranging induces a dependence between first and second terms, giving $\varphi=2.09$). The value of $\mu=0.71$ is defined by eigen values of the diffusion tensor $\mu\beta=4\pi(c/\Omega)^{2/3}(D_1D_2D_3)^{1/3}$. The relation $P_{00}(t)=F(t)$ holds to within $(\beta t)^{1/2}$ at small βt, and it holds to within $(\beta t)^{-2}$ at large βt, if exact D_α are used. The diffusion tensor was calculated numerically basing on the method, developed in Ref. [4]. Eq. (2) has no fitting parameters. Last experimental study [5] revealed that the relation $P_{00}(t)=F(t)G(t)$ should be applied, and parameters for simplest G(t) (giving correction at intermediate time βt near 30 with $\max_t$ G(t) near 1.5) were defined. The method [4] was modified to achieve full quantitative description of the process, and the correction G(t) was calculated for any βt<1000 starting directly from Eq. (1), that gave relative uncertainty ε<0.05 in calculation of G(t) for the system ^{8}Li-^{6}Li, and ε< 0.01 for optical systems. As a result old famous problem of random walks in disordered media with dipole transition rates received full numerical-analytical solution, initiated by experimental β-NMR studies.

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Hybrid density functional dependence of NMR ^{1}JCH coupling constants for 2-halocyclohexanes

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NMR Spin-Spin Coupling Constants (SSCC) are, among all response properties, one of the most difficult to determine theoretically. In recent years, DFT (Density Functional Theory) has been extensively applied to the calculation of indirect nuclear SSCC in large molecules.The performance of hybrid density functionals (which contain a portion of Hartree-Fock exchange) combined with analytical linear response techniques have shown to be a powerful tool for reproducing experimental values in a wide variety of molecules. Extensive and systematic studies of the suitability of the different exchange-correlation functionals for spin-spin calculations have not been carried out yet. In the present work, we show that the calculated $^1J_{CH}$ coupling constants are affected by the choice of the density functional for electron-rich atoms, such as Cl and Br bonded to C. To this end, 2-chlorocyclohexane [Cl-axial (**1a**) and Cl-equatorial (**1b**)] and 2-bromocyclohexane [Br-axial (**2a**) and Br-equatorial (**2b**)] were chosen as model systems.

The theoretical $^1J_{CH}$ coupling constants were calculated by Gaussian 03 package of programs. The FC term was calculated using B3LYP, B3PW91 and MPW1PW91 hybrid functional, and aug-cc-pVTZ-J basis set has been used for hydrogen and carbon atoms and aug-cc-pVTZ for chlorine and bromine. For conformers **1a** and **2a** the calculated B3LYP couplings are overestimated in relation to the experimental values by about 8 Hz. When B3PW91 and MPW1PW91 functionals were applied the difference between calculated and experimental decrease to less than 4 Hz. It is interesting to observe that the B3LYP functional gives an excellent agreement (error less than 3 Hz) when applied for conformers **1b** and **2b.** Theoretical and experimental $^1J_{CH}$ coupling for conformers **1a** and **2a** are larger than for conformers **1b** and **2b**, whose results were discussed in the light of orbital interactions as obtained from NBO calculations. Therefore it can be concluded that B3PW91 and MPW1PW91 are the best functional to calculated $^1J_{CH}$ coupling for compounds contain an halogen atom. (**FAEP, FAPESP)**

Ab initio nuclear quadrupole couplings in noble gas-noble metal halides

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The recent observations of strongly bound noble gas-noble metal halides, such as XeAuF, have raised discussion concerning the nature of the chemical bonding in these systems [1]. One criterion in judging the strength of noble gas-noble metal interaction is to observe changes in the experimentally observed nuclear quadrupole coupling constants (NQCCs) in different complexes.

In the present work, theoretical ab initio NQCCs of noble gas, noble metal, and halogen nuclei are computed for the various complexes for comparison with the experimental ones. As the complexes contain heavy elements, *e.g.* xenon and gold, relativistic effects has to be taken into account. Therefore, fully relativistic four-component Dirac Hartree-Fock (DHF) calculations are carried out at the basis set limit for electric field gradients that couple with the nuclear quadrupole moment at the nucleus. The relativistic effects are obtained by comparing DHF results to the non-relativistic HF calculations. Electron correlation effects are estimated at the second-order Møller-Plesset (MP2) level of theory.

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Prosias, a program for automated PROtein SIdechain ASsignment

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We developed PROSIAS, a program that facilitates and speeds up the assignment of NMR resonance frequencies of protein aminoacid sidechains. Using C(CCO)NH and/or H(CCO)NH peaklists as searchfrequencies, it scans 3D- H(C)CH-, (H)CCH COSY and/or TOCSY and 2D HC-HSQC spectra peaklists for the most likely proton assignment(s) for a given sidechain carbon. It uses the complete sidechain to evaluate whether a particular proton frequency belongs to the spinsystem. After ranking the likeliness of potential proton frequencies, the best scoring protons are used to cross-rank the other protons. Assignments are next imported into the SPARKY suite for NMR spectra evaluation. PROSIAS was tested on five proteins ranging in size from 70 to 150 aminoacids. The accuracy of assignment ranges from 85 to 90%, while the completeness of assignment ranges from 70-85%.

1 GHz NMR and beyond

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This year, a new EC collaboration project has started to explore the possibilities for very high field NMR.

The main goal of this project is to develop the methodology for performing NMR experiments in resistive high-field magnets up to 40 T and in pulsed fields above 60 T. The project unites all high-field NMR initiatives in Europe, and is associated with high magnetic field facilities that provide free access for all users. Medium to high resolution NMR spectroscopy at 1.25 GHz (30 T) will be developed for chemical and biological research. A versatile multi-frequency low temperature spectrometer for fields up to 40 T will be established for the study of physical problems for example with field-induced phase transitions. Finally, a unique pulsed field NMR facility will be established to explore material properties of for example high T_c superconductors at fields above 60 T. The project is coordinated by the Solid State NMR group at the Radboud University in Nijmegen. Partners are the high magnetic field laboratory in Grenoble, the pulsed field facility in Dresden and the National Institute of Chemical Physics and Biophysics in Tallinn. In the poster we will outline the methods that are used to achieve the required resolution in very inhomogeneous and relatively unstable magnets. This includes novel ways of ferromagnetic shimming, the use of efficient microcoils and new very high speed MAS probes. Preliminary results include the first 1 ppm spectra obtained at 1277 MHz in the new high field installation in , the first 2 GHz spectra obtained in pulsed fields and examples of very low temperature NMR analysis of quantum spin phase transitions at magnetic fields above 25 T.

NMR investigation of exchange in some interesting molecules

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Exchange in some molecules (a Ru(II) complex of a hypodentate polypyridine ligand, og two different amfifylic two-station TTF based rotoxanes) is studied by means of a 1D version of the standard exchange experiment as a function of temperature. Results were fitted to the Eyring equation in order to obtain the activation parameters $\Delta H^{\S}$ and $\Delta S^{\S}$.

NMR Analysis of Spermidine-ATP Complex: Dynamic Conformational Change of Spermidine

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Polyamines, represented by putrescine, spermidine and spermine, occur in the wide range of organisms. Particularly, spermidine (SPD, H_2N-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-NH_2) is known to form a complex with ATP under physiological conditions, which may regulate the endogenous functions of these primary metabolites. However, the structure of the complex remains unknown mainly due to their weak interaction and fast association-dissociation equilibrium. In this study, the conformation change of SPD upon complexation with ATP was examined to solve the structure of the complex.

The conformation analysis of SPD is hampered by its acyclic structure, where the absence of asymmetric centers causes unresolved ^{1}H NMR methylene signals. This problem was addressed by the diastereo-specific labeling of each one of two geminal proton pairs with deuterium. Seven labeled SPDs were synthesized for measurement of the dihedral angles formed by N1-C4, C2-C5, C3-N6, C4-C7, C5-C8, N6-C9 and C7-N10, respectively.

When compared with its HCl salt, the spin-spin coupling constant ($^3J_{2\text{-H}\,/\,3\text{-H}}$) of SPD-2,3-$d_{10}$ (analyzing for N1-C4) decreased notably from 9.7 Hz to 8.1 Hz in the ATP complex. For the dihedral angles (C4-C7, C5-C8, N6-C9 and C7-N10), the similar changes were demonstrated by their $^3J_{HH}$ values. The ratios of *anti* and *gauche* conformations were estimated based on these coupling constants as shown in **Table 1**. The data clearly indicate that SPD undergoes conformational changes to increase bent conformation (equal to *gauche* rich) when interacting with ATP. Moreover, the bent population becomes dominant at neutral pH, where the tris-phosphate group of ATP bears the charge of -4. And when SPD interacts with ATP, the butanylene part predominantly takes the bent conformation than does the propanylene part.

Industrial Quantification of different plasticizers in PVC materials by low resolution solid state NMR

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A fast and reliable method has been developed to quantify different plasticizers

[dioctyl phthalate (DOP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP)] in PVC materials by low resolution solid state NMR. The method is based on a mathematical treatment of the FID which has been obtained with an echo solid pulse sequence on a 20 MHz MARAN spectrometer.

We can conclude that the method can be applied to PVC materials containing different types of phthalates, having a concentration range of 10 to 50 % by weight with a precision of about 4 % for the range 15 to 50 % and about 10 % for 10 % by weight.

Non-equispaced Fourier Transforms for Multidimensional Filter Diagonalization Method Evolution Matrices

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The core of the Filter Diagonalization Method (FDM) requires the solution of a generalized eigenvalue problem involving two evolution matrices. The elements of the evolution matrices themselves are formed from Fourier like sums of the data. For multidimensional data sets computing these sums directly takes order $M^2 N^d$ work where M is the dimensionality of the evolution matrix, N the length of the data set and d the number of dimensions. Using a Non-equispaced Fourier Transform (NFFT) the work can be reduced to order M d log N.

Low-Field NMR Using Permanent Magnets

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There are a number of NMR applications, which do not require any chemical-shift resolution, like relaxation and diffusion experiments. In those cases low field NMR has distinct advantages, because susceptibility gradients have lower influence on the field homogeneity.

Here we demonstrate a low-cost magnet system from a Halbach arrangement of permanent magnets following the recently published NMR Mandhalas. With simple permanent magnet bars a 10 MHz magnet is constructed. The field homogeneity achieved by this arrangement is reasonable. As an additional advantage the stray field of this magnet nearly vanishes, whichis advantageous in many possible applications. The whole magnet is of the size of a coffee mug accommodating a 5 mm NMR coil. Our system is connected to a Tecmag Apollo NMR spectrometer providing a truly portable low-cost magnet system equipped with an efficient pulsed magnetic field gradient. Since the static magnetic field is along a diameter of the cylindrical bore, a solenoid is the ideal rf coil, which results in a strong and homogeneous rf field and enhances sensitivity.

First applications include relaxation studies of polyelectrolyte multilayers.

Local structure and molecular mobility in polyelectrolyte multilayers

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Polyelectrolytes allow the adjustment of surface properties such as charge and hydrophobicity. Consecutive adsorption of polycations and polyanions results in multilayers, which can be used as membranes as well as for selective protein adsorption. Multilayers from poly(acrylic acid) and branched poly(ethylene imine)) and poly(styrene sulfonate) and poly(diallyldimethyl ammoniumchloride) have been studied.

The local structure and packing of the polymers in the multilayers is investigated in solid-state NMR. In ^{13}C NMR the conformation of the carboxylic acid groups in the multilayers is studied, which show a significant deviation from the structure in the pure polymers. Because ellipsometry and surface plasmon resonance show, that the thickness of the layers strongly depends on the ionic strength of the initial polymer solutions, differences in the packing are expected. ^{1}H double quantum spectra on the other hand prove molecular mixing in all cases. Molecular dynamics in the multilayers is probed by T_{1r} and WISE experiments, both indicating enhanced mobility in the multilayers compared to the pure polymers, which is attributed to the lower order in the multilayers.

^{1}H-NMR properties of molecules in the structure of tissues in breast cancer

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One of the methods of breast cancer (BC) therapy is a hyperthermia (315.5 – 320 K). Such a kind of treatment rose a problem of precise temperature control using the relaxation times (T_1, T_2) values in a *status localis* region.

Goal of research: to study the T_1, T_2 times and spine populations (P_1,P_2) gradient dependence upon the temperature of tissues in BC.

Samples: postoperational tissues affected by BC (15 samples, $T_{2\text{-}4}$ $N_{0\text{-}1}$ M_0 stages of BC), tissues peripheral towards the *status localis* of BC (PT) (15 samples).

In order to compare data obtained from different patients results were normalized ((parameter at the current temperature (K)/parameter at 310 (K)) *100%/ 4 K).

Results (%/K):

PT (values of parameter gradient in the interval 310 – 314 K): T_{1a} (0.77±0.30), T_{1b} (0.60±0.65), T_{1c} (2.04±0.54); T_{2a} (3.38±0.77), T_{2b} (3.45±0.19), T_{2c} (2.67±0.08), P_{1a}(-0.25±0.02), P_{1b} (0.7±0.43), P_{1c} (-1.17±1.62) , P_{2a} (-2.61±1.16), P_{2b} (-1.04±0.75), P_{2c} (6.8±3.23); 314-320K: T_{1a} (2.28±0.24), T_{1b} (3.38±1.69), T_{1c} (-1.81±2.76), T_{2a} (1.60±0.97), T_{2b} (1.57±0.66), T_{2c} (0.32±0.96), P_{1a} (-5.29±2.36), P_{1b} (3.77±1.12), P_{1c} (0.67±6.10), P_{2a} (2.20±1.9), P_{2b} (-3.05±1.55), P_{2c} (3.62±1.32).

BC (310-314K, values, comparison with PT): T_{1a} (3.33±3.74), T_{1b} (-0.63±7.49), T_{1c} (-1.08±3.16), T_{2a} (3.49±0.42), T_{2b} (2.79±2.32), T_{2c} (5.31±4.53), P_{1a} (-3.36±2.55), P_{1b} (9.50±7.83), P_{1c} (-4.52±7.88), P_{2a} (-1.47±0.14), P_{2b} (2.85±0.18), P_{2c} (-1.35±0.84); 314-320K: T_{1a} (-7.08±2.47) ($p<0.05$), T_{1b} (15.13±8.04), T_{1c} (8.04±4.02) ($p<0.05$), T_{2a} (–2.24±0.26) ($p<0.05$), T_{2b} (-3.09±1.16) ($p<0.01$), T_{2c} (-8.39±4.00), P_{1a} (6.59±2.96) ($p<0.01$), P_{1b} (-10.43±4.10) ($p<0.05$), P_{1c} (15.14±4.01) ($p<0.05$), P_{2a} (0.69±1.54), P_{2b} (-3.10±1.92), P_{2c} (-1.1±3.74).

Conclusion: Concerning the temperature gradient of T_1, T_2 and P_1, P_2 values BC differs from PT in the temperature range 314 –320.

Polarization enhanced low frequency NQR detection

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Almost all explosives and drugs contain nitrogen. ^{14}N NQR spectra and their resonance frequencies depend on the molecular structure and are characteristic for each of these substances, providing a kind of their »fingerprint« and so enabling their detection and identification. The problem is that the ^{14}N NQR spectral lines often lie at low frequencies where the NQR signals are usually very weak and not easy to detect. Possible method to improve the signal is polarization enhancement. Initially protons are polarized in a strong magnetic field so that the proton NMR levels are split considerably more than the nitrogen NQR levels. Due to the Boltzman distribution factor the occupation difference between the high and low NMR levels of protons is much higher than the occupation difference between ^{14}N NQR levels. During adiabatic demagnetization of the measured sample the level crossing between proton NMR and ^{14}N NQR occurs, causing an energy flow from the »hot« nitrogen quadrupolar system to the »cold« proton NMR system which is manifested in the »cooling« of the nitrogen quadrupolar system. Applying standard pulse NQR detection techniques immediately after removing the magnetic field, the signal can be improved according to the ratio of the proton NMR to ^{14}N NQR frequency. We can choose between two possibilities (i) to switch off the magnetic field or to remove the magnet [1,2] and (ii) to remove the sample [3]. Some new experimental results with the polarization enhancement method will be presented.

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Possibility of indirect CW detection of two-frequency NQR

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Multi-frequency NQR as a measuring technique introduces new possibilities in the field of magnetic resonances. In our contribution a simple CW parallel to known pulse versions is tested: two orthogonal RF fields are applied simultaneously at two suitable frequencies to a multilevel quadrupole probe ($I >= 1$, electric field gradient asymmetry $\eta > 0$).

A modified superregenerative NQR detector (SRO) was used, equipped with an additional coil for irradiating the sample at the second frequency. After tuning SRO to a chosen NQR line, the signal proportional to the population difference of the corresponding energy level pair, is monitored. If another suitable transition is saturated simultaneously, sharing one level with the monitored line, the corresponding population change reflects also in the first signal intensity change. The investigated sample was Sb_2S_3 with two abundant quadrupole isotopes ^{121}Sb ($I = 3/2$) and ^{123}Sb ($I = 5/2$) and two chemically inequivalent crystallographic Sb sites, one with appreciably larger asymmetry ($\eta \sim 0.38$).

The aim of our investigation is to test the feasibility of a technically simple and energy saving CW technique and to improve sample specific NQR detection capability for special cases.

New capabilities of NMR spectroscopy for extraction systems study

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The extraction systems based on the BNPC (bidentate neutral phosphorus compounds) and CCD (chlorinated cobalt dicarbollide, $[Co^{III}(B_9C_2Cl_3H_8)_2]^-$) are very promising for radioactive waste processing. Water contained in such mixtures is active and very important participant of extraction equilibrium and plays a key role in extraction effectiveness and selectivity for many extraction systems. There are several types of water molecules in organic phase of such systems. A part of water is constituent of different metal complexes and interacts with the participants of the separation directly, another one is involved in a variety of like molecules associates, for example, reverse micelles, formed by a solvolysis of hard acids (proton, in particularly). There is also water of micro emulsion and no associated (free) water dissolved in organic solvent. Different types of water molecules are distinguished by ^{1}H NMR spectroscopy. The chemical shifts, the magnitudes and the number of these signals do vary in ^{1}H NMR spectra with temperature and amount of water. The use of heavy water for purposes to disturb existing equilibrium in extraction systems performs well and leads to interesting results.

The H/D exchange processes were shown to be completed for free water in a few seconds at room temperature but to be characterized as much more time-consuming for associated water molecules in metal complexes and nano-particles such as reverse micelles.

The most obvious H/D exchange process takes place in BNPC bearing methylene (CH_2) bridge between moieties such as C=O and P=O (or P=O and P=O). This exchange has been revealed by the integral magnitude changing of its signal, whereas all other characteristics of proton spectrum were invariable. The reaction rate depends on the acidity of medium. The deuterium exchange have not been obtained for BNPC wherein C=O and P=O (or P=O and P=O) moieties are spaced by a chain with more then one methylene group. ^{2}H NMR spectroscopy was applied without profit to control the process – there were no signals of CD_2 or CHD groups in the spectrum. ^{13}C NMR spectra were more informative. In parallel with loss in magnitude of methylene bridge signal owing to the deuterium exchange to CD_2 there two pairs (because of ^{31}P is adjacent to CH_2) of corresponding to CH_2 and CHD groups neighboring signals with opposing phases were clearly defined in ^{13}C NMR (dept 135) spectra.

As was obtained by ^{1}H and ^{2}H spectra comparison studies for BNPC systems containing HCCD or HClO4 after their mixing with heavy water, the number of signals in one way or another related to water molecules, their chemical shifts and the dynamic of their changes were not identical. ^{1}H and ^{2}H spectra would differ for a system containing nano-particles with water as a constituent but have to be the same in case of a system including some micro drops of water diffused gradually to the organic layer/water interface. So, the results described above give us the direct and independent confirmation of micelles existing in organic phase.

Immediately after adding of heavy water the part of associated molecules of D_2O is small compare to the amount of free molecules in deuterium spectra and as seen in proton spectra the largest share of ordinary water includes in associates. The trend to the quasi-equilibrium relation of magnitudes between associated water signals and free molecules ones is very slow. This process makes itself evident in decreasing of the magnitude of associated molecules signals in proton spectra and quite the opposite in deuterium spectra. It is necessary to be marked that the degree of H/D replacement can be determined from the signals of no associated water: its concentration in organic phase is nearly constant for every system and can be defined by saturation with ordinary water.

This method of the extraction systems' investigation by the saturation of the organic phase with D_2O and simultaneous monitoring of oppositely directed changes in ^{1}H and ^{2}H spectra we suppose to be uncomplicated and prospective way to study the associates' distribution over the aggregation numbers.

NMR Characterization of cholesteric liquid crystal polymers

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We are interested in the kinetics study of cholesteric liquid crystals polyesters synthesis reaction and its chemical modifications. Some results are presented here for: Poly[oxy(1,2 - dodecane) oxycarbonyl – 1,4 – phenyleneoxycarbonyl – 1,4 – phenylenecarbonyloxy – 1,4 phenylenecarbonyl] PTOBDME. The synthetic method, based on a previously reported one[1], and developed by our group[2], showed an unexpected stereoselective separation of polymer units which incorporate a higher excess of one enantiomer at expenses of the other, although starting from the racemic mixture of the raw materials. The enaniomeric excess is determined by integration of the corresponding set of aliphatic protons Ha, Hb, Hc (where Hc is the hydrogen bonded to the asymmetric carbon atom and Ha and Hb linked to the carbon atom in a to the asymmetric C, showed different chemical shift in the ^{1}H NMR spectrum for each enantiomer that are conveniently well separated[3]. Hd is the hydrogen linked to the another carbon atom in a to the asymmetric C also distingushible by COSY. Differents time of reaction, stirring conditions, time of decantation of toluene, pH control are some of the variables used in our present study to determine the kinetcis of PTOBDE. Structural characterization of the obtained compounds and reagents is performed by ^{1}H, ^{13}C, COSY and HSQC NMR spectra.

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Molecular recognition of saccharides by proteins. A combined NMR and theoretical investigation on the origin of sugar-aromatic interactions.

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Specific recognition of carbohydrates by other biomolecules (enzymes, lectins, nucleic acids) is often involved in the first step of essential biological processes. NMR and X-ray studies on the molecular recognition of oligosaccharides and analogues by lectins and by sugar-processing enzymes have shown that carbohydrate-aromatic interactions take place very frequently. In particular, the existence of stacking interactions between the aromatic rings of Trp, Tyr, and Phe aminoacids of the receptor proteins and the pyranose rings of the carbohydrates have been demonstrated. Herein, we present experimental NMR evidences of the intrinsic tendency of aromatic moieties to interact with certain sugars by using NMR experiments in water solution. Benzene and phenol specifically interact with clusters of C-H bonds of the alpha face of methyl b-galactoside and disaccharides thereof, without requiring the well defined three dimensional shape provided by a protein receptor, therefore resembling the molecular recognition features of carbohydrate-protein complexes. This is not the case for other sugars, *i. e.*, methyl a-mannose, that do not present the corresponding C-H vectors in the proper arrangement.[1]

The geometry of the experimentally based galactose-benzene complexes has been properly accounted for by using a MP2/6-31G(d,p) level of theory, and considering a *Counterpoise* correction during optimization. The theoretical results obtained herein indicate that the carbohydrate-aromatic interactions are stabilizing interactions with an important dispersive component, and that electronic density between the properly located sugar hydrogens and the aromatic ring indeed exists, thus giving rise to three so-called non-conventional hydrogen bonds.1

On the other hand, for carbohydrates bound to glycosidases, distortion of their ground state conformations has also been reported in numerous occasions. In some cases, the attained degree of distortion resemble the oxocarbonium-type transition state of glycoside hydrolysis. Thus, we present the first detailed systematic study of the inversion of simple six-membered ring systems (oxane, thiane and substituted system). One-bond $^1J_{C\text{-}H}$ have been calculated for all the calculated conformers of oxane with the PP/IGLOIII//B3LYP/6-311++(2d,2p) level of theory.[2] Fourier analysis shows that the dependence of $^1J_{CH}$ on HCOC dihedral angle does not parallel the $n_O \rightarrow \sigma^*_{CH}$ stereoelectronic interaction.

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Developments in Magnetic Resonance Force Microscopy

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An emerging method for improving the detection sensitivity of NMR is based on mechanical detection. The first method that was successfully applied to this end is called **M**agnetic **R**esonance **F**orce **M**icroscopy (MRFM) as proposed by Sidles in 1991[1]. It uses a mechanical cantilever as is known from Atomic Force Microscopy (AFM) to detect forces exerted on a spin system in a very inhomogeneous magnetic field. Last year a significant breakthrough was established by Rugar et al. reporting the first successful detection of an individual electron spin by MRFM[2]. This impressive detection sensitivity was achieved at very low temperatures where the mechanical noise of the cantilever is very small. At ambient temperatures the noise increases substantially. Combined with the thermodynamic population and the much lower moment of the nucleus as compared to the electron spin, one cannot hope to achieve such ultimate sensitivity in a routine NMR experiment. On the other hand, it should be able to improve the sensitivity and thus the spatial resolution in a magnetic resonance microscopy experiment by several orders of magnitude. Our focus is to find suitable methods to achieve nanometer scale resolution in materials imaging at ambient conditions. The idea is to image materials with a structural contrast based on the local symmetry at (quadrupolar) nuclear sites[3]. Currently we are exploring the possibilities to image layered semiconductors materials such as GaAs/GaAlAs structures. To this extend these materials are being characterized using MRFM and regular spin-echo and nutation type experiments using high rf field probes. Finally, a new MRFM design is proposed aiming at improving sensitivity in combination with mechanical stability and positional reproducability.

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High-throughput NMR structure determination of ribose nucleic acids

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Current structure determinations of RNAs rely mainly on classical NMR constraints (NOE's and J-couplings) supplemented with Residual Dipolar Couplings. Most time consuming in this structure determination process are acquisition of the multidimensional NMR spectra and the subsequent resonance assignment. The goal of this recently started project is to speed up the processes by a) circumventing the time consuming task of resonance by making extensive use of smart isotope labeling of RNAs in combination with extensive usage of the structural knowledge contained in chemical shifts, b) making use of the fact RNAs contain domains with well-known and well-defined conformations, c) faster acquisition of relevant NMR spectra, and d) automating tasks. We will discuss the proposed methodology and specifically we will discuss and demonstrate the implementation of some fast acquisition schemes for relevant RNA NMR spectra designed for extraction of chemical shifts.

Microstructure of hydration shells of polyatomic ions by NMR-relaxation and quantum chemical methods

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Electrolyte solution attract the attention of many scientists due to their important role in various physical, chemical, biological and technological processes. This work is devoted to investigation of microstructure of aqueous acid solutions: H_3PO_4, H_2SO_4, $HClO_4$, HNO_3, CH_3OOH and HNO_3.

Two methods have been used to study the microstructure of hydration shells of the ions: NMR-relaxation and quantum-chemical calculations. Using the NMR-relaxation method it possible to determine the coordination numbers of ions and to calculate the reorientation times of water molecules in different zones of the solution. Spin-lattice relaxation rates, T_1^{-1}, of the ^2H nuclei in the acid solution were measured using the Bruker spectrometer SXP 4-100. The coordination numbers of anions can be connected with the electron structure of the oxygen atom. It is known that in the case of a single bond of the oxygen atom with another atom in a molecule (or in an ion) the oxygen possesses three negative interaction sites in external space. If the oxygen atom forms a double bond with another atom it possesses only two negative sites. In the anions investigated, the oxygen atoms are characterized by intermediate multiplicity of bonds. Thus, it is possible to suppose that the anion coordination numbers are determined by the superposition of some electron states, or one of them will be predominant. The electronic structure of the ions have also been investigated using quantum chemical calculations. The calculations were performed at the Hartree-Fock level (ROHF-restricted open shell Hartree-Fock), using the GAMESS package. The results of quantum-chemical calculation have been compared with the results of the NMR-relaxation.

Novel STD-NMR pulse sequences for investigating protein isolates

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In general, functional proteomics approaches are based on the assignment of biological functions to individual proteins as components of complex mixtures. One strategy is to assign a specific biological function to a protein is via its binding to known ligands. As this has been shown in numerous examples, STD NMR lends itself well to the detection and characterisation of ligand binding to large protein receptors. In particular, the STD NMR methodology only requires rather small amounts of protein. However, as it has been used so far, it is not tailored for the analysis of crude mixtures of proteins, as these mixtures usually also contain a large number of natural ligands for these proteins. Therefore, the signals from the ligand of interest is likely to be difficult to distinguish from the signals of the other ligands in the sample, and thus complicate the determination of a binding profile for the isolate. Here, we present a number of pulse sequences for STD-NMR, which incorporate homonuclear or heteronuclear filters in order to allow for the selection of a particular ligand in a mixture containing multiple ligands. Consequently we are able to detect binding to a specific ligand and thus identify isolates for further investigation. Compared to other experimental techniques based on either mass spectrometry or antibody-based detection, this approach has the advantage that the protein in question does not need to be covalently modified.

Investigation of peculiarities of ion coordination in aqueous electrolyte solutions by NMR-relaxation method

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The investigation of ion solvation-shell regularity is very complicated, because of the lack of suitable research methods. Solving the problem becomes espesially hard in the case of lable complexes characterized by fast exchange of ligands. On the basis of the NMR-relaxation method it was possible to dertermine the coordination numbers of ions and to estimate the reorientation times of water molecules near ions. This work is the development of such investigations. Spin-lattice relaxation rates of the ^{1}H and ^{2}H nuclei were measured at various temperatures using a home-built relaxometer with operating friequencies of 8, 16, 20, and 60 MHz and Bruker SXP 4-100 spectrometer. It was shown that the nearest vicinity of many ions is constant over a wide temperature range. However it has been found that some ions (Li^{+}, Cl^{-}, Br^{-}) change the microstructure of their hydration shalls in the range of 30 - 40^{0} C. Below 30^{0} C the hydration shalls of the Li^{+}, Cl^{-}, Br^{-} ions consist of 4 water molecules, but at hogher temperatures (>40^{0}C) the hydration shell of of the Li^{+} cation comprises 6 water molecules, the Cl^{-} and Br^{-} anions keep 8 water molecules. The ions play an important role in different biochemical and physiological processes. It is known that the Na^{+}, K^{-}, Cl^{-}, Ca^{+} ions strongly influence on all kinds of metabolism and transport of substances through cellular membranes. A large number of works on the problem of thermoregulation of warm-blooded animals were recently published. However the theory, describing the mechanism of thermoregulation, is absent. Probably, the effect of change of the coordination number of the Cl^{-} anion in the range of 30-40^{0} C can be responsible for the termoregulation of warm-blooded animals.

Effects of Radiation Damping on Z-spectrum

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Researchers have known of the existence of radiation damping since the 1950s [1-3]. Assuming that the NMR probe containing the sample is tuned to electrical resonance, radiation damping manifests itself as additional line broadening [1], and as harmonic peaks in two dimensional spectra [4]. If the probe circuit is not at electrical resonance then additional distortions of spectra may be observed, especially in multiplet signals [5]. It is common to seek to suppress radiation damping through dilution or by physically decreasing the sample size, but in many biological experiments such an approach may not possible. In such cases - one example is the measurement of a z-spectrum [6] - it becomes necessary to model the effects of radiation damping. In biological studies the z-spectrum is used to explore magnetization transfer between the visible water signal and the NMR-invisible macromolecular signal. Information about exchange, i.e., the pseudo-first order rate constant [7], is normally obtained by fitting the steady-state solution of a suitable set of coupled Bloch equations to the experimental data.

In this work the steady state solution of a Bloch equation model including two exchanging pools and off (electrical) resonance radiation damping are presented. In the absence of radiation damping the result is identical to the usual two pool model, and in the absence of exchange and with on-resonance radiation damping the results are identical to those reported by Bloom [1].

The Bloch equation solutions are used to explore the effects of radiation damping on the z-spectrum. For a single pool with the probe at electrical resonance the shape of the Z-spectrum deviates from the usual Lorentzian. However, the effects is surpisingly small for pure water in a 5 mm NMR tube even at 400 MHz field.

Z-spectra were acquired for a 50% H_2O/D_2O mixture and for a 0.4 M solution of urea in the same H_2O/D_2O mixture, using a 5 mm NMR tube and a 2 mm insert tube. The water peak integrals comprising the z-spectrum were fitted using the Bloch equations. The modified Bloch equations provide an excellent description of the z-spectra.

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NMR spectroscopy in ionic liquids: A road towards a better understanding of a new class of solvents

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Ionic liquids (ILs)[1] have gained tremendous attention in recent years as novel solvents for organic synthesis and especially for organometallic catalysis. The physical properties of ILs can be widely varied through choice of the building blocks, which are cation, side chains and anion. ILs commonly used today consist of imidazolium, ammonium, pyridinium or pyrrolidinium cations with alkyl side chains of varying length. Typical anions include halides, tetrafluoroborate and bis(trifluoromethylsulfonyl)amide**.** These compounds are applied as solvents for an ever-increasing number of organic reactions.

However, only a very limited range of analytical techniques for ILs is available. Therefore we systematically develop NMR spectroscopy for use in ionic liquids.[2] This technique will be a valuable tool for investigations on solvent reactivity and catalytic as well as stochiometric reactions in ionic liquids.

At the current stage, NMR measurements can be done routinely in a wide range of ionic liquids and provide spectra comparable in terms of general appearance and resolution to those measured in classical organic solvents. The high viscosities and the lack of deuteration of common ionic liquids are the limiting factors to tackle. Therefore, advanced solvent suppression techniques relying on diffusion ordered spectroscopy were developed[3] to facilitate the study of solutes in ILs and compensate for the large ratio of solvent to solute signals.

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^{13}C MAS NMR study of the reactions of aromatic compounds methylation over zeolite HY.

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Solid-state NMR techniques ware applied to study the mechanisms of the alkylation of aniline, toluene and phenol with methanol on acidic zeolite Y. The samples preparation involved thermo-vacuum pre-treatment of zeolite catalyst, reactants adsorption and sealed under controlled atmospheres in precision glass ampoules, suitable for MAS. 13C MAS NMR has been performed under batch conditions on a Bruker MSL-300 spectrometer operating at 75.15 MHz. Methanol-13C was used as labeled methylated reactant to investigate the general consecutions of alkylating processes. Based on the NMR data obtained, it is established primary N-alkylation of aniline, while toluene is methylated only into aromatic ring; in case of phenol it is observed intermediate picture. These facts are according to the observations obtained in common catalytic experiments (1). But MAS NMR application allows observing the surface species appearing during reaction, such as methoxy groups (2). Elucidation of the role of methoxy groups in alkylation of aromatic compounds of different nature by solid state NMR spectroscopy is the main target of the present investigation. To arrive at a solution of this aim was carried out the special experiments with primary methoxylated zeolite surface by methyl iodide-13C. Collation of the results NMR spectroscopic data obtained with using different methylation agents leads us to the suggestion, that consecutive mechanism, involving two different steps (methoxy groups formation and their reaction with aromatic compounds) is more likely for the aniline methylation than toluene; while in case of phenol can be realized both consecutive and concerted mechanisms.

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Diffusion in lyotropic lamellar systems: Planar lamellae and onions

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Lyotropic lamellar phases under shear flow often form a peculiar defect structure, which resembles close packed multilamellar vesicles [1]. The typical size of the onion-like vesicles depends on the shear rate and can be varied from a few microns to a tenth of a micron. Using rheo-NMR spectroscopy [2], the formation of the shear-induced structure can be followed in situ by observing the line shapes of D2O NMR spectra [3]. While the spectra of planar lamellae show quadrupolar splittings, the onion state is characterized by a single peak. This peak shape results from the motional narrowing due to the diffusive motion of water along the curved lamellae. When the shear rate is increased the diameter of the vesicles becomes smaller and the line width decreases. In order to obtain further information about the diffusion of D2O in the normal lamellar phase and in the onion state pulsed field gradient experiments were performed. The results for aqueous solutions of the nonionic surfactant C10E3 [4] will be presented.

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The Future of Radiospectroscopy.

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Electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), and magnetic resonance imaging (MRI) methods are now widely applied in physics, chemistry, medicine, and biology. However, the following restrictions prevent the further application of these methods:

1. Insufficient sensitivity - A rich variety of samples exist, including materials with low concentration of paramagnetic centers and those having short spin-relaxation times. There is a need to modify and improve both EPR (and NMR) instruments and methodology;
2. Unwieldiness and heaviness;
3. Location - The spectrometers must have an electricity net;
4. Expense;
5. Until recently, a common model of a send-receive coil in NMR and MRI spectroscopy was a birdcage resonator. However, it is difficult to find an optimal L/C - relation for the capacities and inductivities at frequencies above 300 MHz.

In order to resolve these restrictions for applications in radiospectroscopy, we have invented a ferroelectric resonator that is composed of potassium tantalate single crystal [1-3]. Potassium tantalate has a unique property – its dielectric losses decrease as its dielectric constant increases. Improved crystal-growth technology let us to obtain pure and high-quality material for the resonators. Application of the ferroelectric resonator enhances the sensitivity of the EPR method at 10 GHz, and the NMR method at 400 MHz, by a factor of 100.

Because the size of the EPR resonator (for 10 GHz) is very small (1.7x1.7x1.4 mm^3), the spectrometer with this type of resonator will have a size of 10x10x15 cm^3 and a weight of 1.5 kg.

Such a spectrometer may be powered from small battery; therefore, it does not need electrical net and can even work in the fields.

The estimated price for EPR mini-spectrometer will not exceed $1000 - $2000.

In order to provide higher image quality for MRI the proton resonance frequency must be increased. To substitute for the usual birdcage resonator, fully ceramic resonators without from TiO_2 and AL_2O_3 were recently developed. However, they do not reach frequency 300 MHz. To cover a larger range of frequencies we suggest applying ferroelectric materials $KTaO_3$ and $Sr_{3.5}Ba_{1.5}Nb_{10}O_{30}$.

We developed a mini EPR spectrometer with ferroelectric resonator and the prototype of this spectrometer will be demonstrated in action.

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Multivariate modelling of the microstructural quality of food emulsions based on time domain NMR spectroscopy

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Although food quality is often perceived as compositional property, i.e. the presence of molecules that can be tasted or smelled, the dominating factor is often the microstructure of the product. The microstructural quality of food products is often assessed by sensory panels or by simple physical measurements such as texture Firmness or water holding capacity. In food research and product development environments, progress is often hampered by the lack of understanding of the relations between sensory/physical parameters and the underlying microstructures. The current 'deductive' strategies to derive structure-property relationships operate on time-scales, which do not match with the current pace of research and development. We will demonstrate an 'inductive' approach that deploys rapid and 'cheap' relaxometric measurements on commercial benchtop NMR equipment and multi-variate data analysis. Hence, explorative models can be generated that relate microstructure and functional parameters. The approach is also applicable to data generated with the MObile Universal Surface Explorer (MOUSE), hence opening up the possibility of truly non-invasive (i.e. through-package) assessment of the microstructural quality of foods. We will demonstrate this approach on protein-stabilised oil-in-water emulsions, for which physical properties and microstructure have previously been described.

Complexation studies of N-acylimidazolidinones and N-acyloxazolidinones with Lewis acids

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With the aim of rationalizing the relation between complex conformation and the induced stereoselectivity of Lewis acid mediated additions to N-acylimidazolidinones we have made studies on the complexation of N-acylimidazolidinones and N-acyloxazolidinones with several Lewis acids, such as Mg(ClO4)2, AlClEt2 and BF3OEt2.

The conformational dependence of the complexes was studied by means of NMR spectroscopy (1H-NMR and Nuclear Overhauser Enhancement - NMR spectra were acquired in a Bruker ARX400 spectrometer) as well as molecular modelling studies employing DFT theory at the B3LYP/6-31G** level with Gaussian 03.

Our calculations and NMR studies show that the compounds should exist in the anti conformation, both in the uncomplexed and BF3-complexed forms. With Mg(ClO4)2 both compounds adopt the syn conformation. This implies a reaction mechanism different from the one proposed in the literature to explain the diastereofacial control in BF3.OEt2 mediated additions. Work is in progress in order to propose a mechanism in accordance with the experimental data and also to clarify the role of AlClEt2 in similar reactions.

^{1}H NMR-based metabonomics as a tool to elucidate the health benefits of whole-grain cereals

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Consumption of whole-grain cereals has been associated with many health benefits, including a reduction of the risk of cardiovascular disease, diabetes, obesity and some cancers. Apart from being rich in fibre, whole-grain products are also rich in associated bioactive components such as phytochemicals and micronutrients with potential effects within the cells on metabolism and cancer development. The traditional approach for investigating the biological effects of bioactive compounds is selective, cumbersome and time demanding. In this study ^{1}H NMR-based metabonomics have been applied in the search of biochemical effects in blood and urine after the consumption of whole-grain products using the pig as a model.

Two diets with similar levels of dietary fibre and macronutrients, but with contrasting levels of plant lignans, were prepared from rye (high in lignans) and wheat (low in lignans) and fed to four pigs in a cross-over design. Plasma and urine samples were collected after 5 and 7 days on each diet. ^{1}H NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer. Partial least squares regression discriminant analysis (PLS-DA) on spectra obtained for plasma samples revealed that the spectral region at 3.25 ppm dominates the differentiation between the two diets, as the rye-based diet is associated with higher spectral intensity in this region. We suggest that this spectral region should be assigned to methyl groups in betaine, which was supported by spiking experiments and LC-MS analyses of the plasma. PLS-DA on spectra obtained for urine samples revealed changes in the intensities of spectral regions, which most probably should be ascribed to differences in the content of betaine and creatine/creatinine between the two diets.

In conclusion, using ^{1}H NMR-based metabonomics the present study has gained new insight into the biochemical effects of a diet with a high content of whole-grain cereals, which can contribute in elucidating the health benefits of a whole-grain-based diet.

Improving Pulse Sequences for 3D DOSY: internal diffusion encoding (IDOSY) and convection compensation

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Improved pulse sequences for diffusion encoding in HMQC, COSY and 2DJ experiments, HMQC-IDOSY (1), COSY-IDOSY and 2DJ-IDOSY (2) will be presented; these are faster and more sensitive than existing methods, and allow easy compensation for convection.

Information about the sizes of molecules present in a liquid sample can be obtained by determination of apparent diffusion coefficients. In diffusion-ordered spectroscopy (DOSY), diffusion information is obtained by incrementing the amplitudes of pulse field gradients in, for example, a stimulated echo sequence, and fitting the resultant decays of signals to the appropriate expression. The data are presented in 2D plot with a spectral dimension and a diffusion dimension; in the latter the lineshape is a Gaussian centred on the apparent diffusion coefficient and with a width determined by the standard error estimated in the fitting process.

Frequently, overlap is present in the normal spectrum, rendering it impossible to achieve high resolution in the diffusion dimension, as offered by fitting the decay to a monoexponential curve. In such cases a natural extension of the basic DOSY experiment is to add diffusion encoding to a 2D experiment, to form a 3D DOSY experiment, where spectral overlap is much less likely. Such pulse sequences have hitherto been created almost exclusively by concatenating a diffusion encoding sequence with an existing 2D experiment. This is often a practical approach, but in some cases the experiment can be greatly improved by incorporating the diffusion encoding internally in the existing 2D experiment, in a type of pulse sequence we term IDOSY, where I stands for internal. This scheme lends itself particularly easily to experiments that are displayed in absolute value mode, such as COSY or 2DJ spectroscopy. The main advantages of IDOSY experiments are that the minimum experimental time is substantially reduced – up to 32-fold compared with previous 3D-DOSY sequences – and that using a spin-echo instead of a stimulated echo gives an approximate 2-fold increase in signal-to-noise ratio. One potential disadvantage is that systems with short T2's may suffer more signal loss in IDOSY than in conventional sequences.

Convection is highly detrimental to diffusion measurements, and is commonly encountered for samples in low-viscosity solvents (such as chloroform) away from ambient temperature. To achieve convection compensation in a stimulated echo experiment, two stimulated echoes are needed, throwing away 75% of the signal. In IDOSY sequences, convection compensation can be achieved with little or no loss in signal-to-noise ratio.

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NMR Measurements of Diffusion in Concentrated Samples: Avoiding Problems with Radiation Damping

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Pulsed field gradient spin echo NMR is a very effective method for the measurement of diffusion coefficients, and is the basis for mixture analysis by DOSY (Diffusion Ordered Spectroscopy). With the advent of modern high field technology, however, severe problems can arise when it is applied to samples with very high concentrations of spins because of the presence of radiation damping [1, 2]. The mechanism of radiation damping can be the source of many problems in high-resolution NMR of liquids, resulting in a variety of deleterious effects, including excessive line broadening, severe line shape anomalies and signal phase errors. Radiation damping arises when the current induced in the probe coil by transverse magnetization is strong enough to interact with the spin system producing a torque on the magnetization. This torque rotates the magnetization back towards equilibrium at a rate fast compared to spin-spin and spin-lattice relaxation.

A recent paper by Price and Wälchli [3] has described a direct, hardware-based approach to reducing the effects caused by radiation damping, in which the probe tuned circuit is rapidly switched between a high Q and a low Q.

We present an alternative approach, avoiding the need for expensive hardware, in which a slight modification to conventional methods allows the detected magnetization to be scaled down sufficiently to avoid the onset of radiation damping [4]. If the initial pulse flip angle in the oneshot [5] diffusion measurement sequence is sufficiently reduced from 90º, then the net transverse magnetization refocused at the end of the sequence is also reduced. This ensures that at the start of acquisition the net magnetization is small.

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New Techniques in DOSY

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Information about the sizes of molecules present in a liquid sample can be obtained from self-diffusion coefficients. In diffusion-ordered spectroscopy (DOSY), diffusion coefficients are determined by incrementing the amplitudes of pulse field gradients in, for example, a stimulated echo sequence, and then fitting the resultant decays of signals to the appropriate theoretical expression. The data are typically presented in 2D plot with a spectral dimension and a diffusion dimension; in the latter the lineshape is a Gaussian centred on the apparent diffusion coefficient and with a width determined by the standard error estimated in the fitting process.

A variety of effects conspire to make this less straightforward than it sounds. Where signals overlap it is difficult or impossible to extract reliable diffusion information for individual components; many instrumental effects can complicate the analysis; and in concentrated samples the nuclear magnetization itself perturbs the measurements.

Frequently, overlap is present in the normal spectrum, rendering it impossible to achieve the high resolution in the diffusion dimension that is obtainable where the decay can be fitted to a monoexponential curve. In cases of overlap a natural extension of the basic DOSY experiment is to add diffusion encoding to a 2D experiment, to form a 3D DOSY experiment, where spectral overlap is much less likely. Such pulse sequences have hitherto been created almost exclusively by concatenating a diffusion encoding sequence with an existing 2D experiment. This is often a practical approach, but in some cases the experiment can be greatly improved by incorporating the diffusion encoding internally in the existing 2D experiment, in a type of pulse sequence christened IDOSY, where I stands for internal. This scheme lends itself particularly easily to experiments that are displayed in absolute value mode, such as COSY or 2DJ spectroscopy. The main advantages of IDOSY experiments are that the minimum experimental time is substantially reduced – up to 32-fold compared with previous 3D-DOSY sequences – and that using a spin-echo instead of a stimulated echo gives an approximate 2-fold increase in signal-to-noise ratio.

Convection is highly detrimental to diffusion measurements, and is commonly encountered for samples in low-viscosity solvents (such as chloroform) away from ambient temperature. To achieve convection compensation in a stimulated echo experiment, two stimulated echoes are needed, throwing away 75% of the signal. In IDOSY sequences, convection compensation can be achieved with little or no loss in signal-to-noise ratio.

Improved pulse sequences for diffusion encoding in HMQC, COSY and 2DJ experiments, HMQC-IDOSY (1), 2DJ-IDOSY (2) and COSY-IDOSY (3) will be presented; these are faster and more sensitive than existing methods, and allow easy compensation for convection.

Radiation damping can complicate measurements on concentrated samples very significantly, even where special hardware is used to switch the probe Q (4). Diffusion measurements, however, can be carried out even on pure H_2O samples using a very simple modification of existing techniques to to limit the net magnetization used (5).

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^{19}F NMR Measurements of SF_6 gas dissolved in liquid crystals

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Noble gas atoms have been used for years as probe atoms in the investigation of liquid crystals [1]. Particularly popular has been ^{129}Xe due to its exceptionally sensitive NMR shielding. However, the low signal intensity leads often to very long experiment times which may be a significant disadvantage [2].

We have used sulfur hexafluoride (SF_6) gas to study mesophase structures of several liquid crystals. SF_6 is chemically inert and is completely stable in the presence of most materials. Its symmetrical structure and six fluorine nuclei (natural abundance of ^{19}F is 100%) lead to a very intensive singlet in the ^{19}F NMR spectrum. ^{19}F NMR measurements of SF_6 dissolved in liquid crystals revealed a distinct temperature dependence of the ^{19}F NMR shielding. Although the ^{19}F shielding is not as sensitive as that of ^{129}Xe, all the phase transitions of liquid crystals were clearly observed. High intensity of the signal is especially useful in the diffusion measurements of SF_6. It is possible to carry out SF_6 diffusion measurements in a matter of minutes, while measurements with ^{129}Xe take several hours. Diffusion experiments were performed in the direction parallel to the layer normal, i.e. along the external magnetic field.

We believe that ^{19}F NMR spectroscopy of SF_6 gas dissolved in liquid crystals could be a very useful tool to derive information on phase transitions, orientational order parameters and mesophase structures of liquid crystals. In our presentation we will introduce the latest results of ^{19}F NMR shielding and diffusion measurements of SF_6 gas dissolved in a number of thermotropic liquid crystals.

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Advances in the micro-structural characterisation of food emulsions by PFG NMR

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Many food materials and products can be considered as emulsions. Such emulsion can be of the water-in-oil (W/O, e.g. margarine's) and oil-in-water (O/W, e.g. mayonnaise's) type, but sometimes even more complex duplex (e.g. water-in-oil-in-water) emulsions can be encountered. The foods industry heavily relies on the microstructural characterisation of food emulsions for the understanding and prediction of important consumer-related features, such as rheological and microbial stability. Pulsed Field Gradient (PFG) NMR diffusometry has become a key technique for rapid microstructural characterisation of food emulsions, in particular for assessment of droplet size distributions. Within a research setting, such measurement are carried out a high-field NMR spectrometers, but for application within development and manufacturing environments also low-field (20 MHz) benchtop NMR spectrometers are being deployed. Here we report advances within both areas.

Within the benchtop NMR area we have developed a novel acquisition procedure that circumvents the cumbersome routine for balancing of gradient pulses. Hence, it is now possible to record PFG decays almost and order of magnitude more rapidly than the one currently being deployed, and furthermore also a significant gain in sensitivity can be achieved. The procedure has been implemented for determination of droplet sizes in both W/O and O/W emulsions and has been extensively validated for a range of industrially relevant samples. Currently, the methods perform well for unimodal lognormal droplet size distributions.

When food emulsions fall outside the scope of the benchtop NMR methods, we need to take recourse to high-field NMR equipment. Such emulsions involve for example bimodal droplet size distributions, or even duplex emulsions. For the first a constrained lognormal bimodal fitting algorithm was deployed, which was validated by mixing samples with established droplet size distributions at different ratios. This algorithm was recently used to identify bimodal droplet size distributions in several W/O emulsions. For the characterisation of W/O/W duplex emulsions, a procedure adapted from the constrained bimodal fitting routine was explored. Thereby the external water phase was treated as a fraction of large droplets, whereas the internal phase was treated as a fraction of small droplets. Using the bimodal fitting algorithm, but without constraints, the fraction of internal water, as well as the internal droplet size distribution, can be calculated.

Probing grafted ligands at the surface of nano-particles by pulsed field gradient NMR

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Because of their size related physical properties such as quantum confinement and surface plasmon resonance, and their huge surface-to-volume ratio, which is of great interest in the areas of catalysis, reinforcement of polymers, and membranes, nano-particles represent an important research subject from both an academic and a technologic point of view. A good understanding of their surface chemistry is necessary since the adjustment of their size relies sometimes on the controlled poisoning of their growing surface by complexing ligands, and also because their use often needs functionalization of their surface in order to avoid aggregation or to introduce new characteristics such as hydrophobicity, specific recognition of certain chemical functions or an easy dispersion in organic solvents or polymers. However, the *in situ* measurement of the affinity of an organic ligand for an inorganic nano-particle is not an easy task, but is indispensable for a good control over the properties of hybrid organic-inorganic nano-particles.

In consequence, a practical technique, fast and selective for probing the ligands at the surface and for quantifying them against the free ligands is highly desirable. Pulsed field gradient NMR can be that technique since it offers, on top of the classical NMR parameters in solution (chemical shifts and scalar coupling constants), an elegant method for measuring diffusion coefficients, which in turn permit to sort the species as a function of their size. In this way the free ligands can be discriminated from bound ones, even when they have the same chemical shift, since they will display different diffusion coefficients.

The technique has been applied to cerium(IV) oxide nano-particles, presenting a diameter of ~5 nm and functionalized by long chain carboxylic acids. The experiments have been realized by proton NMR with a "LED-BPP" pulse sequence on an Avance 250 spectrometer. The attenuation profiles of each resonance, as a function of the intensity of the gradient, clearly demonstrate the existence of two populations of ligands, one diffusing rapidly corresponding to free ligands and one slowly corresponding to ligands grafted onto the surface of the nano-particles. The analysis of the attenuation profiles has also permitted to determine the relative quantities of the two types of ligands.

NMR diffusion measurements in partly polymerized isobornyl methacrylate-liquid crystal mixtures for modeling of photo-enforced stratification

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To keep pace with customer demand and economic requirements, present liquid crystal display manufacturing technologies require the processing of larger substrates with increasing production speed. Moreover, new display designs require displays that are flexible and non-rectangular in shape. We developed a photo-enforced stratification process[1] that enables the production of *paintable* liquid crystal displays: single-substrate LCDs produced by sequential coating and curing multiple (electro-)optical layers on top of each other. In this technique, a thin film of a mixture of a liquid crystal blend and a polymer precursor. Subsequent photopolymerization-induced diffusion, the result of a UV intensity gradient over the layer, is used to control the location of the phase separation process i.e. at the bottom of the film. Initiating the phase separation at the bottom leads to a stratified morphology: a polymer hard coat on top of a pure liquid crystal (LC) layer. In order to optimize the formulations and the process conditions, we are developing a mathematical model to describe the photo-enforced stratification process. To support the outcome of the model, the value of the diffusion constants as a function of the degree of polymerization of all constituents in the LC/precursor mixture have been quantified experimentally. NMR is a powerful technique for this purpose, since both the diffusion constant and the degree of polymerization can be measured at the same sample. All measured samples contain liquid crystal, a monomer system (consisting of isobornyl methacrylate and bisphenol-A-dimethacrylate), and a photo-initiator. The samples were prepared in a glove box to prevent oxygen-inhibition. For locking purposes, a coaxial insert with deuterated tetrachloroethane ($C_2D_2Cl_4$) was used. After preparation, the sample-tubes were sealed using parafilm and irradiated with UV light in subsequent steps. In this way the degree of polymerization in the sample was increased stepwise. In between the UV irradiation steps the degree of polymerization and the diffusion constants of the liquid crystal and the monomer components were measured. For the measurements of the degree of polymerization, a ^{1}H-spectrum with 16 averages and a delay-time of 5 seconds was recorded at 7T. The double bond signals of the UV-curable precursor were integrated using a signal of the non-reactive LC as a reference. For the diffusion-measurements, a standard stimulated spin-echo sequence with bipolar gradients and a spoiler gradient between the 2nd and 3rd 90^o pulse was used. As expected, diffusion coefficients decrease at higher degrees of polymerization. The rate of this decrease depends on the sample composition. For this application, NMR proves to be an excellent technique to determine the diffusion coefficients as function of the degree of polymerization.

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Conformational Analysis of Cinchonine, Cinchonidine and Their Methoxy Derivatives by NMR and Computational Methods

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Cinchona alkaloids are used as chiral modifiers for heterogeneously catalysed enantioselective hydrogenation reactions of C=O and C=C double bonds. Asymmetric hydrogenation of α-keto esters, keto acetals and diketones using cinchona-modified platinum catalysts is a heterogeneous catalyst system rivaling the enantioselectivity obtained with homogenous hydrogenation catalysts. Previously it has been noted that the conformation of the cinchona alkaloid plays an important role in the enantioselectivity [1].

The conformations of cinchonine (CN) and cinhonidine (CD) have been studied by the NMR spectroscopy and molecular modeling. The conclusions were mainly based on the NOE experiments and coupling constants. Typically CD and CN are present as a mixture of several conformations at room temperature. However, low solubility of these compounds, especially into non-polar solvents, has prevented the detailed study at low temperatures. This is a drawback, since signals of different conformers could be separated in NMR spectra at low temperatures. When the hydroxy group of CN and CD is replaced by a methoxy group, the solubility of the compounds is highly increased and low temperature NMR studies are possible. At the same time, it seems that the conformational equilibrium of methoxy derivatives is analogous to CN and CD. Thus, results obtained from methoxy derivatives can be extrapolated to parent compounds CN and CD.

In the present investigation, methoxy derivatives of CN and CD are synthesized and studied by NMR in several solvents and at low temperatures to receive NMR spectra for individual conformers for the first time. The geometries of the possible conformations are optimized with the density functional theory and Hartree-Fock/MP2 methods. The *J*-coupling constants for crucial proton-proton couplings are calculated based on the Fermi contact contributions. The conformers present in different solutions are solved by the combination of experimental and theoretical results.

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The influence of heteronuclear dipolar interactions on field-cycling NMR measurements of spin-lattice relaxation: ^{13}C, ^{19}F, ^{2}H and ^{1}H studies of proton tunnelling in the hydrogen bond

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A current-switched superconducting field-cycling NMR spectrometer has been designed and built for studying the role of quantum tunnelling in molecular dynamics. [1] The instrument is designed for work in the solid state with sample temperatures extending from 4K up to 300K. The maximum field-switching rate is 10Ts-1. Among the systems studied is molecular rotation in symmetrical groups such as the methyl rotor, the rotations of the hydrogen molecule in various environments and proton transfer in the hydrogen bond. [2,3,4] The latter will form the focus of this paper and the emphasis will be on the methodology of field-cycling relaxometry in cases where the sample possesses two spin species so that heteronuclear dipolar interactions are dominant.

Concerted double proton transfer in the hydrogen bonds of carboxylic acid dimers is well established as the model system for translational quantum tunnelling. Tunnelling matrix elements are exponentially dependent on the barrier properties and the particle mass; therefore isotope effects can provide useful insight into the fundamental mechanism underlying the dynamics. Direct isotope effects, involving deuteration of the hydrogen atoms implicated in the proton transfer, have recently been published. [3] However, of topical interest is the possibility of secondary isotope effects arising from isotopic substitution of the molecular skeleton. Proton transfer in the hydrogen bond is accompanied by the motion of the heavier framework atoms of the molecule; the motion is properly described as one over a multi-dimensional potential energy surface. We have studied the proton transfer tunnelling dynamics via the 13C NMR of 13C substituted benzoic acid with the aim of observing small isotope effects in the tunnelling rate. Such a study has become viable due to advances in field-cycling NMR relaxometry which allow direct mapping of the spectral density and therefore accurate (2-5%) measurement of correlation times. This is experimentally challenging and represents what we believe is the first field-cycling study of 13C. [5]

The introduction of a second spin species has a significant effect on the spin-lattice relaxation of the first spin species (and vice-versa). Compared with homonuclear systems, the spectral density acquires additional components characterised by the sum and difference Larmor frequencies of the two nuclei. Further, instead of a single relaxation rate, the spin-lattice relaxation is characterised by a 2 x 2 relaxation matrix. Therefore, the magnetisation recovery becomes biexponential and the initial polarisation state of the second nucleus strongly affects the magnetisation recovery of the nucleus which is being observed in the spin-lattice relaxation measurement. We shall report on the results of spin-lattice relaxation investigations on 1H-13C, 1H-19F and 1H-2H heteronuclear systems. The role of heteronuclear interactions in spin-lattice relaxation and the methodology of field-cycling relaxometry will be discussed.

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Characterization of the conformational behaviour of the natively unfolded domain of the nucleocapsid-binding domain of Sendai virus using residual dipolar couplings, spin relaxation and small angle scattering

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The Nucleocapsid-binding domain of Sendai virus phosphoprotein, also called Protein X (PX), comprises two structurally and dynamically distinct domains. A 47-residue C-terminal domain forming a three-helix bundle, and the N-terminal fragment consisting of a 48 residue flexible tail. We have measured residual dipolar couplings (RDC), spin relaxation, small angle neutron scattering experiments (SANS) and circular dichroism on PX, indicating in all cases that N-terminal fragment is a highly flexible chain. Our objective was to derive a more detailed description of the structure and dynamics of PX compatible with the wealth of biophysical data available. We have developed conformational sampling models of increasing complexity, based on the previously presented meccano algorithm, modified to introduce amino-acid specific conformational propensities, the presence of secondary structure and a simple representation of side-chain volumes. Different ensembles of conformations were created, and for each of these conformations N-NH, C'-NH and C'-Ca RDC were predicted, as well as the radius of gyration, Rg of the protein. We are thus able to propose a simple model of statistical sampling for the ensemble of conformations of the unfolded domain, with averaged observables that closely reproduce experimentally measured parameters for both the folded and the unfolded domains.This study clearly demonstrates the sensitivity of residual dipolar couplings to detect the presence of more rigid, or potentially more rigid regions of the polypeptide chain, that are otherwise difficult to detect by other biophysical measurements. In the case of PX the local order detected in this way may code for the necessary structuring of the flexible domain prior to interaction with physiological partners. The characterization of the conformational behaviour of this domain is therefore of great interest for the understanding of the structure-function relationship of this protein.

The Use of Transverse Relaxation Decay Deconvolution for the Quantitative Structural Description of Food Based Materials

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Introduction
The phase-composition of food (i.e. relative amount of solid, gel and liquid phases) plays an important role in human perception of food taste and flavour. It has also an impact on food appearance and shelf life-time. The aim of this work was to develop accurate and precise methodology for the phase-compositional assessment of food products and materials, by means of transverse relaxation decay deconvolution (TRDD).

Methodology
Combined FID-CPMG experiments were performed on benchtop time-domain NMR spectrometers, operating at low-field (20 MHz for 1H). The transversal relaxation decays were analysed by deconvolution in semi-empirical mathematical functions. A contrained fitting procedure was applied to fit a population of lineshapes for solid (Pake and gaussian), semi-solid (exponentials) and liquid (exponentials) components. For lipid crystalline polymorphs, a set of fitting constraints was defined that allowed their precise and accurate quantitative assessment. The methodology was extensively tested and validated on a range of lipid based food materials and products from the industrial practice.

Lipid blends:

It was found that for the assessment of Solid Fat Content (SFC), TRDD performs equally compared to the 'classical' methods. In addition, it also provides quantitative information on lipid polymorphism.

Food emulsions: TRDD was applied to Oil-in-Water food emulsions. Solid content of complete product formulations could be assessed in a straightforward manner, as well as crystal polymorphism of solid fat components. Furthermore, also the SFC of the fat phase can be assessed, provided compositional details of the product are specified.

Hydration properties of food materials: FID-CPMG decays of a series of protein and carbohydrate based food ingredients, measured for sample equilibrated at different hydration levels, were analysed in terms of functions representing solid and mobile components. Fractions of the mobile components were correlated with water sorption isotherms to determine correspondence between phase-composition and hydration behaviour.

In-Cell NMR studies of calmodulin protein expressed in E. coli

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In vivo detection of protein dynamics, structural changes or interactions is strongly required for the explicit understanding of structural basis of their functions in living systems. Recent developments of NMR spectroscopy allow us *in vivo* observation of high-resolution heteronuclear multi-dimensional NMR spectra of an overexpressed protein. This "In-Cell NMR" method has been gradually applied for characterization of above mentioned protein behaviour in living cells. In this presentation, we report our recent trial of backbone resonance assignment of calmodulin (CaM) protein overexpressed in living *E. coli* cells. We also describe the evaluation of conditions for sample preparation and the optimization of parameters for the In-Cell NMR measurements.

Backbone resonance assignment was performed by measuring six 3D triple-resonance NMR experiments, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HN(CA)CO and HNCO. In order to overcome the short lifetime and low sensitivity of living *E. coli* samples, we applied nonlinear sampling for indirectly detecting dimensions, which enabled us to measure spectra of equivalent quality with approximately 1/3 duration, when compared with the case employing conventional sampling scheme. The obtained NMR data were processed by using 2D maximum entropy method. By analysing the six 3D triple-resonance NMR spectra, approximately 40-50 % of backbone resonances have been assigned so far, suggesting that the resonance assignment for backbone signals of protein in living cells is possible under optimized condition. Based on the results, we are trying to extend this technique toward the detection of protein-protein interactions, conformational changes in cells.

Study by EPR of the phase transition in KH_2PO_4:$Fe(NO_3)_3$:$9H_2O$

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Electron Paramagnetic Resonance (EPR) has been used to monitory the phase transition in single crystals KH2PO4 (KDP) doped with Fe(NO3)3:9H2O. Fe3+ centre and other radicals are present in these crystals before an irradiation and have been used for following the transition. The line associated with the Fe3+ was useful for monitory this transition and was observed a splitting in this line when the measuring temperature was closed at the phase transition temperature. The EPR spectra of Fe3+ suggest that this ion is substituting a phosphorus ion.

Characterization by electron paramagnetic resonance (EPR) of samples of dump from Cerro Matoso Mine (Colombia).

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In the present work the studied solids are considered as “clayed materials” according to some definitions (soil science sense, grain size sense and geological sense) based on the particle size fraction of the samples (< 2mm). These materials were characterized by EPR, X-ray diffraction and X-Ray fluorescence. The results indicate that the analyzed solids are rich in Fe2O3 and NiO among other oxide. The scum material shows diffraction signals corresponding to minerals called enstatite (pyroxene). Moreover the scum EPR analyses showed a broad line around g = 2.0 corresponding to Fe3+ clusters into a complex glassy matrix. An analysis at different temperature was also performed.

Nanoscale electronic inhomogeneity probed by ^{17}O-^{207}Pb SEDOR in superconducting $BaPb_{1-x}Bi_xO_3$ oxides

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Electron correlations in the conduction band of superconducting oxides $BaPb_{1-x}Bi_xO_3$ ($0.1<x<0.35$) are a subject of many studies during last two decades. Low density of carriers ($\sim 10^{21}$cm^{-3}) and the increased charge fluctuations in Pb/Bi cation sublattice can lead to instability of the uniform ground state of the electron system in metallic phase. As shown in [1] it results in substantial inhomogeneous magnetic broadening of the ^{17}O ($I = 5/2$) and ^{207}Pb ($S = 1/2$) NMR spectra due to distribution of the Knight shifts (K) being proportional to the local spin susceptibility of carriers. In this report we focus on two experiments demonstrating an advantage of the ^{17}O-^{207}Pb spin-echo double-resonance (SEDOR) technique in studies of the nanoscale electron heterogeneity arisen near the Bi cation in metallic phase of the $BaPb_{1-x}Bi_xO_3$ oxides.

- Indirect ^{207}Pb-^{207}Pb, ^{17}O-^{207}Pb nuclear spin couplings. In metal phase the indirect nuclear spin coupling is mediated through the Fermi-contact hyperfine interaction. Sufficient inhomogeneous broadening of the ^{207}Pb line with $^{207}(\Delta K) / K > 1$ allows to consider indirect scalar coupling in the restricted form $J_{ij}S_i^zS_j^z$ with $J_{ij} \sim \chi(r_{ij})$ –non-local spin susceptibility of carriers. For oxides with natural abundance of the ^{207}Pb isotope (~ 21%) the modulated spin-echo envelope $^{207}M(2\tau)$ was observed. The beat frequency $^{SS}\omega_{ex}=J_{1nn}/2 \approx 2.5(2)*^{207}K(ppm)$ s^{-1} is caused by indirect coupling of a pair of the nearest neighboring (*nn*) Pb ions. The *I–S* coupling was studied by ^{17}O-^{207}Pb spin-echo double-resonance (SEDOR) performed in the $BaPb_{1-x}Bi_xO_3$ oxides enriched in ^{17}O isotope up to ~15%. Again we have observed the modulated ^{17}O-^{207}Pb SEDOR signal $M(^{207}\nu;2\tau)$, which is expected for almost isolated ^{17}O-^{207}Pb pairs of nuclei at small concentrations of NMR isotopes involved in SEDOR. The beat frequency $^{IS}\omega= {}^{IS}\omega_{dip}+ {}^{IS}\omega_{ind}$ is controlled by the classic dipolar and the heteronuclear indirect interaction. For the last one we have obtained approximately the same relation between ^{17}K and the beat frequency, namely, $^{IS}\omega= {}^{IS}J_{1nn}/2 \approx 2.3(3)*^{17}K(ppm)$ s^{-1}. The ratio $|J_{1nn}|/\omega_{NMR} \sim 0.07$ is the lower estimate for relative change of $\chi_{s,loc}$ occurring at the interatomic distance [2].

- ^{207}Pb NMR spectra. The NMR spectrum of ^{207}Pb becomes "invisible" by conventional spin-echo technique due to extremely fast rate of the ^{207}Pb spin-echo decay ($^{207}T_2 \sim 1$ μs) occurring in superconducting oxides ($x > 0.2$) [1,3]. In ^{17}O-^{207}Pb SEDOR the relation $^{17}T_2 \sim {}^{207}T_1$, being optimal to detect the ^{207}Pb spectrum, is easy to achieve at $T < 100$ K, since in oxide of the metallic phase $^{207}T_1$ is inverse proportional T. In this work we present the ^{207}Pb spectra measured in superconducting $BaPb_{1-x}Bi_xO_3$ oxides ($x < 0.35$) by ^{17}O-^{207}Pb SEDOR at 20K in magnetic field of 9.4 T. The average Knight shift $<^{207}K> \sim N(E_F)$ was found to vary with *Bi*-concentration *x* in similar manner as $T_C(x)$ behavior, where T_C is the critical superconducting transition temperature in a oxide.

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ESR-Imaging

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We present here our recent results on ESR-imaging via temperature dependent pulsed X-Band ESR and the backprojection reconstruction method focussing on quasi one-dimensional organic conductors, where even pulsed conduction electron spin resonance is possible.

We manipulated the defect concentration in a $(fluoranthene)_2PF_6$-crystal by spatially modulated proton irradiation and used a spatially resolved ESR-analysis to quantify the resulting local defect concentration, spin diffusion coefficient or electron spin concentration. It could be proven that spin diffusion coefficient and Peierls transition can be tailored via the radiation induced defect concentration and monitored via spatially resolved ESR.

Since flow measurement is an established tool in NMR-imaging, the possibility of performing ESR experiments on conduction electrons allows in principle to determine the electron spin drift velocity for given electrical current. We present an appropriate pulsed gradient and current ESR detection scheme and the first successful experiment of detecting the phase shift in the ESR-signal induced by an applied electrical current. The ohmic correlation is demonstrated in a range of more than 0.5 ampere and a maximum drift velocity of 18 m/s was found in the metallic phase of a radical cation salt.

2D imaging of two types radicals by the CW-EPR method

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The CW-EPR method of image reconstruction is based on the sample rotation in a magnetic field of a constant gradient (50G/cm). In order to obtain a projection (radical density distribution) along a given direction the EPR spectra should be recorded with and without the gradient. At the next step the two spectra are subjected to deconvolution, which gives the distribution of the radical density. Having performed this operation for e.g. 36 different angles we get the information necessary for reconstruction of the radical distribution [1]. The problem becomes more complex when there are at least two types of radicals in the sample, then the procedure of deconvolution does not give satisfactory results. We have proposed a solution of getting projections for each radical, based on iterative procedures. The images of density distribution obtained for each radical obtained by our procedure have proved that the method of deconvolution in combination with iterative fitting provides correct results. The test was performed on a sample of polymer PPS Br 111 with glass fibres and minerals. The results indicated a homogeneous distribution of radicals in the sample volume. The images obtained were in agreement with the real shape of the sample.

Another technique of imaging of hyperfine interactions concentration distribution by the electron nuclear double resonance imaging (CW-ENDOR) we would like to propose is based on a step-by-step change of the magnetic field intensity. The procedure was tested on a sample of a monocrystal of succinic acid.

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Investigation of the Nuclear Interactions with the S = 5/2 Electron Spin of the Fe(III) in Aquometmyoglobin by HYSCORE Spectroscopy

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We have investigated the possibility of using HYSCORE (hyperfine sublevel correlation) spectroscopy to study the electronic distribution of systems with an electron spin larger than ½, such as high-spin Fe(III) centers present in some heme proteins. We studied frozen solutions of aquometmyoglobin from equine skeletal muscle by HYSCORE spectroscopy. In this protein, the iron-containing heme group is bound to the protein via a proximal histidine. Since the distal ligand is a water molecule, the Fe(III) is in a high-spin state (S = 5/2). A previous ENDOR (electron nuclear double resonance) study on single crystals of aquometmyoglobin revealed hyperfine and quadrupole tensors of the heme and histidine nitrogens[1]. Since this protein has been already so well characterized, we have chosen it as a model system for our study.

We have used variants of the HYSCORE experiments in order to detect the nitrogen signals over a wide range of magnetic field and therefore of selective orientations. Scholes *et al.* used an effective spin Hamiltonian to simulate the ENDOR spectra. We followed the same approach to simulate the HYSCORE spectra. The features in the HYSCORE spectra are reproduced by the simulations using the hyperfine and quadrupole tensors of the heme and the histidine nitrogen coordinated to the Fe(III) as obtained from the ENDOR study. This shows the possibility to extract hyperfine and quadrupole tensors from the HYSCORE spectra of high-spin systems.

Moreover, the HYSCORE spectra reveal the signals of the remote nitrogen of the hystidine as well as those of the protons interacting with the Fe(III). In order to investigate the proton interactions, HYSCORE experiments have been performed on aquometmyoglobin in deuterated water.

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Virtues of high field/high-frequency EPR: Advantages of multi-frequency approach in EPR

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Multi-frequency EPR is a preferred technique today as it enables one to obtain a more complete picture of the various processes affecting a paramagnetic ion in a given environment. It enables one to distinguish between the processes which depend on the external magnetic field and those which are independent of it. In assessing merit of using a particular EPR frequency, the factors which mask features of EPR spectra, notably linewidth and anisotropy, should also be taken into account. The effects due to second-order terms in perturbation in the energies of the levels become important when the Zeeman and non-Zeeman interactions are of the same order of magnitude. The advantages of high-frequency EPR are that it enhances the field-dependent interactions, while suppressing the second-order effects, in particular, the anisotropy. In addition, it enables observation of EPR spectrum when there exist large zero-field splittings (ZFS), which might not at all be observed, or at best results in a rather complicated angular variation of EPR line positions at low frequencies. As for influence of motion on EPR spectrum, the molecular motion does not completely average out at higher frequency due to faster time scale and thus can be better understood at higher frequencies. Further, the spectral shape is strongly influenced by the relative values of the ZFS and hyperfine (HF) interactions, and is easier to analyze at higher frequencies being simpler. This is because at lower frequencies, ZFS can lead to very broad lines in powders due to anisotropy. Nevertheless, low-frequency EPR is preferred to study field-independent interactions in greater detail, in particular the HF interaction, which is masked at higher frequencies when there exist g-anisotropy and g-strain. As well, aqueous-solution spectra of Cu^{2+} complexes are better resolved at lower frequencies, leading to determination of correlation times. Then there are frequency-dependent effects, e.g. the thermally active direct process of spin lattice relaxation, which can only be studied by multi-frequency approach. On the other hand, local-mode and Raman processes are frequency independent. Samples of different sizes and properties are optimally studied at different frequencies, since different sample sizes are required due to varying size of resonant cavity with frequency. Further, large and/or lossy samples are better studied at lower frequencies and vice versa. All these considerations suggest that multi-frequency approach is necessary to obtain a more complete and unambiguous picture. It includes study of Zeeman (g-value) and HF contributions, differentiating spectra of multiple species from each other, determination of number of nitrogens bound to copper, study of paramagnetic ions with spins greaterer than one-half, distance measurements based on dipolar coupling, study of non-Kramers (even-spin) ions, and measurement of rotational correlations. This presentation will discusses all these multi-frequency features in detail with the help of illustrative examples.

Quantum mechanics of 6-pulse DQC signal in Hilbert space: calculation of signal following the coherence pathways.

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A procedure is described to calculate the DQC (double quantum coherence) signal as a result of successive applications of six pulses on the initial density matrix proportional to $S_{1z} + S_{2z}$, with appropriate time evolutions, in the sequence $\pi/2$, π, $\pi/2$, π, $\pi/2$, π, with selective phase cycling so that only the coherent pathways p = (1,-1), (-1,1), (2,-2), (-2,2), (1), (-1), respectively are retained. The signals obtained after various time intervals following the last π pulse can be overlapped to construct the echo. Appropriate choices of time intervals following the various pulses can be exploited to calculate the two-dimensional DQC signal. The calculations are carried out using the direct-product representation in the 36 x 36 space spanned by the two electronic magnetic quantum numbers ($M_1, M_2 = ½, - ½$), and the two nuclear magnetic quantum numbers ($m_1, m_2 = 1, 0, -1$), describing the two coupled nitroxides in bilabeled membranes. The effect of each pulse is taken into account by subjecting the density matrix to a unitary transformation depending on the effective Hamiltonian, which is the sum of static Hamiltonian and the interaction with the pulse, and the duration of the pulse; whereas each time evolution subsequent to application of a pulse is governed by a unitary transformation depending on the static Hamiltonian and duration of the evolution. The static Hamiltonian includes the dipolar interaction between the two electronic spins, governed by the inter-electron distance. The magnetic quantum number representation appears to be the easiest for following the coherence pathways, since in this case only the elements (12,13,24,34) of the density matrix describe the pathway p=1, whereas their complex conjugate elements of the density matrix (21,31,42,43) describe the p=-1 pathway, using the direct-product representation of the two electronic spins. Likewise, the pathway p=2 is described by the element 14 of the density matrix, whereas its complex conjugate element 41 of the density matrix describes the p=-2 pathway. Finally, it can be easily shown that the unitary transformations used here in the Hilbert space are entirely equivalent to the Louisville-space formalism commonly used, employing superoperators. The calculations are here carried out entirely rigorously by using the eigenvalues and eigenvectors obtained by numerical diagonalization. The background theory of DQC calculation is described in [1,2}.

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Protein microcrystals oriented in a liquid crystalline polymer medium: an EPR spectroscopy study

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A material with oriented molecules can reveal information valuable for computacional and spectroscopic studies of metalloproteins. Correlation between molecular structure and spectroscopic data can be established using single crystals, oriented membrane proteins, paramagnetic molecules linked to DNA fibers and weakly oriented proteins by bicelles, and alligned crystals under intense magnetic fields. Here we report a novel composite solid material with dispersed and partially aligned metalloprotein microcrystals. The thin solid films were obtained with protein microcrystals in a host liquid crystalline polymer medium, with the alignment tuned by a shear flow. A strong orientation effect of the metalloprotein was observed by EPR spectroscopy and optical microscopy. A mathematical model for EPR spectral simulations revealed the orientation distribution function of protein molecules and the orientation of the *g*-tensor within the molecular frame. This new approch provides a general tool for correlating spectroscopic data, with a protein or model compounds structure.

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Magnetic resonance and magnetic anisotropy in nanoscale superlattices Co/Cu

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Magnetic resonance in multilayer $[Co(8Å) / Cu(d_{Cu})(111)]_{20}$ in magnetic fields parallel or a perpendicular film plane on the frequencies f = 9.4 GHz has been investigated. Oscillations of magnetic anisotropy K_A and resonance line width ΔH_{res} were found for thicknesses of copper layer d_{Cu} = 7-19 Å. Extremal values of K_A and ΔH_{res} were observed at $d_{Cu}=nd_{(111)}$, where n - is integer or half-integer and $d_{(111)}$ = 2.087 Å is the distance between Cu (111) planes. The intervals between neighboring maximums and minimums are $1d_{(111)}$, $1.5d_{(111)}$ and $2d_{(111)}$.

Is shown, that the absorption of a microwave capacity is caused by ferromagnetic resonance in the ordered planes of Co atoms. The frequencies of ferromagnetic resonance for directions of field $\theta_H = 0$ (the direction of magnetic field is perpendicular to film) and $\theta_H = \pi/2$ (the direction of magnetic field parallel to film) are described by expressions

$\omega/\gamma = H_{\perp} + H_{eff}$, when $\theta_H = 0$, and $(\omega/\gamma)^2 = H_{\|}(H_{\|} + H'_{eff})$, when $\theta_H = \pi/2$.

$H_{eff} = 2K_A/M - 4\pi M$, $H'_{eff} = 2K_1/M - 4\pi M$, $K_A = K_1 + K_2$.

Oscillations of K_A and ΔH_{res} at change of d_{Cu} are accompanied synchronous oscillations of specific resistance in external magnetic field equal to a field of saturation, and also magnetoresistance.

In thickness region d_{Cu} = 8-11Å and $d_{Cu} \sim$ 18Å the manifestations of interlayer antiferromagnetic exchange interactions between Co layers were revealed.

Results of the presented researches magnetoresonance and magnetoresistance properties of system $[Co(8Å) / Cu(d_{Cu})]_{20}$ demonstrate synchronous oscillations dependence on thickness of copper layers d_{Cu} with a characteristic step in 1-2 copper monolayers. Observed effects are connected with non-monotonous varying of layers borders roughness when d_{Cu} grows, because interlayer diffusion in this system is minimal, (physical properties of samples, including their resistance, are stability during several years).

Characterization by electron paramagnetic resonance (Epr) of samples of dump from Cerro Matoso mine (Colombia).

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The electron Paramagnetic Resonance (EPR) and X Ray Fluorescence were used to study dump samples from Cerro Matoso mine in Colombia. The EPR signal shows an broad line whit a g – factor at 2.1. The fluorescence analysis shows the presence of Fe, MgO, Al_2O_3, MnO in appreciable percentage in these samples. An analysis at different temperature was also performed.

Double electron-electron resonance (DEER) for micelles size determination

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The Pluronic block copolymers, PEO_x-PPO_y-PEO_x, built from chains of polyethylene oxide (PEO) and polypropylene oxide (PPO), are surface active and form micelles. In comparison to normal surfactants, these compounds have the peculiarity that their critical micelle concentration (CMC) and their surface activity depend strongly on temperature. Micellar solutions of various block copolymers were investigated using a variety of techniques including NMR, static and dynamic light scattering (DLS), fluorescence and small angle X-ray scattering (SAXS). Most of these studies concentrated on the determination of the phase diagrams and the CMC of different block copolymers [1,2]. The size of the micelles can be usually determined by neutron and light scattering, which provides the hydrodynamic radius of the micelle. These give an upper limit for the real micelle radius. Here we have applied EPR techniques to study the dimensions of micelles of Pluronic L64 (x=13, y=30), P123 (x=20, y=70) and F127 (x=106, y=70). This was done using the spin-probe 4-hydroxy-tempo-benzoate, which is a hydrophobic spin-probe that is situated at the hydrophobic core of the micelles and does not dissolve in the aqueous solution. The local concentration of the spin-probe was determined from the four pulse Double Election Electron Resonance (DEER) experiment, which measures the dipole-dipole interaction between two spins [3].

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FeAOX-6: A powerful singlet oxygen quencher as detected by spin trap EPR spectroscopy.

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In the present study we investigated the singlet oxygen quenching property of FeAOX-6 using spin trapping EPR spectroscopy. Furthermore $^1O_2$ quenching rate constant (Kq) of FeAOX-6 was measured using time resolved near infrared phosphoresence by flash photolysis. Our results show that FeAOX-6 is a very strong singlet oxygen quencher. The neuroprotective action of FeAOX-6 can be explained partially due to its strong singlet oxygen quenching ability.

Singlet oxygen ($^1O_2$) is one of the reactive oxygen species, which play a key role in important human diseases. Antioxidant strategies in protection against these diseases have been increasingly addressed in recent years. Carotenoids as well as tocopherols are well known quenchers of singlet oxygen. However, the combination of both compounds together has shown additions benefits in treatment of chronic diseases. Recently, we have synthesised a novel chimeric molecule, FeAOX-6 that combine the important properties of carotenoids and tocopherol. Preliminary studies have established that FeAOX-6, exerted both strong neuroprotective and antioxidant activity against peroxyl and superoxide radicals.

Investigating the dynamic properties of PLGA's by solid-state NMR

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Biomaterials have an enormous impact on human healthcare and have been extensively studied for several applications such as resorbable implants, dental surgery, tissue engineering, drug release, *etc*. Although a large range of polymers can be used, aliphatic poly(esters) are the leading candidates in the biomedical and pharmaceutical fields due to their tuneable mechanical properties, hydrolysability, biocompatibility and biodegradability. As carriers, these polymers have several advantages such as improving the therapeutic effect, prolonging the biological activity, controlling the drug release rate, and decreasing the administration frequency.

Although a large variety of organic molecules from low molecular weight synthetic drugs to biological proteins and peptides have been encapsulated successfully, the examples on the molecular level understanding of the mechanism of encapsulation are limited. The studies of the interactions between the polymers and the guest molecules are crucial to explain the mechanism of encapsulation and stabilisation of the drug, and also to evaluate the drug release.

A set of poly(lactide-co-glycolide)'s (PLGA) with different LA/GA ratios and morphologies was studied. Differential Scanning Calorimetry (DSC), high-resolution NMR and Gel permeation Chromatography (GPC) were used to investigate the bulk properties of such systems. In order to correlate these results with local properties of the materials, and to probe the local mobility in PLGA, Solid-State Nuclear Magnetic Resonance (SS NMR) spectroscopy have been extensively used. Different ^{13}C and ^1H solid-state NMR spectroscopic techniques such as variable contact-time cross-polarisation (CP VCT), $T_{1r}{}^H$ measurements and WIdeline SEparation (WISE), were applied to probe the chain dynamics and packing.

The results obtained on PLGA's with different LA/GA ratios suggest that the variations in the monomeric composition are not sufficient to explain the differences in ^1H-^{13}C CP dynamics, and that the aging and processing have a significant influence on the molecular dynamic of the polymers. It has been shown that lactide units exhibited higher local mobility than glycolide units. An increase in lactide content in PLGA's leads to a decrease of the local packing of the materials. Preliminary experiments performed on drug-loaded microsphere with various amino acids and proteins suggested that the incorporation of a guest molecule in a polymeric network of PLGA modified significantly the local dynamics of the polymer.

Experimental and Theoretical Study of the Monomolecular Hyperfine Structure Transformation in MARY Spectroscopy

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It is a common situation in MARY spectroscopy that charge transfer to acceptor is accompanied by instantaneous transformation of the hyperfine structure of one of the partners in the recombining radical ion pair AB. This process can be described as a monomolecular reaction

(1) $A \rightarrow (\tau_c^{-1})C.$

Understanding its manifestations in MARY spectra is very important for correct interpretation of experimental results. Here, the result can considerably depend on the type of the ESR spectra of the radical ions in the initial and the final pair.

In this work we studied two limiting cases of reaction (1), namely, when there is no spin evolution either in the initial or in the final pair. This can be achieved if hyperfine coupling in two of the three partners (B and A or C) is negligible. The case when partner A has a "wide" ESR spectrum, and hyperfine couplings in partners B and C are negligible results in "freezing" of the spin evolution in partner A and thus in the pair as a whole. On the other hand, the case when hyperfine couplings in partners A and B are negligible, and partner C has a "wide" ESR spectrum results in "switching on" of the spin evolution in radical ion pair. The difference between the two cases is in the part of the recombination kinetics that is cut off by time τ_c: if it is the initial part then we have the "freezing" case, and if it is the "tail" then we have the "switching on" case.

Theoretically, MARY spectrum is described as a convolution of the recombination kinetics with time-dependent population of the singlet state of the pair $\rho_{ss}(t)$. Taking into account reaction (1), $\rho_{ss}(t)$ in the case of the "freezing" can be written as

(2) $\rho_{ss}(t) = \rho_{ss}^{(i)}(t) \cdot \exp(-t/\tau_c) + {}^{t}\int_0 \rho_{ss}^{(i)}(\tau) \cdot \exp(-t/\tau_c) d\tau/\tau_c$

and in the case of the "switching on" as

(3) $\rho_{ss}(t) = \rho_{ss}^{(i)}(t) \cdot \exp(-t/\tau_c) + {}^{t}\int_0 \rho_{ss}^{(f)}(t-\tau) \cdot \exp(-t/\tau_c) d\tau/\tau_c,$

where $\rho_{ss}^{(i)}(t)$ is the population of the singlet state in the initial pair AB, and $\rho_{ss}^{(f)}(t-\tau)$ is the population of the singlet state in pair AB that has undergone transition into the pair BC at the moment τ. Performing the convolution it can be shown that in the model of exponential recombination kinetics with characteristic time τ_0 in the first case (2) the hyperfine structure transformation has an effect similar to shortening of the lifetime of the initial radical pair AB, which in turn leads to a broadening of zero field line in MARY spectra. In the second case (3) MARY spectrum is the spectrum of the final pair BC with an intensity proportional to the factor $1/(1+\tau_c/\tau_0)$, and reaction (1) just reduces the intensity of MARY lines due to delay in the development of spin evolution in the pair.

The influence of reaction (1) on MARY spectra was studied experimentally in the solution of 10^{-4} M *para*terphenyl-d_{14} in cyclic and linear alkanes, with radical cations of durene and isooctane being the "wide" partners and radical cation of deuterobenzene being the "narrow" partner. In both cases the predicted behavior of MARY spectra was obtained, and simulation of the experimental spectra yielded information on the rates of process (1).

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Spin Triads in Liquid Irradiated n-Alkanes

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In this work, magnetic effects in three-spin systems (spin triads) in liquid irradiated *n*-alkanes are discussed. Spin triads are the elementary case for the spin catalytic effect, which is the influence of external spin on the spin state of a radical ion pair. A three-spin system is created from a spin-correlated radical ion pair by capturing one of its partners to a spin-labeled charge acceptor/luminophore having a structure **A-Sp-R**. Here **A** is a positive or negative charge acceptor, **R** is a stable radical fragment, and **Sp** is a hydrocarbon bridge. For such systems, in addition to effects of low magnetic fields, a resonance signal in a field equal to the exchange integral in the biradical ion was theoretically predicted, provided that the exchange is not too strong (hundreds of gauss by the order of magnitude). We developed a method of synthesis of a promising class of spin-labeled luminophores with a 3-imidazoline radical fragment, because it seems reasonable to expect that the exchange interaction in the resulting biradical ions will be sufficiently low. In order to check the luminescing properties of this class of compounds, their optical absorption spectra have been studied. This revealed that the absorption band of the 3-imidazoline radical fragment strongly overlaps the radiation spectrum of the luminophore. Therefore, it is necessary to find a way to convert the excitation energy of the recombined spin-labelled luminophore into light to enable optical detection. Currently, a secondary luminophore is being selected, which would be able to absorb the excitation from the primary luminophore and emit a quantum in a longer wave band. The work was supported by Russian Foundation for Basic Research (Grant 03-03-32331) and Russian Programme for Support of High School Research (Project 8318).

Intramolecular Dynamics of Polyfluoroarene Radical Anions as Studied by OD ESR and Quantum-Chemical Methods

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Polyfluoroarene radical anions (RA's) are very interesting objects to study due to peculiarities of their electron and spatial structure caused by pseudo-Jahn-Teller effect. Quantum chemical calculations indicate potential energy surfaces (PES's) of the RA's to be pseudorotational surfaces with a number of local minima corresponding to different non-planar stationary structures [1]. Provided the energy barriers between them are sufficiently low (~kT) it can be supposed that dynamic transitions between these states would result in manifestations of spectral exchange in the ESR spectra of RA's. The ESR study of these phenomena is obstructed with the instability of polyfluoroarene RA's whose fairly high stationary concentration can be obtained only under special conditions (as a rule, frozen solutions at low temperatures). At the same time, the method employed in the present work, Optically Detected ESR (OD ESR), being highly sensitive for radical ion pairs, can register short-lived ion radicals in liquid solutions even at very low stationary concentrations and temperatures close to the room one.

In this work, intramolecular dynamics of two polyfluoroarene (1,2,3-trifluorobenzene and decafluorobiphenyl) RA's was studied both theoretically (using B3LYP/6-31+G* and PBE/6-31+G* calculations) and experimentally by the OD ESR method.

The PES's of both radical anions and values of hfc constants for the structures forming the path of pseudorotation were calculated. The PES of 1,2,3-trifluorobenzene RA includes minima of two types separated by energy barriers of 2 and 4 kcal/mole. The PES of decafluorobiphenyl RA includes four energetically equivalent minima.

The ESR spectra of both radical anions were detected for the first time. The temperature dependence of the spectra in the range from T=243 K to 325 K was measured. The spectrum of 1,2,3-trifluorobenzene RA proved to be temperature-dependent due to fast exchange between the energetically nonequivalent conformations [2]. At low temperatures the observed values of hfc constants of the RA are close to those calculated for the structure corresponding to global PES minimum. As the temperature increases, the contribution of the states of higher energy also increases, which leads to the shift of lines in the spectrum. Temperature dependence of the ESR spectra is in good agreement with the results of quantum chemical calculations of the PES shape. As for the decafluorobiphenyl RA, all over the temperature range we used the experimental spectrum corresponds to fast spectral exchange, with hfc values being averaged over four structures associated with energetically equivalent local minima.

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NMR structure of the glucose-dependent insulinotropic polypeptide

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Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid residue incretin hormone that triggers the release of insulin depending on the level of glucose in blood. This fact, together with the effects of GIP in the metabolism of fats, have made it a potential target for type-2 diabetes and obesity. Here we present the NMR solution structure of GIP in an attempt to further understand its mechanism of action. GIP adopts a well defined alpha-helical conformation between residues Phe^{6} and Gln^{29} when dissolved in a 50% TFE-d_3 aqueous solution. In pure water the helix is only partially formed and less well defined. These results are in agreement with the previously published NMR structure of the biologically active fragment GIP(1-30)amide, which is characterised by a helical segment between residues Phe^{6} and Ala^{28}. These findings may help determine the structural requirements for the biological activity of GIP.

Quadrupole coupling constants of deuterons in molecular clusters with strong hydrogen bonds.

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Electrolyte solutions are very interesting objects but they are still difficult for research due to the presence of many labile substructures with fast exchange of solvent molecules between them. NMR-relaxation method is a unique tool for the investigation of microstructure and molecular dynamics of such substances.

It was earlier shown that information from proton and deuteron resonanses led to the same results in the case of aqueous salt solutions. But in the case of solutions of mineral acids some difficulties appeared which were connected with a change of quadrupole coupling constants (QCC) of deuterons, which belong to hydroxonium ion and water molecules from different substructures of solution. In order to conciliate the experimental data from proton and deuteron resonanses quantum chemical calculations of QCC of deuterons in different molecular clusters modelling different solution substructures were performed.

At first, the QCC values for deuterons of isolated water molecule and hydroxonium ion were calculated at different levels of theory (RHF, MP2, MP4, CISD, CCSD, DFT/B3LYP) using different basis sets. It was established that a combination of DFT/B3LYP method with relatively small double-split basis sets like 6-31G** and cc-pVDZ provides good agreement with experiment. According to these results the B3LYP/6-31++G** combination was chosen for following calculations. The basis set was augmented by diffuse functions because using them is very important for molecular systems with strong hydrogen bonds. The QCC of deuterons were calculated for molecular clusters $(D_2O)_5$, $D_3O^+(D_2O)_3$, $Ca^{2+}(D_2O)_n$ (n = 6, 8, 10, 18) and compared with experimental values. In all cases mentioned above the geometry optimization was made.

These data allows the better interpretation of the experimental results which were obtained concerning molecular mobility and solution microstructure.

Human Chaperone (Hsp70) as Receptor in Ligand-Receptor Interactions Studies by DOSY-NOESY

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Screening ligand-receptor interactions and mapping epitopes with NOE pumping[1] experiments and Diffusion NMR spectroscopy[2] provide valuable information in drug development and protein function studies. However applying these experiments requires a keen eye on the rate of the exchanging systems. For fast-exchanging systems the mentioned NMR techniques are powerful tools, however false negative results, arise in slow exchange regimes[3].

To better investigate these NMR tools and their applications we have human heat-shock Hsp70 protein with still undefined functionality for our experiments. Our choice relied on chaperone Hsp70 of 72,215 Da[4]. The name Hsp70 describes a multi-gene family of chaperones but all members have four commom features: highly conserved sequence, molecular mass about 70 kDa, ATPase activity and an ability to bind and release of hydrofobic segments of unfolded polypeptide chains[5].

We are applying these NMR techniques (1D, 2D and 3D) to the identification of ligand binding epitopes using *D*-glucose, salicylic acid, adipic acid, and caffeine as ligands, and human heat-shock protein (Hsp70) as receptor. The preliminary NOE pumping results show that caffeine, as well as, adipic acid have strong association to Hsp70. Signal enhancement was observed for the adipic acid a-hydrogens suggesting that the carboxylic groups are important in the protein-ligand interaction. In caffeine molecule only two of the three-methyl groups depicted signal enhancement.

With the aim to identify the segments of the biding ligands with Hsp70, the same ligand mixture was analyzed with 3D DOSY-NOESY experiment. The caffeine hydrogens at 3.90, 3.46, and 3.29 *ppm* exhibited cross peak signals indicating binding with Hsp70. A lower binding affinity was observed for the adipic acid with cross peaks of methylene hydrogens at 2.31 and 1.59 *ppm*. T_1 measurements indicate that caffeine and adipic acid are most affected by protein (Hsp70) presence demonstrated by T_1 decreasing.

From these experiments we were able to establish appropriate criteria for the screening protein-ligand binding in fast exchange regime using as model system the human Hsp70 and the four-ligand mixture.

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Resonance investigation and sub-threshold two magnon absorption in low-dimensional magnet C22H18MnO8

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The antiferromagnetic resonance and sub-threshold two magnon absorptions in monoclinic crystals $C_{22}H_{18}MnO_8$ in frequency range 9.6-120Ghz at temperature 1.8K was investigated. The magnetic order type and anysotropy character was established by study of AFMR branches in collinear and noncollinear phases.

The maine line EPR spectra $C_{22}H_{18}MnO_8$ observed at room temperature in magnetic field H_0 (g=2.0) is acompanied by a weak additional line. It's resonance field is equal $H_0/2$ and its angular dependence described by expression $J(\theta)=\sin^2\theta\cos^2\theta$. The transition field $H_0/2$ testify that the magnetic structure of crystal $C_{22}H_{18}MnO_8$ has a low-dimensional. The value of transition field is quantitative measure of one-dimensionality this system.

The AFMR spectrum in one-dimensional $C_{22}H_{18}MnO_8$ is described by two-axice AFM model. The antiferromagnetic vector $\boldsymbol{l}$ align in bases plane of crystal. The values of spin-flop transition fields and energy gapes in magnon spectrum equal H_{sf}=4,1kOe; ν_1=12,2GHz; ν_2=44,3GHz at T=1,8K. The AFMR branche was observed at direction of external magnetic field $H\|\boldsymbol{l}$ and polarization $H\|\boldsymbol{l}$. This branche is softened with increasing H, and become zero at phase transition field. So it's behavior testify spin-flip transition. The behavior AFMR branche described equations $(\omega/\gamma)^2=2H_eH_a[1-(H/2H_e)^2]$ from which follows that value of effective exchange interaction is $2H_e$=102,6kOe.

Additionaly we obsorve high frequency field absorption in spin-flop transitions area in case $H\|\boldsymbol{l}$ and $H\|\boldsymbol{l}$. In this case the microwave field is too small for the nonlinear influence. The two-magnon absorption in this case is due to the photons decay on two magnons with opposite wave vectors ($\mathbf{k_1}$=-$\mathbf{k_2}$) directions [1].

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Control of Asymmetric Electron Transfer Kinethics in Cyanobacterial Photosystem I: TR EPR Study of Methionine to Asparagine Mutation of the Ligand to the Primary Electron Acceptor A_0

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The primary charge separation process in PS I starts after light excitation of the primary donor P700 (chlorophyll heterodimer) by electron transfer (ET) from P700 to the acceptor A0 with the formation of the primary radical ion pair P700+A0-. Two quasi C2 symmetric chains of acceptors exist, named according to the binding protein subunits: PsaA- and PsaB-branch, each with the acceptors: A-1 (accessory chlorophyll), A0 (chlorophyll), A1 (phylloquinone). The two branches converge at the first [4Fe4S] cluster FX. The ET active branch is investigated for the site-directed mutants in Synechocystis sp. PCC 6803. The axial ligand to the central Mg of A0, which is Met residue in wild type, has been substituted in either the PsaA- or PsaB-branch to Leucine (Leu, L) with inert side groups or Asparagin (Asn, N) with charged side groups. These mutations are expected to alter the Red-Ox potential of A0 in either branch and to influence the ET kinetics from A0 to A1 and possibly from A1 to FX. Multifrequency TR EPR techniques are applied to investigate the second P700+A1- and third P700+FX- radical ion pair in the MN and compared with the results for the ML mutants. Only substitution of Met by Asn in the PsaA- (not PsaB-) branch alters the first ET step, similarly to the ML mutant [1]. The effective lifetime of the precursor state P700+A0- increases from 50 ps to the 1-2 ns range with the consequence that spin dynamics (singlet-triplet mixing) can occur and will change the observed spin polarisation patterns. The changes observed in the polarization patterns of the consecutive P700+A1- and P700+FX- radical pairs with temperature can be explained by assuming a distribution of the lifetimes for the P700+A0- precursor state. The comparison of the ML versus MN mutants shows a stronger influence of the latter on both, the magnetic interaction parameters of the P700+A1- state and the A1\u2192 FX kinetics. High field EPR was used to unravel the two kinds of causes for pattern changes. In cyanobacteria, M mutation in the PsaA-branch influences the kinetics of the primary ET steps, while M mutation in the PsaB-branch has no influence on the ET. These results provide additional evidence for asymmetric ET along PsaA-branch in cyanobacteria and allow TR EPR characterization of three consecutive ET processes.

[1] R. Cohen et al. Biochemistry 43 (2004) 4741-4754 PS I-Photosystem I, ET-electron transfer, M/Met-methionin, N/Asn-asparagin, L/Leu-leucine.

New series of mediators in Nitroxide-Mediated Polymerization of Acrylates: TR CIDNP, NMR, EPR and Laser Flash Photolysis Studies

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Stable nitroxyl radicals are widely used as scavengers for carbon-centered radicals. The coupling reaction between nitroxides and carbon-centered radicals has gained additional attention after discovery of the Nitroxide-Mediated Free Radical Polymerizations (NMP)[1]. In NMP the quality (i.e. the molecular weight and polydispersity index) of the polymer formed is being determined by the ratio of coupling and dissociation rate constants of the reversible equilibrium between stable nitroxides, alkyl radicals and alkoxyamines, thus these rate constants are utterly important to know.

In this work we have applied magnetic resonance to the detailed study of the processes, which take place during NMP, and of the structural factors, which may influence on these reactions. The result of this study can be used to formulate the basic requirements for molecular design of the nitroxides for NMP and development of nitroxide-based radical polymerization regulators with improved characteristics. A specially designed nitroxide series (NIO) has been synthesized to reveal the influence of steric and electronic factors upon the reactions mentioned above. This series include 2,5-dihydro- and perhydroimidazole derivatives. The nitroxides within the series differ in steric and/or electronic character of the functional groups surrounding the nitroxide moiety. EPR spectra of these radicals have been measured at different solvents.

In most cases the coupling rate constants can be measured by means of laser flash photolysis (LFP), ESR, and time resolved CIDNP (TR CIDNP) techniques. CIDNP is most informative method because it provides *in situ* information about reaction products. A modified version of TR CIDNP has been developed recently. This original technique is called CIDNP in a Switched External Magnetic Fields (SEMF CIDNP) and it allows measuring rate constants of radical reaction with much higher (~20 ns) time resolution keeping the information about reaction products[2]. Recently we have shown that the SEMF CIDNP method is a good alternative to the TR CIDNP technique due to its better time resolution and it can be also very useful to replace LFP for the systems with low extinction coefficient of transient paramagnetic species[3].

In this work we apply SEMF CIDNP, TR CIDNP and LFP to the measurements of the coupling rate constants between alkyl radicals and series of newly synthesized stable nitroxyl radicals aiming to derive important kinetic parameters for modeling studies of NMP. The carbon-centered radicals were generated by laser pulse photolysis of the corresponding ketone precursors RC(O)R with well-known photochemistry in the presence of stable nitroxyl radicals. Analysis of SEMF CIDNP kinetics with varied concentrations of nitroxide allowed to derive the corresponding k_c values. The dissociation rate constants of alkoxyamines were measured by means of NMR[4]. TR CIDNP was used to study the products of coupling reaction of nitroxides and alkyl radicals. Preliminary results of polymerization of butyl methacrylate in the presence of used nitroxide series reveal narrowing of molecular weight distribution of resulting polymer (PDI ~ 1.25 upon M_w ~ 80 000).

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Orientational Molecular Dynamics in Glasses Studied by Pulsed Multi-Frequency EPR of Spin Probes.

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The origin of glass formation has attracted considerable theoretical and experimental attention, but is still not fully understood. The missing link between the macroscopic behavior of glassy materials and the microscopic dynamic requires the development of physical techniques specifically sensitive to different types of molecular motions.

In our study we exploited the possibilities of advanced high-field EPR to investigate orientational molecular dynamics in glasses near the glass transition temperature. To extract the dynamic parameters of the nitroxide probe Fremy's salt in glycerol glass at 185 K we studied the orientation dependent transverse relaxation times T_2 by means of 2D multi-frequency electron spin echo (2D-ESE) spectroscopy at 3 GHz / 0.1 T (S-band), 9.5 GHz / 0.34 T GHz (X-band), 95 GHz / 3.4 T(W-band), 180 GHz / 6.4 T (G-band).

The relaxation of the electron magnetization is coupled to orientational motion of the spin probe via motion-induced fluctuations of the effective hyperfine (A) and g-values. The rate of motion induced relaxation can be disentangled from other relaxation processes since it depends on the spectral position.

At different external magnetic field different interactions determine nitroxide line shape: At 3 GHz the field independent hyperfine splittings are resolved in the EPR spectrum, at 95 GHz and 180 GHz the field dependent g-tensor components dominate the lineshape, while in X-band both interactions contribute to the spectrum. Multi-frequency 2D-ESE can therefore probe the modulation of the different magnetic parameters with high orientation resolution providing a detailed picture about the motional processes.

Measurements at two different high Larmor frequencies showed a quadratic dependence the anisotropic relaxation rates on the external magnetic field. This allowed us to estimate the time scale of the motional process $10^{-10}\text{s} < t_c < 10^{-7}\text{s}$, i.e. at T=185 K the motion occurs in the fast motion Redfield regime. On the basis of Redfield's relaxation theory we developed motional models which describe different kinds of molecular reorientations and compared them with the experimentally obtained multi-frequency relaxation data.

Fits of the spectral simulations to the experimental results show good agreement for two different relaxation models: (*i*) isotropic orientational motion superimposed by dynamic g-strain and (*ii*) orientational excursions with a larger amplitude about the molecular axis lying in the plane of the nitroxide ring and perpendicular to the NO fragment.

The presented method can not only be applied to investigate motion in isotropic solvent glasses but also to spin labelled proteins to investigate conformational fluctuations that play an important role in the function of proteins.

Financial support by the Deutsche Forschungsgemeinschaft (SFB 498, SPP 1051) and YSF INTAS ((YSF 2002-410/F1b)) is gratefully acknowledged.

Investigation of the active site of the Fe-only hydrogenase from Desulfovibrio desulfuricans, using pulse EPR techniques

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Hydrogen plays an important role in the metabolism of microorganisms, where the reaction $H_2 \rightarrow 2H^+ + 2e^-$ is catalyzed by metalloenzymes, called hydrogenases [1]. It is known, that the active cite of the Fe-only hydrogenase (the so called H-cluster) contains a classical [4Fe-4S] cubane connected via the sulphur ligand of cysteine 382 to a bi-nuclear subcluster [2, 3]. The active site passes several states during the catalytic cycle, which can be detected using EPR. The oxidized form of the H-cluster (H_{ox}) shows a rhombic spectrum (well known '2.1' signal) [4]. The distal iron of the [2Fe] subcluster has a free coordination site. Upon inhibition of the protein by CO this iron is coordinated by the external CO ligand. In this case an axial EPR signal is observed.

The spin distribution of the H-cluster was investigated using pulse EPR techniques. Measurements were done on ^{57}Fe-enriched Fe-only hydrogenase from *Desulfovibrio desulfuricans* at X- and Q-band frequencies for the oxidized (H_{ox}) and the CO-inhibited (H_{ox}-CO) states of the H-cluster at 20K. In this content we present the first Q-band ^{57}Fe HYSCORE study of the H-cluster.

The EPR investigation of the H_{ox}-CO state reveals six different HF couplings with ^{57}Fe, which differ strongly from those found for the H_{ox} state. This indicates a profound delocalization of the unpaired spin density over the H-cluster and a strong influence of the CO ligands on its electronic structure.

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Temperature dependencies of ESR linewidth in quasi -one dimensional systems

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An empirical formula for the temperature dependence of the ESR linewidth is suggested.

Low-dimensional magnets are a subject of intense interest due to the fact that quantum phenomena play an important role in these compounds. An expansion of the EPR linewidth theory to the case of low-dimensional systems at temperatures $kT<J$ was performed, e.g., by Soos [1], who included the temperature dependence of the spin-correlation functions within the tradition formalism. Recently, Oshikawa and Affleck [2] used field-theory methods to derive the temperature dependence of the EPR linewidth deeply in the one-dimensional regime $(kT<<J)$. One relevant result of these papers is that, if the EPR linewidth is due to a single anisotropic interaction, then the temperature dependence of the linewidth is an isotropic function. This means that for all temperatures the relative anisotropy of the EPR linewidth ΔH should be the same as in the high-temperature approximation $T>J$, i.e. $\Delta H(T,\theta,\varphi)=F(T)\Delta H(\theta,\varphi)$, if $T>J$. The orientation of the external magnetic field H is determined by polar and azimuth angle θ and φ.

The quasi-one-dimensional $S=1/2$ antiferremagnet $CuGeO_3$ $(J/k_B\sim120K)$ is the inorganic compound demonstrating a spin-Peierls transition, with a transition temperature $T_{SP}=14.2K$. Temperature dependencies of magnetic susceptibility $LiCuVO_4$ is enhanced with respect to the Bonner-Fisher fit and indicates antiferromagnetic order at $T_N=2.1K$. Corresponding Heisenberg-exchange constant $J/k_B=45K$. In the Quarter-filled spin-ladder system NaV_2O_5 the rungs consist of two VO_5 pyramids along the a axis of the orthorhombic structure. Each rung carrying one electron is connected with the neighboring rungs via additional corners at their bases forming the ladders along the b axis. The magnetic susceptibility NaV_2O_5 is approximately described by a Bonner-Fisher law with $J/k_B=580K$. Below $T_{CO}=34K$ the susceptibility vanishes exponentially due to the opening of the spin gap. In $KCuF_3$ linear antiferromagnetic chains are forming along the c-axis as a result of the orbital ordering of Cu^{2+} holes, with the intrachain exchange interaction $J_c/k_B=-380K$, and turns into a long-range ordered state at temperatures below $T_N=39K$.

The theory of the ESR linewidth is well-developed for conventional three-dimensional exchange-coupled spin systems. It has been show that in the case of sufficiently strong exchange interaction the ESR spectrum is narrowed into a single Lorentz line with a linewidth ΔH (half width at half maximum) determined by the second moment M_2 due to anisotropic interactions and the exchange frequency caused by the isotropic Heisenberg exchange $H=JS_iS_j$ between neighboring spins S_i and S_j.

Here we present investigation of the temperature dependence of the EPR linewidth in $CuGeO_3$, $LiCuVO_4$, NaV_2O_5, $KCuF_3$ in paramagnetic regime. For all samples the linewidth increases monotonically above the phase transition temperatures (T_{PhT}). For a quantitative description of the temperature dependent of ESR linewidths, we tried to empirically approximate at $T>T_{PhT}$ by formula

$$\Delta H=A_1-A_2\log(J/[2(T+T_{PhT})]),$$

where A_1 and A_2- constant which are different for all samples.

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Nuclear Magnetic and Dielectric Relaxation in Polysaccharides

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Cellulose and the other polysaccharides – like methyl cellulose (MC), hydroxypropyl cellulose (HPC) and hydroxypropyl methyl cellulose (HPMC) – were the subject of our study by Nuclear Magnetic and Dielectric Resonance Spectroscopy. These polymers are built of repeating glucose unit which possesses two side groups (-OR) and one group (-CH_2-OR). For Cellulose the "R" symbols mean the hydrogen atoms, thus we have two hydroxyl groups (-OH) and one methylol group (-CH_2-OH) attached to the glucose ring. For MC R=H or R=CH_3 whereas for HPC R=H or R=propylene group. It follows that the studied polysaccharides are characterised by the same repeating glucose units, but with different side groups. The side groups differ in lengths and molecular weight. The main goal of our study was to determine the molecular origin of the relaxation and the influence of the different side groups of glucose unit in the relaxation process.

The proton spin-lattice relaxation times and the proton NMR second moments were studied as a function of temperature for MC, HPC and HPMC at 90 MHz. The two minima are clearly visible in the studied temperature range (100 K to 400 K) in T_1 dependencies. The low temperature minimum, which corresponds to the rotation of the CH_3 groups and the high temperature one assigned to the local motion of the segment of the polymer chain. The Field-Cycling NMR Relaxometry also researched the high-temperature minima where the frequency dependencies of spin-lattice relaxation times T_1 were observed in the range between 20 kHz and 40 MHz for nine different temperatures. The chosen temperatures were above the glass transition temperatures T_g determined for studied polymers by DSC method.

The dielectric spectra for Cellulose, HPC and HPMC were measured in the frequency range from 100 Hz to 1 MHz. It is quite common in dielectric spectroscopy of synthetic polymers to distinguish three types of relaxation: α-relaxation (related to the glass transition), β-relaxation (related to the local motion of the main chain segment or to the larger side groups which makes the side chain) and γ-relaxation (caused by the motion of the side-group). Our results indicate one γ-relaxation process in the Cellulose and two γ and β-relaxation processes in the HPC. In the studied polymers the temperature dependence on the relaxation times follows Eyring's equation and the entropy and enthalpy contributions of free activation energy of thermoactivated motion were determined.

Inter Spin Distance Determination in Proteins Based on Simulations of EPR Spectra in the Frequency Range from 9 to 95 GHz

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EPR spectroscopy in conjunction with site-directed spin labeling has evolved as a powerful tool to study the structure, dynamics and function of proteins. Multi-frequency EPR spectroscopy with the application of microwave frequencies in the range from 9 to 360 GHz is promising for providing quantitative understanding of correlation between nitroxide motion, inter-nitroxide distances and protein structure.

Here we present simulated EPR spectra for different microwave frequencies including dipolar interaction based on two different approaches for the simulation: The more precise approach using exact quantum mechanical theory for the construction of spectra is used for comparison with and evaluation of the widely used approximate approach based on perturbation theory. This approach uses the Pake-Pattern to embed the dipolar interaction between two unpaired electrons in the simulated spectra. Furthermore both types of simulations are used for determination of inter spin distances from EPR spectra of doubly spin labeled mutants of bacteriorhodopsin. For distance determination in proteins experimental data is acquired at low temperatures. The resulting powder spectra of unpaired electrons in randomly oriented proteins are analyzed by fitting simulated EPR spectra to the experimental data. We calculate for the first time EPR powder spectra for different distances between the unpaired electrons using the exact quantum mechanical approach based on diagonalization of the Spin Hamiltonian. However, our results show agreement between both approaches which supports the applicability of the approximate approach based on the Pake-Pattern for data analysis.

A Home Built W-band EPR Spectrometer to Determine Local Polarities at Different Positions in Spin Labeled NpSRII-NpHtrII complexes

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Combining site-directed spin labeling (SDSL) techniques with high-field/high-frequency EPR is known to be a major step forward concerning sensitivity and selectivity of the SDSL method. This applies to studies of molecular motion, polarity and proticity changes of the microenvironment of the spin label (SL), as well as the investigation of conformational changes of spin labeled protein segments [1]. We report of a recently completed home built EPR Spectrometer working at 95 GHz, both in continuous and pulsed mode. We further present first results from CW measurements on spin labeled NpSRII-NpHtrII complex variants reconstituted in purple membrane lipids. EPR spectra of frozen protein solutions (170K) recorded at 95 GHz allow precise identification of the anisotropic *g* tensor and hyperfine *A* tensor components. It was shown that there is a correlation of g_{xx} and A_{zz} which is related to the local polarity of the SL environment [2]. Hence, a most precise determination of tensor components should be aimed at. This is accomplished by fitting experimental spectra with powder spectra, simulated according to the method of van Veen [3]. The values obtained in this way are scaled with results from unbound SL in a polar (water) and in an unpolar (toluene/polystyrene) environment. Different labeling positions at the protein show different polarities suggesting different depths of penetration into the purple membrane lipids, similar to the case of bacteriorhodopsin [4].

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Multifrequency pulsed (triple) ENDOR and ESEEM investigations of Fe-only hydrogenase.

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Hydrogen plays an important role in the metabolism of microorganisms, where the reaction $H_2 \rightleftharpoons 2H^+ + 2e^-$ is catalyzed by metalloenzymes, called hydrogenases [1]. The X-ray investigation of the crystal structure of the Fe-only hydrogenases shows that the active cite (so called H-cluster) contains a classical [4Fe-4S] cubane connected via the sulphur ligand of cysteine (Cys382) to a bi-nuclear subcluster [2, 3]. FT-IR spectroscopic studies on Fe-only hydrogenase show, that this [2Fe-2S] subcluster is coordinated by CO and CN ligands, stabilizing metals in low-oxidation states [4, 5].

The active site passes several states during the catalytic cycle, which can be detected using EPR. The oxidized form of the H-cluster shows a rhombic spectrum (well known 'g_x=2.1' signal) [6]. The distal iron of the [2Fe] subcluster has a free coordination site. Upon inhibition of the protein by CO this iron is coordinated by the external CO ligand. In this case an axial EPR signal is observed.

The spin distribution over the H-cluster was investigated using advanced pulsed EPR techniques including Davies-triple-ENDOR and HYSCORE at 9.5 GHz (X-band) as well as 35 GHz (Q-band). Measurements were done on a ^{57}Fe-enriched Fe-only hydrogenase from *Desulfovibrio desufuricans* in the oxidized state (H_{ox}) and the CO-inhibited (H_{ox}-CO) state. In this context we present the first Q-band ^{57}Fe HYSCORE study of an Iron protein.

For the H_{ox} state we found two ^{57}Fe hyperfine couplings by using Davies ENDOR. According to previous investigations of this state using EPR and Mössbauer spectroscopy, these couplings could be assigned to the [2Fe] subcluster [7, 8].

The EPR investigations of the H_{ox}-CO state revealed all six ^{57}Fe hyperfine couplings of the H-cluster, which indicate a profound delocalization of the unpaired spin density. Four strong ^{57}Fe couplings of similar magnitude, found using Davies-ENDOR, are assigned to irons in the [4Fe-4S] subcluster. Triple-ENDOR experiments enabled the determination of the relative signs of these couplings. Two small ^{57}Fe couplings found using the HYSCORE technique can be assigned to the [2Fe]-couplings.

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EPR at 360 GHz/14 T – a novel tool to investigate protein cofactors with enhanced resolution and sensitivity

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Electron paramagnetic resonance (EPR) at high magnetic fields is especially well suited to study stable or transient radicals, which play an important role as catalytic centres and cofactors of electron transfer reactions. Important examples are ion radicals of chlorophylls in photosynthetic reaction centers where small shifts (10^{-5}) and splittings of the g-tensor components report on the interaction of the radical with its molecular environment.

In order to exploit the benefits of high-field EPR - enhanced sensitivity and spectral resolution - new spectrometers have to be developed. Our laboratory-built high-field/high-frequency spectrometer consists of a 14 T superconducting magnet, a 360 GHz heterodyne mw-board and a semi-confocal Fabry-Perot resonator. A new probehead makes low-temperature experiments and light excitation of the sample in the resonator feasible. The spectrometer works in induction mode and uses a quasi-optical transmission line instead of lossy waveguides. With these improvements, an absolute sensitivity of $1.5*10^{10}$ spins/(mT $Hz^{1/2}$) could be achieved, which is mandatory for measurements on samples which are only available in very small amounts. For the independent determination of phase and amplitude in the 360 GHz/14 T EPR spectrometer at the FU-Berlin a new quasi-optical microwave bridge has been constructed.

Here we present the results of a 360 GHz EPR study on the primary donor, the bacteriochlorophyll-dimer P, in mutant and wild-type bacterial reaction centers of *Rhodobacter sphaeroides*. To characterize the impact of the polarity of the micro-surrounding of the primary donor on the g-tensor, a series of mutations involving the introduction of potentially negative amino acids in the vicinity of P were investigated and compared to wild-type reaction centers and other mutants in which the Mg ligand in the center of a bacteriochlorophyll was changed. Recently this study was extended to wild type and mutant reaction centers of plant photosystem I.

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Pulsed Field Gradient NMR in Combination with Magic Angle Spinning - New Possibilities for Studying Diffusion and Making NMR Microscopy of Heterogeneous Materials

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The **combination of MAS NMR spectroscopy** with **Pulsed Field Gradient (PFG) NMR** opens novel possibilities for **investigating diffusion process**es and also for **NMR microscopy of heterogeneous, non-liquid samples**. The advantages are based on the effect of line narrowing and concern (i) a prolongation of the intervals during which the magnetic field gradients may be applied and a corresponding enhancement in the sensitivity towards small molecular displacements as well as higher spatial resolution, and (ii) an enhanced resolution in the chemical shift scale. MAS PFG NMR facilitates investigations of materials that were hardly accessible by conventional PFG NMR-based techniques so far. In this contribution, recent progress and achievements in this field is demonstrated using a few examples.

1. A remarkable **increase in gradient strength available** is achieved by using a narrow-bore solid-state MAS probe inserted in a triple-axis micro-imaging device. Strong pulsed field gradients up to **2.6 T/m** along the magic angle can be created with extreme high accuracy and stability. The micro-imaging device does not influence the performance of the probe and is not inducing forces on the sample. It is shown that PFG NMR-experiments using stimulated echo sequences can be performed on this system with great accuracy.
2. MAS PFG NMR is demonstrated to have remarkable advantages in comparison with conventional PFG NMR, if applied to **diffusion measurements in beds of nanoporous particles**, notably zeolites. Those investigations mainly profit from the elimination of the disturbing influence of the inhomogeneity of the sample susceptibility. This is illustrated by measuring the diffusion coefficients of organic molecules adsorbed in silicalite-1. The strong gradients in combination with high spectral resolution allow the investigation of mixtures of molecules containing very fast and very slow diffusing molecules. This is demonstrated measuring the diffusion coefficients of water, benzene, ethene, and ethane simultaneously sorbed in NaX zeolite.
3. The described system can be used to create gradients that are following the sample rotation. Under such conditions, as it was shown earlier, **NMR imaging of rotating sample**s becomes possible. The aforementioned equipment opens novel possibilities with respect to spectral resolution and achievable spatial resolution. The accuracy of the triggered MAS images is proofed by a phantom. Spatially resolved spectroscopy was performed using a semi-solid sample and resulted in a resolution never observed before.

The new possibilities for studying diffusion in membranes, catalysts, drug delivery samples, and spatially resolved NMR of biopsy samples are outlined.

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Towards on-line MRI sensors

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NMR has had an enormous impact in both clinical diagnosis and as a research tool in analytical laboratories, and both aspects have given rise to large multinational industries. However, apart from the off-line measurement of simple quality factors such as solid-liquid ratios or oil and water content, NMR has had little impact in the industrial sector as an on-line quality control sensor. This is particularly surprising given the numerous publications showing useful correlations between NMR parameters and quality factors of industrial importance.

In this talk the reasons for this apparent failure of NMR to penetrate the industrial manufacturing sector are analysed and a novel approach based on the hitherto unexplored principle of motional relativity in spin physics is discussed. It is shown how a low-cost sensor based on the motional-relativity principle can be designed in modular form suitable for implementation on a moving conveyor and preliminary results from a prototype sensor are presented. The exciting potential of second-generation developments such as open-access NMR and multiplexed RF transmitter and receiver arrays will also be discussed.

The presentation will include a broad survey of potential industrial applications of the sensor technology and it will be shown how the module design and signal acquisition protocol need to be optimised for each application. This will be illustrated for foreign body detection (1) and for the sorting of fruit and vegetables (2).

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Detection of β-amyloid plaques in vivo by magnetic resonance imaging

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of β-amyloid (Aβ) plaques in the brain. The development of non-invasive *in vivo* methods for imaging amyloid plaques has been a major challenge in recent years. Imaging methods such as SPECT or PET use ionizing radiation and suffer from low resolution. MRI can provide much higher resolution than SPECT or PET without ionizing radiation. Initial efforts to visualize Aβ plaques by MRI in either fixed *ex-vivo* brain[1] or *in vivo* brain after relatively invasive method for contrast delivery[2] or amyloidophilic probes[3] have been reported in recent years. However, imaging of the Aβ plaques *in vivo* with sufficient sensitivity, without contrast agent, has not yet been successful.

Here we report our first results on *in vivo* imaging of Aβ plaques using MRI without contrast agent in a transgenic mouse model of AD. MRI of Aβ plaques was achieved at 9.4 Tesla with careful adjustment of T_2 contrast protocols based on spin echo acquisition (RARE, MSME). Aβ plaques were clearly detected with scan times as short as 25 minutes in the cortex, thalamus and hippocampus of the living mouse brain. The distribution of plaques identified by MRI was in good agreement with those found in the immunohistological analysis of the same brain sections. Furthermore, anatomical changes, such as shrinkage of hippocampus and enlargement of ventricles in the brain of AD mice were clearly visible with *in vivo* MRI. Our results extend the application of MRI as a viable means for detecting Aβ plaques *in vivo,* which is important for the evaluation of disease progression and for testing the efficacy of possible therapeutic agents.

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Advances in Mobile NMR with the NMR-MOUSE

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The conventional NMR-MOUSE employs a u-shaped magnet The sensitive volume of this sensor is ill-defined and changes with the rf frequency not only in depth but also in shape. By adjusting the magnet and the rf coil geometries favourable conditions can be found which result in field profiles suitable for depth profiling planar structures with a spatial resolution better than 5 µm and for measuring chemical shift resolved spectra outside the low field magnet. The construction principles of the sensor and different applications are reported concerning cultural heritage, polymer materials, and medical tissues.

Stray-Field Imaging and Multinuclear Magnetic Resonance Spectroscopy Studies on Triple Curable Hybrid Glass-Ionomers

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Glass-ionomer cements (GIC) are widely used in restorative dental treatment. In chemical curable GIC, the glass component undergoes an acid-base reaction with a polyalkenoate, to form the cement. Recently, new results were reported on GIC formation and structural characterization, using Stray-Field MRI (STRAFI) and multinuclear magnetic resonance [1]. Using STRAFI and solid-state multinuclear magnetic resonance spectroscopy (^{29}Si, , ^{27}Al) a triple curable commercial hybrid glass ionomer (Fuji II LC) was now studied, which contains monomers that undergo light-curing and self-curing reactions. In the absence of light-irradiation, the spatially-resolved kinetics of the hardening paste was similar to the conventional GICs. However, time-constants of the order of seconds were obtained after light-curing over 20 s at about 500 mW/cm^2. Accordingly, the composition of the final cement shows an incomplete leaching out of network forming 4- and 5- coordinated aluminium. The complex structure of these materials will be discussed in terms of the corresponding mechanical properties. [1] R. Pires, T. G. Nunes, I. Abrahams, G. E. Hawkes, C. Morais, C. Fernandez, *J. Materials Science: Materials in Medicine* **15**(3) (2004) 201.

Diffusion Tensor Magnetic Resonance Imaging of Defective Myelination and Recovery in the Rodent Brain

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Both axon and myelin abnormalities have impact on directional water diffusion and tissue anisotropy observed in neurological pathologies in human and in animal models. Diffusion tensor magnetic resonance imaging (DT-MRI), highly sensitive to water molecules movement, has been widely used to study the brain in development and diseases. In the present study, a transgenic mouse line that harbors the conditionally toxic gene *HSV1-tk* in oligodendrocytes was generated. Ganciclovir (GCV) treatment was used to induce a controlled oligodendrocyte cell death and its subsequent dysmyelination in the central nervous system (CNS). DT-MRI was applied for *in vivo*

quantification of myelin loss and recovery. Two mouse models were created, the first one exhibits a severe and irreversible dysmyelination in the brain and the other one is characterized by transient loss of myelin followed by self-myelin reparation. The severity of dysmyelination was assessed by calculating and mapping the radial (perpendicular) and axial (longitudinal) water diffusion to fiber tracts, fractional anisotropy (FA) and averaged principals diffusivities (<D>). Moreover, the recovery was also detected by DT-MRI in the second model.

A significant increase of radial diffusion, reflecting the increased freedom of motion perpendicular to axons due to the lack of myelin was observed in all selected white matter tracts but also in cerebellum. This significant elevation of the diffusion perpendicular was accompanied by a decrease in axial diffusion values, consistent with histological findings of myelin loss and axonal changes including reduced axonal caliber and overexpression of some axonal proteins. We showed clearly that myelination does play a role in the degree of diffusion anisotropy since fractional anisotropy was significantly decreased in white matter and also in regions of mixed grey and white matters in dysmyelinated mice. The recovery was reflected in a decrease in the magnitude of radial diffusion and increase in fractional anisotropy values compared with previous DT-MRI examination of the same treated mice.

Ultrasensitive magnetic resonance imaging by mechanical detection

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Magnetic resonance force microscopy (MRFM) is a three-dimensional, subsurface imaging technique which registers the presence of sample spins via the deflection, or change in mechanical resonance frequency, of a magnet-tipped cantilever. MRFM combines the superior sensitivity of scanned probe microscopies with the chemical specificity of NMR. At single-nuclear-spin sensitivity, MRFM would have numerous exciting applications, such as imaging of single biomolecules or spin-state readout for solid-state quantum computing.

We have previously reported unprecedented sensitivity in nuclear MRFM, and are currently improving our sensitivity by attacking two remaining technical challenges: producing usable nanoscale magnetic tips, and learning to control excess cantilever energy dissipation to the sample surface. We will discuss advances in the nanofabrication of these tips, and a series of recent experiments to elucidate the much debated mechanism of noncontact dissipation between a cantilever and a surface. We will also present our most recent MRFM results.

Sensitivity enhanced ^{13}C MR spectroscopy of the human brain at 3T

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Introduction

In vivo ^{13}C Magnetic Resonance Spectroscopy (MRS) allows continuous, non-invasive monitoring of tissue concentrations of metabolites and metabolic fluxes in humans and therefore it is a very powerful tool for clinical research. However, ^{13}C MRS is limited by sensitivity. A combination of maximization of detection volume with ^{1}H to ^{13}C polarization transfer techniques at high B_0 field strengths can increase this sensitivity. Here we present a method that enables broadband ^{1}H to ^{13}C cross polarization with ^{1}H decoupling of the human brain at 3T within safety guidelines for RF power deposition (SAR).

Methods

A shielded circularly polarized 16-leg ^{1}H birdcage coil was used for homogeneous B_1 field and minimum SAR. Two slightly overlapping, anatomically shaped, ^{13}C coils with a diameter of 13 cm were constructed with loss-less ^{1}H blocking circuits and driven in quadrature. This setup was interfaced to a clinical 3T MRI system (Trio, Siemens, Erlangen). A pulse sequence was used with the spin-locking ^{1}H B_1 field and the cross-polarizing ^{13}C B_1 field realized by a R_4 train with duration of 6.7 ms (i.e. 1/J, using the average coupling of C-H groups in most MR visible metabolites), followed by ^{13}C acquisition with WALTZ16 ^{1}H decoupling for 135 ms. The TR was set to 2 s such that the power deposition remained well below SAR guidelines. The ^{13}C MR signals of myo-inositol were used as in vivo markers to quantify the efficiency of cross polarization in the human brain, while the bandwidth of this cross polarization was validated in phantom measurements and in vivo.

Results

Natural abundance in vivo ^{13}C MR spectra of the human brain with and without cross polarization are acquired. The gain in SNR of the ^{13}C signals of myo-inositol from the brain in the FOV of the ^{13}C coil is about 3.5. The Frequency profile of the gain in SNR by the R_4 cross polarization is determined on a phantom, which shows a bandwidth of 40 ppm. Finally, a ^{13}C MR spectrum, with R_4 cross polarization, of a 21-year-old healthy female subject, is obtained after ^{13}C-1 labelled glucose infusion (TR=2s, 224 averages, 7.5 minutes). A 7.5 minutes ^{13}C MR spectrum acquired prior to the infusion was subtracted for baseline correction. Clear resonances from Glucose, Glutamate, Glutamine, Aspertate, GABA, and Lactate are detected.

Conclusion

We have shown that broadband cross polarization is possible within large volumes of the human brain at 3T. In combination with the efficient coil setup, the sensitivity of ^{13}C MRS substantially improves, while remaining within SAR guidelines.

How MRI reveals plant performance and the unknown part of the water cycle

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Flow of water in plants and trees is key information to study plant performance and the evaporation part by trees in the water cycle, a largely unknow parameter in the present water management models. One can count the number of trees in great detail by remote sensing techniques, but the flow in trees is hard to measure. A number of methods are available to measure water flow in plants and trees, all lacking accuracy and only providing the average linear velocity.

Based on PFG-flow-turbo spin echo imaging and adapted hardware to allow plants and (small) trees (up to 2 m) to be inserted in a dedicated MRI system we have been successful to quantitatively measure xylem (evaporation) and phloem (sugar transport pathway) flow. The experiments result in single pixel propagators, containing both stationary (only diffusing) and flowing water, allowing the amount of stationary and flowing water per pixel to be calculated, as well as the average linear displacement (in both positive and negative direction) and the volume flow. The volume flow correlates very nicely to the water uptake rate of the plants and the calculated cross sectional area in a single vessel containing pixel was within 10 % of the cross sectional area obtained by optical microscopy, demonstrating the accuracy and reliability of the MRI method.

The method has been applied to a tree (poplar), and three different plants (ricinus, tomato and tobacco) and the dynamics of day-night cycles, cold girdling and root chilling has been obtained. This data gives key information on transport mechanisms (Cohesion Tension theory for xylem and Munch counter flow hypothesis for phloem) within plants and trees. The CT theory is heavily under debate. Based on our results for the first time we were able to quantify the Munch counter flow.

For NMR flow measurements to be applicable in situ (field situations) on trees quantitative non-spatially resolved (non-imaging) measuremnts with specifically designed magnets has to be developed. The basic principles and components are now available to develop such NMR bioflow meters.

Combined relaxometry and duffusometry to estimate cell compartments, membrane permeability and cell-to-cell transport

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Both pulsed field gradient NMR (diffusometry) and relaxation time measurements (relaxometry) are widely used to probe the molecular displacements of liquid molecules and the geometry of the microstructures containing them in (multicompartment) porous and biological systems. If the compartments are separated by permeable membranes, exchange due to the membrane permeability and differences in (bulk) relaxation times within the compartments strongly affect the observed T_2, D_{app}, and the extracted compartment radius r_{app}.

To understand the effect of exchange realistic models are available to solve the question in what way the observed parameters are affected and in how far (and in what way) combined diffusion-relaxation time measurements allow to separate signals into a set of multicompartmental sources (1) and to extract the highly important information on membrane permeability *in vivo*.

Here we present a method to extract compartment (cell) sizes and membrane permeability by use of combined relaxometry and diffusion time (D) dependent diffusion measurements, and illustrate it using simulated and experimental measurements. The simulation models are based on finite difference or finite element models for multi-compartment planar, cylindrical (cellular) (2) and tissue geometries. Compartment parameters were afterwards extracted based on (2D) multi-exponential curve fitting of the PFG-CPMG results by SPLMOD, CONTIN and a fitting procedure based an a fast 2D Laplace Inversion algorithm (3,4). Experimental results were obtained in apple and (normal and osmotically stressed) Pearl millet and Maize root tissue, demonstrating different cell dimensions and cell-to-cell transport behaviour.

The apparent diffusion coefficients of the different compartments were used to determine the cell radius and the membrane permeability / cell-to-cell transport by the presented method!

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Improved Paramagnetic CEST MRI Contrast Agents Based on Multiple Ytterbium(III)-DOTAM Complexes Attached to Dendrimers

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In potential, MRI contrast agents based on chemical-exchange dependent saturation transfer (CEST) have several important advantages over T1 and T2* contrast agents that are currently used in the clinical practice. First, CEST MRI contrast agents can be used to achieve pH mapping with MRI [1]. Second, after administration of the CEST contrast agent, the MRI contrast enhancement can be turned on and off at will simply by switching on and off the radiofrequency pulse used for selective saturation of the exchangeable-proton resonance. One of the major limitations for successful medical application of CEST MRI is generally supposed to be its relatively low sensitivity. In this report we investigate the combination of two existing approaches to increase the sensitivity: enhanced proton exchange rates by using paramagnetically shifted amide protons in the Yb(III)-DOTAM complex [2] and enhanced number of exchanging protons by using a polymer [3]. For this purpose we synthesized the zeroth, first, and third generations of a poly(propylene imine) dendrimer loaded with, respectively, one, four, and sixteen yb(III)-DOTAM moieties and assessed the relevant NMR properties of these molecules for application as CEST MRI contrast agents.

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Local intramyocellular lipid content within rat tibialis anterior muscle measured with in vivo 1H MRS correlates with muscle fiber type composition

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Recent ^{1}H MRS studies of rat hind limb showed that the intramyocellular lipid (IMCL) content differs significantly between muscles as a result of different fiber type compositions. However, individual rat hind limb muscles also show regional differences in fiber type distribution. Therefore the variability of the IMCL content within rat tibialis anterior was studied by *in vivo* single-voxel ^{1}H MRS and compared with muscle fiber type composition. The IMCL content in rat tibialis anterior varied by a factor more than two and the highest levels were found in the region containing predominantly oxidative muscle fibers.

Diffusion of solvent molecules in HPMC: Case I, Case II and abnormal diffusion

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Diffusion is the most fundamental form of transport in many chemical and biological systems. This phenomenon is also very important in controlled released drugs based on the hydrophilic polymers. Due to the diffusion of solvent molecules into the polymer matrix the polymer swells and a gel layer is formed about the dry core of the polymer. The gel layer forms a barrier for the drug release and decides about the success or failure of the controlled released devices. It depends on the kinetics of the gel layer formation whether the drug will release at the proper time i.e. in the proper part of the gastrointestinal tracks. The release of the drug can be done by the disintegration of the polymer matrix or by the diffusion of the drug particles throughout the gel layer. Thus, diffusion of body fluids (or another solvent) into the polymer matrix decides about the gel layer formation, but diffusion of the drug particles is one of possible mechanisms of drug release.

Despite the wide range and number of the studies concerning the diffusion of the solvent molecules into the hydrophilic polymer, there remain still the unsolved problems concerning the relations between the gel layer formation and the diffusive transport within the gel matrix. Therefore we undertook the study of solvent diffusion into hydroxypropylmethylcellulose (HPMC). Among many hydrophilic polymers HPMC is characterized by the best physical and chemical properties. It has good swelling properties (i.e. degree and time of swelling), displays good compact properties, can accommodate a large drug load, and is non-toxic. Thanks to these properties HPMC is very commonly used in the pharmaceutical industry since production of coated and controlled release drugs.

Magnetic Resonance Imaging (MRI) was used to investigate the diffusion and swelling processes of HPMC matrices prepared in the form of cylinders and hydrated in a water solvent of pH = 2, 7 and 12 at . The measurements were done with a 300 MHz Bruker AVANCE spectrometer equipped with a micro imaging probehead. The images were taken for solvent protons within the gel HPMC. The gel layer thickness formed around the dry core of the studied samples as a function of hydration time was described by the equation:

$$d = a + bt^{n}$$

where d is the gel layer thickness, n is the exponent characterizing the diffusion mechanism and a, b are constants.

In the literature, the conclusion concerning the mechanism of the diffusion of solvent in polymer matrix is more frequently based on the estimates for n and statistical properties of the regression line (n=0.5 - pure diffusion Fickian or Case I and n=1 - swelling-controlled or Case II transport. We will show that in the case of anomalous transport kinetics a definite conclusion for the diffusion mechanism cannot be justified.

In our study, along with the change in the dimension of the glass core of the polymer the spatially resolved spin-spin relaxations times, spin densities and diffusion coefficients were determined for HPMC tablets in the function of hydration times. On the basis of all these parameters we determined the diffusion mechanism to be Fickian, Case II and anomalous diffusion for solvent of pH = 12, 2 and 7, respectively.

Magnetic Resonance Imaging (MRI) is the only method for the study of the diffusion process of solvent into polymer matrices which gives spatially resolved information of the nature of the process with a resolution of μm. With PGSE methods both transversal and longitudinal (corresponding to the axes of the cylindrical sample) diffusion have been measured in our work Due to the shape of the sample, these two directions were enough to determine the spatially resolved diffusion tensor in a swelling HPMC polymer.

The influence of the solvent pH and the molecular weight of the polymer on the spatially resolved spin-spin relaxations times, spin densities and diffusion tensors will be discussed.

Characterisation of a devitrified glass through homo- and heteronuclear correlation experiments

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Phosphate based glasses have several applications. They can be used as sealing glasses for electronical applications (vanadophosphate), as biomaterials (calcium phosphate) or as encapsulating phase for radioactive waste (iron or aluminium phosphate). In all these applications, devitrification plays an important role and a good understanding of the crystallisation behaviour is therefore necessary. In such a case, both thermal range in which crystallisation occurs and the nature of the formed compounds must be determined. Devitrification often leads to the formation of a large number of compounds with a low cristallinity. Thus, NMR spectroscopy reveals to be a very useful technique, complementary to XRD, especially in the case of remaining amorphous parts which reduce the efficiency of X-ray diffraction techniques.

We have studied a sodium aluminophosphate glass, which has been proposed as a host matrix for treated radioactive waste. Devitrification occurs at 612°C and leads to the formation a several compounds.

The local environment of this devitrified glass has been studied by multinuclear NMR spectroscopy. ^{31}P network connectivity has been investigated "through space" and "through bond" using the following pulse sequences: RFDR (determination of the number of phases), INADEQUATE and double quanta (BABA) techniques (connectivities). High-resolution spectra of quadrupolar nuclei (^{27}Al and ^{23}Na) have been recorded using MQ-MAS and ST-MAS techniques. Finally, the Dipolar and J coupling MQ-HETCOR sequences have been applied to determine ^{31}P/^{27}Al and ^{31}P / ^{23}Na connectivities. The efficiency of the latter experiments has been improved by using the recent development of SPAM techniques. We will present NMR data obtained at different magnetic fields, including triple resonance spectra acquired at very high field (18.8T).

This work confirms the presence of several phosphate compounds and brings new information about the nature of the network (phosphate / aluminophosphate). Moreover, the NMR caracterisation of new phases gives the possibility to expand the chemical shift database of sodium aluminophosphates.

Hydrogen in Ti_3Al intermetallic compound: a proton NMR study

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The Ti_3Al–H system has attracted recent attention because of the wide possibilities of its practical applications, particularly as a hydrogen storage material. This compound can absorb large amounts of hydrogen (up to a content H/M ~ 1.45), and owing to its light molecular mass it corresponds to one of the highest hydrogen weight concentration (~ 3.3 wt. %) for all the studied intermetallic–hydrogen systems. With increasing H content, the lattice structure undergo several transitions and change from the hexagonal structure $D0_{19}$–Ni_3Sn (at H/M ~ 0) through the tetragonal $L6_0$–$CuTi_3$ (at H/M ~ 0.5) to the face centered cubic structure $L1_2$–Cu_3Au (H/M ~ 1) [1]. This suggests a study of the effects of the lattice structure changes on the behavior of H-atoms. Our interest to the Ti_3Al–H system is based also on the fact that the structure $D0_{19}$ is related to the hcp structure of Sc, Y and Lu where the jump motion of dissolved hydrogen is characterized by two frequency scales: the slower jump process being responsible for the long-range diffusion and the very fast localized H motion (see, for example, [2]). The coexistence of two types of H motion has been attributed to the structure of the hydrogen sublattice which consists of pairs of tetrahedral interstitial sites separated by about 1 Å. This suggests the possibility of the fast localized H jumps within such pairs. Hence, the existence of similar features in the case of Ti_3AlH_x system (at least, at low H concentrations) could be expected.

To obtain information on the hydrogen diffusion parameters, we have performed the measurements of the proton spin-lattice relaxation rates, R_1, for the samples $Ti_3AlH_{0.31}$ and $Ti_3AlH_{0.51}$ (with the Ni_3Sn-type structure), $Ti_3AlH_{1.0}$ and $Ti_3AlH_{2.0}$ (with the $CuTi_3$-type dominant phase), and $Ti_3AlH_{4.32}$ (with the Cu_3Au-type dominant phase). The measurements were performed on a modernized Bruker SXP pulse NMR spectrometer at the resonance frequencies $\omega_0/2\pi$= 90, 23.8 and 14.6 MHz using the saturation-recovery method.

In the range $80 < T < 430$ K the temperature dependences of R_1 for $Ti_3AlH_{0.31}$ and $Ti_3AlH_{0.51}$ are characterized by a monotonous increase of R_1 values with no signs of frequency dependence. The behavior of R_1 shows significant deviations from the linear law which is related to the electronic (Korringa) term, $R_{1e} = C_eT$ (where C_e is believed to be proportional to the square of the density of electron states at the Fermi level, $N^2(E_F)$), and which usually dominates in M–H systems with slow hydrogen diffusion. These deviations can be attributed to the temperature dependence of C_e, which is expected for compounds with the Fermi level lying close to a peak in the density of electron states [3]. The shape of the $R_1(T)$ dependence for $Ti_3AlH_{1.0}$ is qualitatively similar to those for Ti_3AlH_x with $x = 0.31$ and 0.51; however the deviations from the linear behavior for $Ti_3AlH_{1.0}$ are stronger.

For samples $Ti_3AlH_{2.0}$ and $Ti_3AlH_{4.32}$ a distinct frequency dependence of the proton relaxation rate appears at $T >$ 320 K (the values of R_1 increase with decreasing ω_0). Such a dependence is typical for the low-temperature slope of the relaxation rate peak which is related to the modulation of dipole-dipole interaction due to hydrogen motion and which is often observed for M–H systems with fast H diffusion. The motional contribution to the relaxation rate, R_{1d}, reaches its maximum value at a temperature at which the characteristic frequency of H jumps, τ_d^{-1}, is approximately equal to the resonance frequency, ω_0. The maximum of R_{1d} has not been found up to 550 K. Thus, our results indicate that the hydrogen mobility in Ti_3AlH_x increases with increasing H content but remains to be rather slow.

The most interesting results have been obtained at low temperatures ($T < 80$ K). For all the samples the proton spin-lattice relaxation rates exhibit distinct frequency-dependent peaks (near 15 K at 90 MHz). The nature of these low-temperature R_1 peaks is not clear at present. The appearance of such peaks may be caused by H motion, so that our results may indicate the coexistence of several H jump processes with strongly differing characteristic frequencies. In this case the motion responsible for the appearance of these peaks must be very fast (the rough estimate yields $\tau_d^{-1} \sim 10^7$ s^{-1} at 10 K, and this is one of the highest values for all known M–H systems).

The other mechanism, which can lead to similar behavior of R_1 is the relaxation due to paramagnetic impurities (R_{1p}). In particular, qualitatively similar $R_1(T)$ dependences were observed for hydrides of some rare earth metals (for example, for LaH_x [4]) and were attributed to the paramagnetic impurity ions of Gd^{3+} and Ce^{3+}.

The position of $(R_{1p})^{max}$ is determined by the condition $\omega_0\tau_i = 1$, where τ_i is the spin-lattice relaxation time of a paramagnetic impurity ion. It should be noted, however, that the low content of Gd and Ce in La and other rare earth elements is a typical situation even for nominally "pure" metals, but this is unlikely for Ti and Al. In order to clarify the situation, additional investigations (including the magnetic susceptibility and ^{27}Al NMR measurements) are required.

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The Use of T_1 Measurements to Study a Model CMP Slurry

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With continuous increase in the complexity of current microelectronic devices and integration of Cu as interconnect, it is required that CMP provides a good surface planarity with minimal surface defectivity. One of the prominent roles of the abrasive particles is its ability to interact with chemical components found in the slurry. Surface adsorption of chemical components on to the abrasive particles can alter the intended chemical and mechanical balance of the slurry. Slurries consisting of abrasive particles of similar characteristics but different surface adsorption characteristic may perform differently in a CMP process. Furthermore, the introduction of copper ions during copper CMP may exacerbate the complexity. These copper ions could interact with the chemical components in the slurry to form copper complexes and also could change the adsorption characteristic of the abrasive particles. The formation of the copper complex and the change in the adsorption characteristic of the particles could have a great impact on the copper CMP performance. Performance will be exemplified with silica based slurry.

The longitudinal relaxation times (T_1) of the different components of CMP slurries were measured using Spin Echo NMR (SE-NMR) at a constant temperature. The fact that NMR is non-invasive and gives information on the molecular level give more advantage to the technique. The model CMP slurry was prepared in D2O to enable monitoring of T1 for the various components' protons. SE-NMR provide a very powerful tool to study the varoius interactions and adsorption processes that take place in a model CMP silica based slurry which contains BTA and/or Glycine and/or Cu^{+2} ions, it was found that BTA is very competitive towards complexation with Cu^{+2} ions and BTA-Cu complex adsorbs on silica surface.

Materials MA 4

Xenon as a Ligand: Using Low Temperature NMR to Characterize an Organometallic Xenon Complex

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The time scale over which NMR measurements are made, typically hundreds of milliseconds or more, has often lead to the belief in the past that this technique could not be applied to probe short lived species such as neutral transition metal complexes with coordinated noble gas atoms, most often xenon.

In this work[1], the first direct observation and characterization by NMR of an organometallic xenon complex will be presented. $Re(^iPrCp)(CO)(PF_3)Xe$ (2) was synthesized and characterized *in situ* following the photolysis of $Re(^iPrCp)(CO)_2(PF_3)$ (1) in liquid xenon at low temperature whilst in an NMR probe. The NMR/UV photolysis setup used in this study will be discussed. The incorporation of a PF_3 ligand allowed a ^{31}P-^{129}Xe *J* coupling to be measured for the first time. ^{19}F, ^{31}P, ^{129}Xe and ^1H chemical shifts with the corresponding indirect *J* couplings permitted deduction of the atomic connectivity and confirmed the identity of **2**. The utility of Broad band Inversion Pulses (BIPs)[2] in the search for the Xe chemical shift in **2** will be discussed. The ^{129}Xe chemical shift of **2**, δ -6,179, is a Xe shift that is significantly shielded, on the order of 1,000 with respect to free Xe. The ability of NMR spectroscopy to probe dynamic processes permitted a lower limit to the life-time of the coordinated Xe of 27 ms to be observed (i.e., a maximum exchange rate between free and bound Xe atoms of $37s^{-1}$) at 163 K. The relatively long life-time of transient **2** was confirmed by IR study which suggested that **2** is the most stable organometallic xenon complex yet reported in comparison to all other related neutral noble gas complexes.

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Measurement of Displacement Autocorrelation Spectrum of Fluid in Porous Media by CPMG Echo Sequence

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The velocity autocorrelation function is a key quantity from which the underlying nature of the molecular dynamics can be revealed. The gradient spin echo is a tool that yields not only macroscopic but also microscopic dynamic variables because of the relation of attenuation to the displacement or the velocity autocorrelation function of spins[1,2]. However, this spin echo potential has been only partially exploited so far[3]. Generally, the modulated gradient spin echo method (MGSE) involves a repetitive train of radiofrequency pulses with interspersed magnetic field gradient pulses or a gradient waveform to periodically modulate the phase of tracking spins The resulting spin-echo attenuation is proportional to the value of the power spectrum of spin displacement or spin velocity at modulation frequency. The rate of phase modulation determines the frequency range of measurement, which is between a few Hz to about 100 kHz at the present state of art. The method is a low frequency complement to the non-elastic neutron scattering method with range above GHz.

A variant of the MGSE sequence is the CPMG echo sequence applied to spins in the constant magnetic field gradient, G, which creates the spectrum of the effective gradient with the dominant sampling peak at the frequency of modulation, ω_m. Using the relation of spin echo attenuation to the displacement autocorrelation spectrum, $I_z(\omega)$, which is after N modulation cycle (t=NT)

$$\beta(t, \omega_m) = 8 \gamma^2 G^2 I_z(\omega_m) t /\pi^2$$

By changing the frequency of modulation, we can trace out $I_z(\omega)$, which is related to the mean squared spin displacement as well as to the power spectra of the velocity fluctuation $D(\omega)=I\_z(\omega).\omega^2$.

In this report, the MGSE-CPMG sequence is used to measure water diffusion through porous silica in order to determine the characteristics of medium such as: the size of the pores, the tortuocity and the intra-pore diffusion constant from the Iz(ω) frequency distribution. The experimental results agree with the predictions of theory.

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Displacement power spectrum and diffusion spectrum of fluidized granular system measured by modulated gradient technique

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It has been noted that the spin echo attenuation is related to the spectrum of velocity autocorrelation function of the spin bearing particles[1], which can be probed by modulated gradient techniques[2]. The frequency sensitivity of the applied modulation is determined by the frequency spectrum of the effective gradient. Thus, the spin echo attenuation is proportional to a value of power spectra of displacement or to a value of velocity autocorrelation spectra at the applied modulation frequency, depending on pulse sequence[3].

The PGSE spin echo method has been applied to study the granular flows[4], but as it is characterized by the zero frequency lobe of the gradient spectrum it has limited potential to measure directly the velocity autocorrelation function. The application of CPMG sequence in combination with steady gradient proved to have an appropriate frequency window for displacement power spectrum measurements $I_z(\omega)$. The spin echo attenuation β is given by $\beta=8/\pi^2(\gamma G)^2 T_e I_z(\omega_m)$, where G is the gradient strength, T_e is the echo time and ω_m is the modulation frequency. The velocity autocorrelation spectrum $D(\omega)$ can be reveled by means of the relation $D(\omega)=I_z(\omega)\omega^2$ and the velocity autocorrelation function can be obtained by Fourier transformation of its power spectrum.

We present the results of autocorrelation spectra measurement of fluidized granular bad, which consists of about 150 mustard seeds. The spectrum was measured in two perpendicular directions at different effective granular temperatures. The measurements have been done in the 100 MHz horizontal bore Oxford magnet with TecMag Apollo MRI spectrometer which enabled us to measure the spectrum in the range between a few Hz and a few kHz.

We have observed a non-exponential form of the velocity autocorrelation function, which agrees with the results of simulations of hard-sphere fluids[5], and can be explained by the model of beads caging by the neighboring grains. The obtained spectrum is similar to the results of positron emission measurement[6], where the shape of spectrum was explained by the constraining nature of the experimental cell and not by the grain caging.

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Velocity autocorrelation function of fluidized granular system measured by the novel spin echo technique

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NMR gradient spin echo is a tool that yields not only macroscopic but also microscopic dynamic variables because the spin echo attenuation is directly related to the displacement or the velocity autocorrelation function of spin bearing particles[1,2], which is the key quantities from which the underlying nature of the dynamical processes can be revealed. However, this spin echo potential has been only partially exploited particularly, when applied to the study of granular flows[3].

The modulated gradient spin echo method involves a repetitive train of radiofrequency pulses with interspersed magnetic field gradient pulses or a gradient waveform that periodically modulate the phase of tracking spins. The resulting spin-echo attenuation is proportional to the value of displacement power spectrum or velocity autocorrelation power spectrum at the modulation frequency, depending on the applied sequence. The frequency range of measurement is determined by the rate of spin phase modulation, which is between a few Hz to about 100 kHz at the present state of art. The method is a low frequency complement to the non-elastic neutron scattering method with range above GHz frequencies.

A variant of the MGSE sequence is the CPMG echo train applied to spins in the constant magnetic field gradient, G. It creates the spectrum of the effective gradient with the dominant sampling peak at the frequency of modulation, ω_m. By changing the modulation frequency, the peak can be adjusted in position in order to samplethe frequency dependence of the displacement autocorrelation power spectrum, $I_z(\omega)$. After N modulation cycles (t=NT), the spin echo attenuation is

$$\beta(t, \omega_m) = 8 \gamma^2 G^2 I_z(\omega_m) t /\pi^2 .$$

Here we report about the first application of this method to obtain the displacement autocorrelation spectrum of air-fluidized granular beads. The granular system consists of pharmaceutical oil-filled, hard plastic spherical beads with the restitution coefficient of 0.85 filled in the cylindrical container. The measurement gives the displacement autocorrelation spectrum, which provides the velocity autocorrelation function of bead motion. An oscillatory decay of function with the long negative tail agrees with the simulation of hard-sphere fluids of Alder and Wainwright[4], who found the exponential decay of velocity autocorrelation function only for very low densities of particles. At higher densities the exponential form breaks down and the velocity autocorrelation exhibits a negative tail. The results of our measurements are similar to the measurement by the positron emission tracking as reported in Ref.[5] where the lobe of spectrum was interpreted as a restriction to bead motion by walls of the experimental cell and not as the result of bead caging within the space of adjacent colliding beads, as follows from the analysis of our measurements.The measurements enable to define the characteristic cage size, the mean collision frequency of beads and the diffusion like constant denoting the beads motion between the cages.

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Continuous Flow Hyperpolarized 129Xe NMR for the study of ordered mesoporous organosilica

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Xenon is a probe for NMR investigation of mesoporous and microporous systems. Optical pumping spin exchange hyperpolarization (HP) greatly enhances the sensitivity of the technique. An apparatus based on a 16 W laser produces a continuous flow (CF) of 4.5% spin polarized Xe (enhancement factor is about 7000 over thermal equilibrium polarization in a 7.04 T field), that is constantly delivered to the sample inside the RF coil volume. CF-HP ^{129}Xe NMR xenon is suited for non-destructive investigation of many kinds of materials, because it is chemically inert, and because of the low exercise pressure. The technique provides a wealth of information on the organization, geometry, chemical nature and interconnectivity of the porous system[1,2]. Much work has been performed in recent years on the synthesis and functionalization of mesoporous materials for catalysis, separation and as template for nanostructures. We synthesized ordered silica-based materials containing nanometric channels with crystalline pore walls and a well-defined hierarchical structure. Scanning Electron Microscopy shows the material is made up by platelets containing thousands of parallel needle-like structures. Needles are usually 1-2 μm long with a diameter of 100nm. At room temperature, CF-HP ^{129}Xe NMR spectra show a broad and very intense symmetric peak near 67 ppm, due to adsorption in mesopores. The signal is even more intense than the free gas peak, a further demonstration of this material's large specific surface. The same experiment was performed at lower temperatures, to discover whether the molecular scale order on the pore walls can be associated to distinct adsorption sites. A specially designed pulse sequence resets the magnetization of the sample at a given time, allowing to plot uptake as a function of time. Uptake data were fitted with a curve compatible with the structure derived from SEM. The proposed values for Xe diffusion constant led to an NMR study of the gas-surface interaction to find bottlenecks in the mesoporous structure.

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^{27}Al-MQMAS study of refractory mortar using 930 MHz solid state NMR

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Optimization of the drying process conditions for a steel-making converter in a steel works is very important since the process is off-line and time-consuming. However, it is very difficult to optimize drying process conditions (temperature, surface active agents, etc.), because the refractory mortar of steel converter is made of very complicated and mixture materials. To help understanding, we have analyzed the refractory mortar using solid state NMR at 16.4T because the refractory mortar is an amorphous material, it means X-ray method can't give useful information. And then, we got new chemical structural information of the refractory mortar using 27Al MQMAS at 16.4T. And also, we have first demonstrated the structural analysis of these complex materials using very high field 930MHz (21.8T) solid state NMR. This paper shows the effect of magnetic field strength in these materials on 27Al MAS and MQMAS spectra. In particular, we find several new chemical sites using 21.8T with compared to the result of 27 Al MQMAS at 16.4T. At the same time, some relaxation information (T1) of the refractory mortar will be discussed because of high resolution MAS spectra at 21.8T. It is clear that solid state NMR at high field give useful information to analyze complicated materials. Using this data, we can adjust and design the drying process and time in steel works.

Charge Transfer in Polymer/Fullerene Solar Cells

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Quasi-one-dimensional (Q1D) polymer semiconductors, poly(3-alkylthiophenes) (P3AT) attract high interest due to their perspective use as active component of molecular electronic devices [1], fullerene-based solar cells with reversible charge separation among them [2].

A charge in P3AT is transferred by polaron along (1D) and between (3D) polymer chains, therefore the photoinduced charge transfer in P3AT/fullerene system should depend on the anisotropic polaron diffusion and on the charge transfer from polymer chain to fullerene molecule. Polaron possesses a spin S=1/2, so EPR spectroscopy is one of the powerful methods for the study of such Q1D systems. This allows, especially at D-band (140 GHz) EPR [3,4], to determine separately all magnetic, relaxation, and dynamics parameters of polarons in organic Q1D solids. First results of X-band (10 GHz) and D-band EPR study of spin/charge dynamics in an initial poly(3-dodecylthiophene) (P3DDT) and in that modified by fullerene, P3DDT/C_{60} composite, are presented.

Temperature dependence of the rates of the polaron 1D and 3D diffusion in P3DDT evidences that the charge in this system moves between its polymer chains in frames of activation mechanism with E_a = 0.18 eV, whereas intrachain charge diffusion depends on interaction of a polaron with lattice phonons with $h\omega_{ph}$ = 0.13 eV. This evidences for correlation of charge and molecular dynamics in the P3DDT sample. The non-illuminated P3DDT/C_{60} system demonstrate single low-field signal attributed to polarons P^+ with g = 2.0032 in P3DDT. The illumination of the system by laser with ν = 488 nm leads to the appearance of an additional signal of polarons and also of high-field line with g = 1.9998 attributed to the fullerene anion-radical C^-_{60}. The intensity A of the two-component signal of the radical pair $P^+C^-_{60}$ depends on both the laser light intensity I_{hv} as $A \sim I_{hv}^{0.16}$ and microwave power. An electron relaxation of the P^+ centers was shown to be much longer then that of the C^-_{60} centers.

Except the intra- and interchain polaron diffusion, the light-induced charge transfer between polymer and fullerene molecules should also be in the system P3DDT/C_{60}. Besides, slow macromolecular librations and fast rotational diffusion of the fullerene molecules are realized in the system studied. As in case of other Q1D polymer systems [3,4], these processes should also be correlated in the system P3DDT/C_{60}. At X-band EPR it is impossible to determine all these processes separately. High spectral resolution provided at D-band EPR allows to study all the dynamics processes in organic polymer system. The peculiarity of light-induced 1D and 3D charge and polaron dynamics in different P3AT/C_{60} systems obtained at D-band EPR is discussed.

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A high resolution NMR setup for coating research

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A novel experimental technique for depth profiling the spatial and temporal evolution of any drying coating is nuclear magnetic resonance (NMR), using a high magnetic field gradient (36 T/m) at a field of 1.4 T at the sample position. This setup has specially designed magnetic pole tips to obtain a gradient in the absolute value of the magnetic field. This allows the NMR-setup to probe the drying of a coating film with a spatial resolution of 5 µm perpendicular to the film. A surface coil of 5mm in diameter is used to excite the sample. The high fill factor and the high field generated by an electromagnet gives a high signal to noise ratio, allowing the profiling to be completed within 10 minutes.

NMR measurements and Confocal Raman Microscopy (CRM) were used to follow the in-depth drying of transparent alkyd coatings. Chemical information can be obtained as a function of position by CRM, but the position that is probed is difficult to determine due to the refraction of light. NMR has two big advantages. First, it allows any non transparent coating to be probed and secondly the position determination is simple. The results from a comparison of MRI and CRM indicate that the disappearance of the double bonds (visible with CRM) in the fatty acid side chains of the alkyd molecules directly results in the formation of cross-links. The cross-linking influences the mobility of the polymers which is visible in the NMR measurements as a decrease in relaxation time (T2).

The in-depth profiling of the coating has allowed us to construct drying models explaining the different drying behavior observed for different coatings with different catalysts or catalysts concentration.

Future possibilities on coating research are moisture transport properties, ageing of coatings, more detailed information on network structure locally (e.g. dangling ends), influence of pigments or nano-particles on the network structures and the influence of the substrate on the drying of the coating.

^{1}H NMR study of gelation process by stilbene oxalamide compound

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N-L-Leucine methyl ester-*N'*-[4-(4'-dodecyloxy)-*trans*-stilbene]-oxamide acts as an efficient low molecular weight organic gelator of various organic solvents. Thermoreversible and stable organogels are preferably formed by intermolecular hydrogen bonding additionally stabilized by π-π stacking and lipophilic interactions. In order to get an insight in to organizational motifs of the molecular self-assembly in polar as well as in apolar aromatic solvents, concentration and temperature dependent ^{1}H NMR spectra of the gelling substance were obtained in dimethylsulfoxide and toluene. Concentration increase leading to aggregation and gel formation results in broad peaks with unresolved *J*-coupling, which is characteristic of molecules dissolved in entrapped solvent [1]. Signals of the assembled molecules are not observed due to the suppressed mobility of the gelator molecule organized in rigid gel network and to long correlation times. On the other hand, temperature increase causes an increase in proton peak intensity implying a collapse of the gel network and, finally, dissolution of the fibrous aggregates. Using an internal standard, concentration of dissolved gelator, c_d, is determined and the dissolution equilibrium constant, K_d, evaluated [2]. From the plot of $\ln K_d$ versus 1/T, the dissolution enthalpy, ΔH_d, and entropy, ΔS_d, are estimated. Moreover, temperature induced chemical shift changes reveal intermolecular interactions involved in gel fibers formation. An up-field trend of NH protons following the gel dissolution indicates hydrogen bonding between oxalamide fragments of the molecules, whereas a down-field shift of the aromatic protons confirms π-π stacking of stilbene moieties.

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^{17}O Solid-State NMR of High-Pressure Silicates: High-Resolution Experiments and First-Principles Calculations

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Structural studies of dense silicates, and in particular their ability to incorporate water, play an important part in understanding the physical and chemical properties of the Earth. Water plays an key role in both surface and crustal geology but its role in the Earth's deep interior is less well understood. However, the synthesis of dense silicate phases is demanding, requiring high pressures and temperatures in a multi-anvil press and, typically, only small quantities (4-10 mg) of material are available. This small sample volume, coupled with the low natural abundance of ^{17}O (0.037%) and the presence of a nuclear quadrupole moment have limited the use of ^{17}O (I = 5/2) NMR in the study of these systems. Recently, the advent of satellite-transition (ST) MAS and the use of high-field magnets has led to great advances in the sensitivity of high-resolution NMR of quadrupolar nuclei.

Here, we show experimental ^{17}O NMR spectra of the three polymorphs of Mg_2SiO_4, the main component of the Earth's mantle, isotopically-enriched with 35% ^{17}O. The three are forsterite (α-Mg_2SiO_4), a low-pressure phase found in the upper mantle, wadsleyite (β-Mg_2SiO_4) and ringwoodite (γ-Mg_2SiO_4), with the latter both being high-pressure polymorphs found in the transition zone (410-660 km) that separates the upper and lower mantle. We use a combination of STMAS and MQMAS at a variety of field strengths (9.4 - 18.8 T) to determine chemical shift and quadrupolar parameters for the distinct oxygen species. Furthermore, we demonstrate a significant correlation between the ^{17}O isotropic chemical shift and the Si-O bond length, as previously observed in other magnesium silicate phases.

Finally, we employ first-principles calculations using the recently-introduced PARATEC and CASTEP-NMR codes, pseudopotential methods which use the guage including projector augmented wave (GIPAW) formalism. The use of a plane wave basis set and periodic boundary conditions make this method ideal for the study of periodic solids, providing an accurate, yet computationally efficient, approach. Excellent agreement is obtained between the experimental and calculated parameters, enabling spectral assignment and offering insight into some of the more unexpected results we obtain.

Modulated gradient spin echo by applied and combination of applied and internal gradients in porous media

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NMR analysis of molecular motion provides information not only on the diffusion constant but also on the geometry of the pores in the case of restricted diffusion. This information is contained in the spectrum of velocity auto-correlation function. Proper temporal modulation of applied gradient field in combination with π RF pulses leads to a spin echo exponential attenuation proportional to the frequency component of the diffusion spectrum:

The range of frequencies that can be examined by NMR is between 10 Hz and 100 kHz, which is in complement to neutron scattering measurements. With a proper application of π RF pulses, the static background gradient contributes at the same frequency and its effect can be estimated and factored out.

$\beta=(a\, G_s^2 + b\, G_a^2)_{D(\omega)}$

Parameter b depends on the temporal gradient modulation and the parameter a is given by the sequence of pi pulses. We used a properly timed sinusoidal gradient modulation.

Analysis of the diffusion spectrum with PGSE

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The pulsed gradient spin echo can be employed for measurements of diffusion spectrum. To determine the spectrum with PGSE, the diffusion constant as a function of the gradient pulse separation Δ needs to be measured. The amplitude of the spin echo is [1]

$E=E_0 \exp(-\int|q(w)|^2 D(w)\, dw/\pi)$

where $q(w)=\int dt \int \gamma G(t')dt' \exp(i w t)$.

For a PGSE sequence

$|q(w)|^2 = 16 \gamma^2 G^2 \sin^2(w \delta/2) \sin^2(w \Delta/2)/w^4$.

PGSE sequence samples the diffusional spectrum around zero frequency, so it is naturally suited for diffusion constant measurements. The width of the zero-frequency peak in q(w) decreases with Δ. The difference of spectra with adjacent Δ's has a peak at a non-zero frequency. Thus the diffusional spectrum can be sampled directly.

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Nuclear Magnetic Resonance evidence of a cluster driven colloidal glass formation

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A number of works (1-3) have recently attempted a connection between the glass and the gel arrested state of matter. Correlations between the dynamical behaviour of gels and glasses suggest that a common understanding of these two disordered states of matter may emerge. In this work we study the arrested phases and the liquid-gel transitions of a synthetic Hectorite clay (Laponite), by means of Triple Quantum Filter (TQF) ^{23}Na Nuclear Magnetic Resonance Relaxation experiments. In isotropic phase, the longitudinal and transverse relaxation of quadrupolar nuclei with spin quantum number I = 3/2 can only be described by single exponential decays if the correlation time is much shorter than the inverse of the Larmor frequency, $\tau_c << \omega_0^{-1}$ (4). If the nuclei under investigation are associated with macromolecules, clusters or slowly tumbling complexes, the relaxation is multiexponential (5-7). In this framework ^{23}Na Nuclear (I = 3/2) is a mighty probe to follow the ageing behavior of isotropic Laponite suspensions (8). We monitored the Laponite colloidal glass formation (9), at ionic strength I = 10^{-4} M, in the isotropic region of the phase diagram, providing estimates of the correlation time τ_c, and the fraction of bound ions during the ageing.

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Multi-Nuclear Solid-State NMR Investigations of Jarosites, Iron Soil Minerals, and their Aluminium Analogues, Alunites

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Jarosites, ($MFe_3(SO_4)_2(OH)_6$, M = H_3O^+, Na^+, K^+), are iron minerals formed in an acidic aqueous environment and stable only at a low pH. They are capable of trapping toxic cations such as lead and cadmium, which may be re-mobilized by an increase in pH. In materials sciences their magnetic properties have resulted in much attention. Jarosites are frustrated magnets containing a so-called Kagomé lattice. Natural and synthetic jarosites contain a significant number of vacancies (5 - 15 %) on the iron and cation (M) sites changing the magnetic properties of these materials. For charge balance an appropriate number of "H^+" are added creating Fe-OH_2 and "H_3O^+" sites, respectively. Several aspects regarding the actual structure of these materials are ambiguous. For example, does the presence of an acidic (H_3O^+) and basic site (Fe-OH) in close proximity lead to an internal neutralization reaction, i.e, Fe-OH + $H_3O^+ \rightarrow$ Fe-OH_2^+ + H_2O? Standard diffraction techniques and vibrational spectroscopy have not been able to answer this question unambiguously. We demonstrate that solid-state NMR spectra can be obtained for these minerals despite the magnetic properties of iron. ^2H MAS NMR spectra of deuterated jarosites shows the presence of several different deuteron species. $\delta(^2H)$ and ^2H quadrupole coupling parameters reflect the number of iron atoms in the second coordination sphere and mobility of the deuterons, respectively. Furthermore, a series of isostructural alunites ($MAl_3(SO_4)(OH)_6$) have been investigated by ^1H and ^{27}Al solid-state NMR techniques.

Amphibian peptides that inhibit nNOS: Binding studies with calmodulin using NMR

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Many Australian amphibians carry biologically active compounds in their skin as protection against predator attack. Most recently, our group has discovered peptides in skin secretions from frogs from the Litoria genus which are active against neuronal nitric oxide synthase (nNOS).[1]

During biological activity testing it was found that these peptides generally have IC50 values in the micromolar range.[2] Addition of calmodulin to the test solutions resulted in partial reactivation of the nNOS. These peptides also inhibit the activity of calcineurin, another enzyme that requires calmodulin for function.

We are interested in the interactions between our amphibian peptides and calmodulin. A series of amphibian peptides has been titrated into uniformly ^{15}N-labelled calmodulin and studied using ^{15}N-HSQC experiments. Three peptides have been tested with differing results. Of these peptides, Caerin 1.8 has been selected for further study in order to determine the 3D structure of the peptide-protein complex using doubly labelled ^{15}N- and ^{13}C-labelled calmodulin. Assignment of the NMR spectra is currently underway and is expected to be completed in time for presentation at this conference.

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Template-aluminosilicate structures in zeolite ZSM-5 formation studied with solid-state NMR

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Species at three stages in the self-assembly of zeolite ZSM-5 have been studied with 1D- and 2D MAS ^{13}C, ^{27}Al, ^{29}Si and ^{1}H NMR spectroscopy and compared with the earlier proposed structures: (1) precursor species containing 33 – 36 T sites around a TPA cation, (2) nano-slabs consisting of a flat 3 x 3 array of such precursors and (3) the final TPA-ZSM-5 zeolite. Synthesis was carried out in D_2O to suppress the water- and silanol protons. Under such conditions, the effective Si-H and Al-H distances measured with ^{29}Si-{^{1}H} and ^{27}Al-{^{1}H} REDOR reflect the interactions between TPA cations and the surrounding aluminosilica. The ^{29}Si-{^{1}H} REDOR curve for Q^4-type silicon atoms at the three mentioned stages are closely similar, as well as the observed ^{27}Al-^{1}H REDOR curve for the precursor species compared to that for the TPA-ZSM-5. This indicates that TPA is already incorporated at an early stage into the aluminosilicate in a similar way as in the final zeolite, in accordance with the earlier proposed MFI self-assembly pathway. However, the effective distances extracted from the initial REDOR curvatures are significantly (10-15%) larger than computed for the model. Since there is no temperature effect, we tentatively assign this difference to a reduction of the ^{29}Si-^{1}H and ^{27}Al-^{1}H interactions by spin-decoherence effects. By assuming the computed model distances and fitting Anderson-Weiss curves to the observed REDOR data, we obtain similar "decoherence times". The observed ^{29}Si-{^{1}H} REDOR dephasing for the Q^3 sites in the precursors is significantly faster than for the Q^4 sites. This is tentatively ascribed to a partial deuteron-proton back-exchange at the silanol positions

Cluster and periodic approaches for ab-initio calculation of NMR parameters in silicates and zeolites

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The direct assignment of the various NMR silicon signals of zeolites may be difficult due to the large number of different sites in the crystallographic unit cell. Furthermore, the small range of chemical shifts in these materials induces many signal superposition. As an example, there are 24 non-equivalent silicon atoms in the ZMS-5 unit cell, while only 21 resonances are observed experimentally. Ab-initio calculation of the NMR shielding tensors is very useful to confirm possible assignments. However, the values obtain in such systems are very dependant on the local geometry of the model used in the calculation [1] and on the reproduction of the electrostatic field in the calculation [2]. The cluster approach can then be useful if the non-local contribution to the shielding tensor is low. However, when electrostatic effects are important, a periodic approach become mandatory. Periodic calculation allows also a direct comparison of all the NMR parameters using an internal reference in the calculation.

In this study we have compared the results of cluster (G03 [3]) and periodic (Paratec [4]) calculations for various silicates and zeolites. As long as purely siliceous zeolites are considered, the cluster approach gives reliable results. Indeed in covalent structures the chemical shielding tensor is mainly influenced by geometrical parameters such as distances, valence and dihedral angles. We present the results obtained with both methods for ZSM-5, and some of the SiO_2 dense polymorphs such as stichovite, keatite moganite.

The results show that periodic calculations give reliable values for these types of systems allowing an assignment of the resonances to the various sites. The cluster approach fails to give reliable results when the definition of the cluster starting from the crystal is not straightforward. Furthermore, all sites are computed in one calculation, at the same precision level, reducing the computational times, although to the expense of huge memory requirements.

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High-frequency EPR and ENDOR Spectroscopy on Semiconductor Nanocrystals

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We have recently shown that ZnO nanocrystals can be doped with shallow donors by the introduction of interstitial Li and Na atoms [1]. This finding opens up the possibility to study the effect of quantum confinement on the electronic structure of these donors. It appears that high-frequency EPR and ENDOR spectroscopy at 95 GHz and 275 GHz is the method of choice to identify the atomic structure of these donors and to probe for the first time the effect of confinement on their electronic wave function.

For the shallow Li donor in ZnO nanoparticles, an increase of the hyperfine interaction with the ^{7}Li nucleus and the ^{1}H nuclei of the $Zn(OH)_2$ capping layer is observed proportional to R^{-3} (R is the radius of the ZnO core) when reducing the radius of the nanoparticles from 2.2 to 1.6 nm. These experiments are carried out at liquid-helium temperatures to ensure that the electron is bound to the positively charged Li^{+} core. When reducing the radius of the particle further to 1.1 nm there is a clear deviation of the R^{-3} dependence. This change in behaviour is attributed to the transition from semiconductor to molecular-cluster-type properties [2].

In addition to the hyperfine interactions with the ^{7}Li and ^{1}H nuclei, information is also supplied by the hyperfine interactions with the ^{67}Zn nuclei. It is concluded that even for the smallest particles the Zn nuclei carry non-zero spin density at the $ZnO/Zn(OH)_2$ interface. Moreover this density increases with decreasing size of the particles. This conclusion is at variance with the results of DFT calculations of Melnikov and Chelikowsky [3] for P-doped Si nanoparticles who concluded that, upon reduction of the size of the nanoparticles, the density at the surface becomes very small and that the electronic density concentrates in the P-Si nearest-neighbour bond.

Remarkably the ^{67}Zn nuclear spins in the ZnO nanoparticles become dynamically polarized when saturating the EPR transition of the shallow donors. This Overhauser effect is thought to be caused by the zero-point vibrations in the phonon system [4] and allows to probe the phonon spectrum in the nanoparticles.

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Structural Elucidation of Aluminium Alkoxides and As-synthesized Zeolite ZSM-5 through {^{27}Al}-^{13}C TRAPDOR and ^{27}Al MQMAS Experiments

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A combination of ^{27}Al Magic-Angle Spinning (MAS) and Multiple Quantum (MQ) MAS, ^{13}C-^{1}H CPMAS and ^{13}C-^{27}Al TRAnsfer of Population in DOuble Resonance (TRAPDOR) NMR has been used for the structural elucidation of three aluminium alkoxides, aluminium ethoxide, aluminium isopropoxide and aluminium tertiarybutoxide. These experiments formed the bases on which the experiments performed on the as-synthesized zeolite ZSM-5 could be interpreted.

Alkoxides are compounds containing acceptor and adjacent donor centers in the same molecule which are capable of forming adducts between molecules to give oligomers like dimers, trimers, and tetramers. The aluminium ion (Al^{3+}) is chemically flexible and form four-, five- and six-coordinated complexes. Therefore, alkoxides form mono, dimer, and oligomeric species with aluminium atoms. Double alkoxides like aluminium siloxides with Al-O-Si linkages are used as precursors for the synthesis of aluminosilicate ceramics and catalysts. Thus, a structural characterization of these compounds is important because of its close connection with chemical reactivity.

The ^{27}Al MAS spectra obtained at 156.34 MHz displayed wide but sharp powder patterns in addition to some broad features. These could be well resolved in the MQMAS 2D spectra and shows that the sharp features arise from aluminium in different environments in the alkoxides while the broad features originate from the already hydrolysed oxidised alkoxide material (Al_2O_3). The ^{27}Al quadrupole coupling constants in the alkoxides ranged from 1 MHz upto 13 MHz.

^{13}C-^{1}H CPMAS spectra showed very narrow lines, resolving the different carbon species (CH_3, CH_2, CH and C) within the various alkoxide structures. Further structural information could be gleaned from the application of the ^{13}C-{^{27}Al} TRAPDOR experiment. In this instance the Q-factor of the X-channel of an HX double resonant probe was spoiled which allowed the channel to be used for carbon and aluminium simultaniously. The TRAPDOR fractions thus obtained correlated to the calculated ^{13}C-^{27}Al second moments, distinguishing between terminal and bridging alkoxides in the structure. The studies characterise the aluminium alkoxides as having a dimeric structure, aluminium tertbutoxide, and a tetrameric structure for aluminium isopropoxide, while aluminium ethoxide appeared to be in a chain-like structure.

Zeolites are porous materials that have a major impact and use in the areas of catalysis (e.g. hydro-cracking), ion exchange (e.g. pollution remediation) and are extensively used as molecular sieves. Despite extensive research, some aspects of zeolites are still not well understood.

In as-synthesized ZSM-5 the interaction between the template molecule around which the zeolite lattice builds and aluminium located in the lattice is clearly illustrated with the TRAPDOR technique. It indicates that although overall the interaction between aluminium and carbon is weak, the aluminium and methyl carbons in one type of channel are much closer to aluminium than in the other type of channel.

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The Non-Local Dispersion tensor and Nuclear Magnetic Resonance

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The importance of mass transport in porous media in a wide range of applications is well documented, and covers systems such as oil and water in rock, contaminants in groundwater and reagents in packed-bed chemical reactors. Dispersion describes the phenomenon whereby particles on the same streamline separate during flow. The physics of dispersion is governed by stochastic processes arising from the interplay between advective velocity gradients, molecular diffusion and boundary layer effects [1].

The measurement of dispersion conventionally involves the use of a set of "tracer" particles to monitor the spreading. Nuclear Magnetic Resonance (NMR) provides a useful tool for non-invasively labelling the molecules uniformly over the sample. To model a porous medium, a cylinder of monodisperse latex or glass spheres is commonly used [2].

The dispersion tensor, $\mathbf{D}^*$, is a local measurement in the sense that it does not depend on positional relationships and is measured as time asymptotes. For situations where the length- and time-scales on which transport occurs are not much larger than the scale of the fluctuations in the velocity field, a non-local description is required [3].

Pulsed Gradient Spin Echo (PGSE)-NMR provides a wealth of information about the velocity correlations in porous media. Presented here is a set of NMR pulse sequences and a superposition designed to extract the velocity correlations necessary to calculate the dispersion as a function of displacement and hence the non-local dispersion. Comparisons to computational results will be made to verify the experimental design as well as comparison with the analytical expression for the non-local dispersion in pipe flow.

The Lattice-Boltzmann algorithm has been shown to successfully predict the flow field in porous media [4], and has been used to model the flow field through our model porous medium. This flow field is used to simulate a large ensemble of virtual tracer particles, from which numerical estimates for both the local and non-local dispersion can be determined. Our implementation of this approach is presented here along with a comparison between the experimental and computational results.

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An Earth's Field Tool for Teaching and Research

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An Earth's field NMR apparatus has been developed to provide a low cost alternative to high field instruments for the demonstration of pulsed NMR techniques, or for the determination of NMR properties of samples while in the field. Conventional high field NMR instruments require an extremely uniform magnetic field for the polarization of the nuclear spins and for the detection of the resultant magnetization. It is difficult and expensive to make a magnetic field of the required homogeneity and so sample sizes are usually quite small. However because the polarization and detection fields are large the resulting signal to noise ratio (S/N) can also be large. Earth's Field NMR works because detection takes place in the highly uniform Earth's magnetic field. This means that large samples can be used, partially compensating for the very low detection field. To improve the initial magnetization, an electromagnet with a field many times larger than the Earth's field is used to initially polarize the sample.

In many ways the Earth's Field NMR apparatus is very similar to a conventional high field laboratory system, except that it operates at very low frequencies (kHz) instead of hundreds of MHz. The control and signal processing part of the apparatus consists of a Digital Signal Processor (DSP) based pulse programmer and data acquisition unit and uses a Universal Serial Bus (USB) interface to communicate with a host laptop computer. A graphical user interface is provided by an application running on Windows XP. Because of the low operating frequency the "RF" transceiver section can operate, with standard operational amplifiers and other audio components. The probe consists of a single transmit/receive coil, a polarizing coil, and a gradient coil. Also included within the system are two constant-current power supplies for driving the polarizing and gradient coils and some auxiliary power supply electronics so that the whole system can be run from a 24 V battery. Buffered versions of the control and received signals have been brought out onto a connector for convenient monitoring.

As an example experiment, a Carr-Purcell-Meiboom-Gill (CPMG) multi-echo pulse sequence was programmed. Sample results of a CPMG single-shot T_2 measurement of water are presented below. Each time-domain echo signal was apodized with a sine-bell-squared filter, zero-filled and then Fourier transformed. The amplitude of each echo was then determined by calculating the integral under the real part of the phased spectrum. A single exponential function was then fitted to the array of echo amplitudes to obtain a T_2 value.

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Behaviour of Thermotropic Nematic N4 (Phase 4) Liquid Crystal Confined to Meso- and Macroporous Controlled Pore Glass Materials as Studied by Xenon-129 NMR Spectroscopy

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The behaviour of nematic liquid crystal N4 (Phase 4), eutectic mixture of p-methoxy-p'-butylazoxybenzenes, in meso- and macroporous controlled pore glass (CPG) materials of average pore diameters varying from 81 to 2917 Å have been investigated on a wide temperature range of ~ 185 - 370 K using ^{129}Xe NMR spectroscopy. The spectra contain lots of information about the changes in orientation of the liquid crystal. The liquid crystal between the particles of porous materials behaves like bulk liquid crystal forming the nematic phase oriented parallel with magnetic field below ~ 347 K. The isotropic-nematic phase transition can be recognized from the large increase (about 7 ppm) of the chemical shift of the signal of dissolved xenon.

In the case of the material with smallest pore sizes (CPG 81), a signal with lorentzian line shape in isotropic chemical shift range is observed below the bulk isotropic-nematic phase transition temperature indicating that xenon experiences isotropic environment inside the pores. On the other hand, an anisotropic powder pattern is observed from the largest pores (CPG 538 – 2917) at those temperatures. It is known that the walls of CPG materials tend to orient the liquid crystal molecules parallel with them. Because the magnetic coherence length in the used field (11.7 T) is much larger (~ 1 mm) [1] than the dimensions of the pores, the director of the nematic phase build up inside the pores is parallel with pore surface. As there is an isotropic distribution of the direction of the pores in the sample, the resonance frequencies of the xenon atoms in different pores form the cylinder symmetric anisotropic (CSA) line shape. The anisotropy increases with degreasing temperature. Both of the previous phenomena are detected from the sample of intermediate pore size (CPG 156); at the highest temperatures of the bulk nematic range, the xenon signal shows isotropic environment inside the pores, but when temperature is lowered, the orientation increases gradually and the powder pattern begins to build up.

At the freezing point of the liquid crystal, the intensity of the signal of dissolved xenon decreases or the signal even disappears. On the basis of the changes in the signals, it has been found that the difference in the melting points of bulk and confined liquid crystals is many times greater than in the case of conventional liquids. This sensitivity can be used to determination of the pore sizes of porous materials.

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Assessment and monitoring of supported organotin catalysts by hr-MAS NMR spectroscopy

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Various organotin compounds, grafted to cross-linked polystyrene by both apolar and polar spacers are investigated as recyclable catalysts in model transesterification and polymerization reactions. It is demonstrated that high-resolution magic angle spinning NMR (hr-MAS) spectroscopy is an invaluable tool to characterize completely the supported catalyst, whenever the support is amenable to swelling by an appropriate solvent.

The 2D ^{1}H-^{13}C HSQC hr-MAS spectra, in particular, allow extensive assignment of the ^{1}H and ^{13}C resonances, as well as the measurement of ^{n}J(^{1}H-$^{117/119}$Sn) and ^{n}J(^{13}C-$^{117/119}$Sn) coupling constants, which are important parameters in the determination of the coordination state of the tin atom.

^{1}H and ^{119}Sn hr-MAS spectroscopy is likewise presented as a monitoring tool for catalytic processes based on organotin compounds, particularly for the investigation of the extent to which polymerization residues are observable in situ in the material pores and of the chemical integrity and recycling conditions of the grafted catalyst.[1]

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Determining porosity and pore size distribution by xenon NMR

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orous materials are solids with very large internal surface areas. They are important in many applications involving catalysis, chemical separation and gas storage. Reliable and efficient methods that are capable of measuring pore volume (porosity) and pore size distribution are of considerable interest.

In the present study, we have applied xenon-129 NMR to investigate silica gels and controlled pore glasses with nominal pore diameters varying from 4 nm to 292 nm. The phase transition phenomena of acetonitrile, cyclohexane, and naphthalene confined in these porous materials have been studied by NMR of xenon dissolved in the samples. The spectra recorded at different temperatures contain detailed information on the materials. The most important result is that the chemical shift of a particular signal obtained below the melting point of confined acetonitrile or naphthalene is highly sensitive to the pore size. The signal originates from the xenon atoms sited in very small cavities built up inside the pores during the freezing transition. The chemical shift of this signal decreases with increasing pore size and this dependence can be used to determine the size distribution function of the pores. In addition, the porosity of the materials can be determined by comparing the intensities of the NMR signals arising from the bulk and confined liquid.

Solid State ^{13}C NMR and XRD studies of Order/Disorder transitions of Deoxycholic Acid inclusion complexes of R_x/S_{100-x}-Camphor (x = 0-100).

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The thermal behaviour of the 2:1 host-guest complexes of deoxychloric acid (DCA) with the chiral guest camphor over a range of enantiomeric mixtures was studied using a combination of solid state ^{13}C NMR and single-crystal X-ray diffraction. The complex undergoes an order/disorder transition; the onset temperature increasing steadily with the amount of S-enantiomer present. In the enantiomerically pure complexes the R- and S-camphor assumes different orientations within the host cavity. In the enantiomerically mixed complexes the camphor assumes the orientation of the dominant enantiomer.

Multinuclear NMR characterisation of phosphosilicate gels

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Solid state ^{1}H, ^{29}Si and ^{31}P MAS NMR have been used to investigate the microstructure of phosphosilicate gels prepared by a modified sol-gel method involving hydrolysis of silicon precursors in a solely aqueous environment at 50°C. Gels with molar compositions 5, 10, 20 and 30 mol% P_2O_5 in P_2O_5 - SiO_2 were studied. After drying to 400°C the gels have very similar structures formed by a siloxane framework containing silanol groups and trapped molecules of orthophosphoric acid together with a very small amount, of pyrophosphoric acid. Unlike the gel samples previously synthesised by the hydrolysis of the silicon precursor in alcoholic solution at room temperature, the co-polymerisation of phosphorus and silicon is much reduced. Although co-polymerisation increases with phosphorus content, it still represents less than 70% of the phosphorus in the 30 mol% P_2O_5 gel. Furthermore there is no evidence for six-coordinated silicon in the glassy matrix.

Determination of ^{31}P Chemical Shielding Tensors of $Na_4P_2O_7$ x 10 H_2O Using Domestically Modified Commercial CP/MAS Probe

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Chemical shielding tensors can be measured by solid state single crystal NMR spectroscopy. It requires a goniometer NMR probe. Modern MAS probes are designed to use rotors with 1 to 4 mm in diameter, so there are several older 7 mm MAS probes used rarely todays in NMR laboratories.

In this poster a simple method is presented how to rebuild a 7 mm Bruker MAS probe to be suitable to measure spatial angular dependence of NMR signals of single crystals.

In the test part ^{31}P chemical shift tensors of sodium pyrophosphate ($Na_4P_2O_7 \cdot 10\ H_2O$) single crystal are determined to demonstrate the functionality of the rebuilt probe.

To determine the chemical shift tensors a freely available computer program called ASICS was used[1]. A small program was written by us to convert Bruker-type data sets for ASICS readable format.

Sodium pyrophosphate was found ideal sample to test goniometer probes:

- It has single crystals in cm size.
- It is non-toxic, inexpensive compound and commercially available.
- It has monoclinic crystals, so it is easy to determine crystal axes by macroscopic geometry, because of well-grown surfaces.
- Refined crystal structure is available in the literature[2,3].
- It has two chemically equivalent ^{31}P nuclei joined by symmetry operations.
- ^{31}P has 1/2 spin and it is easy to detect. One scan was acceptable in each crystal position[4].

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Structure of Melamine Phosphates and Their Condensation as Studied by Solid-State NMR

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Flame retardants are essential additives in consumer products to reduce their intrinsic flammability. In recent years the development of environmentally friendly flame retardants to replace their hazardous halogen based counterparts has received increasing attention. In this respect, melamine phosphates have been identified as a promising group of compounds. For a better understanding of their exact mechanism of functioning, we have determined the structures of melamine orthophosphate (MP) [1], melamine pyrophosphate (MPy) [2] and recently, melamine polyphosphate (MPoly) [3] using a combined approach of X-ray powder diffraction (XRPD) and magic-angle spinning (MAS) solid-state NMR.

Here we present MAS solid-state NMR results obtained on MPoly, which enable us to characterize the inhomogeneities in the sample composition, to determine the average degree of polymerization, and to corroborate the structure and hydrogen-bonding model proposed by XRPD. The route to MPoly proceeds through two consecutive solid-state condensation reactions of the phosphate moieties in MP. We studied the reaction of MP to MPy in detail by ^{31}P MAS solid-state NMR at elevated temperatures.

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^{1}H and Diffusion NMR study of the Capping of InP Colloidal Semiconductor Nanocrystals by TOPO

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We have used trioctylphosphine oxide (TOPO) capped InP colloidal nanocrystals (Q-InP|TOPO) to explore the potential of solution ^{1}H NMR in studying *in situ* the capping and capping exchange of sterically stabilized colloidal nanocrystals. The Q-InP|TOPO spectrum shows resonances of free TOPO, superimposed on broadened spectral features. The latter are assigned to TOPO adsorbed at Q-InP by means of pulsed field gradient diffusion NMR and ^{1}H-^{13}C HSQC spectroscopy. The diffusion coefficient of Q-InP|TOPO nanocrystals is inferred from the adsorbed TOPO's NMRsignal decay. The corresponding hydrodynamic diameter correlates well with the Q-InP diameter. Using the resolved methyl resonance of adsorbed TOPO, the packing density of TOPO at the InP surface can be estimated. Spectral hole burning is used to demonstrate that the adsorbed TOPO resonances are heterogeneously broadened. Exchange of the TOPO capping by pyridine is demonstrated by the disappearance of the adsorbed TOPO resonances and the appearance of pyridine resonances in the ^{1}H NMR spectrum. From the resulting pyridine spectrum, an upper value of the first order rate constant of pyridine desorption can be obtained. These results show that solution NMR should be considered a powerful technique for the *in situ* study of the capping of sterically stabilized colloidal nanocrystals.

Network density in peroxide cured EPDM rubbers studied by solid-state NMR

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Peroxide cross-linking of EPDM is commonly applied in rubber practice as an alternative to sulfur vulcanisation. Although EPM rubber can be cross-linked with peroxides, the introduction of a diene monomer results in a major enhancement of the peroxide cross-linking efficiency. The process of cross-linking proceeds via the two routes: combination of two EPDM macro-radicals and addition of an EPDM macro-radical to the residual EPDM unsaturation. In order to determine contribution to the total cross-link density from the combination and addition reaction, as well as from entanglement density, solid-state NMR relaxation and spectroscopy methods were used in the present study.

We have used solid-state ^{1}H NMR T_2 experiments to quantitatively determine the chemical and entanglement density as a function of the peroxide and diene content.

Magic-angle spinning ^{1}H and ^{13}C spectra of EPDM were recorded for determining the conversion of double bond of diene monomer. The spectra show peaks of the incorporated diene monomer: ENB (2-ethylidene 5-norbornene) and DCPD (5,8-dicyclopentadiene). This permits monitoring the double-bond conversion resulting from the peroxide curing.

Reconstruction of Multidimensional NMR Spectra

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Reconstruction of an image from a set of projections is a well-established science, successfully exploited in X-ray tomography and magnetic resonance imaging(MRI). We have adapted this principle to generate multidimensional NMR spectra, with the crucial difference that these spectra are made up of discrete features. sparsely distributed, rather like the stars in the night sky. Consequently a reliable reconstruction can be made from a small number of projections. This reduces the duration of the measurements by orders of magnitude, of crucial important for structural investigations of biomolecules such as proteins. We explore several practical schemes for reconstruction -- back-projection, iterative least-squares fitting, the lower-value algorithm, and Bayesian methods. The results are compared in terms of their reliability, sensitivity and speed of computation.

A new method for ultrafast high resolution iZQC spectroscopy in vivo

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Localized detection of metabolites is useful for detecting and diagnosing diseases. In many organs, susceptibility differences between tissues and or interfaces, especially air/tissue interfaces, create strong local fields which broaden the resonance frequency lines from each proton species. It then becomes difficult to shim the magnet to create an acceptable linewidth. The broadening can be improved through choosing very small voxels, at a great sacrifice to sensitivity and linewidth.

Intermolecular zero quantum coherences (iZQC) are intrinsically not sensitive to large scale inhomogeneities, and can give sharp lines along the indirect dimension despite variations in the magnetic field[1-4]. Our work on cold-blooded animals at low temperatures has increased resolution by an order of magnitude over a direct acquisition.

Though in vitro ZQC spectroscopy gives narrow lines, in vivo for warm blood animals[5] the t1 noise inherent to the long experimental times of two dimensional spectroscopy decreases the resolution. In principle, ultrafast two-dimensional methods such as those developed by Frydman et al.[6,7] can overcome this problem. However, their approach (time offset excitations of many different narrow slices) will not work for our application, since the molecules involved in an iZQC scan inevitably suffer some chemical shift mis-registration, and their coupling would therefore be eliminated. Recently, we have developed a new pulse sequence that enables the acquisition of 2D ZQC localized spectra within a single data acquisition scan. We have also used the same approach to speed up other standard 2D-spectroscopy pulse sequences such as standard COSY or intramolecular double quantum filtered spectroscopy. Preliminary work in vitro, with a simultaneous acquisition of up to 31 indirect-dimension points per sequence, will be presented.

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Detection of Plant Metabolon formation in Polyamine Biosynthesis by NMR Relaxation Methods

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Polyamines are considered critical regulators of cell growth, differentiation and cell death [1]. The biosynthesis of the polyamines spermidine and spermine takes place from putrescine by two sequential steps catalyzed by the enzymes Spermidine Synthase (SPDS) and Spermine Synthase (SPMS) respectively [2]. Macromolecular complexes of enzymes that effect consecutive metabolic transformations, known as metabolons, provide an effective channeling mechanism between the active sites of the two enzymes. Recent studies performed by Tiburcio and coworkers using a yeast two-hybrid system [3] suggest the formation of a metabolon involving SPDS and SPMS.

Direct detection of protein-protein interactions by NMR is hindered by the need to use concentrations well above the physiological range. This requirement is even more stringent if one wishes to use relaxation effects which are the most sensitive indicators of macromolecular aggregation [4]. Previous attempts to solve this problem have made use of line width measurements in one dimensional spectra [5] or fusion proteins incorporating a reporter domain that is probed through fast exchange with a small molecule spy (SMS) [6].

In this communication we report our ongoing work on the characterization of in vitro metabolon formation through Saturation Transfer Difference (STD) [7] studies of specific small molecule reversible inhibitors acting as SMS for the different enzymes involved in the complex.

Macromolecular association leads to an increase in the correlation time that is sensed by the transient complex involving specific SMSs. Amplification of the signal takes place by fast exchange between bound and free SMS and accumulation of saturated free SMSs due to their long relaxation times. This allows the use of protein concentrations that approach the physiological ones.

Ultimately, the method aims to study complex interactions in multiprotein systems at physiological concentrations, by defining matching pairs of protein-SMS to identify at low which of the possible complexes are actually formed near physiological concentrations.

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Ultra-fast Measurement in Protein NMR: Two-dimensional Simultaneous Measurements to Collect All Information of HACANH/HNCAHA/HCCH-COSY

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In order to reduce total measurement time for resonance assignment of labeled proteins in solution, we developed a new pulse sequence featuring simultaneous acquisition of magnetization transfer pathways of HACANH, HNCAHA and HCCH-COSY. The technique to obtain the information of summation and subtraction of chemical shifts in the indirect axis of the two-dimensional (2D) subset experiment was further developed. These made it possible to assign chemical shifts of $^{1}H_{N}$, ^{15}N, $^{13}C_{a}$,$^{1}H_{a}$ and other side-chain $^{13}C/^{1}H$ correlations on 2D spectra. In addition, by modification of the phase cycling of the sequence and nominal post-experimental signal processing for data separately saved, spectra with normal chemical shifts were also successfully obtained.

The pulse sequence diagram developed to acquire HACANH/HNCAHA simultaneously is composed from four basic blocks making magnetization transfer on *J* coupling. First block was designed to make transfer between $^{1}H_{N}$ and ^{15}N or $^{1}H_{a}$ and $^{13}C_{a}$ simultaneously. In the second and the third blocks, the anti-phase magnetization of 2HN and $2HC_{a}$ are changed to $2HC_{a}$ and 2HN respectively. At last, the magnetizations are changed to $^{1}H_{a}$ and $^{1}H_{N}$ in the fourth block and became observable. In addition to those two pathways, magnetization passed HCCH-COSY pathway is also observable in acquisition period. Furthermore, this pulse sequence was modified to 2D measurements by applying the angle addition/subtraction formulae, with special phase cycling of A1, B1, C1, D1, A2, B2, C2 and D2.

To obtain 3 kinds of signals corresponds to 3 pathways separately and chemical shift summation/subtraction from one measurement, data was stored as eight separated datasets, four of them are to extract 3 kinds of signals by addition or subtraction and each of these four datasets is divided to two datasets which correspond two terms in angle addition/subtraction formulae.

Finally, 2D spectra whose indirect axis correspond the summation or subtraction of chemical shifts of ^{15}N, $^{13}C_{a}$ and ^{13}C were constructed by spectra editing from eight datasets (A1, B1, C1, D1, A2, B2, C2 and D2) with 1mM Chlorella Ubiquitin. On these 2-dimensional spectra, it is possible to make sequential assignment easily.

Ultra-fast Measurement in Multi-dimensional NMR: Spectrum Reconstruction from Folded Spectra with Multi-linear Sampling

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For multi-dimensional NMR measurements, one of the factors to increase measurement time is point resolution in indirect axis. Namely, total measurement time is proportional to the number of points in indirect axis and long time measurements are required to get sufficient resolution in multi-dimensional NMR measurement. I developed a technique to decrease the total measurement time with reconstruction methods from folded spectra. By this technique, I successfully shortened the total measurement time to half for two-dimensional measurements.

As the procedure for processing FIDs acuired with this multi-linear sampling manner, the narrow range spectra were processed same as conventional FFT, and the stacking of narrow range spectra was performed. Then by the comparison of two stacked spectra with different sampling durations, the peak positions in wide spectrum are successfully reconstructed with lowest value method.

By reducing the number of indirect point, the measurement time was reduced less than half of original measurement time even the point resolution was not changed.

Further this technique is applicable for high-dimensional measurements, and their total measurement time can be shortened to less than quarter.

Detection of polymorphic forms by means of SS NMR spectroscopy

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Solid-state NMR spectroscopy is powerful method for the detection polymorphic (crystalline) purity and can easily distinguish different polymorphs and also different solid phases. Therefore this method is one of the few techniques that allow unequivocal identification of polymorphs. Solid state NMR is very often realized as ^{13}C CP MAS. But the ^{13}C CP MAS NMR of drug form gives very complicated spectra with very often overlapped signals of API and placebo.

The case of molecule, which contains fluorine atoms, is presented in this study. The ^{19}F MAS spectra are very sensitive source of information about polymorphs and give us very clear and good results. This advantage is excellently applied in the final solid dosage form. How easily can ^{19}F MAS spectra be used to determine crystalline proportions in amorphous material is also shown in our study. For comparison also ^{15}N CP MAS were measured.

Multiple quantum relaxation: A way of detecting exchange in proteins; application to a C-terminal complexed centrine by using cross correlated dynamics involving modulations of isotropic chemical shifts.

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In proteins, many important biological processes involve motions that span a wide range of time-scales. NMR relaxation measurements provide a detailed description of the dynamics of macromolecules in solution. Most experiments probing protein dynamics have for a long time relied on nitrogen relaxation rates, from which a local order paramater can be extracted. This approach, known as the model-free analysis, allows one to assess fast motions (ps-ns). Unfortunately, using only a single site of the peptide planes does not cover all dynamical features, which are not confined to fast motions but also comprise motions on microsecond-millisecond time scales. These motional processes are probed by complementary methods.

Firstly by measuring the average transverse relaxation rate of in-phase and antiphase nitrogene coherences,using a derivative of a Carr-Purcell-Meiboom-Gill (CPMG) sequence(developped by Loria et al.; 1999), as a function of the CPMG delay. Secondly, by using cross-correlated relaxation methods. Indeed, slow motions can induce correlated fluctuations of the isotropic chemical shifts of two nuclei involved in multiple-quantum coherences (Kloiber et al., 2000; Frueh et al., 2001). The difference between the relaxation rates of double (DQ) and zero-quantum(ZQ) coherences is a good probe of the existence of slow motions. This method has been used to study dynamical conformational changes in a component of the nuclear excision repair (NER) : the C-terminal domain of a calcium-binding protein, Human Centrin 2, associated with a peptide of 17 aminoacids, which is part of the Xeroderma Pigmentosum group C protein (XPC). Studying multiple-quantum relaxation involving different nuclei allowed us to observe significant cross-correlated fluctuations of isotropic chemical shifts for some residues which are part of loops and which exhibit large linewidths.

Combinatorial Selective Labeling for the Assignment of Backbone Amide NMR Resonances

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We have developed a technique, which we have termed Combinatorial Selective Labelling, which uses in vitro translation with differnt patterns of labelled amino acids to allow a large amount of backbone assignment information to be obtained with a small number of very readily prepared samples. The use of in vitro translation allows the method to be cost effective, low in manpower requirements, and avoids scrambling problems. The data analysis is trivial, which makes the method of chemical shift change mapping accessible to non specialists, or allows a large number of targets to be studied without the labour requirements of traditional backbone assignment methods (whether manual or automatic). Furthermore the method is applicable to proteins displaying non-ideal spectra, in that the assignment of one residue is largely independent of other assignments. (Ref. Parker et al., JACS, (2004) v126, pp5020-5021)

Cogwheel phase cycling in triple-resonance liquid-state NMR experiments on proteins

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The concept of cogwheel phase cycling was introduced recently [1,2,3] and has been applied successfully in a number of solid-state NMR experiments. This novel approach represents general and powerful alternative to commonly used "nested" phase cycling [4,5]. The latter one requires the phase of one pulse to be kept constant until any other pulse phase has performed a full cycle. In contrast, cogwheel phase cycling alters all pulse phases simultaneously, which frequently demands fewer steps upon completion. Accordingly, this technique enables the NMR spectroscopist to design significantly shortened experimental setups. In liquid-state NMR this applies especially for multi-dimensional experiments, like those used for protein structure determination. In these experiments the number of scans is not determined by the signal-to-noise requirements but by coherence selection (and desired spectral resolution). This applies especially when cryogenic probes are used. Although pulsed field gradients usually allow for shortest coherence selection schemes, phase cycling has the advantage of permitting parallel coherence transfer pathways. Additionally, for systems with very fast relaxation, like paramagnetic molecules and very large proteins, the use of pulsed field gradients may lead to severe signal loss by relaxation.

We demonstrate the implementation of cogwheel phase cycling both without and in combination with pulsed field gradients in triple-resonance liquid-state NMR experiments. Representative among these are the HNCO, HNCA, HCACO and NOESY-HMQC or NOESY-HSQC experiments. The performance of the modified setups relative to the established schemes is first assessed by simulations. For theoretical comparison we use the CCCP program [6], which allows to display the coherence transfer diagram for a given pulse sequence. After the evaluation of given cogwheel phase cycles the calculated results undergo experimental verification.

The standard NOESY-HMQC pulse sequence as distributed by instrument manufacturers uses "nested" phase cycles of four independently cycled pulse phases with 180° steps. Thus, a total of $2^4 = 16$ phase cycling steps is required upon completion. The modified cogwheel phase cycle-based sequence allows us to complete the coherence selection process in only 5 steps. This corresponds to a time-saving of 69% in each experiment. The quality of the recorded spectra is still satisfying; artefact suppression is good, as is the signal-to-noise ratio. All NOESY-HMQC spectra have been recorded without the application of pulsed field gradients for coherence selection.

In a TROSY-HNCO experiment the time-saving is even more substantial. Here, only 3 instead of 16 steps are required for the completion of the coherence selection procedure, which corresponds to 81% time saving. Again, the artefact-suppression is comparable with respect to a pulse sequence employing "nested" phase cycling. Pulsed field gradients were only used as crushing gradients on zz coherence order. The coherence transfer diagrams for this experiment with "nested" and cogwheel phase cycles are virtually identical.

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Constraints on sample preparation and acquisition parameters for an accuracy and a precision as low as 2‰ in quantitative ^{13}C-NMR

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^{13}C isotopic analysis at natural abundance could be a powerful tool for food authentication or metabolic studies. But a high degree of accuracy, sensitivity and precision is needed due to the restricted range of ^{13}C/^{12}C isotopic ratios in natural substances (50‰ on the isotopic deviation scale).

Reaching a sensitivity of about 2‰ in quantitative NMR spectrometry is a challenging issue due to several constraints, among these :

- It has been shown previously that accuracy was very sensitive to imperfect broad-band decoupling,
- Long longitudinal relaxation times of carbon atoms, associated to the necessity to suppress the Nuclear Overhauser Effects wich are site-specific (inducing errors in surface ratios) require very long experiment times,
- Moreover, the use of an internal reference for isotopic analysis could make the measurement even more complicated.

To study these points, ^{13}C-bilabelled two-carbon molecules (acetic acid and ethanol) were used. The ratio of the two doublets surfaces of such a compound is always supposed to be 1 whatever the enrichment level of each carbon. It is expected that these molecules could be used as a model to evaluate the sensitivity, the precision and the accuracy of a ^{13}C NMR experiment. Using this original approach, the response to the pre-cited constraints was examinated :

- Influence of ^{1}H decoupling schemes was extensively studied and adiabatic pulses were proved to be the more suitable,
- In order to reduce longitudinal relaxation times and therefore the analysis time, addition of a relaxation reagent ($Cr(Acac)_3$) was investigated. The repetition delay should then be adjusted according to the relaxation times of ^{1}H and ^{13}C. Our experiment data are in agreement with theoritical development,
- In order to release from the need of an internal reference, the use of ERETIC (Electronic REference To acces In-vivo Concentrations) was tested.

Proxy Restraints: the Use of Unassigned Signals in NMR Structure Determination

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Obtaining the remaining 10-15% percent of missing resonance assignments is a process that often requires a considerable amount of manual analysis of NOE spectra. Recent developments have shown that in principle it is possible to determine protein structures using NOE data only, without the need for prior resonance assignments (CLOUDS[1]) Thusfar however the applicability of this method seems to be limited to small proteins and NMR data without overlap.

Inspired by the CLOUDs approach we have developed a hybrid approach between the current practice of structure determination and elements of the CLOUDS method. This approach allows using all the available NOE information, including the NOE signals of unassigned resonances, from the start of the structure calculations. The procedure works by assigning the unassigned resonances to arbitrary atoms or molecule fragments, so-called ***proxy residues***.

For the generation of NOE based distance restraints the proxy residues are treated in the same way as the normal protein residues including network anchoring, restraint combination, selection and rejection of assignment possibilities or whatever is provided by the original algorithm. The proxy residues have no vdWaals interaction with the protein and interact only with the protein through NOE based distance restraints and optionally, additional very short ambiguous distance restraints (***identity restraints***) which carry information about the possible assignments.

The use of proxy residues for missing resonance assignments has several advantages:

1. The completeness of the chemical shift list is improved (albeit artificially), which leads to a reduced risk of generation of incorrect distance restraints due to missing assignments.
2. Analysis of the position of the dummy constructs in the resulting structures can in many cases provide the correct assignments or can limit the number of assignment possibilities.
3. It is straightforward to use the identity restraints to incorporate into this procedure additional information that is usually available, like the type of amino acid residue or chemical group (e.g. methyl/methylene/aromatic).

We have implemented our approach in the software packages ARIA/CNS (using cartesian dynamics) and CYANA/CANDID (using torsion angle dynamics). We have tested the approach on several proteins. We find that the resulting structures allow identification of a substantial portion of the unassigned resonances. Moreover we find that in general there is an improvement in the quality of the structures compared to the standard protocols, as judged for example by Ramachandran-plot quality.

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Novel NMR Methods to Study Photoproducts and Interconversion Dynamics

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NMR spectroscopy is perhaps the most powerful spectroscopic method for studying organometallic reactions in solution. It provides a means to characterisation and can also be used to study reactivity, especially processes where two isomers interconvert.

At the university of York a set-up has been built to enable photolysis of samples within the NMR machine. A laser beam is directed inside the NMR machine, using a prism and a mirror attached to the probe.[1] This allows samples to be photolysed and NMR spectra to be recorded at low temperature thereby enabling very novel and unstable products to be formed and their reactivity explored.

We have used this setup to study the photochemistry of $CpCo(C_2H_4)_2$ in the presence of silanes. The study of transition metal silyl hydride complexes is of interest to the organometallic community since the activation of a Si-H bond by a transition metal complex is one of the key steps in hydrosilation and other catalytic reactions.

Two types of metal silyl hydride complexes are known, the 'classical' form with two-centre, two electron bonding and the 'non-classical' form, three-centre, two electron bonding. This form involves a partial interaction of the Si-H bond with the metal centre and can effect the reactivity of such complexes.

When we studied the photolysis of $CpRh(C_2H_3CO_2{}^tBu)_2$ in the presence of silanes, $CpRh(SiR_2R')(H)(C_2H_3CO_2{}^tBu)$ was formed. This complex exists in two different isomeric forms which interconvert. With the help of NMR spectroscopy we were able to propose a mechanism for interconversion, involving an η^2-intermediate.[2]

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Optimization of resolution and sensitivity of 4D NOESY using sparse matched data acquisition and Multi-Dimensional Decomposition

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Highly resolved, multidimensional NOE data are essential for rapid determination of accurate 3D protein structures such as in structural genomics projects. Four-dimensional spectra contain almost no spectral overlap inherently present in lower dimensionality spectra and are highly amenable to application of automated routines for spectral resonance location and assignment. However, a high resolution 4D data set using conventional uniform sampling usually requires unacceptably long measurement time. Recently we have reported that the use of non-uniform sampling and multi-dimensional decomposition (MDD) can remedy this problem. Here we validate accuracy and robustness of the method, and demonstrate its usefulness for fully protonated protein samples. The method was applied to 11 kDa protein PA1123 from structural genomics pipeline. A systematic evaluation of spectral reconstructions obtained using 15-100% subsets of the complete reference 4D ^{1}H-^{13}C-^{13}C-^{1}H NOESY spectrum has been performed. With the experimental time saving of up to 85%, the resolution and the sensitivity per unit time is shown to be similar to that of the fully recorded spectrum. For the 30% data subset we demonstrate that the intensities in the reconstructed and reference spectra correspond with a correlation coefficient of 0.997 in the full range of spectral amplitudes. The spectral reconstructions have no false peaks higher than baseline noise. These translate to accurate distance constrains derived from sparsely sampled, high resolution 4D NOESY data. A small improvement in sensitivity of up to 8% is detected due to the usage of sparse schemes with matched acquisition in indirect dimensions. Possible extensions to other types of triple-resonance NMR experiments used for assignment is considered.

Stretched gelatin gels as chiral alignment medium capable to discriminate enantiomers.

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Residual dipolar couplings contain important structural information about the distance between two nuclei and the angle between internuclear vector and a static magnetic field. Their measurement in high resolution NMR makes necessary the partial alignment of molecules in the sample [1-3]. A number of various media exist for both aqueous (phospholipid bicelles, filamentous phage, polyacrylamide gels) and organic solvents (liquid crystalline phases like poly-γ-benzyl-L-glutamat (PBLG) in $CDCl_3$ or polystyrene gels in various organic solvents).

Chiral alignment media allow enantiomeric discrimination due to the different orientations of enantiomers. The only chiral medium reported so far, in which such discrimination was experimentally observed is PBLG [4]. Here we report a different medium - stretched gelatine gels - suited not only for alignment of molecules in aqueous solutions, but also for enantiomer discrimination. Alignment of a mixture of L- and D-alanines in such a medium resulted in two distinct sets of signals in a J-spectrum.

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Extended Flip-Back Schemes for Sensitivity Enhancement in Out-and-Back Experiments

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In many NMR experiments, only polarisation of a limited sub-set of all protons is converted into observable coherence. As recently shown by the "longitudinal" TROSY implementation (LTROSY[1]), recovery of unused polarisation can be used indirectly and unspecifically to cool the entire proton lattice and, thus, accelerate reequilibration for the selected proton sub-set. We here illustrate transfer of this principle to HSQC-based out-and-back experiments that exploit only polarisation of ^{15}N-bound protons. The presented modifications to the pulse sequences can be implemented broadly and easily, extending standard flip-back of water polarisation to a much larger pool of protons that comprises up to all non-^{15}N-bound protons. The underlying orthogonal separation of H^N polarisation (selected by the main transfer path) from unused H^u polarisation (for extended flip-back) is thereby achieved through positive or negative selection by J-coupling, or using band-selective pulses. In practice, H^u polarisation recovery degrades mostly through cumulative pulse imperfections and relaxtion; we show, however, strategies so substantially minimise such losses. Depending on the extended flip-back scheme employed, we thus recovered up to 55% H^u equilibrium polarisation, affording more than 25% average gains in signal intensity from accelerated $T_1(H^N)$ relaxation.

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qTROSY – A Novel Scheme to Recover anti-TROSY Magnetisation

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In TROSY experiments, spin state selection (S^3) retains only the single HSQC sub-spectrum with minimal T_2 relaxation and maximal resolution, yet at the cost of eliminating half of the available polarisation as undesired anti-TROSY component. We present queued TROSY (qTROSY) as a novel scheme to partially recover and exploit this anti-TROSY polarisation in two concatenated scans. After initial orthogonal spin state separation (oS^3), anti-TROSY polarisation is explicitly stored while its TROSY counterpart follows the desired coherence pathway recorded in a first scan A. The immediately appended scan B then quantitatively converts the recovered anti-TROSY polarisation into a second TROSY spectrum, skipping the time-limiting long reequilibration delay. Both concatenated qTROSY scans thus ideally exploit the full initial polarisation within almost the same measurement time. In practice, T_2 relaxation losses accruing during the coupling evolution delays reduced anti-TROSY polarisation recovery below 40%, obviating sensitivity enhancement through addition of both qTROSY scans; yet, scan B retained a complete scan A spectrum with up to 75% intensity. We therefore propose to employ qTROSY asymmetrically, compacting two separate conventional into one queued TROSY experiment with significantly reduced measurement time, implying primarily the concatenation of different three- or higher-dimensional TROSY experiments. Both anti-TROSY polarisation recoveries and possible time savings are largest for deuterated and for smaller non-deuterated proteins, extending the rentability limit of TROSY towards smaller molecular weights.

Novel Flip-Back Schemes and Queued Spectroscopy: New Methods for Recovery of Lost Magnetisation

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The majority of NMR experiments only converts a part of all 1H polarisation into observable coherence. Recovery of the unused polarisation could obviously make NMR considerably more economical when used in one of two possible ways:

1. indirectly and unspecifically to cool the proton lattice and, thus, accelerate reequilibration of the selected proton subset as in LTROSY[1].
2. directly and specifically, as we propose here, in an immediately (i.e., without reequilibration delay) appended second NMR experiment; such queued experiments on different types of proton polarisation are largely uncorrelated and may therefore differ broadly (in contrast to strictly correlated, specific concatenation schemes recovering lost coherence, such as CoCONOESY[2] or the sensitivity enhancement3 scheme).

For both applicat ions, we present two novel schemes to recover unused polarisation in different types of heteronuclear correlation experiments, based on initial orthogonal separation of observed and lost magnetisations:

1. qTROSY (queued TROSY) recovers anti-TROSY polarisation in TROSY experiments
2. efb (extended flip-back) preserves proton magnetisation not bound to a specific heteronucleus in HSQC-type experiments

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Structure of a BRCT domain - DNA complex determined by NMR and mutagenesis.

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The **BRCT** domain (**BR**CA1 **C-T**erminus) was first identified as tandem repeats of roughly 90 amino acids at the C- terminus of BRCA1 protein and it has, since then, been identified in a large number of multidomain proteins associated with the replication and repair of DNA and with the cell cycle-checkpoint. Most biochemical and structural studies indicate that BRCT domains act primarily as a module for protein – protein interactions in signal transduction regulating DNA metabolism. In contrast to this widely accepted function, the BRCT region of the large subunit of the Replication Factor C complex (RFC) has an unusual dsDNA (double stranded DNA) binding activity, which is not sequence-specific but depends on 5'-phosphorylation. RFC is a multi-subunit protein which plays a crucial role in DNA replication. The dsDNA binding activity is not only unique among the BRCT superfamily but also its specificity to 5' phosphate is unprecedented among DNA binding proteins. The amino acid sequence identity among BRCT domains is very low (~17%), reflecting the diversity of their potential functions. Therefore a good understanding of the DNA recognition by this BRCT domain is of paramount importance in identifying other DNA binders within the BRCT family.

Here the first model structure of a unique BRCT-DNA complex is presented, determined from both NMR and mutagenesis data. Despite the dynamic behaviour of the sample, we determined a relatively high quality solution structure of the BRCT domain from the large subunit (residues 375-480) of RFC, bound to 5' phosphorylated dsDNA. The docking of the BRCT domain to dsDNA was performed with empirical restraints derived from biochemical DNA binding data of a series of mutants. Possible biological implications are discussed.

Double-J-Modulated INEPT-INADEQUATE: A New Highly Efficient Tool for the Structural Elucidation of Small Molecules by NMR

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We describe a new NMR experiment, double-*J*-modulated (DJM)-INEPT-INADEQUATE, for tracing out the carbon skeleton of molecules. This experiment allows simultaneous correlation of directly bonded carbon atoms and those separated by multiple bonds, while at the same time also providing the values of all J_{CC} coupling constants. This is achieved by replacing both fixed carbon-carbon coupling evolution intervals of the INEPT-INADEQUATE experiment by variable time intervals, which are incremented in concert with the DQ evolution period (t_1). We show that the analysis of the fine structure of cross peaks in DJM-INEPT-INADEQUATE spectra leads to accurate values of coupling constants and give guidelines for the proper usage of the method. We have obtained high quality data using a 600 MHz cryoprobe and 71 mg of a monosaccharide. Moreover, our simulations indicate that a similar performance is to be expected for larger molecules and smaller amounts of material. Instead of performing multiple experiments, a single DJM-INEPT-INADEQUATE experiment can thus provide a wealth of information for the structural analysis of small molecules.

In addition we also describe new INEPT-INADEQUATE based methods dedicated to the measurements of long-range carbon-carbon coupling constants and compare their sensitivity with the double-*J*-modulated methods.

J-resolved three-dimensional HSQC exoeriment for accurate determination of one bond coupling constants

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Residual dipolar couplings (RDCs) have become a valuable tool as a complementary data in structure determination of biomolecules [1]. Accurate determination of mostly used one bond coupling constants in both isotropic and anisotropic conditions is essential for reliable structure determination.

For measuring RDCs, different kinds of 2D HSQC derivatives are widely in use. Acquisition of normal coupled HSQCs gives non-satisfactory results due to doubling the amount of signals in spectrum, which increases the possibility of accidental signal overlap. This effect can be minimized by acquiring the data so that the doublet components appear in separate sub-spectra. Still the coupling components appear in the same dimension(s) as chemical shift(s), coupling value extraction becomes complicated as the amount of signals or signal overlap in spectra increases. In a 3D J-resolved HSQC experiment the coupling is placed in the third dimension and the two other dimensions still have a well-resolved HSQC spectrum. In this way, the values of coupling can easily be read from the third dimension.

Our preliminary experiments have shown good applicability of the 3D J-resolved method in RDC determination. The signal assignment and extraction of RDCs is easier and can also be automatized. The process is overall less prone to errors than the same procedure using commonly used 2D HSQC derived methods such as F2-coupled HSQC, IPAP [2], HSQC-α/β [3] and α/β-HSQC-α/β [4]. These improvements are most likely due to the smaller signal overlap in the 3D experiment.

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Automated sidechain assignment from heteronuclear-resolved [^{1}H, ^{1}H]-NOESY spectra

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A novel algorithm for automated resonance assignment that operates directly on the raw NMR data is presented (ASCAN: automated sidechain assignment from NOESY spectra). Minimal input requirements for ASCAN are sequence-specific assignments of at least one proton/heavy atom pair and one additional heavy atom per residue, heteronuclear-resolved [^{1}H,^{1}H]-NOESY and possibly heteronuclear resolved [^{1}H,^{1}H]-TOCSY spectra. In its standard application, ASCAN starts with the complete backbone sequence-specific assignments (including HN/N/Cα/Cβ) and uses them first to guide the peak picking of NMR spectra and then to exploit the covalent polypeptide structure for identifying new side chain resonance assignments.

ASCAN was validated with experimental data sets of the three proteins PBP^A (1-128), TM1442 and FimD (25-125), which consist of single polypeptide chains with 128, 110 and 100 residues, respectively. Proof of principle for ASCAN was established by comparison of the resulting side chain resonance assignment with those obtained by interactive procedures. For each of the three proteins, more than 80% of the side chain protons and heavy atoms were assigned by ASCAN with a correctness rate above 90%. To assess the potential of ASCAN in the context of fully automated protein 3D structure determination, two structure calculations were performed for each of the three test proteins with the software ATNOS/CANDID and one of the common structure calculation algorithms using automated and interactively generated sidechain resonance assignment. In all three cases the two structure bundles coincide closely in terms of precision and accuracy, confirming the reliability of the ASCAN procedure.

Overall ASCAN automates a labor intensive step of NMR data analysis requiring a minimal set of NMR spectra as input.

Automation of NMR spectral analysis and three-dimensional protein structure determination

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This presentation describes work toward a new algorithm for automation of NMR spectral analysis. This software package will combine two previously developed algorithms for automated NMR data analysis, ATNOS (1) for identification of NOE signals in 2D and 3D heteronuclear-resolved [^{1}H,^{1}H]-NOESY spectra based on chemical shift lists and the covalent polypeptide structure, and CANDID (2) for automated NOE assignment. In the new algorithm, a parallelized and fully probabilistic analysis of the NMR spectra will replace the serial use of ATNOS and CANDID, and will thus allow a maximal combined assessment of all properties for a given signal. After establishing an initial, tentative network of mutually supportive NOEs, the quality of all potential signals is re-evaluated using the embedding into this initial NOE network. This iterative procedure of quality assessment based on network-anchoring for both peak picking and NOE assignment allows for robust artefact discrimination, and increases furthermore the number of high-quality signals available in the first cycle of a structure calculation.

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Computer optimisation of spectral aliasing in high-resolution NMR

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Spectral aliasing is an old trick allowing one to increase the number of time increments of multidimensional NMR experiments. It consists in reducing the spectral width either to reach better resolution at constant number of time increments or to decrease the number of time increments needed to reach a given resolution. The application of computer optimisation allows one to rationalise the factor used to reduce the spectral width and reach values larger than the typical factor 2 to 5.

In the case of small molecules having broad ^{13}C spectra and long T_1 relaxation rates, computer optimised aliasing of the carbon dimension of HSQC experiments allows one to reduce the spectral width by a factor between 10 and 50. The experimental time is reduced by the same factor, provided the experimental time is not limited by sample quantity. The applications include HMBC, HSQC edited relaxation measurements and carbon edited diffusion measurements.

When applied to protein NMR where relaxation rates and spectral widths are smaller, the factor of reduction is less than 10 but the importance of being able to reduce experimental times is also higher. Applications include NOESY-HSQC, 3D-HNCO, 4D HSQC-NOESY-HSQC applied to ubiquitin.

Quantitative NMR of Screen Hits without an Internal Standard

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Modern drug and agrochemical discovery relies on the screening of hundreds of thousands of chemicals. The concentration of the chemicals at the time of screening is a critical issue for this process. The presence of impurities reduces the initial concentration below that expected, and instabilities during storage such as degradation and crystallisation reduce it even further. These factors lead to an underestimate of a chemical's true potency which is impossible to predict.

We have adapted 1D proton NMR spectroscopy for quantifying chemicals following screening. Quantitative proton NMR is an attractive method because it can be applied to almost all organic chemicals. Although NMR has been widely used for accurate quantification with internal standards, this is impractical for the throughput we require. Instead, we calibrate the spectrometer using an external standard of known concentration. The reproducibility of modern NMR spectrometers is sufficiently high that this calibration provides a useful estimate of concentration for other samples run in the same conditions.

This poster presents data regarding the reproducibility of NMR spectra and examples of the application of the approach.

Structure of the nucleocapsid protein NCp8 from Simian Immonodeficiency Virus

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In the human immunodeficiency retroviruses type 1 and type 2 (HIV-1 and HIV-2) and Simian immunodeficiency retroviruses (SIV), the nucleocapsid (NC) protein, a small highly basic protein, is characterized by the presence of two zinc finger domains with a common sequence motif, CCHC. NC is associated closely with the dimeric RNA genome in the core of virus particle and was shown to promote the specific encapsidation of the viral RNA into budding virions. NC is also implicated in the reverse transcription. HIV-1 and HIV-2 have resulted respectively from several transmissions of Simian immunodeficiency viruses from chimpanzees (SIVcpz) and sooty mangabeys (SIVsm). It has been shown that the SIVlhoest from l'Hoest monkeys has also the potential to infect human populations. We have determined by NMR the structure of the nucleocapsid protein, NCp8, from this virus to gain insight about the structure modifications that could be induce by three major differences in amino acids, since Glu21, Gly43 and Met46 in NCp7 are replaced respectively by Pro, Glu and Phe in this NCp8. The structure is perfectly well defined, but the global fold is different from this observed for NCp7 although the individual structure of each zinc finger is similar with this observed for the two HIV-1 zinc fingers of nucleocapsid. The main structural change occurs at the level of the linker between the two zinc fingers, and the inter zinc finger interactions are reinforced in NCp8 from SIVlhoest by comparison with that observed in NCp7 from HIV-1. The structure of the nucleocapsid protein NCp8 of SIVlhoest could provide a useful monkey model to investigate the activities of putative antiviral drugs inhibiting the nucleocapsid functions.

High Dynamic Nuclear Polarisation with Biradicals

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A systematic study of the DNP characteristics of biradicals in comparison to the widely used monoradical TEMPO has been performed. The biradicals dissolved in a matrix of protonated polystyrene are of a rigid rod like structure with defined intraradical distances. For three biradical lenghts a series of samples has been studied with decreasing free electron concentration. In all cases the obtained polarisation values are considerably higher than for samples with similar concentrations of TEMPO radicals. The discrepancy increaseswith lower radical concentrations. In the biradical doped samples, even at high interradical distances, pairs of interacting electrons are present which can relax a nuclear spin in a three-spin process, thus supporting the DNP process. The DNP experiments were performed in a continuous flow ^{4}He refrigerator at 1.2 K and 3.5 T.

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Stabilization by interconversion within triads of coherences in multiple refocusing experiments

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We describe a hitherto unknown effect, which has been found to have at least two distinct manifestations. In both cases, the effect helps to rescue multiple-echo experiments from complications.

a) R_2 relaxation experiments are expected to be perturbed by homonuclear couplings when applying only non-selective multiple-refocusing pulses to single quantum coherences. However, offset effects (tilted effective fields) lead to a suppression of the modulation of the echoes, provided the offset effects are large compared to the scalar couplings. As a result, surprisingly simple monoexponential decays are observed, which allow an accurate measurement of the relaxation rates. We show this by simulation and experiment for imino nitrogens in nucleic acids, which experience a scalar coupling of about 7 Hz to the acceptor nitrogen of the hydrogen bond.

b) In applications of multiple-refocusing methods to multiple quantum coherences such as $2N_xH_x$ involving amide nitrogens and protons in protein backbones, cross-correlation leads to a partial interconversion of $2N_xH_x$ into $2N_yH_y$ at the tops of the echoes. The mixture of coherences with *x*- and *y*-phases implies that the experiment is bound to violate the Meiboom-Gill idea, so that self-compensation of inevitable pulse imperfections cannot succeed, no matter which RF phases one chooses. It will be shown how the scalar coupling between the proton and nitrogen nuclei counteracts the effects of pulse errors in a surprising way. The combination of pulse imperfections and scalar couplings cause an oscillatory interconversion between a triad comprising three different coherences in the density operator. When the scalar coupling is strong enough, the desired double quantum coherence is preserved nearly quantitatively (except for true relaxation) in spite of pulse imperfections.

The two cases can be considered as two manifestations of the same effect, working in opposite directions. We give a general description of the underlying perturbation/counteraction principle, which turns out to be applicable also to other constellations occuring in NMR experiments.

Application of Maximum Entropy processing to NMR multidimensional datasets, partial sampling case.

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With the increase in sensitivity of the modern spectrometers, the speeding-up of the acquisition is becoming an important issue in multidimensional NMR. In this purpose, it has been proposed to realize a partial sampling of the acquisition plane, in order to optimize the acquisition time, as well as the sensitivity and the resolution of the final spectra. However this procedure requires the use of alternative processing methods.

In this study we show how the new implementation of Gifa software is efficient in the processing of such partially sampled experiments. The new convergence algorithm, which does not rely on any special user input, affords a very high quality spectral reconstruction, and permits to retain the faint details in high dynamic experiments such as HSQC-NOESY of proteins.

Random and radial spamplings are considered, and we show, on simulated as well as real data, that the Maximum Entropy method is able to recover all the features of the spectra, and permits in the same time gains in acquisition times ranging from 4 to 10.

The design of this new program permits to use the same set-up in an interactive manner as well as in batch mode. It will be shown how the batch mode can be exploited for automatic processing in the frame of the NMRb raw-data NMR repository.

Towards Automated Structure Verification

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The dramatically increasing throughput of modern NMR instrumentation is calling for more automatic data interpretation methods. One application is the verification of structures. We present an approach that is based on the prediction of NMR-spectra based on the know structure and the iterative comparison of the predicted and the experimental spectrum. In contrast to the common linear comparison methods based on static integration we use an iterative fitting process that not only gives a match factor but also retrieves accurate spectral parameters from the experimental data. Examples and applications are discussed.

Stretched Polymer Gels as Alignment Media for All Important NMR Solvents

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Residual dipolar couplings contain important structural information about the distance between two atoms and the angle between a pair of atoms and a static magnetic field. Their measurement in high resolution NMR makes necessary the partial alignment of the molecules in sample. For biological molecules in aqueous solution a number of standard methods exist like phospholipid bicelles, filamentous phage, or other liquid crystalline phases that orient in the magnetic field and partially orient the molecules by steric or electrostatic interactions. In relatively apolar organic solvents liquid crystalline phases like PCBP or poly- -benzyl-L-glutamat (PBLG) and derivatives can be used to weakly orient medium sized organic molecules in the magnetic field. Liquid crystalline phases, however, generally have the disadvantage of a lower limit in the induced anisotropy. An alternative is given by strain induced alignment in a gel (SAG) which is independent of the magnetic field and scalable over a wide range [1,2]. In aqueous solutions mechanically stretched polyacrylamide and an acrylamide/acrylate copolymer were applied. In 2004 we introduced crosslinked polystyrene as a polymer which allows the freely scalable alignment of molecules in chloroform and solvents with similar solubility parameters [3]. Improvements in the spectral quality of partially aligned samples could be achieved by the use of b-irradiation crosslinked polydimethylsiloxane [4]. Finally, our group and the Griesinger group developed independently two gels that can be used for polar organic solvents like DMSO or methanol, for which scalable alignment could not be achieved so far [5,6]. A detailed characterization of the properties of stretched polymer gels will be presented and a number of applications to various molecules will be given. We even will show first spectra on a sample aligned in a chiral gel: gelatin (see also poster presented by K. Kobzar).

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Pattern Pulses: Selective Pulses Dependent on Pulse Amplitude

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Optimum Control Theory was recently applied to the design of the broadband excitation and inversion pulses covering extremely large offset ranges and tolerant to severe miscalibration of RF-amplitude [1]. A slight modification of the calculation routine allows the design of selective excitation and inversion pulses, while extension of that approach onto the dimension of pulse amplitudes makes it possible to design pulses with excitation profile dependent on both pulse amplitude and offset [2].

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(J+D)-Spectroscopy in partially oriented samples: Resolution due to Residual Dipolar Couplings

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In recent years, residual dipolar couplings (RDC) have been introduced and extensively applied for structural studies of proteins, nucleic acids, carbohydrates and more recently of small organic molecules. In NMR spectra, RDCs manifest themselves as an increase or decrease of the splittings due to scalar couplings. Most methods currently applied for the determination of dipolar couplings are based on the measurement of the displacement between cross peaks components in J-coupled spectra, as for example in coupled HSQC spectra. In this kind of experiment twice or even four times the number of signals lead to crowded spectra with reduced resolution. Besides spin state selective techniques like S3CT, S3E or IPAP, J-modulated experiments have been proposed to overcome this inherent problem. Here, we present experiments based on J-evolution periods in a separate dimension for the coupling measurement, called JE-TROSY or JE-HSQC. The method can be further enhanced using GBIRD filters for decoupling of undesired long range couplings, which dramatically reduces linewidth especially in proton rich partially aligned samples. The J-evolution experiments can be used for additional resolution in the (J+D)-dimension, where the dispersion, unlike chemical shift, can be tuned by the strength of alignment. The potential application of these new experiments will be demonstrated on a uniformly 15N,13C-labelled PA4 pentapeptide, as well as on 15N-labelled Saposin C, a medium sized protein.

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Structure refinement of cyclic peptides using RDCs

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Residual dipolar couplings (RDCs) provide important structural information about the distance between two atoms and the angle of the internuclear vector of these atoms with respect to the static magnetic field. In isotropic solution these couplings average to zero but if partial alignment of the molecules is induced by dipolar couplings do not completely cancel out leading to the detectable residual dipolar couplings [1,2]. Meanwhile, RDCs are routinely measured and included in the structure determination process of biological macromolecules. Therefore, numerous standard methods exist for their measurement including techniques like IPAP and alignment media like filamentous phages, phospholipid bicells, or liquid crystalline phases. The media mentioned above are applicable to aqueous solutions but there are just very few alignment media known for organic solvents, e.g. poly-?-benzyl-L-glutamat (PBLG) or polystyrene [3,4].

In this work we present two media for the determination of RDCs in chloroform [5] and dimethylsulfoxide, respectively. They are based on the approach of strain induced alignment in a gel (SAG) and are therefore easily tuneable to the amount of alignment they cause. The background signals of these media can be partially suppressed, e.g. by a relaxation filter, leading to high quality spectra.

For the application of these media we chose two peptides, one soluble in chloroform (Cylosporin A), the other one soluble in DMSO (Veber peptide, cyclo-PFwKTF). For the structure determination the {1H} 1H homonuclear NOEs have been measured as well as the 1DCH-couplings. These restraints were piped into XPLOR-NIH 2.9.4 resulting in different structures if the RDCs are included or not. Although the major fold is conserved and no NOE-restraints are violated, the conformational changes resulting from the inclusion of RDCs leads to a drastic increase of the correlation factor of the experimental RDCs to back-calculated RDCs.

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Automated Protein Structure Determination and Verification from NMR Data

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The reliable automated structure calculation from NMR data is the ultimate goal of the program AUREMOL newly developed by us (1). It is designed for a top-down strategy with the aim to obtain a three-dimensional structure from a minimum of NMR data using information from additional sources. It contains all necessary tools for evaluation and representation of multidimensional NMR data and can also present three-dimensional structures of proteins. Automated assignment of NOESY spectra is a prerequisite for automated structure determination of biological macromolecules. With the program KNOWNOE contained in AUREMOL we present a novel, knowledge based approach to this problem. KNOWNOE is devised to work directly with the experimental spectra without interference of an expert. Besides making use of routines already implemented in AUREMOL, it contains as a central part a knowledge driven Bayesian algorithm for solving ambiguities in the NOE assignments. We can show by the calculation of 2D/3D NMR R-factors that in the cases tested this method works equally well or even better than a human expert since it avoids the bias introduced by the non-random distribution of unequivocally assignable NOE-contacts. Whereas KNOWNOE requires that about 80% of the resonance frequencies in the NOESY-type spectra are assigned, a new algorithm based on an HMM-TA procedure performs the resonance assignments in parallel to the structure determination using a set of NOESY-type spectra supplemented by a small set of spectra containing direct information on the sequential assignment via J-couplings. For obtaining a more reliable fit to the experimental data a T_2-calculation allowing for various modes of internal motion was incorporated into spectra simulation. Additionally, multiplet splitting by J-coupling on the basis of the weak coupling approximation is also calculated. Especially when homology data or mutant data are available only a small set of data is necessary to obtain the spectral assignment together with the three-dimensional structure.

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Protein Backbone Dynamics of M13 Coat Protein in Complex with SDS Micelles from Residual Dipolar Couplings

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Residual dipolar couplings (RDC) have become standard parameters for probing structure and dynamics in biological macromolecules in NMR spectroscopy. Dipolar couplings contain information on the orientation of inter-nuclear, usually one-bond vectors relative to the magnetic field. These dipolar coupling information is averaged out to zero in isotropic solutions, but can be obtained in partially aligned media (e.g. some organic molecules, liquid crystalline bicelles, filamentous phages and strained polyacrylamide gels). However, most of the above media are not compatible with membrane proteins, because they associate with e.g. SDS micelles. The M13 coat protein, studied by us, is a small (50 amino acid) membrane protein with a surface - and trans-membrane helix. To measure RDCs of the M13 coat protein in complex with SDS micelles, we used the method of stretched polyacrylamide gels similar to the one described by Chou et al. (JBNMR 2001) and Jones and Opella (JMR, 2004).

To characterize the methodology for practical use we measured the size of the RDCs (the degree of alignment) of the M13 coat protein in complex with SDS micelles as a function of acrylamide concentration and cross-link ratio of acrylamide/bis-acrylamide. In this way, we were able to determine the optimum conditions for this method to be used for large systems of protein complexes in SDS micelles (~20-25kDa). We have derived practical rules to decide which conditions are suitable for a specific molecule to obtain accurate RDCs combined with good NMR spectra. Using the above method, high precession and reproducible 1H-15N RDCs have been obtained for M13 coat protein in complex with SDS micelles in stretched polyacrylamide gels.

RDCs provide also information about dynamics of the biomolecules and are sensitive to internal motions up to the millisecond timescale. It is possible to determine the alignment tensors from RDCs measured in five different media and subsequently derive local motion tensors (Meiler et al., JACS 2001; Bouvignies et al., JMR 2005). However, for membrane proteins such as the M13 coat protein the choice of media is limited. Alternatively, it is possible to measure five or more RDCs within the (rigid part) of one residue and still derive the local alignment tensor and motion parameters (e.g. Bernado and Blackledge, JACS 2004). We have measured five RDCs within each amino acid residue (HN-N, HN-C', N-C', Cα-C', Cα-Hα) of the M13 coat protein in complex with SDS micelles to derive in this way the dynamics of its surface – and trans-membrane helix.

Oligomers

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Conjugated porphyrin polymers are giant supramolecular chromophores with extraordinary electrooptical and nonlinear optical properties [1]. Several strands of independent work show that the unusual electronic behaviour of these materials can be attributed to strong ground state interporphyrin conjugation, which is amplified in the excited states, and in the oxidised and reduced forms.

We have been investigating the solution behaviour of oligomeric zinc porphyrins by NMR, which comprise one, two, four or eight porphyrin units per molecule. These systems are prone to aggregation in solution and form dimeric, or possibly even larger, complexes which can be disrupted by small molecules that themselves complex strongly with the central zinc, such as pyridine. We have explored the possibility of using diffusion NMR techniques to estimate self-diffusion coefficients as a means of characterizing the dispersed, self-aggregated and complexed states of these oligomers and show these to be effective tools.

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Exploration of peroxiredoxin systems using spin relaxation

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Peroxiredoxins (Prxs) constitute a family of thiol peroxidases that reduce hydrogen peroxide, peroxinitrite, and hydroperoxides using a strictly conserved cysteine. Very abundant in all organisms, Prxs are produced as diverse isoforms characterized by different catalytic mechanisms and various thiol containing reducing agents [1]. The oligomeric state of Prxs and the link with their functionality is a subject of intensive research. Using solution NMR and the study of spin relaxation, we studied two Prx systems that belong to the D-Prx (type II) subfamily [2,4] : the yeast Prx Ahp1 and the *Populus trichocarpa* Prx that were shown to be regenerated *in vitro* by thioredoxin and/or glutaredoxin-glutathione. NMR provides evidences that the reduced proteins are specific noncovalent homodimers in solution. The dimer interface is roughly perpendicular to the plane of the central b-sheet and differs from the interface of A- and B-Prx dimers, where proteins associate in the plane parallel to the b-sheet. We will show that NMR evidences, not only that the proteins exist as homodimers, but also describes the specific types of possible oligomers and their potential interactions with other proteins or molecules.

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Suppression of homonuclear coupling evolution during pulse sequences

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Carr-Purcell-Meiboom-Gill (CPMG) sequences [1,2,3], routinely used for T_2 relaxation measurements, are nowadays applied in various multidimensional NMR experiments. Some applications have exploited the conventional CPMG function, mostly in the analysis of protein backbone dynamics from ^{15}N relaxation times [4,5,6,7]. CPMG has been used also in novel contexts. The implementation of CPMG on INEPT has demonstrated to improve the sensitivity in the studies of fast exchanging spin systems [8,9,10]. One particularly interesting application of CPMG-INEPT has been on the sequential assignment of RNA oligonucleotides, where cross peak phase distortions caused by J_{HH} evolution in ^{1}H-^{31}P polarization transfer steps have been effectively suppressed using CPMG-INEPT with fast 180° pulse rate [11].

The application of CPMG sequences as a general building block for polarization transfer offers intriguing possibilities to evade homonuclear coupling evolution contribution to the quality of spectra [12,13,14]. Some benefits of the approach in quantitative 2D NMR, long-range correlation spectroscopy and isotope editing are presented.

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^{13}C-detected experiments for NMR spectroscopy of nucleic acids

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The routine use of heteronuclear multidimensional NMR spectroscopy for the study of biomolecules was made possible by the introduction of indirect detection in the 1980s and 1990s. Recent technological advances, such as isotope labeling of the samples, commercial availability of magnets with the fields of up to 21.15 T and especially the introduction of cryogenic probe heads has opened a prospect for at least partial renaissance of ^{13}C-detected methods. While the cryoprobes do not change the relative sensitivity of different nuclei, they push the absolute sensitivity of carbon-detected experiments into a range that makes them attractive for biomolecular applications [1]. The lower sensitivity of the carbon detection is partially compensated for by avoiding the transfer of magnetization to a proton for detection, making thus the pulse sequences shorter and less prone to relaxation losses. Other advantages of the ^{13}C-detected experiments include high chemical shift dispersion in the carbon dimension, easy detection of non-protonated carbons, no need for solvent or buffer suppression and less sensitivity to salt concentration or to the presence of paramagnetic nuclei [2].

Specifically, nucleic acid bases contain a number of carbon atoms without directly attached protons. These include carbonyl groups in cytosine, thymine, uracil, and guanine, the C4 atom in cytosine, C5 in thymine, C4, C5, C6 in adenine, and C2, C4, and C5 in guanine. The direct detection of these carbon atoms offers an alternative method for obtaining their chemical shift values and/or data for investigation of the dynamics of nucleic acid bases. Furthermore, the chemical shifts of the amino nitrogen atoms whose protons are broadened beyond detection due to the exchange with water can be obtained using a ^{13}C-detected ^{13}C-^{15}N correlation experiment. In the sugar moiety, the carbon detected experiments are suitable for obtaining ^{1}H-^{13}C correlations in the cases where the proton signal is obscured by the large water peak.

The dense network of coupled carbon spins in ^{13}C-enriched compounds causes a splitting of the signals into multiplets in the directly detected dimension. We have applied the spin-state selection method [3] to remove the splitting when necessary.

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Improved Automated Structure Determination Using Wavelet De-noised NOESY Spectra

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A major time-consuming step of protein NMR structure determination is the generation of reliable NOESY cross peak lists which usually requires a significant amount of manual interaction. We have developed a new algorithm for automated peak picking which couples the identification of NOESY peaks to automated structure determination employing wavelet de-noised NOESY spectra. The core of this method is the generation of incremental peak lists by applying different wavelet de-noising procedures which yield peak lists of a different noise content. In combination with a new scheme for peak integration and additional filters which probe the consistency of the peak lists, good convergence of the NOESY based automated structure determination could be achieved. These algorithms were implemented in the context of the ARIA software for automated NOE assignment and structure determination and were validated for a polysulfide-sulfur transferase protein of known structure. The method should be commonly applicable for efficient protein NMR structure determination and automated NMR peak picking.

Detection of Weak Dipolar Couplings between two CF3-Groups in a Membrane-bound Peptide

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Dipolar couplings contain information about both internuclear distances as well as orientational constraints. To characterize the structure of the antimicrobial peptide gramicidin S and its interaction with lipid membranes, two CF_3-phenylglycine labels were incorporated in the cyclic peptide, providing a direct connection between the fluorine labels and the peptide backbone. By solid state ^{19}F-NMR we measured the couplings of these two trifluoromethyl-groups. Using the CPMG experiment in combination with aligned membrane samples, both the dipolar couplings within each CF_3 group as well as the weak coupling *between* the two CF_3-groups could be resolved. An intra-CF_3 group coupling constant of ~70 Hz and a weak inter-group coupling of ~15 Hz coupling constant were obtained. This approach thus provides the opportunity to explore the large distance range provided by fluorine labels, and obtain weak couplings even in the presence of a stronger intra-CF_3 couplings.

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Excitation Methods for Single-Scan 2D NMR and Applications

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Introduction

Recently techniques for fast acquisition of 2D NMR spectra in a single scan using gradient echoes have been introduced (1). Originally multiple frequency selective pulses were used. Later continuous excitation schemes involving frequency-modulated chirp pulses resulting in amplitude modulation in the indirect dimension through a storage step were proposed (2,3). Very recently schemes employing phase-modulation in the indirect dimension have also been described (4,5). Here we demonstrate two general phase-modulated schemes and outline applications for spectroscopy and spectroscopic imaging.

Methods

The goal of an excitation sequence for single-scan 2D NMR is to provide a range of evolution times (t_1) depending linearly on the position in the sample. A single chirp pulse applied in the presence of a magnetic field gradient fulfils this requirement but gives rise to a quadratic phase roll across the spectral window. This phase roll can be partly compensated by the addition of a chirp 180° pulse and an evolution delay (6). This scheme can be adapted for single-scan 2D NMR by swapping the order of the 180° pulse and the delay and adding a positive and a negative gradient pulse, both of duration τ (5):

chirp RF: $90^\circ_x(\tau)$ $-\frac{1}{2}\tau-180^\circ_y(\frac{1}{2}\tau)$
Gz: $+(\tau)$ $-(\tau)$

A more elaborate scheme using three chirp pulses compensates for remaining B_1-dependent phase deviations to a very high degree (7). In this case adaptation for single-scan 2D NMR can be done by addition of three gradient pulses of duration τ and with alternating positive and negative polarities:

chirp RF: $90^\circ_x(\tau)$ $180^\circ_y(\tau)$ $-\frac{1}{2}\tau-180^\circ_y(\frac{1}{2}\tau)$
Gz: $+(\tau)$ $-(\tau)$ $+(\tau)$

An additional advantage of this scheme is a larger spread of t_1 values resulting in a faster spatial encoding of the indirect detected spectroscopic dimension.

A single-scan COSY sequence can be constructed by adding a hard 90°_y mixing pulse surrounded by equal gradient pulses to facilitate coherence pathway selection and an oscillating gradient train for acquisition of both spectroscopic dimensions.

Further addition of orthogonal phase encoding gradient pulses immediately after mixing gives a fast version of the hybrid 2D spectral/2D spatial experiment previously described as Correlation Peak Imaging (CPI). This technique makes it possible to form metabolic maps from the spatial distribution of the cross peak intensity of individual chemical compounds. Alternatively 2D spectra can be obtained from the same data set for each individual sample voxel (8). In contrast to the conventional CPI technique, which suffers from long experimental duration, our new scheme shows a substantial improvement in the required experimental time.

We present a theoretical comparison of the two excitation strategies including computer simulations and experimental data.

Results

- Single-scan 2D NMR experiments without a mixing element resulting in spectra, where only the diagonal is populated, were simulated to analyze the line shapes. 64x64 spectra without a mixing element and with a mixing element in the form of a 90° pulse to form a COSY experiment were recorded to verify the computer simulations. The 3-pulse excitation scheme was found capable of producing spectra with narrower lines, especially at the base.

- A 4D 31x31x64x64 CPI data set was recorded on a phantom sample containing a 4mm tube with a glucose solution inside an 8mm tube with a glutamine solution in 1h20min with a recycle time of 3s and weighting of the k-space sampling with a 5-step Hanning window. Excellent separation of peaks in the spectral dimensions was achieved. Assuming a recycle delay of 2s, this enables the acquisition of 15x15x64x64 CPI experiments in 10-15min, depending on the k-space weighting scheme, providing sufficient temporal resolution for in-vivo studies.

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Symmetry-based Homonuclear Dipolar Recoupling Using Low RF Fields

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The rotor-synchronized symmetry-based CN pulse sequences for recoupling of ^{13}C-^{13}C and ^{15}N-^{15}N dipolar couplings under magic-angle spinning are generally accepted to be good candidates for accurate measurements of internuclear distances in biological solid-state samples since they posses the quality of gamma-encoding. However, it is also well-known that the applicability of these pulse schemes is hampered by a quite high demand for rf power, especially under high speed spinning conditions. This problem may be of purely technical origin, but also it is known that the combination of high power rf and a certain salt-content may heat up and destroy biological samples unless special coil designs are used.

Here, we present new CN-variants, enabling recoupling at moderate and high speed spinning, applying significantly lower rf fields than typically used. This is accomplished by fine tuning the resonance offset and the rf fields. The new technique has the additional benefit of being highly band-selective which may be useful in the study of uniformly ^{13}C labeled samples.

Different aspects of low-rf CN-recoupling are discussed, and demonstrations are provided by both nummerical simulations and experimental spectra.

Cross Effect Dynamic Nuclear Polarization Using Biradicals

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Dynamic Nuclear Polarization (DNP) is a process that transfers the greater spin polarization of electrons to nuclei and therefore enhances the sensitivity of NMR spectroscopy. With stable paramagnets in insulating solids, both theories and experiments indicated that the cross effect (CE) mechanism is more efficient for DNP at high magnetic fields. CE occurs with two dipolar coupled electron spins and is differentiated from the solid effect (SE) with one electron spin and from the thermal mixing (TM) with multiple electron spins. A theory based on quantum mechanics of spin interactions was developed to understand SE, CE and TM. Specifically, CE requires that the EPR frequency separation matches the nuclear Larmor frequency, i.e. $w_{e1} - w_{e2} \sim w_n$ and that the microwave frequency is close to one of the EPR frequencies, i.e. $w_M \sim w_{e1}$ or w_{e2}. Nonetheless, a proper $e^- - e^-$ dipolar interaction is indispensable in the CE mechanism, and its optimization can be accomplished by tuning the molecular tether between two TEMPO moieties in a *biradical* (BTnE). The BT2E, *bis*-TEMPO tethered by 2 polyethylene glycol chains, was found to yield the maximal proton DNP enhancement of 200 (at 90 K, 5 T), an improvement by a factor of about 4 compared to monomeric TEMPO. While the matched frequencies in the powder EPR lineshape of TEMPO is determined by a random distribution, it can be optimized by using two different radicals, which may be tethered to form a new *biradical* that is utilized to transfer more electron spin polarization to nuclei.Solid-State NMR Spectroscopy of Paramagnetic Transition-Metal Cyanides

Microscale Localized Spectroscopy by Mechanical Detection of NMR.

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The "Magnetic Resonance Force Microscope" (MRFM) combines (nuclear) magnetic resonance with atomic force microscopy (AFM) to perform magnetic resonance imaging on the micro- and nanoscale. A MRFM employs extremely force sensitive AFM cantilevers to measure the tiny attractive (or repulsive) ~aN to ~fN forces between the sample's nuclear magnetization and a small nearby ferromagnetic particle. This mechanical method of detection improves the poor sensitivity of inductively detected NMR - as used in any commercial spectrometer - and has lead to the observation of a single electron spin and to sub-surface nanometer scale imaging in three dimensions.

The combination of the MRFM with high-resolution magnetic resonance spectroscopy is, however, hindered by the large field gradient associated with the ferromagnet. Our strategy to circumvent this problem is to introduce suitable rf-irradiation schemes to study the local spin interactions selectively. This allows to obtain spatially resolved spectroscopic information and chemical contrast while benefiting from the exquisite sensitivity and imaging capacity of the MRFM. In this contribution first examples of such experiments as part of a systematic approach will be presented.

Solid-state NMR: A tool for probing the protein-mineral interface and other structures in bone

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Bone is a highly complex composite material consisting of a protein matrix (predominantly collagen) with calcium phosphate crystals deposited in it. The nature of the calcium phosphate phase in bone has been the subject of considerable discussion in the literature for many years. Of key importance is the structure of the surface of the crystals as it is the surface, which interacts with the surrounding matrix and binds the whole material together. As for any crystal, the surface is disordered and not easily amenable to study by conventional diffraction techniques. Solid-state NMR however is an ideal method for studying both this surface structure and the interface between the surface and protein matrix.

This work will describe the combination of ^{1}H-^{31}P 2D HETCOR, ^{1}H-^{31}P REDOR and other experiments, which have allowed us to characterize the surface of the mineral phase in bone in some detail. Subsequent ^{13}C-^{31}P REDOR experiments have both identified one of the protein groups responsible for binding to the crystal surfaces (a glutamate carboxylate group) and allowed determination of the distance of this group from the crystal surface. This is the first direct experimental evidence of such an interaction in bone and supports a long-standing hypothesis that glutamate coordination to calcium ions in the mineral phase is a primary mechanism for the mineral – protein binding.

The structure of the protein matrix in bone is very important to the material properties of bone. In particular, this matrix is thought to be responsible for dissipating stresses, which might otherwise damage the delicate mineral crystals. Accordingly, we have used solid-state NMR to assess the structure of collagen in bone. The primary method we have used here is measurement of ^{13}C chemical shift anisotropies/ asymmetries via a new method developed in Cambridge which allows the scaling of the effective sample spinning rate, and so measurement of chemical shift anisotropies via spinning sideband patterns whilst magic-angle spinning at high spinning rates. First principles electronic structure calculations enable us to calculate the expected chemical shift parameters for a variety of trial structures. Comparison with the experimental parameters then allows us to determine the range of structures, which can exist in bone collagen.

Other experiments which are being developed in to determine structural parameters for the collagen in bone which utilize ^{14}N (which occurs as every third nucleus in the protein backbone) will also be discussed.

Proton High-Resolution Solid-State NMR for Structure Determination in Powdered Solids

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Proton resolution is largely determined in rotating powders by the quality of homonuclear dipolar decoupling sequences. We show how significantly improved performance in decoupling is provided by application of the recently introduced direct spectral optimization procedure (dubbed eDUMBO). Using the resulting sequences, proton line-width less than 0.2 ppm (after correction for the decoupling scaling factor) can be obtained at 700 MHz for crystalline powdered solids, using standard equipment and moderate magic-angle spinning frequencies.

Efficient proton homonuclear decoupling is critical to the observation of $^{1}J_{CH}$ couplings, and for the efficiency of through-bond correlation experiments in solids. We demonstrate the feasibility of ^{1}H-^{13}C INEPT through-bond polarization transfer to rigid crystalline solids. The use of efficient proton homonuclear dipolar decoupling in combination with direct spectral optimization procedure provides minimization of transverse decoherence, and leads to efficient ^{1}H-^{13}C INEPT transfer for uniformly ^{13}C-labeled, as well as natural abundance, crystalline organic compounds. Application of this technique to 2D heteronuclear correlation spectroscopy provides, for a carbon-13 enriched sample of L-Isoleucine, an enhancement factor up to three in sensitivity in comparison to standard MAS-J-HMQC experiments.

Furthermore, the increased performance in proton homonuclear dipolar decoupling is essential for the acquisition of highly resolved proton spectra in the solid-state, and their applications. We present high resolution ^{1}H-^{1}H correlation experiment, such as high resolution 2D double–quantum single–quantum CRAMPS correlation experiments, and ^{1}H-^{1}H spin diffusion recorded with high resolution in both indirect (t_1) and direct (t_2) dimension of acquisition, using windowed-DUMBO-1 decoupling. We show how those experiments can provide extremely valuable structural information for small powdered compounds at natural abundance. Notably we have recorded a series of directly detected ^{1}H-^{1}H spin diffusion experiments that can be quantitatively exploited to measure the dipolar correlations between sites. The investigation of ^{1}H-^{1}H spin diffusion build-up curves using a rate matrix analysis approach shows that high resolution magic-angle spinning NMR of protons provides a method to probe crystalline arrangements. The comparison between experimental ^{1}H data and simulation is shown to depend strongly on the parameters of the crystal structure, for example on the unit cell parameters or the orientation of the molecule in the unit cell, and those parameters are experimentally determined for a model organic compound, the dipeptide β-L-Aspartyl-L-Alanine

Use of ^{2}H double-quantum MAS NMR for studying molecular motion in solids

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We describe the use of a two-dimensional ^{2}H (I = 1) double-quantum MAS NMR experiment.[1-3] The experiment presented here correlates single- and double-quantum coherences to provide information about molecular motion in solids. Spin I = 1 double-quantum coherences are less sensitive to motional broadening under MAS than single-quantum coherences in certain motional regimes, as they are not subject to quadrupolar broadening to a first-order approximation. Hence, by comparing single- and double-quantum linewidths it is possible to obtain information about motional rates and mechanisms. (This phenomenon is similar to the difference in linewidths recently observed for half-integer quadrupolar nuclei in multiple-quantum (MQ)MAS and satellite-transition (ST)MAS spectra when motion is present[4]).

We present results from a variety of deuterated solids, including oxalic acid dihydrate, $(CO_2D)_2.2D_2O$. In this latter system, the water molecules undergo rapid rotation about their DOD bisector and, as a result, the double-quantum MAS linewidth of this peak is significantly narrower than the single-quantum linewidth. In contrast, this effect is not observed for the OD deuterons.

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Application of Hyperbolic Secant Pulses to Enhance MAS and MQMAS NMR Spectra of Half-Integer Quadrupolar Nuclei in Solids

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More than 70% of the NMR-active nuclei are half-integer quadrupolar nuclei and many of these play an important role in the investigation of materials ranging from ceramics, glasses, catalysts, and superconductors to biological systems[1,2]. Investigating these important classes of materials by solid-state NMR spectroscopy has been hampered by two factors: first, these nuclei often give rise to relatively broad NMR spectra because of the orientation dependence of the nuclear quadrupolar coupling interaction and second, many of these isotopes have small magnetic moments and thus inherently low receptivities which lead to poor signal-to-noise ratios (S/N). Signal enhancement of the central NMR transition of quadrupolar nuclei with half-integer spin may be achieved by applying hyperbolic secant (HS) pulses to the satellite transitions. The HS pulses modify the equilibium Boltzmann populations and provides a signal enhancement of up to 2.7 for spin 3/2 nuclei and 4.1 for spin 5/2 nuclei in MAS powdered solids which is greater than previously reported methods such as rotor-assisted population transfer[3] (RAPT) and double frequency sweep[4] (DFS) pulse experiments. In addition, the use of HS pulses to enhance the intensity of the central transition in MQMAS experiments for spin 3/2 quadrupolar nuclei was investigated by examining ^{87}Rb NMR spectra of a powder sample of $RbNO_3$. Application of HS pulses prior to the triple-quantum (3Q) excitation provides sensitivity enhancements that are superior to those previously reported. For the conversion of 3Q to single-quantum (1Q) observables, the HS pulses have an efficiency similar to that reported for DFS[4] but greater than that of the fast amplitude modulation[6] (FAM) technique[7].

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Signal Enhancement by Repetitive Double-Frequency Sweeps in Solid-State NMR of Half-Integer Quadrupolar Nuclei

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Many pratical applications of solid-state NMR of half-integer quadrupolar nuclei such as ^{23}Na (I=3/2), ^{27}Al, ^{17}O (I=5/2), etc. rely on radio frequency techniques to improve the sensitivity of the studied nuclei. It has been shown that double-frequency sweeps (DFS) [1,2] are an efficient and reliable method to achieve this goal. In this contribution we explore the application of *repetitive* DFS, allowing us to fully deplete the satellite transitions [3], leading to further improvement in the signal enhancement. The method is based on repeating a pulse sequence block consisting of a DFS followed by a selective 90° pulse and signal detection several times prior to letting the spin-system relax to thermal equilibrium during the recovery time interval. Optimal enhancement is obtained by a weighted sum of the individual FIDs [4]. We obtained a maximum enhancement by a factor of 4 on a powder sample of L-tyrosine-[phenol-^{17}O]·HCl at an magic-angle spinning frequency of 25 kHz and an external field of 14.1 T. This is considerably more than reported before in powdered samples. In addition we present results on the same sample at 50 kHz MAS and external field of 18.8 T. We observe that the performance works equally with other nuclei such as ^{27}Al in minerals and ^{23}Na in model compounds. Furthermore the method is compatible with other mechanical averaging schemes such as DOR.

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^{23}Na MQMAS at 21.9T (^{1}H 930MHz)

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We measured MQMAS spectra at world-record magnetic field of 21.9T (1H frequency of 930MHz). In comparison with the 14.1T cases, sensitivity is overwhelming; in the case of 14.1T some of the resonance lines are hidden by noize. 21.9T magnet may afford the opportunity of measurements of large quadrupolar coupling constants.

Developments in Solid-State NMR micro-Spectroscopy

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NMR has developed into one of the most powerful analysis techniques with widespread applications. Sensitivity is still a limiting factor in the applicability of NMR, however. Therefore we witness a renaissance of technique development in magnetic resonance striving to improve its receptiveness. One of the approaches pursued in our laboratory is to investigate to what extent regular inductive methods can be miniaturised in order to study mass-limited samples.

We have built solenoid-based microprobes with coil diameters in the hundred μm regime. Besides lowering the necessary sample volume these allow the generation of rf-fields in the MHz regime. This proves to be extremely useful to efficiently excite spectra with a bandwidth of up to 5 MHz, as will be demonstrated for a number of quadrupolar nuclei. A possible field of application is the study of III-V type semi-conductors. It will be shown that it is possible to study atomic ordering in AlxGa1-xAs, which is important because of its influence on the material's electronic structure. The characterization of order in these compounds is crucial in view of a controlled growth of designed semiconductor structures.

Double tuning is also feasible allowing CP to be performed at powers ~1 Watt. Magic Angle Spinning is needed for high-resolution studies of mass-limited (biological) samples. A CP-MAS micro design will be presented using a 200 μm rotor. This “piggy back” design allows spinning speeds ~ 10 kHz. This opens the way to study orientational order in fibrous biological and biomimicking materials as is demonstrated for a single glycine 13C-enriched spider silk fiber. Here the strong rf-fields can be used for extremely efficient decoupling as is shown for a analinetripeptide.

Surface microcoils are interesting structures for thin film analyses. The concepts for the design of optimal coil geometries, in view of different applications, will be discussed and demonstrated with experimental results.

New very sensitive high-resolution NMR method for quadrupolar nuclei based on the SPAM concept

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Gan and Kwak recently introduced two new ideas in the field of 2D high-resolution NMR methods as applied to quadrupolar nuclei: the double-quantum filtering in STMAS [1], and the Soft Pulse Added Mixing (SPAM) idea [2]. The double-quantum filtering principle allows suppressing all undesired signals in the STMAS method without decreasing its sensitivity. In the SPAM concept, all pathways are added constructively after the second hard-pulse instead of using a single one as previously. We have recently shown that the SPAM idea applied to 2D MQMAS spectra recorded with echo/anti-echo method and data treatment results in a S/N gain of 3 over the classical z-filter MQMAS method [3]. We, here first compare the sensitivity, advantages and drawbacks that can be obtained in DQF-STMAS with respect to 3QMAS. We then show that the SPAM concept can be utilized with the DQF-STMAS method leading to a very large sensitivity gain (10-15) with respect to 3QMAS.

In a second step, we also apply the SPAM concept to HETCOR type of experiments based on dipolar or scalar couplings. This leads to very robust SPAM-HETCOR experiments which allow gaining a factor 3 for the experimental time with respect to previous experiments.

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Quantum information processing via NMR of quadrupolar nuclei: logical gates, relaxation, and quantum state tomography

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Nuclear Magnetic Resonance (NMR) has appeared as a powerful tool for realization of quantum computing, with the successful implementation of several logical gates, full quantum algorithms, quantum simulations, etc. The use of NMR spectroscopy involving quadrupolar nuclei for quantum information processing has recently been investigated as an interesting way of providing a higher number of qubits per nucleus. In this work we discuss the applications of NMR to quantum information processing, focusing on the use of quadrupole nuclei for quantum computing. Various examples of experimental implementation of logical gates are given and compared to calculated NMR spectra and their respective density matrices. The technique of Quantum State Tomography for quadrupole nuclei is detailed, and examples of measured density matrices in a two-qubit $I = 3/2$ spin system are shown. Experimental results obtained from ^{23}Na nuclei in a lyotropic liquid crystal at room temperature are given. The collection of results achieved with this system include: attainment of pseudo-pure as well as pseudo-entangled states, study of relaxation process, implementation of logical gates, quantum state tomography of the density matrices at several steps of simple gates, and finally an analysis of the mutual information changes of theses states. The perspectives for future research on quantum information processing via NMR of quadrupolar nuclei and some insights on future experiments are also discussed.

Some aspects of detection of specific substances by nuclear quadrupole resonance

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Pure nuclear quadrupole resonance (NQR) is a very promising method for the detection and identification of specific substances such as explosives and narcotics. The NQR frequencies depend on the molecular structure of the substances and can be used like “fingerprints” for their identification. The NQR detection technique is very similar to that commonly used in nuclear magnetic resonance (NMR). However unlike the NMR, the direct NQR method normally operates without a strong external magnetic field. Therefore the NQR apparatus is relatively inexpensive, more compact and does not suffer the safety concerns that strong magnetic fields create.

Most of such specific substances as explosives and narcotics contain quadrupolar nuclei, for example the nitrogen-14, with spectral lines usually located in the low frequency range. The low energy difference between excitation levels is a problem because it leads to a relatively poor signal-to-noise ratio (SNR). This problem is common for low frequency NQR spectroscopy. A more specific issue for NQR luggage screening is the remote detection of relatively small amounts of explosives in large volumes which often exceeding 100 litres. This also makes it necessary to detect weak NQR signals. The sensitivity problem is normally solved by using multi-pulse NQR techniques, special equipment and signal processing.

In this contribution we have analysed and discussed specific issues for NQR detection of illicit materials. The NQR detection technique for the different substances have been developed and discussed. We also present some of interesting NQR parameters for number of illicit substances obtained by our research group. In this report the operational features of the NQR scanner are presented. This report also describes the continuing efforts to improve the function of the device through a more sensitive detection, and methods of reducing false alarm events generated from the RF excitation and interference. The basic design, performance and some characteristics of the advanced NQR machines for luggage screening are reported. These machines operate effectively in real world conditions. This was verified by a number of independent trials over a range of illicit materials.

Correlating fast and slow chemical shift anisotropy spinning sideband patterns in MAS NMR

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A pulse sequence is presented that correlates the MAS spinning sidebands of the directly observed dimension with the chemical shift anisotropy (CSA) spinning sideband intensities that would be obtained at an effective spinning frequency ω_r/N in the indirect dimension, where N is a scaling factor that may be varied by changing the number and timing of the π-pulses in the experiment.[1] In essence, the experiment is a combination of the CSA-Amplification experiment[2,3] and the 2D-PASS experiment[4]: the resulting spectrum has the same form as in the CSA-Amplification experiment, but the pulse sequence has the same structure as the 2D-PASS method. Desirable features of the experiment include a fixed-duration evolution period, no loss of signal due to storage pulses, cogwheel phase cycling to select a unique coherence pathway, and the ability to choose a continuous range of scaling factors. The pulse sequence is demonstrated through both simulation and experiment.

Spectra obtained by this experiment may be used to determine CSA tensor parameters since, when the sideband intensities of the direct dimension are summed with the isotropic peak, the lineshape in the indirectly observed dimension corresponds to the CSA sideband intensities at the effective spinning fequency ω_r/N, allowing use of standard methods of analysis. A useful application of the experiment is in the measurement of small CSAs, for which 2D-PASS would require slow spinning frequencies that are prone to instabilities. In addition, a second application is in measuring the CSA of sites in uniformly isotopically labelled samples, this is investigated by numerical simulations and experiments. This may be useful because the ability to scale the CSA in the indirect dimension allows rapid sample spinning to be used to reduce the perturbing effect of homonuclear dipolar coupling, whilst using a sufficiently large scaling factor still allows several CSA spinning sidebands to be obtained in the evolution dimension.

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^{2}H, ^{14}N, ^{31}P Solid-State NMR and Calorimetric Studies of Changes in Lipid Membrane Properties by Branched-Chain Fatty Acids

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Branched-chain fatty acids (BCFAs) are occurring as components in a wide range of food products. BCFAs are found at low concentrations in eukaryotes but predominate in prokaryotes and hence the presence of branched fatty acids in food samples is correlated to the existence of bacteria E. g. Listeria monocytogenes, causing the food-borne often lethal disease listeriosis, can maintain its membrane fluidity at fridge temperatures by increasing its content of BCFAs. In addition, branched-chain fatty acids as part of food intake seem to play a major part in the origin of deadly cardiac irregularities. However, the effect of these BCFAs – as potential diffusion barrier compounds – on the functional and structural organization of cell membranes is unknown. Therefore, a biophysical approach centered around biological solid-state NMR was used to provide the necessary information about the impact of BCFAs on the physiological functioning of model lipid membranes. By utilizing the naturally occurring spin reporters nitrogen-14 and phosphorous-31 present in dimyristoylphosphatidylcholine (DMPC) information about induced changes at the membrane interface corresponding to changes in the lipid headgroup orientation could be extracted using a dynamic NMR model based on the 14N quadrupolar splittings and 31P chemical shift anisotropy obtained for DMPC bilayers containing BCFAs [1,2]. The NMR spectra of DMPC lipid membranes together with either of two different BCFAs, 12-methyltetradecanoic acid (a15:0) and 13-methyltetradecanoic acid (i15:0), revealed differences in their impact on the lipid bilayer. Chain order profiles obtained by 2H NMR using fully deuterated DMPC (DMPC-d67) displays variations in response at different carbon positions along the lipid in the presence of a BCFA but also differences in the lipid matrix when comparing the two BCFAs. The combined differential scanning calorimetry (DSC) study together with the obtained solid-state NMR results indicates an insertion of both BCFAs into the membrane as a kind of spacers between the lipids molecules. A variation in motional restriction is then introduced into the membrane core which affects the fluidity of the lipid bilayer and hence the function of the cell, a fact supporting their hypothetic role as a diffusion barrier. The observed effect is more pronounced if i15:0 is inserted compared to the corresponding a15:0 compound. This deviation between the two BCFAs may origin from a difference in their chain structure where i15:0 has a more localized hydrophobic tail which gives it a stronger interaction with the inner parts of the membrane.

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Comparison of FAM and SPAM pulses and their utility in MQMAS and STMAS NMR spectroscopy of quadrupolar nuclei

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The MQMAS and STMAS techniques have with their invention sought to overcome the difficulties associated with obtaining high-resolution solid-state NMR spectra of half-integer quadrupolar nuclei such as ^{17}O, ^{23}Na and ^{27}Al. However, both techniques suffer from poor efficiency in the final coherence transfer step, that of conversion of triple-quantum coherence into observable single-quantum coherence in MQMAS and of conversion of satellite-transition coherence into central-transition coherence in STMAS. Two techniques that have sought to alleviate this problem are the so-called FAM and SPAM pulses. FAM (fast amplitude-modulated) pulses are inserted into MQMAS pulse sequences as direct replacements for the existing conversion pulses and exist in two forms. FAM-I involves applying a sequence of two pulses of opposite phase, interleaved by free-precession intervals of equal duration to the pulses themselves and repeated an optimum number of times. FAM-II consists of two pulses of opposite phase and non-equal durations, applied just once. SPAM (soft-pulse added mixing) pulses are similarly used to replace existing conversion pulses and involve the addition of a soft 90° pulse to a hard pulse, with the relative phase dependent on whether the conversion effects a change in the sign of the coherence. In this work, both FAM and SPAM techniques are optimised in ^{87}Rb NMR of $RbNO_3$ and applied to the MQMAS and STMAS techniques, with a comparison then made between the signal enhancements they offer. Computational simulations of the performance of FAM and SPAM will also be shown and compared with the experimental results. Finally, the best means of using FAM and SPAM pulses to obtain both signal enhancement and pure-phase two-dimensional absorption lineshapes will be discussed.

Beating Reciprocity S/N Expectations in Triple-resonance MAS by Cryogenic Cooling of Critical Circuit Components in the OptiMAS Probe

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RF circuit efficiencies in 3 to 5 mm triple-resonance "single-coil" MAS probes at very high fields are typically in the range of 25-35% at the low-frequency (LF) and 15-40% at the mid-frequency (MF), which suggests there is considerable opportunity for noise reduction. While higher efficiencies on all channels have been achieved using a cross-coil for ^{1}H and a solenoid for the MF and LF, they are still generally in the range of 30-50% for both the LF and the MF. Although cryogenic cooling of the sample coil in a VT MAS probe appears impractical in a narrow-bore (NB) magnet, cryogenic cooling of the critical LF-MF circuit components other than the sample coil appears practical, and this promises potential noise power reductions exceeding a factor of two, corresponding to a 40% increase in S/N.

We report here progress in the development of an NB probe design for H/X/Y/lock MAS with Magic Angle Gradients (MAG) and automatic sample change in which all the critical LF and MF components (coils and capacitors which have a significant effect on efficiency) other than the sample coil are located in a region that can be readily cooled to ~100 K for reduced thermal noise. The cold zone is immediately below the spinner region, and the circuit design is such that both rf power handling and efficiency are increased compared to most prior circuits, even when the "cold zone" is at room temperature.

The sample coil design uses an improved derivative of the Alderman-Grant saddle coil for ^{1}H with an additional solenoid over it for the two lower-frequency channels.

Novel plug-in connectors allow the five high-power (11 mm x 15 mm x 3.3 mm) ceramic capacitors in the cold zone to be changed as needed to achieve the desired tuning and impedance transformations such that the currents, voltages, and SWR in the transmission line (TRL2) to the variables are relatively low, so rf losses in TRL2 and the tuning variables are only a few percent.

A novel 4 mm sample spinner with drop-in rotors is used for compatibility with automatic sample change via spinner reorientation, which is accommodated by the flexible transmission line (TRL1) above the cold zone. The MAG coil is mounted on the spinner assembly, and a third coil is added between the XC and the solenoid if desired for ^{2}H lock. The design has been dubbed "**OptiMAS**^{TM}".

S/N is no longer governed by the Principle of Reciprocity *in its popular usage*, as not all resistances are created equal – some are noisier than others. Dropping the temperature of the capacitors by a factor of three reduces their noise power by the same factor, even if their Qs (and hence the transmitter power for a given pw90) are unchanged. We will present a numerical method for evaluating S/N in complex circuits containing losses at various temperatures. We hope to report NMR test data from a preliminary version (less sample re-orientation and MAG coil) at 930 MHz, as well as test data at a lower field from the second-generation OptiMAS^{TM} probe.

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Measuring NMR bond distances in single crystals using OSCULANT

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We have derived analytical expressions for determining the orientation of the crystallographic axis system of high-symmetry single crystals, in the goniometer frame, from line-crossings in a single rotation plot. Our new method, which we call OSCULANT (Orientation of Single Crystals Using Linear Approximations to NMR Transits) works for any NMR Hamiltonian of even rank, and is therefore independent of the nature of the interaction or the crystal (so long as it contains the requisite number of symmetry operations). The method eliminates the need to orient to crystals by some other method (e.g. optical goniometry or X-ray), and it eliminates systematic errors due to inaccurate mounting of the crystal. It gives excellent crystal orientations from little more than visual inspection of the rotation plot, and presents a compelling argument for using crystals of high symmetry to determine NMR benchmark information.

We have used our new method, in combination with second-order perturbation analysis of the quadrupolar-perturbed ^{17}O-^{1}H dipolar interactions in single crystals of potassium hydrogen maleate (KHM), to obtain highly accurate measurements of the inverse-cube-averaged internuclear distances ('NMR bond distances') in KHM, which we compare to neutron measurements of the same material. We analyze these distances in terms of the potential surface for this strong hydrogen bond.

Low-Power Homonuclear Dipolar Recoupling in Solid-State NMR Designed by Optimal Control

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During the past decade a lot of effort has been made to improve homonuclear dipolar recoupling sequences for biological solid-state NMR. Dipolar recoupling is typically conducted by using pulse sequences like, e.g., HORROR and C7. These sequences are g-encoded and reach a transfer efficiency of 73 % under ideal conditions. The HORROR pulse sequence is a low-power sequence, but suffers from high dependence on isotropic and anisotropic shielding, and it is sensitive to rf inhomogeneity. The more compensated C7 sequence, and numerous variants of this experiment, solve some of these problems, however, at the expense of dramatically increasing demands on the radio-frequency (rf) power and significant longer transfer times. The high rf power requirements often pose impossible demands to the solid-state NMR probes, in particular if efficient decoupling of the dipolar interactions from the protons is required under high-speed spinning conditions. Furthermore, strong rf may heat the sample and in the worst case destroy it. To alleviate some of these problems, we here introduce novel low-power pulse sequences for homonuclear dipolar recoupling for biological solid state NMR. The sequences are developed numerically using the SIMPSON simulation program in combination with optimal control theory procedures. The sequences are optimized under typical experimental conditions including the request of low power and compensation with respect to rf inhomogenity. The new optimal control dipolar recoupling pulse sequences are demonstrated numerically and experimentally by double-quantum filtered (2QF) MAS experiments.

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