THE ON-LINE EXPERIENCE | 7- 8 DECEMBER 2020

EUROMAR

ORGANIZED BY:

AMPERE

EUROMAR

geRMN

CICbioGUNE

INDEX

INTRODUCTORY REMARKS

Dear Colleagues,

It is my pleasure to welcome you to this short online-EUROMAR meeting, organized by Óscar Millet. Of course, I was looking very much forward to meeting you in person in Bilbao at the planned EUROMAR 2020 meeting – first scheduled at the beginning of July and then at this week of December. Unfortunately, this was not possible due to the high number of Corona cases worldwide. What a pity for all of us, but especially for Óscar, after all the preparations, planning and selection of plenary and invited speakers!

Now, as we are experiencing a long time without any in person meetings, we all realize how important these personal meetings are. Especially for young PhD students or Post-doctoral researchers, this lack of opportunities to present their data and to communicate with senior scientists is discouraging. I am very grateful that Óscar proposed to organize this two-day-online EUROMAR meeting to promote especially work of such young scientists. In addition, we will have lectures from the recipients of the Richard Ernst Prize, the Varian Young Investigator Award, the AMPERE Prize and the Raymond Andrew Prize, as it is tradition at our EUROMAR conferences. I hope very much that you will enjoy this short online meeting – despite the fact that we cannot explore the city of Bilbao and its superb cuisine this time in the evening! I hope there will be another chance sometime in the future!

I wish all of you save and peaceful holidays and am looking very much forward to seeing many of you again next year - hopefully in person!

Best regards,

Thomas Prisner

(Chair of the Board of Trustees of EUROMAR)

Dear Colleagues,

It has been a great pleasure for me to organize an on-line version of the EUROMAR conference. It was a strange conference for a strange year. First of all, I wanted to acknowledge the difficult times we are all undergoing. Most of us are acquainted with people who have suffered, even died from this devastating disease. The implications of the pandemic have shacked the very fabric of our society, including our capacity to casually meet at a conference. Yet, live goes on and we have demonstrated, once more, our ability to endure and bypass adversity. Having an on-line version of EUROMAR aimed to honor this idea as well.

The on-line conference had two main goals; to give a proper scenario for the well-deserved awards and for young scientists as well, in the format of abstract submission and flash presentations. Consistently, the on-line conference becomes completed with the circulation of this booklet, that will give the received abstracts access to the community. I must admit that this conference was an experiment and the reception by the community has been exceptional, for which I would like to personally thank you all. Finally, I wanted to extend my gratitude to the people who actively and efficiently worked to make this conference possible: Sara Gómez, Ganeko Bernardo and, most specially, Beatriz González.

Looking forward to meeting you all in the future (preferably in the real space),

Oscar Millet

SCIENTIFIC COMMITTEE

Inés Garcia Rubio

ICMA, Zaragoza, Spain

Ana M. Gil

University of Aveiro, Aveiro, Portugal

Jesús Jiménez-Barbero

CIC bioGUNE, Derio, Spain

Arno Kentgens

Radboud University, Nijmegen, Netherlands

Antoine Loquet

IECB, Bordeaux, France

Oscar Millet, Chair

CIC bioGUNE, Derio, Spain

Miquel Pons

Universitat de Barcelona, Barcelona, Spain

Thomas Prisner

University of Frankfurt, Frankfurt, Germany

Christina Redfield

University of Oxford, Oxford, UK

Cristina M Thiele

Technische Universität Darmstadt, Darmstadt, Germany

Thomas Vosegaard

Aarhus University, Aarhus, Denmark

Andrew G. Webb

Leiden University, Leiden, Netherlands

AWARDEES

CLARE P. GREY – Richard Ernst Award 2020

She currently is full Professor at the Department of Chemistry from Cambridge University. She has published near 400 papers and she has received many awards including AMPERE award (2010), the Laukien award (2013) and the Vaughn Lecture award (2008). Prof. Grey is a Fellow of the Royal Society. Her research interests include the synthesis, characterization and electrochemical testing of lithium ion batteries electrode materials; the development of new in situ NMR methods for probing lithium-ion battery and supercapacitor function; the application of novel local probes of structure (NMR, pair distribution function analysis) to monitor structural changes in electrode materials during and following cycling; the oxygen-ion and proton conductivity in membranes for solid oxide fuel cells; the structures of solid-water interfaces and the catalysis and sorption.

THOMAS THEIS – AMPERE Prize 2020

He currently is assistant Professor at the Department of Chemistry of the North Carolina State University. Dr. Theis has published close to 50 papers and has received several awards, including the Magnetic Resonance in Chemistry Young Investigator award. His research interests include the development of methodology in hyperpolarization chemistry using para-hydrogen to enhance NMR and MRI signals by up to 6 orders of magnitude and the exploration of new applications such as portable NMR and cost- efficient molecular imaging.

CHRISTIAN BENGS – Raymond Andrew Prize 2020

He did his Ph. D. Thesis in the group of Prof. Malcolm Levitt at the University of Southampton. He has published 14 papers and his research interest include the development of new experimental techniques in NMR spectroscopy and its application to systems of interest in biology and materials science. Specifically, Christian Bengs has focused in understanding the underlying theory associated to the so-called long-lived singlet order and the understanding of the NMR lineshape of fullerenes, among other topics.

PAUL SCHANDA – Varian Young Investigator Award 2020

As of 2021, he will become Full Professor at the Institute of Science and Technology in Austria. He has published 70 papers and he is recipient of an ERC grant among other distinctions and merits. His research interests are the study of dynamic ensembles of protein structures linked to biological function by using multiple biophysical techniques (i. e. ssNMR, lsNMR and cryo-EM) and biochemistry to uncover the link between functional mechanisms of biomolecules and the underlying structural dynamics.

KATHRIN AEBISCHER (JMR award)

Kathrin Aebischer started as a PhD student at the Laboratory of Physical Chemistry at ETH Zürich in December 2020. She obtained both her BSc and MSc in Chemistry at ETH Zürich, examining the effects of radio-frequency field inhomogeneity on MAS solid-state NMR experiments in her master's thesis. Her doctoral research in the group of Prof. Matthias Ernst will focus on methods development in solid-state NMR.

ARNAU BERTÁN (EPR Society award)

Arnau obtained BSc degrees in Physics and Chemistry (2017) from the Autonomous University of Barcelona, Spain, with a final year research project on superparamagnetic composite nanomaterials conducted at the Institute of Materials Science of Barcelona (ICMAB-CSIC). He then moved to London to complete an MSc in Advanced Materials Science and Engineering at Imperial College (2018), with a Master's thesis on nanostructured photoanodes for solar water splitting. After that he joined the Department of Chemistry and Lincoln College at the University of Oxford to start his doctoral studies (DPhil) in Inorganic Chemistry, under the supervision of Dr. Alice Bowen and Prof. Christiane Timmel. His work focuses on the development of new Light-Induced Pulsed Electron Spin Resonance Dipolar Spectroscopy techniques for biological applications.

YURY KUTIN (EPR Society award)

Yury Kutin obtained both Bachelor´s degree and Master´s degree in Physics at the Departament of Physics in Kazan State University. After the Erasmus Mundus PhD Scholarship at Institut für Angewandte Physik (Germany) in 2011, he began his Doctoral thesis at the Institute of Physics in Kazan federal University working on the identification and characterization of impurity centers in ZnO based on high-frequency EPR/ENDOR spectroscopy. Then he works as a postdoctoral researcher at Max-Planck-Institut für Chemische Energiekonversion under the supervision of Dr. W. Lubitz and Dr. A. Schnegg. Now he is in the Physikalische Chemie at the Technische Unviersität Dortmund with the Prof. M. Kasanmascheff working as a postdoctoral researcher in diferent fields as the DEER/RIDME study of dimerization pathways of Cu-labeled DNA G-quadruplexes using an integrates AWG, or the determination of midpoint potencials in 2Fe-2S ferredoxins via EPR-monitored redox tritations.

LAURIANE LECOQ (JMR award)

Lauriane Lecoq began her NMR career during her Ph.D. at the Institute of Structural Biology in Grenoble (France) under the supervision of Jean-Pierre Simorre, working on structural and dynamical characterization of proteins involved in antibiotic resistance using solution state

NMR. After her Ph.D. obtained in 2012, Lauriane continued working in protein solution NMR at the University of Montreal (Canada) as a post-doc. In 2015, she began working in solid state NMR in the group of Anja Böckmann at the MMSB, University of Lyon (France), where she is now working as a CNRS permanent researcher. Her work focuses on viral assemblies, including proteins from the hepatitis B and D, dengue and SARS-CoV-2 viruses.

MARÍA PÍA LENZA (JMR award)

M. Pia Lenza has got her Bachelor degree in Biotechnology and Master degree in Medical Biotechnology at University of Naples "Federico II". After, she attended the residency program in pathology and clinical biochemistry at University of Naples Federico II- Faculty of Medicine. During this period, in 2017 she moved to Bilbao, to do an ERASMUS+ traineeship at CICbioGUNE. In July 2018, she started the PhD in CICbioGUNE in the Chemical Glycobiology Laboratory under the supervision of Jesús Jímenez-Barbero and June Ereño Orbea. Here she is studying the glycan recognition using NMR spectroscopy.

JULIEN MANON (JMR award)

Manon JULIEN is a PhD student in the group of Dr. François-Xavier Theillet and Dr. Sophie Sinn-Justin, in Gif-sur-Yvette, France, since 2017. She studied biology at the ENS Paris-Saclay (France) during which she had the opportunity to spend 10 months of internship in 2016 in the group of Pr. Marc Baldus (Utrecht, Netherlands). During this internship, she started to study membrane systems using ssRMN (Pinto et al., 2019 J Struct Biology). Back in France, she started her thesis thanks to a French ministerial and ARC Foundation fundings. During her thesis, she was interested in the use of liquid NMR to study phosphorylations of disordered regions of proteins. These regions are highly enriched in phosphorylation sites that are important for the regulation of eukaryotic cell signaling and involved in many diseases. With the example of phosphorylation of the BRCA2 protein (frequently mutated in hereditary breast cancers) by the Plk1 kinase, she has published two protocols describing the methodology for monitoring phosphorylation of disordered regions adapted to a wide range of conditions (Julien et al., 2020 Biomol NMR Assign; Alik et al., 2020 Angew Chem Int Ed Engl). Finally, she used this strategy to understand the biological role of BRCA2 protein phosphorylations. Recently, she identified that BRCA2 T207 phosphorylation served as a priming event for the Plk1 kinase that binds to this phosphorylation site to phosphorylate BRCA2 protein more efficiently. Furthermore, in collaboration with Dr. Aura Carreira's group, the group identified that this BRCA2/Plk1 interaction was important to build a quaternary complex important during mitosis (Ehlen et al. 2020 Nat Commun).

ANDREAS MEYER (EPR Society award)

Andreas Meyer studied Chemistry at the University of Bonn (Germany). In 2010 he began his PhD Thesis at the Institute for Physical Chemistry titled "EPR-Based distance measurements on metal nitroxide model complexes as well as EPR Spectroscopic characterization os $[Si_2]$-radicals and titanocene complexes". In 2017, he obtained a year postdoctoral researcher

position in the same department and then he joined the Max-Planck-Institute for Biophysical Chemistry in Göttingen, in which is currently working. His latest publications are focused on the "Measurement of angstrom to nanometer distances with 19F nuclear spins by EPR/ENDOR spectroscopy" and "C-C Cross Coupling reactions of Trityl Radicals: Spin Density Delocalization, Exchange Coupling and a Spin Label".

ON-LINE PROGRAM

December 7

Common session (Room 1)

12:00 GMT (13:00 CET/7:00 EST/20:00 CST) Introduction.*Thomas Prisner, Bernhard Bluemich* and *Oscar Millet*.

12:05 GMT Richard Ernst Prize Lecture Introduction. *Lucia Banci*.

12:10 GMT Richard Ernst Prize Lecture by Clare Grey (Cambridge University).

Prize release information: https://www.bruker.com/news/professor-clare-p-grey-from-the-university-of-cambridge-is-awarded-the-prestigious-richard-e-ernst-prize-in-magnetic-resonance.html

Parallel sessions

Room 1: Biomolecular NMR 1. *Miquel Pons* and *Jesús Jiménez-Barbero*

12:50 GMT (13:50 CET/7:50 EST/20:50 CST) - 13:55 GMT (14:55 CET/8:55 EST/21:55 CST)

12:50 GMT Rebecca B. Berlow, The Scripps Research Institute.

The role of backbone dynamics in modulating competition between disordered ligands.

12:55 GMT Lauriane Lecoq, University of Lyon.

Solution and solid-state NMR to study viral assemblies in Hepatitis B and dengue viruses.

13:00 GMT Joachim Maier, Max Planck Institute - Göttingen.

Dissecting artificially reconstituted immune pre-signaling clusters to study biocondensation.

13:05 GMT Borja Mateos, CIC bioGUNE.

The initial protein oligomeric state dictates the effect of small cosolutes on the oligomerization reaction.

13:10 GMT Beat Vögeli, University of Colorado.

Reducing the Measurement Time of Exact NOEs by Non-Uniform Sampling.

13:15 GMT Tatjana Schamber, Goethe University.

3_SL2 SARS-CoV-2 RNA secondary structure determination by NMR spectroscopy.

13:20 GMT Tiago Gomes, IRB - Barcelona.

Into the structure of human full-length smad proteins.

13:25 -13:55 GMT Q & A for the session.

Room 2: Hyperpolarization. *Arno Kentgens* and *Thomas Prisner*

12:50 GMT (13:50 CET/7:50 EST/20:50 CST) - 13:55 GMT (14:55 CET/8:55 EST/21:55 CST)

12:50 GMT Behdad Aghelnejad, École Normal Supérieure - Paris.

Progress and future plans concerning bullet-DNP.

12:55 GMT James Eills, Johannes Gutenberg University.

Metabolic NMR without the magnet.

13:00 GMT Michael A. Hope, École Polytechnique Fédérale de Lausanne.

A magic angle spinning activated ^{17}O DNP raser.

13:05 GMT Oleg G. Salnikov, Boreskov Institute of Catalysis.

Hyperpolarization of 15N nuclei in nimorazole using signal amplification by reversible exchange at microtesla magnetic fields.

13:10 GMT Saket Patel, Technical University of Denmark.

UV-irradiated 2-keto-(1-^{13}C)isocaproic acid for high performance ^{13}C hyperpolarized MR.

13:15 GMT Kong Ooi Tan, Massachusetts Institute of Technology.

Shedding light on nuclei near the spin diffusion barrier via electron decoupling.

13:20 GMT Théo El Daraï, University of Lyon.

Increasing hyperpolarization lifetime in arbitrary frozen solutions with hyperpolarizing polymers (HYPOP).

13:25 -13:55 GMT Q & A for the session.

Common session (Room 1)

14:00 GMT (15:00 CET/9:00 EST/22:00 CST) Raymond Andrew Prize Introduction. *Anja Böckmann*.

14:05 GMT Raymond Andrew Prize Lecture by Christian Bengs (University of Southampton)

Parallel sessions

Room 1: Solution State Methodology. *Miquel Pons* and *Oscar Millet*

14:35 GMT (15:35 CET/9:35 EST/22:35 CST) - 15:15 GMT (16:15 CET/10:15 EST/23:15 CST)

14:35 GMT Mohamed Sabba, University of Southampton.

Generalized transformation of magnetisation into ultra-longlived eigenorder.

14:40 GMT Jia-Liang Chen, Nankai University.

Dynamic exchange of metal chelating moiety: a key factor in determining the rigidity of protein-tag conjugate in paramagnetic NMR.

14:45 GMT Krzysztof Kazimierczuk, University of Warsaw.

Temperature and concentration as extra dimensions in multidimensional NMR.

14:50 GMT Yamanappa Hunashal, Università di Udine.

Mapping slow and intermediate exchange from structural interconversion or intermolecular interaction in protein NMR spectra.

14:55 -15:15 GMT Q & A for the session.

Room 2: EPR/ESR & F-NMR. *Inés García* and *Thomas Prisner*

14:35 GMT (15:35 CET/9:35 EST/22:35 CST) - 15:15 GMT (16:15 CET/10:15 EST/23:15 CST)

14:35 GMT Yuri Kutin, TU Dortmund University.

Duplex-bridged unimolecular DNA G-quadruplexes: an EPR investigation.

14:40 GMT Shannon Helsper, Florida State University.

Differential hemispherical assessment using multinuclear MR reveals effects of stem cell variance in stroke treatment.

14:45 GMT Andreas Meyer, Max Planck Institute - Göttingen.

High Field ^{19}F-ENDOR for Distance Measurements in the Angstrom to Nanometer Regime in Structural Biology.

14:50 GMT Arnau Bertran, University of Oxford.

Light-induced triplet-triplet electron resonance spectroscopy.

14:55 -15:15 GMT Q & A for the session.

December 8

Common session (Room 1)

12:00 GMT (13:00 CET/7:00 EST/20:00 CST) Introduction. *Beat Meier*.

12:05 GMT AMPERE Prize Lecture by Thomas Theis (North Carolina State University).

Parallel sessions

Room 1: Small Mol. / Metabolomics / Computation. *Christina Thiele* and *Andrew Webb*

12:50 GMT (13:50 CET/7:50 EST/20:50 CST) - 13:55 GMT (14:55 CET/8:55 EST/21:55 CST)

12:50 GMT Juan Carlos Fuentes-Monteverde, Max Planck Institute - Göttingen.

Relative configuration of micrograms of natural compounds using proton residual chemical shift anisotropy.

12:55 GMT Ganeko Bernardo-Seisdedos, CIC bioGUNE.

Metabolic landscape of the mouse liver by quantitative ^{31}P-NMR analysis of the phosphorome.

13:00 GMT Gyula Pálfy, Eötvös Loránd University.

Dynamics plays essential role in drug development against oncogenic K-Ras proteins.

13:05 GMT Arobendo Mondal, Technische Universität München.

Prediction of chemical shifts of 90k antamanide snapshots using machine learning at near experimental accuracy.

13:10 GMT Jacob R. Lindale, Duke University.

Exact Lindblad master equations for chemical exchange.

13:15 GMT Arnab Dey, Université de Nantes.

Hyperpolarized NMR metabolomics at natural 13C abundance.

13:20 GMT Manman Lu, University of Delaware.

Atomic-resolution structure of HIV-1 capsid tubes by magic-angle spinning NMR.

13:25 -13:55 GMT Q & A for the session.

Room 2: Materials & ssNMR Applications. *Thomas Voosegard* and *Antoine Loquet*

12:50 GMT (13:50 CET/7:50 EST/20:50 CST) - 13:55 GMT (14:55 CET/8:55 EST/21:55 CST)

12:50 GMT Alons Lends, Université de Bordeaux.

Capsule and surface organization of cryptococcus neoformans cells in their intact form revealed by ^{1}H detected solid-state NMR at fast MAS regime.

12:55 GMT Amrit Venkatesh, Iowa State University.

Accelerating wideline ^{195}Pt solid-state NMR with fast MAS and dynamic nuclear polarization: application to single-site heterogeneous catalysts.

13:00 GMT Dominik J. Kubicki, University of Cambridge.

Local structure and dynamics in multicomponent tin halide perovskites from Tin-119 solid-state NMR.

13:05 GMT Pinelopi Moutzouri, École Polytechnique Fédérale de Lausanne.

Elucidation of the atomic-level structure of cementitious calcium aluminate silicate hydrate by DNP mas NMR.

13:10 GMT Scholzen Pascal, ESPCI - Paris.

Ferromagnetic NMR for the study of cobalt nanowires and nanoparticle assemblies.

13:15 GMT Thomas Meier, University of Bayreuth.

Nuclear spin crossover in dense molecular hydrogen.

13:20 GMT Monu Kaushik, University of Lyon.

Structural characterization of ultra-thin aluminum oxide layers supported on silica by DNP enhanced solid-state NMR spectroscopy

13:25 -13:55 GMT Q & A for the session.

Common session (Room 1)

14:00 GMT (15:00 CET/9:00 EST/22:00 CST) Varian Young Investigator Award Introduction. *Bernhard Brutscher*

14:05 GMT Varian Young Investigator Award Lecture by Paul Schanda (IBS - Grenoble).

14:35 GMT (15:35 CET/9:35 EST/22:35 CST) Presentation of EUROMAR 2021. *Janez Plavec and Thomas Prisner*.

Parallel sessions

Room 1: Biomolecular NMR 2. *Jesús Jiménez-Barbero* and *Oscar Millet*

14:45 GMT (15:45 CET/9:45 EST/22:45 CST) - 15:25 GMT (16:25 CET/10:25 EST/23:25 CST)

14:45 GMT María Pía Lenza, CIC bioGUNE.

NMR structural characterization of the n-linked glycans in the receptor binding domain of the SARS-CoV-2 spike protein and their interactions with human lectins.

14:50 GMT Manon Julien, CNRS - Gif-sur-Yvette.

Monitoring phosphorylations in the disordered region of BRCA2 and identifying their impact on binding to partners.

14:55 GMT Hans Koss, Columbia University.

Cadherin-11 dimerization multi-site kinetics: combined partial unfolding and strand-swapping.

15:00 GMT Carlos Lima, Universidade Nova de Lisboa.

Deciphering the structural features of LacdiNAc recognition by lectins of immune system using NMR spectroscopy.

15:05 -15:25 GMT Q & A for the session.

Room 2: ssNMR Methods & Instrumentation. *Andrew Webb* and *Arno Kentgens*

14:45 GMT (15:45 CET/9:45 EST/22:45 CST) - 15:25 GMT (16:25 CET/10:25 EST/23:25 CST)

14:45 GMT Nergiz Sahin Solmaz, École Polytechnique Fédérale de Lausanne.

The single-chip dynamic nuclear polarization microsystem.

14:50 GMT Bruno Simões de Almeida, École Polytechnique Fédérale de Lausanne.

Fast remote correlation experiments for 1H homonuclear decoupling in solids.

14:55 GMT Rodrigo De Oliveira-Silva, KU Leuven.

NMR relaxorption: a bimodal instrumentation for simultaneous sorption / NMR detection of vapors inside microporous materials.

15:00 GMT Kathrin Aebischer, ETH Zürich.

Using B1-field selective pulses to improve FSLG-decoupled spectra.

15:05 -15:25 GMT Q & A for the session.

Room 1: JMR & EPR Poster awards. Closing Remarks. *Lucio Frydman, Thomas Prisner and Oscar Millet*

15:40 GMT (16:40 CET/10:40 EST/23:40 CST) - 16:00 GMT (17:00 CET/11:00 EST/24:00 CST)

ABSTRACTS

01. Biomolecular NMR

NMR STRUCTURAL CHARACTERIZATION OF THE N-LINKED GLYCANS IN THE RECEPTOR BINDING DOMAIN OF THE SARS-COV-2 SPIKE PROTEIN AND THEIR INTERACTIONS WITH HUMAN LECTINS

Maria Pia Lenza[1], Iker Oyenarte[1], Tammo Diercks[1], Jon Imanol Quintana[1], Ana Gimeno[1], Helena Coelho[2], Ana Diniz[2], Francesca Peccati[1], Sandra Delgado[1], Oscar Millet[1], Filipa Marcelo[1], Gonzalo Jiménez-Osés[1], Jesús Jiménez-Barbero[1], June Ereño-Orbea[1], Ana Arda[1]

[1] CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.
[2] UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciencias e Tecnologia, Universidade NOVA de Lisboa 2829-516 Caparica, Portugal.

Many viral proteins are found to be glycosylated, which have important implications in viral pathology.[1] These N- and O-linked sugars have been shown, among others, to participate in viral attachment and to modulate immune responses. The spike protein (S) of SARS-CoV-2 contains 22 N-glycosylation sites per monomer, two of them located in the Receptor Binding Domain (RBD).[2]

Herein, we have produced the RBD fragment of SARS-CoV-2 in a human cell culture (HEK293F) achieving ^{13}C-labeling on the N-glycans.[3] This has allowed an unprecedented detailed characterization of the specific glycan structures. Additionally, ^{1}H-^{13}C HSQC spectroscopy on the RBD was exploited as fingerprint in order to dissect the interaction of this glycosylated domain with a variety of human lectins, which are expressed in different organs and tissues that may be affected during the infection, revealing the specific glycan-epitopes responsible for each interaction.[4]

References

[1] Y. Watanabe, T. A. Bowden, I. A. Wilson, M. Crispin Exploitation of glycosylation in enveloped virus pathobiology. *Biochim. Biophys. Acta - Gen. Subj*. **1863**, 1480–1497 (2019).
[2] (a) A. Shajahan, N. T. Supekar, A. S. Gleinich, P. Azadi Deducing the N- and O- glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2, *Glycobiology*, 1-20 (2020). (b) Y. Watanabe, J. D. Allen, D. Wrapp, J. S. McLellan, M. Crispin Site-specific glycan analysis of the SARS-CoV-2 spike, *Science*, **369**, 330 – 333 (2020).
[3] A.W. Barb, D. J. Falconer, G. P. Subedi, Methods in Enzymology, Academic Press, New York, 2019, 239 – 261.
[4] Lenza, M.P., Oyenarte, I., Diercks, T., Quintana, J.I., Gimeno, A., Coelho, H., Diniz, A., Peccati, F., Delgado, S., Bosch, A., Valle, M., Millet, O., Abrescia, N.G.A., Palazón, A., Marcelo, F., Jiménez-Osés, G., Jiménez-Barbero, J., Ardá, A. and Ereño-Orbea, J. Structural Characterization of N-Linked Glycans in the Receptor Binding Domain of the SARS-CoV-2 Spike Protein and their Interactions with Human Lectins*, Angew. Chem. Int. Ed.* (2020) doi:10.1002/anie.202011015.

THE ROLE OF BACKBONE DYNAMICS IN MODULATING COMPETITION BETWEEN DISORDERED LIGANDS

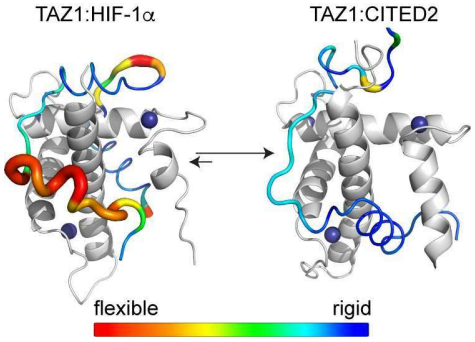
Rebecca B. Berlow[1], Francis D. Appling[1], H. Jane Dyson[1], Peter E. Wright[1,2]

[1] Department of Integrative Structural and Computational Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, U.S.A.
[2] Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, U.S.A.

Intrinsically disordered proteins (IDPs) play important roles in a multitude of cellular processes; however, the mechanisms by which disordered proteins carry out their diverse roles as modulators of cellular signaling are not yet completely understood. IDPs utilize a wide range of mechanisms to interact with folded proteins within the cell, and in many cases, must compete for binding of protein interaction partners. For example, numerous disordered ligands compete for binding of the TAZ1 domain of the general transcriptional coactivators CBP and p300 to control transcriptional responses to a diverse set of stimuli. All of these disordered ligands occupy partially overlapping surfaces of TAZ1, yet little is known about how different IDPs compete for binding of common target molecules. We have previously shown that two disordered proteins, the transcription factor HIF-1α and its negative regulator CITED2, function as a unidirectional, allosteric molecular switch to control transcription of critical adaptive genes under conditions of oxygen deprivation by competing for TAZ1 binding. To gain further mechanistic insight into this process, we have used a combination of structural and biophysical techniques, including NMR relaxation methods and fluorescence approaches, to obtain a detailed molecular description of binding and competition processes involving IDPs. Importantly, we find that in the case of HIF-1α, CITED2, and TAZ1, competition between the IDPs for TAZ1 binding is not only dependent on the physicochemical properties of the IDPs themselves but also on the conformational dynamics of TAZ1. These findings highlight the complexity of binding and competition processes involving disordered proteins and the interplay between highly dynamic folded and disordered proteins in cellular signaling processes.

References

[1] P.E. Wright and H.J. Dyson, Intrinsically disordered proteins in cellular signalling and regulation. *Nat Rev Mol Cell Biol* **16**, 18-29 (2015).
[2] R.B. Berlow, H.J. Dyson, and P.E. Wright, Hypersensitive termination of the hypoxic response by a disordered protein switch. *Nature* **543**, 447-451 (2017).
[3] R.B. Berlow, M.A. Martinez-Yamout, H.J. Dyson, and P.E. Wright, Role of Backbone Dynamics in Modulating the Interactions of Disordered Ligands with the TAZ1 Domain of the CREB-Binding Protein. *Biochemistry* **58**, 1354-1362 (2019).


TAZ1:HIF-1α
TAZ1:CITED2
flexible
rigid

INTO THE STRUCTURE OF HUMAN FULL-LENGTH SMAD PROTEINS

Tiago Gomes[1], Pau Martin-Malpartida[2], Tiago N. Cordeiro[2], Maria J. Macias[1 3]

[1] Institute for Research in Biomedicine, The Barcelona Institute of Science and Technology, Baldiri Reixac, 10, Barcelona, 08028, Spain
[2] Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portugal
[3] ICREA, Passeig Lluís Companys 23, Barcelona, 08010, Spain

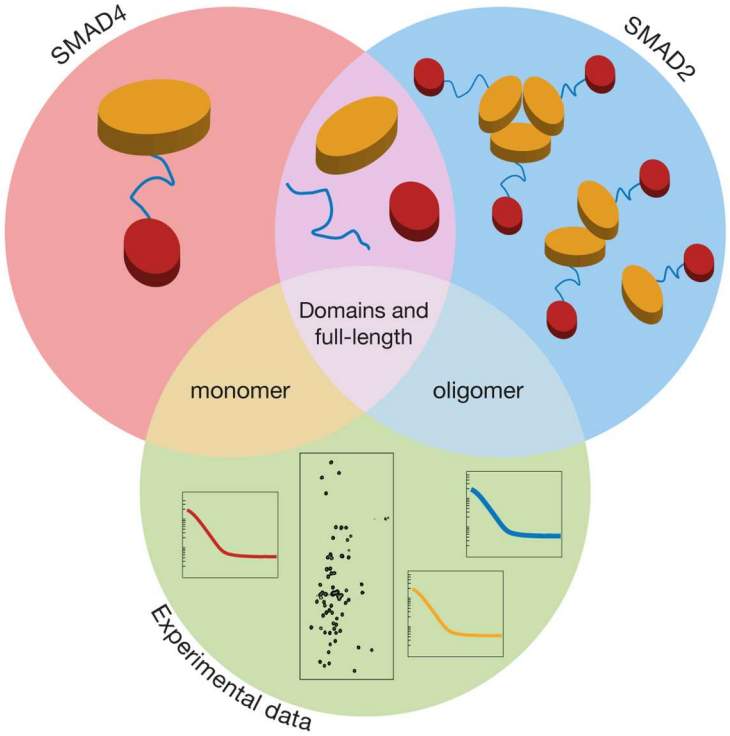
The transforming growth factor β pathway (TGFβ) plays decisive roles in embryo development, tissue homeostasis, and immune responses. When unregulated, it can drive epithelial-to-mesenchymal transitions (EMT) and promote tumor invasion and fibrosis[1]. Smad (mothers against decapentaplegic) proteins are its principal effectors[2]. These proteins are one of the most mutated in gastrointestinal cancers and one of the principal enforcers driving malignancy[3]. Until now, no structural information regarding full-length Smad proteins has been put forward. Using an integrative structural biology approach, using nuclear magnetic resonance (NMR) spectroscopy and small-angle X-ray scattering (SAXS), we have characterized the conformations of full-length Smad2 (461 residues) and Smad4 (552 residues) in solution. We show that Smads inter- domain linker behaves as an intrinsically disordered protein (IDP) tethering the MH1 (DNA binding) and MH2 (protein binding) domains within flexible multidomain Smad2 and Smad4. In solution, Smads display different oligomeric states. Smad4 is monomeric and not predominantly in an auto-inhibited conformation. Smad2 is an oligomeric protein in a monomer-dimer-trimer equilibrium, shaped by phosphorylation and its MH1 domain. We also estimated the latter equilibrium's global affinity by using populations derived from SAXS data analysis, using explicit models. More broadly, this study redefines TGFβ activation by providing the first glimpse into Smads full-length structures, with implications in subsequent TGFβ mechanistic studies and strategies to tackle large and flexible multidomain proteins.

References

[1] David, C.J., and Massagué, J. Contextual determinants of TGFβ action in development, immunity and cancer. Nat. Rev. Mol. Cell Biol. **19**, 419–435 (2018).

[2] Macias, M.J., Martin-Malpartida, P., and Massagué, J. Structural determinants of Smad function in TGF-β signaling. Trends Biochem. Sci. **40**, 296–308 (2015).

[3] Waddell, N., Pajic, M., Patch, A.-M., Chang, D.K., Kassahn, K.S., Bailey, P., Johns, A.L., Miller, D., Nones, K., Quek, K., et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature **518**, 495–501 (2015).


SMAD4
SMAD2
Domains and full-length
monomer
oligomer
Experimental data

MONITORING PHOSPHORYLATIONS IN THE DISORDERED REGION OF BRCA2 AND IDENTIFYING THEIR IMPACT ON BINDING TO PARTNERS

Manon Julien [1,3], Simona Miron [1,3], Asa Elhen [2,3], Aura Carreira [2,3], François-Xavier Theillet [1,3], Sophie Zinn-Justin [1,3]

[1] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, UMR9198, F-91191 Gif-sur-Yvette, France
[2] Institut Curie, PSL Research University, CNRS, UMR3348, F-91405, Orsay, France
[3] Paris Sud University, Paris-Saclay University, France

BRCA2 is an oncoprotein frequently mutated in hereditary breast cancers. BRCA2 is involved in genomic stability pathways such as DNA repair and chromosome segregation. A dysfunction of this protein increases the cancer risk. To improve the diagnosis of these cancers, several molecular studies identified key positions and associated mutations causing a loss of function of BRCA2. However, these studies mainly focused on the C-terminal globular domain of BRCA2.

Here, we characterized the N-terminal region of BRCA2 from aa 48 to aa 284 ($BRCA2_{48-284}$). This well-conserved region from mammals to fishes is disordered, i.e. it lacks stable secondary structure[1]. It is also highly phosphorylated by the kinase Plk1 at the entry into mitosis. However, previous studies using mass spectrometry didn't allow to precisely identify all the phosphorylation sites.

We established real-time NMR protocols to monitor *in vitro* phosphorylation of $BRCA2_{48-284}$[2,3]. We identified that Plk1 phosphorylates BRCA2 at 4 positions, including the 2 highly conserved S193 and T207. From this result, we searched for the functions of phosphorylated S193 (pS193) and T207 (pT207) in mitosis.

First, we identified that pT207 creates a docking site for Plk1 on BRCA2. In collaboration with the group of Dr. Aura Carreira, we showed that this interaction triggers the assembly of a quaternary complex involving BRCA2, Plk1, BubR1 and PP2A at the kinetochore, and contributes to the alignment of chromosomes at the metaphase plate[4]. We also demonstrated that breast cancer variants impact the phosphorylation of BRCA2 and the formation of the complex.

Second, we initiated proteomics experiments to identify new partners specific to phospho-$BRCA2_{48-284}$, and found Plk1 as well as other proteins involved in mitosis.

References

[1] Julien M, Miron S, Carreira A, Theillet FX, Zinn-Justin S. 1H, 13C and 15N backbone resonance assignment of the human BRCA2 N-terminal region. Biomol NMR Assign. 2020 Apr;**14**(1):79-85.
[2] Julien M, Bouguechtouli C, Alik A, Ghouil R, Zinn-Justin S, Theillet FX. Multiple Site-Specific Phosphorylation of IDPs Monitored by NMR. Methods Mol Biol. 2020; 2141:793-817.
[3] Alik A*, Bouguechtouli C*, Julien M*, Bermel W, Ghouil R, Zinn-Justin S, Theillet FX. Sensitivity-Enhanced 13 CNMR.Spectroscopy for Monitoring Multisite Phosphorylation at Physiological Temperature and pH. Angew Chem Int Ed Engl. 2020 Jun 22;59(26):10411-10415.
[4] Ehlen A, Martin C*, Miron S*, Julien M*, Theillet FX, Ropars V, Sessa G, Beaurepere R, Boucherit V, Duchambon P, El Marjou A, Zinn-Justin S, Carreira A. Proper chromosome alignment depends on BRCA2 phosphorylation by PLK1. Nat Commun. 2020 Apr 14;11(1):1819.* co-authors.

CADHERIN-11 DIMERIZATION MULTI-SITE KINETICS: COMBINED PARTIAL UNFOLDING AND STRAND-SWAPPING

Hans Koss[1], Lawrence Shapiro[1], Arthur G Palmer III[1]

[1]Department of Biochemistry and Biophysics, Columbia University, New York, USA

The Cadherin extracellular domain 1 (EC1) is a primary determinant of dimerization specificity of type II Cadherins [1]. Conserved residues Trp2 and Trp4 are crucial to lock the EC1 A-strand into domain-swapped Cadherin type II dimers. Herein, we analyze specific roles of Trp2 and Trp4 in the mechanism and kinetics of dimerization of Cadherin-11 EC1, using various NMR-based methods, including multi-site relaxation dispersion analysis[2], high pressure experiments and mutant analysis. We quantitatively analyze the fast-intermediate and slow-intermediate of the A-strand-bound state with sparsely populated partially and fully strand-exposed states. We propose a framework in which these three folded A-strand-binding states are coupled to unfolded states at slow and very fast dynamic levels. The slow level includes exchange processes between folded and partially unfolded states, which have been recognized as crucial intermediates in domain-swapping proteins other than Cadherin. The very fast level is defined by rapidly exchanging local random coil/folded states of loop residues 30-33. This framework and our methodical approach provide a perspective to study the newly suggested role of unfolded states in Cadherin dimerization, specifically considering the newly discovered destabilizing role of Trp4 exposure in type II Cadherins.

References

[1] Patel, S.D., et al., *Type II cadherin ectodomain structures: implications for classical cadherin specificity.* Cell, 2006. **124**(6): 1255-68.
[2] Palmer, A.G., 3rd and H. Koss, *Chemical Exchange.* Methods Enzymol, 2019. **615**: 177-236.

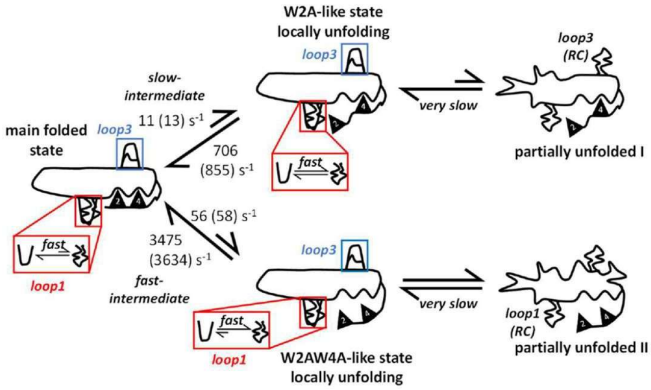


Figure 1. Chemical exchange in Cadherin-11 EC1. The local very fast equilibria for loop 1 are shown in red insets, with the equilibrium arrow indicating general propensity towards the folded or the open random coil state. Slow- intermediate and fast-intermediate exchange have been established by relaxation dispersion. Resulting rate constants for low and high concentrations are shown outside or inside brackets, respectively.

SOLUTION AND SOLID-STATE NMR TO STUDY VIRAL ASSEMBLIES IN HEPATITIS B AND DENGUE VIRUSES

Lauriane Lecoq[1], Marie Dujardin[1], Shishan Wang[1], Maarten Schledorn[2], Marie Bartenschlager[3], Karen Cobas[4], Marie-Laure Fogeron[1], Thomas Wiegand[2], Gerardo Guillen[4], Ralf Bartenschlager[3], Lázaro Gil González[4], Michael Nassal[5], Beat H. Meier[2], Anja Böckmann[1]

[1] Molecular Microbiology and Structural Biochemistry, University of Lyon/CNRS, 69367 Lyon, France.
[2] Physical Chemistry, ETH Zurich, 8093 Zurich, Switzerland.
[3] University of Heidelberg, Department of Infectious Diseases, Heidelberg, Germany.
[4] Centro de Ingenieria Genética y Biotecnologia, Habana 10600, Cuba.
[5] Department of Medicine, University Hospital Freiburg, 79106 Freiburg, Germany.

We present NMR studies of two different viral ribonucleoprotein complexes, one yielding highly symmetric objects, and the other aggregates devoid of visible symmetry. We investigated the Dengue virus (DENV) and the hepatitis B virus (HBV), which both represent a threat to human lives with several hundreds of millions of new infections each year. While the nucleocapsid of HBV is formed by 240 copies of core proteins which assemble into stable and well-ordered icosahedral capsids[1] with the packaged genome inside, DENV (and flaviviruses in general) form less ordered ribonucleoproteins, presenting a yet unknown organization with respect to the icosahedral envelope[2].
We characterized the dimeric and capsid conformational states of the two core proteins; for this we compared carbon and proton-detection solid-state NMR to solution NMR spectra. We observed that the spectra show subtle differences between isolated dimers and assembled capsids, and also that for the HBV capsid, the different asymmetric subunits can be distinguished[3,4]. For the DENV ribonucleoprotein, the aggregates consistently observed under the microscope in a large screen of assembly conditions surprisingly resulted in highly resolved NMR spectra indicative for a structured protein. Comparison with the ^{13}C solution-NMR chemical shifts of the DENV core protein dimers reveals the regions involved in ribonucleoprotein assembly. For both capsids, ^{31}P NMR experiments allowed to observe the respective viral RNAs.
Our work demonstrates that viral capsids can be studied by NMR whether or not they form regular-shaped objects under the electron microscope[5] (see Figure). When large enough quantities are available, they can be investigated with both ^{1}H- and ^{13}C-detected NMR, and ^{13}C chemical shifts are best used to compare isolated core protein and capsid conformations, since they are less sensitive to unavoidable temperature and pH variations between the samples.

References

[1] Wynne, S. A., Crowther, R. A., and Leslie, A. G. The crystal structure of the human hepatitis B virus capsid. *Mol. Cell* **3**, 771–780 (1999).

[2] Kuhn, R. J., Zhang, W., Rossmann, M. G., Pletnev, S. V., Corver, J., Lenches, E., Jones, C. T., Mukhopadhyay, S., Chipman, P. R., Strauss, E. G., Baker, T. S., and Strauss, J. H. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* **108**, 717–725 (2002).

[3] Lecoq, L., Wang, S., Wiegand, T., Bressanelli, S., Nassal, M., Meier, B. H., and Böckmann, A. Localizing Conformational Hinges by NMR: Where Do Hepatitis B Virus Core Proteins Adapt for Capsid Assembly? *ChemPhysChem* **19**, 1336–1340 (2018).

[4] Lecoq, L., Schledorn, M., Wang, S., Smith-Penzel, S., Malär, A. A., Callon, M., Nassal, M., Meier, B. H., and Böckmann, A. 100 kHz MAS Proton-Detected NMR Spectroscopy of Hepatitis B Virus Capsids. *Front. Mol. Biosci.* **6** (2019).

[5] Lecoq, L., Fogeron, M.-L., Meier, B. H., Nassal, M., and Böckmann, A. Solid-State NMR for Studying the Structure and Dynamics of Viral Assemblies. *Viruses* **12**, 1069 (2020).

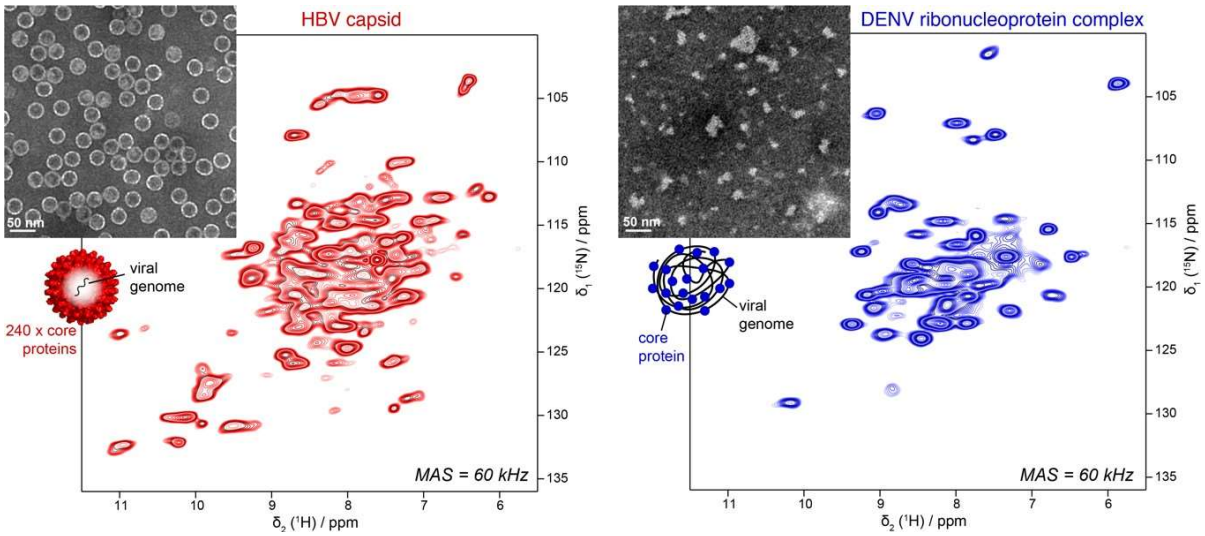


Figure 1. Negative-staining EM pictures of HBV capsid (left) and DENV ribonucleoprotein complex (right) and their corresponding 2D hNH spectra recorded at a MAS frequency of 60 kHz and a magnetic field of 800 MHz.

DECIPHERING THE STRUCTURAL FEATURES OF LacdiNAc RECOGNITION BY LECTINS OF IMMUNE SYSTEM USING NMR SPECTROSCOPY

Carlos Lima[1], Helena Coelho[1], Ana Gimeno[2], Ana Diniz[1], Jorge Dias[1], Filipa Trovão[1], Ana Luísa Carvalho[1], Eurico Cabrita[1], Jesús Jiménez-Barbero[2], Francisco Corzana[3], Filipa Marcelo[1]

[1] UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[2] CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.
[3] Department of Chemistry, University of La Rioja, 26006, Logroño, Spain
cd.lima@campus.fct.unl.pt

The glycome (sugar code) emerges as the 3rd language of life after genome and proteome. The specific sugar codes on pathogens and human glycoproteins are deciphered by carbohydrate-binding receptors (lectins)[1]. Certain diseases, such as cancer, take advantage of this sugar code for its progression and to modulate immune responses[2].

LacdiNAc (GalNAcβ1-4GlcNAc) is a disaccharide that is widespread in invertebrates, being associated with the recognition of parasites, as well as in cancer cells, even though it is not present in healthy human cells[3]. LacNAc (Galβ1-4GlcNAc), on the other hand, is expressed naturally in human cells, but is overexpressed in tumours.[4] Immune-related lectins like human Macrophage Galactose-Type Lectin (MGL) and human Galectin-3 (Gal-3) are known to bound to galactosides derivatives such as GalNAc and Gal units present in LacdiNAc and LacNAc disaccharides, respectively.[2,3] Nevertheless, the molecular determinants that govern the recognition of LacdiNAc and LacNAc by MGL and Gal-3 are poorly understood. Herein, we will report new structural insights on the recognition of LacdiNAc and LacNAc by MGL/Gal-3, unveiled by the combination of $^{1}H/^{15}N$-HSQC NMR based titration experiments, saturation transfer difference NMR (STD- NMR),[4,5] isothermal titration calorimetry (ITC) experiments, molecular dynamics (MD) and X-ray crystallography experiments.

References

[1] Ronald L. Schnaar, J Allergy Clin Immunol. 2015 Mar; 135(3): 609–615;
[2] F. M. Tuccillo, et al, Biomed Res. Int. 2014, (2014).
[3] TK van den Berg, et al. J Immunol. 2004;173(3):1902–7;
[4] M. Nonaka, et al, Mechanisms of Malignant Phenotypes. 2016; 141–161
[5] F. Marcelo et al., J. Biol. Chem., 2019, 294, 1300-1311 and A. Diniz et al., Chem. Eur. J., 2019, 25, 13945-13955.

Acknowledgements

The authors thank to the FCT-Portugal for the Projects IF/00780/2015, PTDC/BIAMIB/31028/2017 and UIDB/04378/2020. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 22161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

DISSECTING ARTIFICIALLY RECONSTITUTED IMMUNE PRE-SIGNALING CLUSTERS TO STUDY BIOCONDENSATION

Joachim Maier[1], Daniel Sieme[1], Leo Wong[1], Arshiya Bhatt[2], Michael Engelke[2], Jürgen Wienands[2], Stefan Becker[1], Christian Griesinger[1]

[1] Department of NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
[2] Institute of Cellular and Molecular Immunology, Georg August University of Göttingen, Humboldtallee 34, 37073 Göttingen, Germany

The adapter proteins SH2-domaincontaining leukocyte protein of 65 kDa (SLP65) and Cbl-interacting protein of 85 kDa (CIN85) act as signal transducers within the BCR signaling pathway[1,2]. In resting B-cells, SLP65 and CIN85 are clustered at VAMP7-positive vesicles[1,3].

Tripartite pre-signaling clusters (Figure: Schematic representation of SLP65-CIN85-vesicle cluster) have been reconstituted *in vitro* at physiological relevant protein concentrations by mixing protein constructs of CIN85, SLP65 and small unilamellar vesicles (SUVs). A homogeneous vesicle distribution within the dense phase is visualized by cryo-electron tomography[5]. The known molecular interactions comprise the interactions of SLP65's N-termini with SUVs (global K_D = ~80 µM), trimerization of CIN85 by the coiled coil domain[4] and promiscuous interactions between CIN85's three SH3 domains and multiple proline-rich motifs (PRMs) of SLP65[5] and CIN85.

To investigate the nature of promiscuous interactions we disentangled contributions of SUVs, PRMs and SH3 domains by applying a new classification method for interaction entities: First, we identified six relevant PRMs of SLP65 and measured the binding affinities of monovalent interactions between 18 combinations of 3x SH3 domains and 6x peptides bearing single PRMs (K_D's ranging from 7 µM to low mM measured by NMR titrations). Next, we designed SLP65 and CIN85 constructs with smaller and larger affinities. Then, the critical concentrations of phase separation was determined for different selection of designed constructs, either with or without binding entity (ϕ^+ and ϕ^-, respectively). E.g. the mixture of $CIN85_{(1-333)}$-3SH3 construct and $SLP65_{(1-330)}$ construct phase separates at a critical concentration ϕ^- of ~100 µM, while mixing both proteins and SUVs reduced the critical concentration ϕ^+ to 2 µM. The ratio ϕ^- / ϕ^+ was analyzed for all binding entities (SUVs, PRM4, 2x weak PRMs replaced by 2x PRM4s and 2 SH3 domains replaced by SH3B) considering its dependence on ϕ^-.

Finally, we ordered the interaction entities according to their contribution to phase separation, starting with the strongest as following: a) Addition of SUVs b) replacing each SH3A and SH3C by SH3B c) including PRM4 d) replacing each PRM5 and PRM6 by PRM4. This report shed light on phase separation propensities and affinities of individual modules and could be a guide to develop a model for binding and phase separation of multimodular proteins.

References

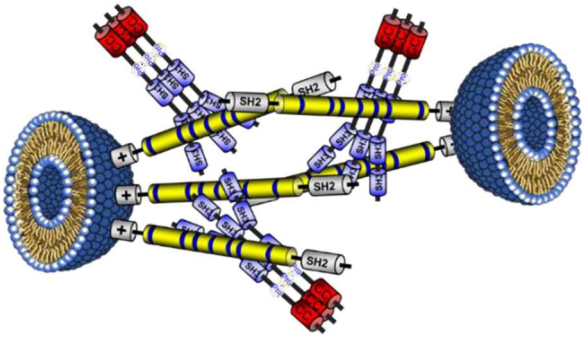
[1] Engelke M, Pirkuliyeva S, Kühn J, Wong L, Boyken J, Herrmann N, Becker S, Griesinger C, Wienands J. Macromolecular assembly of the adaptor SLP-65 at intracellular vesicles in resting B cells. *Sci Signal.* **7**, (339):ra79 (2014).

[2] Keller B, Shoukier M, Schulz K, Bhatt A, Heine I, Strohmeier V, Speckmann C, Engels N, Warnatz K, Wienands J. Germline deletion of CIN85 in humans with X chromosome-linked antibody deficiency. *J Exp Med.* **215(5)**, 1327-1336 (2018).

[3] Oellerich T, Bremes V, Neumann K, Bohnenberger H, Dittmann K, Hsiao HH, Engelke M, Schnyder T, Batista FD, Urlaub H, Wienands J. The B-cell antigen receptor signals through a preformed transducer module of SLP65 and CIN85. *EMBO J.* **30(17)**, 3620-34 (2011).

[4] Kühn J, Wong LE, Pirkuliyeva S, Schulz K, Schwiegk C, Fünfgeld KG, Keppler S, Batista FD, Urlaub H, Habeck M, Becker S, Griesinger C, Wienands J. The adaptor protein CIN85 assembles intracellular signaling clusters for B cell activation. *Sci Signal.* **9**, (434):ra66 (2016).

[5] Wong LE, Bhatt A, Erdmann PS, Hou Z, Maier J, Pirkuliyeva S, Engelke M, Becker S, Plitzko J, Wienands J, Griesinger C. Tripartite phase separation of two signal effectors with vesicles priming B cell responsiveness. *Nat Commun.* **11(1)**, 848 (2020).

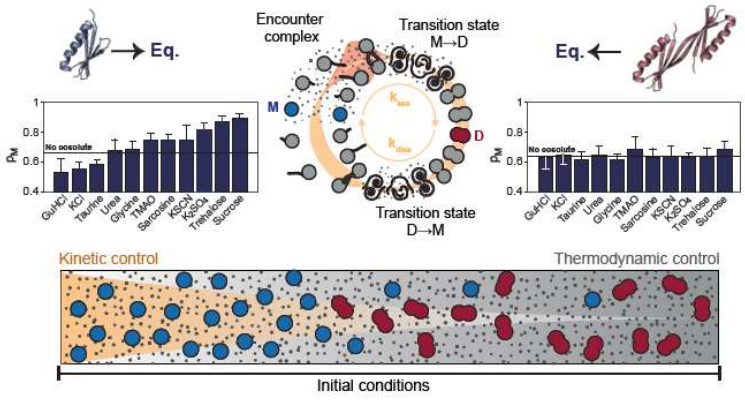


THE INITIAL PROTEIN OLIGOMERIC STATE DICTATES THE EFFECT OF SMALL COSOLUTES ON THE OLIGOMERIZATION REACTION

Borja Mateos[1,2], Ganeko Bernardo-Seisdedos[1], Valentin Dietrich[3], Nicanor Zalba[1], Gabriel Ortega[4], Francesca Peccati[5], Gonzalo Jiménez-Osés[5], Robert Konrat[2], Martin Tollinger[3] and Oscar Millet[1,*]

[1] Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.
[2] Department of Structural and Computational Biology, University of Vienna, Max Perutz Labs, Vienna Biocenter Campus 5. A- 1030 Vienna, Austria.
[3] Center of Molecular Biosciences & Institute of Organic Chemistry, University of Innsbruck, A-6020 Innsbruck, Austria.
[4] Department of Chemistry and Biochemistry, University of California Santa Barbara, CA 93106-9510.
[5]Computational Chemistry Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.

Protein oligomerization processes are widespread and of crucial importance to understand degenerative diseases and healthy regulatory pathways. One particular case is the homo-oligomerization of folded domains involving domain swapping, often found as a part of the protein homeostasis in the crowded cytosol, composed by a complex mixture of cosolutes. Here, we have investigated the effect of a plethora of cosolutes of very diverse nature on the thermodynamics and kinetics of a protein dimerization by domain swapping. Our results show that the oligomerization reaction shifts from a state-function in water to a path- function in the presence of crowders. Specifically, when the reaction is initiated from a large excess of dimer it occurs under thermodynamic control and becomes unsensitive to the effect of cosolutes. Conversely, when the reaction starts from a large excess of monomer, it occurs under kinetic control and it is exquisitely sensitive to the presence and nature of the cosolute. All these effects can be reconciled with the existing crowding theory and highlight the key effect that diffusion has in an oligomerization reaction in a crowded environment.



3_SL2 SARS-CoV-2 RNA SECONDARY STRUCTURE DETERMINATION BY NMR SPECTROSCOPY

Schamber Tatjana[1], Wacker Anna, Harald Schwalbe

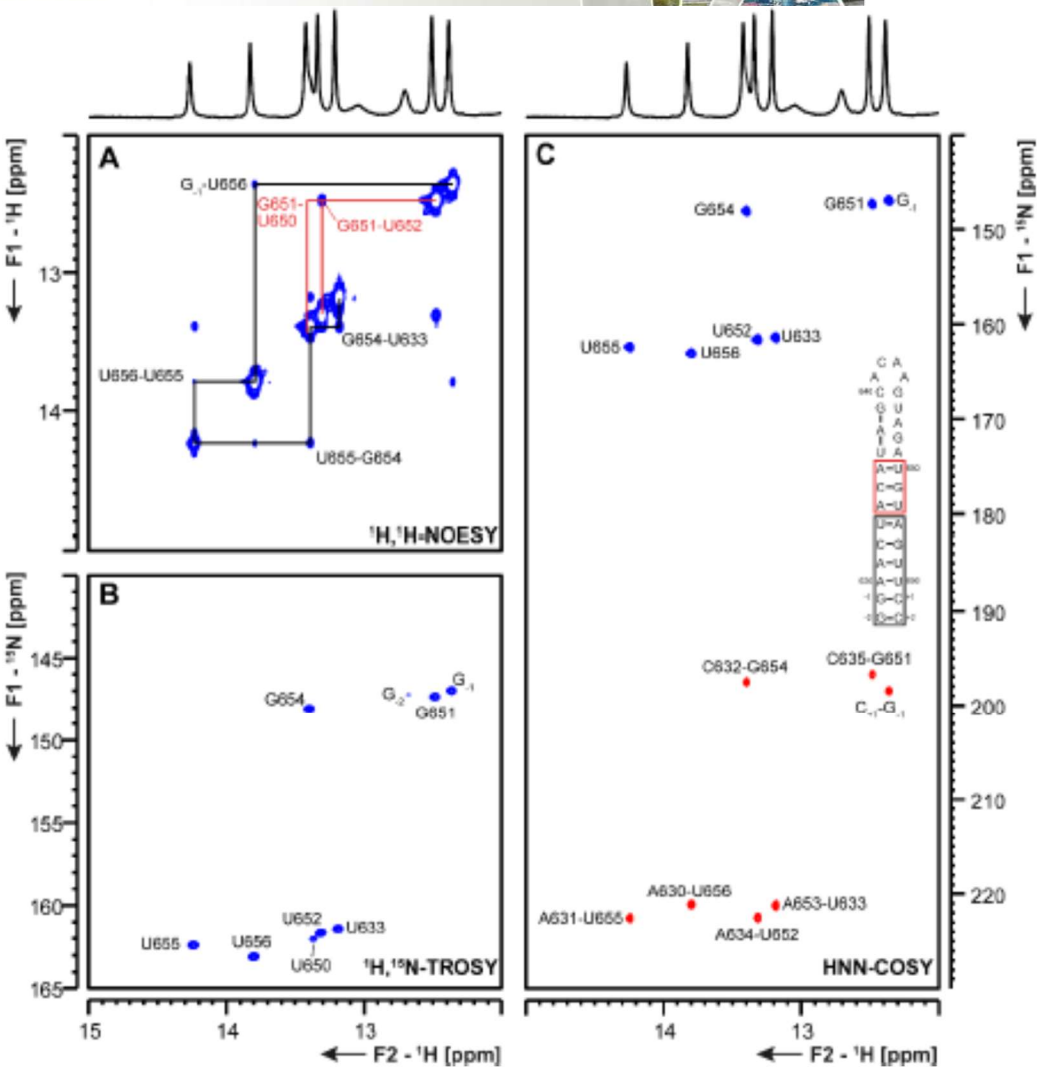
[1] Institute for Organic Chemistry and Chemical Biology, Goethe University, Max-von-Laue-Straße 7, 60438 Frankfurt, Germany

The 3'-UTR of Betacoronaviruses harbors several conserved regulatory RNA elements. 3_SL2 is the second hairpin element in the 3'-UTR with a rather large loop sequence (11 nts) that is complementary to the upstream 3'-most sequence of the bulged stem-loop (3_SL1). We could assign the imino protons by analyzing cross peaks in a ^{1}H,^{1}H-NOESY experiment and ^{1}H,^{15}N-TROSY-and HNN-COSY experiments (**1**). Imino proton spectra show that the A-helical part of 3_SL2 is composed of nine base pairs. In contrast to computational secondary structure predictions, no base pairs between loop nucleotides are observed. In contrast to the NMR data, DMS data show variations in 3_SL2 loop conformations which are highly interesting, as 3_SL2 is supposed to be part of a molecular switch crucial in regulating genome synthesis (**2**). The loop of 3_SL2 is either free or forms a pseudoknot interaction with the basal stem of 3_SL1. Strikingly, both the two-hairpin and the pseudoknot structures are essential for viral replication. While an open loop structure will facilitate pseudoknot formation, a defined folding of the loop might be required for protein interaction in the two-hairpin conformation. Thus, the loop conformation of 3_SL2 is further in investigation.

We further tested the druggability of 3_SL2 by screening all SARS-CoV-2 structured RNA elements.

References

[1] Schwalbe et al, Secondary structure determination of conserved SARS-CoV-2 RNA elements by NMR spectroscopyb Nucleic Acids Research**,** 2020 **(**accepted, breakthrough status).
[2] Goebel, S.J., Hsue, B., Dombrowski, T.F. and Masters, P.S. (2004) Characterization of the RNA components of a putative molecular switch in the 3' untranslated region of the murine coronavirus genome, *Journal of virology,* **78,** 669–682.


A
G-1-U656
G651-U650
G651-U652
G654-U633
U656-U655
U655-G654
¹H,¹H-NOESY
B
G654
G-2
G-1
G651
U655
U656
U652
U633
U650
¹H,¹⁵N-TROSY
C
G654
G651
G-1
U655
U652
U633
U656
C632-G654
C635-G651
C-1-G-1
A630-U656
A631-U655
A653-U633
A634-U652
HNN-COSY
F1 - ¹H [ppm]
F1 - ¹⁵N [ppm]
F2 - ¹H [ppm]

REDUCING THE MEASUREMENT TIME OF EXACT NOES BY NON-UNIFORM SAMPLING

Beat Vögeli[1], Parker J. Nichols[1], Alexandra Born[1], Morkos A. Henen[1,2], Dean Strotz[3], David N. Jones[4], Frank Delaglio[5]

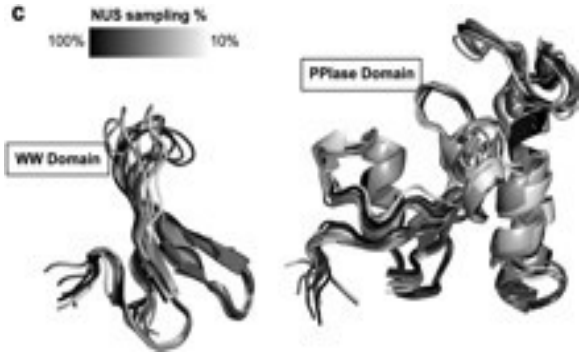
[1] Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, 12801 East 17th Avenue, Aurora, CO 80045, USA.
[2] Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.
[3] Laboratory of Physical Chemistry, ETH Zürich, Zürich 8093, Switzerland.
[4] Department of Pharmacology, University of Colorado Anschutz Medical Campus, 12801 East 17th Avenue, Aurora, CO 80045, USA.
[5] Institute for Bioscience and Biotechnology Research, National Institute of Standards and Technology and the University of Maryland, Rockville Maryland 20850 USA.

We have previously reported on the measurement of exact NOEs (eNOEs), which yield a wealth of additional information in comparison to conventional NOEs. We have used these eNOEs for calculation of high-resolution structures of proteins and RNAs.[1] Due to the need to measure a NOESY buildup series consisting of typically four NOESY spectra with varying mixing times, the 2D series takes a few days and a 3D series up to 10 days. This can be both expensive and problematic in the case of samples that are not stable. One potential method to significantly decrease the required measurement time of eNOEs is to use non-uniform sampling (NUS). The effect of NUS on the extremely tight distance restraints extracted from eNOEs may be very pronounced.

We investigated the fidelity of eNOEs measured from three test cases at decreasing NUS densities:[2] the 18.4 kDa protein human Pin1, the 4.1 kDa WW domain of Pin1 (both in 3D), and a 4.6 kDa 14mer RNA UUCG tetraloop (2D). Our results show that NUS imparted negligible error on the eNOE distances derived from good quality data down to 10% sampling for all three cases, but there is a noticeable decrease in the eNOE yield that is dependent upon the underlying sparsity, and thus complexity, of the sample. For Pin1, this transition occurred at roughly 40% (Figure) while for the WW domain and the UUCG tetraloop it occurred at 20% and 10%, respectively. We rationalized these numbers through reconstruction simulations under various conditions. The extent of this loss depends upon the number of scans as well as the number of peaks to be reconstructed. Based on these findings, we have created guidelines for choosing an optimal NUS density depending on the number of peaks needed to be reconstructed in the densest region of a NOESY spectrum.

References

[1] P. Nichols et al, Extending the applicability of exact nuclear Overhauser enhancements to large proteins and RNA, *ChemBioChem*, **19**, 1695-1701 (2018).
[2] P.J. Nichols, et al, Reducing the measurement time of exact NOEs by non-uniform sampling, *J. Biomol. NMR*, https://doi.org/10.1007/s10858-020-00344-8 (2020).


C
NUS sampling %
100%
10%
WW Domain
PPIase Domain

ANALYSIS OF PROTON-EXCHANGE PROCESS IN AQUEOUS SOLUTIONS OF UREA USING ZERO-FIELD NMR

Seyma Alcicek[1], Piotr Put, Vladimir Kontul, Szymon Pustelny

[1] Institute of Physics Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Krakow, Poland

In recent years, zero-field nuclear magnetic resonance (NMR), an alternative modality of NMR, enabled identification, characterization, and quantification of molecular structures. In isotropic liquids (absence of a direct spin-spin coupling) subjected to zero magnetic field (no Zeeman interaction), J-coupling signals become the main source of information about electronic structure and conformation of molecules, enabling chemical analysis and fingerprinting. [1] Since during chemical reactions (bond-breaking, bond-making) J-coupling strength and relaxation times are modified, measurements of the zero-field spectra become an attractive method to investigate a chemical-exchange process[2].

Urea, the simplest naturally occurring amide and the end-product of protein catabolism, is a good candidate to examine intra- and intermolecular proton exchange. Since, in urea solutions, the proton-exchange rate strongly depends on pH, here we investigated this effect using partially and fully labeled urea solutions via zero-field NMR. We also studied hydrogen-deuterium exchange in solution with various H_2O/D_2O solvent ratios to show the effect of the D/H concentration ratio on the exchange mechanism. We present that the technique enables distinguishing between partially deuterated molecules in J-spectra and predicts the ratio of D/H concentration in solution. This allows us to demonstrate the potential of zero-field NMR for investigating the chemical-exchange process.

References

[1] Blanchard, J. W., Budker, D. Zero- to Ultralow-Field NMR .*eMagRes2016,**5**, 1395 – 1410.* (2016).

[2] L Barskiy, D.A., Tayler, M.C., Marco-Rius, I. et al. Zero-field nuclear magnetic resonance of chemically exchanging systems. *Nat Commun* **10**, 3002 (2019).

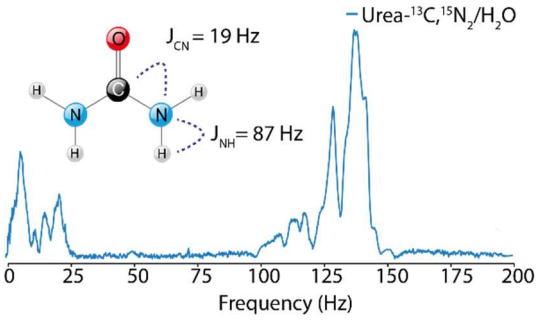


Figure 1. A representative J-spectrum of ^{13}C, $^{15}N_2$ urea in aqueous solution.

OPTIMIZATION OF THE ALGORITHM FOR NMR RESONANCE ASSIGNMENT OF INTRINSICALLY DISORDERED PROTEINS

Paulina Bartosińska[1], Marta Kolonko[2], Anna Zawadzka-Kazimierczuk[1]

[1] Biological and Chemical Research Centre, Faculty of chemistry, University of Warsaw, Poland
[2] Faculty of Chemistry of Wrocław University of Technology, Poland

Intrinsically disordered proteins (IDPs), despite the lack of a rigid structure, show strictly defined functions in the body – they often regulate the activity of cells and are responsible for molecular recognition. NMR, being able to study such dynamic objects, is the method of choice here, however it also faces some difficulties. Rapid changes in the chemical environment cause chemical shift averaging, which results in high peak overlap. The solution to this problem is to use three-dimensional (3D) spectra in combination with four- and five-dimensional (4D and 5D) ones (1). Resonance assignment based on high-dimensional spectra is easy and reliable. It can be performed automatically by the TSAR (2,3) (Tool for SMFT-based Assignment of Resonances) program.

The goal of the present work was to support the assignment with information on predicted chemical shifts for a given protein obtained the Potenci (4) program. The highly automatic testing system allowed also to identify some problematic procedures in the original algorithm. TSAR with the new procedures was tested on a group of 20 natively unstructured proteins from the Biological Magnetic Resonance Bank database. A decrease in the number of incorrectly assigned residues from 0.94% to 0.54% (decrease by 42%), without significantly changing the number of correct assignments (88%), was achieved. The last part of the work was to apply the improved program to the previously unassigned MetC (5) protein. The program correctly assigned 65% of the residues and incorrectly assigned 1% of the residues. The assignment was verified and completed manually - 68 % of the residues were assigned.

References

[1] Grudziąż K, Zawadzka-Kazimierczuk A, Koźmiński W. Vol. **148**, Methods. Academic Press Inc.; 2018. p. 81–7
[2] Zawadzka-Kazimierczuk A, Koźmiński W, Billeter M. J Biomol NMR. 2012 Sep;**54**(1):81–95.
[3] Piai A, Gonnelli L, Felli IC, Pierattelli R, Kazimierczuk K, Grudziaz K, et al. J Biomol NMR. 2016 Mar 1;**64**(3):239–53.
[4] Nielsen JT, Mulder FAA. J Biomol NMR. 2018;**70**(3):141–65.
[5] Kolonko M, Ozga K, Hołubowicz R, Taube M, Kozak M, Ozyhar A, et al. PLoS One. 2016 Sep 1;11(9).

STRUCTURAL INSIGHTS IN THE RECOGNICTION OF GLYCANS BY HUMAN GALECTIN-1

Sara Bertuzzi[1], Ana Gimeno[1], Reyes Núñez[1], Ganeko Bernardo-Seisdedos[1], Sandra Delgado[1], Gonzalo Jiménez-Osés[1], Oscar Millet[1], Jesús Jiménez-Barbero[1,2,3], Ana Ardá[1]

[1] Molecular Recognition and Host-Pathogen Interactions Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.
[2] Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Bizkaia, Spain.
[3] Department of Organic Chemistry II, University of the Basque Country - UPV/EHU, 48940 Leioa, Spain.

In the last decades different families of lectins have emerged as biomedical targets, since the recognition of glycans by these proteins mediates key events in biological processes linked with several diseases, including infections, cancer or autoimmune disorders. This work dissects how human galectin-1 (Gal-1), a β-galactoside binding lectin, recognizes a group of oligosaccharides, from di- (N-Acetyl lactosamine) to tetra-saccharides (blood B type-II antigen). The interest on this specific lectin derives from its involvement in inflammatory responses, differentiation trafficking, survival of immune cells, establishment and maintenance of T-cell tolerance and homeostasis in vivo[1] and cancer progression[2]. As example, it has been reported that Gal-1 overexpression in tumours is positively correlated with a metastatic phenotype[3]. However, the resulting need of molecules able to target and selectively inhibit Gal-1 is a formidable challenge because of the sequence and structural similarities among the members of galectin family. To scrutinize at the molecular level the crucial recognition features of Gal-1/glycan system, a multidisciplinary approach that combine ligand-, receptor-based and relaxation NMR experiments (STD, ^{1}H,^{15}N-HSQC, CLEANEX-PM, Relaxation Dispersion) with biophysical techniques (isothermal titration microcalorimetry, ITC) and computational methods (molecular dynamics simulations, MD) has been adopted. The atomic details of the recognition, the specific binding affinities as well as the thermodynamic and kinetic features of the interactions has been elucidated. Interestingly, the binding events display strikingly different characteristics to those reported for human galectin-3,[4] another member of the family. In particular, it is demonstrated that the entropy term favours the formation of the different complexes. Moreover, the systems display internal motions in different time scales, which are enhanced upon binding of specific ligands, supporting the existence of allostery. Overall, these results shed light on structural and thermodynamic binding features that can be used to guide the rational design of compounds capable of selectively binding Gal-1.

References

[1] I. Camby, M. Le Mercier, F. Lefranc, R. Kiss, Galectin-1: a small protein with major functions. *Glycobiology*. **16**, 137R–157R (2006)
[2] K. Ito, K. Stannard, E. Gabutero, A.M. Clark, S.Y. Neo, S. Onturk, S. J. Ralph, Galectin-1 as a potent target for cancer therapy: role in the tumor microenvironment. *Cancer and Metastasis* Reviews. **31**, 763-778 (2012)

[3] E. Rodriguez, S.T.T. Schetters, Y. van Kooyk, Y, The tumour glyco-code as a novel immune checkpoint for immunotherapy, *Nat Rev Immunol*. **18**, 204–211 (2018)

[4] A. Gimeno, S. Delgado, P. Valverde, S. Bertuzzi, M.A. Berbís, J. Echavarren, A. Lacetera, S. Martín-Santamaría, A. Surolia, F.J. Cañada, J. Jiménez-Barbero, A. Ardá, Minimizing the Entropy Penalty for Ligand Binding: Lessons from the Molecular Recognition of the Histo Blood-Group Antigens by Human Galectin-3, *Angew Chem. – Int. Ed.,* **58**, 7268-7272 (2019)

HIGH-RESOLUTION AND TIME-RESOLVED INSIGHTS INTO 10-23 DNAZYME MEDIATED CATALYSIS

Jan Borggräfe[1,2,8], Julian Victor[1], Hannah Rosenbach[1], Aldino Viegas[1,2], Christoph Gertzen[3,5], Christine Wübben[4], Ralf Biel[6], Helena Kovacs[7], Detlev Riesner[1], Gerhard Steger[1], Olav Schiemann[4], Holger Gohlke[2,3,5,8], Ingrid Span[1], Manuel Etzkorn[1,2,8]

[1] Institute of Physical Biology, Heinrich-Heine-University Düsseldorf, Germany
[2] Institute of Biological Information Processing, IBI-7: Structural Biochemistry, Forschungszentrum Jülich, Germany
[3] Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf, Germany
[4] Institute of Physical and Theoretical Chemistry, University of Bonn, Germany
[5] John von Neumann Institute for Computing (NIC) and Jülich Supercomputation Centre (JSC), Forschungszentrum Jülich, Germany
[6] Jülich Center for Neutron Science (JCNS), Forschungszentrum Jülich, Germany
[7] Bruker Biospin AG, Fällenden, Switzerland
[8] Jülich Center for Structural Biology (JuStruc), Forschungszentrum Jülich, Germany

The 10-23 DNAzyme (Dz) is an artificial DNA oligomer which can be designed to cleave virtually any RNA target with high specificity. It carries a tremendous therapeutic and biotechnological potential, however, so far, it could not fulfill the high expectations, mainly due to lack of structural and functional understanding. Here we used solution NMR spectroscopy to provide high-resolution insights into all stable states occurring in the Dz's catalytic cycle. We also could obtain the first structure of the pre- catalytic Dz:RNA complex using state-of-the-art techniques, such as exact NOEs, paramagnetic spinlabels and residual dipolar coupling, as well as novel approaches, like ^{19}F experiments and homology restraints. We further determined binding sites as well as structural and dynamic effects of metal ions and could link our structural data to key elements of the Dz using systematic activation and mutation studies. Our data reveal that a high amount of cationic metal ions stabilizes the pre-catalytic complex, in which the Dz's catalytic loop is winding around the RNA, bringing catalytic relevant nucleotides in close proximity to the scissile bond. This molecula architecture likely allows the binding of an additional $Mg^{2+,}$ which induces RNA cleavage and leads to multiple exchanging states of the product complex before dissociation. Finally, time-resolved NMR spectroscopy enabled us to follow the DNA-mediated catalytic process in real-time and with atomic resolution and reveals that different steps within the Dz's catalytic cycle occur at different rates. Overall, our mechanistic insights into the 10-23 DNAzyme-mediated catalysis may provide guidance to overcome current limitations of this fascinating system.

THE INTERACTION BETWEEN THE BREAST CANCER 1 PROTEIN AND DS-DNA ENDS MODELLED BY SOLVENT PARAMAGNETIC RELAXATION ENHANCEMENTS AND MOLECULAR DYNAMICS SIMULATIONS

Kateryna Che[1], Gregory L. Olsen[1], Wiktor Koźmiński[2], Dennis Kurzbach[1]

[1] Faculty of Chemistry, Institute of Biological Chemistry, University Vienna, Währinger Straße 38, 1090 Vienna, Austria.
[2]Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland.
E-mail: kateryna.che@univie.ac.at

The human breast cancer susceptibility protein 1 (BRCA1) protein plays key roles in a diverse array of essential cellular processes [1, 2]. In particular, mutations of BRCA1 have been associated with a high risk of both breast and ovarian cancer [1].

Many BRCA1 functions, such as DNA double-strand break repair, involve direct BRCA1-DNA interactions [3]. However, despite the importance of these functions, little is known about the structure of BRCA1-DNA complexes, due in large part to the challenges of studying the DNA-binding site, which is embedded in a large intrinsically disordered domain (IDD).
Here we present results from the joint application of NMR spectroscopy and computational techniques to characterize BRCA1-DNA binding under near-physiological conditions, and present a first model of a BRCA1-DNA complex.

We identify two binding sites, connected by a 20 amino acid-long hinge region, which – in line with the involvement of BRCA1 in double-strand break signaling – bind head-on to exposed ds- DNA ends and the neighboring major grove.

As IDD structural dynamics are strongly influenced by environmental conditions, we performed all experiments in simulated body fluid (mSBF) rather than simple phosphate buffers, to approach conditions more nearly representative of the native cellular environment. To identify the binding sites, chemical shift perturbations were monitored using ^{1}H-^{15}N-^{13}C HNCO spectra acquired for a ca. 300 aa long BRCA1 construct following seven successive additions of a DNA oligomer (Fig. 1b.). Solution paramagnetic relaxation enhancement (sPRE) experiments confirmed the binding sites (Fig. 1c.).

These findings are complemented by structural modeling of the binding region. We predicted the structure of the 67 aa-long binding site using I-TASSER modelling and docked it *in-silico* to the DNA oligomer. The modeled structure corroborated the NMR-derived binding sites (Fig. 1a.).

References

[1] Qiang Wang, Hongtao Zhang, Kiichi Kajino and Mark I Greene. (1998) BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. Oncogene **17**, 1939-1948.

[2] Wen-Ying Mark, Jack C. C. Liao, Ying Lu, Ayeda Ayed, Rob Laister Blair Szymczyna, Avi Chakrabartty and Cheryl H. Arrowsmith. (2005). Characterization of Segments from the Central Region of BRCA1: An Intrinsically Disordered Scaffold for Multiple Protein–Protein and Protein–DNA Interactions. Journal of Molecular Biology **345**(2):275-87.

[3] Riffat Naseem, Alice Sturdy, David Finch, Thomas Jowitt, and Michelle Webb. (2006). Mapping and conformational characterization of the DNA-binding region of the breast cancer susceptibility protein BRCA1. Biochem Journal. 2006 May 1; **395**(Pt 3): 529–535.

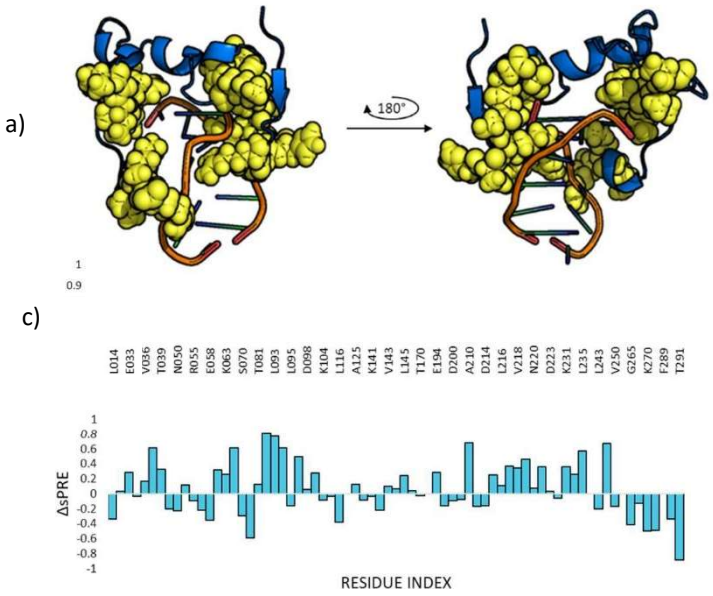


Figure 1. a) Model structure of a $BRCA_1$-DNA oligomer complex (amino acids 444-504). The interacting residues are shown as yellow spheres. b) Chemical shift perturbations upon DNA binding obtained from ^{1}H-^{15}N-$^{13}CHNCO$ spectra. The binding interface from the model structure is indicated by the yellow bars. c) Differential solvent PRE values ΔsPRE from TEMPOL cosolutes illustrating the differing sPRE responses observed for $BRCA_1$ in the presence and absence of DNA. The DNA binding site clearly shows less accessibility upon DNA interaction (negative values; yellow bar), while the rest of the construct experiences somewhat stronger sPRE upon DNA interaction.

EXPLORING THE MOLECULAR MECHANISMS OF ELECTRON SHUTTLING: FROM PROTEIN STRUCTURE TO FUNCTIONAL PROCESSES USING NMR

Anaísa Coelho[1], Bruno M. Fonseca[1], Ricardo O. Louro[1], Catarina M. Paquete[1]

[1]Inorganic Biochemistry and NMR Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal.

The contribution of Fe(II)-oxidizing bacteria (FeOB) to iron cycling in freshwater, groundwater, and marine environments, as well as in most soils and sediments, has been widely recognized in recent years. These organisms perform extracellular electron transfer (EET) i.e. transfer electrons across the cell envelope and establish an electrical contact with extracellular solids, which has been demonstrated to be performed by numerous multiheme *c*-type cytochromes. However, the molecular mechanism for this process remains to be elucidated.

The Gram-negative bacterium *Sideroxydans lithotrophicus* ES-1 (ES-1) oxidizes soluble ferrous iron at the surface of the cell through the Mto redox pathway composed by 4 proteins: the decaheme porin-cytochrome complex MtoAB that functions as an electron conduit through the outer-membrane; the periplasmic monoheme cytochrome MtoD that is proposed to transfer electrons across the periplasmic space to the cytoplasmic membrane; and the inner-membrane protein CymA. This protein is responsible to insert electrons into the quinone pool.

The understanding of how ES-1 couples extracellular electron uptake to metabolic processes is crucial to understand how it performs EET and its impact in the biogeochemical cycle of iron. Toward this objective, the MtoD protein was characterized by NMR spectroscopy.
The assignment of the ^{1}H NMR signals of the heme was performed for the reduced protein and the heme methyl signals in the oxidized state were cross assigned through ^{1}H-NOESY spectra using partially oxidized samples. Triple resonance experiments were used to assign the signals of the protein in the reduced state. To obtain insights on the interaction between MtoD and CymA ^{1}H-1D-NMR spectra were recorded for each addition of CymA to a sample of MtoD. In addition, other NMR experiments, including triple resonance experiments, will be carried out in order to identify the MtoD residues responsible for interacting with CymA. This work defines the molecular details of the injection of extracellular electrons collected from ferrous iron into the bioenergetic metabolism of ES-1. This information is the first step in the complete elucidation of the EET mechanisms of electron uptake by ES-1, a knowledge that is still lacking and is crucial to understand the biogeochemical cycling of metals in the environment.

CATCH ME IF YOU CAN – HOW THE RIBOSOME MELTS MRNA SECONDARY STRUCTURES FOR TRANSLATION INITIATION

Vanessa de Jesus [1], Nusrat S. Qureshi[1], Sven Warhaut[1], Marina Dietz[2], Jasleen K. Bains[1], Julian Langer[3], Mike Heilemann[2], Harald Schwalbe[1], Boris Fürtig[1]

[1] Institute of Organic Chemistry and Chemical Biology - Center for Biomolecular Magnetic Resonance (BMRZ), Goethe-University Frankfurt, Germany
[2] Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, Germany
[3] Max Planck Institute of Biophysics, Frankfurt, Germany

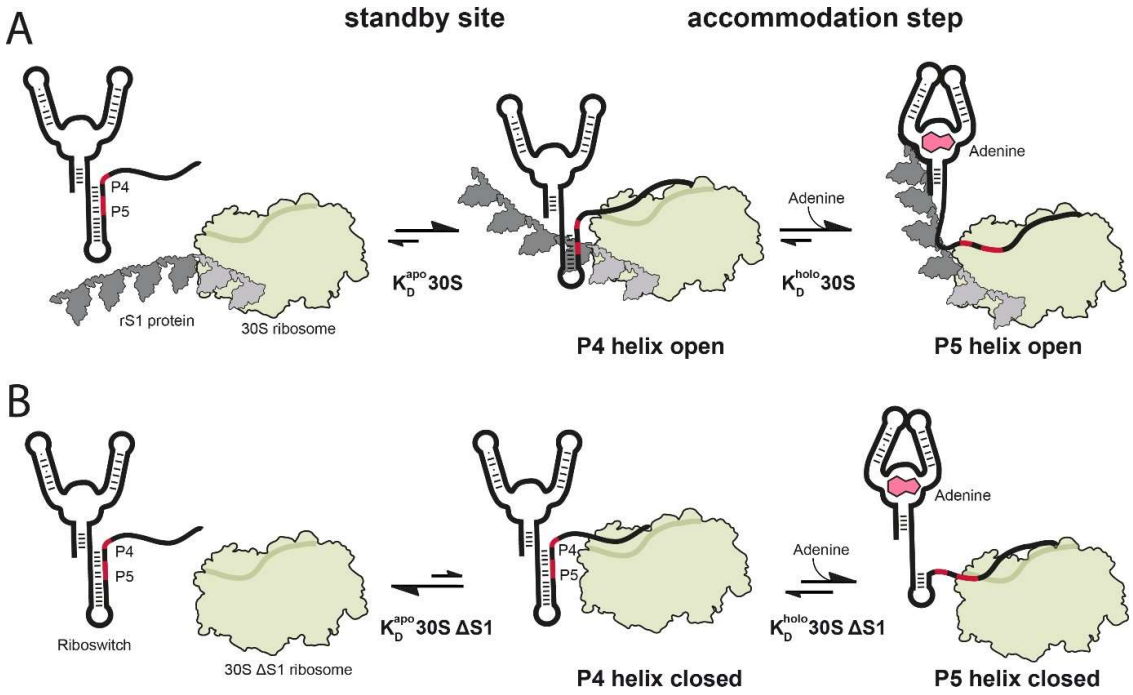
Translation initiation is the rate limiting step in protein synthesis. For translation initiation, the ribosome has to bind to an unfolded translation initiation region TIR (containing SD-sequence and AUG start codon) to form a stabilizing Shine-Dalgarno-anti-Shine-Dalgarno interaction. The standby model describes the relationship between RNA secondary structures around the ribosome binding site (RBS) and the resulting translation efficiency[1]. It explains the paradoxical observations of high translation rates from an mRNA with a stable base paired TIR that should be theoretically completely inhibitory.

Here, we investigate a highly structured 5'-untranslated region (UTR) containing a riboswitch to study the structural dynamics of an mRNA in the context of the standby model. Previous studies have focused on the understanding and characterization of the riboswitch alone. In this adenine-sensing riboswitch from *Vibrio vulnificus*, the apo conformation sequesters the RBS in stable base pair interactions[2]. Adenine binding to the aptamer domain releases the expression platform, providing a single-stranded RBS. We demonstrate that adenine binding is not sufficient to accommodate the mRNA within the ribosomal mRNA-tunnel, as a hairpin flanking the RBS blocks full mRNA accommodation due to steric clashes. By employing an integrated NMR, MST and FCS approach, we find that the chaperone function of the ribosomal S1 protein bound to 30S is essential for a complete melting of the RBS and rS1's presence enables the formation of an initiation complex. In fact, the investigated riboswitch does not function as a stand-alone RNA translation-regulatory system but relies on components of the translation machinery.

Our findings suggest re-evaluating both the translational-regulation mechanism of riboswitches stemming from gram-negative bacteria, as their function appears to be intimately linked to the ribosomal S1 protein as well as the standby model, as parts of the ribosome actively interfere with the unfolding of structured 5'-UTRs.

References

[1] De Smit, M. H. & Van Duin, J. Translational standby sites: How ribosomes may deal with the rapid folding kinetics of mRNA. *Journal of Molecular Biology* **331,** 737–743 (2003).
[2] Reining, A. *et al.* Three-state mechanism couples ligand and temperature sensing in riboswitches. *Nature* **499,** 355–360 (2013).


A
standby site
accommodation step
P4
P5
rS1 protein
30S ribosome
K_D^{apo} 30S
P4 helix open
Adenine
K_D^{holo} 30S
Adenine
P5 helix open
B
P4
P5
Riboswitch
30S ΔS1 ribosome
K_D^{apo} 30S ΔS1
P4 helix closed
Adenine
K_D^{holo} 30S ΔS1
Adenine
P5 helix closed

INTEGRATIVE MODELLING OF THE INTRINSICALLY DISORDERED MYC ASSOCIATED FACTOR X USING 13C DIRECT-DETECTED NMR, PARAMAGNETIC RELAXATION ENHANCEMENTS AND NANOSCALE EPR DISTANCE MEASUREMENTS

Ludovica M. Epasto[1], Gregory L. Olsen[1], Thomas Kress[2], Giuseppe Sicoli[3], Hervé Vezin[3], Karin Ledolter[4], Dennis Kurzbach[1]

E-mail: ludovica.martina.epasto@univie.ac.at
[1]Faculty of Chemistry, Institute for Biological Chemistry, University of Vienna,Währinger Straße 38, 1090, Vienna, Austria.
[2]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, U.K.
[3]Laboratoire Avancé de Spectroscopie pour les Interactions, la Réactivité et l'Environnement (LASIRE), UMR CNRS 8516, Université de Lille, Avenue Paul Langevin - C4, F-59655 Villeneuve d'Ascq, France.
[4]Department for Structural and Computational Biology, Max F. Perutz Laboratories, University Vienna, Campus Vienna BioCenter 5, 1030 Vienna, Austria.

Transcription factors (TFs) play a critical role in numerous physiological and pathological processes. Many important TFs are characterized by a notably high degree of conformational plasticity, suggesting that intrinsic disorder (ID) may be essential to fulfill their functional roles. Indeed, many folds into stable structures only upon DNA binding; in the DNA-free state, conformational heterogeneity often renders characterization of these substrates experimentally challenging. Hence, despite their considerable medicinal importance, atomic-level details of their structures, dynamics, and interactions are often limited.
MAX (MYC associated transcription factor X), a member of the basic helix-loop-helix leucine zipper (bHLH-LZ) family of TFs, is an important example of a partially disordered protein. It forms a stiff elongated coiled-coil helical dimer, adjoining a disordered N-terminal domain that houses the DNA binding site. The dysregulated expression and activity of MYC and MAX is of central importance in several tumor types. However, NMR characterization of MAX is complicated by weak signal intensities and crowded spectra, owing to both the slow and anisotropic rotation of the dimer and the disorder in the binding domain.
Here we address these limitations, using a combination of carbon direct-detected NMR experiments, paramagnetic relaxation enhancements, MD simulations, and nanoscale electron paramagnetic resonance distance measurements (Fig.1A and B).[1-4]
With this approach we could develop detailed models showing how the transition between the inactive monomeric and the active dimeric state of MAX proceeds, and that in both the DNA-free *and* bound states the intrinsically disordered DNA-binding domain of MAX undergoes large scale conformational fluctuations (Fig. 1C). The free state switches between a fully disordered and a partially folded state that promote the DNA binding through the binding-site exposure. The final DNA-bound form samples a continuum of states between opened and closed conformations to enable DNA translation and DNA element recognition.

References

[1] G. Sicoli, H. Vezin, K. Ledolter, T. Kress and D. Kurzbach, *Nucleic Acids Res*, 2019, **47**, 5429-5435.
[2] G. Kizilsavas, K. Ledolter and D. Kurzbach, *Biochemistry*, 2017, **56**, 5365-5372.
[3] G. Sicoli, T. Kress, H. Vezin, K. Ledolter and D. Kurzbach, *J Phys Chem Lett*, 2020, **11**, 8944-8951.
[4] S. Gil, T. Hošek, Z. Solyom, R. Kümmerle, B. Brutscher, R. Pierattelli, I. C. Felli, *Angew. Chem. Int. Ed.* 2013, **52**,11808-11812

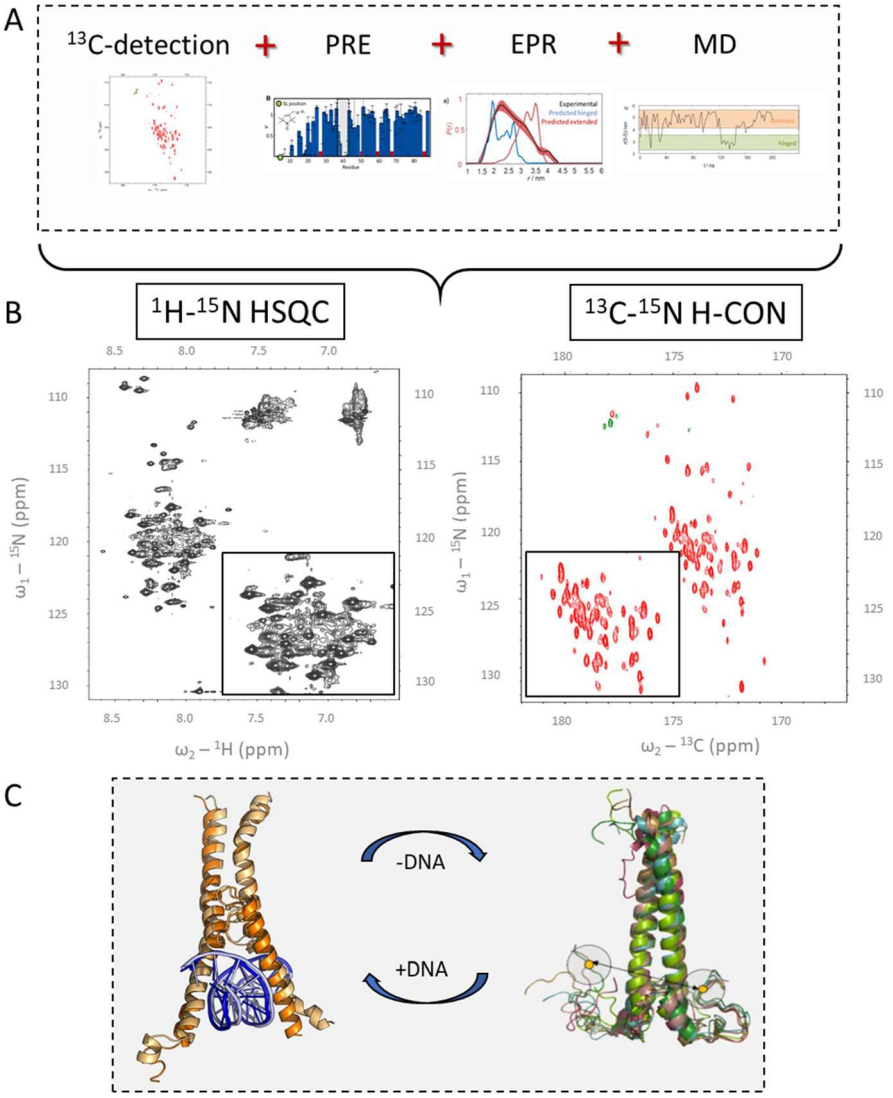


Figure 1. **(A)** Integrated approach for the structural characterization of MAX:MAX and MAX:MAX:DNA as a combination of ^{13}C NMR detections, PRE, EPR measurements and MD simulations. **(B)** Comparison between MAX:MAX spectrum at 35°C in a ^{1}H/^{15}N HSQC experiment (left) vs. a ^{13}C/^{15}N correlation experiment (right). The peaks belonging to the core of MAX are better resolved in the ^{13}C/^{15}N experiment than in the HSQC. **(C)** Structural models representing MAX:MAX in the presence and absence of DNA. The protein can be in an opened conformation or in a closed conformation when bound to DNA in solution. In the absence of DNA the N-terminus remains disordered and interconverts rapidly between several conformations, in which the intrinsically disordered domain (IDD) transiently folds back along the dimer.

STRUCTURAL INSIGHTS ON FUSED IN SARCOMA LIQUID-LIQUID PHASE SEPARATION

Sara S. Félix[1], Philip O'Toole[1], Javier Oroz[2], Douglas V. Laurents[2], Eurico J. Cabrita[1]

[1] UCIBIO, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal
[2] Instituto de Química-Física Rocasolano, CSIC, Spain

Fused in Sarcoma (FUS) is a ubiquitously expressed DNA/RNA binding protein, which is involved in several regulatory nucleic acid processes. In certain stress conditions, FUS is known to undergo liquid-liquid phase separation (LLPS), a mechanism that leads to the formation of membraneless proteinaceous condensates, crucial for cellular survival.[1] These condensates, known as stress granules (SGs) are formed via condensation of stalled mRNA, RNA-binding proteins (RBPs) and intrinsically disordered proteins (IDPs) or proteins containing intrinsically disordered regions (IDRs). A combination of distinct weak and transient interactions are in the core of the formation and dynamics of SGs.[2] This is the case of FUS which is able to interact with RNA via the RNA recognition domain (RRM) and zinc-finger domain (ZnF), and also of participating in promiscuous protein- protein, protein-RNA and protein-ligand interactions via the low complexity (LC) domain and the intrinsically disordered arginine-glycine-glycine (RGG) motifs. Although vital for the cell, SGs can soon evolve into pathological amyloids. FUS inclusion are a hallmark of familial amyotrophic lateral sclerosis (fALS) and frontotemporal lobar degeneration (FTLD), two progressive motor neuron disorders.[1] Currently, there is still low comprehension about the mechanisms and driving forces that lead to controlled protein LLPS and consequent aberrant aggregation in these neurodegenerative diseases. For this reason, deciphering the structural basis of FUS LLPS is a crucial step towards the comprehension of FUS-associated neurodegeneration, which is critical for the development of effective therapies. For this, we used NMR and microscopic methods to study the effect of phase separation on full-length FUS and RGG1-RRM-RGG2, together with the effect of several stress mediators and cellular metabolites. Our preliminary data suggests that contrary to previous studies, that focus on the IDRs and LC domains as the main mediators of phase separation, RNA-binding domains actively participate in the LLPS process.

Our study provides the first structural insight on the full-length FUS biomolecular condensate formation via phase separation.

References:

[1.] Chen, C., Ding, X., Akram, N., Xue, S. & Luo, S. Z. Fused in sarcoma: Properties, self-assembly and correlation with neurodegenerative diseases. *Molecules* **24**, (2019).
[2.] Youn, J. Y. *et al.* Properties of Stress Granule and P-Body Proteomes. *Molecular Cell* **76**, 286–294 (2019).

STRUCTURAL AND FUNCTIONAL INSIGHTS OF AN UNPRECEDENTED MULTIHEME CYTOCHROME FROM *GEOBACTER SULFURREDUCENS*

Tomás M. Fernandes[1], Leonor Morgado[1], Filipe Folgosa[2], Miguel Teixeira[2], Carlos A. Salgueiro[1]

[1]UCIBIO, Chemistry Department, NOVA School of Science and Technology, NOVA University of Lisbon, Campus Caparica, 2829-516 Caparica, Portugal
[2]Instituto de Tecnologia Química e Biológica António Xavier, NOVA University of Lisbon, Avenida da República (EAN), 2780-157 Oeiras, Portugal

Geobacter bacteria have been inspiring the development of many biotechnological applications, which seek improvements in the fields of microbial energy production, bioremediation of contaminated waters and soils, development of sustainable electronic devices and materials, as well as biomedical devices [1-3]. *Geobacter sulfurreducens*, the microorganism with the highest current production in microbial fuel cells known to date, possesses over 100 cytochromes that assure an effective electron transfer between the intracellular components and the cell exterior [4]. The most abundant group of cytochromes in this microorganism is the PpcA-family, composed of five periplasmic c_7 triheme cytochromes with high structural homology and identical heme coordination (His-His) [5]. GSU0105 is a periplasmic triheme cytochrome expressed by *G. sulfurreducens* in Fe(III) reducing conditions, but not expressed at all in cultures grown on fumarate, linking the cytochrome directly with extracellular electron transfer (EET). This cytochrome possesses a low sequence identity with the cytochromes from the PpcA-family and a inevitably different heme-coordination, based on the analysis of its amino acid sequence. This detail, together with the direct linkage with EET pathways, makes GSU0105 an interesting target for functional and structural studies. In this work, several biophysical techniques, including UV-visible, CD, EPR and NMR spectroscopies, were used to characterize the cytochrome. Initial evidences about the secondary structural elements and redox behaviour of the cytochrome were probed. NMR and EPR were used to pinpoint the spin-states of the heme groups in different oxidation states. The results obtained, together with BLAST and structural predictions, support the fact that GSU0105 is a member of a new subclass of triheme cytochromes.

References

[1] X. Liu, H. Gao, J. E. Ward, X. Liu, B. Yin, T. Fu, J. Chen, D. R. Lovley, J. Yao, Power generation from ambient humidity using protein nanowires, *Nature* **578**, 550–554 (2020).
[2] F. Wang, Y. Gu, J. P. O'Brien, S. M. Yi, S. E. Yalcin, V. Srikanth, C. Shen, D. Vu, N. L. Ing, A. I. Hochbaum, E. H. Egelman, N. S. Malvankar, Structure of microbial nanowires reveals stacked hemes that transport electrons over micrometers, *Cell* **177**(2), 361-369 (2019).
[3] A. F. Smith, X. Liu, T. L. Woodard, T. Fu, T. Emrick, J. M. Jiménez, D. R. Lovley, J. Yao, Bioelectronic protein nanowire sensors for ammonia detection, *Nano Research* **13**, 1479–1484 (2020).
[4] D. R. Lovley, T. Ueki, T. Zhang, N. S. Malvankar, P. M. Shrestha, K. A. Flanagan, M. Aklujkar, J. E. Butler, L. Giloteaux, A. E. Rotaru, D. E. Holmes, A. E. Franks, R. Orellana, C. Risso, K. P. Nevin, *Geobacter*: The microbe electric's physiology, ecology, and practical applications, *Advances in Microbial Physiology* **59**, 1-100 (2011).

[5] P. R. Pokkuluri, Y. Y. Londer, X. Yang, N. E. Duke, J. Erickson, V. Orshonsky, G. Johnson, M. Schiffer, Structural characterization of a family of cytochromes c_7 involved in Fe(III) respiration by *Geobacter sulfurreducens*, Biochimica et Biophysica Acta **1797**, 222-232 (2010).

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia (FCT) through the following grants: SFRH/BD/132SFRH/BD/145039/2019 (to TMF), SFRH/BPD/114848/2016 (to LM), PTDC/BIA-BQM/27959/2017 and PTDC/BIA-BQM/31981/2017 (to CAS). This work was also supported by (i) the Applied Molecular Biosciences Unit – UCIBIO, which is financed by national funds from FCT/MEC (UID/Multi/04378/2019), (ii) the ITQB MOSTMICRO (UIDB/04612/2020 and UIDP/04612/2020) Research Unit, cofounded by FCT, through National funds, and by FEDER under the PT2020 Partnership Agreement and (ii) the European Union's Horizon 2020 research and innovation program, under grant agreement 810856. The NMR spectrometers are part of the National NMR Network (PTNMR) and are supported by FCT-MCTES (ROTEIRO/0031/2013 – PINFRA/22161/2016) co-funded by FEDER through COMPETE 2020, POCI, and PORL and FCT through PIDDAC.

THE THERMODYNAMIC PROPERTIES OF TRIHEME CYTOCHROME PPCF FROM *GEOBACTER METALLIREDUCENS* IN THE PRESENCE OF NITRATE

Marisa R. Ferreira[1], Tomás M. Fernandes, Carlos A. Salgueiro

[1] UCIBIO-Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus Caparica, 2829-516 Caparica, Portugal

In the recent years, *Geobacter* bacteria have been presented as microorganisms of high interest in the bioremediation and bioenergy fields, due to their ability for oxidizing several organic and inorganic compounds, as well as for their high current density production in microbial fuel cells. The bacterium G*eobacter metallireducens* (*Gm*) show distinctive features compared the better studied *Geobacter sulfurreducens* (*Gs*), as it has more efficient Fe (III) reduction rates and can convert nitrate to ammonia. While *Gs* is not capable to respire nitrate (1), *Gm* is able to growth with nitrate as soluble electron acceptor (2). Two operons are responsible for the nitrate reductase activity in *Gm*, the *narGYJI*, which are adjacent to the narK-1 and narK-2 genes that encodes for a proton/nitrate symporter and a nitrate/nitrite antiporter, respectively, and the *narGYI* cluster which is missing the noncatalytic subunit (*narJ*). A diheme *c*-type cytochrome (Gmet_0328 and Gmet_1019) is the first gene encoded in both operons which leads to the suggestion that nitrate reductase may be involved in other electron transfer mechanisms besides the menaquinol pool. Then, the product of the ppcF gene (Gmet_0335) of the intact *nar* operon which encodes for a periplamic *c*-type triheme cytochrome involved in the Fe (III) reduction, may be responsible for the electron transfer to the nitrate reductase from extracellular electron donors such as humic substances (3) or graphite electrodes (4). The final two genes of the intact *nar* operon (Gmet_0336 and Gmet_0337), encode the MoeA and MoaA enzymes implicated in biosynthesis of bis-(molybdopterin guanine dinucleotide)-molybdenum, an essential cofactor of the nitrate reductase (5).In the present we used NMR spectroscopy couple to visible redox titrations to determine the redox properties of each heme group in the cytochrome PpcF. The results obtained in this study show that the heme reduction potentials are negative, different from each other. The considerable separation of the hemes' redox potential values facilitates a sequential transfer within the chain of redox centers in PpcF, thus assuring electron transfer directionality to the electron acceptors.

References

[1] Caccavo F, Jr., Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ. Geobacter sulfurreducens sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol. 1994 Oct;**60**(10):3752-9.

[2] Lovley DR, Phillips EJ. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl Environ Microbiol. 1988 Jun;**54**(6):1472-80.

[3] Lovley DR, Fraga JL, Coates JD, Blunt-Harris EL. Humics as an electron donor for anaerobic respiration. Environ Microbiol. 1999 Feb;**1**(1):89-98.

[4] Gregory KB, Bond DR, Lovley DR. Graphite electrodes as electron donors for anaerobic respiration. Environ Microbiol. 2004;**6**(6):596-604.

[5] Aklujkar M, Krushkal J, DiBartolo G, Lapidus A, Land ML, Lovley DR. The genome sequence of Geobacter metallireducens: features of metabolism, physiology and regulation common and dissimilar to Geobacter sulfurreducens. BMC microbiology. 2009;9:109.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia grant PTDC/BIA-BQM/31981/2017 (to CAS).

NMR INVESTIGATION OF THE PHOSPHORYLATION KINETICS AND ACTIVATION STUDIES OF THE GUARDIAN OF THE GERM LINE TAP63A

Jakob Gebel[1], Marcel Tuppi[1,3], Apirat Chaikuad[2], Stefan Knapp[2], Volker Dötsch[1]

[1]Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance and Cluster of Excellence Macromolecular Complexes (CEF), Goethe University, Frankfurt/Main, Germany.
[2]Institute of Pharmaceutical Chemistry, Goethe University, Frankfurt/Main, 60438; Germany.
[3]Present address: The Francis Crick Institute, London, UK.

The decision of life and death of a cell requires “analog” input stimuli, such as DNA damage, to be translated into a binary yes/no answer. In mammalian oocytes the transcription factor TAp63α (hereafter p63), a member of the highly homologous p53 family, is strongly expressed, but shows no transcriptional activity.[1] A meta-stable dimeric state masks the transactivation domains and lowers the DNA binding affinity of the protein, thereby inhibiting transactivation on pro-apoptotic promoters.[2] This meta-stable state is stabilized by a network of charged residues created by a negatively charged N-terminus and a positively charged C-terminus.[3] DNA damage inflicted by γ-irradiation or chemotherapeutic drugs lead to phosphorylation of p63 by two independent kinases. Initially the DNA damage activated checkpoint kinase 2 (CHK2) phosphorylates a single serine in close proximity to the C-terminus of the protein. This “priming” phosphorylation creates a substrate for four consecutive phosphorylation events by casein kinase 1 (CK1). The 3rd phosphorylation event of CK1 is sufficient to overcome the meta-stable state by charge-charge repulsion with the N-terminus and leads to tetramerization and thus activation of the protein.[4]

We could show by NMR and mass spectrometry, for isolated peptides of p63 as well as the complete protein, that the 3rd CK1 phosphorylation is more than an order of magnitude slower than the initial two phosphorylation events. Generally this kinetic behavior is dependent on the exact sequence of the substrate, as p63 mutants as well as a totally different substrate do not show this bi-phasic behavior. Furthermore, by crystallography, we could show that CK1 preferentially binds to p63 in an uncommon product-enzyme complex after the second phosphorylation took place.[5]

Slowing the decisive phosphorylation step enables the cell to repair DNA damage and prevent the activation of p63 by degradation or dephosphorylation of the protein.

References

[1] Suh EK, Yang A, Kettenbach A, Bamberger C, Michaelis AH, Zhu Z, Elvin JA, Bronson RT, Crum CP & McKeon F, p63 Protects the Female Germ Line During Meiotic Arrest. *Nature* **444:** 624–628 (2006)
[2] Deutsch GB, Zielonka EM, Coutandin D, Weber TA, Schäfer B, Hannewald J, Luh LM, Durst FG, Ibrahim M, Hoffmann J, Niesen FH, Sentürk A, Kunkel H, Brutschy B, Schleiff E, Knapp S, Acker-Palmer A, Grez M, McKeon F & Dötsch V, DNA damage in oocytes induces a switch of the quality control factor TAp63α from dimer to tetramer. *Cell* **144:** 566–576 (2011)

[3] Coutandin D, Osterburg C, Srivastav RK, Sumyk M, Kehrloesser S, Gebel J, Tuppi M, Hannewald J, Schï¿½fer B, Salah E, Mathea S, Müller-Kuller U, Doutch J, Grez M, Knapp S & Dötsch V, Quality control in oocytes by p63 is based on a spring-loaded activation mechanism on the molecular and cellular level. *Elife* **5:** 1–22 (2016)

[4] Tuppi M, Kehrloesser S, Coutandin DW, Rossi V, Luh LM, Strubel A, Hötte K, Hoffmeister M, Schäfer B, De Oliveira T, Greten F, Stelzer EHK, Knapp S, De Felici M, Behrends C, Klinger FG & Dötsch V, Oocyte DNA damage quality control requires consecutive interplay of CHK2 and CK1 to activate p63. *Nat. Struct. Mol. Biol.* **25:** 261–269 Available at: http://dx.doi.org/10.1038/s41594-018-0035-7 (2018)

[5] Gebel J, Tuppi M, Chaikuad A, Hötte K, Schröder M, Schulz L, Löhr F, Gutfreund N, Finke F, Henrich E, Mezhyrova J, Lehnert R, Pampaloni F, Hummer G, Stelzer EHK, Knapp S & Dötsch V, p63 Uses a Switch-Like Mechanism To Set the Threshold for Induction of Apoptosis. *Nat. Chem. Biol.* **16**:1078-1086 (2020)

DO WE CORRECT WHAT WE THINK WE DO? A CASE STUDY ON SECONDARY STRUCTURE PROPENSITIES OF A-SYNUCLEIN

Dániel Kovács[1], Andrea Bodor[1]

[1] Eötvös Loránd University, Budapest, Hungary, Faculty of Science, Institute of Chemistry

Intrinsically disordered proteins and protein regions (IDPs, IDRs) constitute a large part of eukaryotic proteomes, yet their physicochemical characterization is hindered by the lack of stable secondary and tertiary structure upon which most theoretical and analytical tools in protein research are based [1]. Instead, IDP's possess certain traits of ordered structure called transient structure, residual structure, structural propensity etc. As a result of the conventional perception of globular proteins, regions with, although mild but detectable, structural propensities are in the focus of efforts aimed at understanding and influencing the behavior of IDPs.

The secondary chemical shift (SCS) is the primary atomic scale observable utilized in the evaluation of the secondary structure propensities of IDP's [2]. For the computation of SCSs, reference values called random coil chemical shifts (RCCSs) are required. Literature is abundant in methods that derive/ calculate RCCSs based on either small peptides or information of protein databases. The obtained RCCSs do not really affect the structural assessment of globular proteins. However, they can have drastic effects in the case of IDPs, where structurally induced deviations from RCCS are of a comparable magnitude with those resulting experimental conditions such as pH, temperature, ionic strength, chemical shift referencing. Despite the vast amount of experimental and computational work done in the field, no thorough and systematic comparison of RCCS calculation methods has been published.

Herein, we present examples of the effect of the pH-dependence of chemical shifts on SCSs of α-synuclein. These are present despite using RCCS values yielded by the two publicly available calculation methods which take pH into account explicitly and on a semi-continuous scale [3,4]. Consequent influences on residual structure evaluation are discussed. Statistical inference is utilized in order to find the RCCS dataset which best represents the consensus of all available RCCS calculation methods.

References

[1] P.E. Wright, H.J. Dyson, Intrinsically unstructured proteins: re-assessing the protein structure–function paradigm. *J. Mol. Biol.* **293** 321–331 (1999).
[2] D.S. Wishart, Interpreting protein chemical shift data. *Prog. Nucl. Magn. Reson. Spectrosc.* **58** 62–87 (2011).
[3] M. Kjaergaard, S. Brander, F. M. Poulsen: Random coil chemical shift for intrinsically disordered proteins: effects of temperature and pH. *J. Biomol. NMR*, **49** 139-149 (2011).
[4] J.T. Nielsen, F.A.A. Mulder, POTENCI: prediction of temperature, neighbor and pH-corrected chemical shifts for intrinsically disordered proteins, *J. Biomol. NMR* **70** 141-165 (2018).

MAS NMR STRUCTURE OF HUMAN COFILIN-2 BOUND TO ADP-F-ACTIN REVEALS ISOFORM SPECIFIC CONFORMATION AND BINDING MODE

Jodi Kraus[1], Elena Kudryashova[2], Ryan W. Russell[1], Dmitri Kudryashov[2], Tatyana Polenova[1]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark DE 19716.
[2]Department of Chemistry and Biochemistry, The Ohio State University, Columbus OH 43210.

Actin, a component of the eukaryotic cytoskeleton, is one of the most ubiquitous and conserved proteins. The cofilin/ADF family of proteins function in the regulation of actin filament disassembly.[1,2] However, there is a lack of atomic-resolution information regarding the structure and dynamics of cofilin/ADF proteins bound to actin filaments. Here we report the atomic resolution structure of human cofilin-2 assembled on ADP-F-actin. From ^{13}C-^{13}C CORD and PAIN-CP spectra acquired on 2-^{13}C-glucose,^{15}N and 1,6-^{13}C- glucose,^{15}N-labeled cofilin, we assigned 1,277 unambiguous and 335 ambiguous distance restraints, which were used for structure calculation. Our structure has a heavy atom precision RMSD of 1.5 Å with an equivalent resolution of 2.3 Å (Figure 1). The structure reveals an extensive network of previously unobserved hydrogen bonding interactions and a novel isoform-specific conformation for human cofilin-2. This unique conformation results in an extended binding pocket that increases the surface contacts with actin. We postulate that the extended binding surface is linked to differences in actin filament severing that are observed in the presence of human cofilin-2 compared to other isoforms.

References

[1] Bernstein, B.W. & Bamburg, J.R., *Trends in Cell Biology* **20**, (2010) 187-195.
[2] Andrianantoandro, E. & Pollard, T.D., *Molecular Cell* **24**, (2006) 13-23.
[3] Yehl, J., Kudryashova, E., Reisler, E., Kudryashov, D. & Polenova, T., *Scientific Reports* **7,** (2017) 44506.

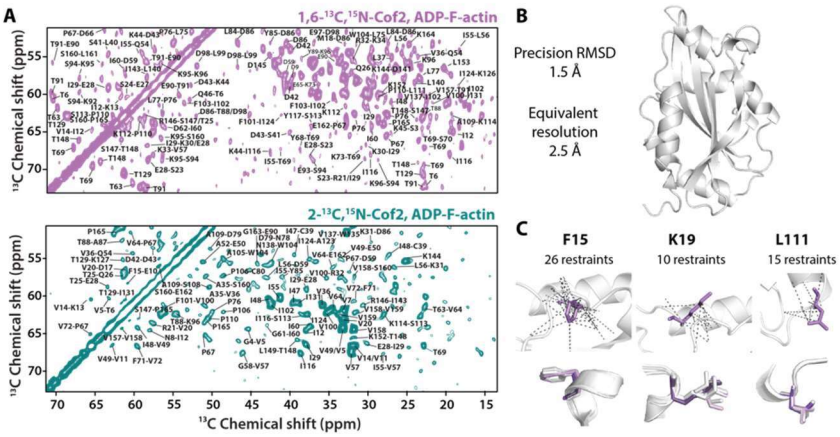


Figure 1. **A**. 2D ^{13}C-^{13}C CORD spectra used for inter-residue distance restraints. **B**. Lowest energy MAS NMR structure of human cofilin-2 bound to F- actin. **C**. Distance restraint network and local alignment of structure ensemble for selected residues.

BRAIN AND ACUTE LEUKEMIA CYTOPLASMIC – AN NMR STUDY

Andras Lang[1], Amit Kumar[1], Jan Jirschitzka[2], Christoph Wiedemann[3], Frank Bordusa[3], Oliver Ohlenschläger[1]

[1] Leibniz Institute on Aging – Fritz Lipmann Institute, Beutenbergstr. 11, D-07745 Jena, Germany
[2] Institute of Biochemistry, Department of Chemistry, University of Cologne, Zülpicher Str. 47, D-50674 Cologne, Germany
[3] Institute of Biochemistry and Biotechnology, Charles Tanford Protein Center, Martin- Luther-University Halle-Wittenberg, Kurt-Mothes-Str. 3a, D-06120 Halle, Germany

The Brain And Acute Leukemia, Cytoplasmic (BAALC) is a recently identified 180-residue-long human protein. BAALC is expressed in cells of either hematopoietic or neuroectodermal origin [1]. As a presumably membrane-anchored protein at the cytoplasmic side, it is speculated that BAALC exerts its function at the postsynaptic densities of certain neurons and might play a role in developing cytogenetically normal acute leukemias (CN-AL) when highly overexpressed by myeloid or lymphoid progenitor cells [2]. In order to better understand the physiological role of BAALC and to provide the basis for a further molecular characterization of BAALC, we have initiated a NMR study and fully assigned the backbone resonances (^{13}C, ^{15}N, ^{1}H) [3]. The [^{1}H,^{15}N]-HSQC spectrum of the protein shows very poor signal dispersion in the amide region of the ^{1}H dimension which is typical for fully disordered proteins. BAALC contains four cysteine residues that were all exploited for single spin-labelling that further proved the intrinsically disordered nature. One of these cysteines is followed by a proline suggesting that the BAALC might bind to the heme paramagnetic centre. In a subsequent NMR interaction study, distant transient heme- binding sites were observed as vanishing cross-resonances suggesting that liberated heme from misfolded proteins in leukemic blasts might have pathological relevance.

References

[1] S.M. Tanner, J.L. Austin, G. Leone, L.J. Rush, C. Plass, K. Heinonen, K. Mrozek, H. Sill, S. Knuutila, J.E. Kolitz, K.J. Archer, M.A. Caliguri, C.D. Bloomfield, A. de la Chapelle, BAALC, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. *Proc. Natl. Acad. Sci. U S A* **98,** 13901-13906 (2001).
[2] S. Weber, T. Alpermann, F. Dicker, S. Jeromin, N. Nadarajah, C. Eder, A. Fasan, A. Kohlmann, M. Meggendorfer, C. Haferlach, W. Kern, T. Haferlach, S. Schnittger, *BAALC* expression: a suitable marker for prognostic risk stratification and detection of residual disease in cytogenetically normal acute myeloid leukemia. *Blood Cancer J.* **4,** e173 (2014).
[3] A. Lang, A. Kumar, J. Jirschitzka, F. Bordusa, O. Ohlenschläger, C. Wiedemann, 1H, 13C, and 15N Backbone assignments of the human brain and acute leukemia cytoplasmic (BAALC) protein. *Biomol NMR Assign.* **14**,163-168 (2020).

THE DEVIL IS IN THE DETAILS: HOW NMR SHEDS NEW LIGHT ON NONRIBOSOMAL PEPTIDE SYNTHETASE CYCLIZATION DOMAINS

Subrata Mishra[1], Aswani Kancherla[1], Kenny Marincin[1], Guillaume Bouvignes[2], Nikolaos Sgourakis[3], Daniel Dowling[4], Dominique Frueh[1]

[1]Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, MD, USA
[2]Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL Université, Sorbonne Université, CNRS, Paris, France
[3]Penn Institute for Immunology, Philadelphia, PA, USA
[4]Department of Chemistry, University of Massachusetts Boston, Boston, MA, USA

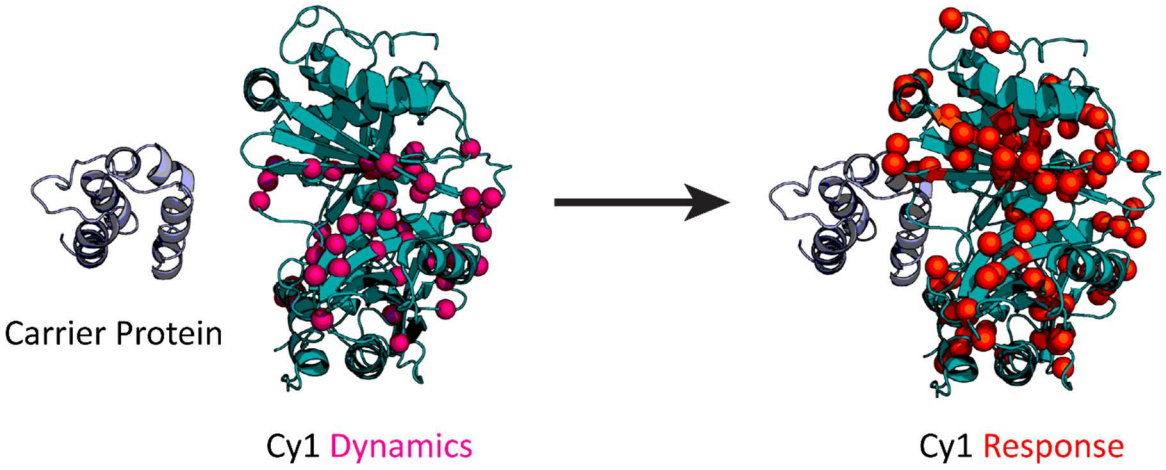
Nonribosomal peptide synthetases (NRPSs) use a modular, multi-domain architecture to assemble a series of substrates into medically relevant secondary metabolites through condensation domains and substrates tethered to partner carrier proteins (CPs). This architecture serves as an attractive route towards generating custom therapeutics. Unfortunately, a dynamic domain architecture has challenged bioengineering approaches of these large systems to produce new bioactive peptides.

Here, we reveal that dynamics within domains should be accounted for when using mutagenesis to engineer NRPSs. We focus on an enhanced condensation domain, the cyclization domain Cy1 from our model system yersiniabactin synthetase, which not only covalently links substrates but also catalyzes heterocyclization – a sought after feature in therapeutic design. Crystallographers have provided many valuable snapshots to derive mechanisms, but various conformations were observed and the significance of these conformations has been debated.[1-5] We now show that these conformational changes do not reflect variations between systems but indicate functional dynamics that must be directly integrated into an interpretation of the domain function.

Using relaxation dispersion NMR and a solution structure, we showed that the conformations seen in crystallography studies reflect ms-µs dynamics in solution. Dynamic residues define an extensive network spanning two distant partner CP binding sites where substrates must engage with Cy1. We then discovered an allosteric response encompassing this network of residues through an *in situ* NMR assay that we designed to monitor the response of Cy1 to one of its partner CP domains in various modified states. Here, we show that introduction of a functionally damaging mutation impairs the ms-µs dynamics observed in wild-type Cy1 and severely reduces its response to its partner CP domain, thereby demonstrating that dynamics mediates the functionally important allosteric response within Cy domains. Dynamics within cyclization and condensation domains must be directly considered when engineering NRPSs to accommodate new substrates in the design ofnovel medicines.

References

[1]Bloudoff K, Fage CD, Marahiel MA, Schmeing TM. Structural and mutational analysis of the nonribosomal peptide synthetase heterocyclization domain provides insight into catalysis. *Proc Natl Acad Sci* **114**, 95–100 (2017).
[2]Dowling DP, Kung Y, Croft AK, Taghizadeh K, Kelly WL, Walsh CT, Drennan C. Structural elements of an NRPS cyclization domain and its intermodule docking domain. *Proc Natl Acad Sci* **113**, 12432–12437 (2016).
[3]Reimer JM, Eivaskhani M, Harb I, Guarné A, Weigt M, Schmeing TM. Structures of a dimodular nonribosomal peptide synthetase reveal conformational flexibility. *Science* **366**, 1-7 (2019).
[4]Bloudoff K, Schmeing TM. Structural and functional aspects of the nonribosomal peptide synthetase condensation domain superfamily: discovery, dissection and diversity. *BBA – Proteins and Proteomics* **1865**, 1587-1604 (2017).
[5]Miller BR, Drake EJ, Shi C, Aldrich CC, Gulick AM. Structures of a Nonribosomal Peptide Synthetase Module Bound to MbtH-like Proteins Support a Highly Dynamic Domain Architecture. *JBC* **291**, 22559-22571 (2016).



REGULATION OF CHAPERONE FUNCTION BY COUPLED FOLDING AND OLIGOMERIZATION

Guillaume Mas[1], Björn M. Burmann[2], Timothy Sharpe[1], Beatrice Claudi[1], Dirk Bumann[1], Sebastian Hiller[1]

[1] Biozentrum, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland.
[2] Department of Chemistry and Molecular Biology, Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, 405 30 Göteborg, Sweden.

Molecular chaperones are central for the survival of the cell (1). For many chaperones, the exact functional mechanisms are not well understood at the atomic level. In particular, it remains mysterious how ATP-independent chaperones can regulate their functional cycles (2). Here, we report a unique activation mechanism regulating the functional cycle of the ATP-independent bacterial chaperone Skp that play a major role for pathogen virulence in host cells (3). The homotrimeric molecular chaperone Skp facilitates the transport of outer membrane proteins across the periplasm. It has been unclear how its activity is modulated during its functional cycle. Combining NMR spectroscopy with biophysical and biochemical techniques, we reveal at an atomic-resolution the monomer- trimer transition that regulate Skp functional cycle. We find that the monomeric state of Skp is intrinsically disordered and that formation of the oligomerization interface initiates folding of the α-helical coiled-coil arms via a unique “stapling” mechanism, resulting in the formation of active trimeric Skp. Native client proteins contact all three Skp subunits simultaneously, and accordingly, their binding shifts the Skp population toward the active trimer. The coupled mechanism is a unique example of how an ATP-independent chaperone can modulate its activity as a function of the presence of client proteins. We show that mutations in Skp affecting proper trimer formation reduce both the growth and survival rate of Salmonella in a mouse infection model demonstrating that Skp activation mechanism is essential for pathogen virulence in the host. Taken together, these finding elucidate a unique chaperone activation mechanism by which bacteria ensure that their outer cell membrane remains intact and functional even under hostile conditions providing a potential new target for the treatment of infectious diseases.

References

[1] F.U Hartl, A Bracher, M Hayer-Hartl, Molecular chaperones in protein folding and proteostasis. Nature **475**, 324–332 (2011).
[2] O Suss, D Reichmann, Protein plasticity underlines activation and function of ATP-independent chaperones. Front Mol Biosci ***2***, 377–10 (2015).
[3] G Mas, B.M Burmann, T Sharpe, B Claudi, D Bumann, S Hiller, Regulation of chaperone function by coupled folding and oligomerization. Sci Adv ***6***, eabc5822 (2020).

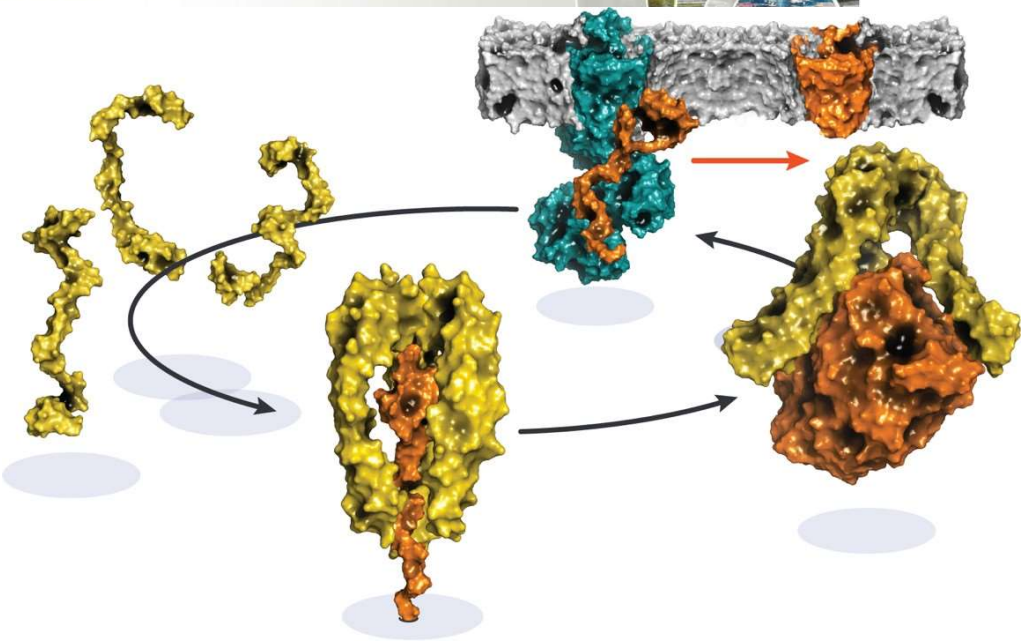


Figure 1. Functional cycle of the periplasmic chaperone Skp. Three Skp proteins (yellow) form a stable structure and transport unfolded OMP (orange) to their destination (from left to right).

THE SL5 ELEMENT LOCATED IN THE 5' GENOMIC END OF IN SARS-COV-2: STRUCTURE OF THE RNA ELEMENT AT THE TRANSLATIONAL START OF ORF1A/B

Andreas Oxenfarth[1], Klara R. Mertinkus[1], Jan-Niklas Tants[2], Andreas Schlundt[2], Christian Richter[3], Robbin Schnieders[1], Sridhar Sreeramulu[1], Anna Wacker[1], Julia E. Weigand[4], Julia Wirmer-Bartoschek[1], Harald Schwalbe[1]

[1] Institute for Organic Chemistry and Chemical Biology,
[2] Institute for Molecular Biosciences,
[3] Institute of Biophysical Chemistry, Center for Biomolecular Magnetic Resonance (BMRZ), Johann Wolfgang Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt/M., Germany.
[4] Department of Biology, Technical University of Darmstadt, Schnittspahnstr. 10, 64287 Darmstadt, Germany

The 5' genomic end of Betacoronaviruses contains several conserved regulatory RNA elements[1]. These structures are critical for the translation initiation efficacy in the SARS- CoV-2 genome[2]. The fifth of these elements, stem-loop 5 (5_SL5), is predicted as a four-way junction, with a stem and three hairpins (Figure 1A). Two of those hairpins, SL5a and SL5b, are each capped by a highly conserved 5'-UUYCGU-3' hexaloop motif. It is assumed that these conserved structures are responsible for RNA-RNA and -protein interactions[3]. In addition, the stem of SL5 of SARS-CoV-2 contains the AUG start codon of open reading frames (ORF1a/b) which encode sixteen different non-structural proteins (Nsps)[2]. 5_SL5 appears to be a promising target for manipulating SARS-CoV-2 subgenomic RNA translation.

We tested the druggability of the SARS-CoV-2 5'UTR by screening individual RNA elements. We found selective unique hits for 5_SL5a and 5_SL5b+c indicating the presence of exclusive RNA structure within SL5. To proceed, we conducted SAXS experiments, combined with FARFAR RNA modelling as model for the structure of the 5_SL5 (Figure 1C). To validate these structural models, the overall NMR structure of the complex SL5 needs to be determined for a detailed analysis of binding sites, and as a rational basis for follow-up interaction studies.

In order to solve the NMR structure of 5_SL5 alone and in complex with potential inhibitors, we divided the construct into 5_SL5stem, 5_SL5a and 5_SL5b+c (Figure 1B). For those constructs, we could assign the imino protons by analyzing cross peaks in a 1H,1H- NOESY experiment as well as correlating nitrogen chemical shifts in 1H,15N-TROSY and HNN-COSY experiments[4]. First hints indicate a similar fold for the UC of the 5'-UUYCGU-3' hexaloop in comparison with the UC of UUCG tetraloop, which was thoroughly analyzed[5].

In our contribution, we will present our on-going results targeting viral RNA of SARS-CoV-2.

References

[1] Madhugiri, R., Fricke, M., Marz, M. & Ziebuhr, J., Coronavirus cis-Acting RNA Elements, *Coronaviruses* (Elsevier), 127–163 (2016).
[2] Miao, Z., Tidu, A., Eriani, G. & Martin, F. Secondary structure of the SARS-CoV-2 5'-UTR. *RNA Biology*, 1–10 (2020).
[3] Sola, I., Mateos-Gomez, P. A., Almazan, F., Zuñiga, S. & Enjuanes, L. RNA-RNA and RNA-protein interactions in coronavirus replication and transcription. *RNA Biology* **8**, 237–248 (2011).
[4] In press.: Wacker, A.; Weigand, J. et al.: Secondary structure determination of conserved SARS-CoV-2 RNA elements by NMR spectroscopy. *Nucleic acids research* (2020).
[5] Nozinovic, S., Fürtig, B., Jonker, H. R. A., Richter, C. & Schwalbe, H., High-resolution NMR structure of an RNA model system: the 14-mer cUUCGg tetraloop hairpin RNA. *Nucleic acids research* **38**, 683–694 (2010).

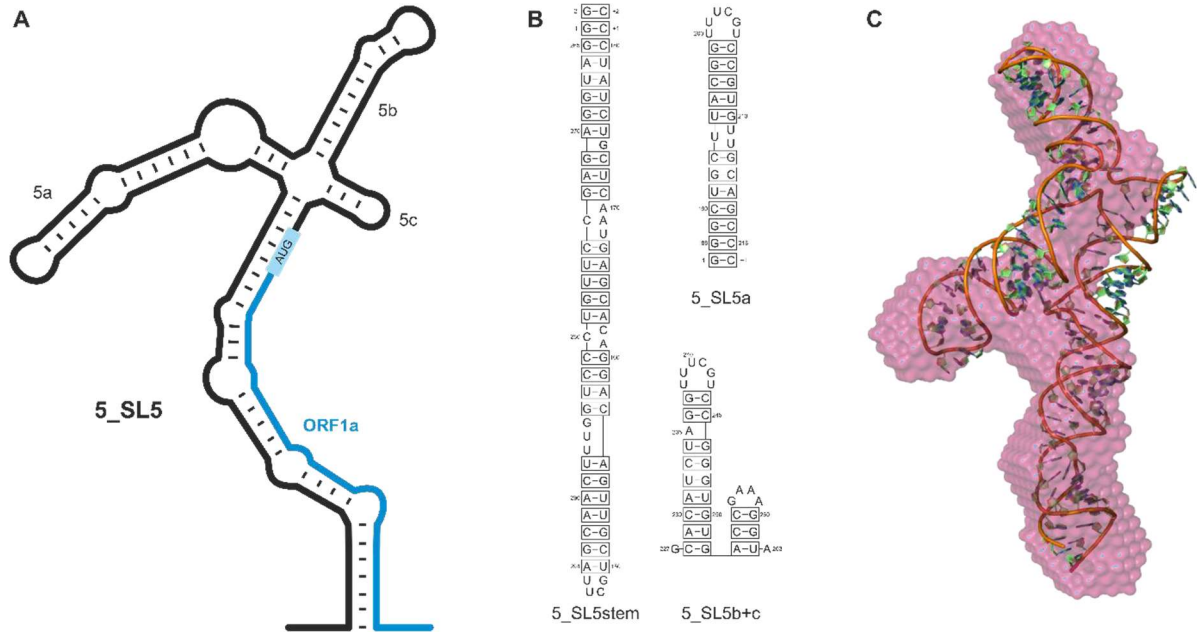


Figure 1. (A) Scheme of the proposed secondary structure of the 5th stem-loop of the SARS-CoV-2 genomic sequence. The annotations highlight the AUG-Start for the open-reading frame 1a (blue) embedded in one of the stem helices. (B) Structures of the constructs 5_SL5stem, 5_SL5a and 5_SL5b+c used for NMR experiments. (C) FARFAR model of the 3D structure of SL5 in overlay with shape of SAXS model (red).

THE FUZZY β-DOMAIN OF MEF2D: ALTERING FUNCTION VIA DYNAMICS

Tamás Milán Nagy[1], Krisztina Fehér[1], Mónika Gönczi[4], Mónika Fuxreiter[3], Katalin E. Kövér[1,2]

[1] Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary
[2] MTA-DE Molecular Recognition and Interaction Research Group, University of Debrecen, Hungary
[3] MTA-DE Momentum, Laboratory of Protein Dynamics, Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary
[4] Department of Physiology, Faculty of Medicine, University of Debrecen, Hungary

The MEF2 transcription factors play crucial roles in muscle cell myogenesis and morphogenesis[1]. Bioinformatical analysis indicates that the alternatively spliced β-domain of MEF2D does not fold into a well-defined structure and likely remains conformationally heterogeneous upon interactions. To study the role of protein dynamics in the biological function a series of dynamically diverse mutants of the wild type MEF2D and 37 AA mini proteins representing the conserved acidic β-domain[2] were designed.

The dynamics of the free peptides has been studied using ^{15}N relaxation measurements (T_1, T_2, heteronuclear NOE) from which reduced spectral density functions were calculated. Random Coil Indexes (RCI) were determined from the chemical shifts (Cα, Cβ, N) to derive model-free order parameters[3] (S^2). Diffusion NMR-spectroscopy (DOSY) showed the differences between the degree of structural compactness and oligomerization properties. Experimental NMR parameters were in good agreement with those obtained from 3x100 ns long classical and scaled molecular dynamics simulations (MD). Different dynamical behavior and level of disorder were observed at both local and global scale due to the impact of mutations on the intramolecular stabilizing interactions (e.g. cation-π, H-bonds, salt- bridges, number of native contacts). Detailed analysis of the dynamical and structural properties of the peptides will give insights into the altered interaction patterns of the MEF2D β-domain with altered biological outcomes.

References

[1] B. Black, E. Olson, Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. *Annu. Rev. Cel.l De.v Biol.* **14**, 167-196 (1998)
[2] B.Zhu, B. Ramachandran, T. Gulick, Alternative Pre-mRNA Splicing Governs Expression of a Conserved Acidic Transactivation Domain in Myocyte Enhancer Factor. *J. Biol. Chem.* **280,** 28749–28760 (2005)
[3] Mark V. Berjanskii, David S. Wishart*, A Simple Method To Predict Protein Flexibility Using Secondary Chemical Shifts J.Am. Chem.* **127**, 14970 -14971 (2005)

Acknowledgements

This research was supported by the National Research, Development and Innovation Office of Hungary (grant NKFI/OTKA NN 128368) and co-financed by the European Regional Development Fund (projects GINOP-2.3.3-15-2016-00004, GINOP- 2.3.2-15-2016-00044 and GINOP-2.3.2-15-2016-00008)

MONITORING THE INTERACTION OF α-SYNUCLEIN WITH CALCIUM IONS THROUGH EXCLUSIVELY HETERONUCLEAR NUCLEAR MAGNETIC RESONANCE EXPERIMENTS

Letizia Pontoriero[1], Marco Schiavina[1], Maria Grazia Murrali[1], Roberta Pierattelli[1], Isabella C. Felli[1]

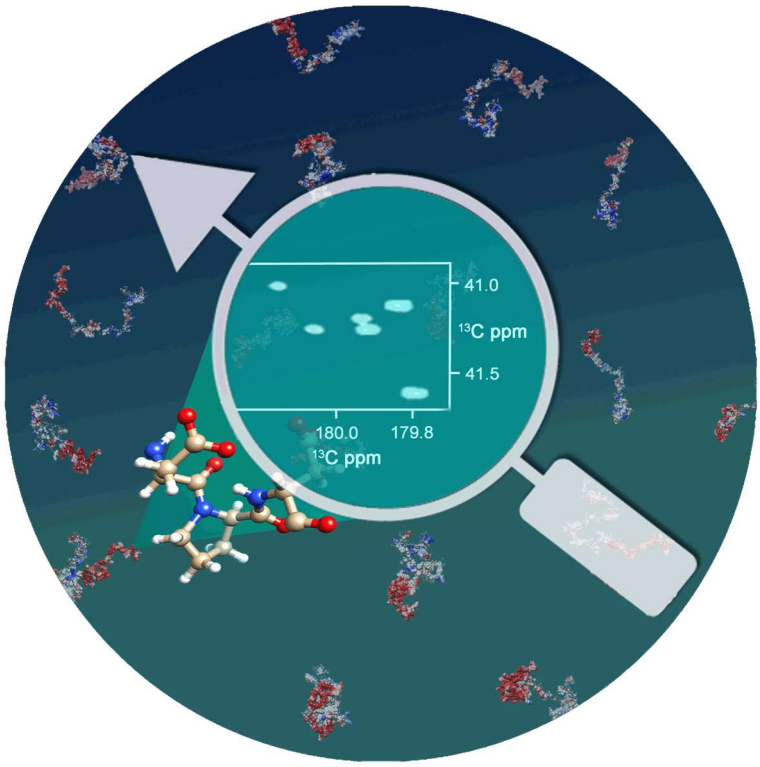
[1] CERM and Department of Chemistry "Ugo Schiff", University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy.

Intrinsically disordered proteins (IDPs) challenge the "structure-to-function" paradigm as they can exert their biological functions without having a stable tridimensional structure [1]. Moreover, the misfuctions of IDPs are often related to the onset of different maladies, such as neurodegenerative diseases [2].

The dynamic properties of IDPs are susceptible to cellular environment therefore experimental tools to study their features in physiologically relevant conditions are very useful to understand how disorder and flexibility modulate protein function. Many of these properties are expected to be modulated by side-chains as well as by local solvent exposure. For their characterization NMR spectroscopy provides a unique investigation tool as it allows to approach in-vitro physiological conditions and in-cell studies [3]. We propose here a set of exclusively heteronuclear NMR experiments to investigate these features in different experimental conditions relevant for function. The ^{13}C direct detected experiments were tested on human α-synuclein that is disordered in its native conditions and it is known to bind calcium ions with its C-terminus in proximity of presynaptic terminals, with a possible link in the onset of Parkinson's Disease[4]. The set was then used to monitor the interaction of the IDP with Ca^{2+} ions . The approach allowed us to obtain a fingerprint of the IDPs and to zoom-in into metal ion coordination sphere, revealing the motifs involved in the interaction [5].

References

[1] V. N. Uversky, Intrinsically disordered proteins from A to Z, Int. J. Biochem. Cell Biol. 43, 1090–1103 (2011).
[2] V. N. Uversky, Wrecked regulation of intrinsically disordered proteins in diseases: pathogenicity of deregulated regulators, Front. Mol. Biosci. 1, 1–24 (2014).
[3] I. C. Felli, R. Pierattelli, Intrinsically Disordered Proteins Studied by NMR Spectroscopy, Springer (2015).
[4] A. Binolfi, R. M. Rasia, C. W. Bertoncini, M. Ceolin, M. Zweckstetter, C. Griesinger, T. M. Jovin, C. O. Fernández, Interaction of α-synuclein with divalent metal ions reveals key differences: A link between structure, binding specificity and fibrillation enhancement, J. Am. Chem. Soc., 128, 9893–9901 (2006).
[5] L. Pontoriero, M. Schiavina, M. G. Murrali, R. Pierattelli, I. C. Felli, Monitoring the Interaction of α-Synuclein with Calcium Ions through Exclusively Heteronuclear Nuclear Magnetic Resonance Experiments, Angew. Chemie - Int. Ed., 59, 18537– 18545 (2020)


41.0
^{13}C ppm
41.5
180.0
179.8
^{13}C ppm

HOW TWO AMINO ACIDS MODULATE THE REDOX PROPERTIES OF THE TRIHEME CYTOCHROME PPCA FROM GEOBACTER METALLIREDUCENS

Pilar C. Portela[1], Marta A. Silva[1], Liliana R. Teixeira[1], Joana M. Dantas[1], Carlos A. Salgueiro[1]

[1] UCIBIO-Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus Caparica, 2829-516 Caparica, Portugal

Geobacter bacteria inhabit anoxic sediments of rivers and aquifers, possessing the remarkable ability to transfer electrons to the exterior of the cell that reduce extracellular electron acceptors such as Fe(III), toxic metals like Cr(VI), and electrode surfaces in microbial fuel cells[1]. Currently, *Geobacter*-based biotechnological applicaZons are being explored for electricity harvesZng from the bacteria's growth in electrodes or for the bioremediaZon of contaminated waters. MulZheme *c*-type cytochromes located in the inner membrane, periplasm and outer membrane play a crucial role in the successful delivery of electrons to the extracellular space. The PpcA family of periplasmic triheme *c*-type cytochromes possesses homologs in the majority of *Geobacter* species, playing a key role in the electron transfer from the inner membrane to the outer membrane. This family (PpcA-E) has been extensively studied in *G. sulfurreducens* (Gs)[2] and, more recently, the funcZonal mechanism of PpcA from *G. metallireducens* (Gm) has been characterized[3]. PpcA from Gs and Gm only differ in 13 amino acids but their funcZonal properZes – namely the heme oxidaZon order and the midpoint reducZon potenZal – are markedly different. In this work, we have assessed the funcZonal impact of residues Lysine 19 and Tryptophan 45 in PpcA from Gm by replacing them with the counterparts in PpcA from Gs – Alanine 19 and Methionine 45. Using a methodology that combined UV-Visible redox ZtraZons and 2D-EXSY NMR experiments we have been able to show, for the first Zme, that the inclusion of an aromaZc residue in a strategic locaZon at the heme core and the presence of a posiZve charged amino acid in the vicinity of a specific heme are able to modify considerably the working funcZonal range in highly homologous proteins.

References

[1] D.R. Lovley, T. Ueki, T. Zhang, N.S. Malvankar, P.M. Shrestha, K.A. Flanagan, M. Aklujkar, J.E. Butler, L. Giloteaux, A.-E. Rotaru, D.E. Holmes, A.E. Franks, R. Orellana, C. Risso, K.P. Nevin, *Geobacter*: The Microbe Electric's Physiology, Ecology, and PracZcal ApplicaZons, in: R.K. Poole (Ed.) Advances in Microbial Physiology, Academic Press, 1-100 (2011).

[2] J.M. Dantas, L. Morgado, M. Aklujkar, M. Bruix, Y. Londer, M. Schiffer, P.R. Pokkuluri, C.A. Salgueiro, RaZonal engineering of *Geobacter sulfurreducens* electron transfer components: a foundaZon for building improved *Geobacter*-based bioelectrochemical technologies, FronZers in Microbiology, 6 (2015).

[3] T.M. Fernandes, L. Morgado, C.A. Salgueiro, Thermodynamic and funcZonal characterizaZon of the periplasmic triheme cytochrome PpcA from *Geobacter metallireducens*, Biochemical Journal **475**, 2861-2875 (2018).

Acknowledgements

This work was supported by PTDC/BIA-BQM/31981/2017 grant from Fundação para a Ciência e Tecnologia (FCT).

DRUG TARGETING OF RIBOSOMAL PROTEIN RPL35 FOR TREATMENT OF RARE SKIN DISEASE EPIDERMOLYSIS BULLOSA MONITORED BY LIQUID NMR SPECTROSCOPY

Adriana Rathner[1], Petr Rathner[1, 2], Andreas Friedrich[1,3], Michael Wießner[1, 3], Christian Manuel Kitzler[4], Jan Krauß[1], Helmut Hintner[1, 3], Michael Breitenbach[1], Johann W. Bauer[1, 3], Hannelore Breitenbach-Koller[1], Jörg von Hagen[5], Norbert Müller[2, 4, 6]

[1] Department of Biosciences, University of Salzburg, 5020, Salzburg, Austria
[2] Institute of Inorganic Chemistry, Johannes Kepler University, 4040, Linz, Austria
[3] Department of Allergology and Dermatology, University Hospital Salzburg, 5020, Salzburg, Austria
[4] Institute of Organic Chemistry, Johannes Kepler University, 4040, Linz, Austria
[5] Technische Hochschule Mittelhessen, Department of Life Science Engineering, 35390, Gießen, Germany
[6] Faculty of Natural Sciences, University of South Bohemia, 37005, Ceske Budejovice, Czech Republic

Epidermolysis bullosa (EB) refers to a family of genetic blistering skin disorders. The most severe, postpartum lethal form is generalized severe junctional EB (gs-JEB). This is caused by premature termination codon (PTC) mutations in the skin anchor protein LAMB3 (laminin subunit beta-3) gene which deliver insufficient levels of Lamb3 protein. We employed NMR titration to verify possible interactions between small binders and ribosomal protein L35 (rpL35) which was identified previously by 2 step specialized RiboScreen (2SSRS) as a ribosomal modifier providing customized increase in the Lamb3 synthesis with minimal interference on the bulk protein expression[1].
Molecular docking studies using SwissDock interface were performed in order to identify possible small binders to rpL35[2]. Based on these results, two FDA-approved drugs, Atazanavir and Artesunate were used for stepwise titration into the solution of rpL35 and changes in the 2D ^{1}H, ^{15}N HSQC spectra of the protein were monitored (chemical shift perturbation, peak intensity). Results from the NMR titration were compared with binding sites predicted by SwissDock and the precise coordination of both molecules by rpL35 could be determined. Atazanavir and Artesunate both bound to rpL35 in solution with their binding sites overlapping to a large degree. Both molecules may now be tested for their potential to trigger a rpL35 ribosomal switch in vivo, ultimately leading to increased production of full-length Lamb3 protein from a LAMB3PTC mRNA for targeted therapy of gs-JEB.

References

[1] J. W. Bauer, C. Brandl, O. Haubenreisser, B. Wimmer, M. Weber, T. Karl et al., Specialized Yeast Ribosomes: A Customized Tool for Selective mRNA Translation. *PLOS ONE*. **8** (7), e67609 (2013)
[2] http://www.swissdock.ch/

Acknowledgements

We acknowledge technical support from KBHB Consult GmbH with 2SSRS and molecular docking.

NMR-DIRECTED MUTATIONS IN A COILED-COIL DOMAIN MODULATE CALCIUM CHANNEL ACTIVATION BEHAVIOR

Petr Rathner[1,2], Marc Fahrner[3], Linda Cerofolini[4], Herwig Grabmayr[3], Agrim Gupta[1], Manuel Kitzler[1], Enrico Ravera[4, 5], Marco Fragai[4,5], Claudio Luchinat[4,5], Christoph Romanin[3], and Norbert Müller[1,6]

[1] Institute of Organic Chemistry, Johannes Kepler University Linz, Linz, Austria
[2] Institute of Inorganic Chemistry, Johannes Kepler University Linz, Linz, Austria
[3] Institute of Biophysics, Johannes Kepler University Linz, Linz, Austria
[4] Magnetic Resonance Center (CERM), Univ. of Florence and Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine (CIRMMP), Sesto Fiorentino, Italy
[5] Department of Chemistry, University of Florence, Sesto Fiorentino, Italy
[6] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

The calcium release activated calcium (CRAC) channel is activated by the calcium sensor protein STIM1. (1) Upon activation, the STIM1 C-terminus undergoes several changes from an inactive to a spatially extended active conformation.(2) We derived an NMR-based solution structure of a coiled coil domain, a three-helix bundle stabilized by interhelical contacts, which were not found in the Stormorken disease-related STIM1. (3) The NMR-directed mutations of interhelical contact sites between within this domain were found to be keys in controlling STIM1 activation. Based on in-vitro NMR, the physiological, store-dependent activation behavior of the pathogenic mutant could be restored in living cells. We show the functional impact of interhelical interactions within a coiled-coil domain for modifying the strength of further inter-domain interactions, which control the activation of the CRAC channel. (3)

References

[1] J . Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno,S. Zhang, O. Safrina, J. A. Kozak, S. Wagner, M. Cahalan, G. Velicelebi, K. Stauderman, STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol.* **169**(3), 435-45 (2005).
[2] M. Muik, M. Fahrner, R. Schindl, P. Stathopulos, I. Frischauf, I. Derler, P. Plenk, B. Lackner, K. Groschner, M. Ikura, C. Romanin, STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. *The EMBO journal***. 30**(9), 1678-89 (2011).
[3] P. Rathner, M. Fahrner, L. Cerofolini, H. Grabmayr, F. Horvath, H. Krobath, A. Gupta, E. Ravera, M. Fragai, M. Bechmann, T. Renger, C. Luchinat, C. Romanin, N. Müller, Interhelical interactions within the STIM1 CC1 domain modulate CRAC channel activation. *Nature Chemical Biology*. (2020)

FAST ACQUISITION OF 2D NMR TITRATION DATA FOR CHARACTERIZATION OF PROTEIN-LIGAND INTERACTION

Javier A. Romero[1], Ewa K. Nawrocka[1,2], Alexandra Shchukina[3], Francisco J. Blanco[4], Tammo Diercks[5] and Krzysztof Kazimierczuk[1]

[1] Center of New Technologies, University of Warsaw, Warsaw, Poland.
[2] Faculty of Chemistry, University of Warsaw, Warsaw, Poland.
[3] Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland.
[4] Structural and Chemical Biology Department, Centro de Investigaciones Biológicas, CIB-CIS, Madrid, Spain.
[5] CIC bioGUNE, Parque Tecnológico de Bizkaia, Ed. 800, Derio, Spain.

NMR spectroscopy offers unique benefits for ligand binding studies on isotopically labelled target proteins. It is actively employed in pharmaceutical industry to access novel ligand classes and for structure-based drug design[1] (SBDD), but it requires large amounts of the target protein and long measurement times to record well-resolved heteronuclear 2D correlation spectra. The time demand is exacerbated by the need to record full titration series to determine the affinity constant (K_D) and quantify the chemical shift perturbation (CSP) amplitudes[2] of individual peak signals as the most accurate and directly accessible NMR constraint for ligand docking and structure-based ligand optimization. In this presentation I will introduce non-stationary complementary non-uniform sampling (NOSCO-NUS)[3], a new method to massively accelerate such exhaustive interaction studies without loss of any information. In NOSCO-NUS titration points are recorded using different NUS schedules. By making the schedules complementary and merging the individual signals one obtains a single, full grid non-stationary[4] 2D NMR signal. Partitioning the t_1 grid into a number of complementary NUS schedules equal to the number of titration points involves very low sampling levels, impossible to realize with conventional strategies. But by picturing the ligand concentration as a non-Fourier dimension and understanding the relationship between it and the NUS schedules one is able to retrieve with high accuracy the values of K_D and the CSP amplitudes. Complete titration series of 2D protein NMR spectra can now be recorded within less than a day, making them more broadly accessible, e.g. in industrial SBDD processes, or for proteins with limited lifetime.

References

[1] J. Stark, R. Powers, Rapid protein-ligand costructures using chemical shift perturbations, *J. Am. Chem. Soc.*, **130**, 535-545 (2008)
[2] M. P. Williamson, Using chemical shift perturbation to characterise ligand binding, *Prog. Nucl. Magn. Reson. Spectrosc.*, **73**, 1-16 (2013)
[3] J. A. Romero, E. K. Nawrocka, A. Shchukina, F. J. Blanco, T. Diercks, K. Kazimierczuk, Non-Stationary Complementary Non-Uniform Sampling (NOSCO-NUS) for fast acquisiton of 2D NMR titration data, *Angew. Chem. Int. Ed.*, **59** (2020)
[4] D. Gołowicz, P. Kasprzak, V. Orekhov, K. Kazimierczuk, Fast time-resolved NMR with non-uniform sampling, *Prog. Nucl. Magn. Reson. Spectrosc.*, **116**, 40-55 (2020)

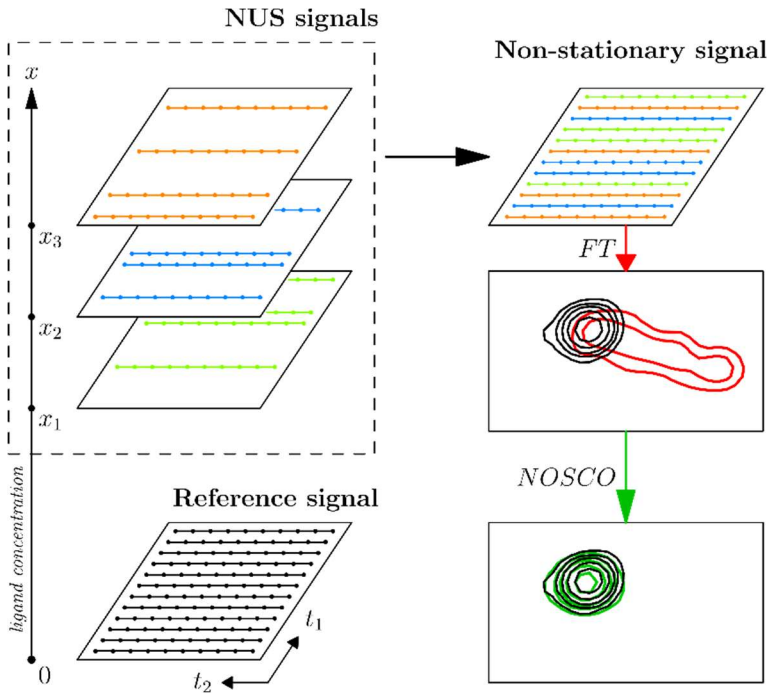


Figure 1. A non-stationary signal is built out of complementary NUS signals from samples at different protein/ligand concentrations (x). Peaks undergoing large CSP will appear distorted in the spectrum. NOSCO applies an artificial correction signal to the non-stationary one, restoring the shape of a distorted peak back to the shape of its reference (protein only signal) and calculating the affinity parameters in the process.

EXTENDED PLATEU OF THE EFFECTIVE CHARGE OF PROTEINS

Mayu Nishizawa[1], Erik Walinda[2], Benjamin Kohn[3], Daichi Morimoto[1], Kenji Sugase[1], Ulrich Scheler[3]

[1] Department of Molecular Engineering, Kyoto University, Kyoto-Daigaku Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[2] Department of Molecular and Cellular Physiology, Kyoto University, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan
[3] Leibniz-Institut für Polymerforschung Dresden e.V. Hohe Str. 6, 01069 Dresden, Germany

In aqueous systems and thus in any living system the electrostatic interaction plays an important role. While it is well known that protein contribute to the stability of pH in solution, we report here on their ability to stabilize their charge well in the pH range, that is physiologically relevant.

The combination of diffusion and electrophoresis NMR yields the effective charge of a macromolecule in solution[1]. The electrophoretic mobility measured in electrophoresis NMR is constant because on the timescale of the NMR experiment a steady state from the force balance between accelerating force in the electric field and the counteracting hydrodynamic friction is present. The hydrodynamic friction is determined from diffusion NMR. The effective charge commonly is smaller than the net charge because of counterions, which condense on the highly charged macromolecule or patches of high charge density[2]. This approach has been applied to study the charge of proteins as a function of pH well beyond the physiologically relevant range. For the intracellular protein ubiquitin an effective charge of zero has been found for pH ranging from 6 to 8. In contrast for the extracellular serum albumin a stable negative charge has been observed. This is understood to be essential for albumin to prevent its removal from the plasma by glomerular filtration in the kidneys.

References

[1] Böhme, U.; Scheler, U. Counterion Condensation and Effective Charge of Poly(Styrenesulfonate). *Adv. Colloid Interface Sci.*, **158**, 63–67 (2010).
[2] Böhme, U.; Scheler, U. Effective Charge of Bovine Serum Albu-min Determined by Electrophoresis NMR. *Chem. Phys. Lett.*, **435**, 342–345 (2007).

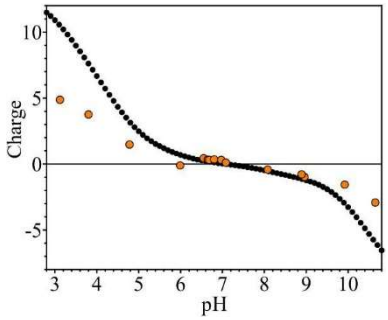


Figure 1. Effective charge, Z_{eff}, of ubiquitin as determined by electrophoresis NMR as a function of solution pH (orange circles) and net charge, Z_{net}, of ubiquitin as calculated by propKa.

DRIVING FORCES IN IDPs: THE CASE OF OSTEOPONTIN COMPACT STATE

Borja Mateos[1], Clara Conrad-Billroth[1], Marco Schiavina[2], Andreas Beier[1], Georg Kontaxis[1], Irene Ceccolini[1], Robert Konrat[1], Isabella C Felli[2], Roberta Pierattelli[2]

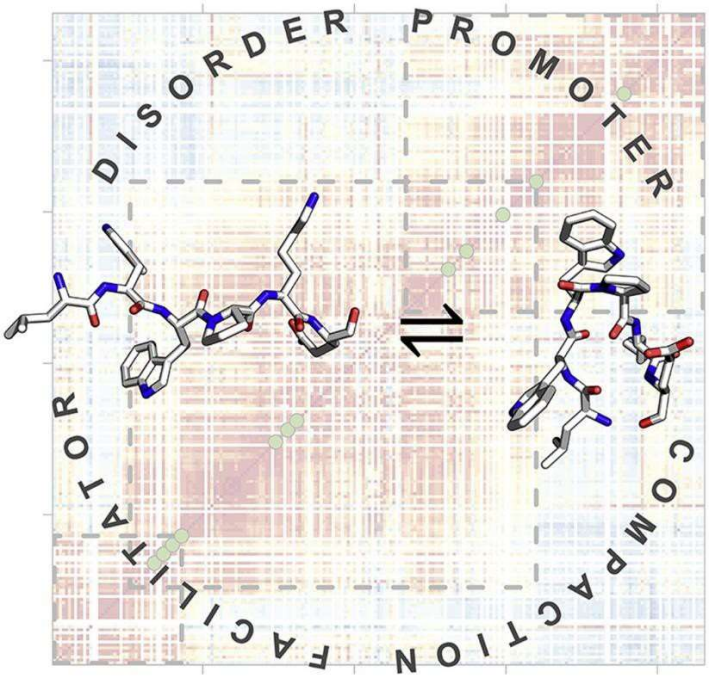
[1] Department of Structural and Computational Biology, University of Vienna, Max Perutz Labs, Vienna Biocenter Campus 5, 1030 Vienna, Austria.
[2] CERM and Department of Chemistry "Ugo Schiff", University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy.

Highly flexible proteins and flexible linkers of complex proteins are present in any living organism and play key roles in a variety of different cellular pathways. They lack a stable three-dimensional structure, but rather adopt many different conformations in dynamic equilibrium retaining biological activity [1]. The interplay between local dynamics and global rearrangements is key for their function and it is strictly dependent on amino acids composition. In IDPs, prolines are much more abundant than in globular proteins and given their unique physicochemical and structural properties, a more detailed understanding of their potential role in stabilizing partially folded states in IDPs is highly desirable. Nuclear magnetic resonance (NMR) spectroscopy, and in particular ^{13}C-detected NMR, is especially suitable to address these questions [2]. Indeed, exploiting the ^{13}C-detection strategy was possible to map minor forms of proline residues and to characterize them.

Thanks to the exquisite sensitivity and spectral resolution of these technique, we gained unprecedented insight into *cis*-Pro populations, their local structural dynamics, and their role in mediating long-range contacts in the context of a whole protein. The study of quail wild type Osteopontin shed a new light on the structural and functional role of proline residues in IDPs [3]. They are not simply *disorder promoter* residues but depending on the primary sequence context can act as nucleation sites for structural compaction in IDPs. In particular, segments that are rich in Aro-Pro motifs result to be more compact than others in the same protein. The study of different mutants of quail Osteopontin provides insights on the relevance of these motifs and their impact on protein features in general.

References

[1] J. Habchi, P. Tompa, S. Longhi, and V. N. Uversky, “Introducing protein intrinsic disorder,” *Chem. Rev.*, **114**, 6561–6588, 2014.
[2] I. C. Felli, Pierattelli, (Eds), "Intrinsically Disordered Proteins Studied by NMR Spectroscopy", Springer, Switzerland, 2015.
[3] B. Mateos, C. Conrad-Billroth, M. Schiavina, A. Beier, G. Kontaxis, R. Konrat, I.C. Felli, R. Pierattelli, “The ambivalent role of proline residues in an intrinsically disordered protein: from disorder promoters to compaction facilitators,” *J. Mol. Biol.*, **432**, 3093-3111, 2019.


DISORDER PROMOTER
COMPACTION FACILITATOR

CHARACTERIZING BICELLE SIZE USING A COMBINED NMR-SAXS METHOD

Fanni Sebák[1,2], András Wacha[3], Attila Bóta[3], Andrea Bodor[1]

[1] Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Pázmány Péter sétány 1/A, H-1117, Budapest, Hungary.
[2] Doctoral School of Pharmaceutical Sciences, Semmelweis University, Üllői út 26, H-1085, Budapest, Hungary
[3] Biological Nanochemistry Research Group, Research Centre for Natural Sciences, Magyar tudósok körútja 2, H-1117, Budapest, Hungary

Physico-chemical characterization of protein-membrane interactions are of key importance in functional characterization of cell membranes. In order to simplify this complex system the most commonly used systems for solution state NMR studies are the PC and PC/PG bicelles, that have ideal behaviour at q>0.5 (long chain/short chain lipid ratio).[1,2]
Translational diffusion experiments enable determination of the diffusion coefficient *D* that serves for the calculation of an effective hydrodynamic radius based on the Stokes-Einstein equation. However, this is only true, if the medium behaves as a continuum. We observed that the translational diffusion coefficient of the solvent water is greatly affected by the viscosity of the solution, thus, in most cases the Stokes-Einstein equation is no longer applicable, and hydrodynamic radii reported in these cases are clearly erroneous. In order to elucidate the discrepancies and to offer a solution for bicelle size determination under these conditions, we started an NMR-SAXS combined investigation for PC bicelles keeping constant q=0.5 and varying the DHPC and DMPC concentrations between 50-300mM and 25-150mM, respectively.

Moreover, we studied the effect on the shape and morphology of the bicelle of selected biologically relevant protein fragments in unlabeled form: the C-terminal part of the vertebrate specific, metastasis associated S100A4, and three, highly hydrophylic K-segments of the plant dehidrin ERD14.

References

[1] A. Bodor, K. E. Kövér, L. Mäler, Membrane interactions in small fast-tumbling bicelles as studied by 31P NMR. *BBA*, **1848(3)**: 760-766 (2015).
[2] E. F. Dudás, A. Wacha, A. Bóta, A. Bodor, Peptide-bicelle interaction: Following variations in size and morphology by a combined NMR-SAXS approach. *BBA Biomembranes*, **1862(2)**, 183095, (2020).

REAL-TIME NMR FOR THE MONITORING OF PRE-NUCLEATION SPECIES EVOLUTION IN MODIFIED SIMULATED BODY FLUID

Albina Selimović[1], Ludovica M. Epasto[1], Gregory L. Olsen[1], Tristan Georges[2], Thierry Azaïs[2], Dennis Kurzbach[1]

albina.selimovic@univie.ac.at

[1] Faculty of Chemistry, Institute of Biological Chemistry, University of Vienna, A-1090 Vienna, Austria

[2] Sorbonne Université, CNRS, Collège de France, Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), F-75005 Paris, France

Calcium phosphates (CaPs), essential bone constituents and bone grafting materials[1], crystallize via a non-classical crystallization pathway involving the formation of transient soluble 'pre-nucleation species' (PNS). The details of this process remain elusive, despite the importance of CaPs in biomineralization and in industrial and biological applications[2,3]. This is largely due to the lack of methods that permit characterization of this dynamic process on sufficiently short timescales. Real-time NMR spectroscopy combines high temporal and spatial resolution and therefore provides a uniquely powerful tool for the study of the process at atomic-level detail[4].

In vivo, apatite formation takes place in physiological fluids. *In vitro*, studies of such phenomena rely on the use of simulated body fluid (SBF), an aqueous solution imitating blood plasma. SBF is also used to assess the bioactivity of bone implants as it enables the deposition of bone-like apatite onto grafting biomaterials[5].

Herein we report NMR observation of the early stages of CaP precipitation, recorded in modified SBF supplemented with polyaspartate (pAsp) or polyethylene glycol (PEG) to provide an improved representation of native physiological conditions while also accounting for macromolecular crowding *in vivo*. Employing RT NMR monitoring of ^{31}P resonances of soluble phosphate species, we are able to directly observe incorporation of free P_i into transient PNS.

Figure 1 shows PNS growth observed in m-SBF-pAsp solution over several hours. Initially, a single sharp ^{31}P resonance corresponding to free P_i is observed. This is followed by broadening and downfield shift of the signal over time, indicative of the progressive change in the local environments of ^{31}P nuclei as they are incorporated into PNS. Notably, when compared to the same experiment conducted in the absence of crowding agents, the onset of PNS formation is delayed. Additionally, in diffusion coefficient measurements the formation of PNS with a significantly lower *D* is observed.

References

[1] Habraken, W., Habibovic, P., Epple, M. & Bohner, M. Calcium phosphates in biomedical applications: materials for the future? *Mater. Today* **19**, 69–87 (2016).

[2] LeGeros, R. Z. Calcium Phosphate-Based Osteoinductive Materials. *Chem. Rev.* **108**, 4742–4753 (2008).

[3] Wang, L. & Nancollas, G. H. Calcium Orthophosphates: Crystallization and Dissolution. *Chem. Rev.* **108**, 4628–4669 (2008).

[4] Weber, E. M. M. *et al.* Assessing the Onset of Calcium Phosphate Nucleation by Hyperpolarized Real- Time NMR. *Anal. Chem.* **92**, 7666–7673 (2020).

[5] Ohtsuki, C., Kokubo, T. & Yamamuro, T. Mechanism of apatite formation on CaO-SiO2-P205 glasses in a simulated body fluid. *J. Non. Cryst. Solids* **143**, 84–92 (1992).

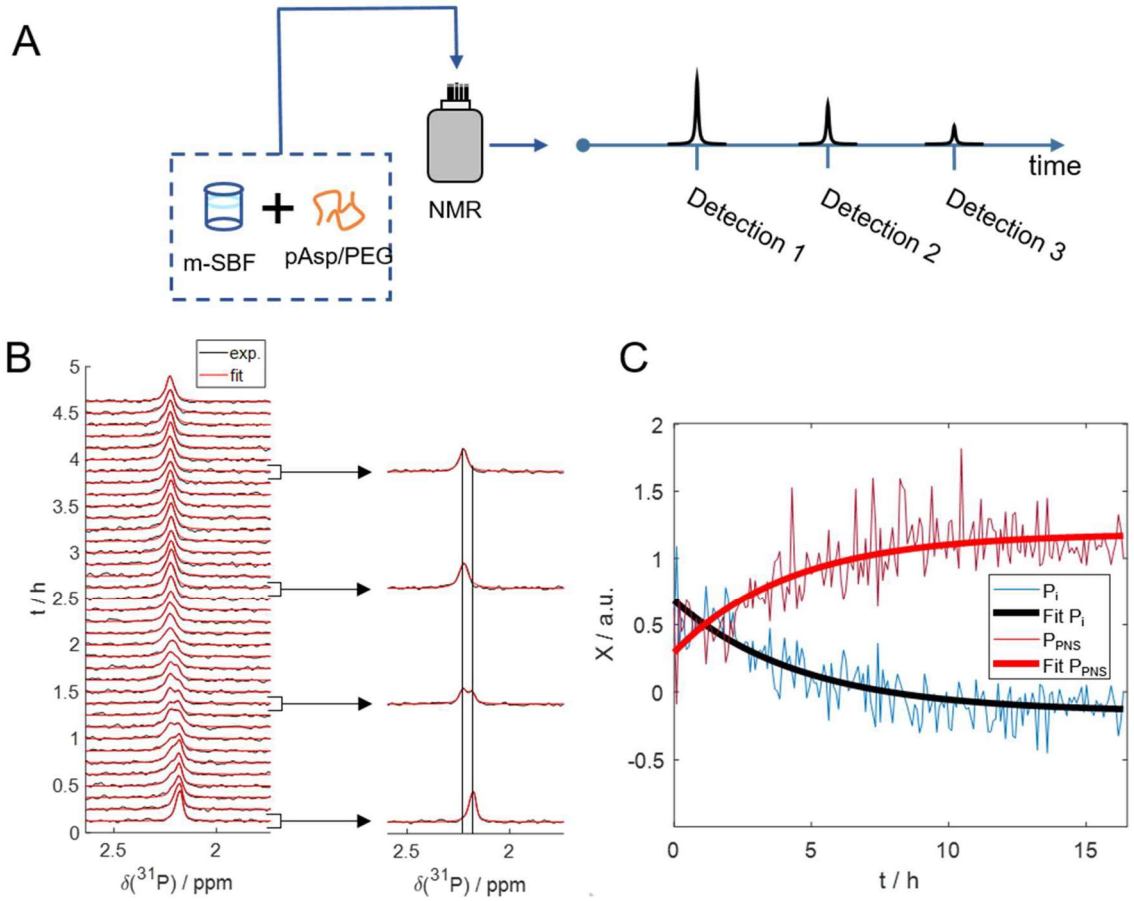


Figure 1. (A) Workflow for m-SBF supplemented with PEG or pAsp and subsequent RT ^{31}P NMR monitoring of calcium phosphate PNS formation. (B) Temporal evolution of ^{31}P signals in m-SBF supplemented with pAsp. An initial signal for free P_i is observed, followed by peak broadening and downfield signal shift, indicative of the progressive change in the local environments of ^{31}P nuclei due to the formation of PNS. (C) Signal intensity time traces of free P_i and bound P_{PNS} in m-SBF-pAsp.

3D TIME-RESOLVED NON-UNIFORM SAMPLING AND ITS APPLICATION FOR PROTEIN NMR

Alexandra Shchuckina[1], K. Kazimierczuk[2]

[1] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[2] Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

Non-uniform sampling (NUS) has become a widely used tool to accelerate time-consuming multidimensional NMR experiments. Some points of the indirect dimensions are in this case skipped and later reconstructed based on various mathematical assumptions about the spectrum. If a sample is undergoing some change during the experiment, time-resolved NUS[1] is highly beneficial. One long NUS experiment is then carried out, the resulting data set is divided into overlapping subsets, and each of them is reconstructed separately. This yields a series of spectra with extremely high time resolution unachievable otherwise. It allows the observation of processes much faster than the experiment duration.

Special signal processing methods are needed to treat the resulting series of spectra. In this work, a software package incorporating such methods is presented. It allows tracking tens of peaks throughout hundreds of spectra. A 2-dimensional version[2] and a 3-dimensional one[3] of this peak-picking and peak-tracking software exist. Both yield the kinetics of peak position changes.

In protein NMR, this can be applied to study temperature coefficients (TC): the dependence of chemical shifts on temperature. Non-linearities in TC, which indicate the presence of a protein compact state, are particularly interesting. The aforementioned approach was applied to temperature-swept 3-dimensional HNCO spectra of two intrinsically disordered proteins - osteopontin and CD44 cytoplasmic tail[3]. Non-linearities of their TC were established based on time-resolved NUS experiments and data processing as well as statistical treatment.

References

[1] Mayzel, M., Rosenlöw, J., Isaksson, L. & Orekhov, V. Y. Time-resolved multidimensional NMR with non-uniform sampling. J. Biomol. NMR 58, 129–139 (2014).
[2] Urbańczyk, M., Shchukina, A., Go lowicz, D. & Kazimierczuk, K. TReNDS - Software for reaction monitoring with time-resolved non-uniform sampling. Magn. Reson. Chem. 57, 4–12 (2018).
[3] A. Shchukina, P. Malecki, B. Mateos, M. Nowakowski, M. Urbanczyk, G. Kontaxis, P. Kasprzak, C. Conrad-Billroth, R. Konrat, and K. Kazimierczuk. Temperature as an extra dimension in multidimensional protein NMR. Chemistry - a European Journal. doi:10.1002/ chem.202003678.

USING NMR SPECTROSCOPY TO UNRAVEL CYTOCHROME *C* MATURATION SYSTEM

Ana V. Silva[1], Andreia F. Veríssimo[2,3], Guillem Hernandez[1], Hugo Monteiro[1], Tiago N. Cordeiro[1], Catarina M. Paquete[1], Fevzi Daldal[2], Ricardo O. Louro[1]

[1] Universidade Nova de Lisboa -Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal
[2] Department of Biology, University of Pennsylvania, Philadelphia, PA USA
[3] Present address: bioMTInstitute for Biomolecular Targeting, Geisel School of Medicine at Dartmouth, Hanover, NH, 03755, USA

Cytochromes-*c* are essential proteins for organisms across all domains of life, playing important roles in electron transfer, a central biological process in biotechnological and health-related applications. Given the importance of this class of proteins, the process that leads to their formation in nature is equally important. The covalent attachment of heme and subsequent folding of the protein requires a dedicated maturation machinery that, despite its recognized biological importance, is far from fully understood. Several maturation systems have been described, including the Cytochrome-*c* maturation (Ccm) System I that is present in most Gram-negative bacteria, including organisms used in bioelectrochemical systems (electroactive organisms) and numerous pathogens.

The aim of this work is to characterize the molecular mechanisms of cytochrome-*c* maturation by the Ccm System I. CD spectroscopy and SAXS were used to probe the overall folding and stability of the apo form of cytochrome *c*' from *Rhodobacter capsulatus.* Using ^{15}N and ^{13}C isotopic labelling, the signals of the protein were assigned and its interactions with components of System I and recognition mechanisms were characterized. Data show that apocytochrome *c*' has characteristics of unfolded protein, specially above 20 ºC, with propensity for alpha-helix elements below that temperature. Small changes in the folding of the apocytochrome occur upon interaction with the System I protein CcmI. Evidence from NMR spectroscopy also points towards the simultaneous occurrence of two distinct binding processes.

These findings are the first step to characterize the binding processes taking place in this maturation system. The full understanding of the functioning of this system is of great relevance for the optimization of the biotechnological and health-related applications of cytochromes-*c*.

RATIONAL MODULATION OF ELECTRON/PROTON TRANSFER MECHANISMS FOR OPTIMAL BIOMASS PRODUCTION IN EXOELECTROGENIC BACTERIA

Marta Silva[1], Carlos Salgueiro[1]

[1] UCIBIO-Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus Caparica, 2829-516 Caparica, Portugal

A family of five periplasmic triheme cytochromes (designated PpcA–E) from *Geobacter sulfurreducens* plays crucial roles in electron transfer pathways and also in extracellular electron transfer[1]. Two members of this family (PpcA and PpcD) were also found to be able to couple e^-/H^+ transfer through the redox Bohr effect observed in the physiological pH range, a feature not observed for cytochromes PpcB and PpcE[2]. This family of cytochromes are constitutively expressed in this bacterium. The redox-Bohr effect may contribute to the ATP synthesis mechanism. Therefore, optimization of this mechanism would contribute to increase bacterial biomass production and to enhance the *Geobacter*-based practical biotechnological applications in bioremediation and bioenergy fields. In attempting to understand the molecular basis for the redox Bohr effect in this structural homologous family of cytochromes, it was observed that residue 6 is a conserved leucine in PpcA and PpcD, whereas in the other two cytochromes (PpcB and PpcE) the equivalent residue is a phenylalanine. The leucine 6 is located in the proximity of the redox-Bohr center in cytochromes PpcA and PpcD. In this work, the phenylalanine 6 residue was replaced by a leucine in PpcB and PpcE and the detailed redox properties of the new mutants were determined using 2D-EXSY NMR experiments and potentiometric redox titrations. In contrast with the wild-type cytochromes, the PpcBL6F and PpcEL6F mutants showed an increased redox-Bohr effect. The results obtained clearly confirm that residue 6 is responsible for the modulation of the redox Bohr effect in this family of cytochromes. This feature enables the rational design of *G. sulfurreducens* strains with optimal redox-Bohr effect that could be explored for various biotechnological applications.

References

[1] T. C. Santos, M. A. Silva, L. Morgado, J. M. Dantas, C. A. Salgueiro, Diving into the redox properties of *Geobacter sulfurreducens* cytochromes: a model for extracellular electron transfer, *Dalton Trans.* **44**, 9335 (2015).
[2] J. M. Dantas, T. Simões, L. Morgado, C. Caciones, A. P. Fernandes, M. A. Silva, M. Bruix, P. R. Pokkuluri, C. A. Salgueiro, Unveiling the Structural Basis That Regulates the Energy Transduction Properties within a Family of Triheme Cytochromes from *Geobacter sulfurreducens*, *J. Phys. Chem. B* **120**, 10221−10233 (2016).

Acknowledgements

This work was supported by PTDC/BIA-BQM/31981/2017 grant from Fundação para a Ciência e Tecnologia (FCT).

SHIFTING PROTEIN FOLDING EQUILIBRIUM WITH IONIC LIQUIDS

Micael S. Silva[1], Aldino Viegas[1], Philip O'Toole[1], Sara Felix[1], Ângelo M. Figueiredo[1,2], Eurico J. Cabrita[1]

[1] UCIBIO, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal
[2] Institute for Structural and Molecular Biology, Division of Biosciences, University College London, London, United Kingdom

Despite several years of intensive research, the topic of protein folding still moves a lot of interest, particularly the understanding of how we can modulate the folding landscape[1]. To accomplish this, and to understand how we can tune the folding and unfolding paths, a complete elucidation of the different accessible states that a protein can explore during this process it is crucial. In this work, using tailored ionic liquids (ILs) as solvents, we were able to modulate the folding pathway of the N-terminal Src homology 3 (SH3) domain of the *Drosophila* adapter protein Drk (drkN SH3). Using choline glutamate ([Ch][Glu]) as a stabilizer and 1-Butyl-3-methylimidazolium dicyanamide ([Bmim][dca]) as a destabilizer and a combination of NMR techniques we have studied the structure and conformational exchange of the drkN SH3 domain on each solvent and provide a comprehensive understanding of the stabilizing and destabilizing effects.

Our data reveals that (de)stabilization of SH3 occurs via electrostatic and hydrophobic IL-protein interactions, and a modification of the water structure around the protein. While [Ch][Glu] interacts preferentially with the folded state of SH3, [Bmim][dca] interacts preferentially with the unfolded state. These interactions lead to different enthalpic and entropic changes that affect the equilibrium thermodynamics of SH3 stability. Quantification of these changes shows that in [Ch][Glu] the magnitude of the entropic stabilization of the folded state is greater than the enthalpic destabilization, leading to an overall stabilization of SH3 while [Bmim][dca] leads to an entropic destabilization compensated by an enthalpic stabilization of the unfolded state. Characterization of the folding/unfolding interconversion rates by ZZ-exchange spectroscopy shows that [Bmim][dca] leads to SH3 destabilization by slowing the folding rate and increased unfolding rate while [Ch][Glu] stabilizes the folded state significantly by slowing the unfolding rate.

Our data provides a thorough understanding of the IL-protein interactions and their effect on the equilibrium thermodynamics and the NMR methodologies used represent a robust and effective way to study the elusive unfolded states of proteins.

References

[1] A. Gershenson, S. Gosavi, P. Faccioli, P. L. Wintrode, Successes and challenges in simulating the folding of large proteins, *J. Biol. Chem.* **295**, 15-33 (2020).

DISSECTING STRUCTURED RNA ELEMENTS OF THE OX40 3'-UTR AS A ROQUIN BINDING PLATFORM BY NMR

Jan-Niklas Tants[1], Lea M. Becker[1], Sophie M. Korn[1], Andreas Schlundt[1]

[1] Institute for Molecular Biosciences, Frankfurt and Center for Biomolecular Magnetic Resonance (BMRZ), 60438 Frankfurt, Germany

Posttranscriptional gene regulation is a prerequisite for cellular homeostasis. Amongst others controlled mRNA decay mediated through *trans*-acting factors is a versatile tool to regulate mRNA levels. The immune regulatory multi-domain RNA-binding protein Roquin (Figure 1A) binds stem-loop structures in the 3'-UTR of its mRNA targets and initiates mRNA degradation. Two classes of target *cis* elements have been described for Roquin: A constitutive decay element (CDE, tri-loop) and an alternative decay element (ADE, hexa-loop) that are either preformed or transiently structured [1]. Structures of the unique Roq core domain with either of these elements have given detailed insights into complex formation [2,3]. An extended Roq domain mediates unspecific contacts to double-stranded RNAs (dsRNA) [4], while the role of the RING and ZnF domains remains unclear. However, many UTRs comprise multiple copies of these elements and functions vary from redundant to additive effects.

We study the 3'-UTR of *Ox40*, a T-cell co-receptor crucial for a balanced immune response. Within its comparably small size of 157 nt it comprises both one ADE and CDE (Figure 1A), plus additional structured RNA elements. We seek to understand how several instances of decay elements affect complex formation with Roquin leading to a concerted functional output.

Due to inherent dynamics, structural investigations of larger RNAs remain challenging. We show how NMR can be used in a divide-and-conquer approach to overcome these limitations. Combining NMR and small-angle X-ray scattering (SAXS) we characterize all structured fragments of the *Ox40* 3'-UTR RNA, their interaction with Roquin and their inter-dependence (Figure 1B). We analyze the contributions of all Roquin domains by electrophoretic mobility shift assays (EMSA), confirm formation of a ternary complex (Figure 1C) and show that affinity increases through binding of the extended Roq domain. Overall, we provide an approach to targeting complete RNA-RBP-hubs in the context of mRNA regulation.

References

[1] Binas, O. et al. Structural basis for the recognition of transiently structured AU-rich elements by Roquin. *Nucleic Acids Res* **48**, 7385-7403 (2020).
[2] Schlundt, A. et al. Structural basis for RNA recognition in roquin-mediated post-transcriptional gene regulation. *Nat Struct Mol Biol* **21**, 671-8 (2014).
[3] Janowski, R. et al. Roquin recognizes a non-canonical hexaloop structure in the 3'-UTR of Ox40. *Nat Commun* **7**, 11032 (2016).
[4] Tan, D., Zhou, M., Kiledjian, M. & Tong, L. The ROQ domain of Roquin recognizes mRNA constitutive- decay element and double-stranded RNA. *Nat Struct Mol Biol* **21**, 679-85 (2014).

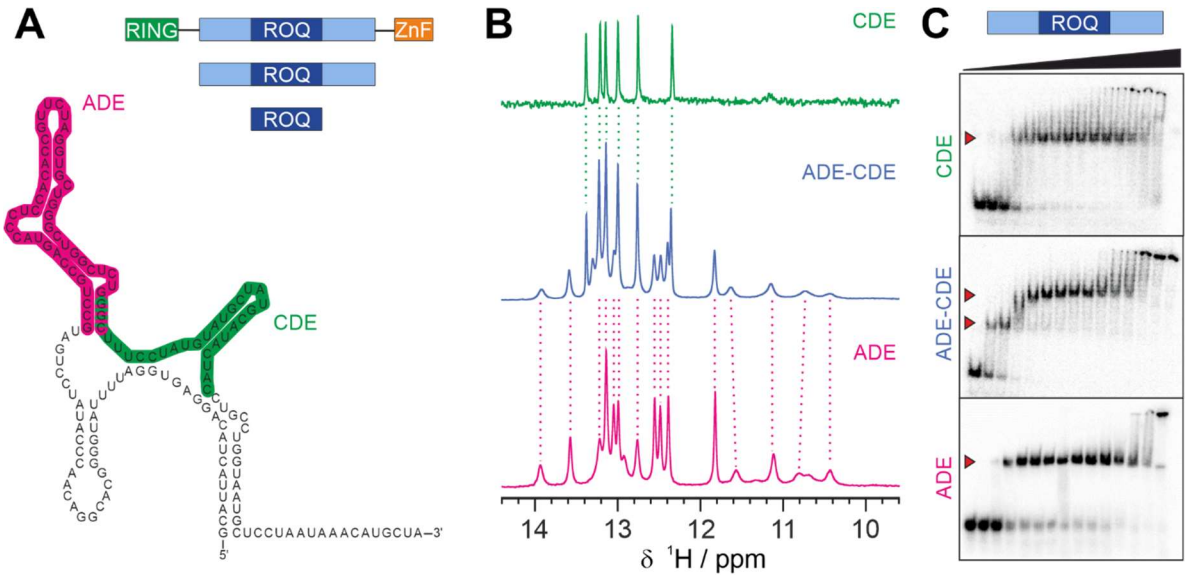


Figure 1. A. Roquin constructs used in this study and secondary structure prediction of Ox40 3'-UTR. ADE and CDE constructs used are coloured in magenta and green. B. The imino proton spectra of CDE, tandem ADE-CDE (blue) and ADE. C. EMSAs of extended Roquin with RNA elements from B.

INHIBITION OF AMYLOID FIBRIL FORMATION BY PREFOLDIN – DECIPHERING THE MECHANISM BY SOLUTION STATE NMR

Ricarda Törner[1], Tatsiana Kupreichyk[2], Lothar Gremer[2], Rida Awad[1], Fenel Daphna[1], Pierre Gans[1], Schoehn Guy[1], Willbold Dieter[2], Wolfgang Hoyer[2], Jerome Boisbouvier[1]

[1] Univ. Grenoble Alpes, CNRS, CEA, Institut de Biologie Structurale (IBS), 71, Avenue des Martyrs, F-38044 Grenoble, France.
[2] Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany

Dysfunction of proteostasis leads to the accumulation of misfolded protein aggregates, as observed in many age-related diseases. Among them is type II diabetes caused by aggregation of amylin/IAPP (Islet amyloid polypeptide) in pancreatic beta cells [1].

We have established that the cytosolic co-chaperonin prefoldin (PFD) [2] inhibits the formation of IAPP amyloid fibrils at sub-stoichiometric ratios and decreases the accumulation of fibril mass. By using an integrative approach, we reveal the action mode of prefoldin during the fibril elongation process, responsible for this strong inhibitory effect. Liquid state NMR interaction studies were used to investigate binding of PFD to IAPP and extract kinetic parameters. Assignment of this heterohexameric chaperonin (90kDa) was achieved with the aid of an in-house developed isotope-aided NMR method. [3] We demonstrate that client binding takes place via two binding regions on IAPP which bind in a highly dynamic fashion inside the PFD cavity.

Analysis of ThioflavinT- fibrillation assays reveal that prefoldin inhibits the secondary nucleation of IAPP fibrils. This result was further confirmed by an EM investigation, where images of prefoldin bound to the fibril surface were obtained.

Our approach gives structural and methodological insights into the process of chaperone inhibition of fibrillation, a first step towards modulation of the interaction for disease-prevention.

References

[1] K. Pillay and P. Govender, “Amylin uncovered: a review on the polypeptide responsible for type II diabetes.,” *BioMed research international*, vol. 2013, 2013, doi: 10.1155/2013/826706.
[2] I. E. Vainberg *et al.*, “Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin.,” *Cell*, vol. 93, no. 5, pp. 863–73, May 1998, doi: 10.1016/S0092-8674(00)81446-4.
[3] R. Törner et al. ”Specific Edition of intra- and inter-chain methyl-methyl NOEs inprotein complexes” *J. Biomol. NMR* vol. 74, pp. 83–94 Jan. 2020, doi: 10.1007/s10858-019-00293-x.

THE DISORDERED INTRACELLULAR SIDE OF THE TRANSLOCATED INTIMIN RECEPTOR

Marta F.M. Viera[1]; Guillem Hernandez[1], Tiago Veloso[1]; Tiago N. Cordeiro[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade NOVA de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

The translocated intimin receptor (Tir) is an essential bacterial effector that spans the host plasma membrane with its N- and C-terminal regions inside the cytosol interacting with several host factors to ensure infection and immunity evasion [1-3]. So, while remaining extracellular, Tir intracellular domains (ICDs) provide a means for the pathogen to establish a direct link to the inside of the host and hijack host signaling by interacting with at least 25 host proteins [4]. Here we report how the solution conformations of Tir ICDs could modulate Tir's virulence activity. Using an integrative structural biology approach, relying on nuclear magnetic resonance (NMR) and small-angle x-ray scattering (SAXS), we show that both regions are structural disordered. The C-terminal ICD of Tir (C-Tir) behaves as an intrinsically disordered protein with non-random coil preferences and long-range contacts at sites phosphorylated by host kinases. We used NMR to map and characterize C-Tir's phosphorylation pattern and host protein interaction, revealing a differential rate and binding selectivity.

The N-terminal domain of Tir (N-Tir) displays an order-disorder interplay: though flexible, it forms a stable dimer in solution. Our study provides the first structural glimpse into the translocated intimin receptor's intracellular side with implications in our understanding of virulence mechanisms at the host-pathogen interface.

References

[1] Kenny, B. et al. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 91, 511–520 (1997)
[2] Hayward, R. D., Leong, J. M., Koronakis, V. & Campellone, K. G. Exploiting pathogenic *Escherichia coli* to model transmembrane receptor signalling. *Nature Reviews Microbiology* 4, 358–370 (2006).
[3] Yan, D., Wang, X., Luo, L., Cao, X. & Ge, B. Inhibition of TLR signaling by a bacterial protein containing immunoreceptor tyrosine-based inhibitory motifs. *Nat. Immunol.* 13, 1063–1071 (2012).
[4] Blasche, S. et al. The EHEC-host interactome reveals novel targets for the translocated intimin receptor. *Sci. Rep.* 4, 7531 (2014).

UNUSUAL RNA Y:Y MOTIFS IN THE SARS-COV-2 3'UTR

Anna Wacker[1], Jenny Vögele[1], Christian Richter[1], RNA Lab Team[1], Boris Fürtig[1], Julia Weigand[1], Harald Schwalbe[1], Jens Wöhnert[1]

[1]www.covid19-nmr.de

The 3'-UTR of Betacoronaviruses harbors several conserved regulatory RNA elements. The 5'-most *cis*-regulatory element is a large, bulged stem-loop (3_SL1, Figure 1), containing a 3'-sequence that forms either the lower part of the stem-loop or base-pairs with a single-stranded sequence within the loop of a downstream, smaller stem-loop (3_SL2). Thus, the SL1-SL2 element is a putative RNA switch, associated with regulation of replication[1,2].
For 3_SL1, we could assign the imino protons by analyzing cross peaks in a ^{1}H,^{1}H-NOESY experiment and ^{1}H,^{15}N-TROSY-and HNN-COSY experiments (Figure 1). In addition to the predicted A-helical parts, we observed three consecutive non-canonical U-U base pairs in the upper part of 3_SL1. These three base pairs give rise to six well-resolved imino proton resonances in the spectral area typical for U imino protons involved in G-U and U-U base pairs (δ ^{1}H: 10-12.5 ppm, δ ^{15}N: 155-160 ppm). Such consecutive Y:Y motif are recurring motifs in the 3'-UTR of SCoV2, also detected in the hypervariable region downstream of the SL1-SL2 element. These structural features of these motifs are further investigated by solution NMR on the respective minimal RNA constructs.
We further tested the druggability of 3_SL1 by screening all SARS-CoV-2 structured RNA elements against a 768-fragment library. We find 5 hits for 3_SL1 which will be further analyzed in the context of RNA structure motif specificity.

References

[1] Stammler, S. N., Cao, S., Chen, S. J. & Giedroc, D. P. A conserved RNA pseudoknot in a putative molecular switch domain of the 3'-untranslated region of coronaviruses is only marginally stable. *RNA* **17**, 1747–1759 (2011).
[2] Williams, G. D., Chang, R. Y. & Brian, D. A. A phylogenetically conserved hairpin-type 3' untranslated region pseudoknot functions in coronavirus RNA replication. *J Virol* **73**, 8349– 8355 (1999).

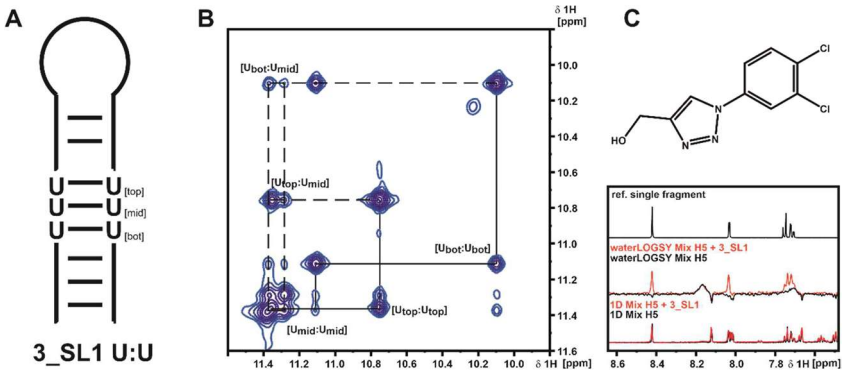


Figure 1. A) Secondary structure cartoon of 3_SL1 highlighting the three U:U base pairs, B) Imino-region of a ^{1}H,^{1}H NOESY showing the connectivities between the three U:U base pair imino protons, C) Representative fragment and corresponding spectra from the fragment- based screening showing the positive WaterLOGSY signal in the middle 1D spectrum.

NMR CHARACTERIZATION OF THE SCoV-2 ELEMENT 3_S2M

Maria Wirtz Martin[1,2], Tobias Matzel[1,2], Julia Wirmer-Bartoschek[1,2], Klara Mertinkus[1,2], J. Tassilo Grün[1,2], Harald Schwalbe[1,2]

[1]Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt am Main, Germany
[2]Center for Biomolecular Magnetic Resonance (BMRZ), Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany

The Betacoronavirus SCoV-2 harbours a 30.000 nt long (+) strand RNA which not only encodes for the viral proteins but also shows a highly structured 5` and 3` untranslated region. The presence of secondary structure elements in the genomic RNA was elucidated by sequence based computational predictions.[1] These highly structured functional RNA elements especially in the untranslated regions are assumed to play an important role in genome replication, translational regulation and have the possibility to impede host cell biomolecular processes in order to ensure virus propagation.

Part of the several conserved regulatory RNA elements of the 3´UTR is a hypervariable region (HVR) in which the conserved RNA element 3_s2m can be found. However, the function of the HVR is still unclear and therefore it is an interesting research target. The structural properties of the 3_s2m conserved motif were analysed extensively using NMR spectroscopy within the BMRZ Covid-19 NMR consortium.

Chemical shift assignment of the imino protons as well as other chemical shifts in the 3_s2m motif from SCoV-2 was performed by analysing ^{1}H,^{1}H-NOESY, ^{1}H,^{15}N-TROSY, HNN-COSY, ^{1}H,^{15}N-CPMG-NOESY and long range ^{1}H,^{15}N-sfHMQC experiments (Figure 1). The findings indicate a secondary structure that is divided into two stem regions separated by an internal asymmetric loop. Interestingly, in 2005 the crystal structure of the s2m motif in SCoV was solved.[2] The 3_s2m motif in SCoV-2 shows two mutations compared to the s2m motif in SCoV which alternate the base pairing pattern of the upper helix (29758G to 29758U) as well as stabilize the lower helix (29732U-29764G to 29732C-29764G).

References

[1] R. Rangan, I. Zheludev, R. Das, *bioRxiv Prepr. Serv. Biol.* 2020, DOI 10.1101/2020.03.27.012906.
[2] M. P. Robertson, H. Igel, R. Baertsch, D. Haussler, M. Ares, W. G. Scott, *PLoS Biol.* 2005, ***3***, 86– 94.

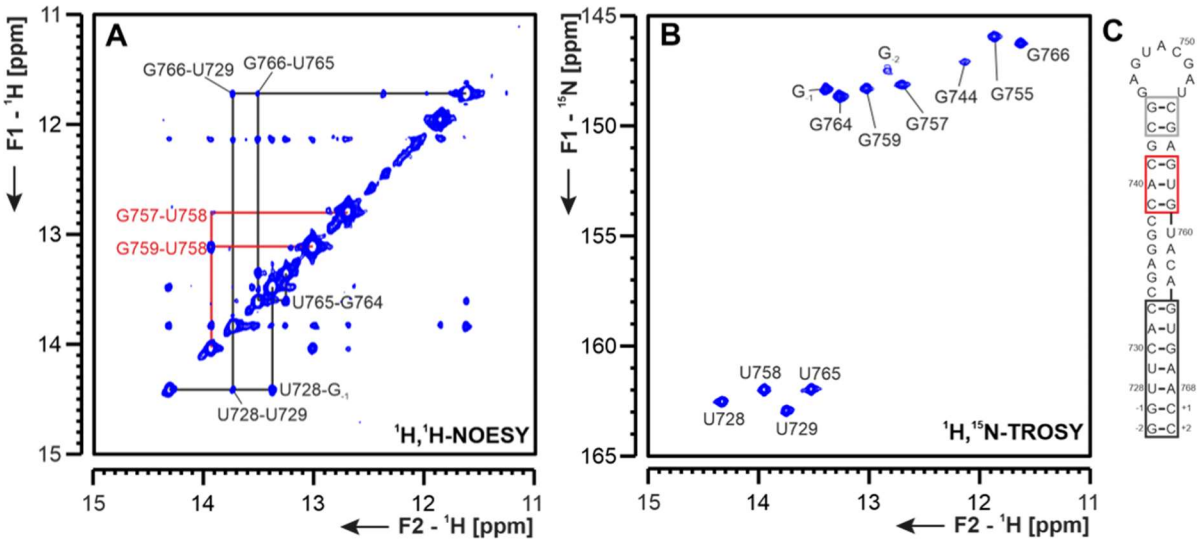


Figure 1. (A) ^{1}H,^{1}H-NOESY, (B) ^{1}H,^{15}N-TROSY and spectra for imino-proton correlation of the 3'-genomic end construct 3_s2m encompassing nts 29,728 to 29,768. Positive contours are given in purple, negative contours in red. The imino-proton correlations are annotated using the genomic numbering, shifted for convenience by 29,000 nts from 5'. Imino proton correlations in (A) between consecutive base pairs are shown in different colors. The experimentally observed secondary structure of 3_s2m is shown in (C). Additional closing base pairs are annotated with "±x". Colors of boxes are according to the correlations in (A). *(data currently in the process of submission).*

02. Computation

EXACT LINDBLAD MASTER EQUATIONS FOR CHEMICAL EXCHANGE

Jacob R. Lindale[1], Warren S. Warren[2]

[1] Department of Chemistry, Duke University, Durham, NC, 27708, USA.
[2] Departments of Physics, Chemistry, Biomedical Engineering, and Radiology, Duke University, Durham, NC, 27708, USA.

Chemical systems often undergo configurational changes (exchange), causing the dynamics to deviate from purely quantum evolution. Accurately modeling these systems provides critical insight into how to control their coherent dynamics in the presence of non-unitary evolution. This is particularly important for the applications of quantum computing, where precise control of qubits in the presence of exchange is essential. Recently[1], we introduced an exact Dissipative Master Equation (DMEx) that expands exchange to infinite order in perturbation theory, greatly improving the convergence of simulations with no additional computational cost. While this is formulated in a way familiar to those in the field of magnetic resonance, the DMEx was constructed in a less convenient form for a more general audience.

The most general Markovian master equation is the Lindblad equation, which is popular in the fields of quantum computing and optics and has recently been used for non-equilibrium spin relaxation theory[2]. Here, we demonstrate that the Lindblad equation is recovered in the case of chemical exchange and, similar to the DMEx, can be extended to include all higher order terms (Figure 1A). In this equation, which we call the exact Lindblad Master Equation (LMEx), the exponential factor in red accounts for all higher order interactions, as the operator portion of the dissipator (blue) satisfies the same infinite-order convergence condition as the DMEx. The Lindblad form unveiled a convenient Fock operator formalism that allowed us to derive LMEx equations for systems with high order Abelian and non-Abelian symmetries, making it extremely flexible. This formalism has already indicated significant improvements over the traditional equation at no added computational cost (Figure 1B). We will report on both the scope of this formalism, exploring dynamic systems with pseudorotation, residual dipolar couplings, and solid state systems, and quantify the stability off the solution relative the traditional Lindblad equation.

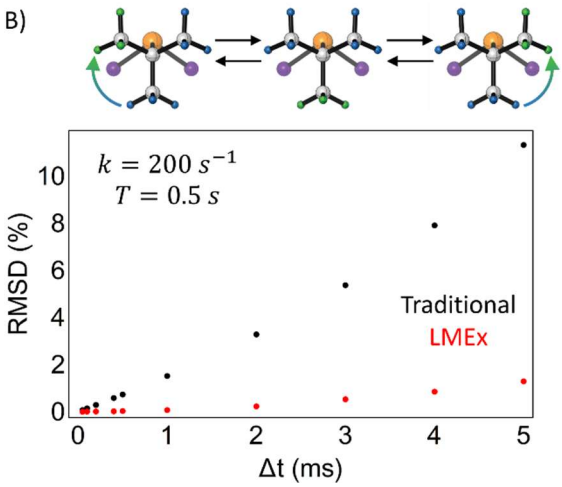
References

[1] J. R. Lindale, S. L. Eriksson, C. P. N. Tanner, W. S. Warren, Infinite-order perturbative treatement for quantum evolution with exchange. *Sci. Adv.* **6**, eabb6874 (2020).
[2] C. Bengs, M. H. Levitt, A master equation for spin systems far from equilibrium. *J. Magn. Reson.* **310**, 106645 (2020).

A)

$$\partial_t \hat{\rho} \ni \sum_k \left(\hat{L}_k \hat{\rho} \hat{L}_k^\dagger - \frac{1}{2} \{ \hat{L}_k \hat{L}_k^\dagger, \hat{\rho} \} \right) \exp\left(\frac{-\Delta t}{2\tau_k} \right) / \tau_k$$

B)



PREDICTION OF CHEMICAL SHIFTS OF 90k ANTAMANIDE SNAPSHOTS USING MACHINE LEARNING AT NEAR EXPERIMENTAL ACCURACY

Arobendo Mondal[1], Karsten Reuter[1,2], Christoph Scheurer[1]

[1] Chair for Theoretical Chemistry and Catalysis Research Center, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany
[2] Theory Department, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, 14195 Berlin-Dahlem, Germany

Nuclear Magnetic Resonance (NMR) spectroscopy is an eminently powerful technique to study structure and dynamics in condensed matter systems non-intrusively. The use of empirical, parametrized, effective-spin Hamiltonians has enabled steady progress in the field for decades. However, the interpretation of NMR spectra for large systems is often challenging due to the free or constrained dynamics within the chemical environment of the the NMR active nucleus. Computed NMR parameters can aid in the interpretation.[1] While the relevant fundamental theoretical expressions for the computation of the Hamiltonian parameters from first principles are sufficiently well-known, it is still challenging to compute chemical shift anisotropy tensors or coupling constants at predictive quality. Computationally intense high-level quantum chemistry is needed to match the high spectral resolution achieved routinely in liquid-state NMR experiments. Simultaneously, multiple such calculations are needed in dynamical simulations to address the local environment's thermal fluctuations around the active nuclei.

Machine learning has recently emerged as an appealing way to overcome the need for a large number of explicit quantum chemical calculations.[2] We here use a combination of kernel ridge regression (KRR) with the smooth overlap of atomic position (SOAP) descriptor[3] to learn NMR parameters for amino-acids from quantum chemical molecular dynamics data. SOAP thereby allows us to efficiently capture the influences of neighboring atoms in the second and third coordination sphere. The established learning protocol enables the quantitative prediction of isotropic chemical shifts at orders of magnitude reduced computational cost. For the experimentally well-studied cyclic peptide antamanide, a full account of dynamical effects yields spectra reaching experimental accuracy.

References

[1] C. Scheurer, N. R. Skrynnikov, S. F. Lienin, S. K. Straus, R. Brüschweiler, R. R. Ernst, Effect of dynamics and environment on 15N chemical shielding anisotropy in proteins. A conbination of density functional theory, molecular dynamics simulation, and NMR relaxation. *J. Am. Chem. Soc.* **121**, 17, 4242-4251 (1999).
[2] F. M. Paruzzo, A. Hofstetter, F. Musil, S. De, M. Ceriotti, L. Emsley, Chemical shifts in molecular solids by machine learning. *Nat. Commun.* **9**, 4501 (2018).
[3] A. P. Bartók, R. Kondor, G. Csányi, On representing chemical environments. *Phys. Rev. B* **87**, 184115 (2013).

DATA-MINING CHEMICAL SHIFTS IN THE SOLID STATE FOR PROBABILISTIC ASSIGNMENT OF ORGANIC CRYSTALS

Manuel Cordova[1,2], Martins Balodis[1], Bruno Simões de Almeida[1], Lyndon Emsley[1,2]

[1] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
[2] National Centre for Computational Design and Discovery of Novel Materials MARVEL, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

Solid-state NMR, in combination with computational methods such as density functional theory chemical shift computation and crystal structure prediction (CSP) procedures, has proven able to determine the crystal structure of organic solids.[1] A key starting point for efficient chemical shift-driven crystal structure determination is the assignment of the experimental NMR spectra. Probabilistic assignments can be obtained without prior knowledge of the three-dimensional structure of molecules through a statistical analysis of large chemical shift databases.[2] However, no such database exists for organic solids.

Here, we combine organic crystal structures obtained from the Cambridge structural database[3] (over 200,000 compounds) with ShiftML, a machine learning model able to predict chemical shifts in molecular solids,[4] in order to construct a statistical basis for the probabilistic assignment of solid-state NMR spectra. Relating the topological representation of molecular fragments to their corresponding statistical distribution of chemical shifts, we propose a probabilistic framework to determine possible assignments of organic crystals, along with their confidence, from the two-dimensional representation of these compounds only.

References

[1] E. Salager, G. M. Day, R. S. Stein, C. J. Pickard, B. Elena,L. Emsley, Powder Crystallography by Combined Crystal Structure Prediction and High-Resolution H-1 Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* **132**, 2564 (2010).
[2] C. Bartels, M. Billeter, P. Guntert, K. Wuthrich, Automated sequence-specific NMR assignment of homologous proteins using the program GARANT. *J. Biomol. NMR* **7**, 207-213 (1996).
[3] C. R. Groom, I. J. Bruno, M. P. Lightfoot, S. C. Ward, The Cambridge Structural Database. *Acta Crystallogr. B*, **72**, 171-179 (2016).
[4] F. M. Paruzzo, A. Hofstetter, F. Musil, S. De, M. Ceriotti, L. Emsley, Chemical shifts in molecular solids by machine learning. *Nat. Commun.* **9**, 4501 (2018).

SEMI-SUPERVISED LEARNING IN METABOLIC ANALYSIS EMPLOYING TWO DIMENSIONAL NUCLEAR MAGNETIC RESONANCE (NMR)

Lubaba Migdadi[1, 2], Jörg Lambert[1], Ahmad Telfah[1], Roland Hergenröder[1] and Christian Wöhler[2]

[1] Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V. 44139 Dortmund, Germany
[2] Image Analysis Group, TU Dortmund, 44227 Dortmund, Germany

Metabolism research has emerged as a new approach for medical diagnostics, disease prediction and the design of medicinal interventions[1]. For the associated task of metabolic profiling, 2D Total Coherence Spectroscopy Nuclear Magnetic Resonance (2D TOCSY NMR) is widely used. Although there are semi-automatic analysis approaches for 2D TOCSY spectra, they are negatively affected by signal overlap as well as signal shifts due to variations of pH and ionic strength in the second frequency dimension[2]. In this work, semi-supervised machine learning approaches are implemented to obtain a fully automatic procedure for signal assignment and deconvolution of 2D TOCSY spectra. A framework for iterative confidence band based classifiers, which autonomously expand their knowledge using limited amounts of manually labelled training samples for automatic metabolic assignments, is proposed. The automatic NMR metabolic profiling avoids the tedious and slow manual analysis, makes the analysis possible for low-resolution measurements and enables metabolic assignment of 2D TOCSY without being restricted to specialists. The outcome of applying the semi-supervised machine learning approaches on NMR spectrum of breast cancer tissues shows a significant recognition of metabolites overlapping in the NMR spectra, which has a strong potential in metabolic profiling.

References

[1] Capati A, Ijare OB, Bezabeh T. Diagnostic Applications of Nuclear Magnetic Resonance-Based Urinary Metabolomics. *Magn Reson Insights.* 2017;10:1178623X17694346-11178623X17694346.
[2] Bingol K, Brüschweiler R. Multidimensional approaches to NMR-based metabolomics. Analytical chemistry. 2014;86(1):47-57.

03. EPR/ESR

LIGHT-INDUCED TRIPLET-TRIPLET ELECTRON RESONANCE SPECTROSCOPY

Arnau Bertran[1], Kevin B. Henbest[1], Marta De Zotti[2], Marina Gobbo[2], Christiane R. Timmel[1], Marilena Di Valentin[*2], Alice M. Bowen[*3]

[1] Centre for Advanced Electron Spin Resonance and Department of Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, UK.
[2] Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy.
[3] National EPR Facility and Department of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK.

The suitability of the porphyrin photoexcited triplet state for nanometre distance measurements by ESR Pulse Dipolar Spectroscopy was demonstrated in combination with nitroxide radicals.[1] Thanks to its non-Boltzmann population, the photoexcited triplet enhanced signal intensity when used as detection spin in PELDOR[2] and improved modulation depth when used as pump spin in LaserIMD.[3] Here we present the new technique of Light-Induced Triplet-Triplet Electron Resonance spectroscopy (LITTER),[4] which uses photoexcited triplet states as both detection and pump spins (Fig. (a)), enabling both the distance and angular distributions between the two triplet moieties to be determined on a nanometre scale. This is demonstrated for a model bis-porphyrin peptide (Fig (b) inset) which renders dipolar traces with strong orientation selection effects (Fig. (c)). Using simulations and DFT calculations, we extract distance distributions (Fig. (c) inset) and relative orientations of the porphyrin moieties, allowing the dominant conformation of the peptide in frozen solution to be identified. LITTER removes the requirement of current light-induced Electron Spin Resonance Pulse Dipolar Spectroscopy techniques to have a permanent paramagnetic moiety, becoming more suitable for in-cell applications and potentially giving access to distance determination in unmodified macromolecular systems containing photoexcitable moieties. LITTER also has the potential to enable direct comparison with FRET and combination with microscopy inside cells.

References

[1] M. G. Dal Farra, S. Ciuti, M. Gobbo, D. Carbonera, M. Di Valentin, Triplet-state spin labels for highly sensitive pulsed dipolar spectroscopy. *Mol. Phys.*, **117**, 2673-2687 (2019).
[2] M. Di Valentin, M. Albertini, E. Zurlo, M. Gobbo, D. Carbonera, Porphyrin triplet state as a potential Spin label for nanometer distance measurements by PELDOR spectroscopy. *J. Am. Chem. Soc.*, **136**, 6582-6585 (2014).
[3] C. Hintze, D. Bücker, S. Domingo Köhler, G. Jeschke, M. Drescher, Laser-Induced Magnetic Dipole Spectroscopy. *J. Phys. Chem. Lett.*, **7**, 2204-2209 (2016).
[4] A. Bertran, K. B. Henbest, M. De Zotti, M. Gobbo, C. R. Timmel, M. Di Valentin, A. M. Bowen, Light-Induced Triplet-Triplet Electron Resonance Spectroscopy. *J. Phys. Chem. Lett.* (2020) (in press).

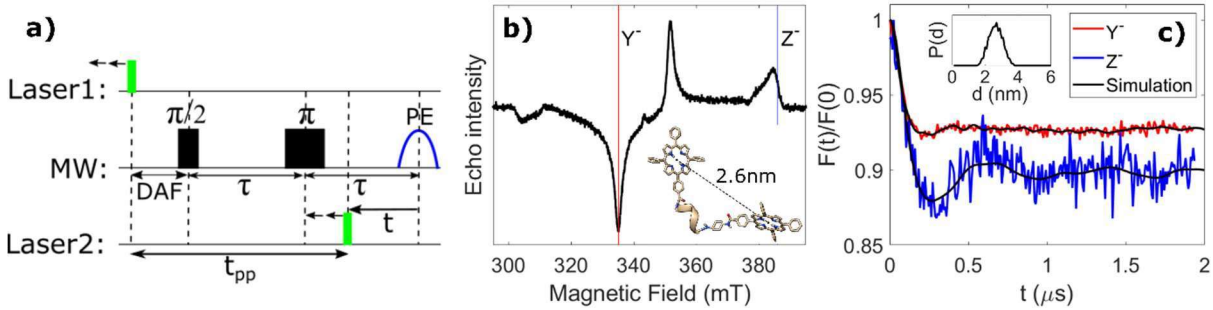


Figure 1. (a) Pulse sequence for LITTER. (b) Echo-detected ESR spectrum of the bis-porphyrin peptide (inset) after photoexcitation, showing the two field positions used for LITTER. (c) Background-corrected LITTER traces (red and blue) and fits leading to the distance distribution (inset).

DUPLEX-BRIDGED UNIMOLECULAR DNA G-QUADRUPLEXES: AN EPR INVESTIGATION

Yury Kutin[1], Lukas M. Stratmann[1], Guido H. Clever[1], Müge Kasanmascheff[1]

[1] Department of Chemistry and Chemical Biology, TU Dortmund University, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

G-quadruplexes (GQs) are DNA secondary structures formed by self-assembly of guanine- rich oligonucleotides *via* Hoogsteen base pairing stabilized by central cations. GQs form *in vivo* in oncogene regulatory regions and within telomeres, which makes them an interesting target for cancer research[1]. Various GQ species are known to assemble higher-order structures, which are believed to affect their biological activity. Thus, understanding these structures and their formation is an important goal, where the EPR spectroscopy (DEER/RIDME techniques) can make a substantial contribution.

Recently, we used rigid square planar $Cu^{II}(pyridine)_4$ complexes covalently incorporated into tetramolecular GQs as spin labels to gain insight into the GQ dimerization *via* π-stacking of terminal G-tetrads.[2] Detection of the dipole-dipole interaction between two Cu^{II} ions each residing in a GQ monomer demonstrated the dimer formation and intercalation of small molecules via exceptionally precise measurement of Cu-Cu distances.

Oligonucleotides adopting mixed duplex/quadruplex conformations have recently gained a lot of attention[3,4]. In the present work we extend the EPR-based approach to novel structures representing two unimolecular GQs connected *via* a duplex bridge (Figure A). Two oligonucleotides were synthesized by solid-phase DNA synthesis, each containing the GQ-forming sequence with pyridine-modified nucleotides capable of binding Cu^{II}. Additional, complementary single-stranded overhangs were attached to the 3' ends, separated by a single nucleotide spacer.

The design allowed the linking of two spin-labeled unimolecular GQs by a duplex bridge, as evidenced by DEER (Figure B-E). The time traces yielded clear dipolar modulations. The mean Cu-Cu distance of 6.3nm showed good agreement with the MD simulations. Our results demonstrate that the Cu^{II} $(pyridine)_4$ spin labels can be successfully applied to complicated DNA structures, containing biologically relevant duplex/quadruplexjunctions.

References

[1] D. Rhodes and H. J. Lipps, G-quadruplexes and their regulatory roles in biology. *Nucleic Acids Res.* **43**, 8627–8637 (2015).
[2] L. M. Stratmann et al., Precise Distance Measurements in DNA G-Quadruplex Dimers and Sandwich Complexes by Pulsed Dipolar EPR Spectroscopy. *Angew. Chem. Int. Ed. doi.org/10.1002/anie.202008618*.
[3] B. Gatto et al., Nucleic Acid Aptamers Based on the G-Quadruplex Structure: Therapeutic and Diagnostic Potential. *Curr. Med. Chem*. **16**, 1248–1265 (2009).
[4] I. Russo Krauss et al., Different duplex/quadruplex junctions determine the properties of anti-thrombin aptamers with mixed folding. *Nucleic Acids Res.* **44**, 983–991 (2016).

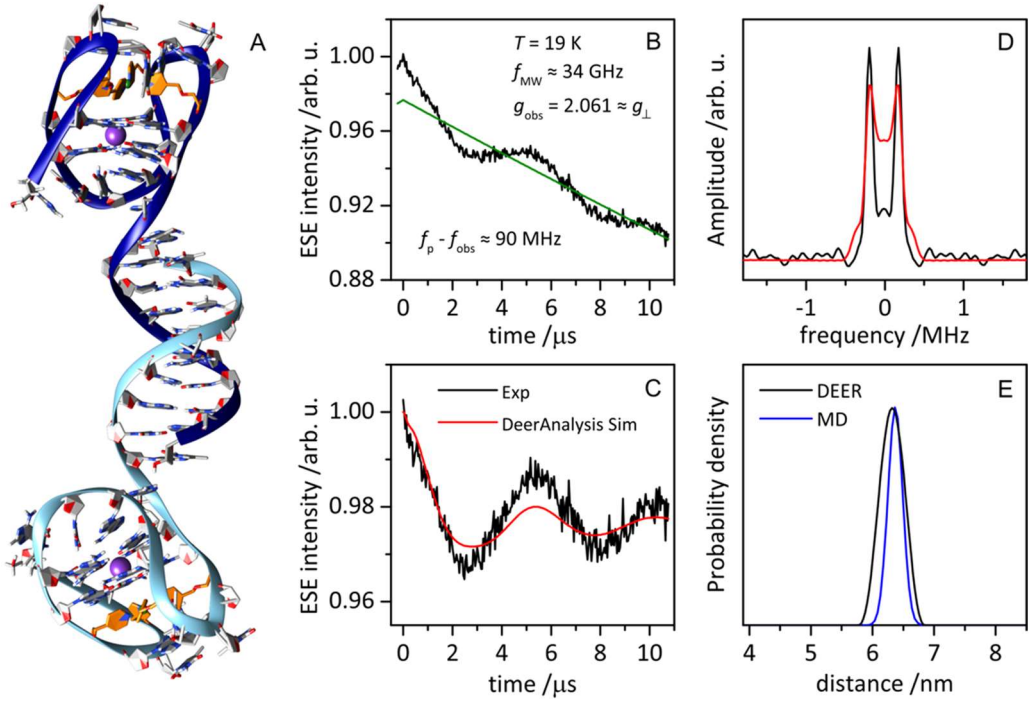


Figure 1. (A) MD-derived model of two duplex-bridged spin-labeled GQs; (B) primary DEER trace with background; (C) form-factor; (D) dipolar spectrum; (E) DeerAnalysis-derived Cu- Cu distance distribution with the MD simulation result.

HIGH FIELD ^{19}F-ENDOR FOR DISTANCE MEASUREMENTS IN THE ANGSTROM TO NANOMETER REGIME IN STRUCTURAL BIOLOGY

Andreas Meyer[1,*], Annemarie Kehl[1], and Marina Bennati[1,2,*]

[1] Research Group EPR Spectroscopy, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany.
[2] Department of Chemistry, Georg-August University of Göttingen, Tammannstr. 2, Göttingen, Germany.

EPR spectroscopy is a powerful method for structural investigations in biomolecular systems that contain unpaired electron spins. The interaction of these unpaired spins with other electron spins can be probed selectively using pulsed dipolar spectroscopy (PELDOR, DQC, etc…) for inter-spin distances of 15 – 100 Å[1] whereas hyperfine spectroscopy (ENDOR, ESEEM, etc…) addresses electron-nuclear spin interactions at distances usually ≤ 5 -7 Å.[2] This restriction to relatively low distances in hyperfine spectroscopy is caused by the low gyromagnetic ratio □ of nuclear spins (^{2}H, ^{13}C, ^{14}N, …) or by spectral crowding in the case of ^{1}H nuclei, which are ubiquitous in biosystems.

This work presents pulsed W-band (94 GHz/3.4 T) ^{19}F-ENDOR as a tool for structural biology. The method benefits from the high gyromagnetic ratio of ^{19}F, which is only ~6% lower than that of protons. This property allows the ^{19}F resonances to be addressed selectively and with high sensitivity at distance ranges clearly exceeding 5 Å. Since fluorine labeling strategies have already been established in other areas of research,[3] W-band ^{19}F-ENDOR has great potential for application in biochemical contexts.

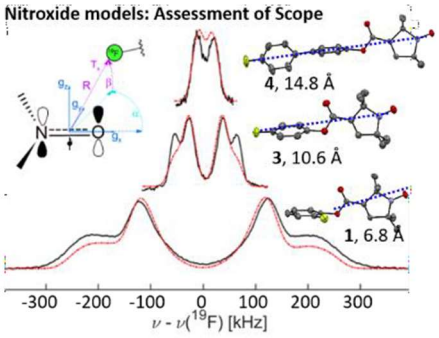
Using a series of small nitroxide model compounds we found that information about the ^{19}F – electron spin distance at atomic resolution can be obtained up to 15 Å.[4]

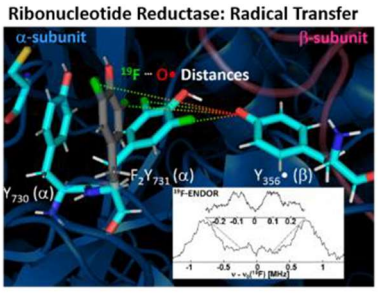
To demonstrate the applicability of the method in a biochemical context, we investigated ^{19}F- and nitroxide-labelled RNA molecules using ^{19}F-ENDOR[4] and were able to refine a previous structural model.[5] As another biochemical example, preliminary results on the ^{19}F-labelled protein ribonucleotide reductase are presented, where the method enables investigating the proton coupled electron transfer in a natural radical transfer chain. Finally, opportunities of employing higher magnetic fields (9.5 T) are discussed.

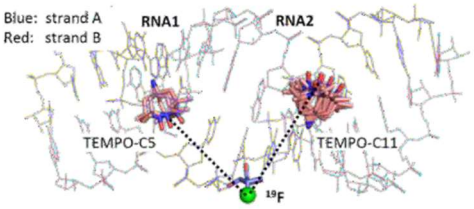
References

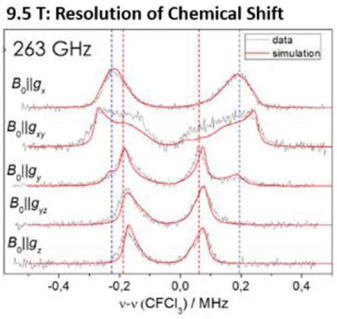
[1] G. Jeschke, The Contribution of Modern EPR to Structural Biology. *Emerg. Top. Life Sci.* **2**, 9–18 (2018).
[2] G. E. Cutsail III, J. Telser, B. M. Hoffman, Advanced Paramagnetic Resonance Spectroscopies of Iron–Sulfur Proteins: Electron Nuclear Double Resonance (ENDOR) and Electron Spin Echo Envelope Modulation (ESEEM). *Biochim. Biophys. Acta BBA-Mol. Cell Res.* **1853**, 1370–1394 (2015).
[3] H. Chen, S. Viel, F. Ziarelli, L. Peng, ^{19}F NMR: A Valuable Tool for Studying Biological Events. *Chem. Soc. Rev.* **42**, 7971–7982 (2013).
[4] A. Meyer, S. Dechert, S. Dey, C. Höbartner, M. Bennati, Measurement of Angstrom to Nanometer Molecular Distances with ^{19}F Nuclear Spins by EPR/ENDOR Spectroscopy. *Angew. Chem. Int. Ed.* **59**, 373–379 (2020).

[5] K. Halbmair, J. Seikowski, I. Tkach, C. Höbartner, D. Sezer, M. Bennati, High-Resolution Measurement of Long-Range Distances in RNA: Pulse EPR Spectroscopy with TEMPO-Labeled Nucleotides. *Chem. Sci.* **7**, 3172–3180 (2016).









MULTIQUANTUM COUNTING OF TRITYL RADICALS

Matthias Bretschneider[1], Phillip E. Spindler[1], Olga Yu. Rogozhnikova[2], Dmitry V. Trukhin[2], Burkhard Endeward[1], Andrey A. Kuzhelev[2], Elena Bagryanskaya[2], Victor M. Tormyshev[2], Thomas F. Prisner[1]

[1] Institute of Physical and Theoretical Chemistry and Center of Biomolecular Magnetic Resonance, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
[2] Novosibirsk Institute of Organic Chemistry, Lavrentiev Avenue 9, Novosibirsk 630090, Russia

Multi-Quantum coherence (MQC) has been used in NMR[1,2] to count coupled spin in clusters. DQC experiments in EPR, first introduced by the Freed group[3], are used to detect the dipolar interaction within a coupled electron spin pair. Here we show that the number of coupled electron spins can also be obtained using this method[4]. To count the number *n* of dipolar coupled spins, experiments which selectively filter the *n*-quantum coherences are proposed. For these experiments a high phase accuracy of the microwave pulses and a sufficient bandwidth to excite homogeneously the electron spins are required. This is achieved using a home built X-band EPR setup based on an arbitrary wave form generator. Here we demonstrate on a series of multi-trityl model compounds that MQ-filtered EPR experiments allow to determine the number of coupled electron spins. The transversal relaxation times of higher quantum coherences are also measured. This approach has the potential to determine the oligomeric state of proteins complexes in their native environment.

References

[1] G. Bodenhausen, R. L. Vold, R. R. Vold, Multiple quantum spin-echo spectroscopy. *J. Magn. Reson.* **37 (1)**, S. 93–106 (1980).
[2] M. Munowitz, A. Pines, Multi-quantum nuclear magnetic resonance spectroscopy. *Science* **233**, 525–531 (1986).
[3] P. P. Borbat, J. H. Freed, Multiple-quantum ESR and distance measurements. *Chem. Phys. Lett.* **313**, 145–154 (1999).
[4] M. Bretschneider, P. E. Spindler, O. Yu. Rogozhnikova, D. V. Trukhin, B.Endeward, A. A. Kuzhelev, E. Bagryanskaya, V. M. Tormyshev, T. F. Prisner, Multiquantum Counting of Trityl Radicals. *J.Phys.Chem.Lett.* **11**, 6286−6290 (2020).

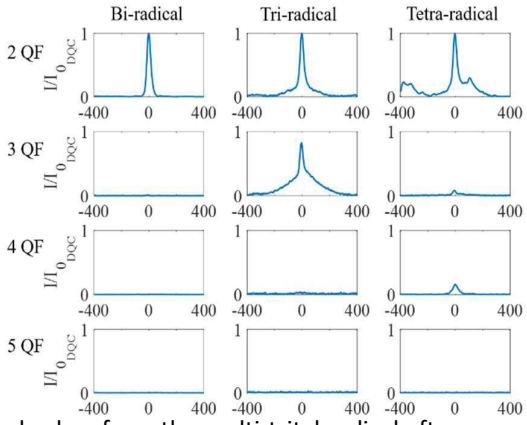


Figure 1. Multi-quantum filtered echos from the multi-trityl radical after appropriate phase cycling to filter the *n*=2-6 quantum.

COMPACTION OF RNA DUPLEXES IN THE CELL

Alberto Collauto[1], Sören von Bülow[2], Dnyaneshwar B. Gophane[3], Haraldur Y. Júlíusson[3], Subham Saha[3], Lukas S. Stelzl[2], Gerhard Hummer[2], Snorri Th. Sigurdsson[3], Thomas F. Prisner[1]

[1] Institute of Physical and Theoretical Chemistry and Center of Biomolecular Magnetic Resonance, Goethe University Frankfurt, Max-von-Laue-Straße 7. D-60438, Frankfurt am Main, Germany.
[2] Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Max-von-Laue-Straße 3. D-60438, Frankfurt am Main, Germany.
[3] Department of Chemistry, Science Institute, University of Iceland, Dunhagi 3. IS-107, Reykjavík, Iceland.

In this work PELDOR/DEER spectroscopy combined with advanced nitroxide-based site-directed spin labelling techniques is used to shed light on the conformation of short RNA duplexes inside Xenopus lævis oocytes. The measurements provide access to distances between labels in the nanometer range at Ångstrom resolution, and, if rigid labels are used, allow furthermore to obtain insights into details of the conformational ensemble.[1] Results are shown for RNA duplexes labelled with strategies differing both by the rigidity of the paramagnetic tag and by its point of attachment to the nucleotide.[2-4] The label was stabilized in the reducing environment of the cell by tetraethyl groups flanking the nitroxide.[5]

Consistently and reproducibly across samples, the experiments showed a reduction of the inter-spin distance upon internalization of the RNA duplexes into cells. To gain insight into the microscopic origin of the observed change, microsecond-scale atomistic molecular dynamics (MD) simulations were performed. Transferring the duplex RNA from water into a dense solution of lysozyme was found to induce a contraction similar to that seen in the experiments.3 Nonspecific electrostatic protein-RNA interactions were identified as the main driver of this compaction by up to 1 Å. On the one hand, the counter-charges on the proteins reduce the effective charge of the RNA backbone; on the other hand, simultaneous interactions with multiple amino acids can exert a mechanical pull on the RNA.

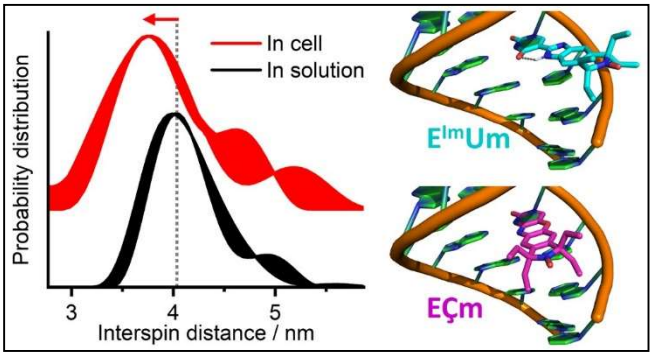
We conclude that the structure of duplex RNA changes in the unique microenvironment inside cells, where nonspecific electrostatic interactions with proteins induce a noticeable compaction.

References

[1] L. S. Stelzl, N. Erlenbach, M. Heinz, T. F. Prisner, G. Hummer, Resolving the conformational dynamics of DNA with Ångstrom resolution by pulsed electron–electron double resonance and molecular dynamics. *J. Am. Chem. Soc.* **139**, 11674–11677 (2017).
[2] S. Saha, A. P. Jagtap, S. Th. Sigurdsson, Site-directed spin labeling of 2'-amino groups in RNA with isoindoline nitroxides that are resistant to reduction. *Chem. Commun.* **51**, 13142–13145 (2015).
[3] A. Collauto, S. von Bülow, D. B. Gophane, S. Saha, L. S. Stelzl, G. Hummer, S. Th. Sigurdsson, T. F. Prisner, Compaction of RNA duplexes in the cell. *Angew. Chem. Int. Ed.* DOI: 10.1002/anie.202009800 (2020).

[4] H. Y. Juliusson, S. Th. Sigurdsson, Reduction resistant and rigid nitroxide spin-labels for DNA and RNA. *J. Org. Chem.* **85**, 4036–4046 (2020).

[5] A. P. Jagtap, I. Krstic, N. C. Kunjir, R. Hänsel, T. F. Prisner, S. Th. Sigurdsson, Sterically shielded spin labels for in-cell EPR spectroscopy: analysis of stability in reducing environment. *Free Radical Res.* **49**, 78–85 (2015).



CONFORMATIONAL PLASTICITY OF AN ABC EXPORTER IN DIFFERENT ENVIRONMENTS: STRUCTURAL INSIGHTS FROM SPIN-LABELED NANOBODIES AND EPR

Laura Galazzo[1], Gianmarco Meier[2], Markus A. Seeger[2], Enrica Bordignon[1]

[1] Faculty of Chemistry and Biochemistry, Ruhr-Universität Bochum, Universitätsstr. 150, 44801 Bochum, Germany.
[2] Institute of Medical Microbiology, University of Zürich, Gloriastr. 30/32, 8006 Zürich, Switzerland.

ABC transporters are proteins that couple the energy deriving from the binding and hydrolysis of ATP to large conformational changes that enable substrate translocation across membranes. MsbA is a homodimeric ABC exporter that flips lipid A - the precursor of lipopolysaccharide (LPS) - across the cytoplasmic membrane and is therefore essential for the survival of *E. coli*[1].

In this study, we used spin-labeled nanobodies (i.e. specific single domain antibodies) as conformational reporters targeting MsbA, after having tested the feasibility of the method in a previous work[2]. The advantage of using spin-labeled nanobodies relies in the possibility of investigating membrane proteins in situ without the need of site-directed mutagenesis. To really understand membrane proteins at the molecular level, they would ideally need to be investigated in situ, namely in the context of the living cell. However, structural elucidation of membrane proteins mostly requires their extraction from the native membrane environment with detergents. Membrane mimics such as liposomes or nanodiscs serve as surrogates of the native lipid bilayer and are thus widely used for structural and functional analyses of membrane proteins.

In our study we made use of DEER (Double Electron Electron Resonance) to reliably measure distances between pairs of Gd(III) spin probes engineered at desired positions in a non-state-specific nanobody targeting the two nucleotide binding domains in the homodimer. Data obtained in detergents, proteoliposomes, nanodiscs and inside-out vesicles from *E. coli* and *L. lactis* will be presented and compared with data acquired with cryo-EM technique. Surprisingly, different environments exhibited different mean distances and especially different degrees of heterogeneity in the ensemble, thus calling the physiological relevance of insights obtained using membrane mimics into question.

References

[1] J. P. Overington, B. Al-Lazikani, A. L. Hopkins, How many drug targets are there? *Nat. Rev. Drug Discov.* **5**, 993-996 (2006).
[2]L. Galazzo, G. Meier, M. H. Timachi, C. A. J. Hutter, M. A. Seeger, E. Bordignon, Spin-labeled nanobodies as protein conformational reporters for electron paramagnetic resonance in cellular membranes. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 2441-2448 (2020).

MICROSECOND SCALE PROTEIN DYNAMICS BY 2D-ELDOR: COMPUTATIONAL IMPROVEMENTS

Pranav Gupta[1], Kevin Chaudhari[1], Jack H. Freed[1]

[1] National Biomedical Center for Advanced ESR Technology and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA 14850.

Two-Dimensional Electron Double Resonance (2D-ELDOR) studies at high frequencies such as 95GHz can offer unique information on molecular motions and dynamics given its better g-tensor resolution. This spectroscopic technique has much potential, since it extends the time-scale of ESR spectroscopy toward slower processes (e.g. conformational changes, chemical exchange, etc), which can be observed by the development of exchange cross- peaks. 2D-ELDOR has been successfully applied at lower frequencies to study phase equilibria in complex membranes, but further use for biological systems, in particular at higher frequencies, was so far limited by several difficulties. One of these lies in spectral analysis, since the usual methods of spectral simulation in the slow motional region are limited for 2D-ELDOR and break down for the case of slow motional 95GHz spectra. ACERT has in the past pioneered the development of computational tools to simulate and interpret dynamic ESR spectra. Here we report on our latest improvement in computational analysis compared to earlier versions of our programs, since it offers much faster computation times in the very slow motional regime through rational Krylov methods that build upon existing Krylov methods used by the ESR community and build compact rational approximations to the Krylov subspace generated by SLE matrices. We demonstrate our results for slow motional 2D-ELDOR spectra corresponding to a prospective biological application of 2D- ELDOR, namely the conformational exchange in biomolecules. Combining the latest theoretical developments[1,2] with upcoming dead time improvements in our 95GHz 2D- ELDOR ACERT spectrometer, we are now well-equipped to obtain key insights into the conformational dynamics of biomolecules at sub-microsecond time scales.

References

[1] P. Gupta, Z. Liang, and J. H. Freed**,** J. Chem. Phys. **152,** 214112 (2020)
[2] P. Gupta, K. Chaudhari, and J. H. Freed (under preparation)

CHEMICAL SHIFT ANISOTROPY IN ^{19}F ENDOR SPECTRA AT 263 GHZ

Annemarie Kehl[1], Markus Hiller[1], Andreas Meyer[1], Marina Bennati[1,2]

[1] Research Group EPR Spectroscopy, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
[2] Department of Chemistry, Georg-August University of Göttingen, Tammannstr. 2, Göttingen, Germany

Fluorine spin labels so far have found many applications, especially the nuclear spin of 1/2, 100% natural abundance, a high gyromagnetic ratio and a large range of chemical shifts make them valuable in magnetic resonance based spectroscopies such as NMR and W-band (94 GHz) ENDOR.[1,2] Using the prototype E780 spectrometer enables ENDOR measurements at 263 GHz with field strengths comparable to NMR (9.4 T), which increases spectral resolution and orientation selectivity.[3]

In this contribution ^{19}F ENDOR spectra at 263 GHz are presented for several nitroxide radical model systems which consist of an aromatic ring substituted with fluorine or a CF_3-group (one example shown in Figure 1). The spectra display an asymmetry, which is not clearly resolved in the spectra at 94 GHz (see Figure 1). It indicates the resolution of the chemical shift anisotropy, which so far has been neglected for EPR and ENDOR spectroscopy.

To simulate the spectra a previously published simulation routine[4] was adapted by implementing the chemical shift tensor and applied to simulate the spectra both at 263 GHz and 94 GHz. For the simulation, the orientation of the chemical shift tensor towards the g-frame is described by Euler angles. This correlation of the chemical shift tensor and the g-tensor as displayed in Figure 1 can provide further structural information on the sample.

In comparison to W-band ENDOR the ^{19}F ENDOR at 263 GHz can provide high resolution spectra and also improve the interpretation by providing data on the chemical shift tensor.

References

[1] K. E. Arntson, W. C. K. Pomerantz, Protein-Observed Fluorine NMR: A Bioorthogonal Approach for Small Molecule Discovery. *J. Med. Chem.*, **59**, 5158 – 5171 (2016).
[2] A. Meyer, S. Dechert, S. Dey, C. Höbartner, M. Bennati, Measurement of Angstrom to Nanometer Molecular Distances with ^{19}F Nuclear Spins by EPR/ENDOR Spectroscopy. *Angew. Chem. Int. Ed.*, **59**, 373-379 (2020).
[3] I. Tkach, I. Bejenke, F. Hecker, A. Kehl, M. Kasanmascheff, I. Gromov, I. Prisecaru, P. Höfer, M. Hiller, M. Bennati, ^{1}H high field electron-nuclear double resonance spectroscopy at 263 GHz/9.4 T. *JMR*, **303**, 17 – 27 (2019).
[4] M. Bennati, C. T. Farrar, J. A. Bryant, S. J. Inati, V. Weis, G. J. Gerfen, P. Riggs-Gelasco, J. Stubbe, R. G. Griffin, Pulsed Electron-Nuclear Double Resonance (ENDOR) at 140 GHz. *JMR*, **138**, 232 – 243 (1999).

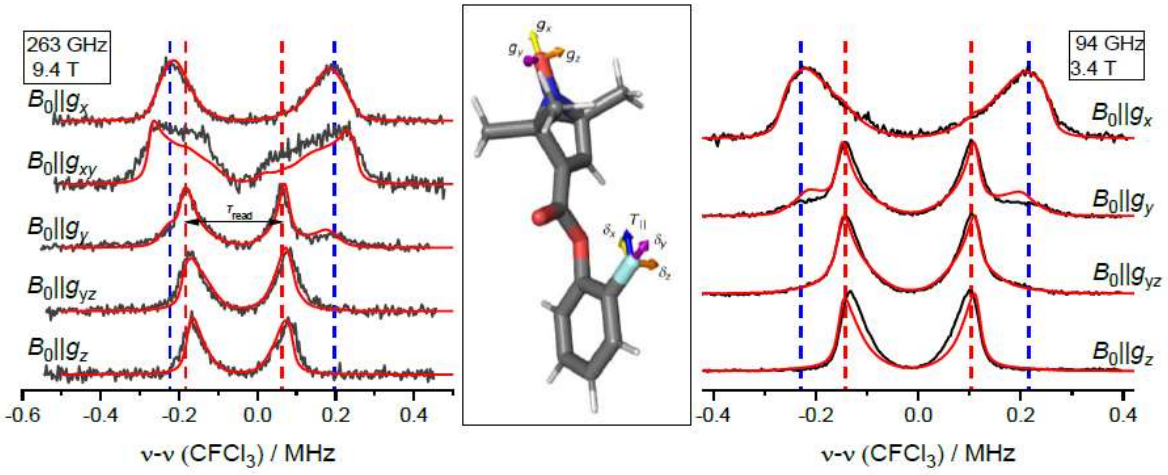


Figure 1. Results for one of the model systems: 263 GHz (left) and 94 GHz (right) ^{19}F ENDOR spectra (black) and their simulation (red) of the compound displayed in the center, the dashed lines indicate the positions of the parallel (blue) and perpendicular (red) component of the hyperfine coupling at $B0||gy$.

T_M EDITED DOUBLE ELECTRON-ELECTRON RESONANCE EPR ON ^{1}H-METHYL LABELED, PERDEUTERATED PROTEINS PERMITS DISTANCE ASSIGNMENT IN PROTEIN COMPLEXES

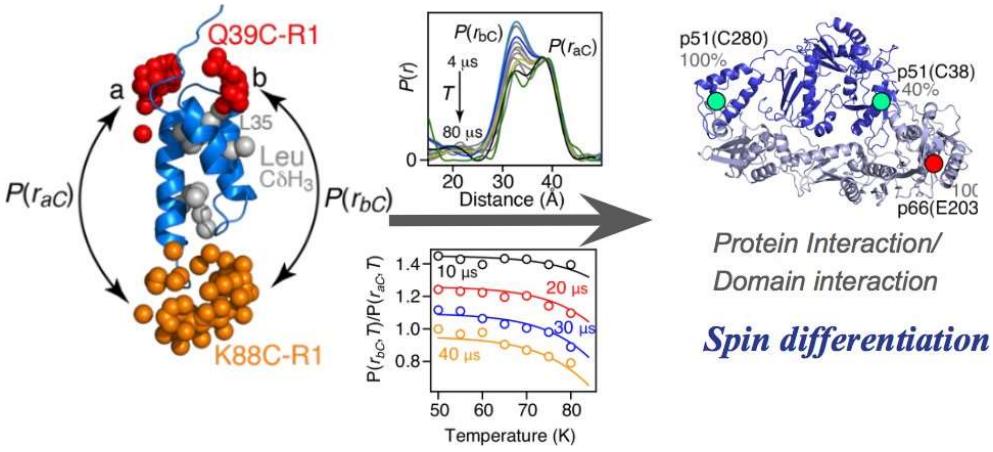
Thomas Schmidt[1], G Marius Clore[1]

[1] Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0520, USA

Modulating the phase-memory relaxation time (T_m) of a spin label by introducing ^{1}H-methyl groups in a perdeuterated protein background is used in DEER experiments to assign interactions in multimodal P(r) distributions.[1] Proof of principle is demonstrated using Protein A where one nitroxide label occupies two distinct regions of conformational space. The presence of a single protonated methyl group in close proximity (4–8 Å) to only one of the two nitroxide rotamer ensembles results in a selective and substantial decrease in T_m, manifested by differential decay of the peak intensities in the bimodal P(r) distance distribution as a function of the total dipolar evolution time.[2] Further spin differentiation occurs due to methyl rotation as function of temperature between 20-100 K. This form of T_m filtering facilitates DEER structural analysis of biomolecular systems with three spin labels, including complexes involving multimeric proteins as evident by studies on HIV-1 reverse transcriptase.[3]

References

[1] T. Schmidt, G. M. Clore, T_m filtering by ^{1}H-methyl labeling in a deuterated protein for pulsed double electron–electron resonance EPR. Chem. Commun., **56**, 10890-10893 (2020).
[2] E. R. Canarie , S. M. Jahn and S. Stoll , Quantitative Structure-Based Prediction of Electron Spin Decoherence in Organic Radicals *J. Phys. Chem. Lett.*, **11** , 3396 —3400 (2020).
[3] T. Schmidt, C. D. Schwieters, G. M. Clore, Spatial domain organization in the HIV-1 reverse transcriptase p66 homodimer precursor probed by double electron-electron resonance EPR. PNAS, 116, **36**, 17809-17816 (2019)



DIPOLAR LINEWIDTH AND DECAY OF A HOMOGENOUS DISTRIBUTION OF POLARIZED ELECTRON SPINS

Sarah R. Sweger[1], Vasyl Denysenkov[2], Lutz Maibaum[1], Thomas Prisner[2], Stefan Stoll[1]

[1]University of Washington, Department of Chemistry, Seattle, WA 98195
[2]Goethe University, Institute for Physical and Theoretical Chemistry, 60438 Frankfurt am Main, Germany

Pulse electron paramagnetic resonance (EPR) spectroscopy, particularly those methods that measure dipolar couplings, are valuable for resolving protein structures. Among these is double electron–electron resonance (DEER) spectroscopy which measures the magnetic dipolar coupling between two radicals in the nanometer range, often on spin-labeled proteins. Such experiments are commonly conducted in the high-temperature regime, where the thermal polarization of the spins is minimal. In this regime, the DEER background signal has a uniform distribution of phases resulting in a single exponential decay function. To develop a more complete understanding of the spin dynamics underlying DEER, we have extended the theory beyond this limit to examine how these dynamics are affected in a highly polarized environment (low temperatures and high fields). This work has led to the discovery of a linear phase dependence of the signal on the magnitude of polarization, with a maximal magnitude of 5% of the in-phase signal. Here we characterize the linear phase dependence through analytical derivation, numerical simulation, and experimental measurements at several fields and temperatures. While the effect comprises only a fraction of the overall DEER signal for these experiments, it highlights a novel aspect of the fundamental spin physics underlying DEER spectroscopy.

References

[1] Marko et al., *Molecular Physics*, **111**, 18-19 (2013).

TOWARDS MILLIWATT DEER ON FAST RELAXING SPIN SPECIES

Markus Teucher[1], Jason W. Sidabras[1], Alexander Schnegg[1]

[1] EPR Research Group, Max Planck for Chemical Energy Conversion, Stiftstraße 34-36, Mülheim an der Ruhr, Germany, 45470

Pulse electron paramagnetic resonance (EPR) experiments rely on very high incident microwave powers (> 300 W) to create the short pulse lengths needed to excite a sizeable portion of the spectrum. However, such high powers result in long dead times (> 50-100 ns) which limit the applicability of pulse EPR. In order to significantly reduce the power needed for these experiments, superior B_1-conversion efficiencies are required. The recently introduced microhelix[1], combines a high B_1-conversion efficiency with an intrinsically large bandwidth (low Q-value) and a high sensitivity. We report deadtimes down to 14 ns achieved using only 300-600 mW of power at X-band. Small build sizes as realized with the microhelix (a) give access to volume-limited samples, while shorter deadtimes allow to investigate fast relaxing spin species. In order to take advantage of this setup, deadtime-limited experiments were explored. We show that the deadtime-limited 3-pulse DEER experiment offers superior sensitivity over the 4-pulse DEER experiment in case of fast transverse relaxation times (b). Resonators like the microhelix can pave the road towards low-cost benchtop pulse EPR spectrometers, which will accelerate the adoption of pulse EPR by a broader community[2].

References

[1] Sidabras, Jason W., et al. "Extending electron paramagnetic resonance to nanoliter volume protein single crystals using a self-resonant microhelix." *Science Advances* 5.10 (**2019**): eaay1394.
[2] Goldfarb, Daniella. "Pulse EPR in biological systems–Beyond the expert's courtyard." *Journal of Magnetic Resonance* 306 (**2019**): 102-108.
[3] Lovett, J. E., Brendon W. Lovett, and J. Harmer. "DEER-Stitch: Combining three-and four-pulse DEER measurements for high sensitivity, deadtime free data." *Journal of Magnetic Resonance* 223 (**2012**): 98-106.

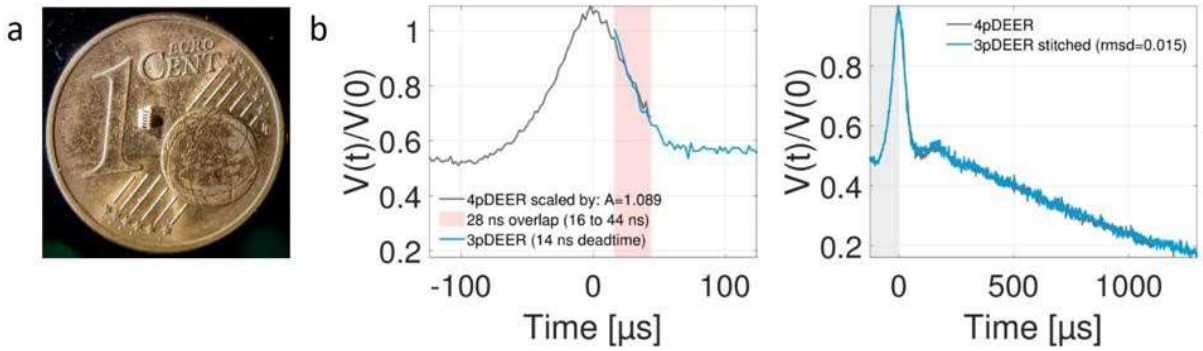


Figure 1. (a) Size comparison of microhelix (0.4 mm inner diameter; 1.2 mm height)[1] and a one Eurocent coin (16.25 mm diameter). (b) 3-pulse and 4-pulse DEER experiments performed with the microhelix. DEER-Stitch[3] can be utilized to combine 3-pulse and 4-pulse data to compensate for deadtimes in 3-pulse DEER.

04. Hyperpolarization

PROGRESS AND FUTURE PLANS CONCERNING BULLET-DNP

Behdad Aghelnejad[1], Mathieu Baudin[1], Geoffrey Bodenhausen[1]

[1] Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France.

Dissolution dynamic nuclear polarization (D-DNP) is currently the subject of many new developments in view of boosting the sensitivity of nuclear magnetic resonance spectroscopy (NMR) and imaging (MRI). Bullet-DNP[1] is a method that is gaining attention recently. A polarized sample is transferred to a spectrometer in the solid state and is dissolved after the transfer, rather than being dissolved in the polarizer, as in the conventional dissolution DNP setup. The bullet-DNP setup has the advantage of significantly decreasing the dilution factor and provides the possibility to use organic solvents for dissolution.

This work was started with the design of a low-temperature broadband NMR probe for multinuclear cross-polarization[2]. This double resonance ^{1}H-X broadband D-DNP probe is specially adapted to the bullet system. The X channel covers a broad range of nuclei from ^{15}N to ^{23}Na (i.e., from 28.9 to 75.5 MHz at 6.7 T). Using the deuterium channel of this probe it was demonstrated that the line shapes due to the quadrupolar couplings of deuterium spins present in virtually all solvents used for such experiments (“DNP juice”) allow a quick yet accurate determination of the deuterium spin temperature or, equivalently, the deuterium polarization[3]. The project continued with the development of a contamination-free insert and fast ejection of the sample (within approximately 1 s) which is now operational. Currently in progress is the construction of a magnetic tunnel with a homogeneous field near 0.34 T to preserve the polarization during the transfer of the solid bullets from the polarizer to the spectrometer, and an efficient dissolution system to dissolve the bullets with adjustable amounts of liquid in the bore of the spectrometer magnet.

References

[1] K. Kouřil, H. Kouřilová, S. Bartram, M.H. Levitt, B. Meier, Scalable dissolution-dynamic nuclear polarization with rapid transfer of a polarized solid, Nat. Commun. **10**, 1733 (2019).

[2] B. Aghelnejad, G. Bodenhausen, S. Marhabaie, A Low-Temperature Broadband NMR Probe for Multinuclear Cross-Polarization, ChemPhysChem **20,** 2830-2835 (2019).

[3] B. Aghelnejad, S. Marhabaie, M. Baudin, G. Bodenhausen, D. Carnevale, Spin Thermometry: A Straightforward Measure of Millikelvin Deuterium Spin Temperatures Achieved by Dynamic Nuclear Polarization, J. Phys. Chem. Lett. **11 (9)**, 3219-3225 (2020).

METABOLIC NMR WITHOUT THE MAGNET

James Eills[1,2], Román Picazo-Frutos[1,2], Dmitry Budker[1,2], John W. Blanchard[2,3]

[1] Johannes Gutenberg University, D-55090 Mainz, Germany
[2] Helmholtz-Institut Mainz, GSI Helmholtzzentrum für Schwerionenforschung GmbH, 55128 Mainz, Germany
[3] NVision Imaging Technologies GmbH, 89081 Ulm, Germany

Zero- to ultralow-field (ZULF) NMR is a modality of NMR experiment performed in the absence of a strong magnetic field. In this regime, Larmor precession is suppressed, and other interactions such as *J*-couplings dominate. This grants three important advantages: the low frequency signals readily penetrate metals and conductive materials, magnetic susceptibility-induced line broadening from sample inhomogeneity is suppressed, and no NMR magnet is needed.

In this work we use two commercially available optical magnetometers in a gradiometric configuration for common-mode noise cancellation. These are positioned against an NMR tube, with Helmholtz coils surrounding the sample to apply magnetic field pulses. This is all contained within a mu-metal shield to attenuate Earth's field, and allow for zero-field detection. This setup is shown in Figure 1a. In this work we form the biomolecule [1-^{13}C]fumarate via parahydrogen-induced polarization in concentrations of ∼120 mM, at up to 45% ^{13}C polarization in aqueous solution[1]. This highly polarized solution is brought into the ZULF spectrometer for signal acquisition. A zero-field spectrum of [1-^{13}C]fumarate is shown in Figure 1b.

Fumarate is a biomolecule which is converted by the enzyme fumarase into malate in one step of the Krebs cycle (Figure 1c). This metabolic step is observed *in vivo* in hyperpolarized magnetic resonance imaging experiments, and has been shown in preclinical studies to be a sensitive marker of medical conditions that involve cell necrosis[2-4]. Now, for the first time, we show that ZULF NMR can be used to study metabolism by observing fumarate conversion to malate. The results are shown in Figure 1d. This opens up exciting possibilities to perform metabolic MRI without the need for a bulky superconducting magnet, or in the presence of metals and conductive materials.

References

[1] Knecht, S.; Blanchard, J. W.; Barskiy, D.; Cavallari, E.; Dagys, L.; van Dyke, E.; Tsukanov, M.; Bliemel, B.; Münnemann, K.; Aime, S.; Reineri, F.; Levitt, M. H.; Buntkowsky, G.; Pines, A.; Blümler, P.; Budker, D.; Eills, J. Rapid Hyperpolarization and Purification of the Metabolite Fumarate in Aqueous Solution. ChemRxiv, **2020**
[2] Miller, J. J.; Lau, A. Z.; Nielsen, P. M.; McMullen-Klein, G.; Lewis, A. J.; Jespersen, N. R.; Ball, V.; Gallagher, F. A.; Carr, C. A.; Laustsen, C.; Bøtker, H. E.; Tyler, D. J.; Schroeder, M. A. Hyperpolarized [1,4-13C2]Fumarate Enables Magnetic Resonance-Based Imaging of Myocardial Necrosis. *JACC: Cardiovascular Imaging* **2018**, *11* (11), 1594–1606

[3] Clatworthy, M. R.; Kettunen, M. I.; Hu, D.-E.; Mathews, R. J.; Witney, T. H.; Kennedy, B. W. C.; Bohndiek, S. E.; Gallagher, F. A.; Jarvis, L. B.; Smith, K. G. C.; Brindle, K. M. Magnetic Resonance Imaging with Hyperpolarized [1,4-13C2]Fumarate Allows Detection of Early Renal Acute Tubular Necrosis. *PNAS* **2012**, *109* (33), 13374– 13379
[4] Mignion, L.; Dutta, P.; Martinez, G. V.; Foroutan, P.; Gillies, R. J.; Jordan, B. F. Monitoring Chemotherapeutic Response by Hyperpolarized 13C-Fumarate MRS and Diffusion MRI. *Cancer Res* **2014**, *74* (3), 686–694

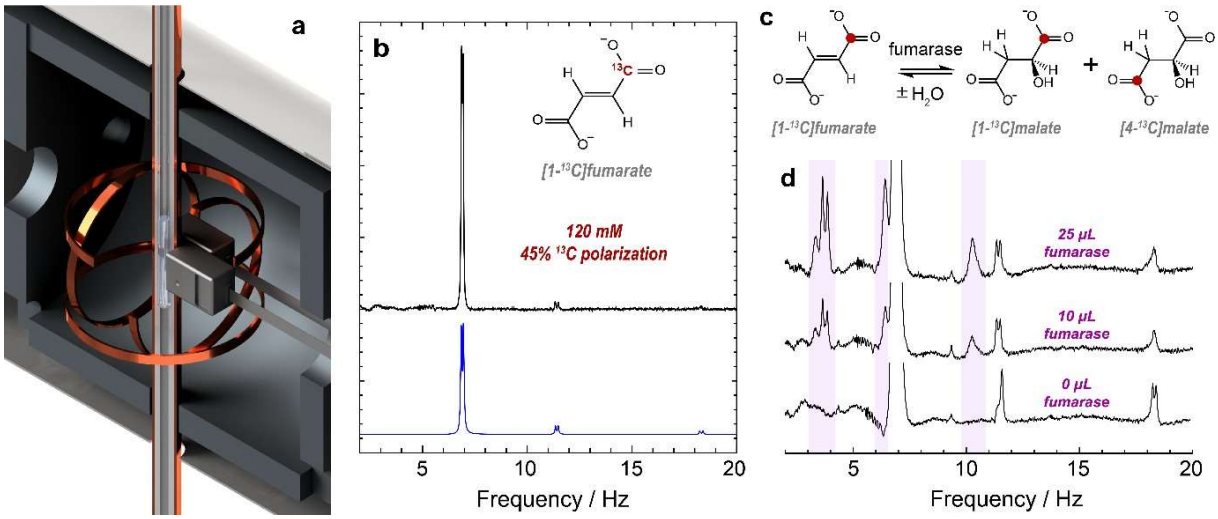


Figure 1. (a) The ZULF setup used in this work, with QuSpin magnetometers shown in black against the NMR tube. (b) A zero-field spectrum of hyperpolarized [1-^{13}C]fumarate (black) with a simulated spectrum beneath (blue). (c) The biochemical transformation of fumarate into malate. (d) Zero-field spectra of a hyperpolarized [1-^{13}C]fumarate solution after addition of the enzyme fumarase to catalyze the formation of malate. Malate peaks are highlighted in pink.

INCREASING HYPERPOLARIZATION LIFETIME IN ARBITRARY FROZEN SOLUTIONS WITH HYPERPOLARIZING POLYMERS (HYPOP)

Théo El Daraï,[1,2] Samuel F. Cousin,[1] Quentin Chappuis,[1] Morgan Ceillier,[1] Olivier Cala,[1] Damien Montarnal,[2] and Sami Jannin[1]

[1] Univ Lyon, Université Claude Bernard Lyon 1, ENS-Lyon, CNRS, UMR 5280, Institut des Sciences Analytiques, 5 rue de la Doua, 69100 Villeurbanne, France
[2] Univ Lyon. Université Claude Bernard Lyon 1, CPE Lyon, CNRS, UMR 5265, Chemistry, Catalysis, Polymers and Processes, 43 Bvd du 11 Novembre 1918, 69616 Villeurbanne, France

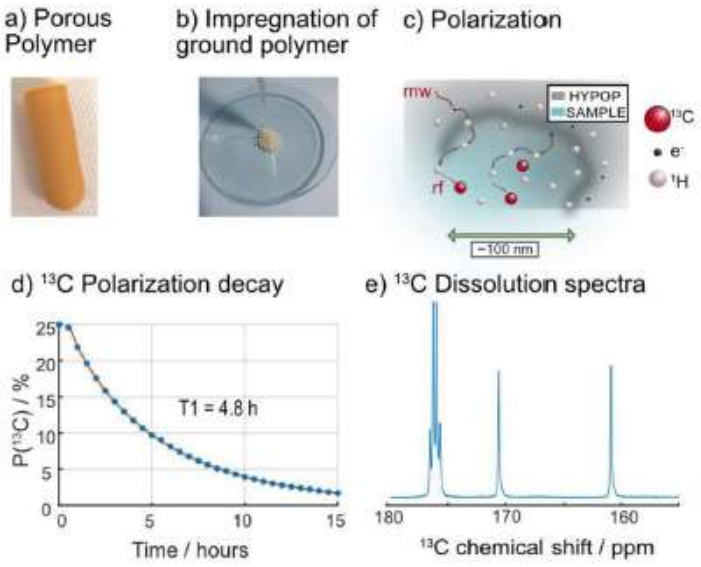
Dissolution Dynamic Nuclear Polarization (dDNP), since its discovery, revealed years after years to be a powerful tool for Magnetic Resonance. This is mainly due to the high performances of the method, and the ability to be coupled with most of Magnetic Resonance technics (MRI, NMR …)[1]. However, those two advantages are counter-balanced by the cost of the equipment and the technical challenges, which can be discouraging. A better solution could be to centralize polarization in a dedicated multiple sample polarizer and then transport the hyperpolarized samples to remote sites. Such possibility would enable a broader democratization of the method. However, conventional dDNP samples cannot be easily transported due to short polarization lifetimes outside of the polarizer.

To overpass this crucial limitation, two methods have emerged few years ago [2,3] and rely on using novel sample formulations, either UV induced radicals or phase separation between a ground salt dispersed in an organic phase doped with radicals. Those solutions suffer from inherent sample specificity and cannot be extended to arbitrary samples and applications. Here, we developed porous hyperpolarizing polymer (HYPOP) matrices containing radicals, which can be impregnated with virtually any kind of solutions, then can be used to polarize the solution and finally transported to another place for dissolution and analysis.

Here, we will present the synthesis of such matrices which allow to reach high level of polarization $P(^1H)$>50% and $P(^{13}C)$~25% while offering the possibility to store or transport the sample after during several hours. A description of how the control of the morphology and porosity is crucial to extend hyperpolarization lifetime will be developed. We will finally show how the partial quenching by ascorbic acid of the material can be performed to inactivated surface radicals and further extend the polarization lifetime.

References

[1] Ardenkjaer-Larsen JH. On the present and future of dissolution-DNP. *J Magn Reson*. **264**, 3-12 (2016).
[2] Ji X, Bornet A, Vuichoud B, et al. Transportable hyperpolarized metabolites. *Nat Commun*. (2017).
[3] Capozzi A, Cheng T, Boero G, Roussel C, Comment A. Thermal annihilation of photo-induced radicals following dynamic nuclear polarization to produce transportable frozen hyperpolarized 13C-substrates. *Nat Commun*. (2017).


a) Porous Polymer
b) Impregnation of ground polymer
c) Polarization
mw
rf
HYPOP
SAMPLE
^{13}C
e$^-$
^{1}H
~100 nm
d) ^{13}C Polarization decay
P(^{13}C) / %
T1 = 4.8 h
Time / hours
e) ^{13}C Dissolution spectra
^{13}C chemical shift / ppm

A MAGIC ANGLE SPINNING ACTIVATED ^{17}O DNP RASER

Michael A. Hope[1], Snædís Björgvinsdóttir[1] Clare P. Grey[2], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
[2] Dept. Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK.

In the raser effect, a sample spontaneously develops transverse magnetisation, which then precesses indefinitely, allowing exceptionally narrow linewidths to be recorded in the NMR spectrum, without applying pulses. Rasers have been demonstrated using e.g. parahydrogen-induced polarisation (PHIP) in solution,[1] optical pumping of ^{3}He or ^{129}Xe gasses,[2] or DNP of Cr^{3+}-doped Al_2O_3 at 1.6 K.[3] To achieve this phenomenon a combination of large negative polarisation, high density of spins, and long nuclear coherence lifetimes is required.

Here we present the first magic angle spinning (MAS) raser (Fig. 1a). At 9.4 T and 110 K, the exceptional ^{17}O enhancements of more than −500 achieved by endogenous solid effect DNP of ^{17}O-enriched Gd-doped CeO_2 provide the large negative magnetisation, while MAS affords the long nuclear coherence lifetime. This allows a 2 millihertz ^{17}O NMR linewidth to be measured, which is limited only by the field stability. The raser effect can be reversibly activated and deactivated by varying the MAS frequency (Fig. 1b) or switching on or off the microwave source. The use of MAS DNP to enable the raser effect should be further applicable to other systems and nuclei to achieve extremely narrow linewidths.

References

[1] Suefke, M.; Lehmkuhl, S.; Liebisch, A.; Blümich, B.; Appelt, S., Para-hydrogen raser delivers sub-millihertz resolution in nuclear magnetic resonance. *Nat. Phys.* **2017**, *13*, 568–572.
[2] Chupp, T. E.; Hoare, R. J.; Walsworth, R. L.; Wu, B., Spin-exchange-pumped 3He and 129Xe Zeeman masers. *Phys. Rev. Lett.* **1994**, *72*, 2363–2366.
[3] Bösiger, P.; Brun, E.; Meier, D., Solid-State Nuclear Spin-Flip Maser Pumped by Dynamic Nuclear Polarization. *Phys. Rev. Lett.* **1977**, *38*, 602–605.

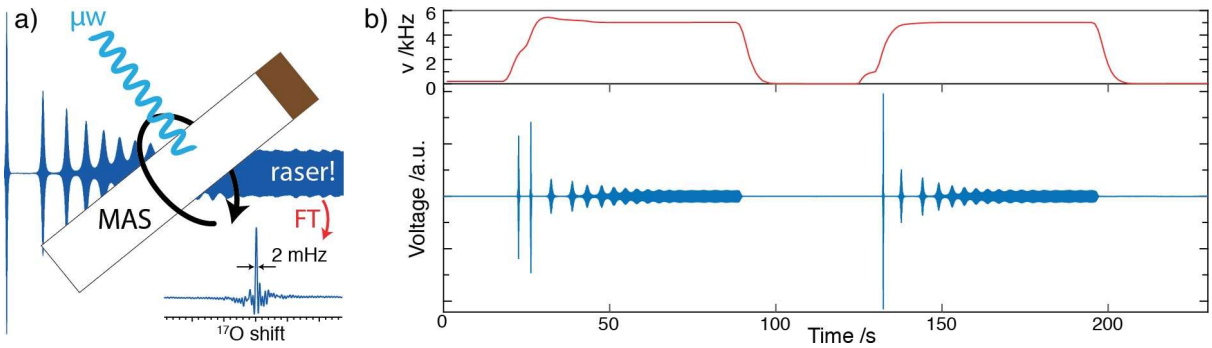


Figure 1. a) Schematic of the MAS raser. b) Reversible switching of the raser by varying the MAS frequency.

UV-IRRADIATED 2-KETO-(1-^{13}C)ISOCAPROIC ACID FOR HIGH PERFORMANCE ^{13}C HYPERPOLARIZED MR

Saket Patel[1], Arthur C. Pinon[1], Mathilde H. Lerche[1], Magnus Karlsson[1], Andrea Capozzi[2], Jan Henrik Ardenkjær-Larsen[1]

[1] Technical University of Denmark, Department of Health Technology, Center for Hyperpolarization in Magnetic Resonance, Building 349, DK-2800 Kgs Lyngby, Denmark.
[2] Ecole Polytechnique Fédérale de Lausanne, School of Basic Science, Institute of Physics, Laboratory for Functional and Metabolic Imaging, Station 6, CH-1015 Lausanne, Switzerland

Enhancing the sensitivity of magnetic resonance spectroscopy/imaging (MRS/MRI) by dissolution dynamic nuclear polarization (dDNP) has enabled new MRS applications such as non-invasive real time monitoring of normal and abnormal cellular physiology with the potential to unravel diseases, develop novel treatment regimes, and quantify enzymatic processes.[1] An important enzyme in this context is branched chain amino acid transaminase (BCAT) that has an important role in nitrogen shuttling and glutamate metabolism in the brain and has been successfully shown to metabolize hyperpolarized (HP) [1-^{13}C]ketoisocaproate (KIC) to leucine in an enzymatic transformation. HP KIC has been demonstrated to efficiently probe BCAT activity and to assess molecular signatures of tumors in rodents based on vastly different levels of BCAT activity.[2] HP-KIC has also been implemented for imaging of cerebral metabolism in normal rats and has been suggested to be a promising substrate for evaluation of cerebral BCAT activity, linked to neurodegenerative diseases.[3] Investigating brain metabolism by means of HP MRS, as a path to identify metabolic signatures for various brain diseases, is of growing interest.

A main limitation of the dDNP technique is short nuclear spin relaxation time (T1) requiring the dDNP equipment near the NMR magnet for fast transfer. An interesting way is to implement thermally labile UV-generated radicals that annihilates above ~ 190 K, makes the extraction of solid sample possible while keeping the HP state alive, and provide a way for storage/transport of HP samples.[4]

Herein, we demonstrate that KIC, an important metabolic brain biomarker, can be highly polarized via dDNP using the endogenous UV-generated ketyl-radical (Figure 1). We investigated precursor molecule and radical properties via UV-vis measurements and ESR measurements at both X-band and DNP field. After optimizing sample preparation and microwave irradiation conditions, we obtained 56% ^{13}C liquid-state polarization in 1 h at 6.7 T and 1.1±0.1 K.[6]

References

[1] JH Ardenkjaer-Larsen et al. Increase in Signal-to-Noise Ratio of > 10,000 Times in Liquid-State Nmr. *PNAS*, **100**, 10158 (2003).
[2] M. Karlsson et. al. Imaging of branched chain amino acid metabolism in tumors with hyperpolarized ^{13}C ketoisocaproate. *Int J. Cancer*, **127**, 729 (2010).

[3] S. Butt et. al. Imaging cerebral 2-ketoisocaproate metabolism with hyperpolarized (13)C magnetic resonance spectroscopic imaging. *J Cereb Blood Flow Metab.* **32**, 1508 (2012)**.**
[4] A. Capozzi et. al. Thermal annihilation of photo-induced radicals following dynamic nuclear polarization to produce transportable frozen hyperpolarized 13C-substrates. *Nat. Commun*. **8,** 15757 (2017)**.**
[5] S. Patel et. al. UV-Irradiated 2-Keto-(1-^{13}C)isocaproic Acid for high performance ^{13}C hyperpolarized MR. *JPCC,* 2020**.**

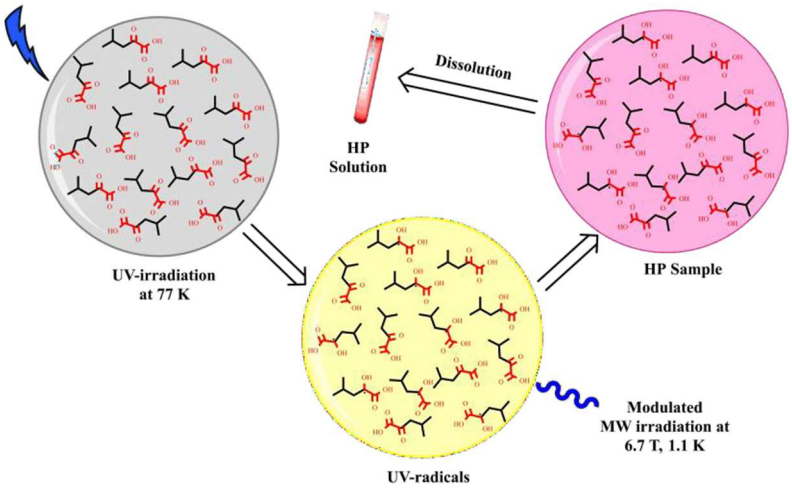


Figure 1. Pictorial depiction of obtaining radical free HP KIC solution via dDNP with UV-generated radicals.

HYPERPOLARIZATION OF ^{15}N NUCLEI IN NIMORAZOLE USING SIGNAL AMPLIFICATION BY REVERSIBLE EXCHANGE AT MICROTESLA MAGNETIC FIELDS

Oleg G. Salnikov[1,2,3], Nikita V. Chukanov[2,3], Alexandra Svyatova[2,3], Ivan A. Trofimov[2,3], Mohammad S. H. Kabir[4], Kirill V. Kovtunov[2,3], Eduard Y. Chekmenev[4], Igor V. Koptyug[2,3]

[1]Boreskov Institute of Catalysis SB RAS, Novosibirsk, Russia
[2]International Tomography Center SB RAS, Novosibirsk, Russia
[3]Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia
[4]Department of Chemistry, Integrative Biosciences, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan, USA

Signal amplification by reversible exchange (SABRE) technique allows one to boost the nuclear spin polarization by several orders of magnitude. SABRE is based on transfer of spin order from parahydrogen to a substrate of interest via their simultaneous reversible coordination to a metal complex. When SABRE experiments are performed at microtesla magnetic fields (SABRE-SHEATH approach[1]), polarization is transferred to heteronuclei with the benefit of significantly longer hyperpolarization lifetimes compared to protons.

Nimorazole is a nitroimidazole-based antibiotic which is now under Phase 3 clinical trial in Europe for potential use as a hypoxia radiosensitizer for the treatment of head and neck cancers. We developed the synthetic approach to isotopically labeled [$^{15}N_3$]nimorazole which was then employed for SABRE- SHEATH studies[2]. The observed ^{15}N polarization levels (P_{15N}) were as high as 3.2% for the $^{15}NO_2$ group (1.9% and 2.4% were obtained for ^{15}N-3 and ^{15}N-1 sites, respectively). The dependence of P_{15N} on polarization transfer field exhibited a maximum at ~0.4 μT for all three ^{15}N sites. At this magnetic field, ^{15}N polarizations build up and decay with similar characteristic exponential times of 23–33 s. At the clinically relevant field of 1.4 T, polarizations of ^{15}N sites relaxed with remarkably long T_1 times of ~51 s, ~1.8 min and ~5.9 min for ^{15}N-3, ^{15}N-1 and $^{15}NO_2$ sites, respectively. Temperature dependence of polarization demonstrated the maximum at ~54 °C with an order of magnitude greater P_{15N} values than at 20 °C. The feasibility of ^{15}N MRI visualization of hyperpolarized [$^{15}N_3$]nimorazole was demonstrated with high spatial and temporal resolution (Figure 1). This proof-of-principle demonstration opens the prospects for utilization of SABRE ^{15}N hyperpolarization of [$^{15}N_3$]nimorazole in bioimaging applications such as reporting on tumor hypoxia status in a manner similar to that of nitroimidazole-based PET tracers.

References

[1] T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, Microtesla SABRE Enables 10% Nitrogen-15 Nuclear Spin Polarization. *J. Am. Chem. Soc.* **137**, 1404–1407 (2015).
[2] O. G. Salnikov, N. V. Chukanov, A. Svyatova, I. A. Trofimov, M. S. H. Kabir, J. G. Gelovani, K. V. Kovtunov, I. V. Koptyug, E. Y. Chekmenev, ^{15}N NMR Hyperpolarization of Radiosensitizing Antibiotic Nimorazole via Reversible Parahydrogen Exchange in Microtesla Magnetic Fields. *Angew. Chem. Int. Ed.* DOI: 10.1002/anie.202011698 (2020).

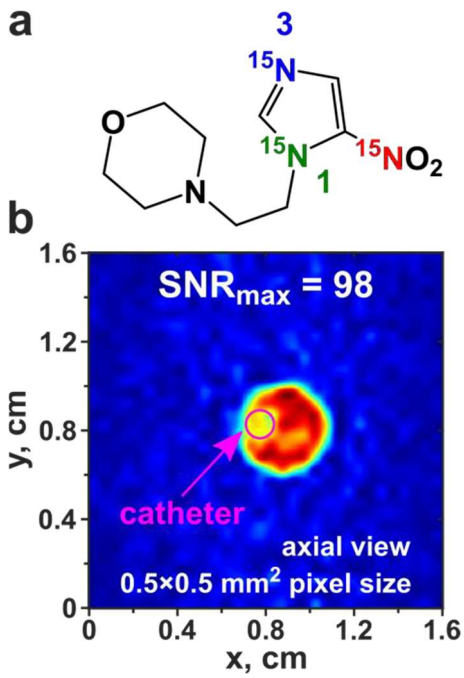


Figure 1. (a) Structure of [$^{15}N_3$]nimorazole. (b) ^{15}N TrueFISP MRI of hyperolarized [$^{15}N_3$]nimorazole (~0.11 M) in a 5 mm NMR tube at 9.4 T (axial view, repetition time = 356 ms).

SHEDDING LIGHT ON NUCLEI NEAR THE SPIN DIFFUSION BARRIER VIA ELECTRON DECOUPLING

Kong Ooi Tan[1,2], Luming Yang[2], Choon Boon Cheong[1,2], Benoit Driesschaert[3], Mircea Dincă[2], Robert G. Griffin[1,2]

[1]Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

[2]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

[3]Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, West Virginia 26506, USA

Dynamic nuclear polarization (DNP) has evolved as the method of choice to enhance NMR signal intensities and to address a variety of otherwise inaccessible chemical, biological and physical information. Despite its success, there is no detailed understanding of how the large electron polarization is transferred to the surrounding nuclei or where these nuclei are located relative to the polarizing agent. We have previously shown using three-spin solid effect that the size of the spin diffusion barrier[1] surrounding the trityl radical in a glassy glycerol-water matrix is < 6 Å.[2] In this contribution, we demonstrate that a combined DNP and electron decoupling approach enables direct NMR detection of intramolecular ^{1}H's on the trityl radicals.[3,4] Although the ^{1}H's reside outside of the spin diffusion barrier, they are still broadened by the electron-nuclei hyperfine interaction, which can be quenched using electron decoupling. We achieved an ~80% improvement in the NMR peak intensity using a ~2 MHz microwave Rabi frequency at 0.35 T and 80 K. The direct detection of ^{1}H's closest to the unpaired electrons allows us to extract the relaxation parameters of the ^{1}H's and shed light on the spin diffusion pathways from the unpaired electron to the bulk nuclei. This information could provide insights important in designing more robust DNP polarizing agents. Finally, we showed that the electron decoupling method can be applied to study magnesium hexahydroxytriphenylene metal-organic framework (MgHHTP MOF).

References

[1] Wolfe, J. P. Direct Observation of a Nuclear Spin Diffusion Barrier. *Phys. Rev. Lett.* **1973**, *31* (15), 907– 910. https://doi.org/10.1103/PhysRevLett.31.907.

[2] Tan, K. O.; Mardini, M.; Yang, C.; Ardenkjær-Larsen, J. H.; Griffin, R. G. Three-Spin Solid Effect and the Spin Diffusion Barrier in Amorphous Solids. *Sci. Adv.* **2019**, *5* (7), eaax2743. https://doi.org/10.1126/sciadv.aax2743.

[3] Saliba, E. P.; Sesti, E. L.; Scott, F. J.; Albert, B. J.; Choi, E. J.; Alaniva, N.; Gao, C.; Barnes, A. B. Electron Decoupling with Dynamic Nuclear Polarization in Rotating Solids. *J. Am. Chem. Soc.* **2017**, *139* (18), 6310–6313. https://doi.org/10.1021/jacs.7b02714.

[4] Alaniva, N.; Saliba, E. P.; Sesti, E. L.; Judge, P. T.; Barnes, A. B. Electron Decoupling with Chirped Microwave Pulses for Rapid Signal Acquisition and Electron Saturation Recovery. *Angew. Chemie Int. Ed.* **2019**, *58* (22), 7259–7262. https://doi.org/10.1002/anie.201900139.

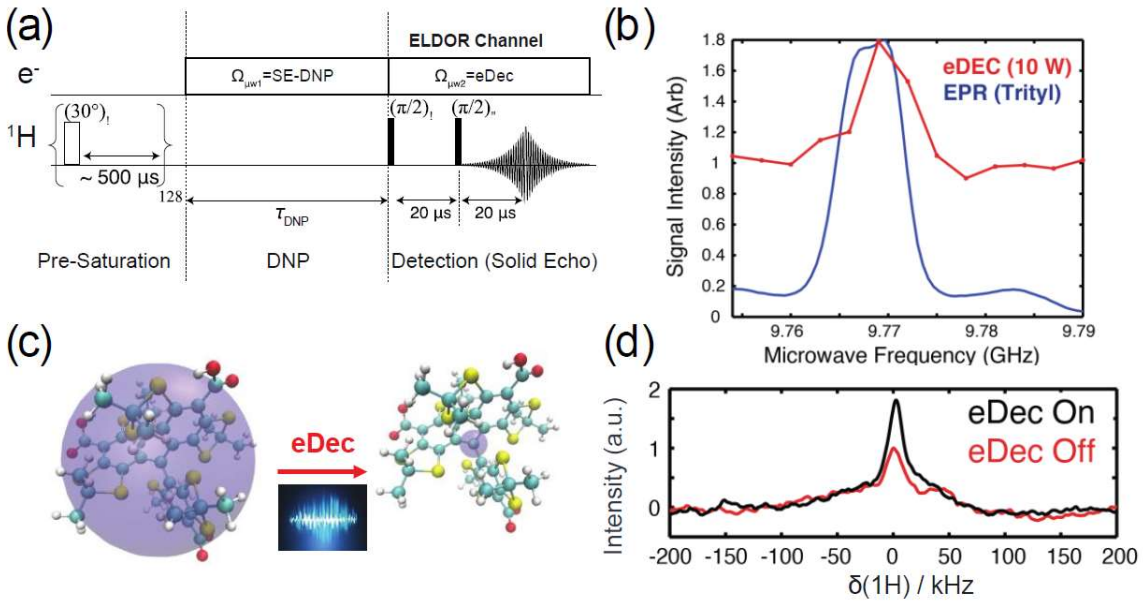


Figure 1. (a) Pulse sequence used in DNP and electron decoupling experiment. (b) Electron decoupling microwave profile. (c) Schematic diagram of hyperfine interaction quenched by electron decoupling. (d) 1D ^{1}H spectrum of trityl radical in deuterated DMSO-water matrix performed at 0.35 T and 80 K.

PARAHYDROGEN HYPERPOLARIZED DIETHYL ETHER FOR MRI

Nuwandi M. Ariyasingha[1], Oleg G. Salnikov[2,3,4], Baptiste Joalland[1], Hassan R. Younes[1], Nikita V. Chukanov[2,3] Kirill V. Kovtunov[2,3], Larisa M. Kovtunova[3,4], Valerii I. Bukhtiyarov[3,4], Igor V. Koptyug[2,3], Juri G. Gelovani[1,5], Eduard Y. Chekmenev[1,6]

[1] Department of Chemistry, Ibio, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48202, USA.
[2] International Tomography Center SB RAS, Novosibirsk, Russia.
[3] Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia.
[4] Boreskov Institute of Catalysis SB RAS, Novosibirsk, Russia.
[5] United Arab Emirates University, Al Ain, United Arab Emirates.
[6] Russian Academy of Sciences, Leninskiy Prospekt 14, Moscow 119991, Russia.

We report successful preparation of hyperpolarized (HP) diethyl ether (DE) anesthetic using a fast and simple approach via Parahydrogen Induced Polarization (PHIP) experiments [1,2]. The existence of long-lived spin states (LLS) in HP DE was also investigated using Spin-Lock Induced Crossing (SLIC) [3].

HP DE was prepared using homogeneous hydrogenation reaction of parahydrogen gas and ethyl vinyl ether precursor and was characterized using ^{1}H NMR spectroscopy at 1.4 T and 47.5 mT magnetic fields. High proton polarization value (>8 %) of HP DE is reported with complete chemical conversion in the weakly coupled regime thus giving rise to radio amplification by stimulated emission of radiation (RASER [5]) conditions. A systematic study of HP DE hydrogenation kinetics and a relaxation study were also performed under homogeneous catalysis. T_1 values were recorded for HP DE in both liquid and vapor phases and the reported lifetimes range are 20-29 s and 1.2 s respectively. Creation of LLS of HP DE gas at the low field (47.5 mT) was successfully achieved with approximately 3-fold increase in the lifetime (T_S ~ 4 s) of the HP molecule at clinically relevant conditions resulting in promising application of HP DE as an inhalable contrast agent for pulmonary imaging in the future.

Although HP DE displays promising results as a good candidate for HP MRI (Figure 1) [2], a key issue preventing it from being used as a contrast agent is its flammability. We also demonstrate the use of fluorination to address the flammability in HP DE and many other structurally similar motifs amenable to PHIP in a recent study where fluorination lowered the flammability levels of the contrast agents while retaining the polarization levels [4]. DE has a long-standing history of inhalable anesthetic utility (still approved in many countries) and we envision the use of HP DE as inhalable contrast agent for functional pulmonary MRI.

References

[1] Ariyasingha, N. M.; Joalland, B.; Younes, H. R.; Salnikov, O. G.; Chukanov, N. V.; Kovtunov, K. V.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Koptyug, I. V.; Gelovani, J. G.; Chekmenev, E. Y., *Chem. -Eur. J.* 2020. doi 10.1002/chem.202002528.
[2] Salnikov, O. G.; et al. *Chem. Eur. J.* **2020**, DOI 10.1002/chem.202003638.

[3] DeVience, S.J.; Walsworth, R.L.; Rosen, M.S. *Phys. Rev. Lett.* **2013,** *111*, 173002.
[4] Joalland, B.; Ariyasingha, N. M.; Younes, H. R.; Salnikov, O. G.; Chukanov, N. V.; Kovtunov, K. V.; Kovtunova, L. M.; Bukhtiyarov, V. I.; Koptyug, I. V.; Gelovani, J. G.; Chekmenev, E. Y., *Chem. -Eur. J*. 2020. Accepted.
[5] Joalland, B.; et al. *Angew. Chem. Int. Ed.* **2020,** *132* (22), 8732-8738

Acknowledgements

We thank our funders: NSF CHE-1904780, DOD CDMRP W81XWH-15-1-0271 and W81XWH-20-1-0576.

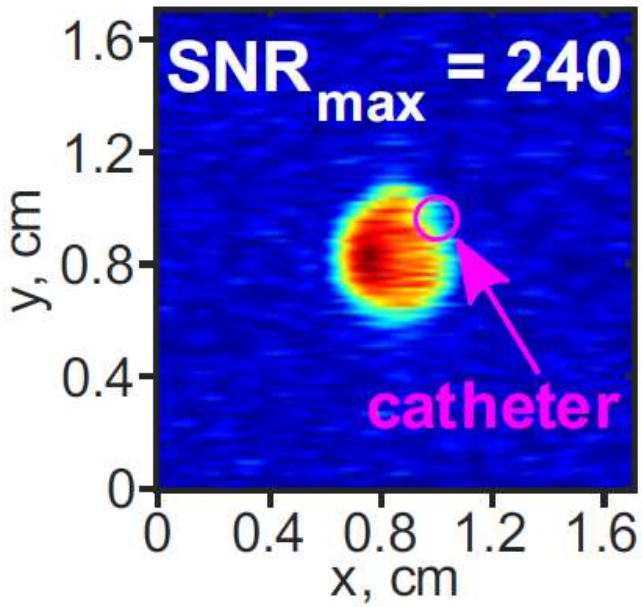


Figure 1. ^{1}H FLASH MRI of diethyl ether vapor in a 5 mm NMR tube (axial view): continuously flowing (5.1 mL/s gas flow rate) hyperpolarized DE. The gas pressure was 3.9 bar. The images were acquired at 9.4 T. Frequency offset was adjusted to the HP signal of CH3 group. The FLASH imaging parameters were: flip angle = 6˚, number of averages = 2, acquisition time = 120 ms, matrix size = 128×16 (zero-filled to 128×128), FOV = 1.7 cm × 1.7 cm, spatial resolution = 0.1×1.1 mm^2/pixel.

DYNAMIC NUCLEAR POLARIZATION AT 21.15 T AND 65 kHz MAS

Snædís Björgvinsdóttir[1], Pierrick Berruyer[1], Andrea Bertarello[1], Gabriele Stevanato[1], Yu Rao[1], Ganesan Karthikeyan[2], Gilles Casano[2], Olivier Ouari[2], Moreno Lelli[3], Christian Reiter[4], Frank Engelke[4], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
[2] Aix Marseille Université, CNRS, ICR, 13013 Marseille, France.
[3] Magnetic Resonance Center (CERM) and Department of Chemistry "Ugo Schiff", University of Florence, 50019 Sesto Fiorentino, Italy.
[4] Bruker Biospin, 76287 Rheinstetten, Germany.

We present the first DNP MAS experiments using 0.7 mm rotors spinning at frequencies up to 65 kHz at 100 K and 21.1 T, the highest magnetic field available for DNP today.[1]

We find that the biradical polarizing agent HyTEK2 provides DNP enhancements as high as 200 at a spinning rate of 65 kHz, and BDPA in an ortho-terphenyl glass yields an enhancement of 106 under the same conditions. In both cases, we observe the ^{1}H DNP enhancements increasing with increasing MAS frequency, providing excellent DNP performance.

The achievable spinning frequencies yield unprecedented ^{1}H resolution in MAS DNP and motivate the use of ^{1}H detected spectra. As an example, we acquired resolved DNP enhanced ^{1}H detected ^{1}H–^{13}C and ^{1}H–^{15}N correlation spectra of microcrystalline histidine·HCl·H_2O.

References

[1] P. Berruyer, S. Björgvinsdóttir, A. Bertarello, G. Stevanato, Y. Rao, G. Karthikeyan, G. Casano, O. Ouari, M. Lelli, C. Reiter, F. Engelke, L. Emsley, Dynamic Nuclear Polarization Enhancement of 200 at 21.15 T Enabled by 65 kHz Magic Angle Spinning. *J. Phys. Chem. Lett.* **11**, 8386-8391 (2020).

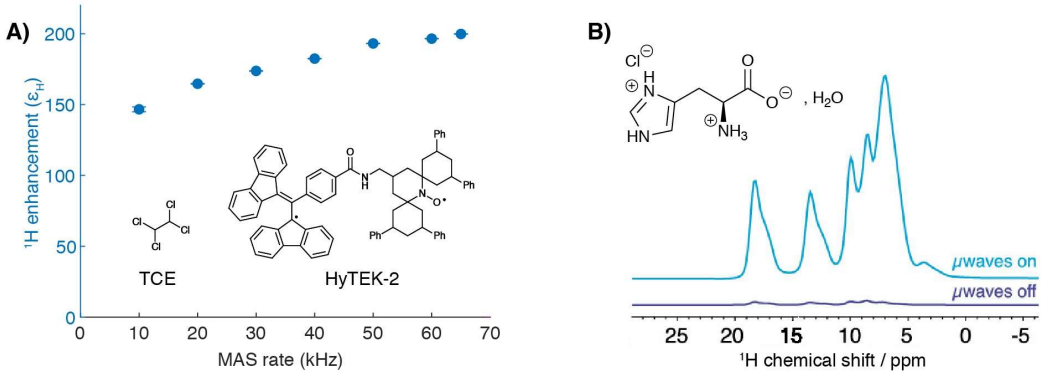


Figure. A) 1H DNP enhancements as a function of MAS frequency, for a 32 mM solution of HyTEK2 in 1,1,2,2-tetrachloroethane (TCE). B) DNP enhanced 1H NMR spectrum of histidine·HCl·H2O impregnated with 32 mM HyTEK2 in TCE.

INVESTIGATING THE SOLVENT EFFECT ON PARA-HYDROGEN HYPERPOLARIZATION OF METABOLITES DERIVATIVES

Oksana Bondar[1], Eleonora Cavallari[1], Carla Carrera[1], Silvio Aime[1], Francesca Reineri[1]

[1] Dept. of Molecular Biotechnology and Health Science, University of Torino
E-mail: oksana.bondar@unito.it

The driving force for the development of hyperpolarization techniques is given by the exploitation of MR hyperpolarized (HP) molecules in the diagnostics of different pathologies. Para-Hydrogen Induced Polarization through Side Arm Hydrogenation (PHIP-SAH) allows to hyperpolarize biologically relevant substrates, such as acetate and pyruvate, on which the H_2 molecule cannot be incorporated directly. This multi-step hyperpolarization procedure (figure 1) is necessary to obtain solutions of HP products suitable for metabolic studies in-cells and in vivo [1, 2]. The HP products are in an aqueous solution, from which the hydrogenation solvent and the catalyst have been removed and their concentration and ^{13}C hyperpolarization are sufficiently high to allow metabolic investigations.

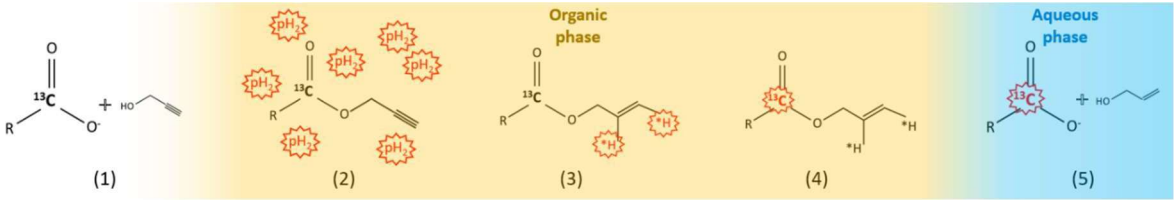
Para-Hydrogen Hyperpolarization of the SAH derivatives is obtained through hydrogenation, using para-enriched hydrogen, of the unsaturated substrate. The high spin order of the parahydrogen protons is, then, transferred to the 13C carboxylate spin, in order to obtain net ^{13}C hyperpolarization. Therefore, it is necessary that parahydrogen spin order is maintained during the catalytic reaction, which is crucial in determining the hyperpolarization level on products.

In this work we have investigated the effect of different hydrogenation solvents on the hyperpolarization level and reaction yield. The use of coordinating solvents, such as methanol-d4 and acetone-d6, that intervene in the hydrogenation pathway, lead to higher polarization level (about 20% ^{13}C polarization), unfortunately these solvents are not compatible with biological applications. Conversely, the use of hydrophobic solvents such as chloroform and toluene, leads to lower polarization level but allows the separation of the aqueous phase from the organic solvent, thus making biological applications possible.

Our results provide important perspectives for using PHIP-SAH method to obtain the HP molecules that portend a new era for in-vivo MRI.

References

[1] Cavallari E, Carrera C, Di Matteo G, Bondar O, Aime S, Reineri F (2020) In-vitro NMR Studies of Prostate Tumor Cell Metabolism by Means of Hyperpolarized [1-13C]Pyruvate Obtained Using the PHIP-SAH Method. Front Oncol 10:1–9.
[2] Cavallari E, Carrera C, Sorge M, Bonne G, Muchir A, Aime S, Reineri F (2018) The 13 C hyperpolarized pyruvate generated by ParaHydrogen detects the response of the heart to altered metabolism in real time. Sci Rep 8:8366.



Schematization of the PHIP-SAH procedure: 1) an unsaturated ester of the carboxylate containing substrate is synthesized; 2) parahydrogenation is carried out in a hydrophobic solvent (chloroform); 3-4) spin order is transferred from parahydrogen protons to the 13C carboxylate spin; 4-5) the ester is hydrolyzed by adding an aqueous solution of Sodium hydroxide (0.1M) and the hyperpolarized product is extracted in the aqueous phase.

EFFECTS OF MICROWAVE IRRADIATION ON NUCLEAR RELAXATION RATES IN DYNAMIC NUCLEAR POLARIZATION

Diego Carnevale,[1] David Guarin,[1] Mathieu Baudin,[1,2] Philippe Pelupessy,[1] Daniel Abergel,[1] Geoffrey Bodenhausen[1]

[1] Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France
[2] Université de Paris, Laboratoire de Chimie et Biologie Pharmacologiques et Toxicologiques, CNRS UMR 8601, Université Paris Descartes, 45 rue des Saints Pères 75006 Paris France

We report the observation of transient surges of the amplitudes of proton spin echoes enhanced by dynamic nuclear polarization[1,2] in a field of 6.7 T in a variety of samples vitrified at 1.3 K when the microwave irradiation is interrupted in the course of the build up of the hyperpolarization. The phenomenon can be attributed to the extension of the lifetime of the transverse proton magnetization when the electrons relax back to their Boltzmann distribution. Consequently, the dipolar flip-flop terms between the electrons and the protons become ineffective. Furthermore, transient slumps of spin echoes are observed when the microwave irradiation is switched back on during a surge.

References

[1] Abragam, A.; Goldman, M. Reports Prog. Phys. 1978, 41 (3), 395–467.
[2] Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. Proc. Natl. Acad. Sci. 2003, 100 (18), 10158–10163.

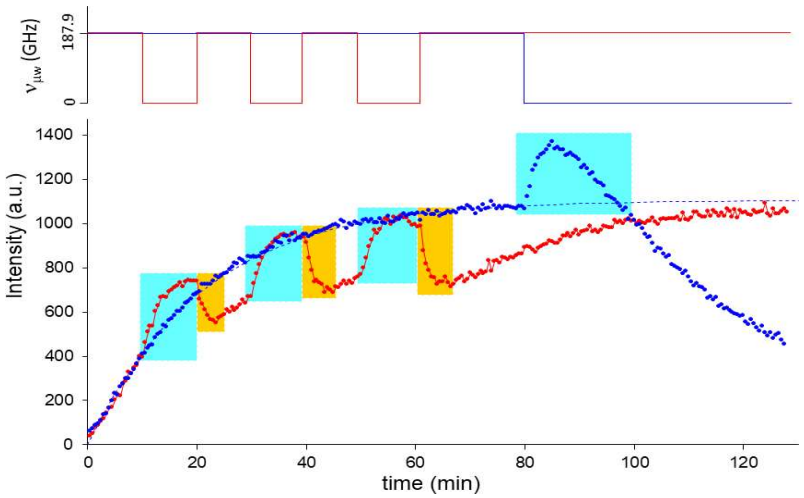


Figure 1. Build-up curves of ^{1}H polarization of the residual protons in deuterated tetrachloroethane containing 35 mM TEMPOL. The top panel indicates how the microwave irradiation is switched on and off. Surges and slumps are highlighted by cyan and orange rectangles, respectively.

EXTENDING THE POTENTIAL OF ZERO-FIELD NMR USING DISSOLUTION DYNAMIC NUCLEAR POLARIZATION

Quentin Chappuis[1], Román Picazo-Frutos[2], James Eills[2], Samuel F. Cousin[1], Morgan Ceillier[1], Stuart J. Elliott[1], Olivier Cala[1], John W. Blanchard[2], Dmitry Budker[2], Sami Jannin[1]

[1] Centre de Résonance Magnétique Nucléaire à Très Hauts Champs, 5 Rue de la Doua, 69100 Villeurbanne, France
[2] Helmholtz Institute Mainz, GSI Helmholtzzentrum für Schwerionenforschung, Mainz 55090, Germany

Zero- and ultra-low field nuclear magnetic resonance (ZULF NMR) is a modality of NMR experiment that does not require strong magnetic fields with applications ranging from molecular spectroscopy and chemical-reaction monitoring to dark-matter searches and study of physics outside of the standard model. [1] In state-of-the-art ZULF experiments, the sample is prepolarized in a permanent magnet (typically 2 T) prior to shuttling to zero field, after which its oscillating magnetization is detected by optical magnetometry. The signals display pure J-spectra features with linewidths on the order of tens of mHz, thanks to the long coherence times achievable at zero field. However, as with most NMR experiments, the approach suffers from a low intrinsic sensitivity arising from the low nuclear spin polarization available even at high magnetic fields. Thus, ZULF NMR detection is usually limited to neat, labelled solutions with molar spin concentrations. Hyperpolarization techniques come to the rescue to overcome this limitation. Parahydrogen is a successful example among them, [2] but it can only be applied to a limited number of compounds. In this work, we used dissolution dynamic nuclear polarization [3] (*d*DNP) to enhance ZULF NMR [4] sensitivity and therefore extend the technique to future applications with arbitrary mixtures of molecules. We report signal enhancements of up to four orders of magnitude with respect to thermal prepolarization at 2 T. We reach a signal-to-noise ratio of 300 for a sample concentration as low as ~0.04 M of analyte in a single shot. Based on relaxation measurements at zero and low fields, we discuss possible improvements of the method. Our study further widens the applicability of ZULF NMR to a broader category of compounds thanks to *d*DNP.

References

[1] J. W. Blanchard, D. Budker. Zero-to ultralow-field NMR. *eMagRes*. **5**(3), 1395–410 (2016).
[2] T. Theis, P. Ganssle, G. Kervern, S. Knappe, J. Kitching, M. P. Ledbetter, D. Budker and A. Pines. Parahydrogen- enhanced zero-field nuclear magnetic resonance. *Nat Phys*. **7**, 571-575 (2011).
[3] J. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. Lerche, *et al*. Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR. *Proc Natl Acad Sci U S A*. **100**(18), 10158–63 (2003).
[4] D. A. Barskiy, M. C. D. Tayler, I. Marco-Rius, J. Kurhanewicz, D. B. Vigneron, S. Cikrikci, *et al*. Zero-field nuclear magnetic resonance of chemically exchanging systems. *Nat Commun*. **10**(1), 1–9 (2019).

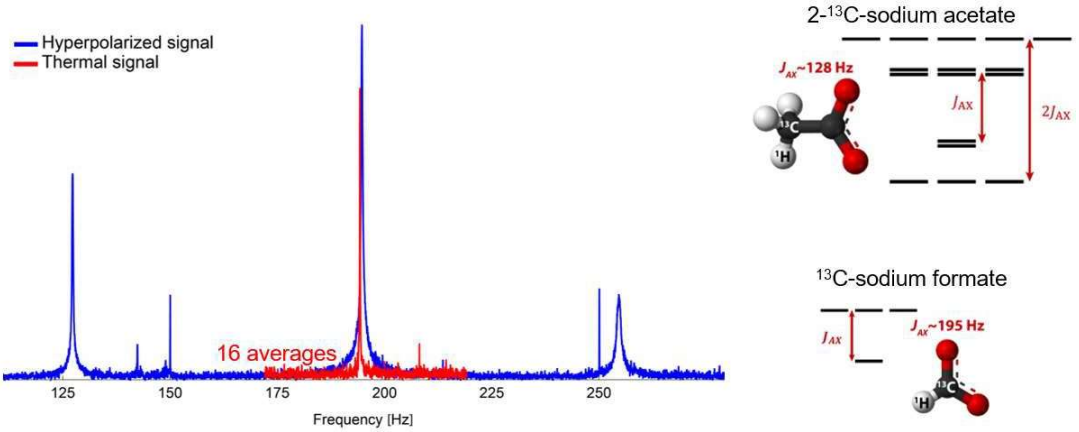


Figure 1. Single scan zero-field NMR spectrum of ~0.04 M ^{13}C-sodium formate and 2-^{13}C- sodium acetate hyperpolarized by *d*DNP (blue) compared with 16 averages of 5 M ^{13}C- sodium formate dissolved in D2O solvent thermally prepolarized at 2 T (red). The energy diagram on the right shows the expected transitions at zero field.

A NUMERICAL ANALYSIS OF OPTICALLY-INDUCED OVERHAUSER DYNAMIC NUCLEAR POLARIZATION

Daniel J. Cheney[1], Christopher J. Wedge

[1] Department of Chemical Sciences, University of Huddersfield, United Kingdom

Overhauser Dynamic Nuclear Polarization (DNP) is a popular approach to achieving signal enhancements in solution-state NMR.[1] It typically relies on the use of microwaves to saturate the electron paramagnetic resonance (EPR) transitions of a radical that has been added to the sample. However, this can lead to significant dielectric heating effects, particularly in aqueous solution at high frequencies. Recently, an optically-driven approach was demonstrated as an alternative to the use of microwaves.[2] A dye is added to the sample alongside the radical, which is photoexcited to its triplet state. This is subsequently quenched by the radical, inducing electronic hyperpolarization in the radical due to the Radical-Triplet Pair Mechanism (RTPM).[3] Initially, an approximately 4-fold signal enhancement for water protons was measured using the dye-radical pair rose bengal and TEMPO.[2,4] This method has the potential to achieve DNP enhancements higher than the theoretical limit of the conventional microwave-driven approach. To aid in the future development of the technique, we have rationalized the experimental results thus far using numerical simulations, solving the kinetic equations for the generation and decay of triplets, and the Bloch equations for the electron and nuclear polarizations.[5] We will present the effects of changing various experimental parameter, including triplet and radical concentrations, laser power, and sample geometry. We have also investigated the possibility of using a pulsed laser, to reduce heating effects further (Figure 1).

References

[1] A. W, Overhauser, *Phys. Rev*. **92**, 411-415 (1953)
[2] M, W. Dale and C. J. Wedge, *Chem. Commum*. **52**, 13221-13224 (2016)
[3] C. Blättler, F. Jent, H. Paul, *Chem. Phys. Lett*. **166**, 375-380 (1990)
[4] H. Takahashi, M. Iwama, N. Akai, K. Shibuya, A. Kawai, *Mol. Phys*. **7**, 1012-1020
[5] D. J. Cheney and C. J. Wedge, *J. Chem. Phys*. **152**, 034202 (2020)

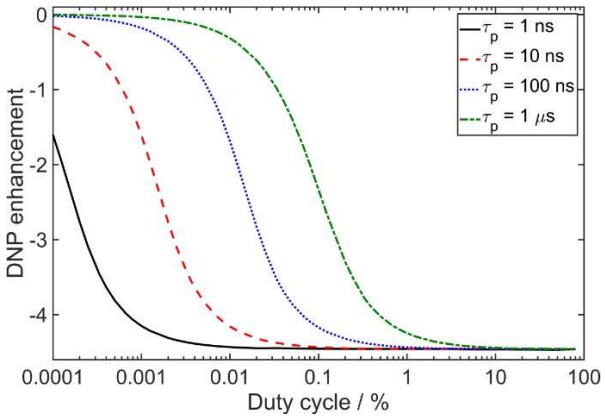


Figure 1. Dependence of the optical DNP enhancement of water on the illumination duty cycle, for various pulse lengths.

FLAVOPROTEINS ALLOW FOR MECHANISTIC STUDIES OF SOLID-STATE PHOTO-CIDNP EFFECT BY FIELD-DEPENDENCE MEASUREMENT AND LEVEL CROSSING ANALYSIS

Yonghong Ding[1], Alexey S. Kiryutin[2], Tilman Kottke[3], Konstantin L. Ivanov[2], Alexandra V. Yurkovskaya[2], Jörg Matysik[1]*

[1] Institut für Analytische Chemie, Universität Leipzig, Linnéstr. 3, 04103 Leipzig, Germany
[2] International Tomography Center, Siberian Branch of Russian Academy of Sciences, Institutskaya, 3a, Novosibirsk, 630090, Russia and Novosibirsk State University, Pirogova 1, Novosibirsk, 630090, Russia
[3] Physikalische und Biophysikalische Chemie, Universität Bielefeld, Universitätsstr. 25, 33615 Bielefeld, Germany.

The solid-state photochemically induced dynamic nuclear polarization (photo-CIDNP) effect[1] generates non-Boltzmann magnetization which can be detected by NMR spectroscopy as significant modification of signal intensity. The effect is well known to occur in reaction centers of photosynthetic systems and in a light-oxygen-voltage (LOV) domain of a biological blue-light receptor called phototropin, in which the conserved cysteine was removed to avoid the natural photo-chemical reactions with the cofactor, flavin mononucleotide (FMN). Thereby under illumination the FMN abstracts an electron from a remote tryptophan residue to form a transient spin-correlated radical pair undergoing spin-evolution resulting in photo-CIDNP[2,3]. Here, we report the successful design of further two LOV proteins able to show solid-state photo-CIDNP effect: a LOV domain of aureochrome from *Phaeodactylum tricornutum* as well as an unusual LOV domain named 4511 from *Methylobacterium radiotolerans* (*Mr*4511) which lacks an otherwise conserved tryptophan in its wild type. Mutating the tryptophan at canonical and novel positions (Figure 1) in *Mr*4511 enables occurrence of ^{15}N and ^{1}H photo-CIDNP effect with characteristic field dependence. Furthermore, we observed a non-tryptophan derived photo-CIDNP effect generated by a cysteine-devoid *Mr*4511 protein in which no tryptophan is present. Our experiments demonstrate possibilities to explore the conditions that allow for solid-state photo-CIDNP and design molecular spin-machines which produce light-induced nuclear spin-hyperpolarization[4].

References

[1] Matysik, J., et al. The solid-state photo-CIDNP effect. Photosynth. Res. **102**, 427 (2009).
[2] Thamarath, S. S., et al. Solid-state photo-CIDNP effect observed in phototropin LOV1-C57S by ^{13}C magic-angle spinning NMR spectroscopy, J. Am. Chem. Soc. **132**, 15542 (2010).
[3] Ding, Yonghong, et al. "Nuclear spin-hyperpolarization generated in a flavoprotein under illumination: experimental field-dependence and theoretical level crossing analysis." Scientific Rep. **9**.1 (2019).
[4] Ding, Yonghong, et al. "Tailored flavoproteins acting as light-driven spin machines pump nuclear hyperpolarization." (Under review)

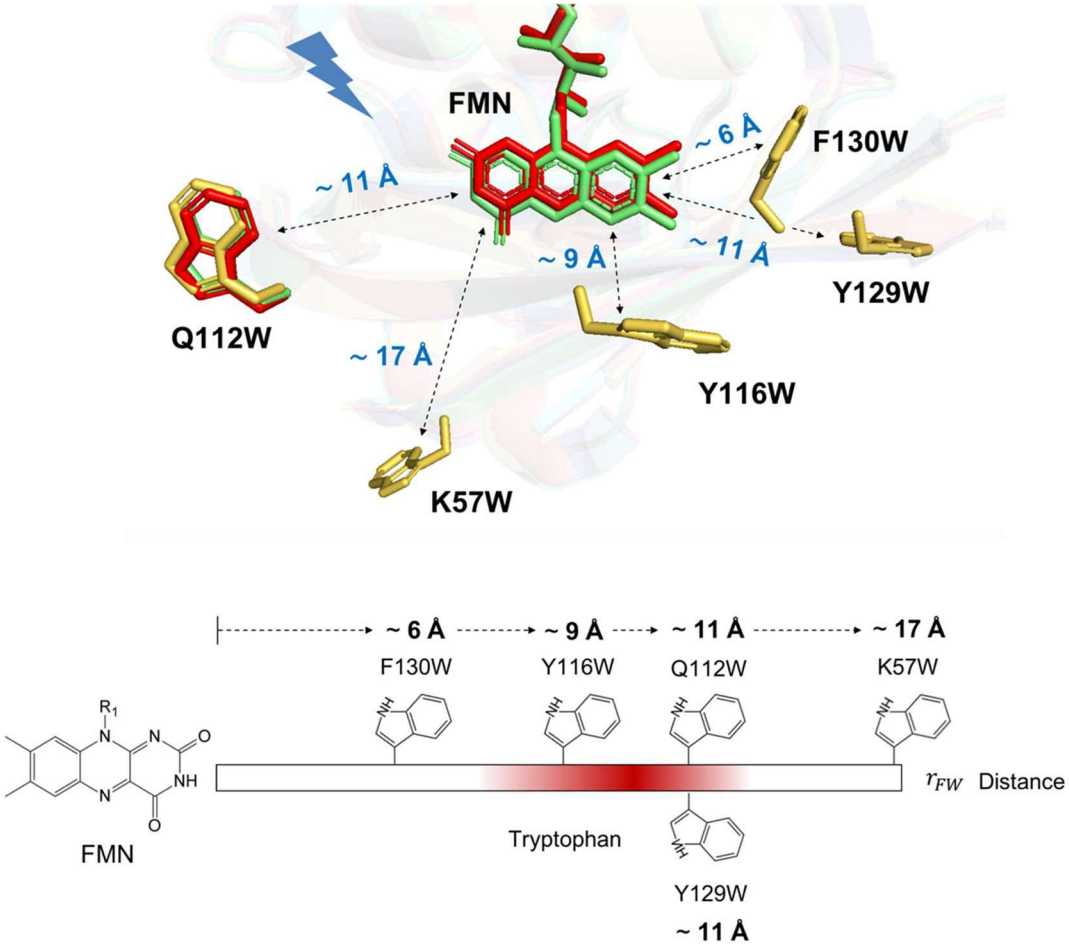


Figure 1. (Top) Alignment of the crystal structures of *Cr*phot-LOV1 (PDB: 1N9L, green), *Pt*aureo1a-LOV (PDB: 5A8B, red), and the simulated structure of *Mr*4511 (yellow) without FMN. The simulation was performed with SWISS-MODEL based on the crystal structure of aureochrome1a-LOV from *Vaucheria frigida* (PDB entry: 3UE6). Five mutants of cysteine- lacking *Mr*4511 were generated, one with tryptophan (W) placed at the canonical position, Q112W, r_{FW}~11 Å, the other four at non-canonical positions F130W, Y116W, Y129W and K57W with increasing distance r_{FW} shown in brackets. (Bottom) Schematic illustration of the protein design experiment in *Mr*4511 from the perspective of the edge-to-edge distance r_{FW} of tryptophan to the cofactor FMN. The relevant r_{FW} for the tryptophan to involve in the formation of the solid-state photo-CIDNP effect is colored in red.

EXPLORATION OF NON-INTUITIVE PULSE SEQUENCES FOR IMPROVED CONTROL OVER SABRE DYNAMICS

Shannon L. Eriksson[1], Warren S. Warren[2]

[1] Department of Chemistry and School of Medicine, Duke University, Durham, NC, 27708
[2] Department of Physics, Chemistry, Biomedical Engineering, and Radiology, Duke University, Durham, NC, 27708

"Hyperpolarization" techniques in magnetic resonance directly address the inherently low sensitivity of this energy regime by artificially inducing large differences in spin populations (commonly >10%). Signal Amplification By Reversible Exchange (SABRE) is one such technique which uses reversible interactions of parahydrogen gas (cheap and simple to produce in large quantities) and a target molecule with an iridium catalyst.

Unfortunately, because SABRE is a relatively new technique, the underlying physics governing the dynamics of polarization transfer are not fully elucidated. SABRE hyperpolarization methods and system dynamics have been designed and interpreted around a traditional magnetic resonance theoretical framework up until now. However, this is a system with non-traditional dynamics in a low magnetic field regime which has not been extensively explored. Spin order is transferred from singlet order to magnetization by consecutive rotation of the system under specific terms in the nuclear spin Hamiltonian.

Without radiofrequency irradiation, some of these rotations may only occur at low magnetic field, while others can be accomplished at any arbitrary field. We may use this separation to exert additional control over our system at low field using large instantaneous manipulation of the leading field. ROtation SElective SABRE (ROSE SABRE) uses a combination of near zero field and low field pulses to strategically control evolution under the different terms in the Hamiltonian and optimize polarization generation under highly non-intuitive field conditions. For example, large polarizations are achieved under fields that are far from normal SABRE SHEATH resonance conditions by refocusing the J coupling terms and controlling the evolution under Zeeman terms. Exploration of these non-intuitive conditions with the aid of a physically accurate computational model[1] was rigorously supported by experiment and clearly demonstrated new conditions under which polarization transfer can be dramatically increased.

References

[1] Lindale, J. R., Eriksson, S. L., Tanner, C. P. N. & Warren, W. S. Infinite-order perturbative treatment for quantum evolution with exchange. *Science Advances* **6**, eabb6874, doi:10.1126/sciadv.abb6874 (2020).

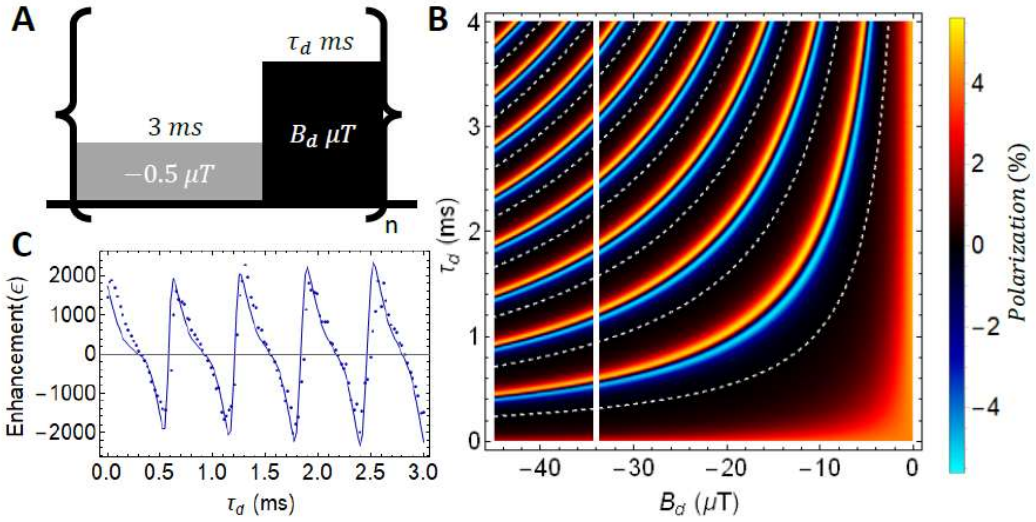


Figure 1. ROSE SABRE pulse sequence application to an Ir(IMes)(H)2(pyr)2(15N-acetonitrile) complex (A) ROSE SABRE pulse sequence with fixed evolution pulse and arbitrary interrupting pulse. (B) DMExFR2 numerical simulation for Polarization achieved after 250 ms of evolution (dotted white contour lines show $\pi \ mod(2\pi)$ rotations about $\omega_H - \omega_N$ under the interrupting pulse). (C) Experimental results (points) compared to numerical simulation prediction (solid line) for interrupting field strength B_d = −34 μT and variable pulse duration τ_d corresponding to a sweep through the white line in the density plot.

INVESTIGATION OF PHOTOINDUCED ELECTRON TRANSFER IN DONOR/ELECTRON ACCEPTOR DYADS BY CIDNP METHOD

Natalya Fishman[1,2], Ivan Zhukov[1,2], Alexey Kiryutin[1,2], Nikita Lukzen[1,2], Mikhail Panov[1,2], Ulrich Steiner[3], Hans-Martin Vieth[1,4], Julian Schäfer[5], Christoph Lambert[5] and Alexandra Yurkovskaya[1,2]

[1] International Tomography Center, Institutskaya 3a, 630090 Novosibirsk, Russia
[2] Novosibirsk State University, Pirogova 2, 630090 Novosibirsk, Russia
[3] Department of Chemistry, University of Konstanz, Universitätsstraße 14, 78457 Konstanz, Germany
[4] Institut für Experimentalphysik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany
[5] Center for Nanosystems Chemistry, Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

The method of chemically induced dynamic nuclear polarization (CIDNP) was used to study the dependence of the reaction in donor-acceptor compounds on the magnetic field in order to obtain detailed information on its effect on the reversible charge separation. The objects of the study were rigidly linked donor-bridge-acceptor dyads, consisting of triarylamine (TAA) as a donor, naphthalenediimide (NDI) as an acceptor, and a meta-conjugated diethynylbenzene moiety as a bridge. Four types of the substituent groups (CN, Cl, Me, OMe) were introduced into the central benzene ring of the bridge at the para- positions, two in each structure. When these dyads are photo-irradiated, intramolecular electron transfer from the donor to the acceptor occurs, and the charge-separated state (short-lived biradical) arises. In the CIDNP field dependence from 1 mT to 9.4 T, the pronounced absorption maxima of the CIDNP were revealed for more than 20 ^{1}H nuclei and 30 ^{13}C nuclei for each of the four dyads studied. A quantitative analysis of the ^{1}H CIDNP field dependence was carried out and information on the positive sign and the magnitude of the exchange interaction in intermediate biradicals was obtained. By the simulation of the 13C CIDNP field dependence 13C hyperfine coupling constants for all polarized 13C nuclei was obtained. Based on the established linear relationship between the amplitude of the proton CIDNP signal of the TAA methyl groups for all dyads at the field dependence maximum and the initial population of the triplet charge-separated state, as well as the positive sign of the CIDNP, it was concluded that the sign electron exchange interaction is positive; CIDNP is formed mainly in the vicinity of the anti-crossing between the electronic T+ and S levels; and coherent triplet-singlet transitions occur near this point, induced by hyperfine interaction, which are accompanied by a simultaneous flip of the spins of electron and nuclei.

Acknowledgements

We thank the Russian Science Foundation (project 19-73-00153) for support of this work.

RECENT PROGRESS IN B-NMR

R. D. Harding[1,2], S. Pallada[1], J. Croese[1,3], A. A. Antušek[4], M. Baranowski[5], M. L. Bissell[6], L. Cerato[3], K. M. Dziubinska-Kühn[7,1], W. Gins[8], F. P. Gustafsson[8], A. Javaji[1,9], R. B. Jolivet[3], A. Kanellakopoulos[8], B. Karg[3], M. Kempka[5], V. Kocman[10], M. Kozak[5,11], K. Kulesz[3,1], M. Madurga Flores[12], G. Neyens[8,1], R. Pietrzyk[5], J. Plavec[10], M. Pomorski[13], A. Skrzypczak[14], P. Wagenknecht[1,9], F. Wienholtz[1], J. Wolak[5], Z. Xu[12], D. Zakoucky[15], M. Kowalska[1,3]

[1] CERN, Geneva, Switzerland
[2] University of York, York, United Kingdom
[3] University of Geneva, Geneva, Switzerland
[4] Slovak University of Technology, Bratislava, Slovakia
[5] Adam Mickiewicz University, Poznan, Poland
[6] University of Manchester, Manchester, United Kingdom
[7] Leipzig University, Leipzig, Germany
[8] KU Leuven, Leuven, Belgium
[9] Oldenburg University, Oldenburg, Germany
[10] National Institute of Chemistry, Ljubljana, Slovenia
[11] Jagiellonian University, Krakow, Poland
[12] University of Tennessee, Knoxville, USA
[13] University of Warsaw, Warsaw, Poland
[14] Poznan University of Technology, Poznan, Poland
[15] Czech Academy of Sciences, Rez, Czech Republic

Recently, the β-NMR technique is gaining traction. This can be attributed to its highly increased sensitivity, as a result from joining both laser-induced hyperpolarization and β-asymmetry detection. As the latest contribution to the field, our setup at ISOLDE/CERN could increase the accuracy of the magnetic moment of ^{26}Na to the parts-per-million using a combination of: low-vapor pressure liquid samples, a ^{1}H field lock system and *ab initio* shielding computations of the reference magnetic moment.[1] As standard chemical shift reference samples for radioactive nuclei in vacuum conditions are scarce, we will use this new accuracy to introduce an absolute shielding scale for ^{26}Na, thus paving the way for β- NMR as a fast and versatile tool for chemistry and biology.

References

[1] https://arxiv.org/abs/2004.02820v2

SPIN DYNAMICS IN EXPERIMENTS ON ORTHODEUTERIUM INDUCED POLARIZATION (ODIP)

Vitaly P. Kozinenko[1,2], Alexey S. Kiryutin[1,2], Stephan Knecht[4], Gerd Buntkowsky[4], Hans- Martin Vieth[1,3], Alexandra V. Yurkovskaya[1,2], Konstantin L. Ivanov[1,2]

[1] International Tomography Center, Siberian Branch of the Russian Academy of Science, Novosibirsk, 630090, Russia
[2] Novosibirsk State University, Novosibirsk, 630090, Russia
[3] Institut für Experimentalphysik, Freie Universität Berlin, Berlin, 14195, Germany
[4] Eduard-Zintl Institute for Inorganic and Physical Chemistry, TU Darmstadt, Darmstadt, 64287, Germany

Experimental methods utilizing non-thermally polarized spin systems have significantly broadened the scope of NMR spectroscopy by alleviating its major weakness – low sensitivity. One of the most promising hyperpolarization techniques up to date is parahydrogen induced polarization of nuclei (PHIP), which exploits the non-equilibrium spin state of para spin isomer of H_2 molecule to produce 10^3-10^4 fold NMR signal enhancement on a timescale of several seconds. To achieve non-thermal spin order of dihydrogen, the H_2 gas is cooled down in the presence of a paramagnetic catalyst, which leads to the enrichment of its para component. However, H_2 is not the only diatomic molecule amenable to such a method of spin isomer enrichment. Bargon and coworkers[1] have demonstrated the possibility of obtaining ^{2}H NMR signal enhancements by using ortho spin isomer of D_2 molecule, consisting of two deuterons (i.e., spin-1 nuclei).

The number of reports on both theoretical and experimental studies of orthodeuterium induced polarization (ODIP) is very limited, not reflecting the intriguing features of the method and its potential for NMR signal enhancement. Here, we present a comprehensive theoretical description of ODIP, which allows predicting ODIP spectral patterns in various experiments and their dependence on the magnetization flip angle. However, ODIP experiments meet difficulties, arising from fast quadrupolar relaxation of ^{2}H nuclei, small J-coupling splitting and complicated patterns of NMR multiplets. Nonetheless, we have found ways how to overcome these issues by optimizing reaction conditions, gas pressure and sample temperature and demonstrate ^{2}H signal enhancement factors up to 1,000 obtained with ODIP for a number of substrate molecules. The reported ODIP experiments are performed at both high and low magnetic fields. Furthermore, we demonstrate the feasibility of more complex ODIP experiments, such as transfer to magnetic heteronuclei, namely ^{13}C, at high magnetic field. The presented ODIP-derived signal enhancement can thus extend the scope of spin hyperpolarization to quadrupolar ^{2}H nuclei, being important probes for molecular structure and mobility.

References

[1] J. Natterer, T. Greve, and J. Bargon, Chem. Phys. Lett. 293, 455 (1998).

THE NMR RASER - A ROUTE TO CHAOS

Sören Lehmkuhl[1], Simon Fleischer[2], Baptiste Joalland[3], Nuwandi M. Ariyasingha[3], Eduard Y. Chekmenev[3], Thomas Theis[1], Stephan Appelt[2,4]

[1] Department of Chemistry, North Carolina State University Raleigh, NC 27695-8204, USA.
[2] ITMC, RWTH Aachen University, D-52056 Aachen, Germany.
[3] KCI, Department of Chemistry, Wayne State University Detroit, MI 48202, USA.
[4] ZEA-2, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany.

With the first parahydrogen pumped RASER, molecular couplings could be determined with micro-Hertz precision.[1] Further studies at higher detection fields showed that also chemical shifts can be probed with a RASER.[2,3] Such molecular parameters could not be probed with a LASER due to the fundamental difference between a LASER and a RASER: In a NMR RASER, spin states can be probed due to their relatively long lifetime. In contrast to that, in a LASER the lifetime of photons in a resonator is on the order of microseconds and therefore adiabatically eliminated.[4]

The crucial point for such high precision measurements and many other applications is multimode operation. Multiple RASER modes are crucial for high sensitivity, to compensate for magnetic field fluctuations. Additionally, this enables the extraction of spectroscopic information. The minimum possible difference between two lines in a RASER spectrum can be obtained from the RASER equations by Appelt et.al[4].

While the difference between two lines can be determined with microHertz precision, their minimum distance is often higher due to their nonlinear coupling. Depending on their distance, two RASER modes undergo several nonlinear phenomena until ultimately collapsing into a single line, which can be described using nonlinear mathematics and synergetics.

Now, we studied different scenarios of mode distance and polarization. This allowed us to observe regions of chaotic behavior for specific sets of parameters.[5] When slowly moving towards conditions for chaos, the modes undergo frequency shifts, frequency combs and multiple period doubling processes until intermittence phenomena and ultimately chaos are observed. An example, where two RASER modes feature chaotic behavior is shown in Figure 1. In this case however, there is still residual order, which stems from phenomena mentioned above.

In conclusion, RASERs not only deliver precise information for analysis, but also allow to study chaos when choosing appropriate experimental conditions.

References

[1] M. Suefke, S. Lehmkuhl, A. Liebisch, B. Blümich, S. Appelt, Para-hydrogen raser delivers sub-millihertz resolution in nuclear magnetic resonance. *Nat. Phys.* **13**, 568-572 (2017).

[2] A.N. Pravdivtsev, F.D. Sönnichsen, J.B. Hövener, Continuous Radio Amplification by Stimulated Emission of Radiation using Parahydrogen Induced Polarization (PHIP-RASER) at 14 T. *ChemPhysChem* **21**, 667-672 (2020). [3] B. Joalland, N.M. Ariyasingha, S. Lehmkuhl, T. Theis, S. Appelt, E.Y. Chekmenev, Parahydrogen-Induced Radio Amplification by Stimulated Emission of Radiation. *Angew. Chem. Int. Edit.* **59**, 8654-8660 (2020).
[4] S. Appelt, A. Kentner, S. Lehmkuhl, B. Blümich, From LASER physics to the para-hydrogen pumped RASER. *Prog. Nucl. Mag. Res. Sp.* **114-115**, 1-32 (2019).
[5] S. Appelt, S. Lehmkuhl, S. Fleischer, B. Joalland, N.M. Ariyasingha, E.Y. Chekmenev, T. Theis, SABRE and PHIP pumped RASER and the Route to Chaos. *Journal of Magnetic Resonance*, in press (2020).

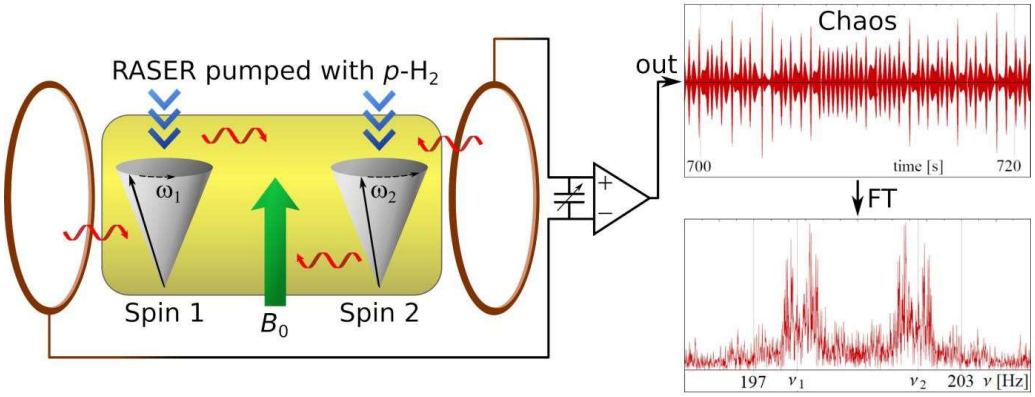


Figure 1. Two nonlinear coupled RASER modes can feature chaos[3]

HYPERPOLARIZATION OF COMMON ANTIFUNGA: AGENTS WITH SABRE

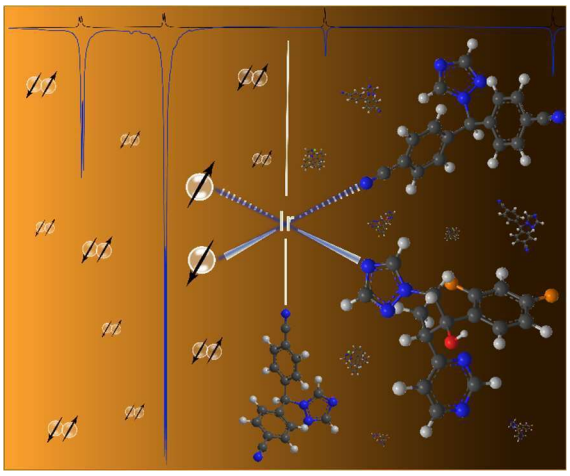
Keilian MacCulloch[1], Austin Browning[1], Patrick TomHon[1], Evan Akeroyd[1], Sören Lehmkuhl[1], Thomas Theis[1]

[1] Department of Chemistry, North Carolina State University, Raleigh, NC 27606, United States.

Signal amplification by reversible exchange (SABRE) is a robust and inexpensive way to enhance nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) signals using parahydrogen(*1*). With this relatively new technique, already a few common drugs and metabolites have been successfully polarized(*2*)(*3*)(*4*). Here, we present the polarization of large antifungal drugs and elicit the detailed hyperpolarization mechanisms for ^{1}H and ^{15}N nuclei. Hyperpolarizing common drugs is of rising interest due to their potential biomedical applications as MRI contrast agents and by enabling studies on protein dynamics at physiological concentrations. We optimize the polarization with respect to temperature and the polarization transfer field for ^{1}H nuclei in the mT regime and for ^{15}N nuclei in the µT regime, which provides detailed insights into exchange kinetics and spin evolution. This work broadens the SABRE substrate scope and provides mechanistic and kinetic insights into the hyperpolarization process.

References

[1] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. Lopez-Serrano, D. C. Williamson, Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer. *Science (80-.).* **323**, 1708–1711 (2009).
[2] A. M. Olaru, M. J. Burns, G. G. R. Green, S. B. Duckett, SABRE hyperpolarisation of vitamin B3 as a function of pH. *Chem. Sci.* **8**, 2257–2266 (2017).
[3] D. A. Barskiy, R. V. Shchepin, A. M. Coffey, T. Theis, W. S. Warren, B. M. Goodson, E. Y. Chekmenev, Over 20% 15N Hyperpolarization in under One Minute for Metronidazole, an Antibiotic and Hypoxia Probe. *J. Am. Chem. Soc.* **138**, 8080–8083 (2016).
[4] H. Zeng, J. Xu, J. Gillen, M. T. McMahon, D. Artemov, J. M. Tyburn, J. A. B. Lohman, R. E. Mewis, K. D. Atkinson, G. G. R. Green, S. B. Duckett, P. C. M. Van Zijl, Optimization of SABRE for polarization of the tuberculosis drugs pyrazinamide and isoniazid. *J. Magn. Reson.* **237**, 73–78 (2013).



INFLUENCE OF THE CHEMICAL STRUCTURE OF NITROXIDE DERIVATIVES ON DNP IN LIQUIDS

Tomas Orlando[1], Marcel Levien, Maik Reinhard, Markus Hiller, Igor Tkach, Marina Bennati

[1] ESR Spectroscopy Research Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

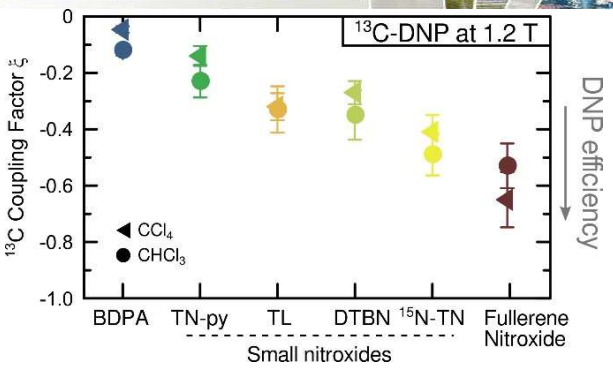
Dynamic nuclear polarization (DNP) in liquids is dominated by the Overhauser effect (OE) and strongly depends on the chosen target molecule/polarizing agent (PA) system as well as on the external magnetic field. Among the different organic radicals that can be used as PAs, small nitroxide derivatives are considered optimal for OE-DNP in the liquid state.[1]
Hereby we show a comparative study that reveals that the OE-DNP performance (i.e. the coupling factor ξ) of nitroxide derivatives in model systems ($^{13}CCl_4$, $^{13}CHCl_3$) varies up to a factor of 3 depending on their chemical structure.

Supported by DFT calculations, we recognized that for an efficient DNP a localized spin density is preferred over a distributed one and the accessibility of the radical site must not be compromised by structural design or conformational rearrangements.

Specifically, we have paid close attention to the case of fullerene nitroxides (FN), which consists of a nitroxide radical rigidly linked to a C_{60} molecule and displays the highest efficiency for ^{13}C-DNP at 1.2. However, FN is not effective for ^{13}C-DNP at higher fields (9.4 T and 14 T). These observations were compared with results obtained in ^{1}H-DNP at 0.34 T with FN as PAs[2], where an opposite trend was observed. This intriguing behavior was analyzed within the framework of the nuclear relaxation theory. We were, therefore, able to disentangle two contributions to the polarization transfer of FNs:[3] 1) the rotational diffusion, which is reducing ξ when $\tau_c > 30$ ps; and 2) a modulation of the Fermi contact interaction between PA and target molecule on the order of 5-10 ps, which is missing in other nitroxide derivatives, such as TEMPONE. Molecular dynamics calculations suggest that the latter arises from a rearrangement of the methyl groups nearby the radical site which is distinctive of the FN molecule.

References

[1] Griesinger, C. et al. Dynamic nuclear polarization at high magnetic fields in liquids. *Prog. Nucl. Magn. Reson. Spectrosc*. **64**, 4–28 (2012).
[2] N. Enkin, G. Liu, M. del Carmen Gimenez-Lopez, K. Porfyrakis, I. Tkach, M. Bennati, A high saturation factor in Overhauser DNP with nitroxide derivatives: the role of ^{14}N nuclear spin relaxation, *Phys. Chem. Chem. Phys.* **17**, 11144–11149 (2015).
[3] M. Levien, M. Hiller, I. Tkach, M. Bennati, T. Orlando, Nitroxide Derivatives for Dynamic Nuclear Polarization in Liquids: the Role of Rotational Diffusion, *J. Phys. Chem. Lett.* **11**, 1629–1635 (2020).


^{13}C-DNP at 1.2 T
^{13}C Coupling Factor ξ
0
-0.2
-0.4
-0.6
-0.8
-1.0
CCl$_4$
CHCl$_3$
DNP efficiency
BDPA
TN-py
TL
DTBN
^{15}N-TN
Fullerene Nitroxide
Small nitroxides

NEW GD(III) COMPLEXES DESIGNED FOR DYNAMIC NUCLEAR POLARIZATION

Yu Rao[1], Gabriele Stevanato[1], Chad T. Palumbo[2], Georges Menzildjian[3], Anne-Sophie Chauvin[2], Anne Lesage[3], Marinella Mazzanti[2], Lyndon Emsley[1]

[1] Laboratory of Magnetic Resonance, Institut des Sciences et Ingeńiere Chimiques, Ecole Polytechnique FedKeŕale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[2] Group of Coordination Chemistry, Institut des Sciences et Ingeńierie Chimiques, Ecole Polytechnique FedKeŕale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[3] Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCB Lyon 1), 69100 Villeurbanne, France

Thanks to their high chemical stability, gadolinium (III) molecular complexes have drawn attention as potential polarization agents (PAs) for magic angle spinning dynamic nuclear polarization (MAS-DNP), especially in reducing environments where nitroxides are not stable. The best Gd (III) PAs to date were designed under the hypothesis that the DNP enhancement is inversely proportional to the square of zero-field splitting (ZFS) parameter *D*,1 which is in turn determined by the nature of ligand. To verify this , we have synthesised three new Gd(III) complexes with different predicted ZFS and tested their MAS-DNP performance at 9.4 T in d8-glycerol:D2O:H2O 6:3:1 (shown as black dots in Figure 1). Comparing the experimentally measured 1H DNP enhancements to the simulated ZFS parameter *D*, the expected negative quadratic relation is established, indicating the validity of screening potential Gd(III) PAs by prediction of ZFS from crystal structures. Nevertheless, the complex with the lowest predicted ZFS, Gd(tpatcnam), did not yield the highest enhancement. This discrepancy might be attributed to a weakness in the prediction model, or to the contribution of other unknown factors that dominates DNP enhancement once the ZFS is sufficiently small.

References

[1] G. Stevanato, D. J. Kubicki, G. Menzildjian, A. Chauvin, K. Keller, M. Yulikov, G. Jeschke, M. Mazzanti, and L. Emsley, A Factor Two Improvement in High-Field Dynamic Nuclear Polarization from Gd(III) Complexes by Design. *J. Am. Chem. Soc.* **141**, 8746-8751 (2019).

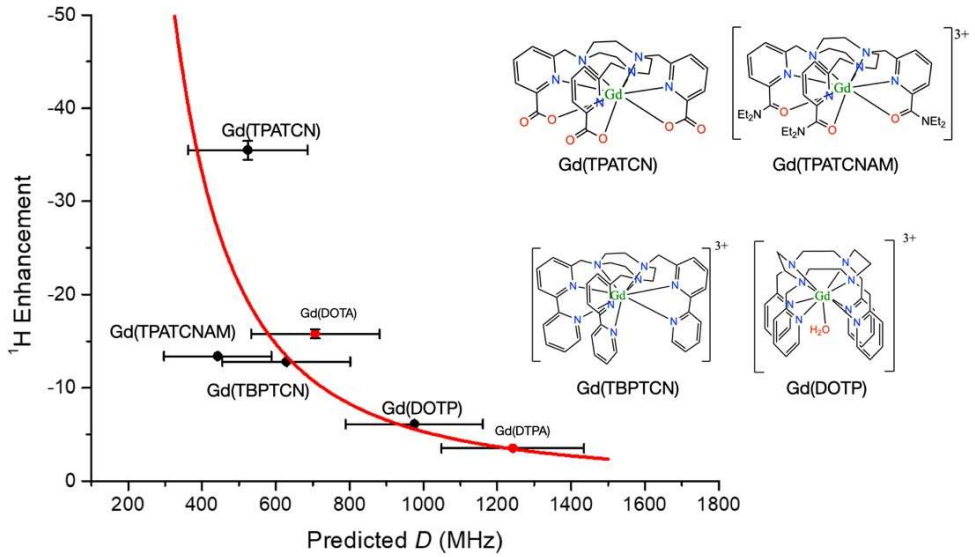


Figure 1. Proton MAS-DNP enhancement measured at 9.4 T, around 100 K and 8 kHz MAS for the new complexes (black dots) plotted as a function of predicted ZFS parameter *D*.

PHOTO-CHEMICALLY INDUCED DYNAMIC NUCLEAR POLARIZATION OF HETERONUCLEAR SINGLET ORDER

Kirill F. Sheberstov[1,2], Liubov Chuchkova[2,3], Ivan V. Zhukov[4,5], Alexey S. Kiryutin[4,5], Artur V. Eshtukov[6], Dmitry A. Cheshkov[6], John W. Blanchard[1,2], Dmitry Budker[1,2,7], Konstantin L. Ivanov[4,5], Alexandra V. Yurkovskaya[4,5]

[1] Institut für Physik, Johannes Gutenberg Universität-Mainz, 55128 Mainz, Germany
[2] Helmholtz-Institut, GSI Helmholtzzentrum für Schwerionenforschung, 55128 Mainz, Germany
[3] Faculté des Sciences Mirande, Université de Bourgogne, Dijon 21078, France
[4] International Tomography Center SB RAS, Novosibirsk 630090, Russia
[5] Novosibirsk State University, Novosibirsk 630090, Russia
[6] State Scientific Research Institute of Chemistry and Technology of Organoelement Compounds, Moscow 105118, Russia
[7] University of California at Berkeley, California 94720-7300, USA

Photo-chemically induced dynamic nuclear polarization (photo-CIDNP) is a method to hyperpolarize nuclear spins using light[1]. Here we demonstrate photo-CIDNP hyperpolarization obtained in the Earth and in zero- to ultra-low fields (ZULF)[2] producing strong enhancement of NMR lines. These experiments have an advantage that light irradiation can be performed efficiently outside the NMR spectrometer. Furthermore, we show that photo-CIDNP in ZULF is a way to hyperpolarize long-lived heteronuclear singlet order[3] between directly coupled ^{1}H-^{13}C spins. Photo-CIDNP of tetraphenylporphyrin (TPP) and *para*-benzoquinone (BQ) is studied here. The sample was placed outside an NMR magnet in a specially designed chamber for irradiation with green light produced by high-power light-emitting diodes positioned around the NMR tube. The sample was then transferred into a 1.4 T benchtop spectrometer for NMR detection. The irradiation chamber was placed either in the Earth magnetic field (~50 μT) or inside a magnetic shield (~1 μT). Figure 1 shows comparison of ^{1}H NMR spectra obtained after achieving thermal equilibrium (top spectrum), after 5 s of irradiation of the sample in the Earth field (middle spectrum), and after 5 s irradiation at ZULF (bottom spectrum). NMR signals corresponding to 2-^{13}C-BQ are strongly enhanced by photo-CIDNP. These lines are not visible in the thermal equilibrium spectrum. Using highly concentrated samples of BQ, the value of $^{1}J_{HC}$ was determined to be 170 Hz; small splitting due to *J*-couplings with other protons in the molecule also become visible. Analysis of the spin dynamics shows that the hyperpolarized states of the ^{1}H-^{13}C spin pair can be characterized by two-spin order (Earth-field case) and singlet order (ZULF case). The heteronuclear singlet order in ZULF is long-lived, having a lifetime of 40 s, which is several times longer than all other relaxation times in the molecule at this field.

References

[1] Goez, M. Photochemically Induced Dynamic Nuclear Polarization. *Advances in Photochemistry* (eds D.C. Neckers, D.H. Volman and G. Von Bünau, 2007).
[2] J. W. Blanchard, D. Budker. Zero- to Ultralow-Field NMR. *eMagRes*. pp 1395–1410 (2016).

[3] A.S. Kiryutin, I.V. Zhukov, A.V. Yurkovskaya, D. Budker, K.L. Ivanov, Singlet Order in Heteronuclear Spin Systems, *Chapter 23 in RSC book of Long-Lived Nuclear Spin Order*, pp. 418–433 (2020).

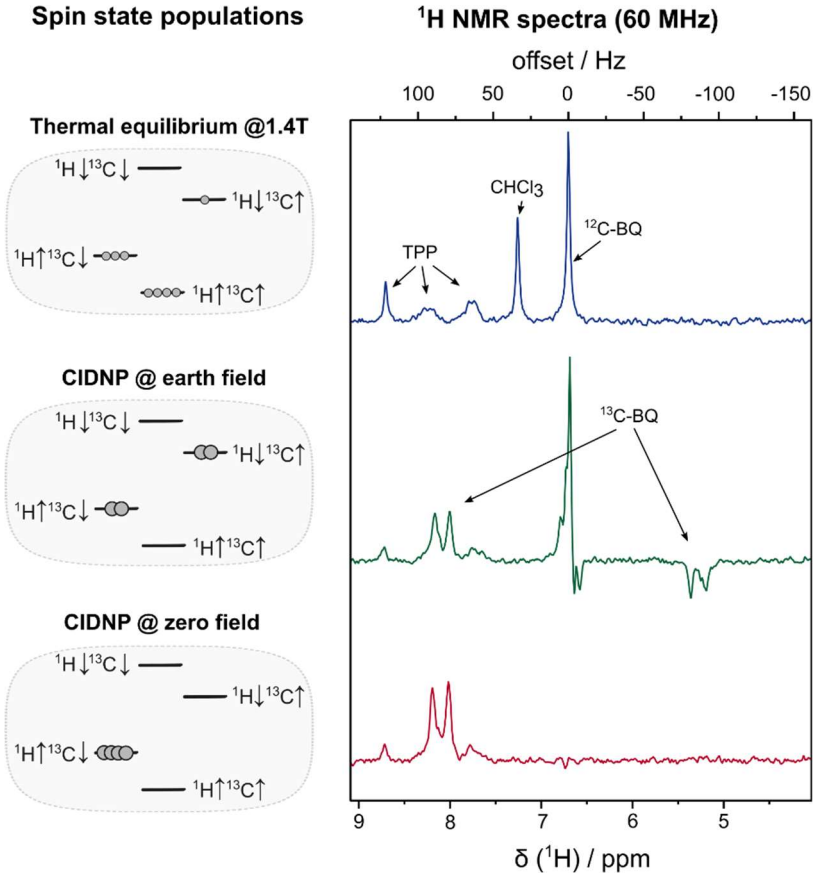


Figure 1. Spin state populations (left) and 60 MHz ^{1}H NMR spectra (right) for three cases. (1) Thermal equilibrium case. (2) photo-CIDNP generated in the Earth field and detected at high field. (3) photo-CIDNP generated at ZULF conditions and detected at high field. All NMR spectra have been obtained by averaging of 10 acquisitions and have the same noise level. Samples contain 0.5 mM TPP, 5 mM BQ diluted in degassed mixture of $CDCl_3$ (70%) and CH_3OH (30%).

ENABLING LOW-CONCENTRATION MEASUREMENTS OF NEW NUCLEAR MAGNETIC EFFECTS BY SABRE

Petr Štěpánek[1], Anu M. Kantola

[1] NMR Research Unit, Faculty of Science, University of Oulu, P.O. Box 3000, Oulu, Finland

Parahydrogen-based hyperpolarization techniques are becoming increasingly more popular in NMR due to their ability to increase the signal intensity by several orders of magnitude compared to thermal polarization, while being comparatively low cost. In particular, the Signal amplification by reversible exchange (SABRE) hyperpolarization technique [1] has stimulated a lot of interest recently as new methods are being developed to broaden the range of its substrates.

In this contribution, we will discuss the application of SABRE in investigating nuclear spin-induced optical rotation (NSOR) [2]. NSOR is a rotation of plane-polarized light beam that arises when the beam passes through a sample with nuclear magnetic moments oriented along the direction of its propagation. NSOR allows to detect nuclear magnetization by optical means instead of electromagnetic induction, as done in typical NMR experiment. Because of this, it encodes different information about the molecule than classical NMR. The strength of NSOR signal is, similarly as in NMR, directly proportional to the nuclear polarization. We show here that using SABRE hyperpolarization allows to enhance the NSOR signal by over two orders of magnitude and facilitate measurements of NSOR in solutions at concentrations that were previously unfeasibly low [3]. In addition, hyperpolarization allows to detect NSOR of substances, which are only measurable in solution. This enables new NSOR experiments and further development of this emerging spectroscopic technique.

References

[1] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. López-Serrano, D. C. Williamson, Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer. *Science* **323**, 1708-1711 (2009).
[2] I. M. Savukov, S. Lee, M. V. Romalis, Optical detection of liquid-state NMR. *Nature* **442**, 1021-1024 (2006).
[3] P. Štěpánek, A. M. Kantola, Low-concentration measurements of nuclear spin-induced optical rotation using SABRE hyperpolarization. *J. Phys. Chem. Lett* **10**, 5458-5462 (2019).

MULTIPLE DNP MECHANISMS IN CARBONACEOUS MATERIALS: FROM EXOGENOUS TO ENDOGENOUS DNP ENABLING SURFACE SENSITIVITY FROM 100 K TO ROOM TEMPERATURE

Asya Svirinovsky-Arbeli[1], Raanan Carmieli[2], Michal Leskes[1]

[1]Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot 7610001, Israel
[2] Department of Chemical Research Support, Weizmann Institute of Science, Rehovot 76100, Israel

Carbonaceous materials are ubiquitous in energy storage and conversion systems, essential for our modern life. Development of these materials relies on our ability to establish structure-property correlations. Solid-state nuclear magnetic resonance (ssNMR) spectroscopy is well suited for providing atomic level structural information, especially when equipped with sensitivity from Dynamic Nuclear Polarization (DNP). Exogenous DNP from nitroxide biradicals is the most efficient approach providing 10^2-10^4 fold enhancement in surface sensitivity. Previously we showed that implementing DNP in conductive carbons is not straightforward due to sample heating and microwave absorption leads to negligible enhancements in DNP if the sample preparation is not refined.[1]

Herein, we consider the application of DNP in the study the surface chemistry of carbonaceous materials through natural abundance ^{13}C detection. We found that with TEKPol biradicals the polarization transfer via ^{1}H-^{13}C cross-polarization is limited to the solvent and does not propagate to the sample surface. We thus investigated in detail polarization transfer directly to ^{13}C nuclei and found multiple interfering mechanisms when employing the exogenous DNP approach: a) ^{13}C cross-effect from the biradicals, b) opposite enhancements due to heteronuclear cross-relaxation leading to solvent Overhauser effect and c) solid-effect from defects and conduction electrons within the carbons.

While the endogenous electron polarization prevents the utilization of exogenous DNP, it provides significant surface sensitivity with signal enhancements of 19 and 50 for ^{13}C and ^{1}H, respectively. Moreover, endogenous DNP as I will show can be used at a wide range of temperatures, providing 9-fold increase in ^{13}C signal at room temperature. This approach opens the way for efficient detection of the carbons' surface chemistry at ambient conditions.

References

[1] A. Svirinovsky-Arbeli, D. Rosenberg, D. Krotkov, R. Damari, K. Kundu, A. Feintuch, L. Houben, S. Fleischer, M. Leskes, The effects of sample conductivity on the efficacy of dynamic nuclear polarization for sensitivity enhancement in solid state NMR spectroscopy. *Solid State NMR.* **99**, 7-14 (2019).

RATIONAL CATALYSTS DESIGN FOR EXCHANGEABLE PROTON HYPERPOLARIZATION

Ewoud Vaneeckhaute[1], Sophie De Ridder[1], Jean-Max Tyburn[3], David Kilgour[3], James G. Kempf[4], Francis Taulelle[2], Johan A. Martens[2], Eric Breynaert[1]

[1] NMRCoRe, KU Leuven, Celestijnenlaan 200F, box 2461, B-3001 Leuven, Belgium.
[2] COK-KAT, KU Leuven, Celestijnenlaan 200F, box 2461, B-3001 Leuven, Belgium.
[3] Bruker Biospin, Silberstreifen 4, 76287 Rheinstetten, Germany.
[4] Bruker Biospin, 15 Fortune Dr., Billerica, Massachusetts 01821, United States.

Nuclear spin polarization, traditionally limited by Boltzmann statistics in nuclear magnetic resonance spectroscopy (NMR) and magnetic resonance imaging (MRI), exhibits drastic enhancement when singlet spin order of parahydrogen (p-H_2) fuels hyperpolarization in target molecules.[1] Signal amplification by reversible exchange (SABRE) transfers polarization stored in the singlet para-state of hydrogen to a target molecule through chemical interaction between p-H_2 and the substrates catalyzed by a transition metal complex.[2] SABRE, in contrast with other Parahydrogen Induced Polarization (PHIP) techniques leaves targets chemically unchanged after interaction with p-H_2 but still alters spin polarization, dramatically enhancing the MR signal.

While spin physics takes a central role in hyperpolarization transfer via SABRE, accurate control over the complexation chemistry is of primordial importance for exploring and extending the capabilities of SABRE.[3] In this contribution, rational design of the transition metal complex through accurate ligand control, broadens the range of hyperpolarized targets towards exchangeable protons and peripheral co-solutes in protic conditions. Ligand control is exerted by controlling the interplay between the traditional target, the aromatic N-containing heterocycle (pyridine =P), and ammonium, modifying pH by dosing ammonium as either of three pH buffering systems: (i) NH_4Cl , (ii) $(NH_4)_2CO_3$ and (iii) NH_4OH. Controlling pH, and thus the concentration of NH_3 (N) in the system, enables creation of three octahedral iridium complexes $[Ir(IMes)(H_2)(PeqNeqPax)]Cl^-$, $Ir(IMes)(H_2)(PeqNeqNax)Cl^-$ and $Ir(IMes)(H_2)(NeqNeqNax)Cl^-$, each with unique properties. Upon p-H_2 bubbling at a low pre-polarization transfer field, hyperpolarization of exchangeable protons is observed both in methanol-d_4 and semi-aqueous conditions. This hyperpolarization occurs together with the production of long-lived hyperpolarized o-H_2 and hydrides (T^* up to 30s), enabling in-situ hyperpolarized 2D LR-COSY at high field, using a single p-H_2 activation step at low field.

References

[1] C. R. Bowers, D. P. Weitekamp, Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment. *J. Am. Chem. Soc.* ***109***, 5541–5542 (1987).
[2] R. W. Adams, J. A. Aguilar, S. B. Duckett et al., Reversible Interactions with Para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer. *Science (80-.)* ***323***, 1708–1711 (2009).
[3] E. Vaneeckhaute, E. Breynaert et al., Hyperpolarized Magnetic Resonance of Exchangeable Protons Using Parahydrogen and Aminosilane. *J. Phys. Chem. C* ***124***, 14541–14549 (2020). Front Cover

HYPERPOLARIZED ^{89}Y-EDTMP AS A CHEMICAL SHIFT-BASED NMR SENSOR FOR pH AT THE PHYSIOLOGICAL RANGE

Qing Wang[1], Christopher Parish[1], Peter Niedbalski[1], S. James Ratnakar[2], Zoltan Kovacs[2], Lloyd Lumata[1].

[1] Department of Physics, University of Texas at Dallas, Richardson, Texas 75080 USA.
[2] Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas 75390 USA.

Yttrium (III) complexes are interesting due to the similarity of their chemistry with gadolinium complexes that are used as contrast agents in nuclear magnetic resonance (NMR) spectroscopy or imaging (MRI). The insensitivity problem in ^{89}Y-based complexes can be circumvented with the use of dissolution dynamic nuclear polarization (DNP)[1] which allows for several thousand-fold enhancement of the NMR signal.

Herein, we report on the feasibility of using hyperpolarized ^{89}Y-complexes with phosphonated open-chain ligands, ^{89}Y-EDTMP and ^{89}Y-DTPMP, as potential chemical shift-based pH NMR sensors. Our data show that hyperpolarized ^{89}Y-DTPMP has pKa~7.01 with a 4 ppm-wide chemical shift dispersion with the signal disappearing at pH below 6.2. On the other hand, pH titration data on hyperpolarized ^{89}Y-EDTMP show that it has a pKa of pH 6.7 and a 16-ppm wide chemical shift dispersion at pH 5-9 range. In comparison, the previously reported hyperpolarized pH NMR sensor 89Y-DOTP has a pKa of 7.64 and ~10-ppm wide chemical shift dispersion at pH 4-9 range. Our data[2] suggest that hyperpolarized ^{89}Y-EDTMP is better than hyperpolarized ^{89}Y-DOTP in terms of pH sensing capability at the physiological range.

References

[1]J.H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin, M. Thaning, K. Golman, Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR, *Proc. Natl. Acad. Sci. USA* **100**, 10158-10163 (2003).

[2]Q. Wang, C. Parish, P. Niedbalski, S. James Ratnakar, L. Lumata, Hyperpolarized 89Y-EDTMP as a chemical shift-based NMR sensor for pH at the physiolocal range, *J. Magn. Reson.* **320**, 106836 (2020).

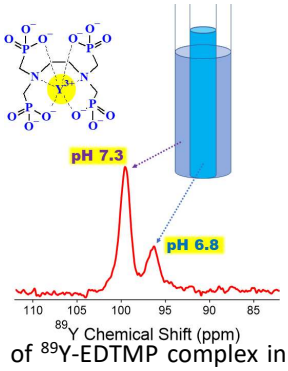


Figure 1. Hyperpolarized ^{89}Y NMR signal of ^{89}Y-EDTMP complex in two-tube compartments with different pH values showing two distinct NMR peaks at 99.7 ppm (pH 7.3) and 96.2 ppm (pH 6.8).

RADIO-FREQUENCY MANIPULATION OF STRONGLY INTERACTING ELECTRON-NUCLEAR SPIN SYSTEMS

Pablo R. Zangara[1,2], Daniela Pagliero[3], Rodolfo H. Acosta[1,2], Carlos A. Meriles[3,4]

[1] Facultad de Matemática, Astronomía, Física y Computación, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000HUA Córdoba, Argentina.
[2] Instituto de Física Enrique Gaviola (IFEG), CONICET, Medina Allende s/n, X5000HUA, Córdoba, Argentina.
[3] Department of Physics, CUNY-City College of New York, New York, NY 10031, USA.
[4] CUNY-Graduate Center, New York, NY 10016, USA.

We use optical spin pumping of NV-hosting diamond to investigate the impact of a continuous radio-frequency (RF) drive on the generation of ^{13}C spin polarization. Since this DNP mechanism is based on electron spin cross relaxation[1,2], we particularly address the manipulation of hybrid electron-nuclear[3] spin states. We focus on strongly hyperfine-coupled carbons and experimentally show that sufficiently strong RF excitation during optical spin pumping can invert the sign of the observed nuclear polarization. With the aid of numerical modeling, we interpret this counter-intuitive finding as a consequence of electron-nuclear spin mixing, allowing us to drive with RF otherwise forbidden transitions between different electron spin manifolds. Our findings can be understood as a form of competition between 'cross effect' and 'solid effect' in an effective four-level system where initialization derives from NV optical pumping, not temperature. Examining the system response at variable magnetic fields, we discuss how this process emerges from a subtle interplay between the number of nuclei featuring a given hyperfine coupling and the type and relative concentrations of paramagnetic defects present in the sample.

References

[1] R. Wunderlich, J. Kohlrautz, B. Abel, J. Haase, J. Meijer, "Optically induced cross relaxation via nitrogen-related defects for bulk diamond 13C hyperpolarization", *Phys. Rev. B* **96**, 220407(R) (2017).
[2] D. Pagliero, K.R. Koteswara Rao, P.R. Zangara, S. Dhomkar, H.H. Wong, A. Abril, N. Aslam, A. Parker, J. King, C.E. Avalos, A. Ajoy, J. Wrachtrup, A. Pines, C.A. Meriles, "Multispin-assisted optical pumping of bulk ^{13}C nuclear spin polarization in diamond", *Phys. Rev. B* **97**, 024422 (2018).
[3] D. Pagliero, P.R. Zangara, J. Henshaw, A. Ajoy, R.H. Acosta, N. Manson, J. Reimer, A. Pines, C.A. Meriles, "Magnetic-field-induced delocalization in hybrid electron-nuclear spin ensembles", *submitted,* arXiv: 2006.07736.

05. Instrumentation - Hardware

NMR RELAXORPTION: A BIMODAL INSTRUMENTATION FOR SIMULTANEOUS SORPTION / NMR DETECTION OF VAPORS INSIDE MICROPOROUS MATERIALS

Rodrigo De Oliveira-Silva[1], Joao Marreiros[1], Rob Ameloot[1], Dimitrios Sakellariou[1]

[1] cMACS, Department of Microbial and Molecular Systems (M2S), KU Leuven, Leuven, Belgium.

Porosimetry is an important and recurrent analysis in material chemistry characterization. Materials such as metal-organic frameworks (MOFs), mesoporous silicas, and zeolites are typically characterized using gas/vapor adsorption and desorption isotherms.[1,2] Through the controlled partial pressure loading of an evacuated sample volume, this technique determines indirectly qualitative and quantitative properties of the pore environment, like BET surface area and pore volume, with limited insight into the quantifiable nature of guest/host interactions. Low-field Nuclear Magnetic Resonance relaxometry is applied commonly to porous media analysis to directly measure the content and physicochemical properties, such as mobility, of the porous environment through nuclear spin relaxation of the contained liquid.[3,4] In this work, we measure molecules of small organic compounds inside the micropores of the sample, a real challenge in terms of signal sensitivity.

We present a first account of simultaneous online physisorption and NMR for the study of microporous MOFs, with vapors of methanol, ethanol and water at 35°C. The instrumentation involved a novel custom-made low-cost homemade permanent magnet designed to be integrated with a commercial physisorption analyzer, without interference, see Figure1(a). The weight of the magnet is low (4 kg) and its homogeneity sufficiently high to allow us for sensitive relaxometric studies over large volumes of sample (~0.1 cm^3). Signal sensitivity of non-hyperpolarized, natural abundance molecules was surprisingly high due to a concentration effect inside the pores of the materials, which act like sponges for such molecules, even at room temperature and low pressure, increasing their local density. CPMG and T_1/T_2 correlation measurements were performed in parallel as a function of the loading under controlled sample and magnet temperature. Samples varying from simple models, to systems with many pore environments, rigid as well as flexible were studied with this combined technique leading to new insights about the interactions during sorption.

References

[1] Tu, M. *et al.,* Engineering Zeolitic-Imidazolate Framework (ZIF) Thin Film Devices for Selective Detection of Volatile Organic Compounds, *Adv. Funct. Mater.* **25**, 4470-4479 (2015)
[2] Marreiros J *et al.*, Active Role of Methanol in Post-Synthetic Linker Exchange in the Metal–Organic Framework UiO-66, *Chem. Mater.* **31**, 1359-1369 (2019)
[3] Judeinstein P *et al.*, Low-field single-sided NMR for one-shot 1D-mapping: Application to membranes, *J. Magn. Reson.* **277**, 25-29 (2017)
[4] D'Agostino, C. *et al.,* Interpretation of NMR Relaxation as a Tool for Characterising the Adsorption Strength of Liquids inside Porous Materials, *Chem. Eur. J.* **20**, 13009-130015 (2014).

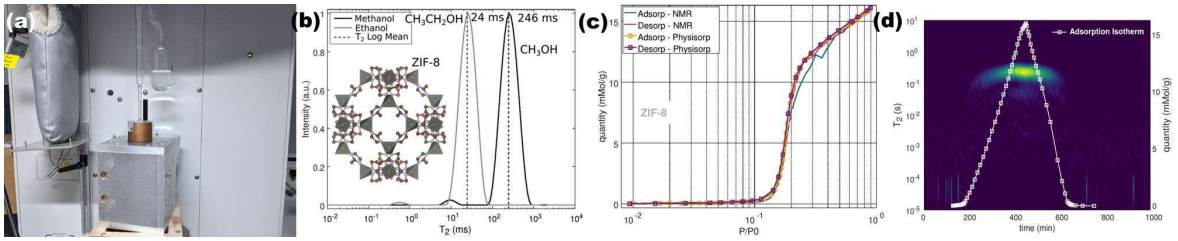


Figure 1. a) NMR relaxorption hyphenated benchtop system. The 0.3T cubic-shape permanent magnet hosts the tube containing the porous solids which is linked to the commercial sorption avalyser. b) T_2 relaxation time distributions from methanol and ethanol vapors adsorbed inside a powdered sample of ZIF-8 metal organic framework showing speciation. c) Isotherms curves (adsorption – desorption) measured simultaneously using both modalities. The agreement between them for the ZIF-8 sample is close to perfect. This behavior is however not observed in all materials. d) Time resolved T_2 relaxometry during adsorption and desorption. The white curse shows the quantity of the adsorbed vapor from the sorption hardware as a function of time. The underlying surface plot shows the intensity of the Laplace transform of the FID as a function of time (horizontal axis) and T_2 value (vertical axis). These curves can be informative about the host-guest interactions and the details of the sorption dynamics.

THE SINGLE-CHIP DYNAMIC NUCLEAR POLARIZATION MICROSYSTEM

Nergiz Sahin Solmaz[1], Marco Grisi[1], Alessandro V. Matheoud[1], Gabriele Gualco[1], and Giovanni Boero[1]

[1]School of Engineering, École Polytechnique Fedérale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland.

Integration of the sensitivity-relevant electronics of nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectrometers on a single chip is a promising approach to improve the limit of detection, especially for samples in the nanoliter and subnanoliter range. We demonstrate DNP-enhanced NMR experiments (Fig. 1a) using a single-chip integrated microsystem having an area of about 2 mm^2.

We have designed a novel single-chip DNP microsystem[1] (Fig. 1b) by cointegrating on a single silicon chip of the front-end electronics of NMR and ESR detectors. The excitation/detection planar spiral microcoils of the NMR and ESR detectors are concentric and interrogate the same sample volume.

We would like to present the details of our recent publication[1] and, Covid-19 permitting, new measurement results at low temperatures.

References

[1] Anal. Chem. 2020, 92, 14, 9782-9789.

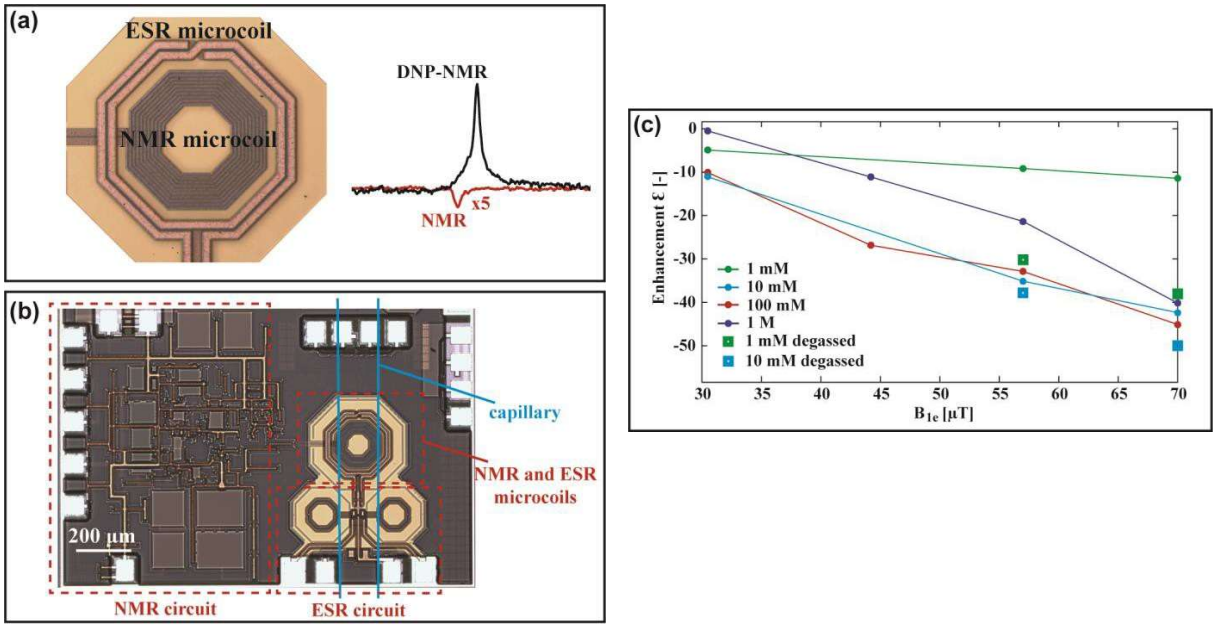


Figure 1. (a) The graphical abstract, (b) the photograph of CMOS DNP microchip, (c) DNP enhancements of TEMPOL/H_2O solutions at different microwave magnetic fields (B_{1e}).

NMR MAGNETS FOR PORTABLE APPLICATIONS USING 3D PRINTED MATERIALS

Belal MK Alnajjar[1], André Buchau[1], Jens Anders[1]

[1] University of Stuttgart, Institute if smart Sensors Pfaffenwaldring 47, 70569 Stuttgart, Germany

In this contribution, we introduce 3D printing as a possibility for realizing lightweight, yet high-precision NMR magnets. Using a commercially available filament containing iron oxide particles allows for the realization of critical components of NMR magnets such as pole pieces and even the flux-conducting yoke. In contrast to shimming structures made of iron, 3D printed structures made of the lightweight filament allow for a robust and inexpensive way of realizing high-performance NMR magnets for future portable NMR applications. We demonstrate the versatility and achievable high performance of the proposed solution with an H-shaped NMR magnet. In this magnet, the 3D-printed filament is used to realize the yoke that guides the magnetic flux inside the magnet as well as 3D-printed pole pieces. The yoke provides an order of magnitude weight reduction compared to a conventional iron yoke. Numerical size and shape optimizations using non-uniform rational basis splines (NURBS) have been applied to obtain the optimal geometry of the passive shim structures.

The manufactured magnets achieve a measured NMR spectral line width of 3 kHz without any additional shimming. Our results clearly demonstrate the efficiency and versatility of the proposed design and optimization approach.

A BACKGROUND-FREE ^{1}H-^{13}C RADIOFREQUENCY PROBE FOR CROSS-POLARIZATION AND LINESHAPE ANALYSIS IN DISSOLUTION-DYNAMIC NUCLEAR POLARIZATION EXPERIMENTS

Stuart J. Elliott[1], Morgan Ceillier[1], Olivier Cala[1], Quentin Chappuis[1], Aurélien Bornet[2], Igor V. Koptyug[3], James G. Kempf[4], Samuel F. Cousin[1] and Sami Jannin[1]

[1] Centre de Résonance Magnétique Nucléaire à Très Hauts Champs - FRE 2034 Université de Lyon / CNRS / Université Claude Bernard Lyon 1 / ENS de Lyon, 5 Rue de la Doua, 69100 Villeurbanne, France
[2] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Batochime, CH-1015 Lausanne, Switzerland
[3] International Tomography Center, SB RAS, 3A Institutskaya St., Novosibirsk, 630090 Russia
[4] Bruker Biospin, 15 Fortune Drive, Billerica, MA 01821, United States

The low sensitivity of conventional nuclear magnetic resonance experiments can be readily overcome by employing hyperpolarization methodologies, in suitable cases. One such technique, dissolution-dynamic nuclear polarization (*d*DNP), provides a robust means of strongly polarizing a variety of small molecules. A drawback of the *d*DNP approach, the excessively long polarization timescales for insensitive nuclei, has been circumvented by the use of cross-polarization (CP) pulse sequences[1,2], which are in general quicker and more efficient (>10× faster polarization of ^{13}C nuclei for *d*DNP, typically improving from ~2 hours to ~10 minutes). However, the capacity to effectively perform CP experiments under *d*DNP conditions remains challenging, and is still plagued by additional complications including spurious background signals from the experimental apparatus. Here we propose a *background-free* ^{1}H-^{13}C radiofrequency coil specifically designed for use in CP experiments at liquid helium temperatures, which allows simplified and "on-the-spot" nuclear spin polarization quantification. We additionally introduce simple guidelines for the optimization and implementation of CP pulse sequences. The *background-free* ^{1}H-^{13}C radiofrequency coil is straightforward to construct[3], fabricated from 3D printed copper/silver wire and ceramic capacitors, and is housed in a rigid PTFE support. Experimental demonstrations are presented for the case of [1-^{13}C]sodium acetate. Furthermore, we use our *background-free* ^{1}H-^{13}C radiofrequency coil for spectral lineshape polarimetry experiments of H_2O and [2-^{13}C]sodium acetate.

References

[1] A. Bornet, R. Melzi, S. Jannin and G. Bodenhausen, *Appl. Magn. Reson.*, **2012**, 43, 107-117.
[2] A. Bornet, A. Pinon, A. Jhajharia, M. Baudin, X. Ji, L. Emsley, G. Bodenhausen, J.-H. Ardenkjær-Larsen and S. Jannin, *Phys. Chem. Chem. Phys.*, **2016**, 18, 30530-30535.
[3] A. Bornet, R. Melzi, A. J. Perez Linde, P. Hautle, B. van den Brandt, S. Jannin and G. Bodenhausen, *J. Chem. Phys. Lett.*, **2013**, 4, 111-114.

ENHANCING BENCHTOP NMR SPECTROSCOPY WITH SAMPLE SHIFTING DEVICE

Javier A. Romero[1], Krzysztof Kazimierczuk[1], Dariusz Gołowicz[1,2]

[1] Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097, Warsaw, Poland
[2] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093, Warsaw, Poland

Fourier Transform benchtop NMR (BT-NMR) spectrometers equipped with permanent magnets benefit from their compactness, low price, and low handling costs. Unfortunately, a lack of resolution and sensitivity strongly limit their applications.

We propose a new way to measure data on the BT-NMR spectrometer to increase the measurement sensitivity. We built and programmed a simple and easy to use device to run NMR experiments with a sequential shifting of the sample during the measurement. This allowed us to almost completely remove relaxation delay from the pulse sequence and still acquire each scan with a high magnitude of initial z-magnetization. After a single scan FID is collected, the excited sample volume is immediately removed from the coil to the other region of the magnet and allowed to relax. The device, which we call SWAPE – Sweeping Apparatus for Polarization Enhancement, allows to obtain a better signal to noise ratio (SNR) in the spectrum, reduce experiment time, and collect quantitative data more efficiently[1]. Notably, the device can be mounted and dismounted within seconds and does not require to modify original spectrometer hardware. We present the performance of the device using several examples: a ^{1}H NMR experiment for 4,4-dimethoxy-2-butanone (Fig. 1), ^{13}C NMR experiments for benzyl salicylate, the liquid pharmaceutical product Acerin, and a mixture of m-anisaldehyde and (R)-(+)-limonene.

References

[1] J. A. Romero, K. Kazimierczuk, D. Gołowicz, Enhancing benchtop NMR spectroscopy by means of sample shifting. *Analyst*, doi:10.1039/D0AN01556C (2020).

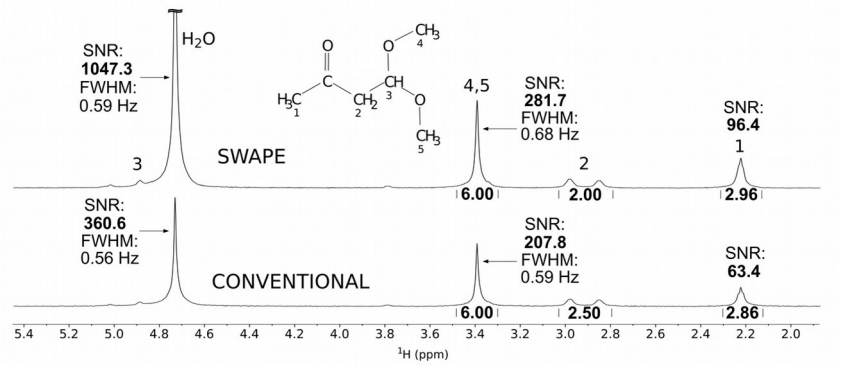


Figure 1. ^{1}H NMR spectra of 4,4-dimethoxy-2-butanone acquired using SWAPE and in a conventional way.

DIGITAL MICROFLUIDICS-NMR INTERFACE: THE NEXT GENERATION MICROVOLUME CHEMICAL REACTION MONITORING PLATFORM

Bing Wu[1], Ian Swyer[2], Sebastian von der Ecken[2], Chunliang Li[3], Amy Jenne[1], Franck Vincent[4], Daniel Schmidig[4], Till Kuehn[4], Armin Beck[4], Falko Busse[5], Henry Stronks[6], Ronald Soong[1], Aaron R. Wheeler[2], André Simpson[1]

[1] Department of Chemistry, University of Toronto Scarborough, 1256 Military Trail, Toronto, ON, CA, M1C 1A4.
[2] Department of Chemistry, University of Toronto, 80 St. George St., Toronto, ON, CA, M5S 3H6
[3] Laboratory of Physical Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, NL
[4] Bruker BioSpin AG. Industriestrasse 26, 8117 Fällanden, CH
[5] Bruker BioSpin GmbH Silberstreifen 4, 76287 Rheinstetten, DE
[6] Bruker Canada Ltd, 2800 High Point Drive, Milton, Ontario, L9T 6P4, CA

High Resolution Nuclear magnetic resonance (NMR) spectroscopy provides unparalleled insights into molecular structure and dynamics. However, compared to many other analytical methods, it usually suffers from relatively low mass sensitivity which is critical for trace and biological analyses. One approach to overcome this limitation is to reduce the size of the NMR coil to sub-mm diameter to create “microcoils”. These microcoils leverage the fact that the magnetic flux density induced by a unit current (and therefore the voltage induced in the coil by nuclear spins) increases at a greater rate due to proximity than the resistive noise, leading to improved signal-to-noise ratios. Although microcoils achieve great success in mass sensitivity, sample handling of small volumes becomes extremely challenging. Digital microfluidics (DMF) involves the movement of droplets (from low nL up to 5 µL) on an open surface of electrodes and can be used in various applications including, liquid-liquid extraction, liquid-solid extraction, cell movement, cell culturing, in chip chromatography, binding assays and many more.
In this study, we introduce an upgraded digital microfluidic (DMF) system capable of interfacing droplets of analyte with microcoils within a high-field NMR spectrometer. Droplets can be moved inside the spectrometer to perform a range of routine DMF protocols, while also permitting the acquisitions of several 1D and 2D NMR experiments. Furthermore, this platform was used to analyze two rapid chemical reactions in real time. We propose that the combination of DMF and NMR will be a useful new tool for a wide range of applications in chemical analysis.[1, 2]

References

[1] Swyer, I.; von der Ecken, S.; Wu, B.; Jenne, A.; Soong, R.; Vincent, F.; Schmidig, D.; Frei, T.; Busse, F.; Stronks, H. J.; Simpson, A. J.; Wheeler, A. R., Digital microfluidics and nuclear magnetic resonance spectroscopy for in situ diffusion measurements and reaction monitoring. *Lab on a chip* **2019,** *19* (4), 641-653.
[2] Wu, B.; von der Ecken, S.; Swyer, I.; Li, C.; Jenne, A.; Vincent, F.; Schmidig, D.; Kuehn, T.; Beck, A.; Busse, F.; Stronks, H.; Soong, R.; Wheeler, A. R.; Simpson, A., Rapid Chemical Reaction Monitoring by Digital Microfluidics-NMR: Proof of Principle Towards an Automated Synthetic Discovery Platform. *Angew Chem Int Ed Engl* **2019**.

APPLICATION OF FLOW-NMR SYSTEM IN ENVIRONMENTAL SCIENCE: REAL-TIME MONITORING OF WASTEWATER TREATMENT

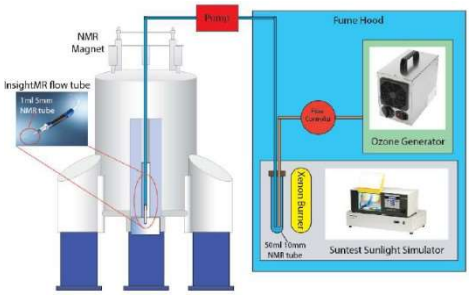
Bing Wu[1], Rudraksha D. Majumdar[1], Daniel H. Lysak[1], Rajshree Ghosh Biswas[1], Maryam Tabatabaei-Anaraki[1], Amy Jenne[1], Xiang You[1], Ronald Soong[1], Daniel Lane[1], Paul A. Helm[2], Anna Codina[3], Venita Decker[4], Myrna J. Simpson[1], Andre J. Simpson[1]

[1] Environmental NMR Centre, Dept. of Physical and Environmental Sciences, University of Toronto, Scarborough, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4.
[2] Environmental Monitoring & Reporting Branch, Ontario Ministry of the Environment, Conservation and Parks, Toronto, ON, Canada, M9P 3V6
[3] Bruker (UK) Ltd., Banner Lane, Coventry, CV4 9GH, UK
[4] Bruker BioSpin GmbH, Silberstreifen 4, Rheinstetten, Germany, 76287

Wastewater contains complex organic signatures, whose components can be harmful to both environmental and human health. NMR is an excellent tool for molecular monitoring, with ability to follow total organics, subcategories (aromatics, aliphatic etc.) and, resolution permitting, even individual compounds. However, due to the relatively low sensitivity of NMR, studies of wastewater to date have involved preconcentration (often many liters) before analysis. In this proof of concept study, we ask “Is NMR sensitive enough for online wastewater monitoring without preconcentration?” In this study, a continuous flow 500 MHz NMR system, coupled with a sunlight simulator and ozone generator, was used on unaltered and unconcentrated wastewater to assess the potential of on-line NMR for understanding wastewater treatment processes. Wastewater from 3 different treatment stages were analyzed. In general, combined ozone and sunlight was more effective at removing organics, while different in behavior of the same molecules within different wastewater fractions suggest that the chemistry is more complex than just the action of “reactants” (light, ozone) on the target chemicals, and to some extent, involves other components in the wastewater. As such, the nature of the effluent itself could also have important impacts on the rate and completion of its degradation. In summary, on-line NMR of wastewater treatment could be carried out without preconcentration of the wastewater, in-line with important future applications for understanding wastewater treatment processes at the molecular level. Over the last decade, there has been considerable development in low field, low footprint, low cost, NMR spectrometers. Benchtop versions of those systems could potentially, in the future, be applied for onsite, online monitoring of waste streams at water treatment plants, as described here.[1]

References

[1] Wu, B.; Majumdar, R. D.; Lysak, D. H.; Ghosh Biswas, R.; Tabatabaei-Anaraki, M.; Jenne, A.; You, X.; Soong, R.; Lane, D.; Helm, P. A.; Codina, A.; Decker, V.; Simpson, M. J.; Simpson, A. J., Towards real-time kinetic monitoring of wastewater treatment: A case study of sunlight and ozone treatment of unconcentrated wastewater using flow NMR. *Chem Eng J* **2021,** *405*, 126696.


Pump
Fume Hood
NMR Magnet
InsightMR flow tube
Ozone Generator
Xenon Burner
50ml 10mm NMR tube
Suntest Sunlight Simulator

06. In cell NMR

A UNIFIED DESCRIPTION OF INTRINSICALLY DISORDERED PROTEIN DYNAMICS UNDER PHYSIOLOGICAL CONDITIONS USING NMR SPECTROSCOPY

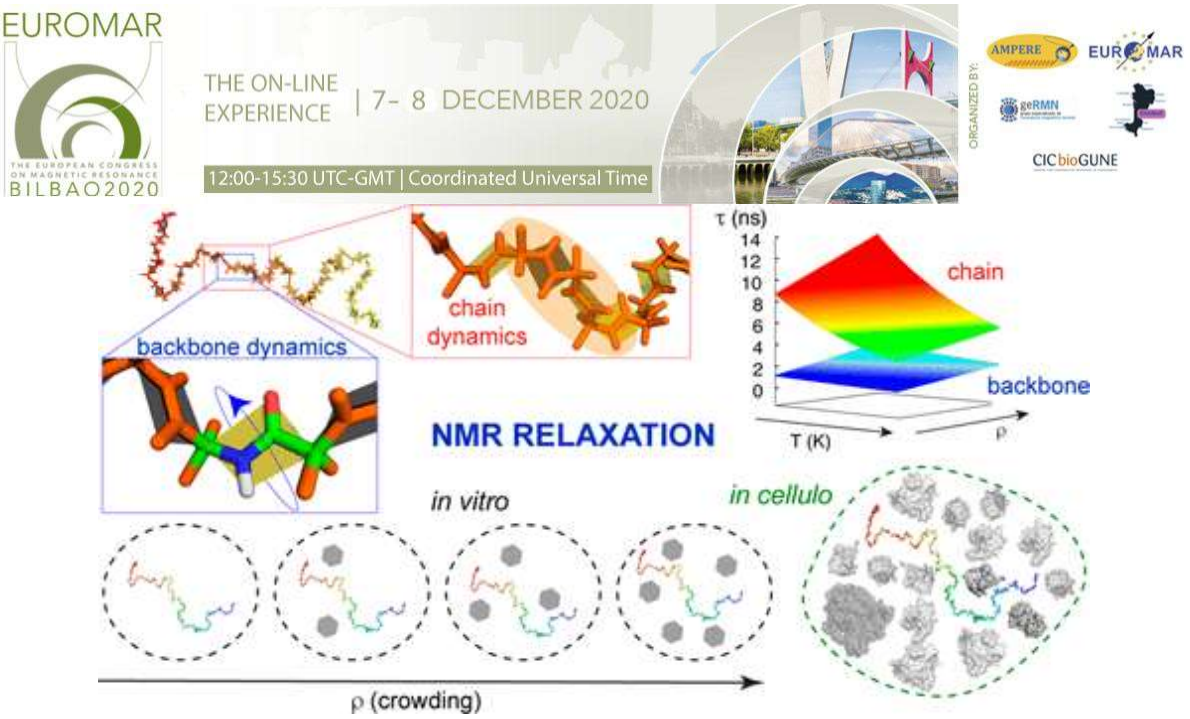
Adamski Wiktor[1], Salvi Nicola[1], Maurin Damien[1], Magnat Justine[1], Milles Sigrid[1], Ringkjøbing Jensen Malene[1], Abyzov Anton[1], Moreau Christophe J.[1], and Blackledge Martin[1]

[1]Institut de Biologie Structurale, Université Grenoble Alpes-CEA-CNRS, 71, Avenue des Martyrs, Grenoble, France.

While intrinsically disordered proteins (IDPs) constitute about half of the human proteome, molecular models of their functional behavior in vivo remain elusive. As proteins of this class do not possess a stable, three- dimensional structure, but sample ensembles of diverse conformations on rather flat energy landscapes, their function is inherently linked to their dynamics occurring on multiple timescales. Recently, we used temperature-dependent ^{15}N spin relaxation to reveal the physical origin of IDPs backbone motions in the ps-ns range. In our model three dominant dynamic modes are relaxation active: fast librational motions, conformational sampling of backbone dihedral angles and slower chain-like segmental dynamics [1, 2]. Herein,we extend the temperature-dependent model-free analysis by systematically modifying viscosity and, consequently, ^{15}N spin relaxation rates, to probe the differential effect of molecular environment on the three identified dynamic modes. We carry out our study on two IDPs with remarkably different dynamic properties: the C-terminal domain of Sendai Virus nucleoprotein, which feature a transiently populated helical recognition motif, and the disordered domain of the cancer-associated mitogen-activated protein kinase kinase (MKK4), which is devoid of any secondary structure. Our results suggest that the effects of temperature and viscosity on dynamics can be combined to develop a unified description of the effect of molecular environment parameters on IDP dynamics. In fact, the proposed model is able to predict molecular environment-induced effects on dynamic modes probed by ^{15}N spin relaxation rates in vitro and in vivo with remarkable accuracy [3]. Overall, we expect that the model will shed some light onto the complex dynamics of IDPs engaged in dynamic complexes in a diverse range of molecular environments.

References

[1] Abyzov A., et al., JACS 2016, **138**, 6240 – 6251
[2] Salvi N., et al., Angew. Chem. Int. Ed. 2017, **56**, 14020
[3] Adamski W.., et al., JACS 2019, **141**, 17817 – 17829


EUROMAR
THE EUROPEAN CONGRESS ON MAGNETIC RESONANCE
BILBAO2020
THE ON-LINE EXPERIENCE | 7- 8 DECEMBER 2020
12:00-15:30 UTC-GMT | Coordinated Universal Time
ORGANIZED BY:
AMPERE
EUROMAR
geRMN
CICbioGUNE
backbone dynamics
chain dynamics
τ (ns)
14
12
10
8
6
4
2
0
chain
backbone
T (K)
ρ
NMR RELAXATION
in vitro
in cellulo
ρ (crowding)

TOWARDS INTRACELLULAR DRUG QUANTIFICATION BY MAS DNP

Andrea Bertarello[1], Pierrick Berruyer[1], Urban Skantze[2], Sepideh Hagvall[3], Samiksha Sardana[4], Malvika Sardana[4], Charles S. Elmore[4], Rodrigo J. Carbajo[5,*], Markus Schade[5], Elisabetta Chiarparin[5], Staffan Schantz[6], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[2] Advanced Drug delivery, Pharmaceutical Science, AstraZeneca, Gothenburg, Sweden
[3] Early CVRM, Biopharmaceutical R&D, AstraZeneca, Gothenburg, Sweden
[4] Pharmaceutical Science, R&D, AstraZeneca, Gothenburg, Sweden
[5] Chemistry, Oncology R&D, AstraZeneca, Cambridge, United Kingdom
[6] Oral Product Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Gothenburg, Sweden
* Now left AstraZeneca

Determining intracellular drug concentration provides a deeper understanding of its function and efficacy. Detection should ideally be performed non-destructively, and without modification of the drug. It requires the ability to detect low amounts of the drug of interest, usually in the µM – nM range. However, none of the available experimental techniques can provide direct intracellular drug concentrations satisfying simultaneously all these requirements.

Solution NMR is a well-established experimental approach for the non-destructive, tag free-quantification of analytes. However, NMR is hindered by low sensitivity, and biased to the study of molecules in the fast motional regime, which is not always the case in the intracellular milieu. Magic-angle spinning dynamic nuclear polarization (MAS DNP) can be applied to overcome these drawbacks, providing higher sensitivity and allowing the detection of immobilized molecules.[1]

Here we present preliminary results showing how MAS DNP can be used for the detection and the quantification of molecules in sub-µM concentration, compatible with intracellular conditions. Our results are a first step towards the use of MAS DNP for the study of intracellular drug chemistry.

References

[1] J. Schlagnitweit, S. Friebe Sandoz, A. Jaworski, I. Guzzetti, F. Aussenac, R. J. Carbajo, E. Chiarparin, A. J. Pell, K. Petzold, *ChemBioChem* **20**, 2474 – 2478 (2019)

07. Materials

STRUCTURAL CHARACTERIZATION OF ULTRA-THIN ALUMINUM OXIDE LAYERS SUPPORTED ON SILICA BY DNP ENHANCED SOLID-STATE NMR SPECTROSCOPY

Monu Kaushik[1], Cesar Leroy[2], David Gajan[1], Zixuan Chen[3], Christoph R. Müller[3], Alexey Fedorov[3], Frank Fayon[1], Christophe Coperet[3], Pierre Florian[2], Anne Lesage[1]

[1] High-Field NMR Center, University of Lyon (CNRS/ENS Lyon/UCB Lyon 1), 69100 Villeurbanne, France.
[2] CNRS, CEMHTI UPR3079, Univ. Orléans, F-45071 Orléans, France.
[3] Department of Chemistry and Applied Sciences, and Department of Mechanical and Process Engineering, ETH Zürich, Switzerland

Dynamic Nuclear Polarization (DNP) enhanced NMR spectroscopy has recently emerged as a versatile approach to investigate the atomic-scale structure of surfaces, opening new analytical opportunities in many areas of material science, including heterogeneous catalysis. In this work, we will present the application of DNP surface enhanced NMR spectroscopy (DNP SENS) to a new family of amorphous silica-alumina (ASA), currently attracting much interest, namely, aluminum oxide layers supported onto silica.

The experiments were carried out on a series of samples prepared by Atomic Layer Deposition (ALD). This approach enables the synthesis of thickness-controlled films whose structure ranges from isolated aluminum sites grafted on a silica support in ASA-like environments to nanometer-sized Al_2O_3 ultra-thin layers.

Firstly, we show how the surface acidity of these catalytic materials can be uniquely probed by ^{15}N DNP SENS using pyridine as a probe molecule to reveal the presence of Lewis and Bronsted acid sites. We will show that the ^{15}N NMR signature of adsorbed pyridine varies with the thickness of aluminum oxide layer. The results will be discussed in light of previous work on alumina[1] and ASA[2] materials. Secondly, we will present ^{27}Al DNP SENS spectra obtained using different pulse sequences under DNP conditions. In particular, we will show that INEPT-based magnetization transfer, recently proposed as an alternative to cross-polarization from ^{1}H to quadrupolar nuclei, significantly enhances ^{27}Al spectral sensitivity[3]. Up to three times sensitivity gain is achieved on the aluminum oxide layers, investigated at a high magnetic field of 18.8 T and fast Magic Angle Spinning (40 kHz). The efficiency of INEPT-based schemes in DNP conditions will be discussed in terms of coherence lifetimes for various catalytic materials.

References

[1] Moroz, I. B.; Larmier, K.; Liao, W.-C.; Copéret, C., Discerning γ-Alumina Surface Sites with Nitrogen-15 Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy of Adsorbed Pyridine. *J. Phys. Chem. C*, **122**, 10871-10882 (2018).
[2] Moroz, I. B.; Lund, A.; Kaushik, M.; Severy, L.; Gajan, D.; Fedorov, A.; Lesage, A.; Coperet, C., Specific Localization of Aluminum Sites Favors Ethene-to-Propene Conversion on (Al)MCM-41-Supported Ni(II) Single Sites. *Acs. Catal.*, ***9***, 7476-7485 (2019).

[3] Raynald G.; Julien T.; Frédérique P; Olivier L; Jean-Paul A., Magnetization transfer from protons to quadrupolar nuclei in solid-state NMR using PRESTO or dipolar-mediated refocused INEPT methods, *J. Magn. Reson.*, **299**, 109-123 (2019).

LOCAL STRUCTURE AND DYNAMICS IN MULTICOMPONENT TIN HALIDE PEROVSKITES FROM TIN-119 SOLID-STATE NMR

Dominik J. Kubicki[1,2], Daniel Prochowicz[4], Elodie Salager[5,6], Aydar Rakhmatullin[5], Clare P. Grey*[2], Lyndon Emsley*[3], Samuel D. Stranks*[1,7]

[1] Dept. of Physics, University of Cambridge, JJ Thomson Avenue, Cambridge, CB3 0HE, UK
[2] Dept. of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK
[3] Laboratory of Magnetic Resonance, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH−1015 Lausanne, Switzerland
[4] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01−224 Warsaw, Poland
[5] Conditions Extrêmes et Matériaux: Haute Température et Irradiation (CEMHTI), UPR 3079 CNRS, Université d'Orléans, 1D Avenue de la Recherche Scientifique, Orléans 45071, France
[6] Réseau sur le Stockage Electrochimique de l'Energie (RS2E), FR 3459 CNRS, 33 Rue Saint Leu, Amiens 80039, France
[7] Dept. of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, Cambridge, CB3 0AS, UK

Organic−inorganic tin(II) halide perovskites have emerged as promising alternatives to lead halide perovskites in optoelectronic applications.[1] While they suffer from considerably poorer performance and stability as compared to their lead analogues, their performance improvements have so far largely been driven by trial and error efforts due to a critical lack of methods to probe their atomic-level microstructure. Solid-state NMR has proven to be perfectly suited for addressing this problem in the context of halide perovskite research.[2–4] Here, I will discuss how we **identified and overcome the challenges associated with the acquisition of solid−state ^{119}Sn MAS NMR** to determine of the local structure of mixed-cation and mixed-halide tin(II) halide perovskites as well as their degradation products.[5] The key experimental consideration is that the longitudinal relaxation of ^{119}Sn in this class of compounds can span 6 orders of magnitude which makes judicious choice of experimental NMR parameters essential for reliable detection of various phases. I will discuss the use of ^{19}Sn to study halide miscibility, degradation pathways of Cs-, MA- and FA-based tin(II) halide materials and show that ^{119}Sn chemical shifts are a sensitive probe of the halide coordination environment as well as of the A-site cation composition. Finally, I will show how to quantify ion dynamics using variable-temperature multi-field relaxation measurements. The comparatively low activation energy for halide transport leads to spontaneous halide homogenization at room temperature whenever two different pure-halide perovskites are put in physical contact.

References

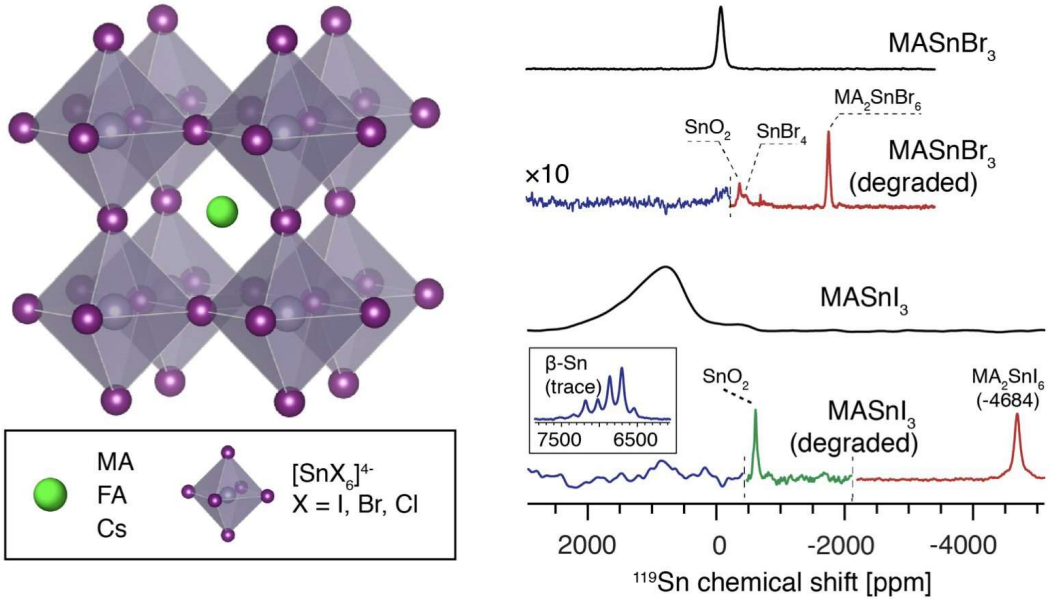
[1] Jena, A. K.; Kulkarni, A.; Miyasaka, T. Halide Perovskite Photovoltaics: Background, Status, and Future Prospects. *Chem. Rev.* **2019**, *119* (5), 3036–3103. https://doi.org/10.1021/acs.chemrev.8b00539.
[2] Rosales, B. A.; Men, L.; Cady, S. D.; Hanrahan, M. P.; Rossini, A. J.; Vela, Javier. Persistent Dopants and Phase Segregation in Organolead Mixed-Halide Perovskites. *Chem. Mater.* **2016**, *28*, 6848–6859.

https://doi.org/10.1021/acs.chemmater.6b01874.

[3] Kubicki, D. J.; Prochowicz, D.; Hofstetter, A.; Zakeeruddin, S. M.; Grätzel, M.; Emsley, L. Phase Segregation in Cs-, Rb- and K-Doped Mixed-Cation (MA)x(FA)1–XPbI3 Hybrid Perovskites from Solid- State NMR. *J. Am. Chem. Soc.* **2017**, *139* (40), 14173–14180. https://doi.org/10.1021/jacs.7b07223.

[4] Franssen, W. M. J.; Kentgens, A. P. M. Solid–State NMR of Hybrid Halide Perovskites. *Solid State Nuclear Magnetic Resonance* **2019**, *100*, 36–44. https://doi.org/10.1016/j.ssnmr.2019.03.005.

[5] Kubicki, D. J.; Prochowicz, D.; Salager, E.; Rakhmatullin, A.; Grey, C. P.; Emsley, L.; Stranks, S. D. Local Structure and Dynamics in Methylammonium, Formamidinium, and Cesium Tin(II) Mixed-Halide Perovskites from 119Sn Solid-State NMR. *J. Am. Chem. Soc.* **2020**, *142* (17), 7813–7826. https://doi.org/10.1021/jacs.0c00647.



NUCLEAR SPIN CROSSOVER IN DENSE MOLECULAR HYDROGEN

Thomas Meier[1], Dominique Laniel[2], Miriam Pena-Alvarez[3], Florian Trybel[1], Saiana Khandarkhaeva[1], Alena Krupp1, Jeroen Jacobs[4], Natalia Dubrovinskaia[2], Leonid Dubrovinsky[1]

[1] Bayerisches Geoinstitut, University of Bayreuth, Bayreuth, Germany
[2] Material Physics and Technology at Extreme Conditions, Laboratory of Crystallography, University of Bayreuth, Bayreuth, Germany
[3] Centre for Science at Extreme Conditions and School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom
[4] European Synchrotron Radiation Facility (ESRF), Grenoble Cedex, France

The laws of quantum mechanics are often tested against the behaviour of the lightest element in the periodic table, hydrogen. One of the most striking properties of molecular hydrogen is the coupling between molecular rotational properties and nuclear spin orientations, giving rise to the spin isomers ortho- and para-hydrogen. At high pressure, as intermolecular interactions increase significantly, the free rotation of H_2 molecules is increasingly hindered, and consequently a modification of the coupling between molecular rotational properties and the nuclear spin system can be anticipated. To date, high-pressure experimental methods have not been able to observe nuclear spin states at pressures approaching 100 GPa and consequently the effect of high pressure on the nuclear spin statistics could not be directly measured. Here, we present *in-situ* high-pressure nuclear magnetic resonance data on molecular hydrogen in its hexagonal phase I up to 123 GPa at room temperature. While our measurements confirm the presence of I=1 ortho-hydrogen at low pressures, above 70 GPa, where inter- and intramolecular distances become comparable, we observe a crossover in the nuclear spin statistics from a spin-1 quadrupolar to a spin-1/2 dipolar system, evidencing the loss of spin isomer distinction. These observations represent a unique case of a nuclear spin crossover phenomenon in quantum solids.[1]

References

[1] Meier, T., Laniel, D., Pena-Alvarez, M., Trybel, F., Khandarkhaeva, S., Krupp, A., Jacobs, J., Dubrovinskaia, N., & Dubrovinsky, L., *Nuclear Spin Crossover in Dense Molecular Hydrogen*. arxiv: 2007.12436, (**2020**).

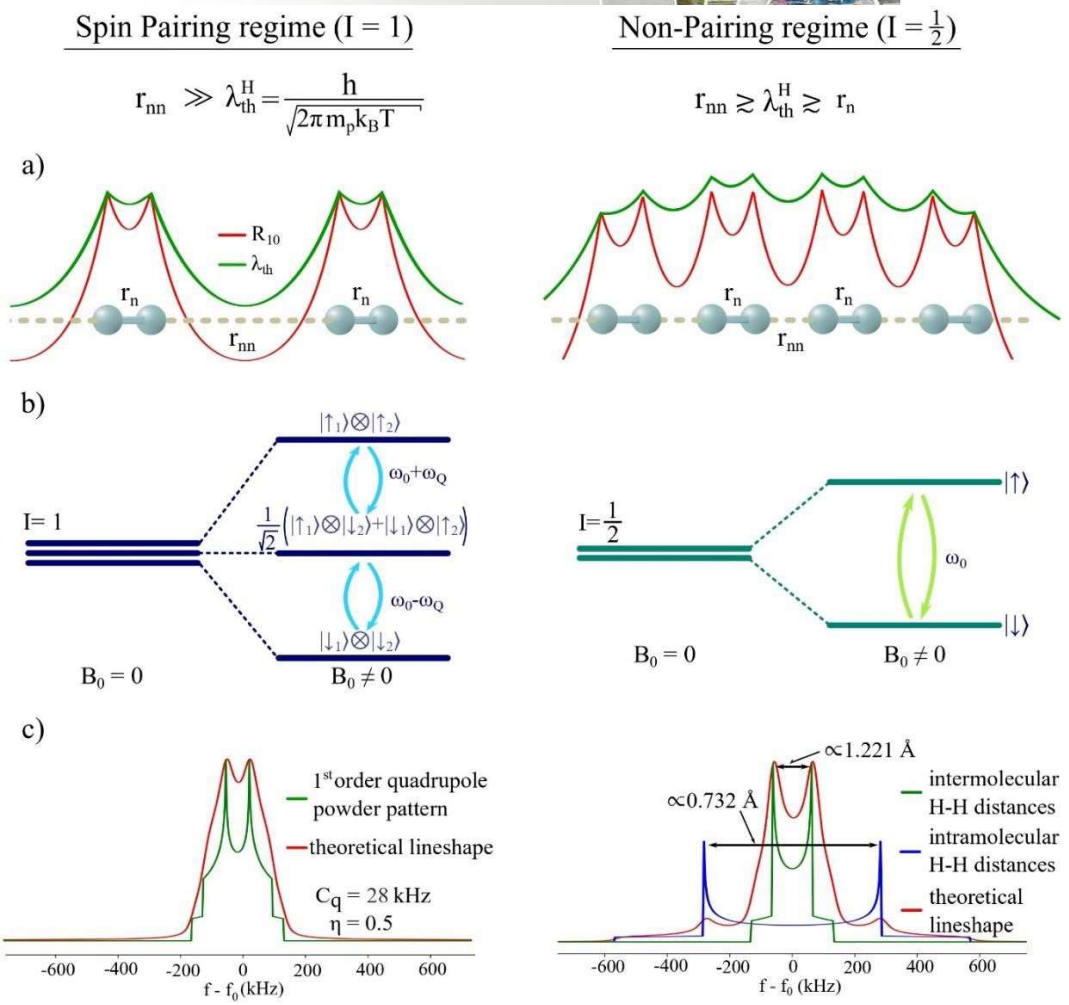


Figure 1. Overview of both spin-pairing and non-pairing regimes. a) Schematic representation of the wave function overlap (red) and the thermal de-Broglie wavelengths (green) of H_2 molecules. b) Schematic representation of the nuclear spin energy levels under the influence of an external magnetic field B_0 for the pairing (i.e. quadrupole interaction) and non- pairing (dipole-dipole interaction) regimes. c) Theoretical line shapes in the pairing and non-pairing regime.

FERROMAGNETIC NMR FOR THE STUDY OF COBALT NANOWIRES AND NANOPARTICLE ASSEMBLIES

Pascal Scholzen[1], d'Espinose de Lacaillerie Jean-Baptiste[1]

[1]Soft Matter Science and Engineering laboratory (SIMM), ESPCI Paris, UMR CNRS 7615, Université PSL, 10 rue Vauquelin Paris 75005, France

An old but little-known NMR technique is ferromagnetic NMR (F-NMR), also called internal field (IF) NMR. In contrast to classical NMR techniques no constant external field is needed, instead, the internal hyperfine field present in ferromagnetic compounds is used. This approach is suitable for the analysis of different nuclei in ferromagnets, but ^{59}Co is particularly responsive to this method. While in recent years ^{59}Co FNMR has been used in depth for the study of cobalt containing catalytic materials and giant magnetoresistance exhibiting multilayers, the goal of this work is to answer fundamental questions by studying model systems, as well as the application to nanowires and electrode materials of conversion reaction batteries.

F-NMR nicely complements X-ray diffraction and magnetometry for the analysis of cobalt nanowires in order to determine their magnetic and crystalline structure, which can be of great interest when analyzing new recording and storage materials. When studying cobalt nanoparticles, it allows to simultaneously give a sample wide overview of the particle size and structures inside the sample. During the analyses of cobalt nanoparticles inside conversion reaction battery materials, it has been observed in some cases that small size nanoparticles, which are supposed to be superparamagnetic at room temperature, exhibit a ferromagnetic signal. A possible explanation for this increase of the so-called blocking temperature are particle interactions. Consequently, a main objective is the validation of this hypothesis and the better understanding of the magnetic behavior of cobalt nanoparticle assemblies by studying controlled model systems.

ACCELERATING WIDELINE ^{195}Pt SOLID-STATE NMR WITH FAST MAS AND DYNAMIC NUCLEAR POLARIZATION: APPLICATION TO SINGLE-SITE HETEROGENEOUS CATALYSTS

Amrit Venkatesh[1,2], Alicia Lund[3], Lukas Rochlitz[4], Ribal Jabbour[3], Christopher P. Gordon[4], Georges Menzildjian[3], Jasmine Viger-Gravel[5], Pierrick Berruyer[6], David Gajan[3], Christophe Copret[4], Anne Lesage[3], Aaron J. Rossini[1,2]

[1] Iowa State University, Department of Chemistry, Ames, IA, USA, 50011.
[2] US DOE Ames Laboratory, Ames, IA, USA, 50011.
[3] Univ Lyon, ENS Lyon, Université Lyon 1, CNRS, High-Field NMR Center of Lyon, FRE 2034, F-69100 VILLEURBANNE, France.
[4] Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland.
[5] Department of Organic Chemistry, University of Geneva, 1211, Geneva, Switzerland.
[6] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015, Lausanne, Switzerland.

Platinum is widely applied in heterogeneous catalysts. Unfortunately, it is challenging to directly determine the structure and symmetry of Pt atoms in catalysts because the Pt atoms are often dilute and located on the disordered surfaces of inorganic support materials. ^{195}Pt solid-state NMR could be a powerful probe of structure in Pt-based catalysts. However, heavy spin-1/2 nuclei such as ^{195}Pt exhibit large chemical shift anisotropy (CSA), leading to solid-state NMR spectra that are 500 kHz - 1 MHz wide, and resulting in low NMR sensitivity. Recently, 2D ^{1}H{^{195}Pt} constant-time dipolar heteronuclear multiple quantum coherence (CT D-HMQC) was shown to enable the acquisition of wideline ^{195}Pt sideband manifolds using fast magic angle spinning (MAS) and ^{1}H detection.[1] But, due to t_1-noise and other mechanisms that reduce NMR sensitivity, CT D-HMQC is not yet applicable to the study of dilute Pt catalysts.

In this contribution, we jointly apply improved fast MAS ^{1}H detection pulse sequences and dynamic nuclear polarization surface-enhanced NMR spectroscopy (DNP-SENS) for the structure determination of a model single-site silica-supported Pt catalyst with a 3.7 wt. % Pt loading.[2] First, we apply fast MAS t_1-noise eliminated (TONE) D-HMQC[3,4] for the rapid determination of ^{195}Pt isotropic chemical shifts of the molecular and surface compounds. A static ^{195}Pt solid-state NMR spectrum, obtained with dynamic nuclear polarization surface-enhanced NMR spectroscopy (DNP-SENS), allowed measurement of the ^{195}Pt chemical shift anisotropy (CSA). The large CSA confirmed that surface Pt-atoms have a square planar geometry, which agreed well with DFT predictions. Finally, ^{1}H-^{195}Pt distance measurements by S-REDOR (symmetry-based resonance echo double resonance) confirm that ligands remain bound to the Pt center upon grafting. The proposed methods pave the way for the rational design of improved Pt-based heterogeneous catalysts and other Pt containing materials such as zeolites and metal organic frameworks, in the future.

References

[1] Rossini, A. J.; Hanrahan, M. P.; Thuo, M. Rapid Acquisition of Wideline MAS Solid-State NMR Spectra with Fast MAS, Proton Detection, and Dipolar HMQC Pulse Sequences. *Phys. Chem. Chem. Phys.* 2016, ***18*** (36), 25284-25295.

[2] Venkatesh, A.; Lund, A.; Rochlitz, L.; Jabbour, R.; Gordon, C. P.; Menzildjian, G.; Viger-Gravel, J.; Berruyer, P.; Gajan, D.; Copéret, C.; Lesage, A.; Rossini, A. J. The Structure of Molecular and Surface Platinum Sites Determined by DNP-SENS and Fast MAS ^{195}Pt Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* 2020, DOI: 10.1021/jacs.0c09101.

[3] Venkatesh, A.; Luan, X.; Perras, F. A.; Hung, I.; Huang, W.; Rossini, A. J. t_1-Noise Eliminated Dipolar Heteronuclear Multiple-Quantum Coherence Solid-State NMR Spectroscopy. *Phys. Chem. Chem. Phys.* 2020, ***22***, 20815-20828.

[4] Venkatesh, A.; Perras, F. A.; Rossini, A. J. *Unpublished Work*

UNVEILING MOLECULAR INTERACTIONS IN IONIC LIQUIDS: THE NMR APPROACH

Raquel Barrulas[1], Tiago Paiva[1], Marcileia Zanatta[1], Teresa Casimiro[2], Marta Corvo[1]

[1] i3N|Cenimat, Department of Materials Science (DCM), NOVA School of Science and Technology (FCT NOVA), NOVA University Lisbon, 2829-516 Caparica, Portugal.
[2] LAQV-REQUIMTE, Chemistry Department, NOVA School of Science and Technology (FCT NOVA), NOVA University Lisbon, 2829-516 Caparica, Portugal.

Ionic liquids (ILs), organic salts with melting points under 100^{o}C, are used as alternative solvents for extraction of natural compounds and CO_2 capture (CC) since they are stable, highly selective and recyclable.[1-3] The possibility of changing ILs properties, namely solvent polarity, acid/base character, density, viscosity and thermal stability by tuning both cation and anion moiety to meet specific requirements, allows the obtention of improved systems.[1,2,4]

Our Nuclear Magnetic Resonance (NMR) approach avoids a laborious process of trial and error, through the study of molecular interactions between ILs and model compounds to tailor IL derived materials. The diffusion and Nuclear Overhauser effect (NOE) experiments provide us the molecular interactions profile to understand the relation between the solvated species and ILs and allow the selection and optimization of the IL's cation/anion identity.[4] This methodology allows the rational development of alternative solvents for polyphenol compounds, CC and biopolymer dissolution, also it may easily be generalized to several materials and applications.[1,2,5]

References

[1] M. C. Corvo *et al*., A Rational Approach to CO2 Capture by Imidazolium Ionic Liquids: Tuning CO2 Solubility by Cation Alkyl Branching. *Chem.Sus.Chem.*, **8**, 1935-1946 (2015).
[2] R. V. Barrulas *et al*., NMR Methodology for a Rational Selection of Ionic Liquids: Extracting Polyphenols. *Sep. Purif. Technol*., **221**, 29-37 (2019).
[3] B. Sreenivasulu *et al*., A journey into the process and engineering aspects of carbon capture technologies, *Renew. Sustain. Energy Rev.*, **41**, 1324–1350 (2015).
[4] M. M. Lopes *et al*., Molecular Interactions in Ionic Liquids: The NMR Contribution towards Tailored Solvents, *IntechOpen*, (2019).
[5] T. Paiva *et al*., On the influence of imidazolium ionic liquids on cellulose derived polymers. *Eur. Polym. J.*, **114**, 353-360 (2019).

Acknowledgments

This work was funded by National Funds through FCT – Portuguese Foundation for Science and Technology, reference UIDB/50025/2020-2023 and PTDC/QUI-QFI/31508/2017, POR Lisboa and PTNMR (ROTEIRO/0031/2013; PINFRA/22161/2016), co-financed by ERDF through COMPETE 2020, Portugal, POCI and

PORL and FCT through PIDDAC (POCI-01-0145-FEDER-007688). LAQV is financed by national funds from FCT/ MCTES (UID/QUI/50006/2019) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER- 007265). R.B. acknowledges FCT for the SFRH/BD/150662/2020 PhD fellowship. T.P. acknowledges FCT for the SFRH/BD/133447/2017 PhD fellowship. M.C. gratefully acknowledges PTNMR for the researcher contract.

CHALLENGING THE ASSIGNMENT OF ^{31}P NMR RESONANCES FOR THE INTERACTION OF TRIMETHYLPHOSPHINE OXIDE WITH ZEOLITE ACID SITES

Carlos Bornes[1], Jeffrey A. Amelse[1], Carlos F. G. C. Geraldes[2,3], João Rocha[1], Luís Mafra[1]

[1]CICECO, Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[2]Department of Life Sciences and Coimbra Chemistry Center, Faculty of Science and Technology, University of Coimbra, 3000-393 Coimbra, Portugal
[3]CIBIT/ICNAS - Instituto de Ciências Nucleares Aplicadas à Saúde, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

Trimethylphosphine oxide (TMPO) has been suggested as a reliable and practical molecular probe to study the acid-base interaction with zeolite acid sites, providing information about acid properties such as site nature (Brønsted or Lewis), amount, accessibility and strength, which play a major role on predicting the overall catalytic activity of zeolites.[1] TMPO is an attractive molecular probe for NMR studies because it has 100% natural abundant nuclei (^{31}P) with a wide chemical shift range, allowing an easy identification of TMPO's interactions.

Most literature reports state that i) the interaction with Brønsted and Lewis acid sites result in ^{31}P resonances spread over distinct chemical shift range and ii) the ^{31}P chemical shifts is linearly correlated with the strength of the Brønsted acid sites. Our recent work shows that this may not be the case and that the interpretation of 1D ^{31}P NMR spectra of TMPO-loaded zeolites is quite inconsistent across the literature. 2D ^{1}H–^{31}P heteronuclear correlation (HETCOR) NMR of TMPO-adsorbed zeolites, in tandem with DFT calculations, challenges previous assignments based on 1D ^{31}P NMR.[3] Data enabled an unambiguous discrimination of Brønsted and Lewis acid sites, and extended our understanding of TMPO:Brønsted complexes formed and the proton-transfer mechanism. We show that 31P and 1H chemical shifts of TMPO-loaded zeolites encode information beyond acid site strength and nature (Brønsted or Lewis), providing information about the stabilization of neutral (TMPO···ZeoH) and charged ($TMPOH^+\cdots Zeo^-$ and $(TMPO)_2H^+\cdots Zeo^-$) ion pairs, whose geometry and amount depends on the pore structure/volume. The formation of higher-order complexes in TMPO-loaded zeolites raises questions regarding studies using TMPO and other probe molecules where similar complexes may form, which may lead to misinterpretation of NMR data.

References

[1] A. Zheng, S.-B. Liu, F. Deng, Chem. Rev. 2017, **117**, 12475–12531.
[2] M. Boronat, A. Corma, ACS Catal. 2019, **9**, 1539–1548.
[3] C. Bornes, M. Sardo, Z. Lin, J. Amelse, A. Fernandes, M. F. Ribeiro, C. Geraldes, J. Rocha, L. Mafra, Chem. Commun. 2019, **55**, 12635–12638.

SYNTHESIS, CHARACTERIZATION, AND MAGNETIC PROPERTIES OF $MAl_4(OH)_{12}SO_4 \cdot 3H_2O$ WITH M = Co^{2+}, Ni^{2+} AND Cu^{2+}: A NOVEL CLASS OF 1D SPIN CHAINS

Anders B. A. Andersen[1*], Rasmus Tang[2], Sofie Janas[2], Nicolai D. Skjold[1], Dorthe Bomholdt Ravnsbæk[1], Lars Pilsgaard Hansen[3], Juha Vaara[4], Kim Lefmann[2], Ulla Gro Nielsen[1]

[1] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense
[2] Nano-Science Center, Niels Bohr Institute, University of Copenhagen, Denmark
[3] Haldor Topsøe A/S, Kgs. Lyngby, Denmark.
[4] NMR Research Unit, University of Oulu, Finland
*E-mail: andersbaa@sdu.dk

Low-dimensional spin systems (1D and 2D) are of great interest due to their pronounced quantum behavior. 1D spin chains may potentially exist in the so-called MAl_4-LDH, which has the general formula of $[M^{2+}Al_4(OH)_{12}A_{2/x}{}^{x-} \cdot 3H_2O]$, based on their crystal structure (Fig 1a).[1,2] The MAl_4-LDH are obtained by insertion of divalent cations into half the vacant sites in aluminum hydroxide, $Al(OH)_3$, and several different M(II) ions can be incorporated, albeit synthesis of pure MAl4-LDH is challenging.[1,2] After extensive synthesis optimization, we have successfully obtained isostructural MAl_4-LDH with Co^{2+} (d^7, S=3/2), Ni^{2+} (d^8, S=1), and Cu^{2+} (d^9, S=1/2). This allowed for detailed studies on how the choice of magnetic ion (electron spin, S) affects the magnetic properties. The hyperfine shift $\delta_{iso}(^{27}Al)$ of the MAl_4-LDH in ^{27}Al MAS NMR show the local magnetic order and give hints to the low-temperature magnetic order. This is due to the $\delta(^{27}Al)$- ranges which are 5 to -130 ppm for Co^{2+} (S=3/2), -240 to -580 ppm for Ni^{2+} (S=1), and -70 to -300 ppm for Cu^{2+} (S=1/2), i.e., Co^{2+} has the largest number of unpaired electrons but the lowest hyperfine shift in $\delta(^{27}Al)$, hinting towards a local antiferromagnetic coupling. In contrast, the Ni^{2+} and Cu^{2+} exhibit stronger magnetic interactions despite fewer unpaired electrons, hinting towards local ferromagnetic couplings. A key feature of 1D-spin chains is weak inter- and intra- chain interactions at low temperatures, which is observed below ca. 30 K by studying C_p and χ from room temperature down to a few Kelvin. These observations were combined with DFT calculations of the exchange couplings and provide us a valuable insight into the magnetic properties of MAl_4-LDH.

References

[1]E. Sokolova *et al*, Can. Mineral. **43**, 1511-1519 (2005).
[2]N. D. Jensen *et al*, Inorg. Chem. **58**, 6114-6122 (2019).

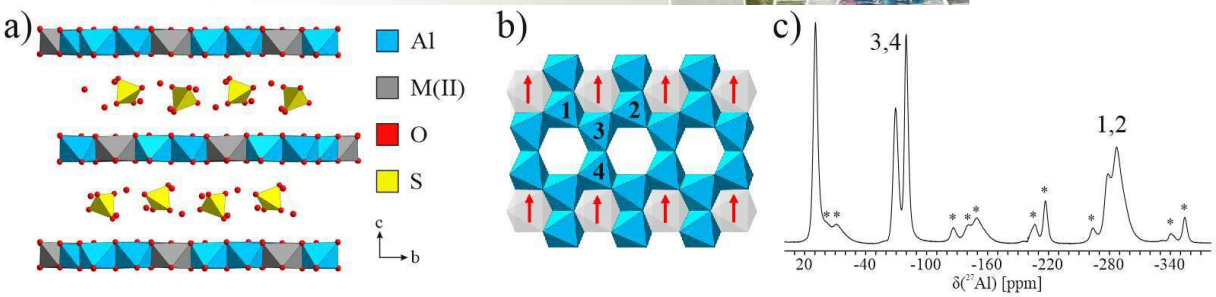


Figure 1. a) Crystal structure of MAl_4-LDH, b) 1D-spin chains, c) ^{27}Al MAS NMR of $CuAl_4$-LDH, spinning sidebands marked with *.

NATURAL ABUNDANCE OXYGEN-17 SOLID-STATE NMR OF METAL ORGANIC FRAMEWORKS ENHANCED BY DYNAMIC NUCLEAR POLARIZATION

Diego Carnevale[1],Georges Mouchaham[2], Sujing Wang[2], Mathieu Baudin[1,3], Christian Serre[2], Geoffrey Bodenhausen[1], Daniel Abergel[1]

[1] Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France.
[2] Institut des Matériaux Poreux de Paris, Ecole Normale Supérieure, ESPCI Paris, CNRS, PSL University, 75005 Paris, France.
[3] Université de Paris, Laboratoire de Chimie et Biologie Pharmacologiques et Toxicologiques, CNRS UMR 8601, Université Paris Descartes, 45 rue des Saints Pères 75006 Paris France.

We present natural abundance ^{17}O spectra obtained through DNP-enhanced MAS NMR experiments of a mesoporous Zr-oxo-cluster carboxylate-based MOF.[1] The ^{1}H nuclei are hyperpolarized by DNP prior to cross-polarization from ^{1}H to ^{17}O to obtain the sensitivity required to detect different ^{17}O sites in the material. Moreover, the use of DFT methods to calculate the chemical shielding anisotropy (CSA) and electric-field gradient (EFG) tensors allowed us to identify and assign all ^{17}O environments. Our observations prove that the ^{17}O sites are very sensitive to local structure and feature characteristic lineshapes for different chemical environments.

References

[1] S. Wang, L. Chen, M. Wahiduzzaman, A.Tissot, L. Zhou, I. A. Ibarra, A. Gutiérrez-Alejandre, J. S. Lee, J.- S Chang, Z. Liu, J. Marrot, W. Shepard, G. Maurin, Q. Xu, C. Serre. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.12661310.v1.

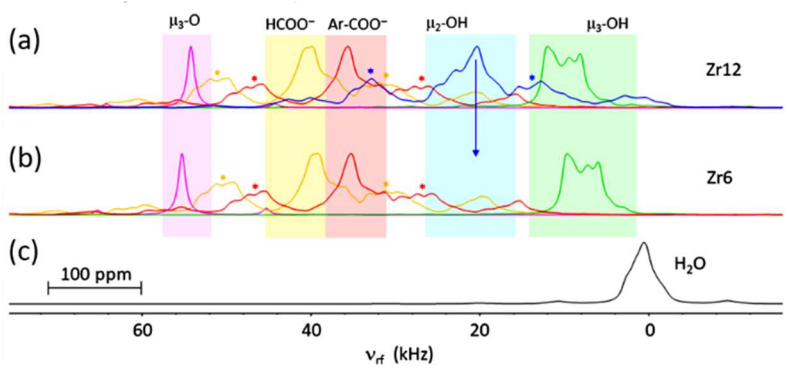


Figure 1. (a) Superposition of simulated ^{17}O MAS spectra for the 6 ^{17}O sites of the Zr12-oxo-cluster assuming NMR parameters calculated with DFT methods. (b) Spectra analogous to (a) for the Zr6-oxo-cluster. (c) Simulated signal of $H_2{}^{17}O$ in DNP juice. The signal of the bridging μ_2-OH sites that are present in Zr12-oxo- cluster (I) but absent in Zr6-oxo-cluster (II) is highlighted by a vertical blue arrow.

UNRAVELLING THE CATION MICROSTRUCTURE IN MIXED MA/FA LEAD HALIDE PEROVSKITES

Helen Grüninger[1], Menno Bokdam[2a,b], Nico Leupold[3], Paul Tinnemans[1], Ralf Moos[3], Gilles A. de Wijs[1], Fabian Panzer[3,4], Arno P.M. Kentgens[1]

[1] Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands.
[2a] Faculty of Physics and Center for Computational Materials Sciences, University of Vienna, Sensengasse 8/12, 1090 Vienna, Austria.
[2b] Faculty of Science and Technology and MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands.
[3] Department of Functional Materials, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany.
[4] Soft Matter Optoelectronics, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany.

Lead halide perovskites have attracted great interest due to their possible application in solar cells, providing excellent power conversion efficiencies (PCE) of currently up to 25.5 % combined with simple and low-cost production procedures. Recent developments in the field of high efficient perovskite solar cells are based on stabilization of the perovskite crystal structure of $FAPbI_3$, while preserving its excellent optoelectronic properties. Compositional engineering of e.g. MA or Br ions mixed into the $FAPbI_3$ structure results in the desired effects. However, detailed knowledge of local structural features, such as local (dis)order or cation interactions of formamidinium (FA^+) and methylammonium (MA^+) is still limited, but crucial for further optimization of the PCE. Here, we elucidate the microscopic distribution of MA^+ and FA^+-ions in mixed perovskites $MA_{1-x}FA_xPbI_3$, and $FA_{0.85}MA_{0.15}PbI_{2.55}Br_{0.45}$, by combining high-resolution double-quantum ^1H solid-state nuclear magnetic resonance (NMR) spectroscopy with state-of-the-art near-first principles molecular dynamics (MD) simulations using machine-learning force-fields (MLFF). We show that on the scale of a few nearest-neighbor coordinations, partial MA and FA clustering takes place over the whole MA/FA compositional range. While similar MA and FA ordering as in the $MA_{1-x}FA_xPbI_3$ systems was observed for $FA_{0.85}MA_{0.15}PbI_{2.55}Br_{0.45}$, the average cation-cation interaction strength increased significantly indicating a restriction or partial immobilization of the cation dynamics upon Br^- incorporation. Our results shed light on the heterogeneities in composition due to the cation microstructure of mixed halide perovskites, helping to further exploit their full optoelectronic potential.

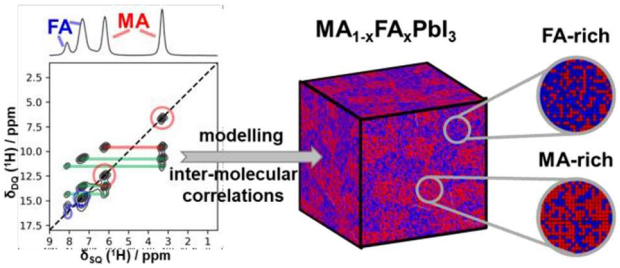


Figure 1. 2D ^{1}H-^{1}H DQSQ NMR spectra in combination with MLFF MD simulations allow for a quantitative analysis of ^{1}H-^{1}H correlations between MA and FA cations in mixed $MA_{1-x}FA_xPbI_3$ systems. Our results show that partial MA and FA clustering takes place causing local heterogeneities in the perovskite composition, i.e. MA- and FA-rich domains.

NANOSCALE PHASE SEGREGATION IN SUPRAMOLECULAR π-TEMPLATING FOR HYBRID PEROVSKITE PHOTOVOLTAICS FROM NMR CRYSTALLOGRAPHY

Aditya Mishra[1], Michael A. Hope[1], Manuel Cordova[1], Toru Nakamura[2], Paramvir Ahlawat[3], Dominik J. Kubicki[1], Farzaneh Jahanbakhshi[3], Marko Mladenović[3], Ursula Roethlisberger[3], Jovana V. Millć[2], Michael Grätzel[2], Lyndon Emsley[1]

[1] Laboratory of Magnetic Resonance, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland.
[2] Laboratory of Photonics and Interfaces, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland.
[3] Laboratory of Computational Chemistry and Biochemistry, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland.

Photovoltaics based on metal-halide perovskites (e.g. $FAPbI_3$: FA^+ = Formamidinium ion) have demonstrated optoelectronic properties which are already providing solar conversion efficiencies exceeding 25%. However, their instability under operating conditions remains a significant challenge for commercialization. To improve stability, layered perovskites have been the subject of recent intense research.[1,2] Layered perovskites incorporate an organic spacer layer between $[PbI_6]^{4-}$ perovskite slabs. The organic spacer structure must be optimized to control the properties of the resulting material. For this the organic spacer structure must be determined. This is a significant challenge for diffraction methods due to the polycrystallinity of samples and the presence of heavy atoms which obscure the signatures from the organic spacer molecules. NMR, on the other hand, as a local element-specific probe is the perfect tool to study the organic spacers. In particular, by combining NMR with chemical shift calculations and molecular dynamics simulations, NMR crystallography methods allow the supramolecular structure to be determined.

Here we employ multinuclear (^1H, ^{19}F, and ^{13}C) solid-state NMR to reveal the atomic-scale molecular structure of layered hybrid perovskites[2,3,4]. We focus on a class of layered perovskites based on $A_2FA_{n-1}Pb_nI_{3n+1}$ compositions comprising of Ruddlesden–Popper phases, with n layers of $[PbI_6]^{4-}$ octahedra within each perovskite slab, where the spacer (A^+) could be either phenyl-ethyl ammonium (PEA^+), pentafluorophenylethylammonium (FEA^+), or their 1:1 mixture. Cross-polarization and 2D HETCOR experiments reveal the proximity of the two aromatic units, and the probable structure for the spacer layer is revealed by NMR crystallography. We show that in contrast to the expected templating effect, the PEA^+ and FEA^+ units form nanoscale phase segregated domains. Overall, we show how NMR crystallography enables determining the supramolecular structure of layered hybrid perovskites, thereby advancing properties and applications of hybrid materials.

References

[1] Smith, I. C.Hoke, E. T.Solis-Ibarra, D.McGehee, M. D.Karunadasa, H. I. Angew. Chem., Int. Ed.,**2014**, 53, 11232.
[2] Ruiz-Preciado, Marco A.,Kubicki D.J.,Hofstetter A., McGovern L.,Futscher H.,Ummadisingu A., Gershoni R.,Zakeeruddin SM. Ehrler, Bruno Emsley L., Milić, J., Grätzel M. J. Am. Chem. Soc. **2019**, 142, 1645.

[3] Alanazi, A. Q.; Kubicki, D. J.; Prochowicz, D.; Alharbi, E. A.; Bouduban, M. E. F.; Jahanbakhshi, F.; Mladenović, M.; Milić, J. V.; Giordano, F.; Ren, D.; Alyamani, A. Y.; Albrithen, H.; Albadri, A.; Alotaibi, M. H.; Moser, J.-E.; Zakeeruddin, S. M.; Rothlisberger, U.; Emsley, L.; Grätzel, M. J. Am. Chem. Soc. 2019, 141, 17659.
[4] Bi, D.; Li, X.; Milić, J. V.; Kubicki, D. J.; Pellet, N.; Luo, J.; LaGrange, T.; Mettraux, P.; Emsley, L.; Zakeeruddin, S. M.; Grätzel, M. *Nat. Commun.* **2018**, *9*, 4482.

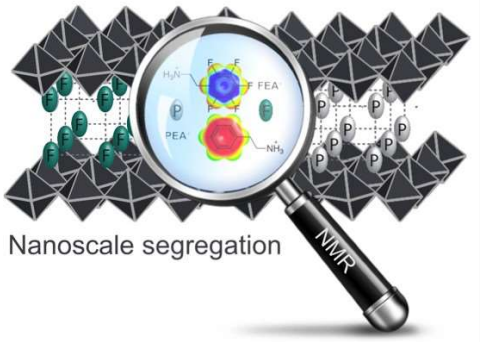


Figure 1. Schematic representation of layered hybrid perovskites undergoing nano-scale phase-segregation.

ZN INCORPORATION IN SYNTHETIC C-S-H AND ITS EFFECT ON CEMENT HYDRATION THROUGH DNP ENHANCED SOLID STATE NMR

Anna Morales-Melgares[1,2], Pinelopi Moutzouri[1], Paul Bowen[2], Karen Scrivener[2], Lyndon Emsley[1]

[1] Laboratory of Magnetic Resonance (LRM), École Fédérale Polytechnique de Lausanne (EPFL), Lausanne, Switzerland
[2] Laboratory of Construction Materials (LMC), EPFL, Lausanne, Switzerland

The demand of concrete-based infrastructures worldwide is very high and is expected to keep increasing in the next decades. As a result, the contribution of CO_2 emissions from cement production are expected to increase. One of the possible solutions is the partial substitution of the clinker by Supplementary Cementitious Materials (SCMs). These are materials which production does not imply the formation of CO_2, but tend to lower the early-age strength of Portland cement. Therefore, a fair tradeoff between clinker and SCMs must always be met.

It has recently been demonstrated that the addition of minor elements (i.e Zn) can enhance the mechanical strength of a clinker and that, presumably, this enhanced characteristic is acquired during the hydration of the anhydrous cement blend. But the hydration process of a concrete is extremely complex. Therefore, the kinetics and the thermodynamics of cement hydration are difficult to study in real systems.

The goal of this project is to understand the role of Zn in synthetic single-phase C-S-H. It is important to study pure C-S-H, synthesized in a controlled and isolated system, in order to reduce the number of experimental variables and to be able to understand the main product of hydration in isolation. The study of these systems implies their synthesis and their characterization through solid state Nuclear Magnetic Resonance (NMR), since chemical analysis from NMR is the most promising technique to help elucidate the role and the position of Zn in the C-S-H structure at the atomic level. Cross-polarization (CP) and INADEQUATE MAS NMR experiments have been performed on C-S-H samples containing different concentrations of Zn and have helped us develop a hypothesis on the exact position of this element in the C-S-H matrix.

References

[1] Kumar, Abhishek, et al. "The atomic-level structure of cementitious calcium silicate hydrate." *The Journal of Physical Chemistry C* 121.32 (2017): 17188-17196.
[2] Kunhi Mohamed, Aslam, et al. "The Atomic-Level Structure of Cementitious Calcium Aluminate Silicate Hydrate." *Journal of the American Chemical Society* (2020).

NMR-BASED APPROACH FOR THE DEVELOPMENT OF POLYMERIC DEEP EUTECTIC SYSTEMS (PRIDES) FOR CO_2 CAPTURE

Débora Nunes[1], Marta C. Corvo[2], Ana S. D. Ferreira[1]

[1] UCIBIO, Chemistry Department, NOVA School of Science and Technology (FCT NOVA), NOVA University Lisbon, 2829-516 Caparica, Portugal.
[2] i3N|Cenimat, Department of Materials Science (DCM), NOVA School of Science and Technology (FCT NOVA), NOVA University Lisbon, 2829-516 Caparica, Portugal.

Global warming is one of the most important and time-sensitive problems Mankind faces today. More than ever an aggressive approach to reduce the anthropogenic CO_2 in the atmosphere is imperative. Higher-performance sustainable CO_2 capture systems, particularly for near atmospheric pressure, are targeted by the scientific community. Deep Eutectic systems (DES) have emerged as sustainable ionic liquid analogs, sharing IL properties but having lower cost, easier-to-prepare and lower toxicity, showing also promising results towards CO_2 capture (CC). Opposite to ILs which are single compounds, DES are formed by combining at a specific ratio two or more compounds, through complexation of hydrogen bond donor/ acceptor. Their name arises from the steep decrease in the melting point compared to their starting molecular components, due to the low lattice energy arising from the charge delocalization that occurs through the hydrogen bond.

Polymeric Deep Eutectic Systems (PRIDES) combine the peculiar features of DES with the complexity and the mechanical properties of a polymeric framework, which boosts the system CO_2 absorption ability.

In our work, we present choline chloride-based PRIDES and their CO_2 capture performance. Nuclear Magnetic Resonance (NMR) techniques enable us to study and quantify *in-situ* of CO_2 allowing a rational approach towards the rational design of these new systems.

References

[1] M. C. Corvo, J. Sardinha *et al. ChemSusChem*. 8, 1935–1946 (2015).
[2]N. M. Simon, M. Zanatta *et al.*, *ChemSusChem*, 10, 4927-4933 (2017).
[3]A.R.C. Duarte, A.S.D. Ferreira *et al.*, *Eur. J. Pharm. Biopharm.*, **114**, 296-304 (2017).
[4] A. Krishnan, K.P. Gopinath *et al.*, *Environ Chem Lett*, 18, 2031–2054 (2020).

Acknowledgment

This work was funded by National Funds through FCT – Portuguese Foundation for Science and Technology, reference UIDB/50025/2020-2023 and PTDC/QUI-QFI/31508/2017, POR Lisboa and PTNMR (ROTEIRO/0031/2013; PINFRA/22161/2016), co-financed by ERDF through COMPETE 2020, Portugal, POCI and PORL and FCT through PIDDAC (POCI-01-0145-FEDER-007688). M.C. gratefully acknowledges PTNMR for the researcher contract. This work was supported by the Applied Molecular Biosciences Unit – UCIBIO which is financed by national funds from FCT (UIDB/04378/2020).

BOND LABILITY AND INSTABILITY IN ZEOLITE FRAMEWORKS UNDER AQUEOUS CONDITIONS EVIDENCED BY ^{17}O NMR SPECTROSCOPY

Cameron M. Rice[1], Daniel M. Dawson[1], Russell E. Morris[1,2], Sharon E. Ashbrook[1]

[1] EaStCHEM and School of Chemistry, University of St Andrews, Fife, KY16 9ST, United Kingdom.
[2] Department of Physical and Macromolecular Chemistry, Faculty of Sciences, Charles University, Hlavova 8, 128 43 Prague 2, Czech Republic.

Zeolites are the most widely used inorganic supports in industry and of particular value is their tolerance to harsh operating conditions, particularly in processes where nucleophiles, such as water, are involved.[1,2] Although their metastable frameworks display high stability, they are known to be dynamic and several, sometimes advantageous, cases of zeolite framework instability exploited by water, have been reported.[3] The reactivity of these zeolites with water is often characterized by a change in long-range order of the material, evidenced by X-ray diffraction.

Recently, a more widespread instability of zeolite frameworks under aqueous conditions has been reported, evidenced by solid-state NMR spectroscopy.[4,5] A sensitive probe of local structure, NMR shows changes to the local geometry and bonding in aluminosilicate zeolites, caused by the action of water. A particular nucleus of interest here is ^{17}O; the NMR-active isotope of oxygen. ^{17}O NMR is challenging owing to its extremely low natural abundance (0.037%), moderate gyromagnetic ratio and quadrupolar (I = 5/2) spin. However, the prevalence of oxygen in zeolites and the availability of ^{17}O- enriched reagents provide an opportunity to track how water interacts with zeolite frameworks and the effect this has on framework oxygen species. By exposing aluminosilicate zeolites to small quantities of $H_2{}^{17}O$ and studying their interaction *in-situ* using ^{17}O NMR we have observed rapid ^{17}O enrichment of framework Si-O-Si and Si-O-Al linkages in zeolites, without framework degradation.

This experimentally observed bond lability for a protonated chabazite has been predicted to proceed *via* a hydrogen-bonded chain of water molecules, with energetic barriers to bond cleavage as low as ~30 kJmol^{-1}.[4] The present work explores and extends the general applicability of this mechanism, by investigating the effect that countercation substitution, silicon to aluminium ratio, prevalence of defects and framework history has on the rate and extent of this surprising oxygen exchange.

References

[1] P. A. Wright, *Microporous Framework Solids*, Royal Society of Chemistry, Cambridge, 2008.
[2] L. Zhang, K. Chen, B. Chen, J. L. White and D. E. Resasco, *J. Am. Chem. Soc.*, 2015, **137**, 11810-11819.
[3] C. J. Heard, L. Grajciar, F. Uhlík, M. Shamzhy, M. Opanasenko, J. Čejka and P. Nachtigall, *Adv. Mater.*, 2020, *in press*, 10.1002/adma.202003264.
[4] C. J. Heard, L. Grajciar, C. M. Rice, S. M. Pugh, P. Nachtigall, S. E. Ashbrook and R. E. Morris, *Nat. Commun.*, 2019, **10**, 4690-4697.
[5] S. M. Pugh, P. A. Wright, D. J. Law, N. Thompson and S. E. Ashbrook, *J. Am. Chem. Soc.*, 2020, **142**, 900- 906.

POLYMER CHAIN DYNAMICS UNDER SHEAR – A RHEOLOGICAL NMR STUDY

Benjamin Kohn[1], Vincent Körber[1], Enno Stündel[1], Petrik Galvosas[2], Ulrich Scheler[1]

[1] Leibniz-Institut für Polymerforschung Dresden e.V. Hohe Str. 6, 01069 Dresden, Germany
[2] School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand

Rheological NMR is the in-situ combination of NMR with external mechanical shear. Here we report on the investigation of the effect of shear in a Searle cell on the chain dynamics of an entangled polymer. The NMR transverse relaxation T_2 is sensitive to the slow motion of polymer chain segments. In entangled polymers at least two components are observed, a short one for chain segments with restricted motion due to entanglements and a longer component for the more free chain segments. For short polymer chains little effect of shear in a Searle cell is observed while for high molecular weight with clear signs of entanglement both components become longer. This is indicative of longer chain segments between entanglements, which could result from loss of entanglements or rearrangement resulting in longer chain segments between entanglements[1].
A new experimental setup permits measuring NMR relaxation under oscillatory shear varying both amplitude and frequency. The resulting strain-rate dependence shows that the prolongation of the relaxation time indicating longer chain segments between entanglements starts at a strain of at least 500 for entangled PDMS. The effect requires both a minimal deformation and a minimal frequency. When the prolonged T_2 is observed apparently the fraction of the chain segments and thus the signal intensity contributing the short T_2 assigned to the restricted motion decreases. This shows a loss of entanglements.
The combination of PFG NMR with NMR imaging in the same setup allows to measure flow pattern. At the turning point of the oscillation for low-viscosity fluids a counterflow is observed.

References

[1] Brigitte Wiesner, Benjamin Kohn, Mandy Mende, Ulrich Scheler Polymer Chain Mobility under Shear—A Rheo-NMR Investigation, Polymer **10(11)**, 1231; https://doi.org/10.3390/polym10111231 (2018).

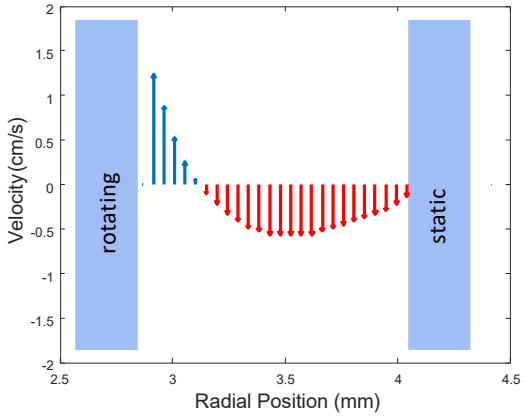


Figure 1. Flow profile in the Searle cell after turning in the oscillatory shear showing the counterflow effect.

RATIONALIZING MATERIALS FOR CO_2 CAPTURE THROUGH NMR ANALYSIS

Marcileia Zanatta[1], Raquel V. Barrulas[1], Tiago G. Paiva[1], Marta C. Corvo[1]

[1] LabNMR Cenimat|i3N, Department of Materials Science (DCM), NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal. (m.zanatta@fct.unl.pt)

The mitigation of climate change effects requires the use of alternative materials and technologies, to control CO_2 atmospheric levels through its capture, storage and use. Traditionally, aqueous alkanolamines solutions are widely used for CO_2 removal industrially. However, there are some drawbacks of this process causing environmental and economic problems. Ionic liquids (ILs) have attracted much attention in the field of CO_2 capture (CC) due to their gas transport properties and negligible vapor pressure. ILs are multifunctional materials, composed entirely of ions, that present the possibility to modulate their physical and chemical properties by changing the combination of cation and anion. With the appropriate IL structure is possible to create a tailored material that present elevated sorption capacity. Nuclear Magnetic Resonance (NMR) is a powerful technique to observe the CO_2-IL interaction *in-situ*, quantify the sorption capacity and evaluate the sorption mechanism. In this context, in the last years, our group has been working in the development of NMR protocols for CC in high-pressure (HPNMR) and, atmosphere pressure in IL and poly(ionic liquids) (PILs). Combining different experiments, as Nuclear Overhauser Effect (NOE), quantitative ^{13}C, diffusion, and HMBC, we can select the best IL achieving high sorption capacities. [1-3] Recently, we have demonstrated the positive effect of water in the CC by IL and PIL composites and extended our methodology for ion gel materials.[4-5] Herein, our progress in NMR analyses applied to materials for CC will be revisited, presenting a rationalization to create even more effective and designed sorbents.

References

[1] M. Zanatta, *et. al*. Comment on Preorganization and Cooperation for Highly Efficient and Reversible Capture of Low-Concentration CO2 by Ionic Liquids. *Angew. Chem. Int. Ed., **58**, 2, 382 (*2019).
[2] N. M. Simon, M. Zanatta, *et. al*. Cation-Anion-CO2 interactions in imidazolium based ionic liquid sorbents. *ChemPhysChem*, **19**, 21, 2879-2884, (2018).
[1] N. M. Simon, M. Zanatta, *et. al*. Carbon dioxide capture by aqueous ionic liquid solutions. *ChemSusChem*, **10**, 4927 (2017).
[3] M. Zanatta, *et. al*. Handling CO_2 sorption mechanism in PIL@IL composites *J. CO2 Util.* **41**, 10122, (2020).
[4] M. Lopes, *et. al*., Molecular interactions in Ionic Liquids: The NMR contribution towards tailored solvents. Chapter - NMR Spectroscopy Book, Intech Open (2019).

Acknowledgements

This work was supported by Portuguese funding through FCT- Fundação para a Ciência e a Tecnologia, Portugal (PTDC/QUI-QFI/31508/2017, ROTEIRO/0031/2013, PINFRA/22161/2016), co-financed by FEDER, European funds, through COMPETE 2020, POCI, and PORL and FCT through PIDDAC (POCI-01-0145-FEDER-007688; UID/CTM/50025/2019).

08. Metabolomics

METABOLIC LANDSCAPE OF THE MOUSE LIVER BY QUANTITATIVE ^{31}P-NMR ANALYSIS OF THE PHOSPHOROME

Jon Bilbao[1], Ganeko Bernardo-Seisdedos[1,2], David Fernández-Ramos[1,3], Fernando Lopitz-Otsoa[1], Virginia Gutierrez de Juan[1], Maider Bizkarguenaga[1], Borja Mateos[1,4], MarcosF. Fondevila[5,6], Jordi Abril-Fornaguera[7], Tammo Diercks[8], Shelly CLu[9], Rubén Nogueiras[5,6], José M Mato[1,3] and Oscar Millet[1,2].

[1] Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. 48160, Derio, Spain.
[2] ATLAS Molecular Pharma S. L., Parque Tecnológico de Bizkaia, Ed. 800. 48160, Derio, Spain.
[3] CIBERehd, Instituto de Salud Carlos III, Madrid, Spain.
[4] Department of Structural and Computational Biology, University of Vienna, Max Perutz Labs, Vienna Biocenter Campus 5. 1030 Vienna, Austria.
[5] Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, Spain.
[6] CIBER Fisiopatologia de la Obesidad y Nutrición (CIBERobn), Santiago de Compostela, Spain.
[7] Liver Cancer Translational Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Barcelona, Catalonia, Spain.
[8] NMR Platform, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. 48160 Derio, Spain.
[9] Division of Digestive and Liver diseases, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, US.

The liver plays a central role in all metabolic processes in the body. However, precise characterization of liver metabolism is often obscured by its inherent complexity. Phosphorylated metabolites occupy a prominent position in all anabolic and catabolic pathways. Here we develop a ^{31}P-NMR-based method to study the liver "phosphorome" through the simultaneous identification and quantification of multiple hydrophilic and hydrophobic phosphorylated metabolites. We applied this technique to define the metabolic landscape in livers form a mouse model of the rare disease disorder congenital erythropoietic porphyria (CEP), as well as two well-known murine models of nonalcoholic steatohepatitis, one genetic, methionine adenosyltransferase 1A knockout mice, and the other dietary, mice fed a high-fat choline deficient diet. We report alterations in the concentrations of phosphorylated metabolites that are readouts of the balance between glycolysis, gluconeogenesis, the pentose phosphate pathway, the tricarboxylic acid cycle and oxidative phosphorylation, and of phospholipid metabolism and apoptosis. Moreover, these changes correlate with the main histological features: steatosis, apoptosis, iron deposits and fibrosis. Strikingly, treatment with the repurposed drug ciclopirox improves the phosphoromic profile of CEP mice, an effect that was mirrored by the normalization of liver histology.

In conclusion, these findings indicate that NMR-based phosphoromics may be used to unravel metabolic phenotypes of liver injury and to identify the mechanism of drug action.

References

[1] Chang, M. L. & Yang, S. S. Metabolic Signature of Hepatic Fibrosis: From Individual Pathways to Systems Biology. *Cells* (2019) doi:10.3390/cells8111423.

[2] Emwas, A. H. et al. Nmr spectroscopy for metabolomics research. *Metabolites* (2019) doi:10.3390/metabo9070123.

[3] Bell, J. D. et al. A 31P and 1H-NMR investigation in vitro of normal and abnormal human liver. *BBA - Mol. Basis Dis.* **1225**, 71–77 (1993).

[4] Harvey, P. J., Gready, J. E., Hickey, H. M., Le Couteur, D. G. & McLean, A. J. 31P and1H NMR spectroscopic studies of liver extracts of carbon tetrachloride-treated rats. *NMR Biomed*. **12**, 395–401 (1999).

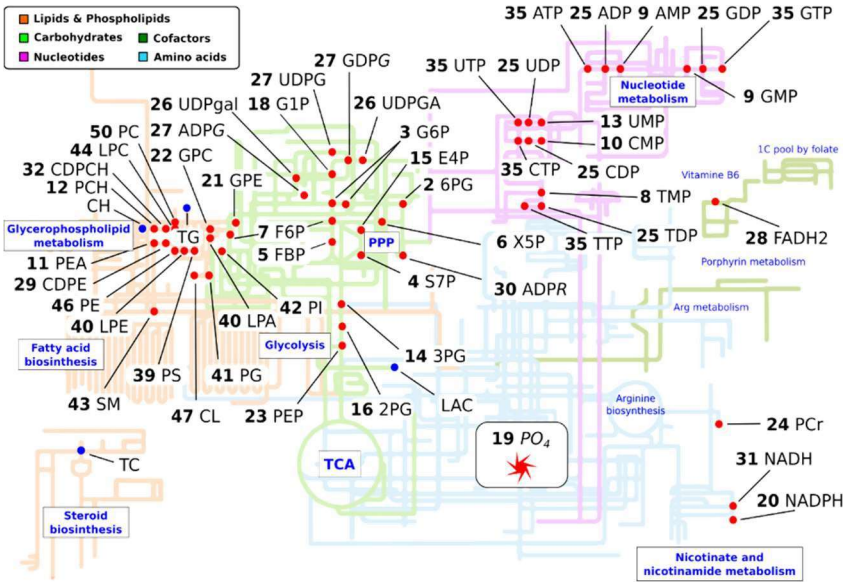


Figure 1. Map of the phosphorylated metabolism. Metabolites measured in liver extracts are represented as red circles, when measured and detected, or black circles, when measured but not detected. Phosphorome gives information on central metabolism (glycolysis, PPP, and the TCA cycle), glycogenesis, phospholipid, nucleotide, nicotinamide and energy metabolism.

HYPERPOLARIZED NMR METABOLOMICS AT NATURAL ^{13}C ABUNDANCE

Arnab Dey[1], Benoît Charrier[1], Estelle Martineau[1,2], Catherine Deborde[3,4], Elodie Gandriau[1], Annick Moing[3,4], Daniel Jacob[3,4], Dmitry Eshchenko[5a], Marc Schnell[5a], Roberto Melzi[5b], Dennis Kurzbach[6], Morgan Ceillier[7], Quentin Chappuis[7], Samuel F. Cousin[7], James G. Kempf[5c], Sami Jannin[7], Jean-Nicolas Dumez[1], Patrick Giraudeau[1]

[1] Université de Nantes, CNRS, CEISAM UMR 6230, F-44000 Nantes, France.
E-mail: arnab.dey@univ-nantes.fr.
[2] SpectroMaitrise, CAPACITES SAS, Nantes, France.
[3] INRAE, Univ. Bordeaux, UMR Biologie du Fruit et Pathologie, Centre INRAE de Nouvelle Aquitaine-Bordeaux, F-33140 Villenave d'Ornon, France
[4] Bordeaux Metabolome, MetaboHUB, Centre INRAE de Nouvelle Aquitaine-Bordeaux, F-33140 Villenave d'Ornon, France. INRAE 2018 doi:10.15454/1.5572412770331912E12
[5] Bruker Biospin, [a]Industriestrasse 26, 8117 Fällanden, Switzerland, [b]Viale V. Lancetti 43, 20158 Milano, Italy, [c]15 Fortune Dr., Billerica, mA 01821 USA
[6] University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria
[7] Université de Lyon, CNRS, Université Claude Bernard Lyon 1, ENS de Lyon, Centre de RMN à Très Hauts Champs (CRMN), FRE 2034, F-69100 Villeurbanne, France.

Standard NMR metabolomic studies play an important role to extract crucial information on biological systems owing to the high reproducibility and repeatability of NMR. For sensitivity reasons, this application relies mostly on 1D ^{1}H NMR spectroscopy. However, 1D ^{1}H NMR is limited by the strong peak overlap that is a feature of complex biological mixtures.

Recently, developments of hyperpolarization methods have given a significant boost to applications of NMR. Hyperpolarization ^{13}C NMR, especially dissolution Dynamic Nuclear Polarization[1] (d-DNP) offers an appealing solution for metabolomics studies as it improves the sensitivity of solution-state NMR by factors up to 10^5 while retaining the intrinsic advantage of wide spectral range and high resolution. In a preliminary d-DNP study,[2] we showed the unique ability to detect ^{13}C signals on plant and cancer cell extracts in a single scan at natural abundance, results that are inaccessible by conventional state-of-the-art high field NMR. Later, we reported a repeatability better than 4% for ^{13}C signals on such extracts[3], as suitable for analytical metabolomics.

Here, for the first time, we introduce d-DNP into a complete workflow for untargeted metabolomics. This consists of sequential steps starting from biological extract preparation, d-DNP hyperpolarization, solution-state NMR acquisition, spectra processing to statistical analysis for discriminating metabolic marker identification. We demonstrate the approach on two groups of samples, obtained from extracts of the same tomato variety at two stages of fruit development[4]. A principal component analysis (PCA) applied tohyperpolarized ^{13}C NMR spectral data resulted in very clear group separation and highlighted several biomarkers in

full agreement with reported studies[5]. We also optimize parameters of the semi-automated d-DNP system, where most steps can be performed by a single operator within a time compatible with high-throughput studies. These results pave the way towards future ^{13}C-based "omics" studies for a variety of samples and applications.

References

[1] J.H. Ardenkjaer-Larsen *et al. Proc. Natl. Acad. Sci.,* 2003, ***100*** 10158.
[2] Dumez *et al. Analyst,* 2015**,** ***140****,* 5860.
[3] Bornet *et al. Anal. Chem.* 2016, ***88***, 6179−6183.
[4] Dey *et al.* manuscript in review.
[5] Lemaire-Chamley *et al.*, *Metabolites*, 2019, ***9***, 93.

NMR-BASED ASSAY TO REVEAL MYOTONIC DYSTROPHY 1 BIOMARKERS IN SKELETAL MUSCLE CELLS

Marc Azagra[1], Juan Manuel Fernández-Costa, Javier Ramón-Azcón, Irene Marco-Rius

[1] Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Barcelona, Spain

Myotonic dystrophy 1 (DM1) is a life-threatening and chronically debilitating disease – the most common cause of muscular dystrophy in adults – afflicting 1 in 8000 people worldwide[1]. Two main challenges in day-to-day patient management and discovering efficient anti-DM1 drugs are: 1) necessity of muscle biopsies, with their associated pain and risks; and 2) the long delay between treatment and evaluation of efficacy[2,3]. A knowledge of reliable biomarkers for DM1, especially those measuring early treatment response, may help overcome these.

To identify metabolic biomarkers of DM1, lysates were collected from human myotubes (differentiated from myoblasts) and mouse biopsies from quadriceps, in DM1 and healthy subjects. The intracellular metabolic pool of cell and tissue lysates was measured by 500 MHz ^{1}H-NMR. In human myotubes, we identified several intracellular metabolites of the glucose metabolic pathway, such as glutamine, glucose, lactate, valine, leucine or isoleucine. The main difference between healthy and DM1 myotubes was a 10-fold increase of lactate in DM1. In mouse biopsies, lactate was also identified, but there was no apparent increase between control and DM1. We will discuss lactate and other NMR-identified DM1 biomarkers to highlight key differences between patient and mouse data, and the potential role in diagnosis.

References

[1] Kumar, A., Agarwal, S., Agarwal, D., & Phadke, S. R. (2013). Myotonic dystrophy type 1 (DM1): a triplet repeat expansion disorder. *Gene*, ***522***, 226-230 (2013)

[2] Overby, S. J., Cerro-Herreros, E., Llamusi, B., & Artero, R. (2018). RNA-mediated therapies in myotonic dystrophy. *Drug Discov. Today*, ***23***, (2013)

[3] Marshall, D. D., & Powers, R. (2017). Beyond the paradigm: Combining mass spectrometry and nuclear magnetic resonance for metabolomics. *Progr. Nucl. Magn. Reson. Spectrosc.*, ***100***, 1-16 (2017).

Acknowledgements

We thank Dr. Vincent Mouly and Dr. Anne Bigot for providing the cell lines and Dr. Ariadna Bargiela and Dr. Ruben Artero for the mouse biopsies. Also to Jose Yeste Lozano and Alba Herrero Gómez for discussions. This work is part of a project that has received funding from the European Union's Horizon 2020 Future and Emerging Technologies Open under grant agreement 863037 (BLOC), the European Union's Horizon 2020 European Research Council (ERC Starting Grant) under grant agreement No 714317 (DAMOC), and the Junior Leader Postdoctoral Fellowship Programme from "la Caixa" Banking Foundation (LCF/BQ/PI18/11630020).

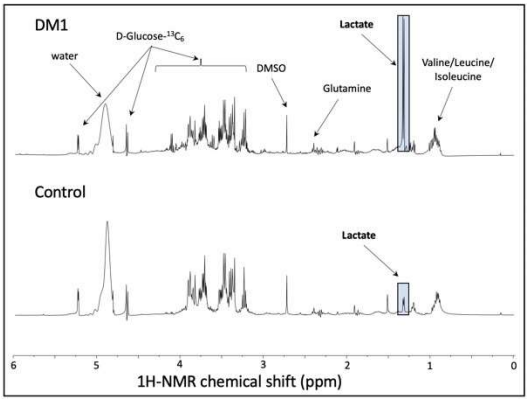


Figure 1. Typical 1H NMR spectrum revealing a large difference in lactate between healthy and DM1.

COVID-19 PATIENTS PRESENT A SEVERE METABOLOMIC AND LIPIDOMIC DYSREGULATION AND AN INCREASED PORPHYRIN ACCUMULATION IN SERUM

Maider Bizkarguenaga[1], Chiara Bruzzone[1], Itxaso San Juan[1], Rubén Gil-Redondo[1], Ana Laín[1], Ganeko Bernardo-Seisdedos[1], David Fernández-Ramos[1], Fernando Lopitz-Otsoa[1], Nieves Embade[1], José M. Mato[1] and Óscar Millet[1]

[1] Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Science and Technology Park of Bizkaia building 800, 48160, Derio, Spain

SARS-CoV-2 virus, which produces the COVID-19 disease, is a novel coronavirus infection currently affecting the worldwide population. At first, this pathology was classified as a respiratory disease, but recent studies have shown that it also affects many other different organs. Our studies suggest that COVID-19 produces important changes in the metabolism and alterations in the blood. A big serum cohort with 263 COVID-19 patients and 280 control patients, recollected before the pandemic started, were analysed by NMR spectroscopy and HPLC chromatography. With these analyses of the serum samples 112 lipoprotein subclasses and 47 metabolites were identified and quantified, and statistical analysis by OPLS-DA was able to completely separate the COVID-19 positive patients from the control ones. The metabolomic analysis of COVID-19 patients showed an increased concentration of ketone bodies, succinic, citric, glutamic and pyruvic acids related to the dysregulation of hepatic central metabolism. Also, COVID-19 patients showed an altered redistribution of lipoprotein particle size and composition. The analysis of different porphyrins for a subset of samples of COVID-19 patients showed an increased level, especially uroporphyrin I (URO I), coproporphyrin I (COP I) and III (COP III). These mayor changes in the metabolism and alterations in the blood showed that SARS-CoV-2 infection induces liver damage associated with dyslipidemia and oxidative stress and haematological disorder.

References

[1] Bizkarguenaga M, Bruzzone C, Gil-Redondo R, Diercks T, Arana E, García de Vicuña A, Seco M, Bosch A, Palazón A, San Juan I, Laín A, Gil-Martínez J, Bernardo-Seisdedos G, Fernández-Ramos D, Lopitz-Otsoa F, Embade N, Lu S, Mato JM, Millet O. SARS-CoV-2 Infection Dysregulates the Metabolomic and Lipidomic Profiles of Serum. *iScience.* **23**(10):101645 (2020).

[2] San Juan I, Bruzzone C, Bizkarguenaga M, Bernardo-Seisdedos G, Laín A, Gil-Redondo R, Diercks T, Gil-Martínez J, Urquiza P, Arana E, Seco M, García de Vicuña A, Embade N, Mato JM, Millet O. Abnormal concentration of porphyrins in serum from COVID-19 patients. *Br J Haematol.* **190**(5):e265-e267 (2020).

NMR-METABOLOMICS OF URINE AND SERUM SAMPLES FOR A BETTER UNDERSTANDING OF THE METABOLIC SYNDROME MOLECULAR BASIS.

Chiara Bruzzone [1], Rubén Gil-Redondo [1], Maider Bizkarguenaga [1], Ana Laín [1], Laura delaCruz-Villar [1], Dolores Corella [2,3], Nieves Embade [1], José M. Mato [1], Óscar Millet [1]

[1] Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800. E-48160, Derio, Spain.
[2] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain.
[3] Department of Preventive Medicine, University of Valencia, 46010 Valencia, Spain.

Metabolic syndrome (MetS) is a complex condition that include a series of metabolic alterations that are responsible for an increased risk of developing different disorders like cardiovascular disease (CVD), liver disease and type 2 diabetes. There are different definition of the metabolic syndrome but the most accepted one is the one of the World Health Organization (WHO) that define the metabolic syndrome as the presence of diabetes and other two of the following conditions: obesity, dyslipidemia, hypertension or microalbuminuria[1]. The role of each of the individual factor in the development of this complex syndrome has not been unraveled. The number of people affected by this condition is increasing worldwide, therefore it is extremely important to find new diagnostic tools and biomarkers for an early diagnosis. NMR-based metabolomics of urine samples from a huge cohort of more than 10.000 people from the working population in the Basque Country and from a specific study enriched in metabolic syndrome patients was used to provide a metabolic fingerprinting of the metabolic syndrome. Taking into consideration samples metadata, volunteers were classified into 16 different subgroups that describe the presence or absence of all the different conditions that can lead to the development of the metabolic syndrome. Multivariate analysis of median spectra profiles showed clear differences between the subgroups emphasizing the significant weight of diabetes and hypertension in the MetS molecular definition. The relevant metabolites involved in the MetS condition were also identified. Ongoing analysis of the matched serum samples from the same volunteers allow to identify 115 lipoprotein related parameters that will lead to a better understanding of different aspect of this pathology.

References

[1] S. Monnerie, B. Comte, D. Ziegler, J. A. Morais, E. Pujos-Guillot, and P. Gaudreau, Metabolomic and Lipidomic Signatures of Metabolic Syndrome and its Physiological Components in Adults: A Systematic Review. *Sci. Rep.*, **10**, 1–13 (2020).

NMR-BASED METABOLOMICS OF URINE SAMPLES REVEALS SIGNS OF ENHANCED CARBON AND NITROGEN RECYCLING IN PROSTATE CANCER.

Chiara Bruzzone[1], Ana Loizaga-Iriarte[2,3], Pilar Sánchez-Mosquera[4], Rubén Gil-Redondo[1], Ianire Astobiza[2,4], Tammo Diercks[5], Ana R. Cortazar[2,4], Aitziber Ugalde-Olano[2,6], Hartmut Schäfer[7], Francisco J. Blanco[8,9], Miguel Unda[2,3], Claire Cannet[7], Manfred Spraul[7], José M. Mato[1], Nieves Embade[1], Arkaitz Carracedo[2,4,9,10], Oscar Millet[1]

[1] Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800, 48160 Derio, Spain.
[2] CIBERONC, Madrid 28025, Spain.
[3] Department of Urology, Basurto University Hospital, Bilbao 48013, Spain.
[4] Cancer Cell Signaling and Metabolism Lab, CIC bioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, Ed. 800, 48160 Derio, Spain.
[5] Structural Biology Unit, CIC bioGUNE, Basque Research and Technology Alliance, Ed. 800, 48160 Derio, Spain.
[6] Department of Pathology, Basurto University Hospital, Bilbao 48013, Spain.
[7] Bruker Biospin GmbH, 76287 Rheinstetten, Germany.
[8]Structural Biology of Cancer Lab, CICbioGUNE, Basque Research and Technology Alliance, Ed. 800, 48160 Derio, Spain.
[9] Ikerbasque, Basque Foundation for Science, Bilbao 48011, Spain.
[10]Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Bilbao 20018, Spain.

Prostate cancer is the second most common tumor and the fifth cause of cancer-related death among men worldwide. PC cells exhibit profound signaling and metabolic reprogramming that account for the acquisition of aggressive features. Although the metabolic understanding of this disease has increased in recent years, the analysis of such alterations through noninvasive methodologies in biofluids remains limited. Here, we used NMR-based metabolomics on a large cohort of urine samples (more than 650) from PC and benign prostate hyperplasia (BPH) patients to investigate the molecular basis of this disease. Multivariate analysis failed to distinguish between the two classes, highlighting the modest impact of prostate alterations on urine composition and the multifactorial nature of PC. However, univariate analysis of urine metabolites unveiled significant changes, discriminating PC from BPH. Metabolites with altered abundance in urine from PC patients revealed changes in pathways related to cancer biology, including glycolysis and the urea cycle. We found out that metabolites from such pathways were diminished in the urine from PC individuals, strongly supporting the notion that PC reduces nitrogen and carbon waste in order to maximize their usage in anabolic processes that support cancer cell growth.

References

[1] C. Bruzzone, A. Loizaga-Iriarte, P. Sánchez-Mosquera, R. Gil-Redondo, I. Astobiza, T. Diercks, A. R. Cortazar, A. Ugalde-Olano, H. Schäfer, F. J. Blanco, M. Unda, C. Cannet, M. Spraul, J. M. Mato, N. Embade, A. Carracedo and O. Millet, ^{1}H NMR-Based Urine Metabolomics Reveals Signs of Enhanced Carbon and Nitrogen Recycling in Prostate Cancer. *J. Proteome Res.* **19**, 2419–2428 (2020).

ANTS LIPIDS RESERVE CORRELATED WITH TASK ALLOCATION IN *MYRMICA RUBRA*

Baptiste Busi[1], Sean McGregor[2], Emilie Baudat[1], Laurent Keller[2], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fèdèrale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[2] Department of Ecology and Evolution, Université de Lausanne (UNIL), CH-1015 Lausanne, Switzerland

Ants have been described as one of the major pests in agriculture, and can damage certain ecosystems, although they are also essential in others.[1]

Ants typically can have two different "jobs", either caring for the broods in the nest (nurses) or foraging outside (foragers). This distribution of labor is possible due to social stomachs, which allow ants to regurgitate food or water to each other. By this mechanism the entire colony can be fed with only a portion of the individuals actually risking their lives by leaving the nest.[2] In the present work we investigate task allocation in *Myrmica rubra*.

The bimodal allocation of tasks is correlated to nurses having large lipids reserves, and foragers being leaner. Our hypothesis is that lipids reserve will drive the division of labor in *Myrmyca rubra*. [3] To confirm this requires a protocol to measure the ants' metabolism without killing them, which was not previously possible.

Here we present a MAS NMR approach for the determination of fat contents in live *Myrmyca rubra* ants through their lifecycle. We determine fat contents from ^{1}H spectra at 1.2 kHz MAS, the brief centrifugal force of which ants can experience without damage. Even at such low speed, the almost liquid state of the inside of the ants gives a resolution high enough to track the lipids reserve of ants thorough their lives.

In addition to this metabolic record, a tracking of the ants task allocation and social network is constructed by placing the ants in an artificial environment with a nest and an outside world, and tracking their position, work and interaction using an automated camera recording QR codes glued to the back of the ants.

Preliminary findings and correlation of this approach are presented.

References

[1] Chan, K. H.; Guénard, B., Ecological and socio-economic impacts of the red import fire ant, Solenopsis invicta (Hymenoptera: Formicidae), on urban agricultural ecosystems. *Urban Ecosyst* **2020,** *23* (1), 1-12.
[2] Hölldobler, B.; Wilson, E. O., *The Ants*. 1990.
[3] Blanchard, G. B.; Orledge, G. M.; Reynolds, S. E.; Franks, N. R., Division of labour and seasonality in the ant Leptothorax albipennis: worker corpulence and its influence on behaviour. *Animal Behaviour* **2000,** *59*, 723-738.

^{1}H NMR BASED COMPREHENSIVE METABOLIC PROFILING IN SEPTIC SHOCK PATIENTS WITH AND WITHOUT COMORBID CONDITIONS AT DIAGNOSIS

Swarnima Pandey[1], Adnan Md. Siddiqui[1], Afzal Azim[2], Neeraj Sinha[1]

[1]Centre of Biomedical Research, Lucknow, India
[2]SGPGIMS, Lucknow, Uttar Pradesh

Background: Septic shock with co-morbid conditions like hypertension, coronary artery disease, chronic kidney disease and diabetes are major risk factors, accompanied by marked alterations in metabolic processes. Management of patients with septic shock is still a major challenge for clinicians because of its considerable mortality amongst the hospitalized patients. Therefore, a better understanding of the characteristics governing septic shock in the context of comorbidities is crucial for understanding their combined pathophysiology and thus enhancing the treatment strategies and reducing the mortality.

To decipher the metabolic alterations brought about by co-morbid conditions in septic shock, one must have a picturesque view of the metabolic profile; that is small metabolite and lipid molecules must be studied together to gain a comprehensive insight into alternation of the metabolites caused due to comorbidities. The aim of this study is to identify the metabolic alterations due to co-morbid conditions with septic shock after analyzing the complete metabolic information.

Method: Metabolic profiling of sera obtained from 50 septic shock patients, out of which 20 patients are septic shock patients with co morbid conditions and 30 are septic shock patients without any comorbid conditions by using high resolution 1D ^{1}H CPMG and diffusion edited NMR spectra. Univariate and multivariate statistics were applied to identify the discriminatory metabolites between the groups.

Results: We found that the serum level of 3 hydroxybutyrate, 3 hydroxyisovalerate, methionine, myoinositol, carnitine, phenylalanine, HDL, LDL and lipoprotein with phosphocholine head group were significantly lower in septic shock with comorbidities whereas propanediol, alanine, N acetylglycine, choline, and bile acids were significantly lower.

Conclusion: These metabolic alternations could thereafter be validated in larger cohort studies and can be helpful for personalized treatment strategies.

References

[1,2,3,*] Henna Jaurila, et al.^{1}H NMR Based Metabolomics in Human Sepsis and Healthy Serum. Metabolites. 2020 Feb; 10(2): 70.

09. MRI – *In vivo*

DIFFERENTIAL HEMISPHERICAL ASSESSMENT USING MULTINUCLEAR MR REVEALS EFFECTS OF STEM CELL VARIANCE IN STROKE TREATMENT

Shannon Helsper[1,2], Xuegang Yuan[1,2], F. Andrew Bagdasarian[1,2], Samuel C. Grant[1,2]

[1] FAMU-FSU College of Engineering, Chemical & Biomedical Engineering, Tallahassee, Florida.
[2] National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida.

Human mesenchymal stem cell (hMSC) therapy is a primary focus of preclinical research and clinical treatment of stroke. hMSC variance, however, can lead to clinical trial failure. High field MRI/S reveals therapeutic potency as early as 24 h post-implementation by displaying physiological impacts in the presumably healthy, non-ischemic hemisphere. A transient MCAO[2] was instituted in Sprague-Dawley rats and treated by immediate administration of healthy or compromised hMSC in saline compared to saline alone (control). Cerebral biochemical markers were measured over 21 d using sodium chemical shift imaging (^{23}Na CSI) and relaxation enhanced spectroscopy (RE-MRS).[1] Combining these measurements at 21.1 T provided insight into ionic and metabolic homeostasis, and demonstrated that differential impacts of ischemic recovery based on hMSC variance also manifested as effects in the non-ischemic hemisphere.

Rigorous *in vitro* analysis was completed on hMSC from multiple donors to assess their therapeutic potential. Prior to injection, cellular assays did not reveal differences between cell lines at early passage. Extended culture, however, revealed possibly early and irreversible senescence in compromised donor cells, which was confirmed with PCR, macrophage co-culture and oxygen/glucose deprivation.

^{23}Na CSI showed high specificity in lesion detection with robust group differences by day 3 for total lesion size, reduction and ^{23}Na signal intensity. ^{23}Na signal measurements in the non-ischemic hemisphere provided evidence of ionic regulation only for the healthy hMSC group compared to compromised hMSC and control rats at 24 h. Elevated lactate, NAA and creatine ratios (to Cho) for compromised hMSC and control support metabolic remodeling, even beyond the lesion.

^{23}Na MRI provided reliable metrics for assessing the impact of hMSC variance in stroke treatment, both at the lesion and contralaterally. ^{1}H RE-MRS provides complementary data about energetic/metabolic activity, permitting for corroboration of more widespread cerebral impacts of hMSC treatments applied to stroke.

References

[1] Shemesh, N. et al. Nat. Commun. 5, (2014).
[2] Longa, E.Z., et al. Stroke 20, 84-91 (1989).

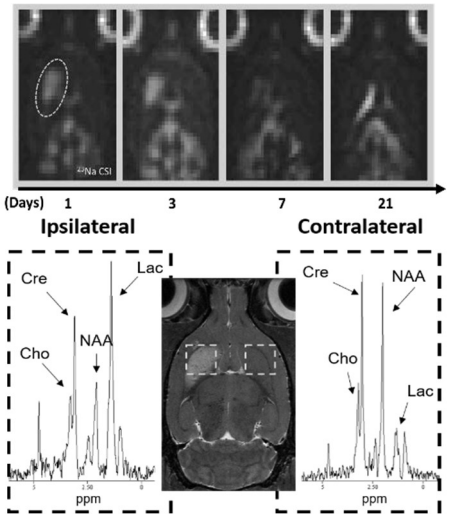


Figure 1. Representative ^{23}Na MRI and ^{1}H MRS.

INVESTIGATING TISSUE MICROSTRUCTURE IN THE LIVING HUMAN BRAIN WITH 6D DIFFUSION-RELAXATION IMAGING

Jan Martin[1], Alexis Reymbaut[1], Michael Uder[2], Frederik B. Laun[2], Daniel Topgaard[1]

[1] Department of Chemistry, Lund University, Lund, Sweden
[2] Institute of Radiology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

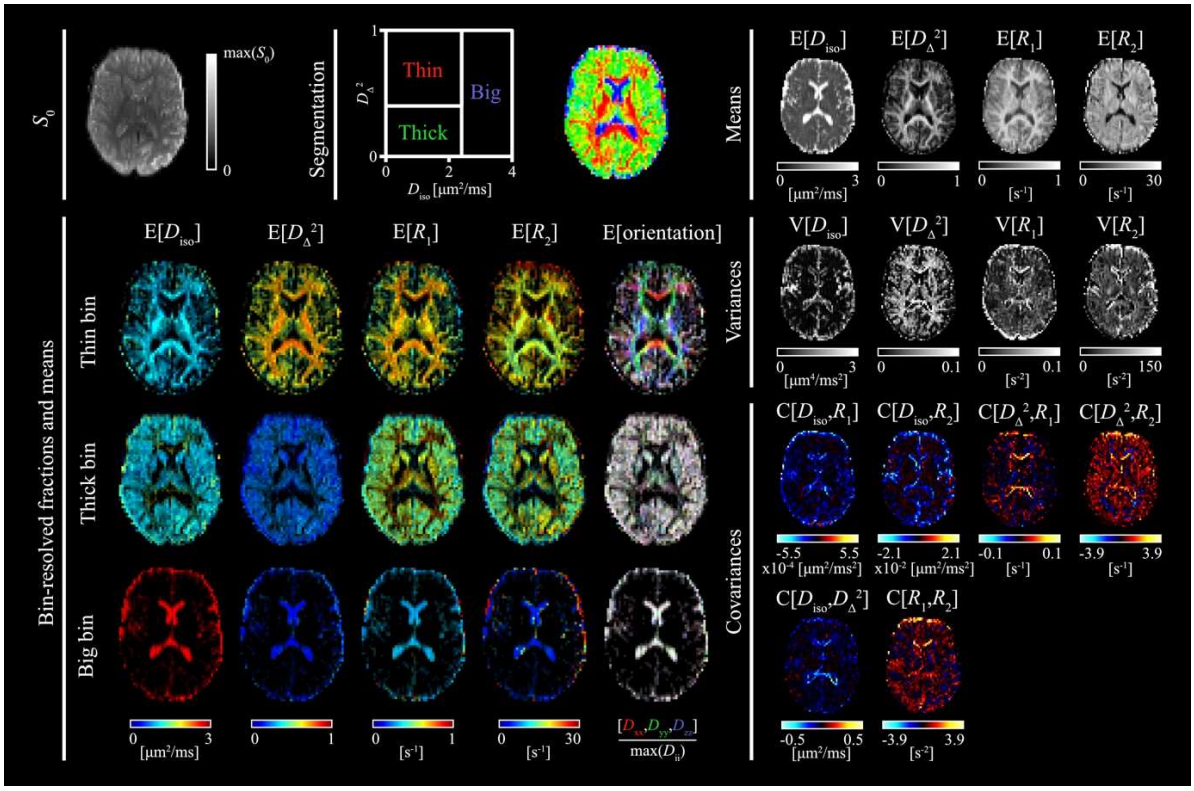
Brain tissue contains microscopic water compartments several orders of magnitude smaller than the typical resolution in clinical MRI. As a result, each voxel comprises a distribution of relaxation constants and diffusion tensors that characterizes its tissue content. Recent works have succeeded in measuring these distributions by translating methods that are well-established in diffusion NMR on small bore systems to clinical MRI systems[1,2]. It thereby became possible to correlate the information contained in the diffusion tensor $\mathbf{D}$ with either the transversal or the longitudinal relaxation constant R_2 and R_1, respectively[2,3].

We present a novel experimental protocol to simultaneously correlate both relaxation rate constants with the diffusion tensor, thus achieving spatially resolved 6D diffusion-relaxation distributions ($\mathbf{D}$- R_1- R_2) on a clinical MRI system. Data was acquired with a single-shot EPI pulse sequence modified for tensor-valued diffusion encoding[4]. Diffusion encoding was performed using linear, planar, and spherical b-tensor shapes. Correlation of the diffusion-weighted signal with R_1 and R_2 was realized by repeating a subset of measurements at different echo times and repetition times. Diffusion-relaxation distributions were fitted using an unconstrained Monte-Carlo inversion algorithm and distinct microscopic tissue compartments were isolated by binning the $\mathbf{D}$- R_1- R_2 space[2]. The corresponding parameter maps are shown in Figure 1. Here, the diffusion tensor is expressed in terms of isotropic diffusivity D_{iso}, normalized anisotropic diffusivity D_Δ, and its principal eigenvalues D_{xx}, D_{yy}, and D_{zz}.

Our approach shows great potential for clinical MRI as it offers an abundance of highly specific information on the probed tissue without making any assumptions about the underlying microstructure.

References

[1] J. P. de Almeida Martins, D. Topgaard, Multidimensional correlation of nuclear relaxation rates and diffusion tensors for model-free investigations of heterogeneous anisotropic porous materials. *Sci. Rep.* **8**, 2488 (2018).
[2] J. P. de Almeida Martins, C. M. W. Tax, F. Szczepankiewicz, D. K. Jones, C.-F. Westin, D. Topgaard, Transferring principles of solid-state and Laplace NMR to the field of in vivo brain MRI. *Magn. Reson.* **1**, 27-43 (2020).
[3] A. Reymbaut, J. Critchley, G. Durighel, T. Sprenger, M. Sughrue, K. Bryskhe, D. Topgaard, Towards non-parametric diffusion-T_1 characterization of crossing fibers in the human brain. *arXiv:2006.07881 (2020)*
[4] J. Martin, S. Endt, A. Wetscherek, T. A. Kuder, A. Doerfler, M. Uder, B. Hensel, F. B. Laun, Contrast-to-noise ratio analysis of microscopic diffusion anisotropy indices in q-space trajectory imaging. *Z. Med. Phys.* **30** (1), 4- 16 (2020)


S_0
max(S_0)
0
Segmentation
Thin
Big
Thick
D_Δ^2
D_{iso} [µm²/ms]
Means
E[D_{iso}]
E[D_Δ^2]
E[R_1]
E[R_2]
[µm²/ms]
[s⁻¹]
Bin-resolved fractions and means
Thin bin
Thick bin
Big bin
E[orientation]
Variances
V[D_{iso}]
V[D_Δ^2]
V[R_1]
V[R_2]
[µm⁴/ms²]
[s⁻²]
Covariances
C[D_{iso},R_1]
C[D_{iso},R_2]
C[D_Δ^2,R_1]
C[D_Δ^2,R_2]
x10⁻⁴ [µm²/ms²]
x10⁻² [µm²/ms²]
C[D_{iso},D_Δ^2]
C[R_1,R_2]
max(D_{ii})

10. Small molecules & drug design

RELATIVE CONFIGURATION OF MICROGRAMS OF NATURAL COMPOUNDS USING PROTON RESIDUAL CHEMICAL SHIFT ANISOTROPY

Juan Carlos Fuentes-Monteverde[1,2], Nilamoni Nath[1,3], Dawrin Pech-Puch[2], Michael Reggelin[4], Armando Navarro[4], Jaime Rodríguez[2], Carlos Jiménez[2], Christian Griesinger[1]

[1] NMR Based Structural Biology, MPI for Biophysical Chemistry, Göttingen, Germany
[2] Centro de Investigacións Científicas Avanzadas (CICA) e Departamento de Química, Facultade de Ciencias, Universidade da Coruña. A Coruña, Spain
[3] Department of Chemistry, Gauhati University, Gopinath Bardoloi Nagar, Guwahati, India
[4] Department of Chemistry, Technical University of Darmstadt, Alarich Weiss Straße 4, 64287, Darmstadt, Germany
[5] Departamento de Química Fundamental, CCEN, Universidade Federal de Pernambuco, Cidade Universitária, Recife, Brazil

3D molecular structure determination is a challenging task for organic compounds or natural products available in minute amounts. While the constitution can be derived from proton/proton and proton/carbon correlations, *J*-couplings, NOEs, residual dipolar couplings (RDCs) or ^{13}C residual chemical shift anisotropies (RCSAs) are used to determine the relative configuration. Nevertheless, for compounds in the range of few microgram these RDC or ^{13}C RCSAs are difficult to collect because of rely on 1% natural abundance of ^{13}C.[1,2,3] Herein, we demonstrate the use of the highly sensitive ^{1}H RCSAs that provides spatial orientation of different structural moieties within a molecule. The power of the methodology was demonstrated in rigid and flexible natural compounds swollen in different alignment media. The major obstacles that come of protonated alignment media is the dominant presence of polymer background signals masking the signals of compound being investigated. Together with the micro stretching device from Hilgenberg, the use of deuterated gel such as PMMA-*d*8 gel can evade the problem (Figure 1). ^{1}H RCSA methodology was successfully applied to elucidate the correct relative configuration of 35 microgram Briarane B-3. This Briarane B-3 was isolated from *Briareum asbestinum* for which relative configuration was unknown since 1993.[4] The absolute configuration was also accomplished by comparison of calculated electronic circular dichroism and experimental spectra.[5] In summary, we reported that ^{1}H RCSAs as powerful parameters that complemented *J*-couplings and NOEs, without the necessity to use another anisotropic parameter at microgram scale.

References

[1] N. Nath, M. Schmidt, R. R. Gil, R. T. Williamson, G. E. Martin, A. Navarro-Vázquez, C. Griesinger, Y. Liu, Determination of Relative Configuration from Residual Chemical Shift Anisotropy. *J. Am. Chem. Soc*. **138**, 9548- 9556 (2016)
[2] N. Nath, J. C. Fuentes-Monteverde, D. Pech-Puch, J. Rodríguez, C. Jiménez, M. Noll, A. Kreiter, M. Reggelin, A. Navarro-Vázquez, C. Griesinger. Relative configuration of micrograms of natural compounds using proton residual chemical shift anisotropy. *Nat. Commun*. **4372**, 1-9 (2020)
[3] Y. Liu, A. Navarro-Vázquez, R. R. Gil, C. Griesinger, G. E. Martin, R. T. Williamson. Application of anisotropic NMR parameters to the confirmation of molecular structure. *Nat. Protoc.* **14**. 217-247. (2018)

[4] C. D. Harvell, W. Fenical, V. Roussis, J. L. Ruesink, C. C. Griggs, C. H. Greene. Local and geographic variation in the defensive chemistry of a West Indian gorgonian coral (*Briareum asbestinum*). *Mar. Ecol. Prog. Ser*. **93**. 165- 173 (1993)

[5] G. Pescitelli, T. Bruhn. Good Computational Practice in the Assignment of AbsoluteConfigurations by TDDFT Calculations of ECD Spectra. Chirality. **28**. 466-474.

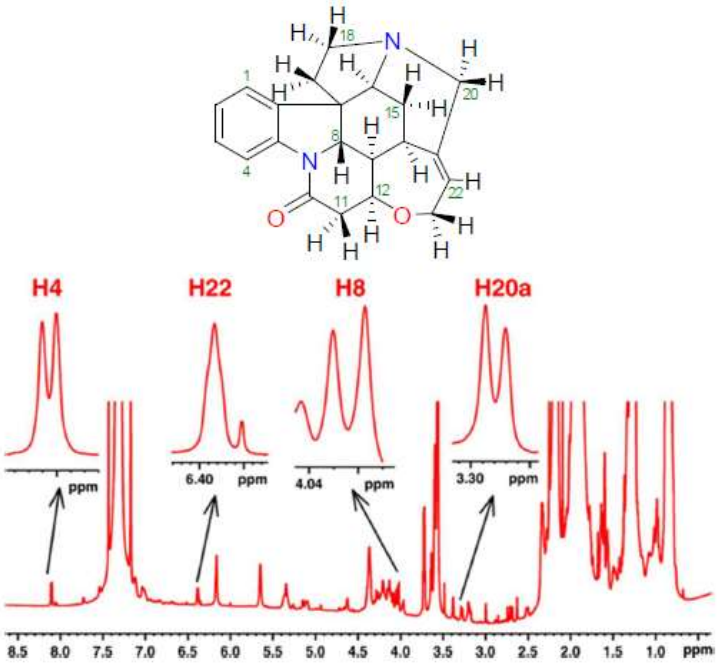


Figure 1. 1D ^{1}H NMR spectrum of 10-µg strychnine acquired with 8192 scans in PMMA-*d*8 gel under maximum alignment condition. For clarity, expansions for some of the proton signals (H4, H22, H8, and H20a) are also shown. Alignment induced by using a stretching device. Resonances become visible due to the removal of proton signals of the polymer.

DYNAMICS PLAYS ESSENTIAL ROLE IN DRUG DEVELOPMENT AGAINST ONCOGENIC K-RAS PROTEINS

Gyula Pálfy[1,2], Dóra K. Menyhárd[2], Orsolya Tőke[3], István Vida[1], Zoltán Orgován[4], Péter Ábrányi-Balogh[4], László Petri[4], György M. Keserű[4], András Perczel[1,2]

[1] Eötvös Loránd University, Laboratory of Structural Chemistry and Biology, Budapest, Hungary
[2] MTA-ELTE Protein Modelling Group, Budapest, Hungary
[3] Laboratory for NMR Spectroscopy, Research Centre for Natural Sciences, Budapest, Hungary
[4] Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Budapest, Hungary

Decoding molecular flexibility in order to understand and predict biological processes—applying the principles of dynamic-structure-activity relationships (DSAR)—becomes a necessity when attempting to design selective and specific inhibitors of a protein that has overlapping interaction surfaces with its upstream and downstream partners along its signaling cascade. Ras proteins are molecular switches that meet this definition perfectly. They are membrane-bound small GTPases which act as a molecular switch and play a key role in many signal transduction pathways regulating cell proliferation, differentiation, and survival. They alternate between GTP-bound active and the GDP-bound inactive conformers. The close-lying P-loop and the highly flexible switch-I and switch-II regions are the site of nucleotide-, assisting-, and effector-protein binding. The most frequent oncogenic mutants are G12C, G12D, and G12V, all „frozen" in the active form and thus, induces malignant tumors. The oncogenic mutations do not cause easily characterized overall structural changes, due partly to the inherent conformational heterogeneity and pliability of these segments.[1] To obtain detailed dynamics information we developed a new approach enabling us to work with the native K-Ras-GTP-complex without the artifacts caused by GTP analogues.[2] Comprehensive backbone dynamics analysis of GDP- and GTP-bound forms of K-Ras and its oncogenic mutants both at the ps-ns (fast) and µs-ms (slow) timescales of motion revealed that switch-I and -II regions are highly dynamic showing R_{ex}-exchange in switch I region, while a mutation in the P-loop alters its mobility. According to HetNOE measurements, the behavior of Tyr32 of switch-I turned out to be more rigid in its GTP-bound form. This new finding reveals the key importance of Y32 in hydrolyzing GTP. Residue-specific information of these oncogenic proteins let us perform binding tests of a Cys-specific covalent small molecule library against K-Ras-G12C-GDP examined by both protein-based (HSQC) and drug-based methods (^{19}F-NMR) in parallel.[3,4]

References

[1] G. Pálfy, D. K. Menyhárd, A. Perczel, Dynamically encoded reactivity of Ras enzymes: opening new frontiers for drug discovery, *Cancer Metastasis Rev*, ahead of print, doi: 10.1007/s10555-020-09917-3.
[2] D. K. Menyhárd, G. Pálfy, Z. Orgován, I. vida, G. M. Keserű, A. Perczel, Structural impact of GTP binding on downstream KRAS signaling. *Chem. Sci.* **11**, 9272–9289 (2020).

[3] G. Pálfy, I. Vida, A. Perczel, 1H, 15N Backbone Assignment and Comparative Analysis of the Wild Type and G12C, G12D, G12V Mutants of K-Ras Bound to GDP at Physiological pH. *Biomol. NMR Assign.* **14(1)**, 1–7 (2020).
4 L. Petri, P. Ábrányi-Balogh, T. Imre, G. Pálfy, A. Perczel, D. Knez, M. Hrast, M. Gobec, I. Sosič, K. Nyíri, B. G. Vértessy, N. Jänsch, C. Desczyk, F.-J. Meyer-Almes, I. Ogris, S. G. Grdadolnik, L. G. Iacovino, C. Binda, S. Gobec, G. M. Keserű, Assessment of tractable cysteines by covalent fragments screening. *Chembiochem*. ahead of print, doi: 10.1002/cbic.20200700.
[5] H. R. Kalbitzer, I. C. Rosnizeck, C. E. Munte, S. P. Narayanan, V. Kropf, M. Spoerner, Intrinsic allosteric inhibition of signaling proteins by targeting rare interaction states detected by high-pressure NMR spectroscopy. *Angew. Chem. Int. Ed. Eng.* **52(52)**, 14242–14246 (2013).

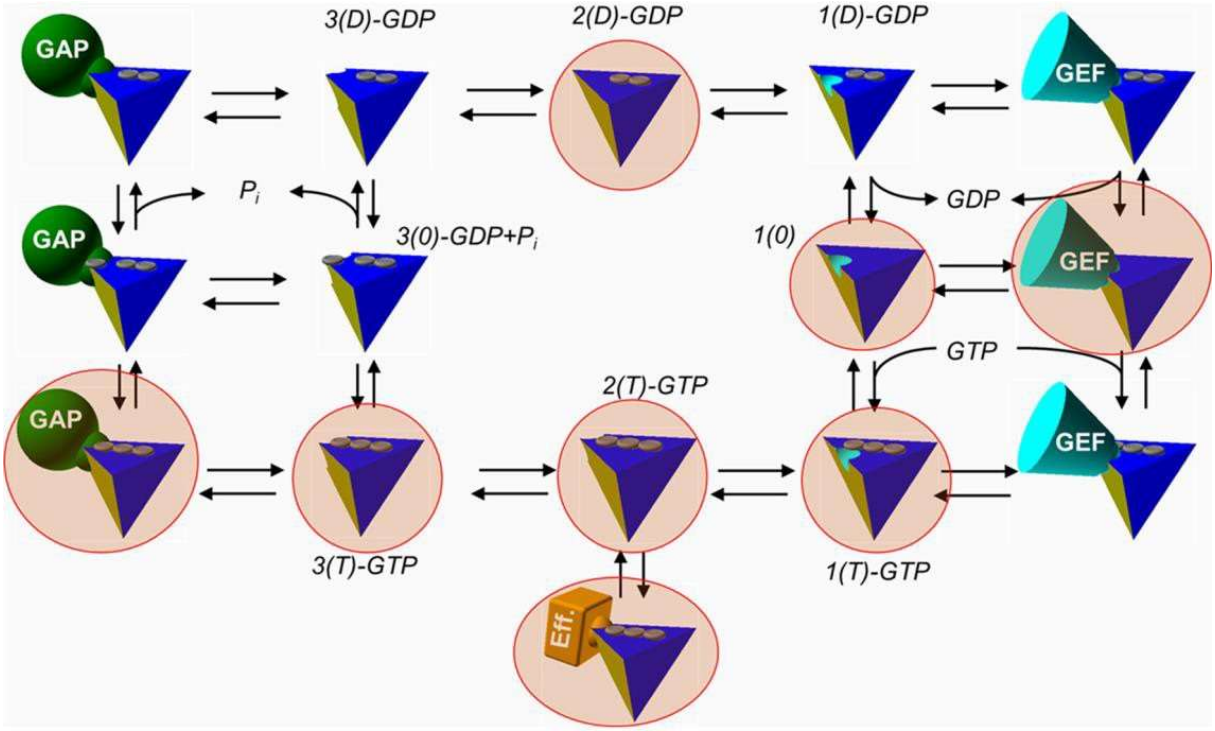


Figure 1. Hypothetical conformational states of Ras proteins in Ras cycle according to Kalbitzer *et al.*[5] Those states which were identified and studied experimentally (by NMR or X-ray methods) are encircled and designated by light red background.

SORPTION OF THE CARBON DIOXIDE BY POLYMER MATRIX OF PMMA: NMR SPECTROSCOPY STUDIES OF KINETICS

Ilya Khodov[1], Michael Kiselev[1]

[1] G.A. Krestov Institute of Solution Chemistry, Russian Academy of Sciences, 153045, Ivanovo, Russia

Creating composite materials based on polymers and drug compounds is a promising field of research. For example, implants made of a composite material containing PMMA and carbamazepine are a good alternative for titanium implants, which may decrease pain caused by compression of basillary arteria on the trigeminal nerve[1]. Investigation of kinetics of processes occurring in the polymer phase is one of the most important problems of studying the behavior of polymers in supercritical carbon dioxide ($scCO_2$). Solving these problems is important for optimization of processes of polymer impregnation. The saturation degree of the polymer matrix depends on its residual swelling; studying kinetic parameters of sorption in polymers allows estimating time necessary for achieving the equilibrium concentration of the supercritical fluid in the pores of the polymer matrix. To estimate the degree of residual swelling, high-pressure[2] ^{13}C NMR spectroscopy was applied.

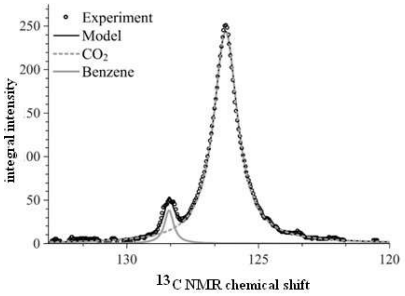
Obtained data on the sorption kinetics of $scCO_2$ in PMMA may be used to estimate the impregnation parameters of drug compounds in a polymer matrix.

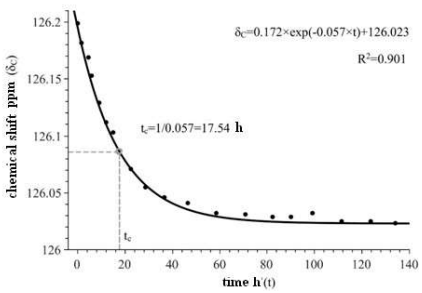
References

[1] P. Banczerowski, G. Czigléczki, I. Nyáry, Long-term effectiveness of an ad hoc tailored titanium implant as a spacer for microvascular decompression in the treatment of trigeminal neuralgia caused by megadolichoectatic basilar artery anomaly: 9-year follow-up. *J. Neurosurg.* **121**, 1492–1496 (2014).
[2] I. Khodov, A. Dyshin, S. Efimov, D. Ivlev, M. Kiselev, High-pressure NMR spectroscopy in studies of the conformational composition of small molecules in supercritical carbon dioxide. *J. Mol. Liq*. **309**, 113113 (2020).

Acknowledgements

This work was financially supported by the Ministry of Science and Higher Education of the Russian Federation (grant no. RFMEFI61618x0097). The NMR spectroscopy experiment was performed using the molecular fluid spectroscopy facility (http://www.ckp- rf.ru/usu/503933/) of G.A. Krestov Institute of Solution Chemistry of the Russian Academy of Sciences (ISC RAS) (Russia).





EXPRESSION AND PURIFICATION OF A DOMAIN-SWAPPED CO-FOLDED PROTEIN DOMAIN BY STRUCTURE-GUIDED TANDEM FOLDING IN AUTOINDUCING CULTURES

Erik Walinda[1], Daichi Morimoto[2], Tomoki Sorada[2], Masahiro Shirakawa[2], Kenji Sugase[2], Kazuhiro Iwai[1]

[1] Department of Molecular and Cellular Physiology, Graduate School of Medicine, Kyoto University, Kyoto 606-8306, Japan.
[2] Department of Molecular Engineering, Graduate School of Engineering, Kyoto University, Kyoto, Japan

The linear ubiquitin chain assembly complex tethering motif (LUBAC-LTM) is a uniquely formed tertiary structure due to co-folding of the LTM polypeptides from two accessory LUBAC components, HOIL-1L and SHARPIN, into a single globular domain. Disruption of the intricate LTM-LTM interaction between HOIL-1L and SHARPIN by stapled peptides destabilized LUBAC in lymphoma patient-derived cells, thereby attenuating activity of the key transcription factor NF-kB and impairing cell survival[1]; thus, targeting the LTM is a promising strategy for the development of novel anticancer therapeutics. To further aid screening of small-molecule inhibitors that can selectively disrupt the LTM-LTM interaction, it is necessary to obtain high-purity samples of soluble, folded LTM domain. Ideally such a sample would not contain LUBAC components other than the LTM itself, so that molecules binding to other parts of the complex can rapidly be eliminated from the screening process. Here we report a simple strategy that enabled successful production of the isolated LTM domain in high yield and purity based on the following five points: (1) structural analysis of the LUBAC core highlighting the possibility of the LTM regions of SHARPIN and HOIL-1L as a single tandem protein; (2) in-silico verification that the fusion protein remains folded in the absence of other LUBAC domains; (3) bacterial expression downstream of EGFP to efficiently screen expression and solubility; (4) slow transcription enabling gentle folding at ambient temperature using Studier's autoinduction method; (5) selective protease cleavage to remove the covalent linkage between the two motifs. Formation of stably folded LTM was verified by size-exclusion chromatography and heteronuclear NMR spectroscopy. From 200 ml culture sufficient protein for structural studies was obtained. The presented strategy may prove beneficial for LUBAC LTM-centered drug-screening efforts and likely serve as a useful primer for similar cases, i.e. when a structured fragment of a larger complex is to be obtained for site-specific downstream applications such as site-targeted drug screening or antibody generation.

References

[1] Fujita, Hiroaki, et al. "Cooperative domain formation by homologous motifs in HOIL-1L and SHARPIN plays a crucial role in LUBAC stabilization." *Cell Rep.* **23.4**, 1192–1204 (2018).

11. Solid State NMR - Methods

USING B_1-FIELD SELECTIVE PULSES TO IMPROVE FSLG-DECOUPLED SPECTRA

Kathrin Aebischer[1], Nino Wili[1], Zdeněk Tošner[2], Matthias Ernst[1]

[1] Physical Chemistry, ETH Zürich, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland.
[2] Department of Chemistry, Faculty of Science, Charles University, Hlavova 8, 12842 Prague 2, Czech Republic.

The inhomogeneity of the radio-frequency (rf) field is a prevalent problem in solid-state NMR and one of the major contributions to the residual linewidth achieved with homonuclear decoupling sequences under magic-angle spinning (MAS)[1]. Experimentally, the detrimental effects of the rf inhomogeneity can be reduced by physically restricting the sample to the central part of the coil. Alternatively, sample restriction can be achieved using radio-frequency selective pulses. Such B_1-field selective pulses have been demonstrated some years ago, however, the numerically optimized rf selective pulses proposed showed many sidebands[2].

Here we present the implementation of band- selective pulses in the spin-lock frame byusing a modulation of the pulses that is resonant with the spin-lock field. Thus, arbitrary nutation-frequency selective pulses can be applied to spins experiencing selected parts of the rf- field distribution. In our measurements the family of I-BURP[3] pulses was chosen, but in principle any band-selective pulses can be used. The implementation of these pulses is straightforward and presents a simpler and more effective alternative to spatial sample restriction. Using such B_1-field selective pulses, significant improvements in homonuclear decoupled proton spectra under MAS using frequency-switched Lee–Goldburg decoupling were achieved. The otherwise prominent zero-frequency artefact is almost eliminated, and substantially narrower lines are obtained due to the reduction of the rf-field distribution. These spectral improvements coincide with a loss in signal intensity and a compromise between resolution and sensitivity must be found.

References

[1] J. Hellwagner, L. Grunwald, M. Ochsner, D. Zindel, B. H. Meier, M. Ernst, Origin of the residual line width under frequency-switched Lee–Goldburg decoupling in MAS solid-state NMR. *Magnetic Resonance* **1**, 13-25 (2020).
[2] P. Charmont, D. Sakellariou, L. Emsley, Sample restriction using radiofrequency field selective pulses in high-resolution solid-state NMR. *Journal of Magnetic Resonance* **154**, 136-142 (2002).
[3] H. Geen, R. Freeman, Band-selective radiofrequency pulses. *Journal of Magnetic Resonance* **93**, 93-141 (1991).
[4] K. Aebischer, N. Wili, Z. Tošner, M. Ernst, Using nutation-frequency-selective pulses to reduce radio- frequency field inhomogeneity in solid-state NMR. *Magnetic Resonance* **1**, 187-195 (2020).

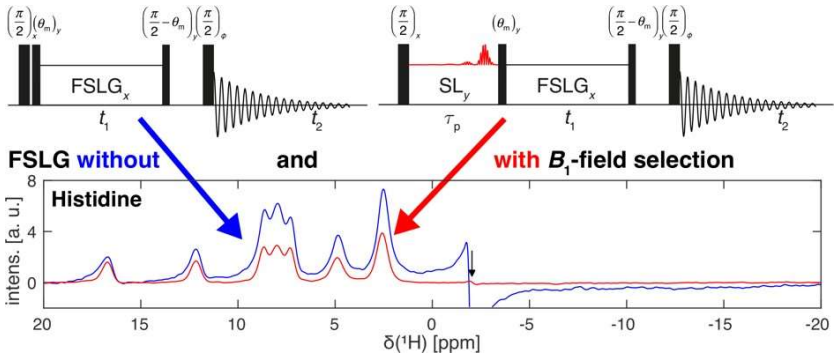


Figure 1. Comparison of experimental FSLG decoupled proton spectra of histidine with and without B_1-field selection at a static field of 11.7 T recorded using a 1.9 mm Bruker MAS probe spinning at 14 kHz. The spectral improvements are evident. The zero-frequency peak is eliminated and narrower lines with reduced feet on the high-frequency side are obtained.

FAST REMOTE CORRELATION EXPERIMENTS FOR ^{1}H HOMONUCLEAR DECOUPLING IN SOLIDS

Bruno Simões de Almeida[1], Pinelopi Moutzouri[1], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fedérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

In ^{1}H MAS spectra, the residual homogeneous broadening under MAS is due to a combination of higher-order shifts and splittings.[1] We have recently shown how the two-dimensional anti-z-COSY experiment can be used for the removal of the splittings.[2] However, this requires spectra with high resolution in the indirect dimension (t_1), leading to experiment times of hours. Here, we show how the anti-z-COSY [3] can be adapted in order to be combined with the two-dimensional one pulse (TOP) transformation[4] and lead to significantly reduced experimental time while retaining the line narrowing effect[5]. More specifically, this modification allows the remapping of the data in a way that the interactions sampled in t_1 can after two active shearing transformations be transformed onto the direct dimension and inherit the sampling properties of this dimension, therefore allowing narrow spectral widths to be acquired in t_1. This approach accelerates the acquisition time by a factor of 15 or more. As seen in Figure 1, the experiment is demonstrated on a powdered sample of L-histidine monohydrochloride monohydrate, where the new TAZ-COSY sequence at 100 kHz MAS, yields between a factor 1.6 and 2.3 increase in resolution compared with the equivalent one-pulse experiment, in just 20 min, compared with a similar resolution achieved by the fully sampled anti-z-COSY in 5 h and 30 min.

References

[1] M. M. Maricq, J. S. Waugh, J. Chem. Phys. 1979, 70, 3300-3316; M. H. Levitt , D. P. Ral eigh, F. Creuzet, R. G. Griffin, *J Chem Phys* ,**1990**, 92, 6347-6364.; Nakai, C. A. M cDowell, *J Chem Phys,* **1992,** 96, 3452-3466.
[2] P. Moutzouri, F. M. Paruzzo, B. Simoes de Almeida, G. Stevanato, L. Emsl ey, Angew. Chem. Int. Ed. Engl. 2020, 59, 6235-6238.
[3] H. Oschkinat, A.Past ore, P. Pfandler, G. Bodenhausen, J. M agn. Reson. 1986, 69, 559-566.
[4] M. C. Davis, K. M. Shookman, J. D. Sillaman, P. J. Grandinetti, J. Magn. Reson. 2011, 210, 51-58; F. M. Paruzzo, B. J. Walder, L. Emsley, J. M agn. Reson. 2019, 305, 131-137.
[5] P. Moutzouri, B. Simoes de Almeida, L. Emsley, J. Magn. Reson. 2020, in press, pre-proof.

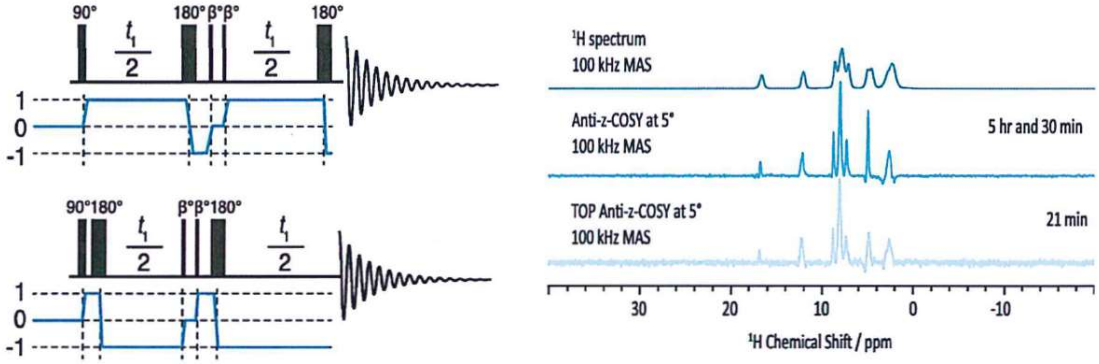


Figure 1. (left) TAZ-COSY pulse sequence and coherence-transfer pathways for the acquisition of echo and anti-echo part of the signal. Here, β indicates a low-flip angle pulse; (right) echo-detected 100 kHz MAS spectrum of powdered microcrystalline L-Histidine monohydrochloride monohydrate (top); 1D spectrum obtained as integral projections of an anti-z COSY spectrum acquired with a full spectral width in the indirect dimension with a β of 5° (middle); 1D spectrum obtained as integral projections from a TAZ-COSY acquired with an indirect spectral with of 1000 HZ with a β of 5°.

RADIAL DISTRIBUTION FUNCTIONS OF COMPLEX PARTICLES BY RELAYED DNP

Pierrick Berruyer[1], Arthur C. Pinon[1], Jasmine Viger-Gravel[1], Glenna L. Drisko[2], Clément Sanchez[3,4], Lyndon Emsley[1]

[1] Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
[2] ICMCB - UMR5026, 87, Avenue du Docteur Schweitzer, 33608 PESSAC, France
[3] Sorbonne Universités UPMC Univ Paris 06, CNRS, Collège de France UMR 7574, Chimie de la Matière Condensée de Paris, F-75005, Paris, France
[4] Chaire USIAS Chimie de la matière ultra divisée, University of Strasbourg Institut of Advanced Studies, France

We have recently established approaches to measure domain sizes and thus reveal key aspects of morphology in materials using the approach dubbed Relayed Dynamic Nuclear Polarization (R-DNP). Prior to R-DNP, the material is impregnated with a solution containing the polarizing agent, and upon microwave irradiation, protons of the frozen solvent are hyperpolarized almost instantaneously and act as a bath of hyperpolarization. The hyperpolarization then *slowly* diffuses into the core of the materials through ^{1}H-^{1}H spin diffusion.[1] By modelling the dynamics of spin diffusion, it has been demonstrated that domain sizes can be measured in different classes of materials, such as microcrystalline powders, mesoporous materials, or two- component polymer film coatings.[2-4] The approach has been recently extended to more complex morphologies including the validation of core-shell structures of organic crystalline nanoparticles for example.[5]

Here, we show that R-DNP can be used to produce a radial distribution function (i.e. a ^{1}D image or depth profile) of core-shell particles. Specifically, R-DNP allows the depth profiling of nanoparticles composed of an amorphous core containing polystyrene, coated with a shell of porous silica containing the surfactant CTAB.

References

[1] A. C. Pinon *et al.*, Measuring Nano- to Microstructures from Relayed Dynamic Nuclear Polarization NMR. *J. Phys. Chem. C*. **121**, 15993–16005 (2017).
[2] L. Zhao, *et al.* DNP-enhanced solid-state NMR spectroscopy of active pharmaceutical ingredients. *Magn. Reson. Chem.*, 1–27 (2018).
[3] S. Björgvinsdóttir *et al.*, Bulk nuclear hyperpolarization of inorganic solids by relay from the surface Bulk nuclear hyperpolarization of inorganic solids by relay from the surface (2018).
[4] A. C. Pinon, *et al.* Core–Shell Structure of Organic Crystalline Nanoparticles Determined by Relayed Dynamic Nuclear Polarization NMR. *J. Phys. Chem. A*. **122**, 8802–8807 (2018).

ON THE USE OF RADIO-FREQUENCY OFFSETS FOR IMPROVING DOUBLE QUANTUM HOMONUCLEAR DIPOLAR RECOUPLING OF HALF-INTEGER QUADRUPOLAR NUCLEI

Nghia Tuan Duong[1,2,#], Daniel Lee[1,⊥], Frédéric Mentink-Vigier[1,$], Olivier Lafon[2,3], Gaël De Paëpe[1]

[1]Univ. Grenoble Alpes, CEA, IRIG-MEM, 38000 Grenoble, France
[2]Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181 – UCCS – Unité de Catalyse et Chimie du Solide, F-59000 Lille, France
[3]Institut Universitaire de France, Paris 75231, France

Current affiliations:
[#] NMR Science and Development Division, RIKEN SPring-8 Center, and Nano-Crystallography Unit, RIKEN-JEOL Collaboration Center, Yokohama, Kanagawa 230-0045, Japan
⊥ Department of Chemical Engineering and Analytical Science, University of Manchester, Sackville Street, Manchester, M13 9PL, UK
[$] National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr, Tallahassee, 32301, FL, USA

Detecting proximities between nuclei is crucial for atomic-scale structure determination with NMR spectroscopy. Different from spin-1/2 nuclei, the methodology for quadrupolar nuclei is limited for solids due to the complex spin dynamics under simultaneous magic-angle spinning (MAS) and radio-frequency irradiation. Herein, the performance of several HORROR-based homonuclear dipolar recoupling sequences are evaluated for ^{27}Al (spin-5/2). It is shown numerically and experimentally on mesoporous alumina that $BR2_2^1$ [1] outperforms the supercycled S_3 sequence [2] and its pure double quantum (DQ) (bracketed) version, [S_3], both in terms of DQ transfer efficiency and bandwidth. This surprising result, given the S_3 sequence is among the best schemes for spin-1/2, is thoroughly explained, highlighting the crucial role of radio-frequency offsets during the $BR2_2^1$ spin dynamics. The analytical approximation of $BR2_2^1$, derived in an offset-toggling frame, clarifies the interplay between offset and DQ efficiency, namely the benefits of off-resonance irradiation and the trough in DQ efficiency for $BR2_2^1$ when the irradiation is central between two resonances, both for spin-1/2 and half-integer-spin quadrupolar nuclei with small quadrupolar interactions. Additionally, density matrix propagations show that the $BR2_2^1$ sequence, applied to quadrupolar nuclei with larger quadrupolar interactions, can create single- and multiple quantum coherences for near on-resonance irradiation. This significantly perturbs the creation of DQ coherences between central transitions of neighboring quadrupolar nuclei, and explains the DQ efficiency trough for on-resonance irradiation. Overall, this work aids experimental acquisition of homonuclear dipolar correlation spectra of half-integer-spin quadrupolar nuclei and provides theoretical insights towards improving recoupling schemes at high magnetic field and fast MAS.

References

[1] Q. Wang, B. Hu, O. Lafon, J. Trébosc, F. Deng, J. P. Amoureux, Double-Quantum Homonuclear NMR Correlation Spectroscopy of Quadrupolar Nuclei Subjected to Magic-Angle Spinning and High Magnetic Field. *J. Magn. Reson.* **200**, 251–260 (2009).

[2] G. Teymoori, B. Pahari, B. Stevensson, M. Edén, Low-Power Broadband Homonuclear Dipolar Recoupling without Decoupling: Double-Quantum ^{13}C NMR Correlations at Very Fast Magic-Angle Spinning. *Chem. Phys. Lett*. **547**, 103–109 (2012).

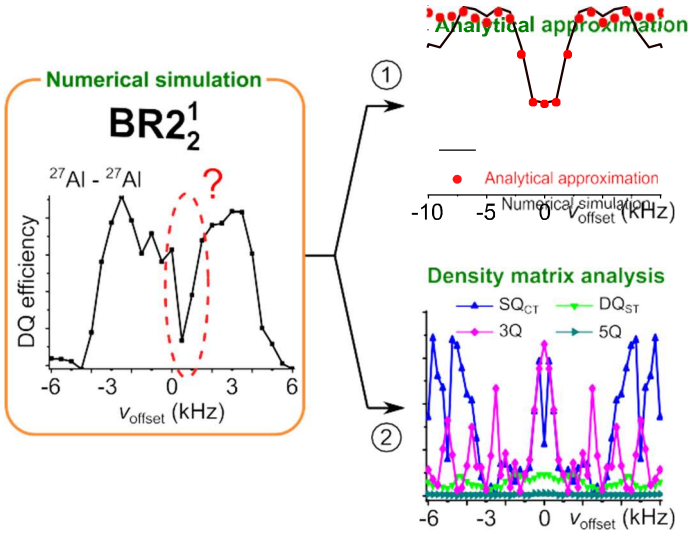


Figure 1. Two theoretical approaches for an insight into the spin dynamics during dipolar recoupling between ^{27}Al nuclei.

THEORY OF COHERENT AVERAGING IN MAGNETIC RESONANCE USING EFFECTIVE HAMILTONIANS

Rajat Garg[1], Ramesh Ramachandran

[1] Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER) Mohali, Sector 81, Manauli P.O. Box-140306, Mohali, Punjab, India

A perturbative approach based on multimode Floquet theory [1] is proposed to explain the coherent averaging effects of radio frequency pulses on nuclear spins in magnetic resonance experiments. In contrast to the contemporary descriptions, RF interaction frame transformation is defined in the Floquet space circumventing the need of evaluating time dependent integrals in the Hilbert space. The present framework not only facilitates continuous detection of time domain NMR signal but also yields faster results desirable for maximizing the efficiency of multiple pulse sequences in a particular experiment [2].

Employing Effective Hamiltonians [3], the utility of the present framework in the context of multiple pulse based Heteronuclear decoupling is discussed.

References

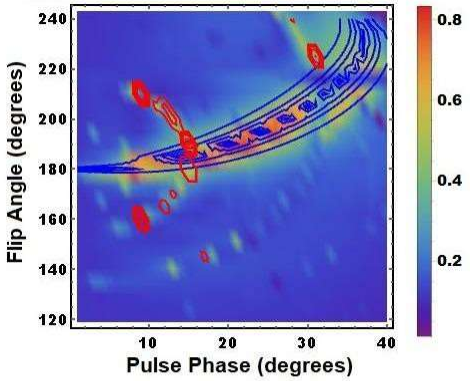
[1] J. H. Shirley, Solution of the Schrödinger Equation with a Hamiltonian Periodic in Time, *Phys. Rev*. **138**, 979 (1965).
[2] Rajat Garg and Ramesh Ramachandran, Theory of coherent averaging in magnetic resonance using effective Hamiltonians, *J. Chem. Phys*., **153**, 034106 (2020).
[3] Ramesh Ramachandran and M. S. Krishnan, Effective Hamiltonians in Floquet theory of magic angle spinning using Van Vleck transformation, *J. Chem. Phys*., **114**, 5967 (2001).

$$\bar{H}_{int}(t) = e^{i\int_0^t H_{RF}(t')dt'} H_{int}(t) e^{-i\int_0^t H_{RF}(t')dt'}$$
$$= H_{int}(t) + i\int_0^t \left[H_{RF}(t'), H_{int}(t)\right] dt' + \ldots\ldots$$

RF interaction frame transformation in the Hilbert space

$$H^M_{F,int} = e^{i\lambda S_M} H_{F,int} e^{-i\lambda S_M}$$
$$= H_{F,int} + i\lambda \left[S_M, H_{F,int}\right] + \ldots\ldots$$

RF interaction frame transformation in the Floquet Hilbert space



In the figure depicted, lines tracing the minimum of residual dipolar coupling (coefficient of I_ZS_{JZ} (blue line) and $I_ZS_{1Z}S_{2Z}$ (red line)) are overlaid the numerically simulated ^{13}C peak height as a function of pulse phase and flip angle for a CH_2 system under TPPM decoupling at (ν_r, ν_{RF}) = (25kHz, 150kHz). From a practical perspective, the residual dipolar couplings (computed analytically) facilitate faster optimization of pulse parameters (computational time = 1 min.) as compared to numerical simulations (computational time = ~6 hours).

PROTON-DETECTED FAST-MAGIC-ANGLE SPINNING NMR OF PARAMAGNETIC INORGANIC SOLIDS

Arthur L. Lejeune[1,2], Jan Blahut[1], Ladislav Benda[1], Kevin J. Sanders[1], Benjamin Burcher[2], Leonor Catita[2], David Proriol[2], Pierre-Alain R. Breuil[2], Anne-Agathe Quoineaud[2], Andrew J. Pell[1,3], Guido Pintacuda[1]

[1] Université de Lyon, Centre de RMN à Très Hauts Champs de Lyon (FRE 2034 - CNRS, ENS Lyon, UCB Lyon 1), 5 rue de la Doua, 69100 Villeurbanne, France.
[2] IFP Energies Nouvelles, Rond-point de l'échangeur de Solaize, 69360 Solaize, France
[3] Stockholm University, Department of Materials and Environmental Chemistry, Svante Arrhenius väg 16C, SE- 106 91 Stockholm, Sweden

^{1}H-NMR spectroscopy is the most obvious avenue to fast routine characterization of chemical compounds and biomolecules due to the ubiquitous presence of ^{1}H nuclei, their large gyromagnetic ratio γ_H and high natural abundance. Nonetheless, these same properties lead to a strong dipolar coupling network which prevents identification of the different ^{1}H sites and limits the utility of the technique. In the case of paramagnetic samples, the problem is further exacerbated by the hyperfine interaction of the high-γ ^{1}H nuclei with the unpaired electrons of the paramagnetic center(s), which introduces further problems in the acquisition and interpretation of the spectra. Here, we show that, by using MAS rates of 60-100 kHz, in combination with tailored RF irradiation schemes and modern computational approaches, it is possible to record and interpret resolved ^{1}H NMR shifts in a powdered sample of a paramagnetic organometallic complex with less than 1 mg of sample at natural abundance. Specifically, we characterize a tetracoordinated 14-valence-electron high-spin (S=2) Fe(II) complex, a newly synthetized catalyst for unsaturated compound transformations such as olefin oligomerization and polymerization. The combination of carefully-tuned pulse sequences and high MAS rates permits extensive experimental constraints for resonance assignment, and marked improvements in sensitivity and resolution, allowing a reduction in sample quantities and experiment time for obtaining NMR spectra of paramagnetic materials. This embodies a dramatic expansion of the experimental capabilities in paramagnetic NMR. We anticipate that the continued development of MAS probes capable of ever higher MAS rates and of the associated RF schemes will extend the horizons of experimental NMR and allow the study of even more complicated systems.

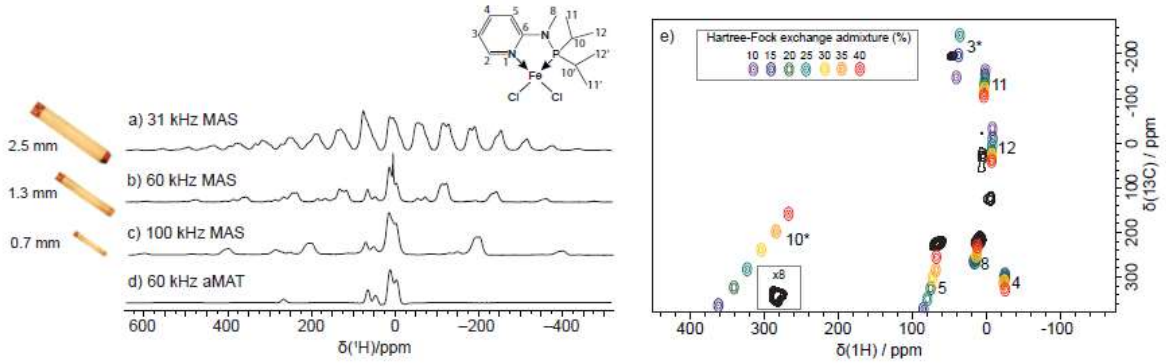


Figure 1. 1D ^{1}H NMR spectra of a high-spin (S=2) Fe(II) complex acquired at different MAS rates at 11.7 T (left) and ^{1}H-detected HSQC-TEDOR spectrum acquired at 100 kHz (right). Superposed to the experimental spectrum (black contours) are the positions of the cross-signals calculated on the basis of the crystal structure using variable Hartree-Fock exchange admixtures (rainbow contours).

OBSERVATION OF LOW-γ QUADRUPOLAR NUCLEI BY SURFACE-ENHANCED NMR SPECTROSCOPY

Hiroki Nagashima,[1*] Julien Trébosc,[2,3] Yoshihiro Kon,[1] Kazuhiko Sato,[1] Olivier Lafon,[2,4*] Jean-Paul Amoureux[2,5,6*]

[1] Interdisciplinary Research Center for Catalytic Chemistry, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan. [2] Univ. Lille, CNRS UMR 8181, UCCS, F-59000 Lille, France.
[3] Univ. Lille, CNRS FR 2638, IMEC: Institut Michel-Eugène Chevreul, F-59000 Lille, France.
[4] Institut Universitaire de France.
[5] Bruker Biospin, 34 rue de l'industrie, F-67166 Wissembourg, France.
[6] Riken NMR Science and Development Division, Yokohama, 230-0045 Kanagawa, Japan.

DNP surface-enhanced NMR spectroscopy (DNP-SENS) has become a powerful technique to probe atomic-level structure of surfaces.[1] However, this approach based on indirect DNP is often inefficient for quadrupolar nuclei with small dipolar coupling to protons, including those with low gyromagnetic ratio (^{67}Zn, ^{95}Mo, ^{47,49}Ti, etc) or located in subsurface region.[2]

In order to circumvent this limitation, we have recently introduced a sequence to efficiently transfer DNP-enhanced ^{1}H polarization to half-integer quadrupolar nuclei.[3] This new method is based on the refocused INEPT scheme with adiabatic symmetry-based dipolar recoupling and continuous-wave irradiation between recoupling blocks in order to limit the losses due to ^{1}H-^{1}H dipolar interaction (Fig.a). This improved robustness allows polarization transfer through small heteronuclear dipolar coupling. Hence, we have leveraged on this novel sequence to enhance the NMR signals of surface low-γ quadrupolar nuclei using indirect DNP and have reported the first DNP-enhanced ^{67}Zn, ^{95}Mo and ^{47,49}Ti NMR spectra. This robust indirect DNP method have also allowed the observation of unprotonated ^{17}O sites located below the surface. These novel capabilities have been applied to characterize the Brønsted acid sites at the surface of titania-supported MoO_3, a widely used heterogeneous catalyst, and to identify the phases near the surface of Al-doped ZnO nanoparticles used in optoelectronics device (Fig.b).

References

[1] A. Lesage, M. Lelli, D. Gajan, M. A. Caporini, V. Vitzthum, P. Miéville, J. Alauzun, A. Roussey, C. Thieuleux, A. Mehdi, G. Bodenhausen, C. Coperet, L. Emsley, *J. Am. Chem. Soc.,* **2010**, *132*, 15459–15461.
[2] W. Li, Q. Wang, J. Xu, F. Aussenac, G. Qi, X. Zhao, P. Gao, C. Wang, F. Deng, *Phys. Chem. Chem. Phys.* **2018**, 20, 17218−17225.
[3] H. Nagashima, J. Trébosc, Y. Kon, K. Sato, O. Lafon, J.-P. Amoureux, *J. Am. Chem. Soc.,* **2020**, *142*, 10659–10672.

Acknowledgements

This presentation is based on results obtained from a project (JPNP16010) commissioned by the New Energy and Industrial Technology Development Organization (NEDO).

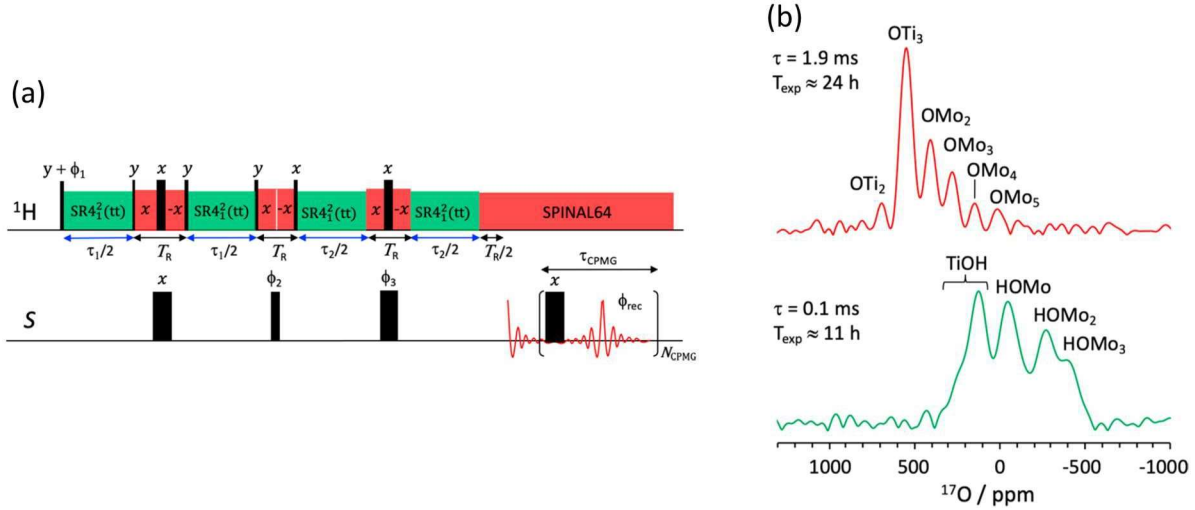


Figure 1. (a) Schematic diagram of adiabatic D-RINEPT pulse sequence. (b) DNP-enhanced ^{17}O MAS spectra of MoO3/TiO2 acquired using adiabatic D-RINEPT transfer with recoupling time τ = 1.9 ms (top) and 0.1 ms (bottom) at ν_R = 10 kHz.

STEADY-STATE FREE PRECESSION IN SOLID-STATE NMR

Tamar Wolf[1], Lucio Frydman[1]

[1] Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot 7610001, Israel

Solid-state nuclear magnetic resonance (ssNMR) provides uniquely rich structural and dynamical insights on both crystalline and amorphous solids. ssNMR is hampered by low sensitivity, for unreceptive nuclei in general, and many half-integer quadrupolar nuclei in particular. Thus, methods to improve the sensitivity of ssNMR spectroscopy are of prime importance.

One widely used approach of data acquisition is the Carr-Purcell Meiboom-Gill (CPMG) sequence[1,2], which was found useful to increase the sensitivity of quadrupolar solids NMR spectra affected by significant inhomogeneous broadenings[3]. Here, we explore the implementation of steady-state free precession (SSFP) experiments, another proposal derived from Carr's early work[4], in the realm of ssNMR of quadrupolar nuclei on static and spinning samples. SSFP sequences provide optimal SNR per unit time, and are widely utilized in magnetic resonance imaging (MRI) acquisitions[5], as well as in nuclear quadrupole resonance (NQR).

We have examined SSFP's ability to enhance the sensitivity of wideline spectra, and in particular the regimes when SSFP could be advantageous compared to CPMG acquisitions. Sensitivity enhancements of between one and two orders of magnitude are theoretically predicted and experimentally demonstrated in certain instances (Fig. 1). Aspects in need ofimprovement to enable a wider use of SSFP-based approaches, particularly to wideline quadrupolar studies, are also inspected.

References

[1] H. Y. Carr, E. M. Purcell, Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Phys. Rev.* **94**, 630–638 (1954).
[2] S. Meiboom , D. Gill, Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Rev. Sci. Instrum.* **29**, 688–691 (1958).
[3] F. H. Larsen, H. J. Jakobsen, P. D. Ellis, N. C. Nielsen, Sensitivity-enhanced quadrupolar-echo NMR of half- integer quadrupolar nuclei. Magnitudes and relative orientation of chemical shielding and quadrupolar coupling tensors. *J. Phys. Chem. A* **101**, 8597–8606 (1997).
[4] H. Y. Carr, Steady-State Free Precession in Nuclear Magnetic Resonance. *Phys. Rev.* **112**, 1693–1701 (1958).
[5] K. Scheffler, S. Lehnhardt, Principles and applications of balanced SSFP techniques. *Eur. Radiol.* **13**, 2409– 2418 (2003).

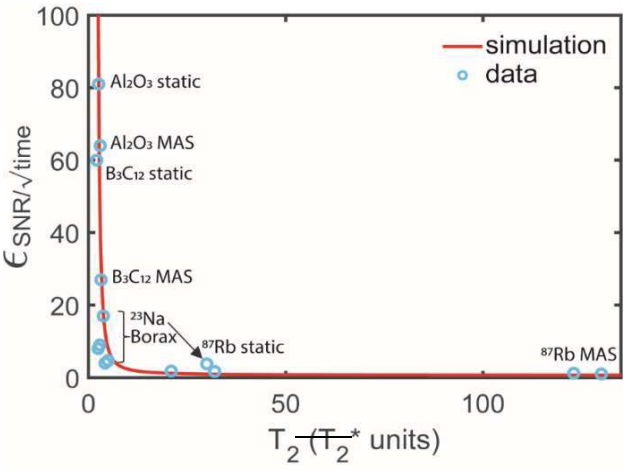


Figure 1. Comparison of the experimentally observed enhancements in $SNR/\sqrt{time}$ afforded by SSFP over CPMG, with a simulation.

12. Solid State NMR - Applications

CAPSULE AND SURFACE ORGANIZATION OF *CRYPTOCOCCUS NEOFORMANS* CELLS IN THEIR INTACT FORM REVEALED BY ^{1}H DETECTED SOLID-STATE NMR AT FAST MAS REGIME

Alons Lends[1], Gaelle Lamon[1], Axelle Grélard[1], Estelle Morvan[1], Inaki Guijarro[2], Birgit Habenstein[1], Vishu Kumar Aimanianda[2], Antoine Loquet[1]

[1] Univ. Bordeaux, CNRS, CBMN IECB, Pessac, France
[2] Institut Pasteur, CNRS, Paris, France

Pathogenic fungal, bacterial or yeast cells possess a molecular barrier at their surface called the cell wall, a critical architecture in constant evolution during the pathogen life cycle and that constitutes a preferential target for drug development. Here we show that solid-state NMR can provide molecular details of the surface of cells in their intact form, based on minimal sample requirement through the use of ^{1}H-detection at 100 kHz magic-angle spinning. The approach is successfully demonstrated on ^{13}C, ^{15}N labeled *Cryptococcus neoformans*, a human pathogen infecting immunocompromised individuals. ^{1}H detection with INEPT based sequences facilitate the observation of an unconventional external structure called the capsule, which was highly mobile (spectrum shown in Fig.1A). The rigid cell wall was probed using CP based experiments (spectrum shown in Fig.2B). Both experiments showed a good resolution and line-widths which are comparable with fully protonated protein samples. Using tailored ^{1}H detected MAS experiments we manage to identify the polysaccharides, proteins and lipids which constitutes to the structure of intact cells and build possible architectural model from the identified molecules (Fig.1C). Our approach enables the establishment of spectroscopic fingerprints to rapidly access pathogenic cell surface composition on intact cells.

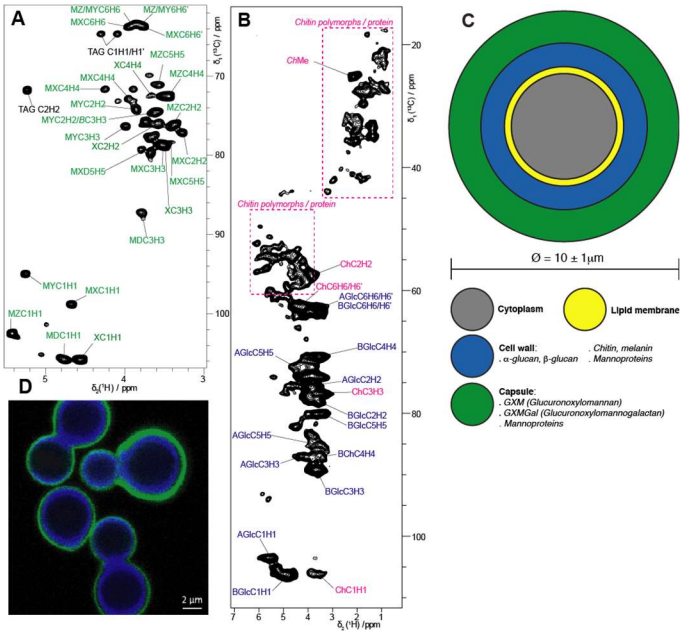


Figure 1. A – hCH CP 2D spectrum, B – hCH INEPT 2D spectrum, C – the overall model of the *C. neoformans* cells with possible location of polysaccharides and lipids based on the NMR data, D – Fluorescence microscopy of C. neoformans cells.

ATOMIC-RESOLUTION STRUCTURE OF HIV-1 CAPSID TUBES BY MAGIC-ANGLE SPINNING NMR

Manman Lu[1,2,3], Ryan W. Russell[1,2], Alexander J. Bryer[1,2], Caitlin M. Quinn[1], Guangjin Hou[1], Huilan Zhang[1], Charles D. Schwieters[4], Juan R. Perilla[1,2], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1] Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA.
[2] Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
[3] Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
[4] Imaging Sciences Laboratory, Center for Information Technology, National Institutes of Health, Bethesda, MD, USA.

The conical mature HIV-1 capsid is assembled from 1000-1500 individual capsid CA proteins. It encloses the viral genome and plays important roles in the viral life cycle[1]. Here, we present the atomic-resolution structure of CA tubes, determined by MAS NMR, combined with low-resolution cryo-EM and data guided molecular dynamics (MD) simulations[2]. The structure of a single CA chain was calculated from experimental MAS NMR restraints comprising 1311 non-redundant ^{13}C-^{13}C distance restraints, 390 torsion angle restraints and 1126 ^{13}C and ^{15}N chemical shifts. A CA hexamer unit was generated by integrating NMR-derived restraints and the low-resolution (8.0 Å) electron density map of a tube. The final structure of a CA tube was created by NMR data-guided MD simulations. Notable differences from the crystallographic structure of flat hexamers[3] were found and novel and unique atomic resolution information on flexible and functionally important regions of the capsid, that are inaccessible by other structural techniques was obtained. Such regions comprise the cyclophilin A-binding loop, the interdomain linker and residues lining the hexamer pore. This novel structural data can be exploited in complementary capsid-targeted therapeutic strategies.

References

[1] E. M. Campbell, T. J. Hope, HIV-1 capsid: the multifaceted key player in HIV-1 infection. Nat. Rev. Microbiol. **13**, 471–483 (2015).
[2] M. Lu, R. W. Russell, A. J. Bryer, C. M. Quinn, G. Hou, H. Zhang, C. D. Schwieters, J. R. Perilla, A. M. Gronenborn, T. Polenova, Atomic-resolution structure of HIV-1 capsid tubes by magic-angle spinning NMR. Nat Struct Mol Biol. **27**, 863–869 (2020).
[3] A. T. Gres, K. A. Kirby, V. N. KewalRamani, J. J. Tanner, O. Pornillos, S. G. Sarafianos, X-ray crystal structures of native HIV-1 capsid protein reveal conformational variability. Science. **349**, 99-103 (2015).

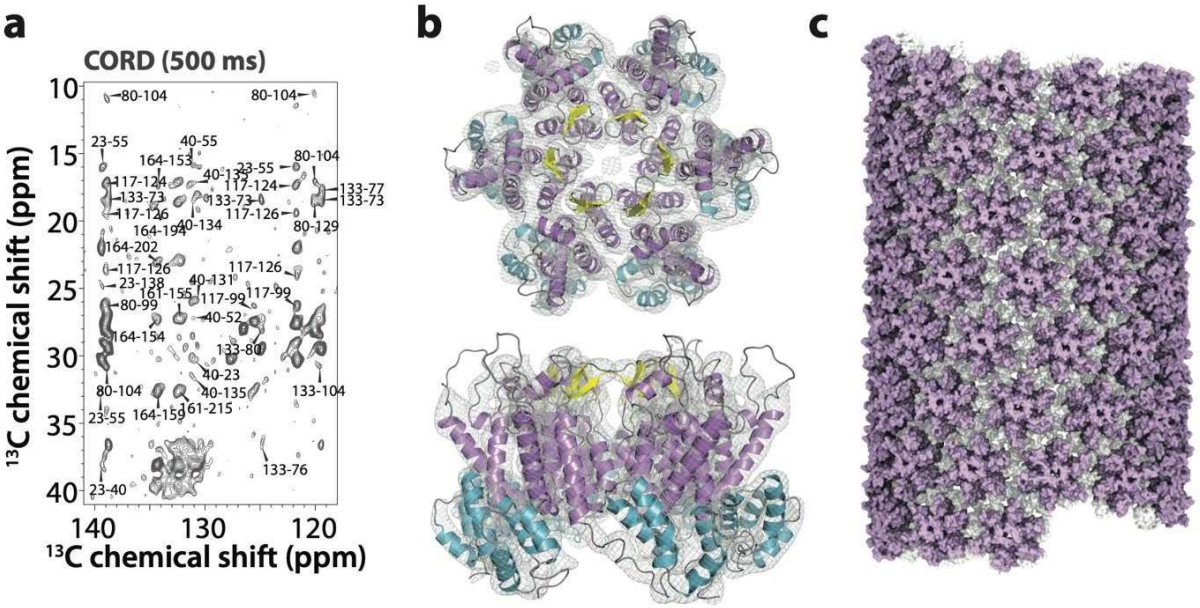


Figure 1. Atomic-resolution structure of the HIV-1 capsid tube by MAS NMR. (a) A selected region of 2D CORD spectra of [1,6-^{13}C]-glucose,U-^{15}N-labeled CA tubes. (b) Top and side views of the superposition of the NMR structure of a CA hexamer unit and the 8-Å resolution cryo- EM map. (c) Molecular surface of a (-12,11) helical symmetry tube derived from NMR data- guided MD simulations.

ELUCIDATION OF THE ATOMIC-LEVEL STRUCTURE OF CEMENTITIOUS CALCIUM ALUMINATE SILICATE HYDRATE BY DNP MAS NMR

Pinelopi Moutzouri[1], Aslam Kunhi Mohamed[2,5], Pierrick Berruyer[1], Brennan J. Walder[1], Jirawan Siramanont[2,3], Maya Harris[2], Mattia Negroni[1], Sandra C. Galmarini[3], Stephen C. Parker[4], Karen L. Scrivener[2], Paul Bowen[2] and Lyndon Emsley[1]

[1]Institut des Sciences et Ingénierie Chimiques, EPFL, Lausanne, Switzerland
[2] Institut des Matériaux, EPFL, Lausanne, Switzerland
¢SCG CEMENT Co., Ltd., Saraburi 18260, Thailand
[3] Building Energy Materials and Components, EMPA, Switzerland
[4] Department of Chemistry, University of Bath, UK
[5] Department of Civil, Environmental and Geomatic Engineering, ETH Zürich, Switzerland

Calcium silicate hydrate (C-S-H), the main hydration product of ordinary Portland cement, has a layered calcium-silicate sheet structure with a disordered interlayer space containing water molecules, hydroxyl groups and calcium ions. When present, aluminum can be incorporated into the C-S-H as aluminate species with coordination number of four, five, and six, forming calcium aluminate silicate hydrate (C-A-S-H). Here, we use first principles calculations to predict that at high Ca:Si and H_2O ratios, the stable coordination number of aluminum is six and that it is incorporated into the bridging sites of the linear silicate chains. The prediction is confirmed experimentally by one- and two-dimensional Dynamic Nuclear Polarization (DNP) enhanced ^{27}Al and ^{29}Si solid-state NMR experiments.[1] In some of the highest field DNP MAS NMR experiments to date, we show that a narrow peak appearing at 5 ppm in the ^{29}Si filtered ^{27}Al NMR spectrum at 21.14 T can be assigned to the silicate-bridging $[AlO_2(OH)_4]^{5-}$ sites. In order to confirm the assignment of this peak a natural abundance DNP enhanced 2D $^{27}Al/^{29}Si$ refocused dipolar INEPT MAS NMR experiment was performed to correlate NMR signals from ^{27}Al nuclei that are less than ~4.3 Å from ^{29}Si. As shown in Figure 1, a correlation of the 5 ppm ^{27}Al NMR signal with ^{29}Si NMR signals at -77 ppm, a ^{29}Si chemical shift which by DFT shielding calculations is consistent with six-coordinate aluminate inserted as a bridge between silicate chains, is observed. We therefore answer the debated and long-standing question of the structural nature of aluminum in C-A-S-H.

References

[1] Mohamed A. K., Moutzouri., P., Berruyer, P., Walder, B.J., Siramanont, J., Harris, M., Negroni, M., Galmarini, S.C., Parker, S.C., Scrivener, K.L., Emsley, L., Bowen, P., *J. Am. Chem. Soc.*, **2020**, 142, 11060

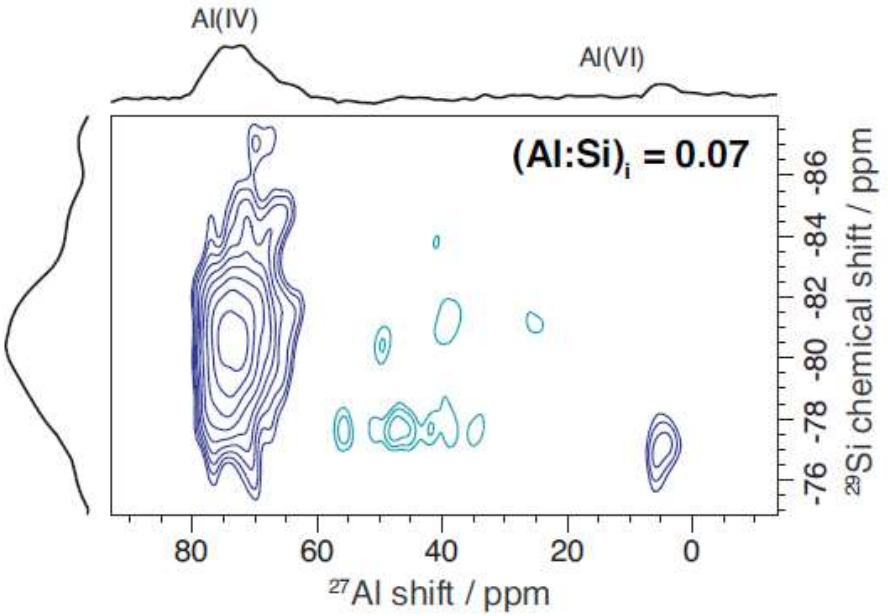


Figure 1: DNP enhanced two-dimensional {^{29}Si}^{27}Al refocused dipolar INEPT MAS spectrum acquired for a synthetic C-A-S-H sample (Ca:Si ratio of 2.0 and (Al:Si) ratio of 0.07) at 9.40 T, 100 K, and 10 kHz MAS.

STRUCTURAL CHARACTERIZATION OF p53 DBD FIBRILS

Sahil Ahlawat [1], Sreejith Rarankurussi [1], Ambuja Navalkar [2], Samir K Maji [2], Vipin Agarwal [1]

[1] TIFR Centre for Interdisciplinary Sciences, Tata Institute of fundamental research, Hyderabad, 500046, India,
[2] Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India

p53 is a transcription factor that inhibits genes required for cell cycle progression and activates genes leading to apoptosis. The function of p53 is to prevent the cell from becoming cancerous by controlling its cell division, and hence it is called “guardian of the genome”. 50% of the reported cancers have mutations in p53. Apart from mutations, another way by which p53 becomes inactive inside cells is its aggregation. The reports of p53 aggregation inside cell lines and cancerous tissues are published [(1)].

We are interested in the structural characterization of the p53 DNA binding domain (DBD) aggregates. The fibrillization of purified p53 DBD was setup at 37˚C with chondroitin sulfate A (CSA) with rotation as mentioned earlier [(2)]. The fibrils obtained were not homogeneous, as p53 DBD is unstable at 37˚C. The p53 DBD stability depends on Zn^{2+} ions, reducing agent and DNA. After binding to the specific DNA sequences, the stability of p53 DBD increases showing higher T_m. The ratio of p53 DBD to its cognate DNA sequence in bound state is 2:1 with two monomer binding to one dsDNA. The p53 DBD was fibrilized in the most stable condition (with Zn^{2+}, TCEP and cognate DNA) and fibrils formed appear to be curvilinear. The fibrils formation takes about five weeks and is not present in the soluble fraction. During fibrils formation, p53 DBD dissociates from the cognate DNA. We performed ssNMR experiments on the uniformly labeled samples. The chemical shifts showed fibrils have β-sheet as the secondary structure. For the assignment of spectra, specific (un)labelling schemes are required.

References

[1] Ano Bom, A. P. D., Rangel, L. P., Costa, D. C. F., de Oliveira, G. A. P., Sanches, D., Braga, C. A., Gava, L. M., Ramos, C. H. I., Cepeda, A. O. T., Stumbo, A. C., De Moura Gallo, C. V., Cordeiro, Y., and Silva, J. L. (2012) Mutant p53 aggregates into prion-like amyloid oligomers and fibrils: implications for cancer. *J. Biol. Chem. 287*, 28152–28162.
[2] Ghosh, S., Salot, S., Sengupta, S., Navalkar, A., Ghosh, D., Jacob, R., Das, S., Kumar, R., Jha, N. N., Sahay, S., Mehra, S., Mohite, G. M., Ghosh, S. K., Kombrabail, M., Krishnamoorthy, G., Chaudhari, P., and Maji, S. K. (2017) p53 amyloid formation leading to its loss of function: implications in cancer pathogenesis. *Cell Death Differ. 24*, 1784–1798.

^{1}H-DETECTION AND DYNAMIC NUCLEAR POLARIZATION-ENHANCED NMR OF Aβ_{1-42} FIBRILS

Salima Bahri[1], Robert Silvers[1,2], Brian Michael[1], Kristaps Jaudzems[3], Daniela Lalli[3], Gilles Casano[4], Olivier Ouari[4], Anne Lesage[3], Guido Pintacuda[3], Sara Linse[5], Robert G. Griffin[1]

[1] Department of Chemistry and Francis Bitter Magnet Laboratory Massachusetts Institute of Technology, Cambridge, MA 02139
[2] Department of Chemistry and Biochemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306
[3] Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCB Lyon 1), 69100 Villeurbanne, France
[4] Aix Marseille Universite, CNRS, ICR, 13013 Marseille, France
[5] Department of Chemistry, Lund University, Lund, SE

Research on Alzheimer's disease (AD) needs rapid and reliable high-throughput characterization, so to address the structural diversity of Aβ plaques and rationalize the role of peptide length, mutations, and post-translational modifications in their formation. These problems are beyond the capability of currently available techniques. Magic-angle spinning (MAS) NMR spectroscopy is an ideal technique for studying amyloids at atomic resolution but suffers from low sensitivity, requiring relatively large amounts of samples and extensive signal acquisition periods. Recent advances in ^{1}H-detection at fast MAS and dynamic nuclear polarization (DNP) have ushered in a new era for NMR-based structural biology, but their potential has not yet been demonstrated for the structural investigation of complex amyloid assemblies. Here we show that resolved and sensitive 2D and 3D correlations are obtained on ^{13}C,^{15}N-enriched and fully-protonated samples of M$_0$Aβ_{1-42} fibrils by ^{1}H detected NMR at $\omega_r/2\pi$ =110 kHz and high field (23.4 T/ 1000 MHz for ^{1}H) at room temperature and ^{13}C detected DNP MAS NMR at $\omega_r/2\pi$=40 kHz and high field (18.8 T/ 800 MHz for ^{1}H) at low temperature. These spectra enable nearly complete resonance assignment of the core of M$_0$Aβ_{1-42} (K16-A42) using sub-mg sample quantities, as well as the detection of numerous unambiguous inter-nuclear proximities defining both the structure of the core and the arrangement of the different monomers. Overall, this work demonstrates the possibility of expeditious structural analysis of amyloid fibrils without requiring preparation of large sample amounts, and illuminates the path to the study of unlabeled AD peptides derived from tissue samples available in limited quantities.

SOLID-STATE NMR STUDIES OF THE DNAJB8 HSP40 CHAPERONE IN ITS OLIGOMERIC STATE

Irina Matlahov[1,2], Bryan D Ryder[3,4], Patrick CA van der Wel[1,2], Lukasz A Joachimiak[4,5]

[1] University of Pittsburgh, Department of Structural Biology, School of Medicine, Pittsburgh, PA 15213, United States.
[2] University of Groningen, Zernike Institute for Advanced Materials, Nijenborgh 4, Groningen, 9747 AG, Netherlands.
[3] University of Texas Southwestern Medical Center, Molecular Biophysics Graduate Program, Dallas, TX 75390
[4] Center of Alzheimer's and Neurodegenerative Diseases, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States.
[5] Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States

Molecular chaperones facilitate cellular protein homeostasis by controlling protein folding, misfolding and aggregation. The Hsp40/Hsp70 chaperone families are central in this crucial process whose age-dependent decay may underpin age-associated diseases such as Huntington's and Alzheimer's disease[1]. It is the Hsp40 "co-chaperones" that tailor the chaperone response to specific disease-associated proteins, and recruit Hsp70 via their characteristic J-domains. Two such oligomeric chaperones, DnaJB6 and DnaJB8, are notable due to their unusually efficient inhibition of polyglutamine (polyQ) aggregation[2] associated with Huntington's disease[3], which is a longstanding focus of ours[4]. These oligomeric proteins have been challenging to understand in detail, due to their propensity to form heterogeneous oligomers that are not amenable to the usual techniques of structural biology.

We present here our use of solid-state NMR (ssNMR) to understand the oligomeric state of DnaJB8, in terms of its domain architecture, structure and dynamics. Various complementary ssNMR experiments show that the oligomeric protein is heterogeneous, as expected, but also shows regions of notable order. The degree of order varies along domains of the chaperone, with parts of the crucial J-domain being particularly well ordered. Despite a high degree of static disorder elsewhere in the protein, the oligomers lack the dynamically flexible regions expected based on sequence analysis and solution phase studies. In higher ionic strength, the protein shows an increase in mobility pointing to an important role of electrostatic interactions in stabilizing the oligomeric assemblies. The ssNMR and other data permitted apparent localization of charge-matching binding interfaces, which could be mapped onto known protein structures of DnaJB8 and homologous proteins. These data pointed to potential auto-inhibitory interactions in these oligomers that control its interactions with Hsp70.

Combined with complementary techniques, our studies of these oligomeric DnaJB8 chaperones provide insights into the architecture and dynamics of this unique member of the Hsp40 family.

References

[1] Kampinga, H. H.; Craig, E. A., The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **2010,** *11* (8), 579-92.
[2] Hageman, J.; Rujano, M. A.; van Waarde, M. A.; Kakkar, V.; Dirks, R. P.; Govorukhina, N.; Oosterveld-Hut, H. M.; Lubsen, N. H.; Kampinga, H. H., A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. *Mol Cell* **2010,** *37* (3), 355- 69.
[3] Gillis, J.; Schipper-Krom, S.; Juenemann, K.; Gruber, A.; Coolen, S.; van den Nieuwendijk, R.; van Veen, H.; Overkleeft, H.; Goedhart, J.; Kampinga, H. H.; Reits, E. A., The DNAJB6 and DNAJB8 protein chaperones prevent intracellular aggregation of polyglutamine peptides. *J Biol Chem* **2013,** *288* (24), 17225-37.
[4] Matlahov, I.; van der Wel, P. C. A., Conformational studies of pathogenic expanded polyglutamine protein deposits from Huntington's disease. *Exp Biol Med* **2019,** *244* (17), 1584-1595.
[5] Matlahov, I.; van der Wel, P. C. A., Hidden motions and motion-induced invisibility: Dynamics-based spectral editing in solid-state NMR. *Methods* **2018**, *148*, 123-135.

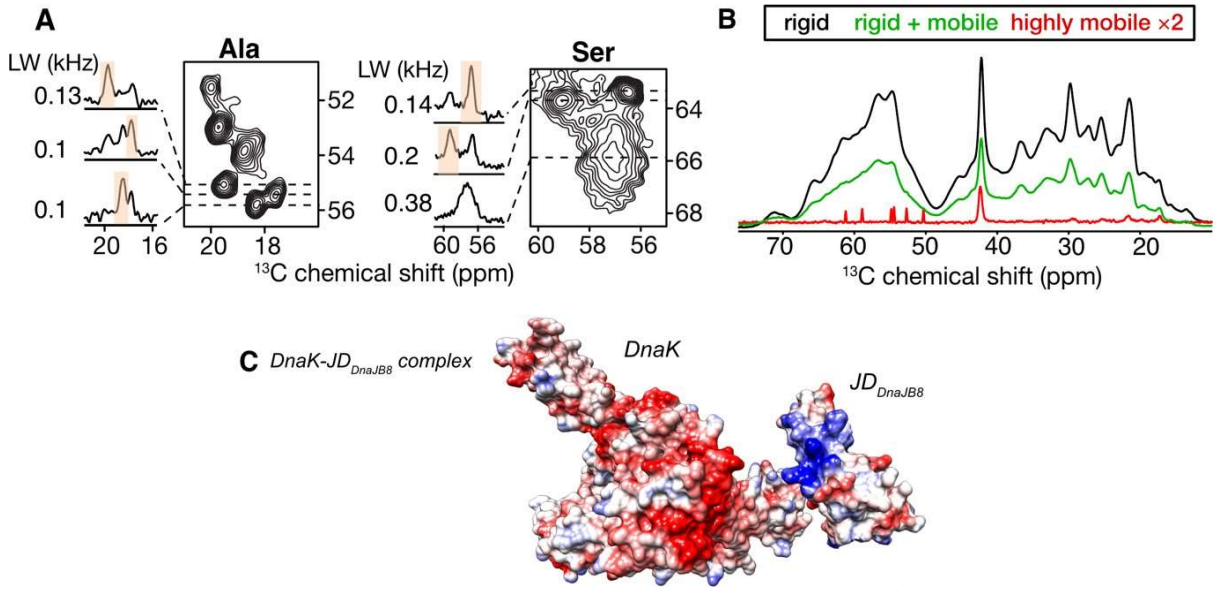


Figure1. Solid-state NMR of DnaJB8 oligomers. **(**A**)** Ala and Ser regions from ^{13}C- ^{13}C DARR 2D spectrum, along with 1D slices through the experimental 2D data. Dramatic variations in linewidth indicated regions of high- and low order in the oligomers. (B) ^{13}C 1D DYSE[5] spectra showing residues with variable levels of mobility, indicating a surprising lack of flexible regions. (C) Electrostatic surface potential of the J domain of DnaJB8 in complex with Hsp70. Highly positive and highly negative potentials are shown as deep blue and deep red respectively. White color represents neutral charge (PDB: 5NRO, 2DMX).

MULTI-HIGH-FIELD ^{27}Al MAS NMR AUGMENTED BY DFT: NEW INSIGHTS ON ALKYLALUMINUM CO-CATALYST STRUCTURE FOR EFFICIENT Ni-CATALYZED ETHENE OLIGOMERIZATION

Ilia B. Moroz[1], Pierre Florian[2], Jasmine Viger-Gravel[3], Christopher P. Gordon[1], Anne Lesage[3], Christophe Copéret*[1]

[1] Department of Chemistry and Applied Biosciences, ETH Zürich, Vladimir-Prelog-Weg 1-5, 8093 Zurich, Switzerland
[2] CEMHTI CNRS UPR3079, Université d'Orléans, 1D Avenue de la Recherche-Scientifique, 45071 Orléans Cedex 2, France
[3] Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCB Lyon 1), 69100 Villeurbanne, France

The oligomerization of ethene is one of the key technologies for the production of α- olefins (e.g. 1-butene, 1-hexene, etc.) that are co-monomers for the synthesis of linear low- density polyethylene. This reaction is catalyzed by Ni-based catalysts and requires addition of co-catalysts, typically alkylaluminum reagents.[1] Despite tremendous efforts there is still a lack of efficient heterogeneous catalysts that could simplify the existing chemical processes. One approach to heterogenize the catalyst is to support molecular aluminum compounds on oxide supports, e.g. on silica (SiO_2), and contact the molecular Ni complex with the obtained solid co-catalyst.[2] However, a poor understanding of the supported co-catalyst structure prevents further improvement of the heterogeneous systems.

Here we will show how multi-high-field ^{27}Al MAS NMR augmented by DFT computations can shed light on the co-catalyst structure. The supported co-catalyst is prepared by exploiting the Surface Organometallic Chemistry approach that consists of (i) a rational design of the molecular Al precursor and (ii) a controlled reaction of the precursor with the surface of silica support. This enables us to provide the first experimental evidence for *monomeric* Al species to be the efficient co-catalysts for Ni-catalyzed ethene oligomerization. More precisely, we were able to identify *three types* of Al surface species, each characterized by a specific isotropic ^{27}Al chemical shift (δ_{iso}) and quadrupolar coupling constant (C_Q). These parameters were obtained from the ^{27}Al MAS NMR measurements at different high magnetic fields (16.4 T and 23.5 T) and compared with the computed values for the cluster models with distinct Al environment. We will discuss the exact structure of these Al species and propose the species that are responsible for the high catalytic activity in ethene oligomerization.

References

[1] E. Y. X. Chen, T. J. Marks, Cocatalysts for Metal-Catalyzed Ofefin Polymerization: Activators, Activation Processes, and Structure-Activity Relationships. *Chem. Rev.* **100**, 1391–1434 (2000).
[2] J. R. Severn, J. C. Chadwick, R. Duchateau, N. Friederichs, "Bound but Not Gagged" – Immobilizing Single-Site α-Olefin Polymerization Catalysts. *Chem. Rev.* **105**, 4073–4147 (2005).

Acknowledgements

This work was supported by Equipex contract ANR-10-EQPX-47-01 and ANR contract ANR-17-CE29-0006-01. I.B.M. is also grateful to Swiss Government Excellence scholarship for financial support.

TRACE LEVEL DETECTION AND QUANTIFICATION OF CRYSTALLINE SILICA IN AN AMORPHOUS SILICA MATRIX WITH NATURAL ABUNDANCE ^{29}SI NMR

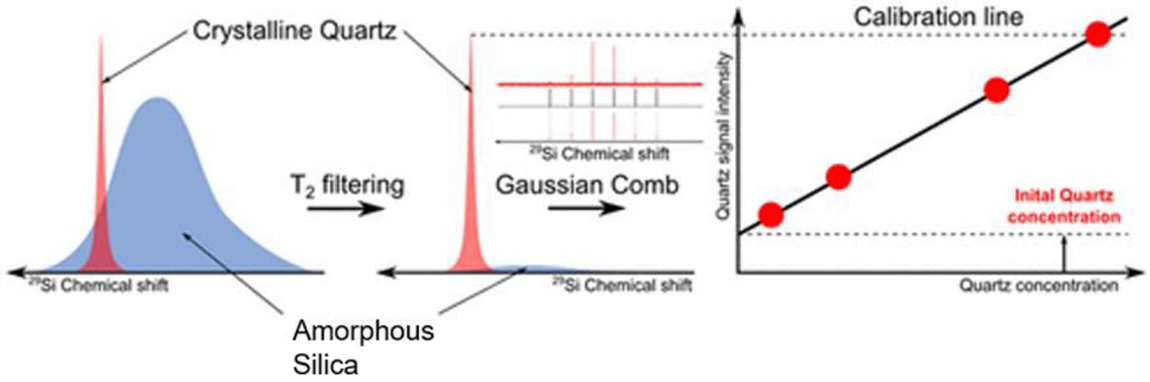
Sambhu Radhakrishnan[1,2], Henri Colaux[2], C. Vinod Chandran[1,2], Dirk Dom[1,2], Loes Verheyden[2], Francis Taulelle[1,2], Johan Martens[1,2], Eric Breynaert [1,2,*]

[1] NMRCoRe, Celestijnenlaan 200F, box 2461, 3001 Leuven, Belgium.
[2] Center for Surface Chemistry and Catalysis – Characterisation and Application Team (COK-kat), Department of Microbial and Molecular Systems (M2S), KU Leuven, Celestijnenlaan 200F, box 2461, 3001 Leuven, Belgium.

A protocol for the detection by NMR spectroscopy of trace amounts of quartz in amorphous silica gels was developed and tested on commercially available samples. Using ^{29}Si MAS NMR spectroscopy with CPMG acquisition and standard addition of crystalline quartz, quantitative detection of quartz concentrations up to 0.1 %wt. was achieved. CPMG permitted to suppress the amorphous silica derived signal, benefitting from the extremely long T_2 relaxation time of quartz in ^{29}Si, and hence dramatically increasing the sensitivity. Dedicated post-processing exploiting the known CPMG spikelet frequencies allowed to probe the near-absence of quartz in commercial, 100 % silica samples, enabling to assess conformity of unknown samples to EU legislation (REACH).

References

[1] S.Radhakrishnan, H. Colaux, C. Vinod Chandran, D. Dom, L. Verheyden, F. Taulelle, J. Martens and E. Breynaert; Trace level detection and quantification of crystalline silica in an amorfous silica matrix with natural abundance ^{29}Si NMR, *Anal. Chem.* **92**, 19, 13004-13009 (2020).



DYNAMICS OF A DIVALENT CATION CHANNEL BY >100 KHZ MAS NMR

Tobias Schubeis[1], Marta Bonaccorsi and Guido Pintacuda

[1] Université de Lyon, Centre de RMN à Très Hauts Champs de Lyon (FRE 2034 - CNRS, ENS Lyon, UCB Lyon 1), 5 rue de la Doua, 69100 Villeurbanne, France.

In recent years, magic-angle spinning (MAS) NMR has developed as a powerful technique for structural biology of membrane proteins, enabling the study of these systems in their native like environment. In particular, faster MAS rates have paved the way for proton-detection in the solid state, allowing the acquisition of resolved proton resonances in fully protonated samples in sub-milligram amounts. This technical progress revolutionises the atomic-level investigation of proteins, expanding the range of information exploitable for the determination of structures and opening new horizons for the investigation of dynamics.

Here we demonstrate that MAS rates of 100 kHz and above, coupled to ultra-high magnetic fields, permit the site-specific measurement of observables connected to local and global dynamics in the bacterial divalent cation channel CorA reconstituted in lipid bilayers. CorA is a 5x42 kDa pentamer comprised of two transmembrane helices and a large cytoplasmic domain hosting a metal binding site. The measurement of residue-specific dynamic parameters provides insights into the conformational equilibria underlying the transport mechanism of cations through the CorA channel. Our data is challenging models previously formulated on the basis of cryo-EM structures.

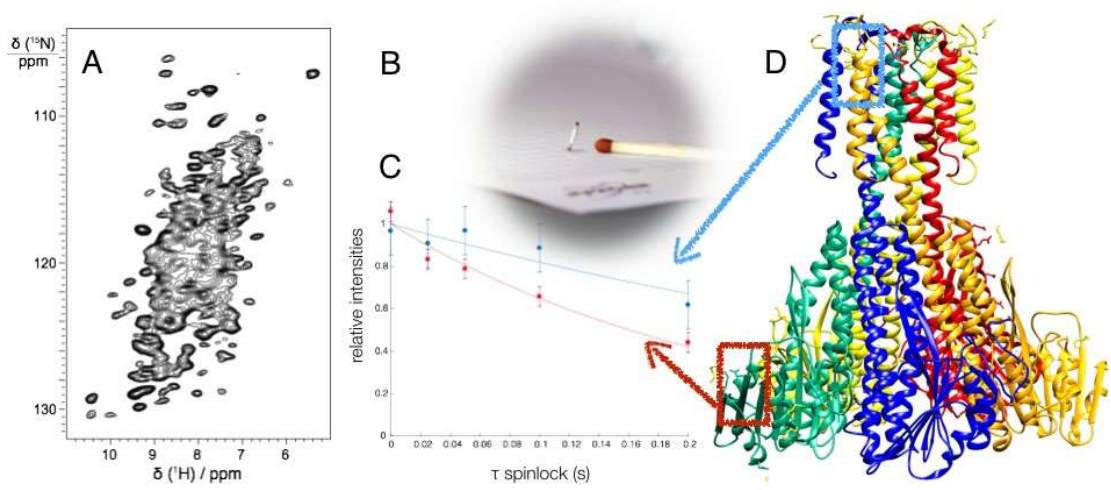


Figure 1. (A) 2D ^{1}H,^{15}N dipolar correlaXon spectrum of Mg^{2+}-bound ^{15}N,^{13}C-CorA in DMPC bilayers, obtained on a 1 GHz spectrometer at 110 kHz MAS. (B) The sample in a 0.7 mm Bruker rotor. (C) Representative ^{15}N R_{1rho} relaxation decays for two Gly residues in the transmembrane and in the cytoplasmic domains of the channel. (D) Ribbon diagram of the CorA cryo-EM structure.

NATURAL ABUNDANCE MAS-DNP NMR SPECTROSCOPY REVEALS HIDDEN INTERACTIONS OF COLLAGEN IN BONES AND CARTILAGE

Nidhi Tiwari[1,2], Sungsool Wi[3], Frederic Mentink-Vigier[3], Navneet Dwivedi[1,4], and Neeraj Sinha

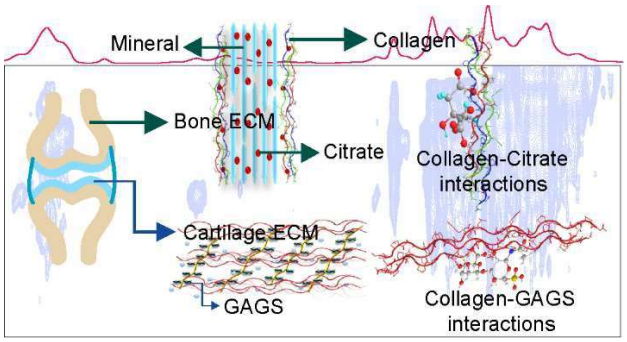
[1]Centre of Biomedical Research, SGPGIMS Campus, Raebarelly Road,Lucknow – 226014, INDIA
[2]Department of Chemistry,Institute of Sciences, Banaras Hindu University, Varanasi – 221005, INDIA
[3]National High Magnetic Field Laboratory, Tallahassee, Florida 32304, USA
[4]Department of Physics, Integral University, Lucknow-226026, INDIA

Higher vertebrates' skeleton is made up of a highly specialized form of connective tissue, which consists of bone and cartilage.[1] These connective tissues are primarily composed of fibrous extracellular matrix (ECM), involved in diverse physiological roles, including nutrient storage, endocrine function, and providing structural integrity.[2] It is the nature of the extracellular matrix (ECM) that define the physicochemical properties of these connective tissues Collagen is an integral component of bone and cartilage ECM, poses an extra challenge to the conventional biophysical spectroscopic methods to probe various interactions responsible for its complex multiscale hierarchical structure.[3,4]

In our proposed study, we shall look at the various interaction among the extracellular matrix of bone and cartilage in their native state. We present a significant advancement to probe the interaction of collagen with other extracellular matrices (ECM) components of native bone and cartilage along with specific/non-specific interactions inside collagen assembly at the nanoscopic level through natural abundance 2D ^{1}H-^{13}C/^{15}N HETCOR and ^{13}C DQ-^{13}C SQ dynamic nuclear polarization (DNP) based solid-state nuclear magnetic resonance (ssNMR) spectroscopy. The detected molecular interactions such as between citrate – collagen and glycosaminoglycan (GAGs) - collagen inside the native bone and cartilage matrix along with other backbone and side-chain interactions in the collagen assembly are responsible for the structural stability of these important class of biomaterial.

References

[1] Hall, B. K. Bones and Cartilage: Developmental and Evolutionary Skeletal Biology: *Second Edition; Elsevier Inc.*, (2015).
[2] McKee, T. J.; Perlman, G.; Morris, M.; Komarova, S. V. Extracellular Matrix Composition of Connective Tissues: A Systematic Review and Meta-Analysis. *Sci. Rep.* 9 (1), 1–15, (2019).
[3] Mroue, K. H.; Viswan, A.; Sinha, N.; Ramamoorthy, A. Solid-State NMR Spectroscopy: The MagicWand to View Bone at Nanoscopic Resolution, *1st ed.; Elsevier Ltd.*, Vol. 92, (2017).
[4] McKee, T. J.; Perlman, G.; Morris, M.; Komarova, S. V. Extracellular Matrix Composition of Connective Tissues: A Systematic Review and Meta-Analysis. *Sci. Rep*. 9 (1), 1–15, (2015)


Mineral
Collagen
Bone ECM
Citrate
Collagen-Citrate interactions
Cartilage ECM
GAGS
Collagen-GAGS interactions

HIGH RESOLUTION ^{1}H SOLID-STATE NMR SPECTROSCOPY OF MATERIALS

Y. T. Angel Wong[1], Roel van de Ven[1], Ruud Aspers[1], Arno P. M. Kentgens[1]

[1]Magnetic Resonance Research Center, Institute of Molecules and Materials, Radboud University, 6500 GL Nijmegen, Netherlands.

Solid-state nuclear magnetic resonance (SSNMR) spectroscopy has played a significant role in materials science due to its ability to provide valuable structural and motional information. In particular, ^{1}H SSNMR spectroscopy is a promising materials characterization method as ^{1}H is ubiquitous, and has a high natural abundance and NMR sensitivity. Nevertheless, these advantages are often counteracted by the presence of strong homonuclear dipolar interactions, resulting in severe line broadening and therefore poor spectral resolution. As such, the applicability of ^{1}H SSNMR spectroscopy is greatly hindered in many cases. Here, we show that high resolution ^{1}H SSNMR spectra can be acquired at moderate spinning speeds via combined rotation and multiple pulse spectroscopy (CRAMPS)[1]. Extraordinary ^{1}H linewidths were obtained for glycine when CRAMPS was combined with micro magic-angle spinning (μMAS) technology.[2] We will discuss ^{1}H spectra of an active pharmaceutical ingredient (API) that show higher resolution at moderate magnetic field strength than those recorded using ultra-fast magic-angle spinning (MAS) at high magnetic field strengths. Our results suggest the possibility of a comprehensive ^{1}H SSNMR-based materials characterization without the need of state-of-the-art equipment (*i.e.*, ultra-fast MAS and ultra-high magnetic field strengths). Continuing work is focusing on further improving the achievable ^{1}H spectral resolution, and utilizing the μMAS method on structurally complex systems.

References

[1] B. C. Gerstein, R. G. Pembleton, R. C. Wilson, L. M. Ryan, High Resolution NMR in Randomly Oriented Solids with Homonuclear Dipolar Broadening: Combined Multiple Pulse NMR and Magic Angle Spinning. *J. Chem. Phys.* **66**, 361-362 (1977). [2]H. Janssen, A. Brinkmann, E. R. H. van Eck, P. J. M. van Bentum, A. P. M. Kentgens, Microcoil High-resolution Magic Angle Spinning NMR Spectroscopy. *J. Am. Chem. Soc.* **128**, 8722-8723 (2006).

PARAMAGNETIC SOLID-STATE NMR TO LOCALIZE THE METAL ION COFACOR IN AN OLIGOMERIC DnaB HELICASE

Johannes Zehnder[1], Riccardo Cadalbert[1], Laurent Terradot[2], Peter Güntert[1], Anja Böckmann[2], Beat H. Meier[1] and Thomas Wiegand[1]

[1] Laboratorium für Physikalische Chemie, ETH Zürich, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland.
[2] IBCP BMSSI, 7 Passage du Vercors, 69007 Lyon, France.

Solid-state NMR is a versatile tool to study conformational and dynamic changes of a protein during enzymatic reaction cycles such as the hydrolysis of ATP occurring in motor proteins. ATP-binding is accompanied by the binding of a Mg^{2+} cofactor which can be exchanged with paramagnetic ions (e.g. Mn^{2+} and Co^{2+}). Such bound paramagnetic metal ions deliver structural information in solid-state NMR experiments, since the paramagnetic effects, such as paramagnetic relaxation enhancements (PREs) or pseudo-contact shifts (PCSs), scale with the distance between the metal and the nuclei of the protein. We herein present a strategy using paramagnetic solid-state NMR to determine the metal position in the bacterial DnaB helicase (672 kDa) complexed with ADP:AlF_4^-, a metal ion and single-stranded DNA. DARR, NCA and NCACB spectra of the paramagnetic protein samples were analyzed to extract PREs allowing accessing the radii of the blind spheres of the different metals ions in which protein resonances are broadened beyond detection [3]. PCS were extracted from the Co^{2+}-containing samples.

The determination of the metal ion position in our starting model of apo DnaB based on a low-resolution X-ray structure was done with CYANA. We developed a protocol for the optimization of the metal positions in such a large oligomeric assembly based on solid-state PRE and PCS distance restraints. This not only allowed us to localize the metal ion in the nucleotide-binding domain of DnaB, but also enabled the verification of the only available low-resolution structural model. In contrast to chemical-shift perturbations occurring upon nucleotide binding, information from paramagnetic NMR is not biased by allosteric effects which influence chemical-shift values often used to monitor nucleotide binding.

References

[1] Thomas Wiegand, Riccardo Cadalbert, Carole Gardiennet, Joanna Timmins, Laurent Terradot, Anja Böckmann, Beat H. Meier, *Angew. Chem. Int. Ed.*, **2016**, *55*, 14164-14168.
[2] Thomas Wiegand, Riccardo Cadalbert, Denis Lacabanne, Joanna Timmins, Laurent Terradot, Anja Böckmann, Beat H. Meier, *Nat. Commun.*, **2019**, *10* (31).
[3] Andrew J. Pell, Guido Pintacuda, Clare P. Grey, *Prog. Nucl. Magn. Reson. Spectrosc.*, **2019**, *111*, 1-271.

13. Solution NMR - Methods

MAPPING SLOW AND INTERMEDIATE EXCHANGE FROM STRUCTURAL INTERCONVERSION OR INTERMOLECULAR INTERACTION IN PROTEIN NMR SPECTRA

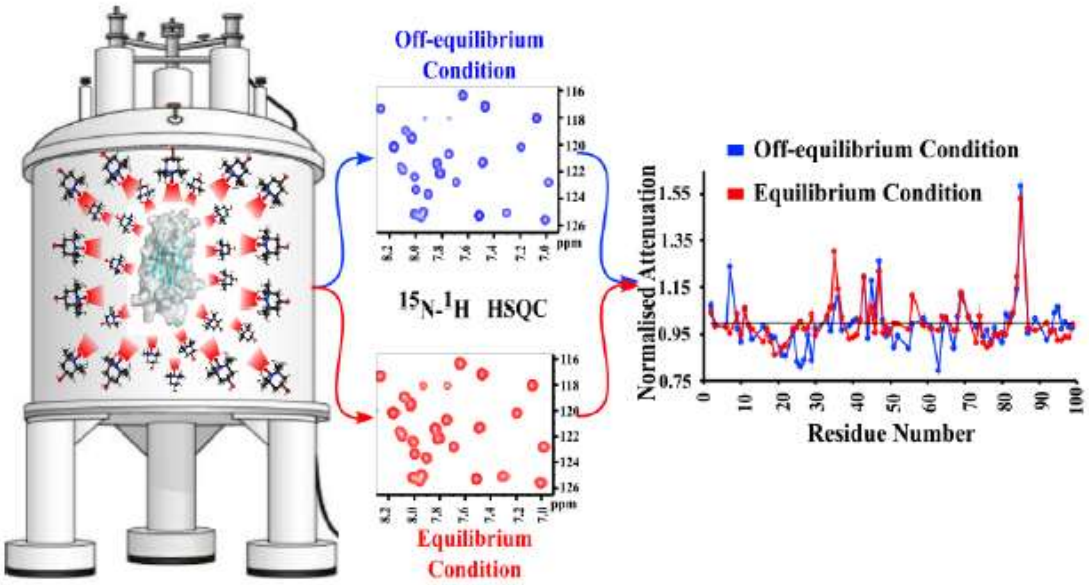
Yamanappa Hunashal[1,2], Cristina Cantarutti[3], Sofia Giorgetti[4], Loredana Marchese[4], Henriette Molinari[5], Neri Niccolai[6], Federico Fogolari[7,8], Gennaro Esposito[2,8]

[1] DAME, Università di Udine, 33100 Udine, Italy
[2] Science Division, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates.
[3] Institute of Chemistry, UMR CNRS 7272, Université Côte d'Azur, University of Nice Sophia Antipolis, Parc Valrose, 06108, Nice Cedex 2, France
[4] Dipartimento di Medicina Molecolare, Università di Pavia, Via Taramelli 3, 27100 Pavia, Italy
[5] Istituto di Scienze e Tecnologie Chimiche (SCITEC), CNR, Via A. Corti, 12, 20133, Milano, Italy
[6] Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, Via Moro 2, 53100 Siena, Italy
[7] DMIF, Università di Udine, 33100 Udine, Italy
[8] INBB, Viale Medaglie d'Oro 305, 00136 Roma, Italy

A new method based on the assessment of the paramagnetic perturbation on the NMR spectra of proteins is described. The paramagnetic perturbation is applied through water-soluble extrinsic probes such as Tempol, a nitroxide forming a stable free radical. The method relies on the measurement of equilibrium and off-equilibrium attenuations due to the effect of the radical unpaired electron. In particular, the individual backbone amide signals observed in HSQC or TOCSY NMR spectra of proteins report the nitroxide accessibility to the molecular surface in terms of specific extent of attenuation (1,2). When paramagnetic attenuation is sampled under non-equilibrium conditions, the signal intensities also reflect additional relaxation processes arising from slow or intermediate exchange events due to structural interconversion or intermolecular interaction. Thus, besides the local conformational dynamics, one can also map the protein-protein association equilibria that are relevant for oligomerization or precede the nucleation steps of amyloidogenic proteins. Applications to amyloidogenic and ordinary protein models such as β2-microglobulin and hen-egg-white lysozyme will be presented.

References

[1] G. Esposito, A.M. Lesk, H. Molinari, A. Motta, N. Niccolai, A. Pastore. *J. Mol. Biol*. **224**, 659–670 (1992).
[2] H. Molinari, G. Esposito, L. Ragona, M. Pegna, N. Niccolai, R.M. Brunne, A.M. Lesk, L. Zetta, *Biophys. J*. **73**, 382–396 (1997).


Off-equilibrium Condition
15N-1H HSQC
Equilibrium Condition
Off-equilibrium Condition
Equilibrium Condition
Normalised Attenuation
Residue Number

TEMPERATURE AND CONCENTRATION AS EXTRA DIMENSIONS IN MULTIDIMENSIONAL NMR

Krzysztof Kazimierczuk[1]

[1] Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

NMR experiments are often acquired in series to study how external conditions (temperature, pH, pressure, ligand concentration) affect spectra and draw conclusions about molecular structure and dynamics. The acquisition is particularly time-consuming in the case of multidimensional NMR experiments since sensitivity and resolution have to be sufficient in each spectrum separately. Although non-uniform sampling (NUS) can help to accelerate each experiment in a series, it is still sensitivity-limited. Also, NUS requires more sampling points for crowded spectra, e.g. large or disordered proteins.

In my talk, I will present three very recent works from my group. All allow accelerating serial NMR experiments far beyond the NUS limit and all are inspired by the Radon transform[1,2]. I will discuss the common concept behind the following methods:

- fast acquisition of pseudo-2D pure-shift spectra in variable-temperature studies[3]
- temperature-swept 3D NUS HNCO spectra to study dynamics of intrinsically disordered proteins[4]
- rapid acquisition of 2D HSQC spectra to study protein-ligand binding[5]

References

[1] E. Kupče, R. Freeman Mapping molecular perturbations by a new form of two-dimensional spectroscopy. J. Am. Chem. Soc. 2013;135:2871–2874. doi: 10.1021/ja310107e.
[2] R. Dass, P. Kasprzak, K. Kazimierczuk Quick, sensitive serial NMR experiments with Radon transform. 2017; 282: 114-118. doi: 10.1016/j.jmr.2017.07.011.
[3] M. Rytel, P. Kasprzak, P. Setny, and K. Kazimierczuk. Quick temperature-sweep pure-shift NMR: The case of solvent effects in atorvastatin. Physical Chemistry Chemical Physics, 21(35):19209–19215, 2019.
[4] A. Shchukina, P. Malecki, B. Mateos, M. Nowakowski, M. Urbanczyk, G. Kontaxis, P. Kasprzak, C. Conrad-Billroth, R. Konrat, and K. Kazimierczuk. Temperature as an extra dimension in multidimensional protein NMR. Chemistry - a European Journal. doi:10.1002/chem.202003678.
[5] J.A. Romero, E. K. Nawrocka, A. Shchukina, F. Blanco, T. Diercks, and K. Kazimierczuk. Non-stationary complementary non-uniform sampling (NOSCO NUS) for fast acquisition of serial 2D NMR titration data. Angew. Chem. Int. Ed. 10.1002/ange.202009479.

GENERALIZED TRANSFORMATION OF MAGNETISATION INTO ULTRA-LONG-LIVED EIGENORDER

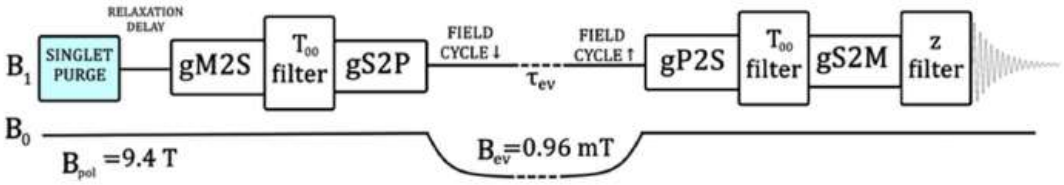
Mohamed Sabba[1], Christian Bengs[1], Johannes Colell[2], Andrew Hall[3], Laurynas Dagys[1], Weidong Gong[1], Lynda J. Brown[1], Malcolm H. Levitt[1]

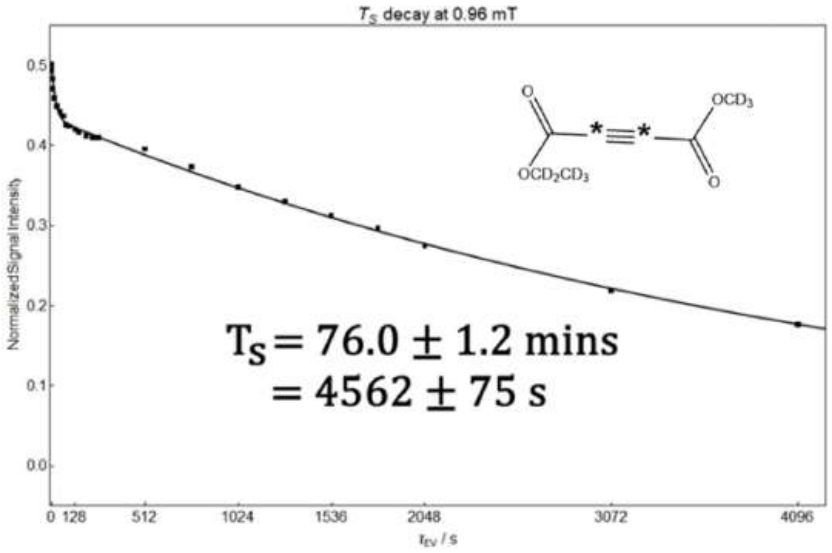
[1] School of Chemistry, University of Southampton, SO17 1BJ, United Kingdom
[2] Institut für Technische und Makromolekulare Chemie, RWTH Aachen University, Worringer Weg 1, 52074 Aachen (Germany)
[3] School of Chemistry, University of Edinburgh, EH9 3JJ, United Kingdom

Various schemes have been described for the generation of the most ubiquitous form of long-lived order- **singlet order** - in 2-spin systems in the presence of chemical shift inequivalence. These schemes have varying performance - recently, we have described the gM2S pulse sequence which accomplishes efficient, robust generation of singlet order across a wide range of inequivalence regimes[1]. However, for singlet order to relax as an eigenorder, strong rf fields (spin-locking) or fast-field cycling is required, which may be impractical or even dangerous to implement when the inequivalence is large; this presents an obstacle to biomedical applications. We present a new sequence, gS2P, which efficiently converts singlet order into long-lived eigenorder, requiring no spin-locking and being ideally suited for adiabatic processes such as slow field-cycling. We illustrate the utility of the gS2P sequence in generating long-lived eigenorder in a series of derivatives of $^{13}C_2$-butynedioc acid (an important molecule in pyruvate metabolism), coupling it to gM2S/field-cycling and discovering exceptional ultra-long-lived lifetimes in excess of one hour – the first such discovery since 2015[2]. This new methodology is expected to pave the way to extended applications of long-lived order in NMR.

References

[1] Christian Bengs, Mohamed Sabba, Alexej Jerschow, and Malcolm H. Levitt; Generalised magnetisation-to-singlet-order transfer in nuclear magnetic resonance. *Phys. Chem. Chem. Phys.*, ***22**, 9703-9712 (2020)*
[2] Gabriele Stevanato, Joseph T. Hill-Cousins, Pär Håkansson, Soumya Singha Roy, Lynda J. Brown, Richard C. D. Brown, Giuseppe Pileio, Malcolm H. Levitt; *Angew. Chem.*, ***54**, 3740-3743 (2015)*


RELAXATION DELAY
B1
SINGLET PURGE
gM2S
T00 filter
gS2P
FIELD CYCLE ↓
τev
FIELD CYCLE ↑
gP2S
T00 filter
gS2M
z filter
B0
Bpol =9.4 T
Bev=0.96 mT

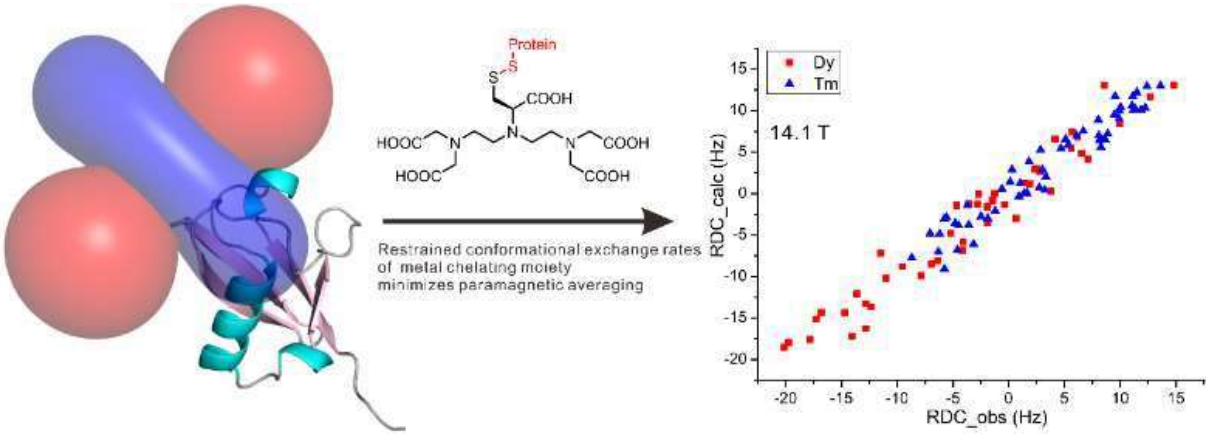

TS decay at 0.96 mT
Normalized Signal Intensity
0.5
0.4
0.3
0.2
0.1
0.0
0 128 512 1024 1536 2048 3072 4096
τEV / s
OCD3
OCD2CD3
TS = 76.0 ± 1.2 mins
= 4562 ± 75 s

DYNAMIC EXCHANGE OF METAL CHELATING MOIETY: A KEY FACTOR IN DETERMINING THE RIGIDITY OF PROTEIN-TAG CONJUATE IN PARAMAGNETIC NMR

Jia-Liang Chen, Bin Li, Xun-Cheng Su[1]

[1]State Key Laboratory of Elemento-organic Chemistry, Nankai University, Tianjin 300071, China

The paramagnetic effects manifested in NMR spectroscopy generally contain paramagnetic relaxation enhancement (PRE), pseudocontact shift (PCS) and residual dipolar coupling (RDC), and these effects correlate tightly to the flexibility of protein-tag conjugates. Rigidity of paramagnetic tag is of great importance in decoding the structural details of macromolecular complexes, since paramagnetic averaging reduces the PCSs and RDCs. Methods in increase the rigidity of paramagnetic tag have been proposed by shortening the tether or using a double arm linkage between a protein and the paramagnetic tag, and coordination of paramagnetic center with protein sidechains. Here we show that the dynamic exchange of metal chelating moiety is a key factor in determining the rigidity of a paramagnetic tag in the protein conjugates. Slowing down the conformational exchange rates in metal chelating moiety greatly minimizes the paramagnetic averaging and increases PCSs and RDCs, and this effect has been demonstrated in an open-chain tag, Py-L-Cys-DTPA. In the protein-L-Cys-DTPA conjugates, this tag generates large PCSs and RDCs that are comparable to the reported cyclic DOTA-like tags. The proposed method sets a new way to design high-quality paramagnetic tags for the applications in biological systems.



PREFERENTIAL SOLVATION OF CARBOHYDRATES IN WATER - TRIFLUOROETHANOL MIXTURES: A SOLVENT DETECTED HETERONUCLEAR NMR APPROACH

Bhawna Chaubey[1], N. Chandrakumar[2], Samanwita Pal[1]

[1] Department of Chemistry, Indian Institute of Technology Jodhpur, Jodhpur, Rajasthan, India-342037.
[2] MRI-MRS Centre, Indian Institute of Technology Madras, Madras, Tamil Nadu, India- 600036,

The present study aims to establish a simple approach involving multiple field (MF) multinuclear longitudinal relaxation analysis of the solvents to decipher solute-solvent interactions during solvation. Aqueous trifluoroethanol (TFE) co-solvent systems (TFE: D_2O) containing model carbohydrates are chosen. Carbohydrates undergo marked preferential solvation in several solvent mixtures.[1,2] On the other hand TFE-water demonstrates extremely interesting solvent dynamics in terms of TFE clustering effect, hydrogen bonding etc. as a function of solvent compositions. In literature preferential solvation of carbohydrates such as cyclodextrins (CD) has been investigated by mapping the intermolecular cross-relaxation or Nuclear Overhauser Effect (NOE) between solute- solvent[3,4]. As opposed to these well-established methods, we have determined ratio of longitudinal relaxation rates (R_1^{carbo}/R_1^{free}) of solvents in the presence and absence of carbohydrates to monitor the dynamics of the solvents around β-CD and glucose. Low field (0.34 T) ^{19}F longitudinal relaxation rates (R_{1F}) of TFE along with high field (11.7 T) R_{1D} for D_2O are measured for this purpose. Figure 1 exhibits the behavior of relaxation rate ratio as a function of TFE % (v/v) composition. Correlation times (τ_c) are further extracted for both TFE and D_2O for various compositions of % (v/v) TFE: D_2O co-solvent systems considering intramolecular dipolar interactions in case of low field ^{19}F and quadrupole interaction in case of high field ^{2}H as the sole relaxation mechanisms. The differential trends of R_1 or τ_c ratio for TFE and D_2O (in presence & absence of carbohydrates) indicate that test carbohydrates undergo selective solvation by TFE. The maximum transfer of solute bound water to bulk solvent is achieved in 20-30% (v/v) TFE range. MF relaxation study of solvents emerges as a straightforward approach unraveling selective solvation for the first time.

References

[1] Vishnyakov, A., Laaksonen, A. Carbohydrates Exhibit a Distinct Preferential Solvation Pattern in Binary Aqueous Solvent Mixtures. *Angew Chem Int Ed Engl.* **112**, 144–146 (2000).
[2] Bagno, A., Rastrelli, F., Scorrano, G. Detecting Intermolecular NOEs by Means of a Novel DPFGSE Pulse Sequence. Application to the Solvation of Carbohydrates in Binary Mixtures. *J. Magn. Reson.* **167**, 31– 35 (2004).
[3] Halle, B. Cross-Relaxation between Macromolecular and Solvent Spins: The Role of Long-Range Dipole Couplings. *J. Chem. Phys.* **119**, 12372–12385, (2003).
[4] Guerrero-Martínez, A.; Berger, S.; Tardajos, G. Selective Solvation of Cyclodextrins by Small Molecules: A NOE Study. *ChemPhysChem*, **7**, 2074–2076, (2006).

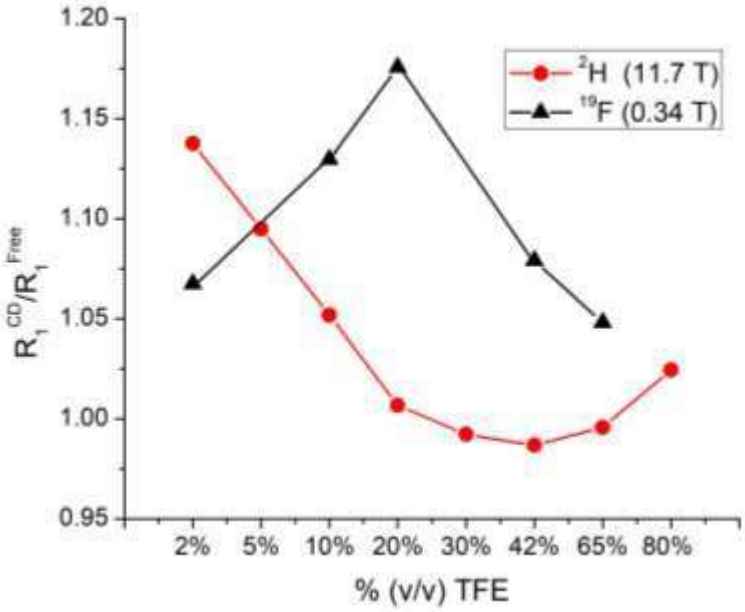


Figure 1. R_{1D} and R_{1F} ratio for D_2O (^2H at 11.7 T) and TFE (^{19}F at 0.34 T) respectively in the presence of β-CD to free co-solvent mixture (without β-CD) as a function of % (v/v) TFE composition in D_2O at 298 K.

NANOPARTICLE-ASSISTED NMR CHEMOSENSING: ENHANCED DETECTION OF ANALYTES THROUGH A WATER-MEDIATED SATURATION TRANSFER

Federico De Biasi[1,2], Daniele Rosa-Gastaldo[1], Xiaohuan Sun[1], Fabrizio Mancin[1], Federico Rastrelli[1]

[1]Department of Chemical Sciences, University of Padua, via Marzolo 1, 35131 Padova (PD), Italy
[2]Department of Chemistry, University of Florence, via della Lastruccia 3-13, 50019 Sesto Fiorentino (FI), Italy

The self-organization of thiolate organic molecules onto the surface of gold nanoparticles (AuNPs) provides a simple route for the creation of coating monolayers in which the physico-chemical properties of each coating molecule are replicated tens to hundreds of times. By exploiting different non-covalent interactions (namely hydrophobic, H-bonding, ion pairing…), such monolayer-protected AuNPs can in turn provide tailored host sites for virtually any class of guest molecules.[1,2] Notably, the reduced translational and rotational diffusion rates of AuNPs induced by their bulky metallic core offer the possibility to selectively manipulate the magnetization of those species that bind to the monolayer through diffusion- and relaxation-based NMR protocols.[3] These so-called nanoparticle-assisted "NMR chemosensing" experiments represent an alternate route in the context of mixtures analysis compared to the more conventional chromatographic approaches, allowing the detection and identification of target molecules within multi-analyte matrices using solely NMR spectroscopy.

The advantages of NMR chemosensing are manifold and include the possibility to unambiguously identify the interacting analytes based on their spectral fingerprint, even in samples of unknown composition.[4] However useful in the analysis of mixtures, the intrinsic sensitivity of this approach remains a challenge, as in many other NMR experiments. For aqueous systems, we have recently demonstrated that water spins in long-lived association at the nanoparticles' monolayer offer an additional source of magnetization which, in combination with high-power saturation transfer experiments, can deliver a boost in sensitivity.[5] This allowed us to probe analytes down to 50 µM in 1 h of acquisition time using standard instrumentation (a 500 MHz spectrometer equipped with a non-cryogenic probe). The presented approach is general and can be applied to host-guest systems of any composition.

References

[1] *J. Am. Chem. Soc.*, **2005**, *127* (47), 16384 – 16385
[2] *Angew. Chem. Int. Ed.*, **2019**, *58* (23), 7702 – 7707
[3] *Prog. Nucl. Magn. Reson. Spectrosc.*, **2020**, *117*, 70 – 88
[4] *Chem. Sci.*, **2018**, *9*, 4777 – 4784
[5] *J. Am. Chem. Soc.*, **2019**, *141* (12), 4870 – 4877

THE PRACTICAL ASPECTS OF NON-STATIONARY NMR MEASUREMENTS

Ewa Klaudia Nawrocka[1,2], Krzysztof Kazimierczuk[1]

[1]Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland.
[2]Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-089 Warsaw, Poland.

Serial 2D NMR experiments help to understand the essence of many chemical and physical phenomena such as the reaction mechanism[1] or ligand binding[2]. Their duration, however, may be prohibitive.

As shown in our recent works[1,2,3] (Figure 1), the serial measurements can be accelerated by a joint sampling of indirect evolution times and the parameter that is varied (reaction progress, concentration, temperature, etc.). Here, we will present the practical aspects of such measurements - reducing their duration, collection of reference "stationary" spectra[1,2], generation of sampling[2], and the possible automation. The procedures needed to set up a non-stationary experiment will be shown, as well as the practical examples of the processing approaches[4,5].

References

[1] E. K. Nawrocka et al. Non-stationary 2D NMR – a novel method for studying reaction mechanism in situ. *Anal. Chem*. **91**, 11306−11315 (2019).
[2] J. A. Romero et al. Non-Stationary Complementary Non-Uniform Sampling (NOSCO NUS) for Fast Acquisition of Serial 2D NMR Titration Data. *Angew. Chem.* **132**, 1–5 (2020).
[3] D. Gołowicz, P. Kasprzak, Vladislav Orekhov, K. Kazimierczuk; Fast time-resolved NMR with non-uniform sampling. *Progress in Nuclear Magnetic Resonance Spectroscopy* **116**, 40–55 (2020).
[4] M. Urbańczyk et al.; TReNDS—Software for reaction monitoring with time-resolved non-uniform sampling. *Magn. Reson. Chem.* **57 (1)**, 4–12 (2012) (web page: http://trends.spektrino.com).
[5] https://doi.org/10.1002/chem.202003678.

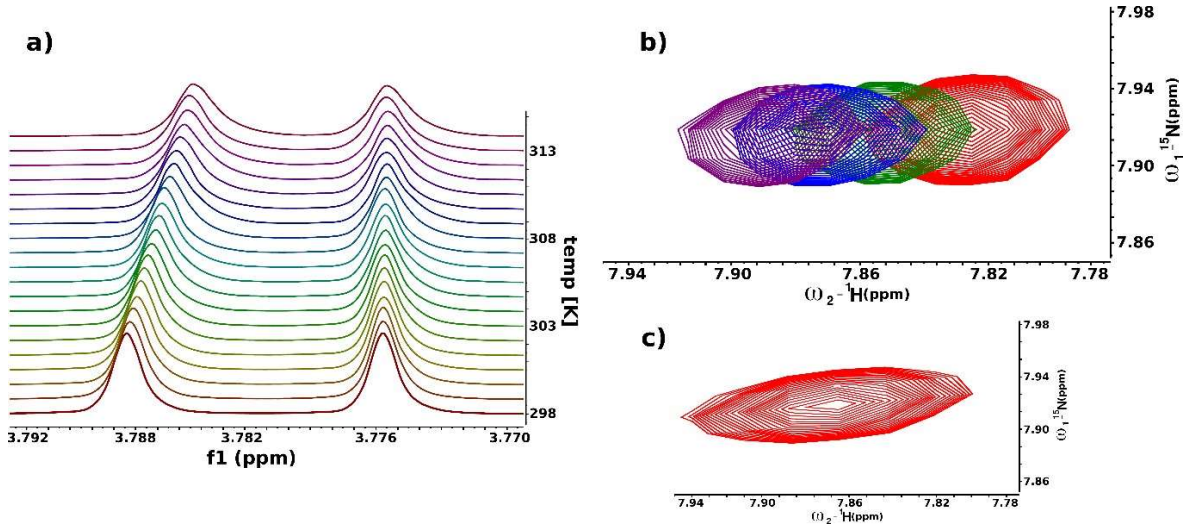


Figure 1. Examples of non-stationarity: a) 1D experiments (stacked spectra in different temperatures); b) 2D NMR spectra from titration series; c) the same experiment but as a combination of these signals produces a single 2D broadened peak.

OPTIMIZED ADIABATIC PULSES FOR TRANSFERRING SINGLET ORDER TO HETERONUCLEAR MAGNETIZATION: APPLICATION TO FUMARATE HYPERPOLARIZED WITH PARAHYDROGEN

Bogdan A. Rodin[1,2], Vitaly P. Kozinenko[1,2], James Eills[3], Konstantin L. Ivanov[1,2]

[1] International Tomography Center SB RAS, Novosibirsk, 630090, Russia
[2] Novosibirsk State University, Novosibirsk, 630090, Russia
[3] Helmholtz Institute Mainz, Johannes Gutenberg University, 55099 Mainz, Germany

Singlet order is can be defined for a coupled spin pair as the difference between the singlet state population and the mean population of triplet states. Singlet order often relaxes much slower than magnetization, i.e., it is often long-lived, which allows one studying various slow processes and preserving hyperpolarized spin order in the singlet state. A particularly interesting and relevant problem in this area is given by transfer of the singlet order of two protons to polarization of a neighboring heteronucleus. Several methods were proposed achieve such a transfer, including techniques using adiabatic adiabatic rf-pulses or B_0-field pulses. Here we propose a significant improvement of such adiabatic methods by using "constant-adiabaticity" temporal profiles of the pulses,[1] which were originally proposed to generate singlet order in a spin pair. All constant-adiabaticity methods were implemented for the important metabolite [1-^{13}C]fumarate, representing an AA'X-type spin system.
First of all, we have generalized and improved the procedure to evaluate constant-adiabaticity ramps by using a pertinent restriction of the space of spin states. With this method, we have introduced constant-adiabaticity rf-field ramps to manipulate spin order in high-field experiments, allowing us to reach an excellent efficiency of 96.2% for singlet order to heteronuclear polarization transfer. In experiments with parahydrogen, such rf-pulses allowed us to obtain 6.8% ^{13}C polarization. Last but not least, the same optimization method has been utilized in ultralow-field experiments, using field cycling (FC) and field sweeping (FS) to convert the proton spin order into the ^{13}C polarization. The constant adiabaticity profiles have demonstrated a much better performance in comparison with their linear analogues used before: one can achieve efficient polarization transfer in much shorter times, getting around the problem of relaxation losses of spin order during the field pulse.

The highly efficient polarization transfer techniques used here are of a more general scope and can be exploited to generate heteronuclear spin hyperpolarization in various molecules, notably, in metabolites.

References

[1] B.A. Rodin, K.F. Sheberstov, A.S. Kiryutin, J.T. Hill-Cousins, L.J. Brown, R.C.D. Brown, B. Jamain, H. Zimmermann, R.Z. Sagdeev, A.V. Yurkovskaya, K.L. Ivanov, Constant-adiabaticity RF-pulses for generating long- lived singlet spin states in NMR, J. Chem. Phys., 150 (2019) 064201

Acknowledgements

This work has been supported by the Russian Science Foundation (project No. 20-62-47038).

ACCELERATING STRUCTURAL ELUCIDATION OF NATURAL ABUNDANT CARBOHYDRATE BY RELOADED THREE-DIMENSIONAL NMR SPECTROSCOPY

Qi Shi[1,2],Jinyu Yan[3], Bin Jiang[4], Jihui Wang[1,*], Xinmiao Liang[3,*], Xuanjun Ai[2,*]

[1] School of Biological Engineering, Dalian Polytechnic University, Dalian 116034.
[2] Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[3] Key Lab of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[4] State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China

Structure-function relationships of carbohydrates are well established at least for polysaccharides, glycans and human milk oligosaccharides (HMOs). NMR is one of the most frequently used but challenging methods in carbohydrate structural characterization as which is suffered from serious signal overlapping. We applied three-dimensional (3D) HSQC- TOCSY spectroscopy to solve the long-lasting overlapping problem. Replacing shaped pulses in the 3D pulse program with rectangle pulses resulted in undistorted signals and 2-4 times sensitivity improvement by using lactose as a model compound. The 3D method was then applied to a HMO hexasaccharide LNDFH-I and 34 out of 43 fingerprint signals could be determined directly, together with the other 9 signals being classified into 4 temporarily undefined residues plus an isolated peak of the N-acetyl group. Further analysis of two-dimensional heteronuclear correlation spectra lead to fast determination of intra- and inter-residue connection in the hexasaccharide. Validity of the 3D method was also checked for other oligosaccharides and polysaccharides. In conclusion, the reloaded 3D HSQC-TOCSY method significantly accelerates structural elucidation of carbohydrate.

References

[1] R. Raman, S. Raguram, G. Venkataraman et al., Glycomics: an integrated systems approach to structure-function relationships of glycans. *Nat. Methods* **2**, 817-824 (2005).
[2] S.S. Ferreira, C.P. Passos, P. Madureira, Structure function relationships of immunostimulatory polysaccharides: A review. *Carbohydr. Polym.***132**, 378-396 (2015).
[3] Kobata, Akira, Structures and application of oligosaccharides in human milk. *Proc. Jpn. Acad. Ser. B-Phys. Biol. Sci.***86**, 731-747 (2010).

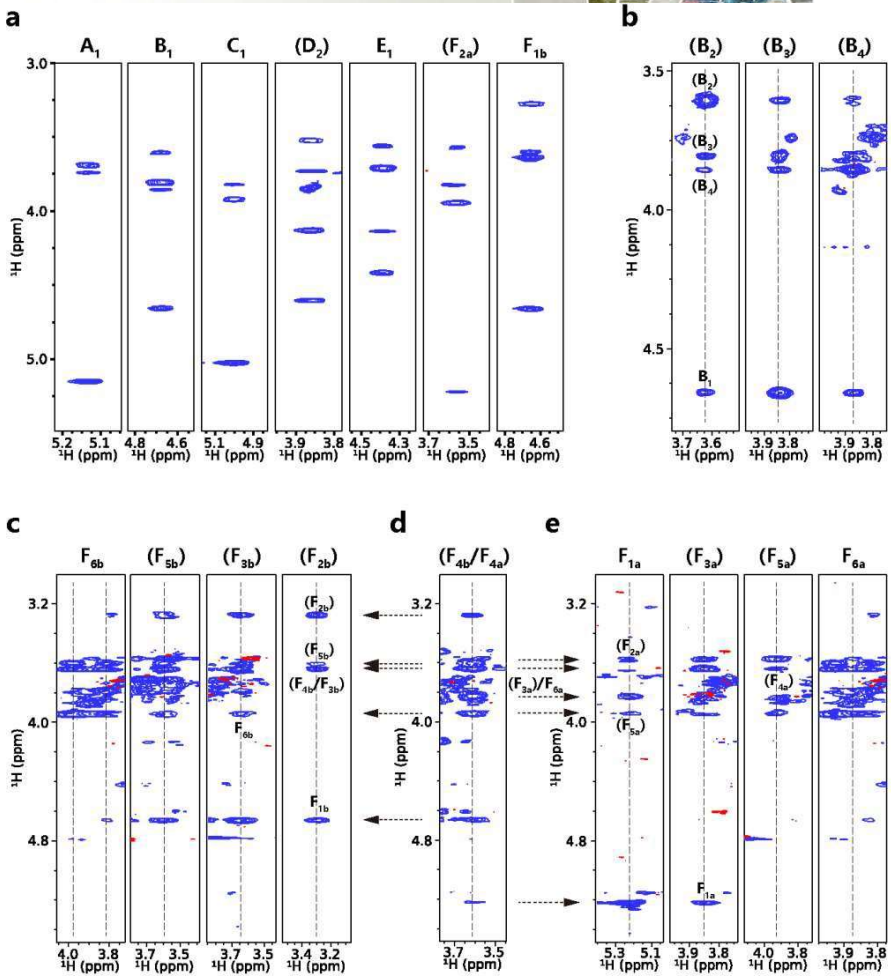


Figure 1. ^{1}H-^{1}H TOCSY spectra of fingerprint peaks from 3D HSQC-TOCSY.

REACHING COHESIVE STRENGTH AND FREE VOLUME BY NMR

Wagner Silva[1,2], H.I.M. Veiga[2], M. Tariq[2], J.M.S.S. Esperança[2], J.N. Canongia Lopes[3], L.P.N Rebelo[2], E.J. Cabrita[1,2]

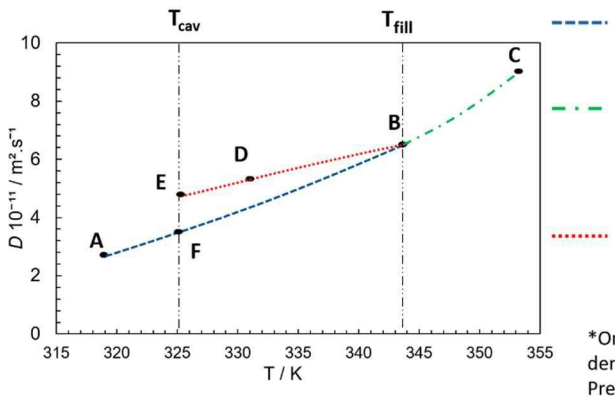
[1] UCIBIO, Chemistry Department, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; wm.silva@campus.fct.unl.pt (WS), ejc@fct.unl.pt (EC).
[2] LAQV/REQUIMTE, School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; jmesp@fct.unl.pt (JE), luis.rebelo@fct.unl.pt (LR).
[3] Chemistry Department, CQE, IST-UL, 1049-001 Lisbon, Portugal.

The cohesion forces and the diffusion mechanisms of the molecules that constitute a given liquid have a direct influence on the physicochemical properties and consequently on their possible applications. An unprecedented method was developed to study the cohesion forces and the effect of free volume in pure liquids and mixtures by NMR.

The strategy consists of subjecting the liquid to negative absolute pressure (NEG) in an isochoric system and observing the change in the behavior of the liquid using the NMR toolbox. Under this condition the molecules are "stretched" according to the magnitude of the pressure to which the liquid is subjected. Although the concept of NEG sounds weird, it occurs in nature e.g. fluid inclusions in minerals[1], can also be associated to important biological functions such as the transport of sap in trees[2] and can even be found in the human body.[3] There are several strategies to force a liquid entering into NEG regimes. In this work we use the Berthelot method.[4] Three distinct ionic liquids (ILs) were stretched, and studied by NMR according to the Berthelot cycle. It was possible to observe that according to the magnitude of the stretching, the increase in free volume exponentially increases the diffusion speed of the ions, Figure 1. Through the analysis of the chemical shift it is possible to identify the relationship between the structure of the ions that constitute the IL and the cohesion force, also through NOESY it was possible to identify that the ionic species are moving away according to the magnitude of the stretching.

References

[1] Roedder, E. Metastable Superheated Ice in Liquid-Water Inclusions under High Negative Pressure. Science (80). 1967, **155** (3768), 1413–1417.
[2] Wheeler, T. D.; Stroock, A. D. The Transpiration of Water at Negative Pressures in a Synthetic Tree. Nature 2008, **455** (7210), 208–212.
[3] Scholander, P. F.; Hargens, A. R.; Miiller, S. L. Negative Pressure in the Interstitial Fluid of Animals. Science (80-). 1968, **161** (3839), 321–328.
[4] Imre, A.; Martinás, K.; Rebelo, L. P. N. Thermodynamics of Negative Pressures in Liquids. J. Non-Equilibrium Thermodyn. 1998, **23** (4), 351–375.



Isobaric Heating Stage; A to B (T_{fill}).
Pressure is equal to vapor pressure of the liquid.

Isochoric Heating Stage, B to C;
and Isochoric Cooling Stage C to B.
Pressure on the green line period is Positive.

Isochoric Cooling Stage, B crossing D to E;
Point E the system collapse (T_{cav}) and return to the isobaric line, point F.
Pressure on the red line is the absolute negative pressure, the region correspondent to the increase of free volume.

*On the isochoric system, the volume of the liquid, density and molar concentration is constant. Pressure change according to increase or decrease according to the temperature.

Figure 1. Schematic representation of Berthelot Cycle followed by NMR Self-Diffusion.

THE TRANSLATIONAL DIFFUSION COEFFICIENT: A WAY TO FOLLOW PROTEIN DENATURATION

Csenge Lilla Szabó[1,2], Fanni Sebák[1,3], Andrea Bodor[1]

[1] Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Budapest, Hungary
[2] Hevesy György PhD School of Chemistry, Eötvös Loránd University, Budapest, Hungary
[3] Doctoral School of Pharmaceutical Sciences, Semmelweis University, Budapest, Hungary

Understanding protein unfolding is important in revealing mechanisms of folding and misfolding – some of the key points in pharmaceutical and medical research. PFG NMR is a powerful tool to study the hydrodynamic properties of biomacromolecules, thus it enables us to distinguish between folded and unfolded states of proteins. In our previous work, we established empirical correlations between the molecular mass and the translational diffusion coefficient for both folded and disordered proteins.[1]

Here we present the extension of the previously reported correlations and its application on the monitoring of protein unfolding. We studied two well-known model proteins, hen egg white lysozyme and bovine ubiquitin in two different chaotropic media: DMSOd6-water mixtures, which may present pharmacological relevance, and the widely applied 8M urea at various pH. As the translational diffusion coefficient (*D*) depends on the temperature and the viscosity of the media, we established correction formulas to compare the results collected under various conditions. We discuss the possibilities and potential errors of using small molecules as diffusion standards. The continuum model fails in case of small molecules[2] and we proved that it causes an unexpectedly high error for the most preferred dioxane in mixtures of DMSOd6 and water. In order to establish a more accurate viscosity correction method, we tested some other small molecules and compared them with the directly measured viscosities of the samples.

Our results showed that DMSO can completely unfold proteins even if they are stabilized by disulphide bridges. The denaturing ability of 8M urea depends strongly on the pH, the increase in the hydrodynamic radius resulting from the loss of stable 3D fold can be observed only in acidic media (pH < 3).

References

[1] E. F. Dudás, A. Bodor, Quantitative, Diffusion NMR Based Analytical Tool To Distinguish Folded, Disordered, and Denatured Biomolecules. *Anal. Chem.* **91**, 4929–4933 (2019).
[2] R. Evans, G. Dal Poggetto, M. Nilsson, G. A. Morris, Improving the Interpretation of Small Molecule Diffusion Coefficients. *Anal. Chem.* **90**, 3987–3994 (2018).

OPTICALLY DETECTED, FAST-ULTRALOW-FIELD-CYCLING RELAXOMETRY

Sven Bodenstedt[1], Morgan W. Mitchell[1], Michael C. D. Tayler[1]

[1] Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels, Barcelona, Spain.

Dynamic processes such as adsorption and exchange are often probed by nuclear magnetic relaxation dispersion (NMRD), where field-dependent relaxation rates of nuclear spins can quantify correlation times for molecular motion. Correlation times τ_c are obtained if relaxation is measured across Larmor frequencies $\gamma B << \tau_c$ to $\gamma B >> \tau_c$, where γ is the gyromagnetic ratio and B is the field strength. Extremely slow correlations thus require an ultralow field environment, e.g. MuMetal chamber. Yet, conventional NMRD methods rely on fast-field-cycling (FFC) magnets to switch between high fields for prepolarization and inductive detection and low fields for relaxation[1]. Since these are not compatible with magnetic shielding, and unshielded FFC requires Earth's-field compensation strategies that are at best elaborate[2,3], at worst haphazard, τ_c is limited at the high end.

Here, we combine the speed of FFC with sensitive detection of NMR signals at very low field using an optical magnetometer (^{1}H frequency from 1 Hz to 10 kHz, tunable). Rapid (<1 ms) and accurate (<1 nT) FFC enables measurements of T_1 and T_2, thus correlation times, that cannot be probed with conventional methodology. The technique is applied to n-alkanes adsorbed on porous alumina. Below 10-20 Hz ^{1}H frequency, T_1 and τ_c have similar magnitude, prompting investigation of whether molecular diffusion or spin diffusion limits the relaxation process within the pores. Another example concerns relaxation in paramagnetic ion solutions. Limits of achievable relaxation rates ($1/T_1 < 50\ s^{-1}$) and magnetic fields (B < 0.02 T) for the technique, plus progress towards reaching fundamental limits, are discussed.

References

[1] R. Kimmich, Field-cycling Relaxometry: instrumentation, model theories and applications, (2018) ISBN 978-1-78801-154-9
[2] E. Anoardo, G.M. Ferrante, Magnetic field compensation for NMR field-cycling relaxometry in the ULF band, *Appl. Magn. Reson.* **24**, 85 (2003).
[3] B. Kresse, A.F. Privalov, F. Fujara, NMR field cycling at ultralow magnetic fields, *Solid State Nucl. Magn. Reson.* **40**, 1926-2040 (2011).

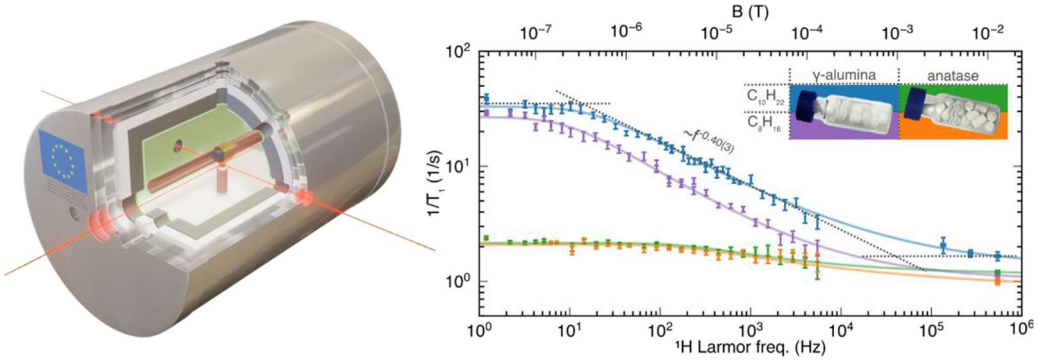


Figure 1. (L) Magnetometer and fast-field cycling coils for NMRD, located in a MuMetal shield; (R) 1H T1 dispersion for C8H18 and C10H22 in porous alumina/titania. The power-law exponent indicates restricted motion, while the shoulder frequency indicates the slowest diffusion rate.

EUROMAR
THE ON-LINE EXPERIENCE | 7- 8 DECEMBER 2020
12:00-15:30 UTC-GMT | Coordinated Universal Time
BILBAO2020

DIVERSIFYING NOAH SUPERSEQUENCES WITH NEW HSQC-BASED MODULES

Jonathan R. J. Yong[1], Tim D. W. Claridge[1], Ēriks Kupče[2]

[1] Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK
[2] Bruker UK Ltd., Banner Lane, Coventry, CV4 9GH, UK

In recent years, there has been growing interest in techniques which accelerate NMR data acquisition. A notable example is the NOAH technique (NMR by Ordered Acquisition using ^{1}H detection),[1,2] in which multiple 2D experiments (referred to as "modules") are directly concatenated without interleaved recovery delays to form "supersequences" (*Figure 1*). To date, many standard 2D experiments such as HSQC, HMBC, COSY, TOCSY and NOESY/ROESY have been successfully incorporated into NOAH supersequences. Depending on the number of modules in one supersequence, time savings of up to 3–4× may be accomplished, with no special hardware or experiment setup required.

The time savings provided by fast NMR acquisition techniques are generally greatest when sensitivity is not a limiting consideration. Increasing the sensitivity of NOAH modules will therefore allow the NOAH technique to be more widely applicable. To this end, we introduce a newly designed sensitivity-enhanced HSQC[3] sequence which can be used as a NOAH module. This provides up to 1.6× gains in signal-to-noise ratio (SNR) for XH groups as compared to the original HSQC module, while at the same time preserving the magnetization of uncoupled spins for subsequent module(s). This module is especially useful for ^{15}N correlation spectra, where up to 5× gains in SNR can be realized relative to the originally published HMQC module. The utility of these modules can be further increased by utilizing non-uniform sampling or linear prediction. We also demonstrate how various artefacts in NOAH modules may be effectively suppressed by appropriate placement of pulsed field gradients. Altogether, this proves that fast data acquisition need not come at the expense of spectral sensitivity or purity.

References

[1] Kupče, Ē.; Claridge, T. D. W. NOAH: NMR supersequences for small molecule analysis and structure elucidation. *Angew. Chem. Int. Ed.* **56,** 11779–11783 (2017).
[2] Claridge, T. D. W.; Mayzel, M.; Kupče, Ē. Triplet NOAH supersequences optimised for small molecule structure characterisation. *Magn. Reson. Chem.* **57,** 946–952 (2019).
[3] Palmer, A. G.; Cavanagh, J.; Wright, P. E.; Rance, M. Sensitivity improvement in proton-detected two-dimensional heteronuclear correlation NMR spectroscopy. *J. Magn. Reson.* **93,** 151–170 (1991).

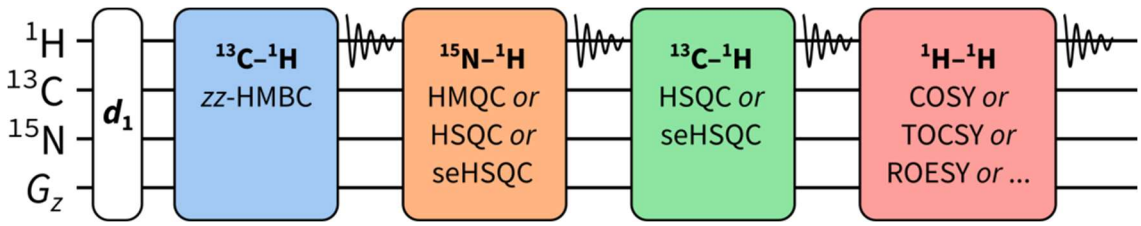


Figure 1: Schematic overview of the modular nature of NOAH supersequences. All four modules can be recorded with only one recovery delay (d_1).

TWO-DIMENSIONAL NMR EXPERIMENTS EXPLOITING SPIN MIXING UNDER ZERO- OR ULTRA-LOW FIELD CONDITIONS

Ivan Zhukov[1,2], Alexey Kiryutin[1,2], Fabien Ferrage[3], Gerd Buntkowsky[4], Alexandra Yurkovskaya[1,2], Konstantin Ivanov[1,2]

[1] International Tomography Center SB RAS, Novosibirsk 630090, Russia
[2] Novosibirsk State University, Novosibirsk 630090, Russia
[3] Laboratoire des Biomolécules, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France
[4] Technical University of Darmstadt, Institute of Inorganic and Physical Chemistry, D-64287 Darmstadt, Germany

Multi-dimentional correlation spectroscopy in liquid-state NMR provides invaluable information about properties of complex molecular systems[1]. For example, the 2D TOCSY experiment[2] is a useful tool for identification of substances in complex mixtures, notably, metabolites in biological samples[3], and further assignment of the signals. However, even when state-of-the-art high-resolution NMR methods are used, the problem of overlapping spectral peaks imposes limitations on analytical capabilities of 2D-NMR. Employing heteronuclear correlation NMR methods improves the situation by providing information on chemical shifts of heteronuclei. In our project, a new heteronuclear 2D experiment is developed, which combines spin mixing at Zero or Ultra-Low Field (ZULF) and standard inductive detection at high field. We propose to name this experiment ZULF-TOCSY. The method allows one to achieve correlation across all NMR-active nuclei also taking advantage of high-resolution NMR detection. The features of the ZULF-TOCSY technique has been demonstrated using solutions of ^{13}C,^{15}N labeled amino acids[4]. By utilizing ZULF-TOCSY it is possible to render sequential assignment of a short peptide based on long-range J-couplings between carbonyl carbons and α-protons, using peptides with natural magnetic isotope abundance. It is worth noting, that sequential assignment based on ZULF-TOCSY spectra is possible even for proline-rich peptides.

References

[1] R. R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions. *Clarendon Press ; Oxford University Press: Oxford Oxfordshire, New York* (1987).
[2] L. Braunschweiler, R. R. Ernst, Coherence Transfer by Isotropic Mixing - Application to Proton Correlation Spectroscopy. *J. Magn. Reson.*, **53**, 521-528. (1983)
[3] K. Bingol, L. Bruschweiler-Li, D. W. Li, R. Brüschweiler, Customized metabolomics database for the analysis of NMR ^{1}H–^{1}H TOCSY and ^{13}C–^{1}H HSQC-TOCSY spectra of complex mixtures. *Anal. Chem.*, **86**, 5494-5501 (2014).
[4] Zhukov, A. S. Kiryutin, F. Ferrage, G. Buntkowsky, A. V. Yurkovskaya, K. L. Ivanov, Total correlation spectroscopy across all NMR-active nuclei by mixing at zero field. *J. Phys. Chem. Lett.*, **11**, 7291-7296 (2020).

THE FUTURE OF EUROMAR

EUROMAR

PORTOROŽ 2021

SPINNING INTO THE FUTURE

4th - 8th JULY 2021

Euromar 2021 will be held at the Congress center at hotel Bernardin in Portorož, Slovenia as a hybrid event combining live interactions in a wonderful scenic environment and virtual participation.

EUROMAR AMPERE NATIONAL INSTITUTE OF CHEMISTRY SLONMR CENTRE OF EXCELLENCE

www.euromar2021.org

Utrecht

3 – 10 July 2022