EUROMAR
EUROPEAN CONFERENCE ON MAGNETIC RESONANCE
5TH—8TH JULY 2021
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THOMAS PRISNER
— Chair of the Board of Trustees of EUROMAR

DEAR COLLEAGUES,

as Chair of the Board of Trustees of EUROMAR I want to welcome you all to the EUROMAR meeting 2021. Unfortunately, like last year, the pandemic situation does not allow us to meet in person. I am sure you all regret not being at the beautiful Slovenian seaside in Portoroz as much as I do. Within the last year, we all painfully realized how important direct personal interaction is for scientific research, as well as for scientific education. Of course, virtual meetings cannot compensate all aspects of conventional physical meetings. On the other hand, they also offer some new opportunities and possibilities. I am very grateful to Janez Plavec, who agreed in 2019 (well before the pandemic situation) to organize the EUROMAR meeting 2021 and who courageously and unwaveringly continued his commitment even in this difficult situation. Organizing an event like the EUROMAR conference under these circumstances is not only much more work but also a 'high risk' endeavor. We all more or less know how 'normal' conferences work, but many things are different and have to be different with online meetings. As experimental scientists, thankfully we are all somewhat used to an uncertain situation like this, so we are not afraid to explore the different parameters and are interested in the results. I hope that we will all enjoy this online EUROMAR meeting as much as possible, listen to new and exciting scientific talks, use the opportunity to discuss with poster presenters, keep our interaction with vendors active and enjoy to see each other, if only through the screen of our computers. I would very much like to thank all the sponsors and vendors for their financial support and their solidarity in these special times, the organization and scientific committees for their work and, last but not least, I would like to thank Janez Plavec once again for his vigor and determination to get it going. I wish you all a successful meeting and hope very much to see you in person again soon.

Best regards,

Thomas Prisner

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JANEZ PLAVEC
— Chair Euromar 2021

DEAR PARTICIPANTS OF THE EUROMAR 2021 CONFERENCE, DEAR COLLEAGUES,

It is a great pleasure to welcome you all on behalf of the organizing committee to this year's EUROMAR conference. A warm welcome to plenary and invited speakers, presenters of promoted talks and poster presenters. We are about to start an exciting four-day conference with many contributions from groups all over the world. EUROMAR 2021 conference had to be transformed a few times during past last two years. Local organizers, members of the Slovenian NMR centre, are sorry that we were not allowed to gather everyone in person and to enjoy your company and private discussions in a scenic and stimulating as much as the refreshing environment of the northern Adriatic.

In early Spring this year a very unfortunate decision had to be made. With all the optimism and enthusiasm needed to organize a major international conference, we were forced to move EUROMAR 2021 to virtual format only. On the positive side, all lectures have been uploaded into the conference platform and are available on demand. This may be of great advantage for our colleagues in the far East and far West who will not be forced to stay up very late or to get up very early in order to follow presentation of their favorite speaker. Registered attendees will be able to watch recordings of oral Q & A sessions that will take place on-line at the end of each session. Vendor webinars will be accessible for later viewing as well. New communication methods now allow everyone to get in touch with anyone with a click and a short note.

Our lives and everyday activities were affected in so many ways. Interactions, teaching, sharing excitement over new results, etc. have been taking new forms as the very essence of our society has changed, including our ability to meet and discuss freely at a conference. Yet, life goes on and the magnetic resonance community has demonstrated, once more, our ability to endure and overcome difficulty. A virtual version of EUROMAR 2021 aims to provide a well-deserved recognition of award winners and especially young scientists. The organizers admit that this year's conference can be considered an experiment. Every poster presentation is supported with a short video presentation in addition to the standard abstract that will hopefully stimulate interactions through various media platforms. You, the participants of the conference, will have the opportunity to provide feedback on these new ways to organize an international event.

May I personally thank you all for your support and understanding. Finally, I want to extend my gratitude to the members of the International Scientific Committee, who have helped to shape the scientific program, vendors and supporters, who contributed through financial support, and my colleagues that actively participated in organization of EUROMAR 2021 conference. Their names are listed on the conference webpage and in the booklet. A sincere and big appreciation for their support and contribution. Thank You all.

Looking forward to meeting you all in the near future live,

Janez Plavec

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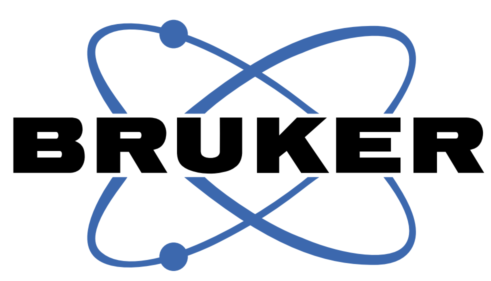

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MONDAY, JULY 5

- 09:00 — **OPENING AND PRIZE CEREMONY**
- 10:55 — Promotional video
- 11:00 — PLENARY 1: Biomolecules and interactions
- 11:30 — PLENARY 2: NMR of quantum materials
- 12:20 — SESSION 1: Biomolecules and interactions I
- 12:20 — SESSION 2: NMR of quantum materials
- 13:45 — Break
- 13:50 — User meeting BRUKER
- 15:20 — Workshop Jeol
- 15:30 — Break
- 15:35 — PLENARY 3: In-cell magnetic resonance
- 16:05 — PLENARY 4: Small molecules I
- 16:55 — Break
- 17:00 — SESSION 3: In-cell magnetic resonance
- 17:00 — SESSION 4: Small molecules I

TUESDAY, JULY 6

- 9:00 — PLENARY 5: Field-cycling NMR relaxometry
- 9:30 — PLENARY 15: Organic and composite solids
- 10:20 — Promotional video
- 10:25 — SESSION 5: Field-cycling NMR relaxometry
- 10:25 — SESSION 6: Energy storage and conversion materials, catalysts
- 11:50 — Break
- 11:55 — User meeting JEOL
- 12:55 — Webinar Magritek
- 13:10 — Break
- 13:15 — PLENARY 7: Hyperpolarization
- 13:55 — Webinar Merck
- 14:10 — Workshop Bruker
- 14:40 — SESSION 9: Benchtop and low-field NMR
- 14:40 — SESSION 7: Hyperpolarization
- 14:40 — SESSION 8: Methods development
- 15:40 — Break
- 16:00 — Webinar Oxford Instruments
- 15:45 — Hyperpolarization
- 15:45 — Methods development
- 16:30 — Break
- 16:35 — SESSION 10: Biomolecules and interactions II
- 16:35 — Hyperpolarization
- 16:35 — Methods development

WEDNESDAY, JULY 7

- 9:00 — PLENARY 8: MRI in material science and biomedical applications
- 9:30 — PLENARY 9: Integrated structural biology
- 10:20 — Promotional video
- 10:25 — SESSION 11: MRI in material science and biomedical applications
- 10:25 — SESSION 12: Integrated Structural biology
- 11:50 — Break
- 11:55 — Workshop Bruker
- 12:25 — Webinar Magritek
- 12:40 — Workshop Jeol
- 13:00 — Webinar Merck
- 13:15 — Break
- 13:20 — PLENARY 10: EPR in biomolecular and material science
- 13:50 — PLENARY 11: EPR in biomolecular and material science
- 14:40 — Break
- 16:00 — Webinar RS2D
- 14:45 — SESSION 13: EPR in biomolecular and material science
- 14:45 — SESSION 14: Drug design and combat against COVID-19
- 16:30 — Break
- 16:35 — TUTORIAL LECTURE (40 min lecture + 20 min Q&A)
- 17:45 — Webinar Quad System
- 17:15 — SESSION 15: Biomolecular dynamics
- 17:35 — SESSION 16: Small molecules II
- 19:05 — BRUKER night

THURSDAY, JULY 8

- 9:00 — PLENARY 12: Frontiers in magnetic resonance
- 9:30 — PLENARY 13: Biosolids
- 10:20 — Promotional video
- 10:25 — SESSION 17: Frontiers in magnetic resonance
- 10:25 — SESSION 18: Biosolids
- 12:30 — Break
- 12:35 — Webinar Silantes
- 12:50 — Workshop Jeol
- 13:10 — Webinar Mikro + Polo
- 13:20 — Break
- 13:25 — PLENARY 14: Metabolomics
- 13:55 — PLENARY 6: Energy storage and conversion materials, catalysts
- 14:45 — Break
- 14:50 — SESSION 19: Metabolomics
- 14:50 — SESSION 20: Organic and composite solids
- 17:15 — **CONCLUDING REMARKS**

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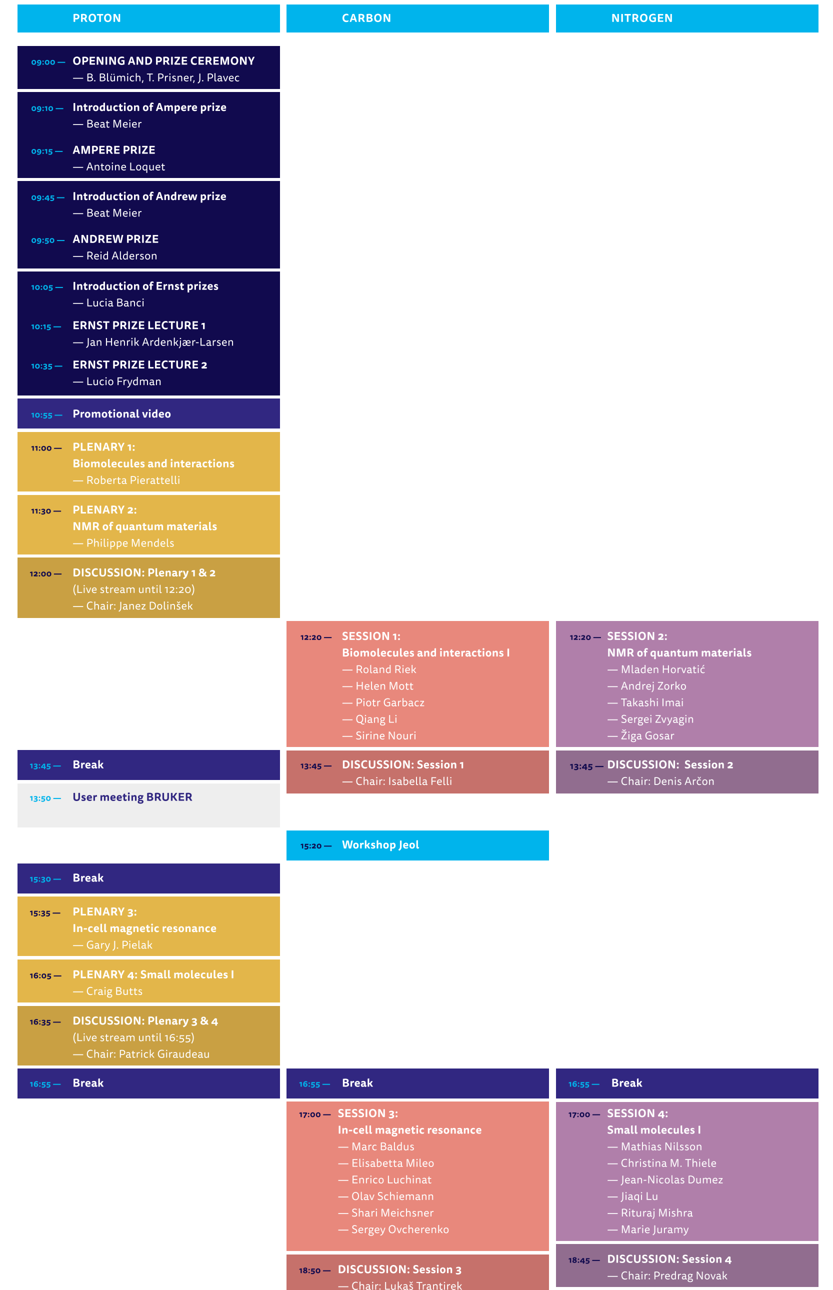

Time	PROTON	CARBON	NITROGEN
09:00	OPENING AND PRIZE CEREMONY — B. Blümich, T. Prisner, J. Plavec		
09:10	Introduction of Ampere prize — Beat Meier		
09:15	AMPERE PRIZE — Antoine Loquet		
09:45	Introduction of Andrew prize — Beat Meier		
09:50	ANDREW PRIZE — Reid Alderson		
10:05	Introduction of Ernst prizes — Lucia Banci		
10:15	ERNST PRIZE LECTURE 1 — Jan Henrik Ardenkjær-Larsen		
10:35	ERNST PRIZE LECTURE 2 — Lucio Frydman		
10:55	Promotional video		
11:00	PLENARY 1: Biomolecules and interactions — Roberta Pierattelli		
11:30	PLENARY 2: NMR of quantum materials — Philippe Mendels		
12:00	DISCUSSION: Plenary 1 & 2 (Live stream until 12:20) — Chair: Janez Dolinšek		
12:20		SESSION 1: Biomolecules and interactions I — Roland Riek — Helen Mott — Piotr Garbacz — Qiang Li — Sirine Nouri	SESSION 2: NMR of quantum materials — Mladen Horvatić — Andrej Zorko — Takashi Imai — Sergei Zvyagin — Žiga Gosar
13:45	Break	DISCUSSION: Session 1 — Chair: Isabella Felli	DISCUSSION: Session 2 — Chair: Denis Arčon
13:50	User meeting BRUKER		
15:20		Workshop Jeol	
15:30	Break		
15:35	PLENARY 3: In-cell magnetic resonance — Gary J. Pielak		
16:05	PLENARY 4: Small molecules I — Craig Butts		
16:35	DISCUSSION: Plenary 3 & 4 (Live stream until 16:55) — Chair: Patrick Giraudeau		
16:55	Break	Break	Break
17:00		SESSION 3: In-cell magnetic resonance — Marc Baldus — Elisabetta Mileo — Enrico Luchinat — Olav Schiemann — Shari Meichsner — Sergey Ovcherenko	SESSION 4: Small molecules I — Mathias Nilsson — Christina M. Thiele — Jean-Nicolas Dumez — Jiaqi Lu — Rituraj Mishra — Marie Juramy
18:45			DISCUSSION: Session 4 — Chair: Predrag Novak
18:50		DISCUSSION: Session 3 — Chair: Lukaš Trantirek	

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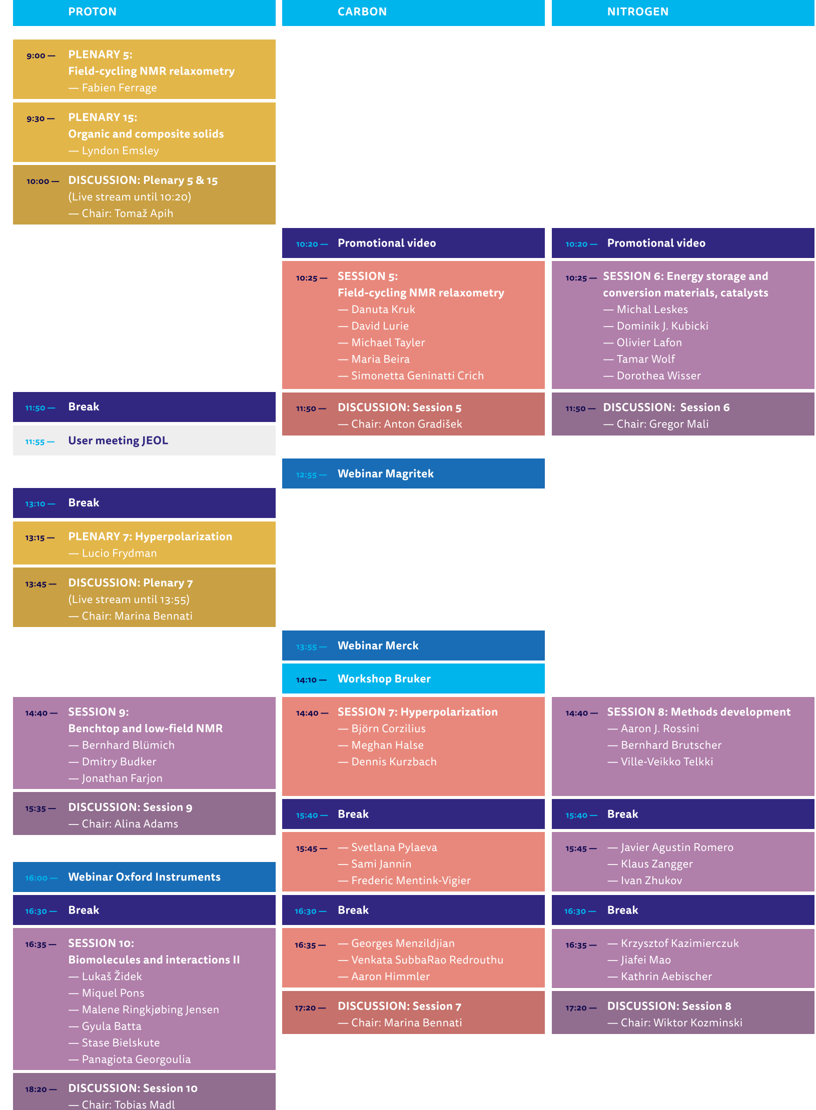
TUESDAY, JULY 6



PROTON

- 9:00 — PLENARY 5: Field-cycling NMR relaxometry — Fabien Ferrage
- 9:30 — PLENARY 15: Organic and composite solids — Lyndon Emsley
- 10:00 — DISCUSSION: Plenary 5 & 15 (Live stream until 10:20) — Chair: Tomaž Apih
- 11:50 — Break
- 11:55 — User meeting JEOL
- 13:10 — Break
- 13:15 — PLENARY 7: Hyperpolarization — Lucio Frydman
- 13:45 — DISCUSSION: Plenary 7 (Live stream until 13:55) — Chair: Marina Bennati
- 14:40 — SESSION 9: Benchtop and low-field NMR — Bernhard Blümich — Dmitry Budker — Jonathan Farjon
- 15:35 — DISCUSSION: Session 9 — Chair: Alina Adams
- 16:00 — Webinar Oxford Instruments
- 16:30 — Break
- 16:35 — SESSION 10: Biomolecules and interactions II — Lukaš Žídek — Miquel Pons — Malene Ringkjøbing Jensen — Gyula Batta — Stase Bielskute — Panagiota Georgoulia
- 18:20 — DISCUSSION: Session 10 — Chair: Tobias Madl

CARBON

- 10:20 — Promotional video
- 10:25 — SESSION 5: Field-cycling NMR relaxometry — Danuta Kruk — David Lurie — Michael Tayler — Maria Beira — Simonetta Geninatti Crich
- 11:50 — DISCUSSION: Session 5 — Chair: Anton Gradišek
- 12:55 — Webinar Magritek
- 13:55 — Webinar Merck
- 14:10 — Workshop Bruker
- 14:40 — SESSION 7: Hyperpolarization — Björn Corzilius — Meghan Halse — Dennis Kurzbach
- 15:40 — Break
- 15:45 — Svetlana Pylaeva — Sami Jannin — Frederic Mentink-Vigier
- 16:30 — Break
- 16:35 — Georges Menzildjian — Venkata SubbaRao Redrouthu — Aaron Himmler
- 17:20 — DISCUSSION: Session 7 — Chair: Marina Bennati

NITROGEN

- 10:20 — Promotional video
- 10:25 — SESSION 6: Energy storage and conversion materials, catalysts — Michal Leskes — Dominik J. Kubicki — Olivier Lafon — Tamar Wolf — Dorothea Wisser
- 11:50 — DISCUSSION: Session 6 — Chair: Gregor Mali
- 14:40 — SESSION 8: Methods development — Aaron J. Rossini — Bernhard Brutscher — Ville-Veikko Telkki
- 15:40 — Break
- 15:45 — Javier Agustin Romero — Klaus Zangger — Ivan Zhukov
- 16:30 — Break
- 16:35 — Krzysztof Kazimierczuk — Jiafei Mao — Kathrin Aebischer
- 17:20 — DISCUSSION: Session 8 — Chair: Wiktor Kozminski

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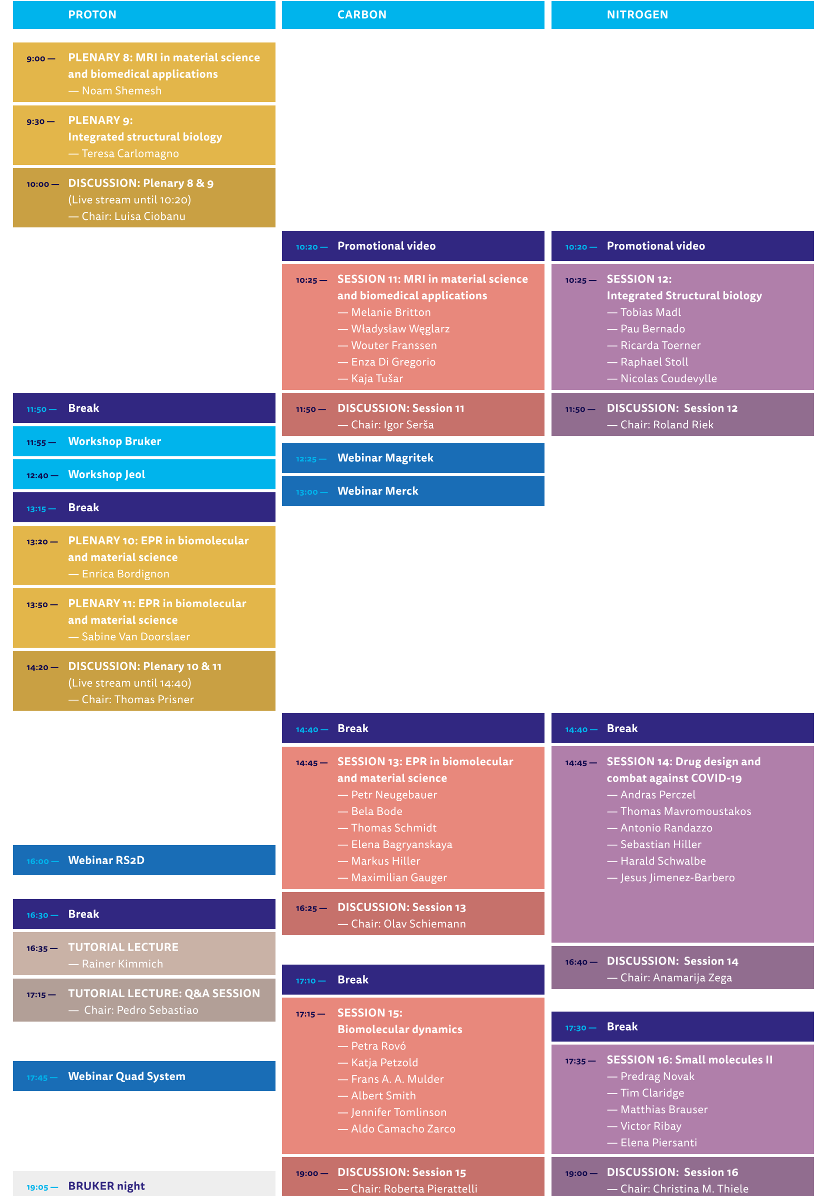

PROTON

Time	Event
9:00	PLENARY 8: MRI in material science and biomedical applications — Noam Shemesh
9:30	PLENARY 9: Integrated structural biology — Teresa Carlomagno
10:00	DISCUSSION: Plenary 8 & 9 (Live stream until 10:20) — Chair: Luisa Ciobanu
11:50	Break
11:55	Workshop Bruker
12:40	Workshop Jeol
13:15	Break
13:20	PLENARY 10: EPR in biomolecular and material science — Enrica Bordignon
13:50	PLENARY 11: EPR in biomolecular and material science — Sabine Van Doorslaer
14:20	DISCUSSION: Plenary 10 & 11 (Live stream until 14:40) — Chair: Thomas Prisner
16:00	Webinar RS2D
16:30	Break
16:35	TUTORIAL LECTURE — Rainer Kimmich
17:15	TUTORIAL LECTURE: Q&A SESSION — Chair: Pedro Sebastiao
17:45	Webinar Quad System
19:05	BRUKER night

CARBON

Time	Event
10:20	Promotional video
10:25	SESSION 11: MRI in material science and biomedical applications — Melanie Britton — Władysław Węglarz — Wouter Franssen — Enza Di Gregorio — Kaja Tušar
11:50	DISCUSSION: Session 11 — Chair: Igor Serša
12:25	Webinar Magritek
13:00	Webinar Merck
14:40	Break
14:45	SESSION 13: EPR in biomolecular and material science — Petr Neugebauer — Bela Bode — Thomas Schmidt — Elena Bagryanskaya — Markus Hiller — Maximilian Gauger
16:25	DISCUSSION: Session 13 — Chair: Olav Schiemann
17:10	Break
17:15	SESSION 15: Biomolecular dynamics — Petra Rovó — Katja Petzold — Frans A. A. Mulder — Albert Smith — Jennifer Tomlinson — Aldo Camacho Zarco
19:00	DISCUSSION: Session 15 — Chair: Roberta Pierattelli

NITROGEN

Time	Event
10:20	Promotional video
10:25	SESSION 12: Integrated Structural biology — Tobias Madl — Pau Bernado — Ricarda Toerner — Raphael Stoll — Nicolas Coudevylle
11:50	DISCUSSION: Session 12 — Chair: Roland Riek
14:40	Break
14:45	SESSION 14: Drug design and combat against COVID-19 — Andras Perczel — Thomas Mavromoustakos — Antonio Randazzo — Sebastian Hiller — Harald Schwalbe — Jesus Jimenez-Barbero
16:40	DISCUSSION: Session 14 — Chair: Anamarija Zega
17:30	Break
17:35	SESSION 16: Small molecules II — Predrag Novak — Tim Claridge — Matthias Brauser — Victor Ribay — Elena Piersanti
19:00	DISCUSSION: Session 16 — Chair: Christina M. Thiele

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PROTON

- 9:00 — **PLENARY 12: Frontiers in magnetic resonance** — Eugenio Coronado
- 9:30 — **PLENARY 13: Biosolids** — Guido Pintacuda
- 10:00 — **DISCUSSION: Plenary 12 & 13** (Live stream until 10:20) — Chair: Marc Baldus
- 12:30 — **Break**
- 12:35 — **Webinar Silantes**
- 13:20 — **Break**
- 13:25 — **PLENARY 14: Metabolomics** — Ana Gil
- 13:55 — **PLENARY 6: Energy storage and conversion materials, catalysts** — Arno P.M. Kentgens
- 14:25 — **DISCUSSION: Plenary 14 & 6** (Live stream until 14:45) — Chair: Tim Claridge
- 17:15 — **CONCLUDING REMARKS**
  - **Tatyana Polenova**, JMR
  - **Thomas Prisner**, IES Award
  - **Anja Böckmann**, President of the AMPERE Society
  - **Marc Baldus**, EUROMAR 2022
  - **Janez Plavec**, closing remarks

CARBON

- 10:20 — **Promotional video**
- 10:25 — **SESSION 17: Frontiers in magnetic resonance**
  - Fedor Jelezko
  - Joerg Wrachtrup
  - Tjerk Oosterkamp
  - Roberta Sessoli
  - Petr Štěpánek
  - David Korenchan
  - Bruno Simões de Almeida
- 12:30 — **DISCUSSION: Session 17** — Chair: Yishay Manassen
- 13:10 — **Webinar Mikro + Polo**
- 14:45 — **Break**
- 14:50 — **SESSION 19: Metabolomics**
  - Daniel Raftery
  - Benedicte Elena-Herrmann
  - Kavita Dorai
  - Jan Sykora
  - Ewa Nawrocka
  - Miriam Pérez Trujillo
- 16:35 — **DISCUSSION: Session 19** — Chair: Claudio Luchinat

NITROGEN

- 10:20 — **Promotional video**
- 10:25 — **SESSION 18: Biosolids**
  - Markus Weingarth
  - Clemens Glaubitz
  - Miguel Mompean
  - Clara Nassrin Kriebel
  - Xavier Falourd
- 11:50 — **DISCUSSION: Session 18** — Chair: Helen Mott
- 12:50 — **Workshop Jeol**
- 14:45 — **Break**
- 14:50 — **SESSION 20: Organic and composite solids**
  - Klaus Schmidt-Rohr
  - Andraž Krajnc
  - Martin Dračínský
  - Manuel Cordova
  - Bing Wu
- 16:15 — **DISCUSSION: Session 20** — Chair: Axel Gansmüller

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- BIOSOLIDS
- BIOMOLECULAR DYNAMICS
- INTERGRATED STRUCTURAL BIOLOGY: NMR IN HAND WITH COMPLEMENTARY METHODS
- IN-CELL MAGNETIC RESONANCE
- SMALL MOLECULES
- DRUG DESIGN AND COMBAT AGAINST COVID-19
- METABOLOMICS
- HYPERPOLARIZATION
- FIELD-CYCLING NMR RELAXOMETRY
- MRI IN MATERIAL SCIENCE AND BIOMEDICAL APPLICATIONS
- ENERGY STORAGE AND CONVERSION MATERIALS, CATALYSTS
- ORGANIC AND COMPOSITE SOLIDS
- METHODS DEVELOPMENT
- BENCHTOP AND LOW-FIELD
- EPR IN BIOMOLECULAR AND MATERIAL SCIENCE
- FRONTIERS IN MAGNETIC RESONANCE
- NMR OF QUANTUM MATERIALS

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PRIZE LECTURES

ANTOINE LOQUET

CNRS, IECB, CBMN,
University of Bordeaux,
Bordeaux, France

STRUCTURAL BIOLOGY OF PROTEIN ASSEMBLIES AND PATHOGEN CELL SURFACE BY SOLID-STATE NMR SPECTROSCOPY

We will present the NMR activity carried out in the laboratory, focusing on recent and unpublished results:

- solid-state NMR-based structure determination of amyloid fibrils using ^{1}H detection and ^{1}H-^{1}H distance restraints at fast magic-angle spinning;
- multidimensional ^{1}H-detected solid-state NMR of protein assemblies at fast magic-angle spinning combined with DNP;
- development of 1H detection at fast magic-angle spinning to study the surface of pathogens.

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T. REID ALDERSON[1,2]

[1]Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, UK
[2]Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD, USA

PROTEIN FOLDING INVESTIGATED BY NMR SPECTROSCOPY

In my thesis research, I used NMR spectroscopy, which simultaneously provides atomic-level insight into both the structures and dynamics of biomolecules, to investigate two fundamental aspects of biochemistry: how do proteins fold from linear chains of amino acids into complex three-dimensional structures, and how do the class of proteins known as molecular chaperones prevent pathological protein misfolding and aggregation? Dysregulated protein folding contributes to the etiology of protein misfolding diseases, including Alzheimer's, Parkinson's and type II diabetes. Understanding the precise molecular mechanisms that underpin these processes could guide the design of future therapeutics that help mitigate protein misfolding diseases.

During my PhD, I determined a redox-controlled mechanism that regulates the function of the molecular chaperone HSP27 [1, 2], and I elucidated the structural basis for mutations in HSP27 that cause incurable motor neuropathies [3]. I also contributed to the development of novel NMR methodology involving rapid pressure changes to study protein folding at atomic resolution on the millisecond timescale, enabling unprecedented insight into the early stages of folding and misfolding [4-6]. By performing complementary static pressure experiments, I quantified the compaction of an unfolded polypeptide chain as the difference in free energy between the unfolded and folded states is lowered [7]. In natively unfolded and denatured proteins, I studied the formation of *cis*-proline bonds, which often must isomerize to the *trans* conformation before folding can occur, and I found that model peptides overestimate the fraction of the *cis* conformation [8]. Finally, I aided in the development of NMR software that automatically assigns methyl resonances from highly deuterated, selectively methyl-$^{13}CH_3$ labeled proteins up to 1 MDa in aggregate mass, with assignments obtained based on a comparison of through-space experimental restraints (NOEs) and high-resolution structures [9, 10].

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DISSOLUTION DYNAMIC NUCLEAR POLARIZATION

Hyperpolarized Metabolic MR is a novel medical imaging modality that offers exceptional possibilities to follow changes in metabolism *in vivo* in real time [1]. The method is enabled by a more than 10,000 fold enhancement [2] of the signal from metabolic contrast agents that probe central metabolic pathways. The contrast agent is typically enriched in ^{13}C and polarized by dissolution Dynamic Nuclear Polarization (dDNP). The contrast agent circulates via the vasculature to the tissue of interest, where it is taken up by the tissue cells and metabolized into specific products. MR is unique in several ways: 1) it already provides anatomical and morphological images with high resolution and contrast based on the tissue water protons, 2) it is does not expose the patient to any ionizing radiation, and 3) it is a spectroscopic method that allows quantification of the individual metabolites. The first tracer in clinical development is ^{13}C-pyruvate. Pyruvate is at a pivotal point in glycolysis and allows us to directly probe the Warburg effect in cancer through the elevated lactate-to-pyruvate ratio, but also other pathologies. The hope is that more accurate diagnosis and staging can be made, and that the method will provide an early read-out of response to treatment. The first clinical studies have been published [3–6] with encouraging results.

In this talk I will review dDNP of ^{13}C and ^{1}H with trityl and UV radicals with the aim of reaching higher polarization in a faster manner. Direct polarization of ^{13}C with trityl is robust and efficient, however, subtle differences in the chemical structure of the trityl and change of matrix, affects the DNP efficiency. In contrast, UV radicals are labile, and quench in the solid state well below room temperature, which may provide a means to hyperpolarized solids with long relaxation for storage and transportation.

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BASKING IN ERNST'S LEGACY: PULSES, SPECTRA, IMAGES

Even for those of us that never had the privilege to work with Richard Ernst (1937 – 2021), the man and his persona stand out among the greatest of our generation. In fact Ernst's greatness can probably be best appreciated by none better than us, the practitioners of Nuclear Magnetic Resonance. His experiments, his formalisms and derivations: they all carry a stamp of ingenuity, depth and rigorousness that is typically Ernst's. Whether revisiting a previous proposition or making a completely new one, whether being the sole senior author or pushing the envelope of Science in a collaboration, most of us would recognize "an Ernst" just by reading its pages. In the present short talk I will present how three such classical "Ernsts" –the Ernst/Anderson pulsed approach to high resolution NMR, his proposition to collect 3D images non-invasively by Fourier NMR Zeugmatography, and the Jeener/Ernst proposition to unravel complex information by multidimensional NMR– have molded our own research. I will specifically focus on how pulsed NMR led us to propose an alternative wat to collect 1D NMR spectra by spatiotemporal encoding, opening in turn the possibility to acquire arbitrary multidimensional NMR spectra / images in a single scan. The talk will center mostly on the latter's potential, highlighting ongoing preclinical and clinical projects dealing with understanding and diagnosing breast and pancreatic cancers, following fetal and neonatal development in normal and abnormal pregnancies, and tackling new functional / structural aspects of the brain with very high definition.

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PLENARY LECTURES

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UN-STRUCTURAL BIOLOGY BY NMR SPECTROSCOPY

Intrinsically disordered proteins (IDPs) and regions (IDRs) that do not adopt a well-defined three-dimensional structure under physiological conditions are now well recognized in structural biology. The last two decades have seen increasing evidence for the involvement of IDPs and IDRs in many complex biological pathways, complementing globular proteins' molecular functions. Historically understudied, intrinsic protein disorder is nowadays central in an increasingly large number of studies.

The structural properties of highly dynamic polypeptides cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in the characterization of highly flexible regions in multi-domain proteins and entire proteins characterized by the lack of a 3D structure. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information.

I will illustrate how NMR can help in describing the importance of intrinsic disorder to encode in a relatively short polypeptide many functional modules and to orchestrate protein-protein interactions.

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NMR IN QUANTUM MATERIALS: FROM MILESTONES TO THE INTRIGUING "KAGOME" CASE

In recent years, there has been a growing interest in material systems where quantum effects play a major role in emergent functionalities or as a seed for novel concepts. This vast field of research from fundamental to applications encompasses superconductors, graphene, topological insulators, Weyl semimetals, quantum spin liquids...

My talk will be on the fundamental side, focusing on systems where quantum effects are manifest in spin systems. Very simple Hamiltonians accounting for the interactions between spins are at work, yet many uncovered phases can be generated by a combination of a reduced dimensionality, of the geometry of the lattice and of frustration. The topic has expanded a lot over the last 30 years.

After introducing the field, I'll focus on the case of quantum spin liquids. This quantum disordered ground state was proposed by Anderson in 1973 as an alternative to the standard antiferromagnetic state "à la Néel". The discovery of high temperature superconductivity in 1986 acted as a revival for the search of such a quantum spin liquid, based on his ideas again, of a resonating valence bond state. Soon after, the antiferromagnetic kagome lattice decorated with quantum spins was proposed as the best candidate for stabilizing such a state in dimension higher than one which triggered the search, still very active, for both new theories and materials with a kagome geometry. In my talk, I will mainly focus on a few among a growing number of kagome compounds where such a state is now stabilized but resists to a definite interpretation. Herbertsmithite, $ZnCu_3(OH)_6Cl_2$, which has been known since 2005 as one of the best representative of that spin liquid physics has been at the center of our activity on quantum materials in Orsay, Recently, working on high quality single crystals considerably improved the accuracy of NMR measurements and its ability to address fundamental issues such as the class of models relevant to the description of the ground state. Despite the existence of out-of-kagome planes defects which mask the intrinsic physics, one can indeed isolate the ^{17}O NMR spectral signature of kagome spins S=1/2 at Cu^{2+} sites. This illustrates all the power of NMR as a local probe aimed at imaging the central physics at work through a wise selection of the probe nucleus. The shift measurements give access to the local susceptibility and spins dynamics, through the prism of T_1 relaxation, shed light on excitations in a low energy range specific to NMR. This a common working scheme to all NMR groups studying quantum materials. I'll finally complement my presentation by showing how ESR can help in characterizing deviations to the Heisenberg model which have to be taken into account in the comparison to models.

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PROTEIN & PROTEIN-COMPLEX STABILITY IN LIVING CELLS

The crowded and complex environment in cells is predicted to affect protein behavior compared to dilute buffer. My laboratory and our collaborators are examining crowding effects on the stability of proteins and their complexes in cells and under physiologically relevant crowded conditions using NMR. A challenge in these endeavors is detecting the test protein in a sea of crowders. NMR is ideally suited to overcome this challenge and provide high quality data on folded- and unfolded- proteins as well as free and bound forms of complexes. I will focus on equilibrium data acquired in living *Escherichia coli* cells, *Xenopus laevis* and *Danio rerio* oocytes as well as *in vitro* in concentrated cosolute solutions using ^{19}F NMR. The cosolutes include synthetic polymers and their monomers, other proteins and lyophilized cytosol. The results show that crowding affects folding and binding in ways not always correctly predicted by simple theory. The differences point to opportunities for theoretical efforts and simulations.

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NMR-BASED STRUCTURE ELUCIDATION – DENSITY FUNCTIONAL THEORY, MACHINE LEARNING AND A LOT OF DATA

Interpreting NMR spectra has become somewhat of an art form in chemistry, particularly when applied to molecular structure elucidation. Skilled practitioners might pore over several, often complex, NMR spectra for hours, days and even weeks to work out the connectivity (2D structure), stereochemistry and conformation (3D structure) of challenging new molecules.

Our team develop tools that help with the steps in this process: creating new NMR experiments that provide different or more quantitative information than existing methods; applying quantum chemical calculations (usually Density Functional Theory) to accurately predict the NMR properties of candidate 3D molecular structures; developing machine learning tools that can accelerate predictions with 3D structural relevance by 10,000-fold [1] and thus potentially allow us to screen hundreds of thousands of molecular structures to find good fits to the experimental NMR spectra.

The potential (and limits!) of these approaches will be illustrated by the case of the structure reassignment of the baulamycin natural products [2] - molecules with 128 diastereoisomers, wach with around 1 billion potential 3D conformations - which we solved by combining elegant chemical synthesis (which I won't talk about much) with quantitative NMR and computational methods (which I will).

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MULTISCALE DYNAMICS FROM HIGH-RESOLUTION RELAXOMETRY AND TWO-FIELD NMR

High magnetic fields have empowered NMR spectroscopists to probe large complex systems with atomic resolution. Yet, investigating dynamics at very high magnetic fields is often challenging. Line broadening from chemical exchange worsen dramatically while relaxation becomes uninformative about nanosecond motions at very high fields. We have developped, in collaboration with Bruker Biospin, a series of instruments that couple the sensitivity and resolution of high magnetic fields with the evolution of nuclear spins at a lower fields, down to a few mT. Here, we will show how coupling high- and low-field NMR has allowed us to decipher nanosecond motions in protein side chains [1-2], improve models of motions, detect transient interactions in complex mixtures [3] and observe spin systems under intermediate chemical exchange, with spectra broadened beyond detection at high magnetic fields [4].

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SOLID-STATE NMR STUDIES OF MATERIALS FOR ENERGY CONVERSION AND STORAGE

Research and development in energy conversion and storage from renewable sources is increasingly important. As the function of these materials relates to their structure and dynamics, NMR has a relevant role to play in this research.

In the last decade, interest in hybrid halide perovskite materials has skyrocketed due to their remarkable photoluminescent and photovoltaic (PV) properties. Here NMR is particularly attractive as almost all isotopes that constitute the materials are accessible [1]. Compositional variation of the (organic) cations (A) or halides (X) mixed in $A^{+}B_{2}^{+}X_{3}$ results in desired effects in optimizing their optoelectric properties and improving their stability. We study cation dynamics, compositional variations and local ordering using solid-state NMR, NQR, XRD and DFT calculations. Mixing halides influences cation dynamics. The halides also display intriguing dynamics themselves allowing us to monitor spontaneous mixing of $MAPbI_3$ and $MAPbBr_3$ parent compositions.

All-solid-state lithium batteries are recognized as promising next generation energy storage techniques as they have the potential to provide higher energy density, assured safety, prolonged lifespan and easy processability. To realize them, high ion-conductive solid electrolytes have to be developed. NMR is deployed to study Li^{+} dynamics and characterize local structure [2]. Aluminum doping has been widely used in oxide electrolytes to stabilize the structure and enhance the Li ionic conductivity. We investigate Al-incorporation in β-Li_3PS_4, interpretation of the NMR spectra provides insights in the effect of Al-doping on the structure and dynamics in Li-Al-P-S systems. Alternatively, nanoconfined lithium borohydride shows high lithium-ion mobility at ambient temperatures. $LiBH_4$ in silica nanopores is clearly divided into two distinct fractions at all temperatures, one being highly dynamic. NMR reveals this is not just due to the nano-confinement, but there is a specific interaction with the silica pore walls.

Finally, we study rare earth oxyhydrides, such as $YO_xH_{(3-2x)}$, that change color and transparency when irradiated with UV light. Although their optical properties have been studied extensively, the understanding of the relationship between photochromism, chemical composition, structure, and in particular the role of hydrogen, is limited [3]. As these materials are grown as 1μm thin films they are difficult to characterize by NMR. Nevertheless, we could quantify the hydrogen content, get insight in hydride dynamics and identify different yttrium environments in the lattice. A better understanding of the structure function relation can lead to applications in smart windows or a novel class of electrolytes based on H^{-} ion conductivity.

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SENSITIVITY ENHANCED NMR SPECTROSCOPY WITHOUT DNP? C'EST POSSIBLE!

Chemical exchange saturation transfer, or CEST, is a central tool in modern metabolic imaging. CEST exploits the fast exchanges that labile hydrogens undergo with water in order to amplify the signatures of hard-to-observe metabolites. At the same time, it has often been assumed that fast exchanges with the solvent complicate or altogether preclude the observation of the labile sites. In the present study we discuss how exchanges with the solvent can actually be put to good use in order to enhance certain valuable molecular signatures –particularly those involving NOE and TOCSY correlations between labile and non-labile protons in biomolecules. Using relatively simple manipulations tunable by straightforward calibrations, it is shown that solvent exchanges can be used to amplify imino, amino, amide and hydroxy peaks in the 2D NOESY/TOCSY NMR spectra of nucleic acids, proteins and saccharides, by factors ranging from 2 to 10-fold. Even larger gains in sensitivity per unit time –up to two orders of magnitude– can be attained when the spectra are sparse, in which case the information can be efficiently encoded via selective manipulations. Heteronuclear information can also be incorporated into this kind of experiments, leading to high resolution and enhanced sensitivity, in minimal acquisition times. In fact solvent exchanges are not the sole mechanism capable of amplifying NMR spectra in such manner: also spin-diffusion effects among abundant spins, can be used to a similar end when trying to sensitize the spectra of dilute, insensitive species. The talk will conclude with a demonstration of how this can be brought to bear in order to impart high sensitivity/unit_time gains in wideline solid state NMR of ^{15}N, ^{17}O, ^{33}S and other "tough" nuclides.

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INSIGHTS INTO MICRO-ARCHITECTURE FROM DIFFUSION-DRIVEN CORRELATION TENSOR MRI

Diffusion MRI (dMRI) plays an important role in characterising micro-architectural features in diverse systems ranging from porous media [1,2] to *in-vivo* tissues [3]. Non-gaussian diffusion effects, typically quantified from diffusional kurtosis [4], have been shown to provide higher sensitivity towards microstructure than their diffusion tensor counterparts [5]; however, the specificity of diffusional kurtosis is limited [6] since diffusion non-gaussianity can arise from fundamentally different sources. In particular, diffusional kurtosis can arise from [7,8]: anisotropy of diffusion tensors (K_{aniso}); variance in the magnitude of diffusion tensors (K_{iso}); and restriction effects [9] (*e.g.*, full or partial restriction, tortuosity, surface-to-volume effects, diffusion in heterogeneous cross-sections, etc.), here collevtively termed microscopic kurtosis (μK). Exchange can also modulate the extent of the different sources. Contemporary methods based on conventional single-diffusion-encoding (SDE) NMR or MRI cannot resolve these underlying kurtosis sources, as they are all conflated in the MR diffusion-driven signal decay [10]. Valiant attempts have been made for resolving isotropic and anisotropic kurtosis sources [7,8]; however, strict assumptions are invoked, namely, diffusion time-indepdenence [11] and identically zero μK [10] – assumptions which likely are not met in many realistic systems.

In the talk, we will explore a new general approach – termed "correlation tensor MRI" (CTI) [10] – for resolving the different diffusional kurtosis sources. CTI [10] is based on displacement correlation tensor theory [12] and double-diffusion encoding (DDE) [13] waveforms, which enable the explicit measurement of the *Z* displacement correlation tensor [12] as well as the conventional *W* kurtosis tensor [5]. Hence, the different kurtosis sources can be disentangled without relying on strong model (or otherwise unverifiable) assumptions (n.b. exchange remains a potential confounding factor,). CTI theory and validation will be presented, followed by the first imaging results in *ex-vivo* and *in-vivo* rat brains revealing striking K_{aniso}, K_{iso} and μK contrasts. The first application of CTI for characterizing ischemia reveals insights into potential underlying biological correlates, including neurite beading and edema. Higher CTI sensitivity towards the ischemic lesion and potential for resolving its heterogeneity were observed. All these features bode well for future davances in CTI methodology and for applications in diverse porous systems.

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HIGH MOLECULAR-WEIGHT COMPLEXES IN THE REGULATION OF GENE EXPRESSION: A VIEW BY INTEGRATIVE STRUCTURAL BIOLOGY

The Regulator of Ty1 Transposition protein 106 (Rtt109) is a fungal histone acetyltransferase required for histone H3 K9, K27 and K56 acetylation. These acetylation sites have been linked to processing and folding of nascent H3 and play an integral role in replication- and repair-coupled nucleosome assembly. Rtt109 is unique in its activation, performed by two structurally unrelated histone chaperones, Asf1 and Vps75. These proteins stimulate Rtt109 activity via different mechanisms [1]. Rtt109–Asf1 association has been proposed to be responsible for K56 acetylation, while the Rtt109–Vps75 interaction is required for K9 acetylation [2,3].

In our work we find that Rtt109, Vps75 and Asf1 are capable of assembling as a previously uncharacterized complex onto the substrate H3-H4 dimer. Using an integrative structural biology approach based on a powerful combination of solution state NMR and small angle neutron scattering (SANS) we solve the structure of this complex and provide a mechanistic explanation for the enzyme activity [4]. We show that Vps75 promotes acetylation of residues in the H3 N-terminal tail by engaging it in fuzzy electrostatic interactions with its disordered C-terminal domain, thereby confining the H3 tail to a wide cavity faced by the Rtt109 active site. These fuzzy interactions between disordered domains achieve localization of the H3 tail to the catalytic site with minimal loss of entropy, and may represent a common mechanism of enzymatic reactions involving highly disordered substrates.

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NOT ALL MEMBRANE-MIMICKING ENVIRONMENTS ARE THE SAME. AN EPR CASE STUDY FROM *IN VITRO* TO IN CELL.

Nanobodies (*i.e.* single-domain antibodies) are promising new tools for in-cell applications due to their low molecular weight, protein- and state- specificity, nano- or sub-nano-molar affinity to their target and the possibility to be inserted into cells. We show here how spin-labeled nanobodies can be used as conformational reporters of wild type ABC transporters via Double Double Electron Resonance (DEER), a pulsed EPR technique that accurately measures inter-spin distances. First, we show a proof of principle of the use of gadolinium-labeled nanobodies against the heterodimeric exporter TM287/288 [1]. Caveats regarding the nanobody state specificity at micromolar concentrations will be discussed. Second, we present a systematic study on the homodimeric ABC exporter MsbA using non-state-specific nanobodies targeting the two nucleotide binding domains. We gathered structural information in detergent, proteoliposomes, nanodiscs, inside-out vesicles from *E. coli* and in living cells. We found that there is a remarkable modification of the conformational landscape of the transporter in specific membrane-mimicking environments, proving the need of in-cell structural studies [2]. Advantages and challenges of using biocompatible Gd-labels down to nanomolar concentrations in cells will be also addressed [3].

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PROBING ORGANIC AND HYBRID PEROVSKITE SOLAR CELL MATERIALS WITH EPR

The constantly growing energy demand and its related negative impact on the environment drives the search for alternative renewable energy sources. Organic solar cells (OSCs) have great potential for future applications. While fullerene acceptors seemed for a long time unavoidable as acceptor components, non-fullerene acceptors (NFAs) have recently taken the lead with power conversion efficiencies (PCEs) reaching over 18% for single-junction solar cell design. With these rapid advances in material synthesis and device performance, the long-term stability of OSCs has become the main remaining obstacle hampering commercialization. In order to overcome this last hurdle, a thorough understanding of the photostability of the blend materials in OSCs is needed. In the first part of the talk, the strength of combining different EPR techniques with DFT computations and optical spectroscopy will be demonstrated in order to analyse the light-induced formation of positive and negative polarons in blend materials and to study the stability of donor and acceptor materials in fullerene-free bulk heterojunction solar cells [1,2].

In parallel with the rapid recent rise in the PCEs of OSCs, lead-halide perovskites have been shown to perform excellently as photovoltaic materials. While 3D lead-halide perovskites already abundantly demonstrated their performance in thin-film photovoltaics [3], the 2D hybrid perovskites, consisting of layers of lead-halide octahedra separated by long organic linker cations, have recently emerged as being very promising for functional material design tailored towards specific optical and electronic properties. In the second part of the talk, it will be demonstrated how the earlier mentioned EPR approach can capture the light-induced charge transfer in a series of novel 2D perovskites.

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SPIN MOLECULES FOR QUANTUM COMPUTING

Spins provide one of the simplest platforms to encode a quantum bit (qubit), the elementary unit of future quantum computers. A challenge in this topic is to control the quantum decoherence in these spin qubits by minimizing the sources of decoherence (dipolar spin-spin interactions, hyperfine interactions and spin-phonon interactions). This loss of quantum information by interaction with the environment can be quantified by the phase memory time T_2, which for electronic spins can be estimated from pulsed EPR measurements. Here, I will show how a molecular approach can compete with, or even be advantageous to a conventional approach, which is based on extended inorganic lattices. Thus, the versatility of chemistry can be exploited to design robust molecular quantum spin systems showing enhanced decoherences or that host more than one spin qubit in order to implement quantum logic gates [2].

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STRUCTURAL DYNAMICS OF MEMBRANE PROTEINS BY MAGIC-ANGLE SPINNING NMR

In recent years, magic-angle spinning (MAS) NMR has developed as a powerful technique to investigate structure and dynamics of membrane proteins, enabling the study of these challenging systems in their native-like environment. Faster (100 kHz and above) MAS rates have paved the way for the direct detection of proton resonances, enabling a boost in sensitivity and resolution with respect to the more traditional approaches. In combination with high magnetic fields, this technical progress revolutionizes the atomic-level investigation of proteins i) by enlarging the molecular size of the systems that can be investigated with site specificity; ii) by reducing the requirements in terms of isotopic labeling, notably deuteration; iii) by speeding up the tedious processes of resonance assignment and acquisition of dynamical parameters.

Here we review the strategies underlying this leap forward and describe its potential for the detailed characterization of different transmembrane channels and transporters reconstituted in lipid bilayers. By measuring extended sets of site-specific dynamic observables involving both the backbone and the side chains, we unveil the presence of localized variations in conformational flexibility in regions responsible for substrate selectivity and transport and shed new light on the relation between conformational plasticity and function.

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INCURSIONS OF NMR METABOLOMICS IN DISEASE RESEARCH

This talk will cover examples of applications of NMR metabolomics in two different contexts: 1) stem cell (SC) metabolism for guidance of differentiation onto pure lineages for tissue regeneration improved strategies, and 2) understanding and following-up breast cancer (BC) therapy, in two *in vivo* murine models of BC (triple-negative (poor prognostic) and endocrine (one of the most common).

The first part of this talk is based on SC differentiation as a means for specific tissue regeneration, an ongoing important research field with multiple clinical applications. As well as using metabolomics to characterize endo- and exometabolome stepwise adaptations during SC differentiation, issues such as inter-donor variability or SC aging effects are not yet fully known or understood. Here, NMR cell metabolomics is shown to unveil the full metabolome of adipose tissue SCs and their progression through osteogenesis. Transversal osteogenesis metabolic markers are suggested, as are apparent donor-specific metabolites and corresponding pathways. Furthermore, the effects of SC aging, seldomly considered as important underlying contributions, are identified and used to refine global progression markers into osteogenic-only markers. These results show how NMR metabolomics can help in understanding and, potentially, optimizing SC behavior in tissue regeneration medicine. Future perspectives to enhance such knowledge will be discussed.

Secondly, this talk will address the process of acquisition of endocrine therapy resistance in a medroxyprogesterone acetate (MPA) mouse model of BC, and the use of NMR metabolomics of tumor tissue to find resistance metabolic signatures. Implications of the results on therapy schemes for endocrine BC will be briefly discussed. In addition, metabolomics of a xerograph mouse model of triple-negative BC will also be addressed, including the characterization of the tumors themselves upon treatment with cisplatin and a novel potential palladium complex, as well as the effects of those drugs on different mouse organs, to assess toxicity effects. The above results exploit the use of different *in vivo* murine models of BC, to unveil tissue metabolic signatures and their contribution to understanding tumor metabolic dynamics during disease BC progression or therapy.

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NEW METHODS IN NMR CRYSTALLOGRAPHY

Structure elucidation of amorphous materials and microcrystalline solids presents one of the key challenges in chemistry today. While techniques such as single crystal diffraction and cryo-electron microscopy are generally not able to characterize such materials, we will show how an approach based on measured NMR chemical shifts in combination with Machine Learned calculation of shifts from candidate structures can rapidly determine full three-dimensional structures from powders. Further, since the method does not require any significant long-range order, we have recently shown ho it can be extended to determine structures in non-crystalline, amorphous, and hierarchical or composite materials.

In the talk we will focus on some of the most recent key methodological advances, which include:

- Super-resolution ^{1}H NMR spectroscopy in solids;
- Bayesian probabilistic assignment of chemical shifts in organic solids; and
- Sensitivity gains provided by dynamic nuclear polarization (DNP).

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TUTORIAL LECTURE

RAINER KIMMICH

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FIELD-CYCLING NMR RELAXOMETRY: ELUCIDATION OF COMPLEX MOLECULAR DYNAMICS

In this tutorial, we will outline the unique strength of field-cycling NMR relaxometry for the characterization of molecular dynamic processes in ordered, macromolecular or porous media [1]. The methodological idea behind and typical experimental set-ups will be described in terms of technical and physical limits at low and high fields. This in particular refers to the Redfield limit, *i.e.* the condition that the spin-lattice relaxation rates must be less than the fluctuation rates of spin interactions in order to be compatible with the Bloch/Wangsness/Redfield (BWR) relaxation theory. With restricted molecular motions leaving secular spin interactions partially unaveraged, so-called local fields will further limit the experimental range at low fields.

Fluctuations of spin interactions, *i.e.* the thermal phenomena causing spin relaxation, are described and characterized by correlation functions or – via Fourier transform – by spectral densities. We will outline how these functions can be derived for given model scenarios of molecular dynamics in diverse complex systems. The basis of such probability treatments is in each case an adequate equation of motion plus topological constraints imposed by the system. Relevant equations of motion are normal or anomalous rotational or translational diffusion equations and Langevin-type equations predicting distributions of modes in elastically coupled systems.

Applications to be discussed in more detail are (i) order director fluctuations in nematic liquid crystals both in bulk and subjected to surface ordering in porous media, (ii) reptation, *i.e.* the snakelike displacement of polymer segments through chain-entangled or pore-confined systems, and (iii) dynamic exchange and displacement processes of fluid adsorbate molecules at surfaces of porous media. The dominating mechanism of the latter scenario will be shown to be “reorientation by translational displacments” (RMTD) along the topology of pore surfaces. This process can be influenced in a non-thermal way by flow-enhanced transport of adsorbate fluids through pore networks. Corresponding experimental and computer-simulated data will be presented. Finally, a list of general take-home rules summarizing this tutorial will be discussed.

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TOWARDS AUTOMATED AND MULTI STATE NMR STRUCTURE DETERMINATION

NMR belongs to one of the main methods for atomic resolution structure determination of biomolecules. In order to automate the 3D structure determination of proteins we implemented machine learning in peak picking and sequential assignment in concert with FLYA and CYANA to fully automatize protein structure determination from amino acid sequence and multi-dimensional NMR spectra (including 2D correlation and 3D backbone triple resonance experiments, side chain correlation and NOESY experiments) as input with almost complete success rate on 100 protein systems (with a molecular weight up to ca 20 kDa) tested within 5 hours of calculation time.

The exact NOE approach is further discussed as it enables multi-state structure determination of proteins opening along with relaxation measurements an avenue towards a movie of a protein at atomic resolution. Applications therein on 4 different systems (*i.e.* PDZ, WW domain, cyclophilin, KRAS) are presented.

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INVESTIGATION OF THE LIPIDATED C-TERMINUS OF RALA: MUTUALLY EXCLUSIVE BINDING TO MEMBRANES OR CALMODULIN.

RalA is a small GTPase and a member of the Ras family. This molecular switch is activated downstream of Ras and is widely implicated in tumour formation and growth. The C-terminus of RalA is isoprenylated, which, along with a polybasic motif, anchors the protein to the lipid bilayer. We have used nanodiscs to assess the interactions of RalA with the membrane and found that the bound nucleotide modulates the extent of the interactions, with the inactive, GDP-bound RalA binding more tightly to the nanodisc. Previous work has shown that the ubiquitous Ca^{2+}-sensor calmodulin (CaM) binds to small GTPases such as RalA and K-Ras4B but a lack of structural information has obscured the functional consequences of these interactions. We have investigated the binding of CaM to RalA and found that CaM interacts exclusively with the C-terminus of RalA. Biophysical and structural analyses show that the two RalA membrane-targeting motifs (the prenyl anchor and the polybasic motif) are engaged by distinct lobes of CaM and that CaM binding leads to removal of RalA from its membrane environment. The structure of this complex, along with a biophysical investigation into membrane removal, provides a framework with which to understand how CaM regulates the function of RalA.

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CROSS-RELAXATION INDUCED BY THE ANTISYMMETRIC PART OF THE CHEMICAL SHIFT TENSOR

One of the NMR-based methods of structure determination of molecules, especially those of biological activity such as proteins and nucleic acids, is utilizing interferences between nuclear relaxation mechanisms. In particular, the interference due to the dependence of the magnetic field nearby the nuclei on the orientation of the molecule with respect to the external magnetic field, *e.g.*, that which is caused by the chemical shift anisotropy (CSA), namely the CSA cross-relaxation, may be used for this purpose. For instance, CSA cross-correlation provides the dihedral angles of the protein backbone, facilitating the determination of the overall three-dimensional structure of the biomolecule [1].

We show that an overlooked part of the CSA interaction, the so-called vector antisymmetry of chemical shift tensor ($\boldsymbol{\sigma}^*$), defined as $(\sigma^*)_\alpha = \epsilon_{\alpha\beta\gamma}\sigma_{\beta\gamma}/2$, contributes to the CSA cross-relaxation rate constant, and its neglection may cause an erroneous attribution of the molecular conformation.

The rate constant of the CSA cross-correlation caused by the antisymmetric part of CS tensor (anti-CSA) was found using the Bloch-Redfield-Wangsness theoretical description of nuclear relaxation processes [2]. We found that this rate constant for the cross-correlation of anti-CSA of nuclei *I* and *S* is proportional to the factor $\gamma_I\gamma_S B^2 \boldsymbol{\sigma}_I^* \cdot \boldsymbol{\sigma}_S^*$, where $\gamma_{I/S}$ is the gyromagnetic ratio of the nucleus I/S and B is the strength of the magnetic field. Therefore, the most favorable orientation of anti-CSA represented as a vector is when antisymmetries are parallel to each other, *e.g.*, most planar molecules fulfill this condition.

The dramatic influence of anti-CSA is demonstrated using a combined computational and experimental approach in studies of *cis*-difluorodichloroethene. It follows from the coupled-cluster quantum chemical computations performed in the CFOUR computer program that the symmetric parts of ^{19}F nuclei in *cis*-difluorodichloroethene molecule are oriented relative to each other in such a way that almost only anti-CSA contributes to the ^{19}F-^{19}F cross-relaxation rate. For this simple model molecule, the magnitude of anti-CSA reaches 30 ppm for ^{19}F nuclei, and the contribution of anti-CSA to the total CSA cross-relaxation rate is at least 90%. Our theoretical results are supported by experimentally determined rate constants of ^{19}F-^{19}F cross-relaxation in the ^{19}FCl^{13}C=^{12}C^{19}FCl molecule and measurements of cross-correlated relaxation constants involving interaction between the CSA and dipole-dipole relaxation mechanisms.

Moreover, form the computational studies of amino acids, we found that anti-CSAs of ^{15}N/^{13}C$_\alpha$ nuclei are of the order of several ppm. Therefore, the anti-CSA contribution to CS-induced cross-correlation affects the structure determination of an oligopeptide. Consequently, the proper analysis of molecular conformation using CSA cross-correlation requires introducing a new component that depends on the antisymmetric part of the chemical shift tensor.

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4'-F URIDINE-A SENSITIVE LABEL FOR PROBING RNA STRUCTURE AND FUNCTION BY ^{19}F NMR

Due to the high sensitivity in chemical shift, the 100% natural abundance and the pronounced bioorthogonality of ^{19}F, ^{19}F NMR has been successfully used to investigate secondary structure and function of nucleic acids for more than a decade [1]. There are several main strategies in the field of fluorine-labeling, one of which is the introduction of fluorine(s) at sugar, usually C2'-position [2]. Among them, 2'-F-labeled RNA is powerful tool, but it directly affect ribose puckering [2].

Structurally, C4'-position should be an attractive modification site in ^{19}F-labeling. First of all, C4'-substitutions locate on the edge of the minor groove upon duplex formation, with no significantly affecting the helical conformation [3]. Secondly, C4'-position is a member of the phosphate backbone (P_0-O5'-C5'-C4'-C3'-O3'-P_{-1}) which has directly interactions with proteins, such as cGAS and ribonucleases. Unfortunately, due to the instability of 4'-F-modified nucleosides, scientists have not successfully introduced them into the RNA strands [4]. The phenomenon, cleavage of glycosidic bonds in conventional experimental solutions, is the main reason for limiting its continued research.

Herein, we reported a more mature strategy through a selective protection of the hydroxy groups in stages to synthesize 4'-F-modified uridine phosphoramidite monomers, which was stable enough to then be incorporated into longer oligonucleotides through standard solid-phase synthesis. Therfore, we incorporated $U^{4'\text{-}F}$ into oligos and investigated their biophysical and biochemical properties. The 4'-F modification does not significantly alter the nature of RNA and therefore can mimic the state of native RNA for structural studies. ^{19}F NMR results show that this modification can not only distinguish between ssRNA and dsRNA, but also identify mismatches and binding of RNA-processing proteins with chemical shift dispersion up to 4 ppm, indicating that this modification can be widely used through NMR spectroscopy for determination various RNA structures [5].

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BACTERIAL TYPE 1 PILI AS A NEW VERSATILE ALIGNMENT MEDIUM FOR NMR SPECTROSCOPY

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The measurement of NMR Residual Dipolar Couplings (RDCs) is a powerful tool to gain information on atomic-level structure and dynamics of biomolecules. RDCs become measurable when a molecule is partially aligned with respect to the NMR magnetic field. To reach this partial alignment, a variety of anisotropic media such as bicelles, strained gels or filamentous phages can be used to induce predominantly steric or electrostatic alignment [1,2]. However, finding suitable alignment media compatible with various types of molecules and a broad range of experimental conditions remains a challenge.

Here we present a new liquid-crystalline alignment medium composed of purified type 1 pilus rods from *Escherichia coli*, which are filamentous protein polymers extending from the surface of the cell into the extracellular space [3,4]. Remarkably, under physiological conditions type 1 pilus rods have practically infinite kinetic stability against spontaneous dissociation/unfolding [5]. Using a recombinant system to elongate the wild-type pilus rod from an average length of ~670 nm to ~2100 nm, we show that alignment is achieved at a minimum pilus concentration of ~15 mg/ml, which together with a yield of ~17 mg pili obtained per liter of bacterial culture allows for efficient NMR sample preparation. In agreement with the high stability of the pilus against denaturation, the alignment medium was stable at all temperature tested (4 - 50°C) and withstood challenging conditions such as pH 1.5, the presence of commonly used detergents and lipids (including 1% (w/v) SDS), or 50% (v/v) DMSO, DMF or methanol. To demonstrate the applicability of the type 1 pilus alignment medium, we measured RDCs of three different systems - a protein (human ubiquitin), an RNA (HIV-1 TAR) and a small organic molecule (camphor) – and show that in each case, experimental RDCs were highly correlated with RDC values back-calculated from high-resolution structures of these molecules. Overall the alignment induced by type 1 pili appears to arise mainly from steric interactions with a negative electrostatic contribution.

In summary, the pilus-based medium is compatible with challenging experimental conditions and is usable with both biological macromolecules and small organic molecules. These results suggest that type 1 pili should represent a very useful complement to existing alignment media and may assist molecular structure or dynamics determination by NMR.

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DISTINGUISHING THE DIMENSIONALITY OF A SPIN SYSTEM: DETERMINATION OF THE TOMONAGA-LUTTINGER PARAMETER *K* IN QUASI-1D SYSTEMS

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The nuclear magnetic resonance (NMR) in antiferromagnetic quantum spin materials has recently provided a major result regarding quasi-one-dimensional (quasi-1D) systems, namely an important improvement of the standard, "Tomonaga-Luttinger liquid" (TLL), low-energy description of these systems in their gapless regime (typically induced by magnetic field). This purely 1D description is characterized by power-law dependence of the response/correlation functions, whose exponents are defined by the TLL parameter *K*. However, at low temperature the effects of 3D exchange couplings significantly modify the TLL response, which can be theoretically described by a random phase approximation (RPA). We confirm the validity of the recently derived RPA correction for the spin-spin correlation functions [1], by applying the corresponding formula to the temperature dependence of the NMR relaxation rate T_1^{-1} in the two representative quasi-1D spin compounds, $(C_7H_{10}N)_2CuBr_4$ (DIMPY) and $BaCo_2V_2O_8$. A successful fit of the T_1^{-1} data provides the first direct experimental determination of the *K* values that perfectly agree with the theoretically calculated ones [2].

In general, by taking into account the RPA correction, we strongly broaden the scope of the TLL description, which becomes applicable to less quasi-1D compounds/systems approaching the 3D regime. The RPA correction can be easily calculated for any geometry of the 3D exchange couplings, and provides a $T_1^{-1}(T)$ dependence that is clearly different from those observed in either quasi-2D [3] or in 3D spin systems. NMR T_1^{-1} data thus provide an exceptionally simple way to distinguish the effective dimensionality of a gapless spin system. In quasi-1D systems, from T_1^{-1} data we directly determine the *K* parameter, providing insight into the nature and the strength of the quasiparticle interactions.

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INTRIGUING SPINON-METAL STATE OF AN INSULATING KAGOME LATTICE ANTIFERROMAGNET

Spinon metal is a fascinating state of mater found in certain charge insulators that feature strong geometrical frustration of the underlying spin lattice. Contrary to more common ferromagnetically or antiferromagnetically ordered states, it belongs to a class of states known as quantum spin liquids. These highly quantum entangled states remain magnetically disordered down to zero temperature due to strong quantum fluctuations and feature exotic magnetic excitations known as spinons. In the case of a spinon metal, spinons form a Fermi surface and may, in a way, behave similarly as itinerant electrons behave in ordinary metals.

Realizations of spinon metals in real materials are extremely rare. I will present such case that has been recently confirmed in the kagome lattice antiferromagnet Zn-brochantite, $ZnCu_3(OH)_6SO_4$, via local-probe techniques including muon spectroscopy and nuclear magnetic resonance. Quite unexpectedly, these investigations have revealed that the spinon-metal state in Zn-brochantite undergoes a magnetic-field induced instability due to spinon pairing at low temperatures [1]. This phenomenon is analogous to the formation of Cooper pairs of electrons in superconductors. Moreover, another phenomenon traditionally associated with itinerant electrons has been recently discovered in this material [2] in which the charge degrees of freedom are frozen. Namely, magnetic moments of impurities present in Zn-brochantite become screened by spinons at low temperatures in an event that resembles Kondo screening of magnetic impurities by conduction electrons in metals. In this spinon Kondo effect that was theoretically predicted a while ago but has been observed experimentally for the first time in Zn-brochantite [2], the role of electrons in Kondo screening is taken over by chargeless spinons. Both phenomena, spinon pairing and spinon Kondo effect, make spinon metals highly attractive for emerging quantum technologies.

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INVERSE LAPLACE TRANSFORM (ILT) T_1 ANALYSIS AS A NEW PROBE OF QUANTUM MATERIALS WITH DISORDER

Magnetic materials generally undergo a phase transition into a magnetically ordered ground state at low temperatures. When the spin-spin interactions are geometrically frustrated, such a phase transition may be prevented and a novel quantum spin liquid (QSL) sate may be realized instead, in which spins are highly entangled but remain paramagnetic [1]. Examples of such QSL's include Kitaev lattice formed by Ising spins arranged on the honeycomb structure, and spins interacting with Heisenberg's exchange interaction on the the kagome lattice (*i.e.* corner-sharing triangular lattice). Identifying such a QSL material is a holy grail of today's quantum condensed matter physics, and NMR techniques plays a pivotal role in characterizing their local magnetic properties [2]. A common trait shared by various QSL candidate materials is that their ground state properties are sensitive to structural disorder, which makes proper characterization of the magnetic ground state difficult due to the distribution of the NMR parameters. In this talk, we will demonstrate how one could overcome this difficulty by deducing the density distribution function $P(1/T_1)$ of the NMR spin-lattice relaxation rate using the inverse Laplace transform techniques [3-6].

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PRESSURE-TUNED MAGNETIC INTERACTIONS IN A TRIANGULAR-LATTICE QUANTUM ANTIFERROMAGNET

Quantum triangular-lattice antiferromagnets are important prototype systems to investigate numerous phenomena of the geometrical frustration in condensed matter. Apart from highly unusual magnetic properties, they possess a rich phase diagram (ranging from an unfrustrated square lattice to a quantum spin liquid), yet to be confirmed experimentally. One major obstacle in this area of research is the lack of materials with appropriate (ideally tuned) magnetic parameters. Using Cs_2CuCl_4 as a model system, we demonstrate an alternative approach, where, instead of the chemical composition, the spin Hamiltonian is altered by hydrostatic pressure [1]. The approach combines high-pressure electron spin resonance and r.f. susceptibility measurements, allowing us not only to quasi-continuously tune the exchange parameters, but also to accurately monitor them.

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^{75}AS NQR STUDY OF QUASI ONE-DIMENSIONAL $A_2MO_3AS_3$ SUPERCONDUCTORS

$A_2Mo_3As_3$, where A stands for K, Rb or Cs, is a family of quasi one-dimensional conductors, where MoAs form quasi one-dimensional double-walled subnanotubes, with A ions in between chains. It is isostructural to the $A_2Cr_3As_3$ family, which was shown to be superconducting at low temperatures, with $T_c = 6.1$ K [1], $T_c = 4.8$ K [2] and $T_c = 2.2$ K [3], for A = K, Rb and Cs respectivelly. The Molybdenum family achieves higher critical temperatures of 10.4 K [4], 10.5 K [5] and 11.5 K [6] for A = K, Rb and Cs respectivelly.

In this talk, a comprehensive ^{75}As NMR study of of $A_2Mo_3As_3$ for A= K, Rb, Cs is presented [5]. The ^{75}As NQR spectra show surprising results, indicating four NQR lines, not conforming to the generally accepted structure and space group ($P\bar{6}m2$), which suggests just two chemically inequivalent As sites. Recent density functional theory and neutron total scattering studies of $K_2Cr_3As_3$ propose structural instability in the original structure and a local deformation to a space group Amm2 [7]. A possibility of a similar occurance in $K_2Mo_3As_3$ is thus explored here.

Both $A_2Cr_3As_3$ and $A_2Mo_3As_3$ show a power-law temperature dependencies of spin-lattice relxation rates, which are fingerprints of the Tommonaga-Luttinger liquid behaviour above T_c. However, there is an important difference between the two families of materials with $A_2Cr_3As_3$ showing repulsive interactions and $Rb_2Mo_3As_3$ showing attractive interactions. Interestingly, ^{75}As NQR and ^{87}Rb NMR show different spin-lattice relaxation rate dependence with temperature in $Rb_2Mo_3As_3$. In the $K_2Mo_3As_3$, even the different As sites show different spin-lattice relaxation rates, suggesting the importance of multi-orbital physics.

The importance of multi-orbital physics is again shown in the superconducting state. No Hebel-Slichter cohrenece peak was observed in both $Rb_2Mo_3As_3$ and $K_2Mo_3As_3$, the first sign of unconventional superconductivity. ^{75}As NQR in $Rb_2Mo_3As_3$ shows BCS gap, while ^{87}Rb NMR shows a reduced gap. In the $K_2Mo_3As_3$ again, even different As sites show different behaviour in the superconducting state, with one site observing the BCS gap, and the others following the Korringa relation even below T_c.

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CELLULAR SOLID-STATE NMR SPECTROSCOPY: RECENT PROGRESS AND APPLICATIONS

Solid-state NMR (ssNMR) spectroscopy provides increasing possibilities to study biomolecules in their native pro– or eukaryotic cellular setting. To conduct such studies, we have designed preparative procedures that reduce unwanted background labelling by dedicated expression [1,2], purification [3] and protein delivery [4] methods. In addition, we have addressed challenges regarding spectroscopic sensitivity by either using ^{1}H-detection (see, *e.g.* [5]) or Dynamic Nuclear Polarization (DNP) to enhance spectroscopic signals. In the latter case, we have investigated the use of hydrophilic tritryl-nitroxides together with Liu *et al*. [7] because of their favourable magnetic-field strength dependence.

In our presentation, recent methodological progress will be discussed. In addition, we report on applications in the context of whole-cell biocatalysis [8] and the study of protein-protein interactions in bacterial and human cells. In particular, we demonstrate the use of cellular ssNMR to study the supramolecular structure and dynamics of the lipoprotein BamC as part of the β-barrel assembly machinery (BAM) complex that catalyzes -barrel protein insertion into the outer membrane of E.coli (see, *e.g.* [8,9]). Using ^{1}H ssNMR we have studied BAM complexes containing BamC in lipid bilayers and cellular environments. This method allowed us to probe the structural and dynamic changes of BamC in different protein complexes and membrane environments on a residue-specific level. Our results reveal a remarkable topological variability of BamC, which may be critical during different stages of the BAM-mediated substrate insertion process in bacteria [10].

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PROBING PROTEIN STRUCTURAL DYNAMICS IN-CELL BY NITROXIDE-BASED SDSL-EPR

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Understanding how the intracellular medium modulates protein structural dynamics and protein-protein interactions is an intriguing but required topic scientists search to address by studying biomolecules in their native environment. As the cellular environment can not be reproduced in vitro, investigation of biomolecules directly inside cells has attracted a growing interest in the past decade. Indeed, efforts in magnetic resonance spectroscopies have enabled important improvements in the study of structural dynamics directly in the cellular context.

Among magnetic resonances approaches, site-directed spin labeling coupled to electron paramagnetic resonance spectroscopy (SDSL-EPR) has demonstrated to be one of the powerful approaches to study structural properties of biomolecules[1,2]. In particular, nitroxide-based SDSL-EPR couples the benefits of high sensitivity and the lack of size constraints for the biomolecule of interest with the ability to study protein structural transitions and interactions at physiological temperature.

In this talk, we will discuss the results achieved in the investigation of the structural dynamics features of several cytosolic proteins directly inside cells, by combining the use of nitroxide labels and EPR (cw-EPR and pulsed dipolar experiments) spectroscopy. Furthermore, we will focus on NarJ protein [3], for which, beside reaveling its structural features directly in *E. coli*, we verify its activity inside cells, by evaluating its ability in restoring the activity of its biological partner, the nitrate reductase.

These results represents a step forward in the development of EPR-based cellular structural biology that goes beyond the feasibility and opens new perspectives to address biological questions directly inside the cell.

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PROTEIN-DRUG INTERACTIONS MONITORED BY TIME-RESOLVED NMR IN HUMAN CELLS

In-cell NMR can investigate protein conformational changes and chemical modifications at atomic resolution directly in living cells. The approach has recently shown great potential in the context of drug development, as it can investigate directly the interaction between a ligand and its target protein in its physiological environment. As such, in-cell NMR can provide precious insights on the intracellular drug-target interaction that can be beneficial to assess the efficacy of active compounds at an early stage of drug development and to increase the chances of success in the subsequent pre-clinical and clinical studies. Furthermore, NMR bioreactors can greatly improve the cell sample stability over time and allow monitoring the evolution of intracellular processes, including protein-drug interactions, by time-resolved in-cell NMR. Here, the latest developments of in-cell NMR applied to protein-drug interactions in human cells are overviewed. First, drug screening in human cells is applied to investigate a set of novel carbonic anhydrase (CA) inhibitors, which are found to bind the intracellular target in a dose- and time-dependent manner [1]. The same approach is applied to study the off-target binding to CA of drugs originally developed to interact with other targets [2]. Then, an optimized design of in-cell NMR bioreactor is applied to measure drug diffusion and binding in real time [3,4]. Finally, a novel application of in-cell NMR is reported, where intracellular ligand binding affinities are measured in human cells by competition binding experiments.

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TRITYL SPIN LABELS: STUDYING BIOMOLECULAR CONFORMATIONS WITHIN CELLS BY PULSED DIPOLAR EPR

The understanding of biomolecular function is coupled to knowledge about the structure and dynamics of these biomolecules, preferably acquired under native conditions. In this regard, pulsed dipolar EPR spectroscopy (PDS) in conjunction with site–directed spin labeling (SDSL) is an important method in the toolbox of biophysical chemistry. However, the currently available spin labels have diverse deficiencies for in–cell applications, for example, low radical stability or long bioconjugation linkers.

In the talk, synthesis strategies are introduced for the derivatization of trityl radicals with a maleimide–group, which enables bioconjugation of the trityl labels to cysteins [1-4]. Especially, the resulting trityl spin labels **SLIM** [3] and **oxSLIM** [4] yield narrow distance distributions, enable highly sensitive distance measurements down to concentrations of 45 nM, and show high stability against in cell reduction. Using these labels in combination with the Double Quantum Coherence experiment (DQC), the guanine–nucleotide dissociation inhibitor (GDI) domain of *Yersinia* outer protein O (YopO) is shown to adopt in eukariotic cells a different conformational ensemble than in vitro [3].

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IN-CELL CHARACTERIZATION OF THE STABLE TYROSYL RADICAL IN *E. COLI* RIBONUCLEOTIDE REDUCTASE VIA ADVANCED EPR SPECTROSCOPY

Determining the structure of biomolecules in their intracellular environment is fundamental to understand their function. Yet, achieving high resolutions within the cell possesses a great challenge. In this work, we employed advanced electron paramagnetic resonance (EPR) spectroscopic methods to characterize the structure and dynamics of Y_{122}•, the stable tyrosyl radical in E. coli ribonucleotide reductase (RNR) in whole cells at atomic resolution.

The *E. coli* RNR, a paradigm for class Ia enzymes including human RNR, catalyzes the biosynthesis of DNA building blocks and requires a di-iron tyrosyl radical (Y_{122}•) cofactor for activity.[1] The knowledge on *in vitro* Y_{122}• structure and its radical distribution within β2 subunit has accumulated over the years; yet, little information exists on *in vivo* Y_{122}•. Therefore, determining *in vivo* structure and distribution of Y_{122}• in RNR is essential to understand the function of the protein in its native intracellular environment. First, we employed multi-frequency EPR (9, 34, and 94 GHz) spectroscopy to characterize the generated Y_{122}• radical in whole *E. coli* cells. Our experimental data combined with spectral simulations demonstrated that the structure and environment of Y_{122}• in the cells are highly similar to those of Y_{122}• *in vitro*. Next, we performed orientation-selective electron-nuclear double resonance (ENDOR) spectroscopy at 34 GHz to probe the H-bonding environment of Y_{122}• in living cells. The analysis of our ENDOR data with a significantly high signal-to-noise ratio displayed that the number of hydrogens coupled to Y_{122}• in the cells and *in vitro* is the same. At last, we detected distances between two Y_{122}•s residing in each β monomer of *E. coli* RNR via double electron-electron resonance (DEER). These orientation-selective experiments revealed insights into not only the *in vivo* structure and dynamics of Y_{122}• and β2 subunit but also the *in vivo* radical distribution within β2.[2]

Additionally, we have site-specifically incorporated 2,3,5-trifluorotyrosine (F_3Y) at residue 122, generated and identified its radical form F_3Y_{122}• in the cells, and obtained *in vivo* distances between F_3Y_{122}•s. This marks the first spectroscopic verification of the generation of an unnatural amino acid radical in whole cells providing a new possibility for investigating the structure and role of tyrosyl radicals involved in fundamental processes such as photosynthesis, reduction of O_2 to water, and DNA repair in living cells.

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UPTAKE OF RL2 BY HUMAN LUNG CANCER CELLS: MONITORING BY EPR AND CONFOCAL MICROSCOPY

A recombinant κ-casein fragment – intrinsically disordered recombinant lactaptin 2 (RL2) has been shown penetrating both cancer and normal cells and inducing apoptosis of cancer cells [1, 2]. Since penetration into cells is known to be an essential step for biological action of any therapeutic molecules the knowledge of RL2 penetration pathways is relevant. Cell-penetrating peptides are known for their effective penetration into cells via direct penetration through cell membrane or endocytosis and for their ability to deliver cargo molecules.

We applied confocal microscopy and CW-EPR methods to examine RL2 penetration into human lung A549 cancer cells. RL2 was labeled by a reduction resistant nitroxide [3-((2,5-dioxopyrrolidin-1-yloxy)carbonyl)-2,2,5,5-tetraethyl-1-oxyl] spin label to perform in-cell EPR measurements. A549 cells incubated with spin labeled RL2 showed the presence of three EPR spectral components related to different spin label mobility and rotation correlation times differing by more than an order of magnitude. The weight ratio of these components was changing with time during the cells monitoring by CW-EPR. Confocal microscopy results and EPR spectra simulations allowed us to assign these components to 1) spin labeled RL2 which is sticking on the membrane surface of endosomes and having spin label rotation correlation time 4.78 ns, 2) spin labeled RL2 with mobility similar to buffer solution and rotation correlation time 2.95×10^{-1} ns, 3) spin labeled amino acids formed in cells due to protein digestion with rotation correlation time 4.1×10^{-2} ns. Thus, CW-EPR and the spin label utilized allowed us to follow the kinetics of different forms of spin labeled RL2 during more than 10 hours after cells incubation with minimal spin concentration 10-60 μM in cells. The results of the set of experiments will be shown.

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MULTIVARIATE AND MULTIWAY NMR

Nuclear magnetic resonance (NMR) spectroscopy is the single tool most widely used by chemists for determining the molecular structures of unknown compounds. It is a wonderfully versatile and sensitive tool, but it has one major drawback: it often struggles to analyze mixtures. Many of the most challenging problems are presented to us as mixtures, so a great deal of effort goes into separating the individual components so that they can be identified and characterized.

Fortunately, powerful NMR methods for the analysis of intact mixtures are making its way into the standard set of experiments available to the spectroscopists. One of the most potent is diffusion-ordered spectroscopy (DOSY) in which the signals from different components can be separated by their different diffusion properties. The information available from the basic DOSY experiment can be enhanced by advanced, e.g. multivariate, processing and extended by incorporating information from other sources such as relaxation.

This lecture will be focussed on using multivariate and multiway statistics to extract information that is otherwise very hard to obtain. Two-dimensional multivariate techniques, such as PCA, can be very powerful for NMR data, but the so-called rotational ambiguity often results in spectra and components that are linear combinations of the pure components. This can, in suitable cases, be overcome by constraining the solution to match known physical phenomena, such as the shape of the signal decay in a DOSY experiment (SCORE [1,2]) or the form of the kinetics for a chemical reaction. When additional dimensions can be added the data is said to be multiway (e.g. three-way, four-way). When the dimensions are mathematically independent, the rotational ambiguity is no longer affecting the data and pure components can be extracted directly from the multi-way decomposition. Examples of this includes, classic chemical reactions [3,4], pathogenic fibril formation [5], and beer analysis [6].

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DE NOVO STRUCTURE DETERMINATION OF ORGANIC COMPOUNDS FROM MULTI-ALIGNMENT DATA SETS

Residual Dipolar Couplings (RDCs) have proven useful tools in organic structure determination for the determination of configuration and/or conformation. [1] Previous approaches for obtaining structures from RDCs in organic compounds mainly rely on fitting data to structural proposals (e.g., the different diastereoisomers) to verify or falsify them. The process becomes intricate or might even fail in determining the structure, if flexibility plays a (significant) role in the unknown compound. The number of structural proposals grows exponentially with increasing flexibility as then configuration and conformation need to be taken into account (all conformers of each respective diastereoisomer). More recent approaches use RDCs as (pseudo)forces in structure optimisation processes to account for this. [2]

Here we present a novel and completely different approach for organic structure determination, which allows for **directly obtaining vector and dynamics information** from measurements of RDCs in several linearly independent media. While based on an approach from biomolecular NMR [3], it was unclear whether such an approach would be feasible at all for organic compounds.

The talk will answer the questions whether in cases of unknown conformation and configuration it is possible to discriminate structures, whether it is possible to induce the many different orientations necessary, and whether it is possible to measure enough couplings per compound. [4]

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DEVELOPMENTS IN ULTRAFAST 2D NMR FOR THE ANALYSIS OF OUT-OF-EQUILIBRIUM MIXTURES

NMR spectroscopy is a powerful tool for the analysis of mixtures, and the complexity of mixtures often calls for the use of multidimensional experiments. For samples that evolve in time, the duration of these experiments can be a limitation. Ultrafast 2D NMR based on spatial encoding is the fastest approach to collect 2D NMR data [1-2]. It has been used for the monitoring of chemical reactions, and the analysis of hyperpolarised substrates. However, the use of spatial encoding is challenging for samples that experience flow. This is a limitation for online monitoring, and for samples hyperpolarised with methods that involve rapid displacements.

Over the past few years, we have developed an array of ultrafast 2D NMR experiments for the analysis of out-of-equilibrium mixtures that experience different types of flow. We have characterised the effects of flow on the spatial encoding process, and proposed several methods to improve the experiments' accuracy in flow conditions [3-5]. Two types of applications are desribed in this presentation.

Online monitoring using flow NMR is a growing area with applications in organic chemical synthesie and process monitoring, that would benefit from fast 2D NMR methods. Interference between flow and spatial encoding can result in significant variations of the peaks' intensities of UF 2D ^{1}H-^{1}H correlation spectra, and these variations are a severe obstacle for reaction monitoring. We showed that different spatial encoding schemes are all affected by the process when a longitudinal axis is used for encoding. On the other hand, using a transverse axis for spatial encoding, accurate results can be obtained. This makes it possible to collect 2D spectra in a single scan for flow rates of up to 2.5 mL/min that are typical for online monitoring.

Hyperpolarisation methods provide a much needed sensitivity boost for NMR spectroscopy, which could notably benefit to the analysis of mixtures by diffusion-ordered spectroscopy (DOSY). For dissolution dynamic nuclear polarisation (D-DNP), and Signal Amplification By Reversible Exchange (SABRE) implemented with the sample-shaking approach, rapid displacement of the sample just before acquisition induces significant internal motion.We showed that, for UF DOSY, the effect of flow can be compensated by a double-diffusion encoding strategy, as well as by transverse encoding. With UF DOSY sequences that compensate or avoid flow effects, accurate results can be obtained in a single scan, thus combining sensivity enhancement through hyperpolarisation and extra information through diffusion.

Overall, these methods have the potential to increase the information that can be obtained from mixtures that evolve in time.

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NOVEL DARK STATE PHOSPHATE ASSEMBLY FORMATION IN AQUEOUS SOLUTIONS

Phosphate-containing species are in constant flux throughout the phosphorous cycle and are pooled within the cells of all living organisms. Besides, they also play a potental role in quantum neuroscience. An understanding of the phosphate equilibrium with phosphate-containing species and structures under varying conditions allow for the manipulation of biological energy and the formation and dissolution of biological structures. ^{31}P NMR is commonly used to characterize the composition, dynamics and structural properties of biomolecules and lipid interfaces. While performing ^{31}P NMR to investigate the native state of phosphate species as a function of temperature, we encountered peculiar line broadening and relaxation effects that cannot be explained by typical dynamical processes of small molecules. We will present results showing that phosphate containing species reversibly assemble into unreported spectroscopically “dark” species, whose fractional population increases with temperature.

The NMR-based observation is shown to be consistent with the formation of soft phosphate assemblies. Dynamic Light Scattering further provides evidence for the formation of micron-sized assemblies. The formation of spectroscopically dark states appears largely reversible (by cooling to 20 °C), with a minor irreversible (and filterable) component. These findings suggest an entropically driven association mechanism, with a small population becoming kinetically trapped. We show that assembly can be facilitated by the addition of depletants and modulated by changing counterion salt type, with trends found in line with the Hofmeister series. ^{31}P NMR Diffusion Oriented Spectroscopy revealed an increase in diffusion coefficients at elevated temperatures of 70 °C, indicative of dehydration. To further explore the equilibrium of this dark population, we performed chemical exchange saturation transfer experiments, which can identify weakly populated states in proteins [1]. Our spectra were wider than expected (by a factor of 2-3), indicating exchange with a broad spectroscopic signature population. This population appears to increase with temperature as shown by a dip width increase, thereby supporting that phosphates assemble and are in exchange with detectable phosphate species.

This study presents a surprising discovery that phosphate molecules ubiquitously present in biological systems possess a tendency to form soft assemblies largely invisible to NMR. The conditions investigated under which phosphate-containing molecules in water assemble should be relevant to a variety of processes that use phosphates as building blocks.

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ULTRAFAST DIFFUSION-BASED UNMIXING OF ^{1}H-NMR SPECTRA

Utilization of the spatial-parallelization concept in diffusion-ordered NMR spectroscopy (DOSY), which is known for separation-free identification of mixtures and molecules [1] reduces the acquisition-time of the experiment from few minutes to few hundreds of milliseconds. Recent years have witnessed the use of this spatially encoded-DOSY (SPEN-DOSY) method [2] in mixture analysis via univariate processing methods, where each peak is fitted individually to extract its diffusion constant. However, such analysis on overlapping-peaks of complex mixtures restricts the identification/separation of molecules and leads to wrong values of diffusion constants. This work addresses this problem by using Direct Exponential Curve Resolution Algorithm (DECRA) [3]; a fast multivariate processing algorithm, which separates components of a mixture. This is illustrated with an ethanol-butanol mixture and a sucrose-propanol mixture.

Applying DECRA to SPEN data required the design of a radio-frequency (rf) pulse that provides a quadratic spacing of the spatially parallelized gradient area. In addition, clean unmixing of components required additional pre-processing step, to account for the effect of chemical shifts during spatial encoding and during acquisition. Once designed, these new tools are straightforward to implement and use. Combining existing SPEN-DOSY pulse sequence with this newly designed rf pulse, 2D data was acquired for model mixtures, with experiment durations of less than 500 ms. All processing steps are then completed in less than 2 s, out of which only 100 ms required for DECRA. Together these tools provide an "ultrafast" unmixing of 1H NMR spectra, which should be useful for the analysis of reaction mixtures and hyperpolarized substrates.

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MONITORING CRYSTALLIZATION PROCESS IN CONFINED POROUS MATERIALS BY DYNAMIC NUCLEAR POLARIZATION SOLID-STATE NMR

Establishing mechanistic understanding of crystallization processes at the molecular level is challenging, as it requires both the detection of transient solid phases and monitoring the evolution of both liquid and solid phases as a function of time [1]. Here, we demonstrate the application of dynamic nuclear polarization (DNP) enhanced NMR spectroscopy [2,3] to study crystallization under nanoscopic confinement, revealing a viable approach to interrogate different stages of crystallization processes [4]. We focus on crystallization of glycine within the nanometric pores (7-8 nm) of a tailored mesoporous SBA-15 silica material with wall-embedded TEMPO radicals. The results show that the early stages of crystallization, characterized by the transition from the solution phase to the first crystalline phase, are straightforwardly observed using this experimental strategy. Importantly, the NMR sensitivity enhancement provided by DNP allows the detection of intermediate phases that would not be observable using standard solid-state NMR experiments. Our results also show that the metastable β polymorph of glycine, which has only transient existence under bulk crystallization conditions, remains trapped within the pores of the mesoporous SBA-15 silica material for more than 200 days.

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DYNAMICS OF IONIC SYSTEMS BY MEANS OF NMR RELAXOMETRY

Nuclear Magnetic Resonance (NMR) relaxometry is highly appreciated as a method revealing not only the time-scale but also the mechanism of molecular and ionic motion. Fast Field Cycling (FFC) technology has revolutionized this area of NMR relaxation studies enabling them to be performed as a function of the amplitude of the magnetic field. Such studies are referred to as "NMR relaxometry" and they bring a new dimension to NMR by probing different frequencies of molecular motion. The accessible range of magnetic fields spanning from hundreds of nT to 3T (from about 5kHz to above 100MHz, referring to ^{1}H resonance frequency) implies that one can investigate, in a single experiment, motional processes across a huge range of time scales from ms to ns.

According to spin relaxation theories, relaxation rates are given as linear combinations of spectral density functions being Fourier transforms of corresponding time correlation functions characterising the dynamical processes leading to stochastic fluctuations of the spin interaction that causes the relaxation. The mathematical forms of the correlation functions (and, hence, the spectral densities) depend on the mechanism of motion. Consequently, the shape of frequency dependences of spin-lattice relaxation rates (often referred to as relaxation dispersion profiles) is a fingerprint of the nature of the molecular and ionic motion. In this way one can not only unambiguously distinguish between translational and rotational dynamics, but also access the dimensionality and anisotropy of these dynamical processes (for instance, reveal the dimensionality of ionic diffusion).

The development of batteries is a vital goal of material science and technology. There is a growing awareness that further progress in the development of electrolyte systems heavily relays on a thoroughgoing understanding of the mechanisms of ionic dynamics. At present, confinement of ionic liquids into nano-porous matrices is the versatile strategy to overcome disadvantages of liquid electrolytes. Nanoconfined ionic liquids constitute composite (hybrid) systems with dynamical properties strongly affected by the nature of the confinement (solid or polymeric matrices), the size and geometry of the pores and the interactions between ions and pore walls. The price for confining ionic liquids is a significant slowing down of their mobility, especially long-range translational diffusion determining the conductivity of electrolytes. Consequently, the effort in the area of electrolytes based on ionic liquids is strongly focused on developing composites in which fast ionic diffusion is preserved.

In this lecture an overview of applications of NMR relaxometry to reveal dynamical properties ionic liquid based systems with focus on the theoretical challenges and achievements will be presented.

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FAST FIELD-CYCLING MAGNETIC RESONANCE IMAGING

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Most contrast in conventional MRI arises from differences in T_1 between normal and diseased tissues. Studies on small tissue samples have shown that extra information could be obtained from T_1-dispersion measurements (plots of T_1 versus magnetic field), but this information is invisible to standard MRI scanners, which operate only at fixed magnetic field (*e.g.* 1.5 T). We have developed Fast Field-Cycling (FFC) MRI to exploit T_1-dispersion as a novel biomarker [1].

FFC relaxometry is conventionally used to measure T_1-dispersion, by switching the magnetic field rapidly between levels [2] (polarisation at high field, followed by evolution (relaxation) at low field, and finally detection at high field. FFC-MRI obtains spatially-resolved T_1-dispersion data, by collecting MR images at a range of evolution magnetic fields [3].

We have built two whole-body human sized FFC scanners, operating at detection fields of 0.06 T [4] and 0.2 T [5]. The 0.06 T device uses a double magnet, with field-cycling being accomplished by switching on and off a resistive magnet inside the bore of a permanent magnet; this has the benefit of inherently high field stability during the detection period. The 0.2 T FFC-MRI system uses a single resistive magnet, bringing the advantage of increased flexibility in pulse sequence programming, at the expense of lower field stability during the detection period, necessitating more complex instrumentation.

We have demonstrated that FFC relaxometry can detect the formation of cross-linked fibrin protein from fibrinogen in vitro [6]. We have also shown that FFC can detect changes in human cartilage induced by osteoarthritis [7] and differences between normal tissue and tumour [8]. We have performed *in vivo* FFC-MRI studies on patients with acute ischaemic stroke; FFC-MRI images exhibited increased intensity in stroke-affected regions, with maximum contrast typically at the lowest evolution field used (0.2 mT) [9]. All human studies were conducted following approval of the relevant Research Ethics Committees and with the informed consent of patients.

Other work has focused on improving pulse sequences and data analysis, as well as speeding up the collection of FFC-MRI images [10,11]. Work to improve the hardware and software is ongoing, including the implementation of improved RF coils and receiver coil arrays [12].

FFC-MRI has significant potential for the generation and use of novel biomarkers arising from ultra-low field MRI contrast and from low- and ultra-low field T_1-dispersion phenomena.

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NMR DETECTION AT ULTRALOW FIELD: TAME OR STILL WILD?

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Recently, many groups have jumped onto the bandwagon of ultralow-field magnetic resonance following advances in heteronuclear hyperpolarization via para-enriched H_2 and sensitive magnetometers (e.g. optically pumped alkali vapors) to detect spin coherences in the audio band.

The current popularity of ultralow field suggests there is undoubtedly "room at the bottom". However, what remaining problems are really unique to NMR detection in ultralow field, and cannot be solved by mainstream approaches, say rf-detection of NMR in high magnetic field? In this presentation we discuss methodological advances in the following areas:

Heteronuclear dynamical decoupling at ultralow field: $^{1}H \rightarrow {}^{2}H$ and $^{14}N \rightarrow {}^{15}N$ isotopic replacement is often performed to mitigate intramolecular dipole-dipole relaxation pathways. In ultralow field, the advantage may be outweighed by scalar relaxation effects, potentially compromising spectral resolution [1] and polarization transfer around level anticrossings [2]. Here we demonstrate trains of dc (not ac!) field pulses to induce a spin selectivity that averages out J couplings between ^{2}H and ^{1}H. Composite pulses and a careful choice of phase cycle ensure robustness up to several thousand pulses.

Fast-field cycling relaxometry: We recently showed NMR relaxation measurements [3] in the microtesla field range via a simple setup comprising a water-cooled prepolarizing coil (20 mT), a solenoid (0-100 µT, homogeneity ~1 ppk) and an optical magnetometer (<20 fT/rtHz sensitivity across 10-1000 Hz frequency range). Coil switching times below 1 ms allow probing of ^{1}H T_1 and T_2 relaxation rates up to several tens of s^{-1}. Examples include study of relaxation in liquid molecules near to paramagnetic pore surfaces, and fluids inside thick-walled metal tubing.

Single scan relaxometry: The NMR line width in ultralow field is limited by natural coherence lifetime. We show this property can be used to investigate relaxation of long-lived multinuclear coherences for small molecules such as $^{13}CH_3OH$ in the strong coupling regime between 0-100 µT [3]. We also suggest windowed acquisition methods to measure T_1 in the direct dimension.

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TUNING THE ^{1}H NMR PARAMAGNETIC RELAXATION ENHANCEMENT AND LOCAL ORDER OF [ALIQUAT]+-BASED SYSTEMS MIXED WITH DMSO

Ionic Liquids are an ever-growing field, not only due to the diversity of known structures, but mainly because, by choosing the proper cation-anion combination, it is possible to tune the physicochemical properties of these systems for a wide range of applications. To enable a controlled optimization of these properties, it is important to assess both the macroscopic properties of ILs, such as viscosity, as well as to understand these compounds at a molecular level.

More recently, a novel group of ILs, having a paramagnetic metal-based ion incorporated into their structure, was described as Magnetic Ionic Liquids (MILs). Some properties of MILs, such as viscosity and diffusion coefficient, can be magnetic field dependent, opening a new range of important applications for this type of materials.

The presence of a paramagnetic metal in MILs induces an effective NMR relaxation pathway to ^{1}H nuclei, even for metal concentrations in the millimolar range. This effect is the basis for applications such as MRI contrast agents and is known as paramagnetic relaxation enhancement (PRE).

This study relies on ^{1}H NMR relaxometry and diffusometry and complementarily uses wide-angle X-rays scattering profiles, which elucidate the effect of DMSO on the local organization of the [Aliquat]+-based ionic liquids. As far as the authors know, there are no similar studies performed on these systems, and this is the first ever attempt to access local order dynamics on an ionic liquid [1].

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RELAXOMETRY OF CANCER: ROLE OF INTRACELLULAR WATER LIFETIME AS A TUMOUR BIOMARKER BY *IN VIVO* FAST FIELD CYCLING

Conventional diagnostic magnetic resonance imaging (MRI) techniques have focused on the improvement of the spatial resolution by using high magnetic fields (1-7T). High field allows the visualization of small tumour mass but lacks to give a precise evaluation of tumour grading and metastatic potential. Recently, we showed that the intracellular water lifetime represents a hallmark of tumour tissue cells status that can be easily monitored by measuring T1 at different and relatively low magnetic field strengths, ranging from 0.2 to 200 mT. A fast exchange through cell membranes indicates a high metabolic rate and thus a high activity of the tumor cells. Thus it is possible to measure the high metabolic pressure by an enhance water exchange with the exterior of the cell. Therefore, intracellular water lifetime can be considered an important tumour biomarker directly depending on the rate of cell proliferation, cell migration and in responding to external stimuli as hypoxia or extracellular acidosis.[1-3] Moreover, currently tumour responses to therapy are monitored primarily by imaging evaluating essentially the decrease of tumor size. This approach, however, lacks sensitivity and can only give a delayed indication of a positive response to treatment. In our study, we propose the use of FFC-NMR to provide relevant information about response to treatment by monitoring changes of water exchange rates through cell membranes that are directly dependent on the metabolism alterations caused by the chemo- or radio-therapy. Finally, T_1 relaxation, measured at vey low magnetic fields, can be exploited to support the surgeon in real time margin assessment during breast concerving surgery (BSC). It was found that a good accuracy in margin assessment, i.e. a sensitivity of 92% and a specificity of 85%, can be achieved by using two parameters, namely *i)* the slope of the line joining the R_1 values measured at 0.02 and 1 MHz and *ii)* the sum of the R_1 values measured at 0.39 and 1 MHz. The obtained results suggest that a simplified, low cost, automated instrument might compete well with the currently available tools in BCS margins assessment.

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STRUCTURAL INSIGHT INTO LAYERED ELECTRODE INTERPHASES FROM EXO- & ENDOGENOUS DNP

The electrode – electrolyte interface in rechargeable batteries has a central role in the battery's performance. A plethora of degradation processes, including electrolyte decomposition, structural and mechanical transformations lead to loss of energy and power densities. These processes can be controlled through deposition of thin protective layers at the electrode interface which prevent degradation, while enabling efficient ion transport across the interface. However, rational design of such interphases is limited by the scarcity of analytical tools that can probe few nanometers thick, heterogenous and disordered layers.

In this talk I will describe how Dynamic Nuclear Polarization (DNP) can be used to efficiently probe thin interphases which are otherwise invisible in NMR. Using different DNP polarization sources – exogenous nitroxide biradicals and endogenous Fe(III) dopants, we are able to gain detailed insight into the composition of the interface as well as its 3D structure. We demonstrate this approach on an alkylated $Li_xSi_yO_z$ interface, deposited through molecular layer deposition, that leads to performance enhancement in high energy cathodes. The combination of exo- and endogenous DNP as well as lithium isotope experiments provide unique insight at the atomic-molecular level which is crucial for designing new materials for high energy battery cells.

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SURFACE NMR CRYSTALLOGRAPHY OF METAL HALIDE PEROVSKITES

Surface modification strategies play an essential role in enhancing the optoelectronic performance and stability of metal halide perovskites.[1] Among the various surface modifiers reported during the past few years, water vapour is one likely to be encountered accidentally. However, it has been challenging to study the formation and elucidate the atomic-level structure of the surface-bound species, including hydration layers, owing to their extreme dilution. The conventional approach employing DNP SENS to study surfaces has proven comparatively inefficient in this class of materials so far.[2]

To overcome this challenge, we have identified Na^+ as a spy cation which binds specifically with the hydrated surface layer of the perovskite while it has no propensity to interact with the pristine non-hydrated bulk. Owing to its high NMR receptivity (545 times that of ^{13}C), ^{23}Na is perfectly suited for studying dilute surface species, including real-time monitoring of hydration. We employ a combination of ^{23}Na MAS NMR and chemical shift calculations to elucidate the speciation of sodium within the hydrated surface layer and show that it provides a unique atomic-level picture of its complex interior.

This strategy broadens the range of materials research questions that have been successfully addressed using solid-state MAS NMR in the context of metal halide perovskite research.[3,4]

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OBSERVATION OF QUADRUPOLAR NUCLEI IN SOLIDS BY CONTROLING INTERNUCLEAR TRANSFERS

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Half-integer quadrupolar nuclei, such as ^{11}B, ^{17}O and ^{27}Al, represent about two thirds of stable NMR-active nuclei and are present in organic, inorganic and biological solids . Nevertheless, the NMR observation of these nuclei in solids remains often more challenging than those of spin-1/2 nuclei. This difficulty comes from the larger size of the density matrix for quadrupolar nuclei as well as the large anisotropic quadrupolar interaction, which complicates the spin dynamics. In particular, the cross-polarization under magic-angle spinning (CPMAS) lacks of robustness for quadrupolar nuclei, which limits the observation of proximities with other isotopes but also the enhancement of NMR signals by dynamic nuclear polarization (DNP).

In the past year, we made significant progresses to circumvent these issues. We introduced robust symmetry-based heteronuclear dipolar recoupling built from adiabatic or composite inversion pulses to transfer the polarization of protons to quadrupolar nuclei at MAS frequencies ranging from 10 to 60 kHz [1-3]. These transfers were combined with DNP to detect the NMR signals of quadrupolar isotopes with low natural abundance (^{17}O) and low gyromagnetic ratio (^{47,49}Ti, ^{67}Zn, ^{95}Mo), notably near the surface of the materials. Furthermore, we acquired high-resolution NMR spectra of surface ^{17}O nuclei by combing these transfers with multiple-quantum MAS sequence.

For the observation of homonuclear proximities between quadrupolar nuclei, we have shown that the high robustness of symmetry-based $\mathrm{BR}2_2^1$ recoupling stems from the role of offset for the reintroduction of dipolar interactions [4]. Furthermore, for this recoupling, the intensity of correlations is reduced on-resonance by the creation of unwanted coherences. We also investigated pulse sequences for the observation of heteronuclear proximities between half-integer quadrupolar nuclei [5]. We notably showed that the recoupling scheme must be applied to the channel for which the magnetization is parallel to the B_0 field.

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QUADRUPOLAR ISOTOPE CORRELATION SPECTROSCOPY: RESOLVING OVERLAPPING WIDELINE NMR SPECTRA OF QUADRUPOLAR NUCLEI UNDER STATIC CONDITIONS

Solid-state NMR spectra of quadrupolar nuclei provide a wealth of information pertaining to molecular structure, dynamics, chemical identity, and purity. Extracting this information, which is encoded in the quadrupolar coupling constant (C_Q), the asymmetry parameter (η_Q) and the chemical shift parameters, is generally straightforward when investigating samples that possess a single chemical site. However, significant difficulty in spectral analysis arises when investigating samples possessing multiple non-equivalent chemical sites, which give rise to overlapping NMR spectra of each site, and drastically reduce individual site resolution. Current methods that address these problems are useful only in cases where the C_Qs are relatively small, under magic-angle spinning (MAS) [1-2], yet are not well suited for large C_Qs –for which static acquisition (*i.e.*, no MAS) is preferable [3]. Hence, there is a necessity for robust methods capable of resolving and deconvolving overlapped broad NMR spectra that are characterized by large C_Q values.

To this end, herein we introduce QUadrupolar Isotope Correlation SpectroscopY (QUICSY) as a potential method for resolving overlapped NMR spectra under static conditions, by correlating – *via* cross polarization – the central-transition NMR spectra arising from two quadrupolar isotopes of the same element. Several elements that are important and ubiquitous across materials science, pharmaceuticals, and inorganic chemistry possess at least two NMR-active quadrupolar isotopes, which are amenable to QUICSY. Examples include the isotopes of chlorine (^{35,37}Cl) and bromine (^{79,81}Br), which are often present in active pharmaceutical ingredients [4]; the isotopes of gallium (^{69,71}Ga), which comprise semiconductors and metal-organic frameworks [5], and the isotopes of rubidium (^{85,87}Rb). This approach is demonstrated here using two model rubidium-containing compounds, $RbClO_4$ and Rb_2SO_4, which possess one and two chemical sites, respectively. The resulting 2D QUICSY NMR spectra, provide rich information about the magnitude and geometry of the quadrupolar and the chemical shift interactions, as well as details concerning the relative orientation of their respective interaction tensors. We validate our results by comparing experimental 2D QUICSY spectra to 2D analytical calculations based on previous literature parameters. Thus, 2D QUICY NMR might offer a way to extract the NMR parameters for overlapping wideline spectra of quadrupolar nuclei when conventional methods cannot be applied.

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ADSORPTION OF PHOSPHATE ON γ-ALUMINA ELUCIDATED BY DNP-ENHANCED SOLID-STATE NMR SPECTROSCOPY AND DFT CALCULATIONS

Transitional γ-aluminas are widely used in industrial processes as heterogeneous catalysts and catalyst supports. Numerous empirical studies have shown that inorganic additives, such as phosphate, have a strong impact on the formation of catalytically active phases on γ-alumina and on the resulting activity of the material. [1-3]

However, an atomic-scale description of the chemical interactions of phosphates with γ-alumina is still missing, but is essential to obtain a rational understanding of the role of this additive. The combination of MAS NMR and DFT calculations has recently allowed us to disentangle the nature and location of surface hydroxyl groups on facets and edges of γ-alumina crystallites.[4] Here, we present an unprecedented insight into phosphate speciations and localizations on γ-alumina by combining cutting-edge Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy (DNP SENS) techniques with Density Functional Theory (DFT) calculations. Using ^{31}P double- and triple-quantum filtered NMR experiments, the presence of bi- and polyphosphates is investigated. By ^{27}Al-^{31}P dipolar- and scalar-based correlation spectra, Al-O-P connectivities are established. These experimental results are combined with DFT calculations, which allow to determine in detail the thermodynamically most stable binding modes of phosphate and preferential binding sites on different crystallite facets. ^{31}P NMR chemical shifts were calculated and compared to the experimental data. The evolution of phosphate speciations and binding sites is studied in a broad range of phosphate coverages up to 4.6 P/nm^2. The results will support future knowledge-driven development of catalytic materials, by better targeting surface modifier concentration and binding modes in view of their specific role in the catalysis itself or in nanoparticle formation.

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LOCALIZED DNP ENHANCEMENT IN BIOMOLECULES

With increasing complexity of biomolecular assembly, sensitivity as well as specificity play a major role in NMR-based structural biology. Dynamic nuclear polarization (DNP) has shown tremendous potential to increase sensitivity in numerous applications [1]. Even though in conventional DNP experiments uniform signal enhancements are typically obtained, DNP itself can act as a source of specificity as well [2]. In this presentation, two methods allowing to introduce a large degree of specificity to DNP-enhanced MAS NMR spectra will be presented.

The first method utilizes the distance dependence of the dipolar hyperfine interaction between the electron spin (source) and nuclear spin (target). The hyperfine interaction is mediating the initial step of the complex mechanism of the overall DNP transfer. By microwave irradiation, electron-nuclear coherences are generated which finally result in nuclear hyperpolarization. If subsequent spin diffusion is restricted, this transfer dynamic can act as a measure for hyperfine interaction. However, the competing paramagnetically enhanced spin-lattice relaxation counteracts the creation of a polarization gradient, theoretically leading to uniform, distance-independent DNP enhancement. Nevertheless, the direct-DNP build-up rate can act as a direct measure of this interaction and can thus yield distance information in biomolecules. We will present experimental evidence for this elusive DNP distance dependence on a ubiquitin model protein spin-labeled with a Gd^{3+} chelate tag [3]. By correlating the ^{15}N build-up rates with computationally assisted structural modeling of the paramagnetically labeled protein, the theoretically r^{-6} dependence is observed. However, the paramagnetic Gd^{3+} generates a bleaching volume with a radius of 12 Å within which no nuclear spin contributes to the measured rate. This opens up the possibility to extract distance restraints directly from direct DNP which can be further used in structural modeling.

The second method employs efficient indirect DNP (via ^{1}H), but introduces methyl groups as localized polarization transfer pathways between ^{1}H and ^{13}C. The introduction of specifically ^{13}C-labeled methyl groups via different biomolecular methods enables the discrete placement of antennas for hyperpolarization within the carbon network. Based on heteronuclear cross-relaxation, SCREAM-DNP (specific cross-relaxation enhancement by active motions under DNP) can thus probe the local environment around these highly dynamic functions [4]. This is especially interesting in the context of biomolecular complexes and specific contacts between binding partners [5]. Examples and applications on proteins, RNA, and ribonucleoproteins (RNPs) will be presented and future implications discussed.

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ENHANCING PORTABLE NMR SPECTROSCOPY USING *PARA*HYDROGEN HYPERPOLARISATION

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Increasing NMR sensitivity through hyperpolarisation holds great promise for integration with portable NMR spectrometers, whose relatively low magnetic fields (≤ 2 T) limit both signal strength and chemical shift dispersion. *Para*hydrogen-induced polarisation (PHIP) methods are of particular interest because they can deliver high levels of hyperpolarisation (up to tens of %), which are independent of the detection field and build up over a period of seconds. Importantly, PHIP methods can be implemented with relatively inexpensive and compact instrumentation. The key to unlocking the hyperpolarisation potential of *para*hydrogen (p-H_2) is a chemical reaction that breaks the symmetry of the protons in p-H_2 that exist in a singlet state, either through hydrogenation of an unsaturated molecule (e.g. an alkene or alkyne) [1] or through reversible binding to a metal centre (the signal amplifcation by reversible exchange (SABRE) approach) [2]. Both hydrogenative PHIP and non-hydrogenative SABRE have the potential to unlock new applications for low-field, portable NMR spectroscopy. In addition, ultra-low-field NMR can provide insights into the spin dynamics of p-H_2 hyperpolarisation and so can be used to develop new optimised polarisation transfer strategies.

This presentation will explore a range of applications for parahydrogen-enhanced portable NMR including: monitoring photochemical reactivity using benchtop NMR detection and p-H_2 hyperpolarisation, observing the spin dynamics of a range of iridium di-hydride complexes that provide continuous hyperpolarisation in the ultra-low-field regime through reversible exchange with p-H_2, and the use of field-cycled NMR with Earth's field detection [3] to explore SABRE polarisation transfer mechanisms in strongly-coupled ^{1}H-^{19}F spin systems.

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INTEGRATED DISSOLUTION DNP, NMR, AND EPR TO DERIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF INTRINSICALLY DISORDERED PROTEINS

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.

Many fold into stable structures only upon DNA binding; in the DNA-free state, conformational heterogeneity often renders characterization of these substrates experimentally challenging. Hence, atomic-level details of their structures, dynamics, and interactions often lack despite their considerable medicinal importance. Here we address this question by integrating dissolution dynamic nuclear polarization (D-DNP) [1], carbon direct-detected NMR experiments, paramagnetic relaxation enhancements, MD simulations, and EPR nanoscale distance measurements. With this approach, we could develop detailed models of the role of intrinsically disordered TF domains and how their heterogeneous conformational sampling modulates transcriptional activity [2-4].

We focus on MAX (MYC associated transcription factor X) as a model system, a member of the basic helix-loop-helix leucine zipper (bHLH-LZ) family, is a well-known 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 are of central importance in several tumor types. However, the characterization of MAX is complicated, in particular under physiological conditions and concentrations, by weak signal intensities and crowded spectra, owing to both the slow and anisotropic rotation of the dimer and the intrinsic disorder of the DNA-binding site. As a consequence, the functional role of intrinsic disorder for MAX has remained unclear until now.

Our studies relate in particular to the long-standing dilemma between spatial plasticity of transcription factors and their generally high binding affinities, which counteract relocalization in DNA-bound states. 'Facilitated diffusion' and 'facilitated dissociation' models try to answer how TFs move along DNA, how they pass other bound molecules while diffusing along the double-strands, and how they detach from DNA strands. We show how MAX solves these tasks by involving its intrinsically disordered domain enabling (i) rapid switching between loosely and tightly DNA-bound states and (ii) widening its sampling space to recruit DNA to the binding site efficiently.

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MIXED-VALENCE POLARIZING AGENTS FOR EFFICIENT OVERHAUSER EFFECT DNP IN INSULATING SOLIDS AT HIGH MAGNETIC FIELDS

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Overhauser effect DNP in insulating solids [1] has a number of beneficial properties for applications: it requires less microwave power than other methods, the enhancement was shown to scale favourably with magnetic field strength [2].

Mixed-valence character of the BDPA radical was recently investigated using state-of-the-art quantum-chemistry methods. We have vigorously proven that the radical belongs to a specific class of mixed valence compounds: spin density is localized on one part of the molecule, but can tunnel to the other side with a frequency in a range of hundreds of GHz [3]. Interestingly, same conclusions for BDPA were reached in 1975 by Watanabe *et al.* [4].

Now we have performed theoretical scanning of large number of mixed-valence molecules, looking for ones with properties similar to those of the BDPA (g-tensor anisotropy, large values of hyperfine coupling constants, similar electron transfer rate). Those molecules have been then synthesized and investigated by high field EPR. Their DNP field profiles were measured at 18.8 Tesla (527 GHz) at 100 K in tetrachloroethane (90D:10H) matrix under 8kHz MAS, concentration of the radicals was 10 mM. We have indeed observed large signal enhancement for all three radicals when irradiated on EPR resonance. Other properties of the signal, such as MAS and microwave power dependence are similar to the BDPA [5].

Our finding opens a whole class of mixed-valence radicals to prospective applications as polarizing agents at ultra-high magnetic fields. Organic mixed-valence compounds make up a large and well-studied group of molecules, their versatile nature allows for adaptation for various applications.

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POROUS FUNCTIONALIZED POLYMERS ENABLE GENERATING AND TRANSPORTING HYPERPOLARIZATION

More than fifteen years after the invention of dissolution dynamic nuclear polarization (d-DNP) [1], it was demonstrated that d-DNP could potentially be performed remotely, off-site [2], thus without the need of a polarizer on-site. In our group, we have in the past years, merely been working at improving efficiency [3], compatibility [4], and repeatability [5] of d-DNP. Our candid objective basically was to enable (or at least to improve) applications; nonetheless it has led us to develop a new concept i) to dramatically extend hyperpolarization lifetimes from minutes to days and, ii) to enable transport to far distant MRI or NMR sites [2].

We are now generalizing this new concept to a broad range of systems, such as neat endogenous tracers, mixtures of metabolites, or amino acids, by developing new hyperpolarizing solids such as our silica-based HYPSO materials, or more recently hyperpolarizing porous polymers (HYPOP). These can be impregnated with arbitrary solutions that are then hyperpolarized efficiently and stored and transported over hours, before being melted and released.

We will present DNP results on the very first generation of these new materials [6]. We'll also show how the porosity and morphology of this material can be tuned to further improve performances. Finally, we also show how hyperpolarization lifetimes can be further extended by treating the HYPOP surfaces before impregnation, and performing cold-crystallization of the sample after impregnation.

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THE DISTANCE BETWEEN G-TENSORS OF NITROXIDE BIRADICALS GOVERNS MAS-DNP PERFORMANCE IN THE BTUREA FAMILY

Limited sensitivity of solid-state NMR experiments makes it challenging to study systems where the isotope of interest is in low concentration. To overcome the limitations, bis-nitroxide radicals [1] are common polarizing agents (PA) that are used to enhance the nuclear polarization during Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) [2,3]. These biradicals efficiently increase the proton spin polarization through the Cross-Effect mechanism [4,5]. The relative orientation of the bis-nitroxide moieties is critical to ensure efficient polarization transfer. [6] Recently, we have defined a new quantity, the distance between g-tensors, that correlates the relative orientation of the nitroxides with the ability to polarize the surrounding nuclei. [7] Here we analyse experimentally and theoretically a series of biradicals belonging to the bTurea family, namely bcTol [8], AMUPol [9] and bcTol-M [10]. They differ by the degree of substitution on the urea bridge that connects the two nitroxides. Using quantitative simulations developed for moderate MAS frequencies, we show that these modifications mostly affect the relative orientations of the nitroxide, i.e. the length and distribution of the distance between the g-tensors, that in turn impacts both the steady state nuclear polarization/depolarization as well as the build-up times. The doubly substituted urea bridge favours a large distance between the g-tensors, which enables bcTol-M to provide $\epsilon_{on/off} > 200$ at 14.1 T/600 MHz/395 GHz with build-up times of 3.8 s using a standard homogenous solution. The methodology described herein was used to show how the conformation of the spirocycles flanking the nitroxides in the recently described c- and o-HydrOPol biradicals [11] affects the relative orientation of the nitroxides and thereby the polarization performance.

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BDPA-NITROXIDE BIRADICALS FOR EFFICIENT DNP ENHANCED SOLID-STATE NMR AT HIGH FIELDS AND ELEVATED TEMPERATURE

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Over the last decade, dynamic nuclear polarization (DNP) has become a cornerstone technique to circumvent the inherent sensitivity limitations of solid-state magic angle spinning (MAS) NMR. At very high magnetic fields (typically 18.8 T and above) however, the gold standard binitroxide polarizing agents developed at 9.4 T and yielding 250-fold signal enhancements fail to produce such significant gains.

A new class of hybrid polarizing agents based on BDPA-nitroxide biradicals, HyTEKs, was introduced in 2018 by Wisser and co-workers [1] and shown to successfully circumvent the limitations of binitroxides at high fields, yielding enhancements as high as 180-fold at 18.8 T spinning at 40 kHz MAS in a 1.3 mm probe, or more recently 200-fold at 21.1 T spinning at 65 kHz in a 0.7 mm probe [2]. These hybrid radicals take advantage of the narrow (and therefore easy to saturate) isotropic BDPA EPR line, strong electron-electron magnetic interactions and long electronic relaxation times thanks to the functionnalization of the nitroxide moiety.

In this context, we will first present a new family of BDPA-nitroxide radicals based on a 5-membered ring nitroxide. The performances of the best radical of the series will be presented at 18.8 and 21.1 T and compared to the HyTEK series. While the DNP efficiency of the Hy-5 radicals do not exceed those reported for HyTEK polarizing agents, this study provides new clues to fine-tune and modulate the structure of hybrid radicals.

We will then discuss the performance of BDPA-nitroxide radicals at elevated temperatures. One of the main limitation of DNP MAS NMR is its limited applicability to cryogenic temperatures. By incorporating BDPA or TEKPol in ortho-tertphenyl (OTP), a rigid matrix with a glass transition temperature (T_g) about 243 K, Lelli and co-workers were able to improve the performance of these radicals at elevated temperatures [3]. In this work, we will show that by formulating HyTEK2 in OTP enhancements as high as 65 at 230 K at 18.8 T were maintained. The DNP performance of this system as a function of temperature will be presented and discussed in the light of variable temperature EPR relaxation measurements and spin dynamics simulations using the SPINACH software [4].

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TPPM DNP

In pulsed DNP, microwave irradiation is applied to an NMR sample in the form of a pulse sequence with the aim to enhance the bulk nuclear polarization, and thereby the sensitivity of the NMR experiment. This relatively new way of doing DNP may have several advantages over existing, classical forms of DNP, which all rely on continous microwave irradiation. These include an effective Hamiltonian that does not depend on the static magnetic field, thus holding the promise of a field-independent DNP efficiency, and the ability to generate DNP at a low duty cycle, which reduces sample heating, particularly at the highest magnetic fields.

The first DNP pulse sequence, NOVEL (Nuclear spin Orientation Via Electron spin Locking),[1] dates back to the 1980s. NOVEL works well in chemical systems compatible with DNP/MAS NMR of biomolecular systems, e.g. trityl in DNP juice, but the sequence is technically challenging to implement at high magnetic fields, because during the contact period the electron nutation frequency (ω_{1S}) must match the ^{1}H Larmor frequency (ω_{0I}). Fortunately, in recent years two new DNP sequences have been introduced, for which the requirement on the electron nutation frequency is considerably relaxed. These are: Off-resonance NOVEL,[2] with the matching condition

$$\sqrt{\omega_{1S}^2 + \Omega_S^2} = \omega_{0I}$$

and TOP (Time-Optimized Pulsed) DNP,[3] with the matching condition

$$k\omega_m + l\omega_{eff} = n\omega_{0I}$$

In Off-resonance NOVEL, a low electron nutation frequency is compensated by irradiating off-resonance. In TOP DNP, which consists of a train of pulses and delays $((t_p)_x - d - (t_p)_x - d - \cdots)$, a combination of the modulation frequency ($\omega_m, k = \pm1, \pm2, \ldots$) and the effective frequency of the electron spins under the influence of the pulse train ($\omega_{eff}(\Omega_S, \omega_{1S}, t_p, d), l = \pm1$) must equal the nuclear Larmor frequency, which further increases the possibilities when the electron nutation frequency is low.

Here we introduce two new DNP pulse sequences: TPPM DNP (Two Pulse Phase Modulation, $(t_p)_{-\varphi/2} - (t_p)_{\varphi/2} - (t_p)_{-\varphi/2} - (t_p)_{\varphi/2} \ldots$) and XiX DNP (X-inverse-X, $(t_p)_x - (t_p)_{-x} - (t_p)_x - (t_p)_{-x} \ldots$), named after the analogous, well-known heteronuclear decoupling sequences. For both sequences, the matching condition is the same as for TOP DNP, but because the trajectories of the electron spins differ, $\omega_{eff}(\Omega_S, \omega_{1S}, t_p)$ will be different, as well as the scaling factors. In experiments at Q band (51 MHz/1.2 T/34 GHz), using a nutation frequency of 18 MHz, we have found that XiX DNP is superior to TOP DNP. Numerical simulations of polarization transfer in an ensemble of dipolar coupled e^--^{1}H spin systems were set up with Spinach[4] and were extremely helpful for screening the XiX and TPPM matching conditions.

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IN-SITU EPR AND DNP-ENHANCED NMR OF SILICON NANO PARTICLES

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Nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) are two established methods for studying the structure of engineered materials such as silicon-based micro- and nano- particles [1]. Direct ^{29}Si NMR detection is challenging due to its low thermal polarization; however low-temperature (<4 K) dynamic nuclear polarization (DNP) can slowly enhance sensitivity. Albeit, the endogenous free-radicals (lattice defects) driving the DNP process are in low concentrations [2]. Consequently, EPR sensitivity is low thus requiring intense microwave irradiation and frequency modulation to sufficiently saturate the target electron transition. But unfortunately, solid-state microwave sources are inefficient at high frequencies.

We present a technical solution for NMR and EPR detection to study the DNP efficiency of crystalline silicon particles in our 7 T DNP polarizer [3]. The over-sized steel WR28 waveguide coupling microwaves from the source (197 GHz Tx, Virginia Diodes) is electroplated with a layer of gold, palladium and finally silver to reduce transmission losses at 197 GHz. The method involves reducing metallic cations on the waveguide walls using a specially constructed plating anode that holds the desired electrolyte. Unlike traditional electroplating methods, the electrolyte solution doesn't require heating nor intermittent pH and surface layer treatment, allowing for use on a regular lab bench. To facilitate EPR detection, a 750-turn solenoid coil fabricated from 0.1 mm diameter copper wire is fitted onto the DNP. The ^{29}Si DNP spectra and longitudinal-EPR for the crystalline silicon particles (P28A002, Alfa Aesar), are illustrated in Fig 1B. Transmission loss was characterized on the bench using a diode detector (WR5.1ZBD-F, Virginia Diodes). Plating the 655 mm long waveguide resulted in a decrease of the loss by 3.1 dB. Testing eight months later yielded similar results suggesting good plate adhesion during electroplating and low corrosion.

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INDIRECT DETECTION METHODS FOR THE RAPID MEASUREMENT OF ISOTROPIC AND ANISOTROPIC ^{195}PT CHEMICAL SHIFTS IN HETEROGENEOUS CATALYSTS

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Platinum is commonly found in heterogeneous catalysts in the form of dispersed atoms/ions, immobilized organometallic compounds and metal nanoparticles. ^{195}Pt is a 33% naturally abundant spin-1/2 nucleus with a gyromagnetic ratio close to that of ^{13}C, making ^{195}Pt solid-state NMR is an appealing technique to study the molecular structure of platinum catalysts. However, ^{195}Pt solid-state NMR experiments are often challenging, if not impossible, because ^{195}Pt chemical shift anisotropy (CSA) can easily exceed 8000 ppm. Furthermore, many heterogeneous catalysts feature low metal loadings on the order of 0.5-4 wt.%. We have previously shown that fast MAS, ^{1}H{^{195}Pt} dipolar heteronuclear multiple quantum correlation (D-HMQC) experiments can be used for the sensitive and high-resolution detection of isotropic and anisotropic ^{195}Pt chemical shifts. [1-3] In this presentation we describe new solid-state NMR methods that enhance sensitivity and facilitate high-resolution detection of wideline ^{195}Pt solid-state NMR spectra.

First, we will show new t_1-noise eliminated (TONE) D-HMQC that provide order of magnitude gains in sensitivity by suppressing t_1-noise.[4] The TONE D-HMQC experiments exploit short high-power adiabatic pulses (SHAP) for broadband inversion of ^{195}Pt. SHAP pulses can also be implemented into ^{1}H{^{195}Pt} symmetry-based rotational echo double resonance (S-REDOR) experiments to increase dipolar dephasing and facilitate more accurate measurement of ^{1}H-^{195}Pt dipolar coupling constants and internuclear distances,[4] including in model catalysts.[3] We then present a novel fast MAS, indirect detection approach to rapidly measure ^{195}Pt CSA. The high power ^{195}Pt pulses used in D-HMQC/S-REDOR are replaced with long-duration, low-power, sideband-selective excitation/saturation pulses. The ^{195}Pt spinning sidebands can then be mapped out by varying the offset of the ^{195}Pt sideband selective pulses, enabling the rapid measurement of the chemical shift tensor parameters with high sensitivity. Using these approaches, we were able to measure ^{195}Pt CSA exceeding 8000 ppm in only a few minutes for pure compounds and a few hours for a Pt compound grafted on silica with ca. 4 wt.% Pt loading. In comparison, DNP-enhanced wideline ^{195}Pt CPMG experiments required many days of acquisition to measure the CSA. Therefore, these indirect detection methods pave the way for the analysis of complex Pt-based heterogeneous catalysts with low loadings.

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LIGHT NMR: A HIGH-RESOLUTION TOOL FOR THE MECHANISTIC UNDERSTANDING OF PHOTOPHYSICAL PROPERTIES OF FLUORESCENT PROTEINS

Fluorescent proteins with distinct photo-transformation properties are crucial for a wide range of applications in advanced fluorescence microscopy and biotechnology. However, using photo-transformable fluorescent proteins (PTFPs) remains highly challenging because environmental conditions such as pH, temperature, salt, oxygen level, reducing agents, and viscosity affect their brightness, photostability and photo-transformation landscape. Rational fluorescent protein design currently exploits the mechanistic information available from structural studies, mainly X-ray crystallography, in order to design new PTFP variants with improved properties for particular applications. Yet, protein crystals only grow under environmentally-restrictive crystallization conditions and crystallographic data, often recorded at cryogenic temperatures, lack important information on structural heterogeneity and conformational dynamics. Thus crystallographic structures may poorly account for photophysical properties observed in biological samples at physiological temperature. Multidimensional solution NMR spectroscopy provides an unique tool to investigate at atomic resolution the conformational dynamics of PTFPs in different photo-stationary states, under a variety of physicochemical conditions, and over a wide range of time scales.

We have set up a portable NMR in-situ illumination device that is compatible with high-field NMR spectrometers, allowing NMR sample illumination at 3 different wavelengths (405, 488, and 561 nm) that can be synchronized with NMR pulse sequences. A set of multidimensional and multinuclear (^{1}H, ^{13}C, ^{15}N) NMR experiments, including ^{15}N relaxation, H/D exchange, pH titration measurements, light-driven EXSY, and specific chromophore experiments, were setup to build an "NMR investigation toolbox" for fluorescent proteins [1]. Studying rsFolder, a reversibly switchable green fluorescent protein, we have identified four distinct configurations of its p-HBI chromophore, corresponding to the *cis* and *trans* isomers, with each one either protonated (neutral) or deprotonated (anionic) at the benzylidene ring. The relative populations and interconversion kinetics of these chromophore species depend on sample pH and buffer composition that alter in a complex way the strength of H-bonds that contribute in stabilizing the chromophore within the protein scaffold [2], and determine its fluorescence and switching quantum yields.

Light-NMR spectroscopy provides a powerful tool to investigate the effects of environmental conditions or protein mutations on chromophore state populations and dynamics, and to correlate them with altered photophysical properties as a prerequisite for rational design of phototransformable fluorescent protein variants with improved properties.

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ULTRAFAST DIFFUSION EXCHANGE SPECTROSCOPY

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Relaxation and diffusion NMR (called Laplace NMR, LNMR) experiments offer insights into molecular dynamics. Multidimensional approach improves the resolution of LNMR. Furthermore, it enables one to correlate diffusion and relaxation parameters and study exchange phenomena through the relaxation or diffusion contrast. Multidimensional LNMR experiments are, however, very time consuming, as the experiment must be repeated many times with an incremented evolution time delay or gradient strength.

Recently, we have demonstrated that multidimensional LNMR can be accelerated by one to three orders of magnitude by spatial encoding [1,2], which was originally exploited in ultrafast (UF) NMR spectroscopy [3]. We call the method UF LNMR. We have exploited UF LNMR in the investigation of porous media, chemical analysis, identification of intra- and extracellular metabolites as well as mobile NMR [1,2,4-7]. The single-scan approach also significantly facilitates the use of hyperpolarization to boost sensitivity by many orders of magnitude [2,4,7].

This talk concentrates on the description of a novel ultrafast diffusion exchange spectroscopy (UF DEXSY) method [8]. Conventional DEXSY is very time consuming, as both the indirect and direct dimensions are collected point by point. In the UF DEXSY, complete data is measured in a single scan by exploiting spatial encoding in the indirect dimension and CMPG based detection accompanied by gradients, which serve both as read and diffusion gradients. We have exploited the method in studying exchange phenomena in surfactant solutions relevant to aerosol research. Furthermore, we have preliminary data showing that the experiments are feasible also with a low-field single-sided NMR spectrometer (NMR-MOUSE), and the significant sensitivity boost provided by dissolution dynamic nuclear polarization (dDNP) method allows single scan analysis of molecular exchange in a yeast cell solution.

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ACCELERATED ACQUISITION OF 2D TITRATION DATA WITH NON-STATIONARY COMPLEMENTARY NON-UNIFORM SAMPLING (NOSCO-NUS)

The versatility of NMR and its broad applicability to several stages in the drug discovery process is well known and generally considered one of the major strengths of NMR. Indeed, NMR is the only biophysical technique which can detect and quantify molecular interactions, and at the same time provide detailed structural information with atomic-level resolution [1]. This powerful NMR application requires long measurement times to record well-resolved heteronuclear 2D correlation spectra of the protein, where the signals of amino acids affected by ligand binding gradually shift or disappear with increasing ligand concentration. The time demand is heightened by the need to record full titration series to determine the affinity constant and quantify the chemical shift perturbation (CSP) amplitude.

We propose a method for complementary non-uniform sampling (NUS) of entire titration series of heteronuclear 2D correlation spectra within less time than even required for a *single* conventional spectrum [2]. For this we sample the indirect t1 dimension as in a standard NUS experiment, yet with complementary sampling schedules for the different titration points such that each ligand-to-protein ratio is sampled for a unique set of t_1 times. A separate reference spectrum of the protein before adding ligand is also recorded. The various NUS 2D time-domain data of a titration series can be combined and compressed to yield a single 2D correlation spectrum. Signals that shift strongly (i.e. show large CSP) appear smeared out and broadened due to superposition of their variable positions in the different underlying 2D spectra of the titration series. Such NMR signals can be modeled as non-stationary signals [3]. The idea powering NOSCO-NUS is to use the traceable dependence of t_1 times with each titration point in the series to treat the smeared signals, refocusing them into the sharp reference signal with a concomitant increase in intensity. This is achieved by multiplying each selected NMR signal by a synthetic signal which obeys the same single-site binding model followed by the shifting peaks. Accurate binding parameters (affinity constant and CSP in both dimensions) are found when peak height difference between reference and corrected signals is minimal. The precision of NOSCO-NUS is shown to be comparable to conventional methods.

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MONITORING FAST CHEMICAL PROCESSES BY REACTION-INTERRUPTED NMR SPECTROSCOPY

NMR spectroscopy is a powerful, widely used tool for the investigation of chemical reactions. It has been used to study kinetics of unstable reaction intermediates, mechanisms in organic chemistry and for process control in so-called inline/online NMR [1]. A further advantage compared to other methods is that there is no necessity for a prior calibration, as the integral of a signal is directly proportional to the quantity of the material present. NMR Spectroscopy is generally used to study stable molecules under equilibrium conditions. Here we present a suite of experiments, that we developed in order to study fast reversible and irreversible reactions, which are ongoing during the pulse-sequence.

Despite recent technological and methodological developments to study on-going reactions, tracing the fate of individual atoms during an irreversible chemical reaction is still a challenging and elaborate task. Reaction-interrupted excitation transfer (ExTra) NMR provides a selective tracking of resonances from atoms, which undergo chemical conversion [2]. We show that reactions, triggered either by rapid mixing or by photo-excitation can be conveniently followed at a sub-second time scale using standard NMR equipment. In ExTra NMR we use the selectively inverted magnetization of a selected atom to follow its conversion in the course of a fast reaction. The speed of recording irreversible reactions by in situ NMR can be significantly enhanced by combining rapid injection devices with very fast data acquisition schemes, which rely on relaxation independent observation of individual NMR tube slices by spatially selective excitation [3].

In contrast to ExTra NMR, reversible photoinduced reactions can be investigated by an light pulse interrupted NOESY/EXSY experiment. Irradiation during the mixing period of a modified EXSY experiment leads to additional peaks in the resulting two-dimensional spectra [4]. The photo-induced exchange of magnetization occurring in photo-switchable (Z) and (E) forms of azo compounds provides information on the corresponding dynamic equilibria. We report on the dependence of the diagonal-to-cross-peak ratio on concentration, light intensity and mixing time. Reversible chemical processes can also be investigated by a 2D experiment, which allows the monitoring of CEST between any two signals at once. This is achieved by a combination of slice-selective excitation with obersavation during a weak pulsed field gradient.

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TOTAL CHEMICAL SHIFT CORRELATION AMONG ALL MAGNETIC NUCLEI BY ISOTROPIC MIXING IN ZERO- TO ULTRA-LOW FIELD

Any two-dimensional NMR pulse sequence contains the same functional intervals, or principal building blocks: (i) preparation; (ii) evolution; (iii) mixing; (iv) detection [1]. In a well-known TOCSY experiment [2], which highlights the network of coupled nuclei, the mixing block is given by a sufficiently strong spin-lock pulse. In this experiment, an isotropic mixing of polarization due to scalar spin-spin coupling between locked nuclear spins occurs during the spin-locking pulse application. The heteronuclear version of TOCSY known as HEHAHA (Heteronuclear Hartmann-Hahn) [3] relies on polarization transfer between heteronuclei once Hartmann-Hahn conditions are met. However, in practice, the application of the HEHAHA pulse sequence is limited by technical problems since strong radiofrequency irradiation is necessary to cover the entire distribution of heteronuclei chemical shifts.

A few years ago, a cost-efficient shuttling add-on compatible with commercial NMR spectrometer was built in our lab [4]. Ultra-wide field variation from 5 nT to 9.4 T was made possible by sample positioning along the bore of NMR magnet compiled with electromagnet coil system located in a μ-metal magnetic shield. Armed with such unique equipment, we realized an attractive idea to fulfil the condition of strong coupling for any heteronuclei by utilizing zero- to ultra-low field while keeping high-resolution NMR detection at high field. Here we present this novel approach to obtain a 2D chemical shift correlation spectrum with polarization transfer between heteronuclei named ZULF-TOCSY [5]. The mixing block of ZULF-TOCSY contains an interval of isotropic mixing at zero- to ultra-low field. This experiment in principle allows observing all possible correlations between signals of all magnetic nuclei with different gyromagnetic ratios. This unique feature assigns the application of the ZULF-TOCSY experiment for the straightforward determination of mixture components. In addition, ZULF-TOCSY allows rendering sequential assignments in peptides at natural abundance then no amide protons are present using a single 2D spectrum [6]. The isotropic mixing at zero- to ultra-low field can be used to construct more sophisticated NMR pulse sequences in the future.

† *Konstantin Ivanov passed away on March 5th, 2021 at the age of 44 years as a consequence of Covid-19.*

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POISON-GAP SAMPLING EXPLAINED

Non-uniform sampling (NUS) became a popular way of accelerating time-consuming multidimensional NMR experiments. Among various non-conventional sampling schemes, the Poisson-gap (PG) schedules [1,2] became particularly popular, especially when combined with compressed sensing (CS) reconstruction of the missing data points [3]. However, the effectiveness of PG is based mainly on practical experience and has not been so far explained based on the CS theory. Moreover, there is an apparent contradiction between the observed potential of PG and CS theory which states, that a "flat" pseudo-random generator is the most optimal way of generating sampling schedules. In this presentation, we explain why and when PG reveals its superior features in NMR spectroscopy. We support our theoretical considerations with simulations and analysis of experimental data from BMRB. The latter reveals (so far unnoticed) feature of NMR spectra explaining the success of PG and related schedules. We name this feature the "clustered" sparsity. In other words, the NMR spectra are not only sparse, but the peaks often form groups in the indirect dimension.

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ROOM-TEMPERATURE DNP NMR SPECTROSCOPY OF SMALL BIOLOGICAL MOLECULES IN WATER

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful and popular technique for probing molecular structures, dynamics and chemical properties. However the conventional NMR spectroscopy is bottlenecked by its low sensitivity. Dynamic nuclear polarization (DNP) boosts NMR sensitivity by orders of magnitude and resolves this limitation. In liquid-state this revolutionizing technique is still restricted to a few specific model molecules in organic solvents. Here we present that, for the first time, a full scheme of small biological molecules, ranging from carbohydrates to amino acids, have been hyperpolarized efficiently in water directly at room temperature and at high-field. A trend between observed ODNP enhancement factor and paramagnetic shifts have been revealed, which instructs us to revisit paramagnetic NMR literatures and to discover new class of molecules (heterocyclics) that can be hyperpolarized by Overhauser DNP. The QM/MM MD simulation underscores the dynamic intermolecular hydrogen bonds as the driving force for Overhauser DNP. Our work reconciles DNP, paramagnetic NMR and computational chemistry, illuminates unexplored molecular space for modern liquid-state DNP NMR spectroscopy, and reveals new molecular and chemical mechanism of Overhauser DNP in liquids.

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RADIAL RADIO-FREQUENCY FIELD INHOMOGENEITY IN MAS SOLID-STATE NMR EXPERIMENTS

Radiofrequency-field (rf) inhomogeneity is a prevalent problem in NMR experiments that often leads to the deterioration of the performance of pulse sequences. The extent of the inhomogeneity is largely determined by the coil geometry and different coil designs have been proposed to improve rf homogeneity. In solid-state NMR probes, solenoid coils wound around the rotor inside the MAS stator are still most commonly used, even though they suffer from comparatively large rf inhomogeneity. The spatial rf-field distribution over the sample volume in such MAS probes is mainly characterized by the distribution along the rotor axis. However, significant radial contributions are also present [2]. Under MAS, these radial contributions lead to a time-dependent modulation of the rf amplitude and phase experienced by a spin packet [3]. In contrast to the static part of the rf inhomogeneity, the effects of these time-dependent modulations are not well-studied. Here we present an investigation of the influence of such MAS-induced rf amplitude and phase modulations on building blocks commonly encountered in solid-state NMR pulse schemes. Potential effects were studied using numerical simulations and analytical approaches based on Floquet theory.

In nutation spectra, additional sidebands at multiples of the rotor frequency arise due to modulation of the rf phase. Significantly weaker sidebands due to amplitude modulations are observed around the nominal rf amplitude. The intensity of such sidebands can help to characterize the rf-field modulations and thus give insight into the radial contribution to the rf-field inhomogeneity for the MAS probe used. For polarization transfer sequences such as Hartmann-Hahn cross polarization, REDOR and symmetry-based C sequences only minor effects of the rf amplitude and phase modulations were observed that should be of no consequence for experimental implementations. In simulated homonuclear spectra under FSLG decoupling, significant line broadening occurred when MAS modulation of the rf amplitude was taken into account. Floquet analysis of the effective Hamiltonian up to second order revealed that this broadening is most likely due to the re-introduction of homonuclear dipolar coupling terms in the first-order effective Hamiltonian. However, no experimental characterization of this additional line broadening was possible, as experimentally obtained line widths were not decoupling limited.

Overall, our findings suggest that the effects of the radial contribution to the rf-field inhomogeneity on solid-state NMR pulse sequences are small and thus negligible for experimental implementations. They could, however, play a more important role in the numerical optimization of new pulse sequences.

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PROGRESS IN COMPACT NMR

The era of compact NMR started in the first half of the 1970ties with the advent of tabletop NMR instruments, most notably the Bruker Minispec relaxometer and the Newport Analyzer, a relaxometer from a company sunsequently acquired by Oxford Instruments [1]. In took about 50 years to figure out how to cope with the tolerances of permanent magnet blocks so that they can be assembled reliably to make small permanent magnets with field homogeneity sufficiently good to resolve the ^{1}H chemical shifts of small molecules in solution. Today multi-nuclear NMR spectroscopy with compact tabletop instruments is an established analytical method in many academic and industrial laboratories [2]. Arguably the miniaturization of compact permanent magnets benefitted from the advances in profiling the strayfield of inside-out NMR sensors used for logging oil wells and related compact strayfield relaxometers for nondestructive materials testing [3,4].

Recent progress in instrumentation and applications of compact NMR is reported. In particular, methods of mapping the sensitive volume of strayfield-sensors like the NMR-MOUSE and compact magnets have been developed [5], the NMR-MOUSE has been employed in unconventional outdoor scenarios like Yellowstone National Park and the fire damaged library of the Glasgow School of Art [6], a compact and open permanent magnet has been built and passively shimmed for chemical shift resolved Overhauser DNP experiments [7], and a high-pressure setup has been constructed and tested for compositional analysis of gas mixtures at pressures of up to 200 bar [8].

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ZERO- TO ULTRALOW-FIELD (ZULF NMR): RECENT PROGRESS AND NEW DIRECTIONS

In this talk, we will recall how it is possible to do NMR without magnets, survey the recent progress, and present new directions being pursued in our laboratory and elsewhere.

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OPTIMIZING ENZYME- AND CELL-BASED BIOPROCESSES WITH MOBILE FLOW NMR

Better optimizing biochemical processes induced by free enzymes or by cells, is crucial to improve the production of high added value commercial products in pharmaceutics, cosmetics, food and energy sciences. NMR is essential to the optimization and control of bioprocesses since it provides both structural and quantitative information in a non-destructive fashion. In this context, emerging benchtop NMR instruments are particularly attractive since they are cheap, mobile and compatible with industrial settings. However, at lower fields, the limits of NMR are worsened: low sensitivity and weak spectral resolution are detrimental to the analytical performance. To overpass such limitations, we implemented NMR techniques using pulse field gradients in diffusion NMR, pure-shift methods, ultrafast 2D NMR and schemes for removing the water signal in biological samples, on a 1 T apparatus equipped with a gradient coil [1].

1- In the field of food processes, we showed that quantitative WET-180-NOESY [1] is the most suitable tool able to selectively remove the water signal to monitor under flow two key enzyme-catalyzed bioprocesses in food industry: sucrose inversion for enhancing the sweetness [2] or for producing lactose free milk [3] for intolerant persons.

2- Several microalgae have the ability to produce lipids thanks to a metabolic redirection provoked by nitrogen starvation conditions. In the context of following bioprocesses by low field NMR [4], a 43 MHz apparatus was used for the first time for non-invasive monitoring of entire microalgae in their cultivation medium. Thanks to the implementation of WATERGATE W5 scheme [5], the water peak representing more than 95% of the culture could efficiently be removed: 27,000 times less than in ^{1}H. The main peak from *in vivo* lipids is then observable after 1h of W5 acquisition and can be assigned and quantified as the total lipids [6]. To monitor lipid in-cell accumulation, a benchtop spectrometer was coupled to a photobioreactor, an automated device for microalgae cultivation. For the first time, the real-time online and *in vivo* access to lipid production kinetics during 3 weeks by compact NMR was obtained with a very similar profile to GC offline reference method [7]. Our recent results with compact flow NMR monitoring open the avenue for improving enzymatic and *in vivo* cell bioprocesses involving the real time detection and quantification of valuable metabolites.

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NMR REVEALS STRUCTURE-FUNCTION RELATIONSHIP IN DISORDERED PROTEINS: MAP2C AS AN EXAMPLE

It is well documented that intrinsically disordered proteins (IDPs) play important biological functions, especially in signalling processes. However, it is less clear how is the biological activity of IDPs related to their structure and dynamics. Favourable relaxation properties of IDPs and development of non-uniform sampling methods [1] make NMR an excellent tool for atomic-resolution studies of the structure-function relationship in large IDPs. Investigation of a microtubule associated protein 2c (MAP2c) is presented as an example.

MAP2c is a splicing variant of the MAP2 gene expressed in developing brain neurons. MAP2c resembles Tau in its ability to control dynamics of microtubules, but MAP2c and Tau differ in amino-acid sequences of their N-terminal domains and in cellular localization. Compared to Tau and despite of its biological importance, MAP2c is much less studied than Tau.

Almost complete resonance assignment of MAP2c, relying on 5D non-uniformly sampled experiments [2], made detailed studies of MAP2c structure possible. The chemical shifts were converted to populations of secondary structures by the ASTEROIDS analysis [3]. The results revealed numerous local pre-structured alpha-helical or polyproline II motifs [4], in contrast to another IDP studied in the group, C-terminal domain of delta subunit of bacterial RNA polymerase, with almost uniform preference of polyproline II conformations [5]. Analysis of relaxation and paramagnetic relaxation enhancement showed formation of a transient compact structure, not observed in Tau.

Features related to the biological function of MAP2c were also studied by NMR. Line-broadening due to interaction with well-ordered proteins allowed us to quickly identify interaction sites for several binding partners. Overlap of the regions interacting with tubulin and with regulatory proteins (14-3-3, plectin) explains the mechanism of modulation of the regulatory activity of MAP2c by additional protein-protein interactions [4]. Distinct amide frequencies of phosphorylated serine and threonine, and selective labelling of tyrosine make measurement of phosphorylation kinetics possible for all phosphorylated residues. Distinct phosphorylation patterns and kinetics of MAP2c and Tau represent another level of specific regulation of biological activity [6]. Furthermore, MAP2c, but not Tau, specifically binds regulatory subunit of an important protein kinase, PKA. Positions of residues involved in the mentioned regulatory events strongly correlate with the identified local pre-structured motifs, and with the formation of the large compact structure.

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A FOUR-DOMAIN INTRAMOLECULAR FUZZY COMPLEX IN C-SRC

The study of the myristoylated, intrinsically disordered region of c-Src, the first discovered oncogene, is a representative example of the paradigm shift towards understanding the integration of intrinsically disordered regions and globular folded domains present in the majority of eucharyotic proteins implicated in high-order regulatory processes.

In this talk we shall present unpublished work on the use of NMR to characterize the entire 250-residue regulatory region of c-Src, including the disordered SH4 and Unique domains, and the two globular regulatory domains SH3 and SH2.

This work extends our previous findings on the intramolecular fuzzy complex formed by the disordered regions and the SH3 domain [1, 2], and also on the modulation of c-Src membrane anchoring by competition between intra- and intermolecular interactions involving the myristoylated N-terminus of c-Src[3,4].

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CAPTURING THE HIGH-RESOLUTION STRUCTURE OF A LOW-POPULATED AROMATIC RING FLIPPING INTERMEDIATE

Aromatic residues cluster in the core of folded proteins, where they stabilize the structure through multiple interactions. NMR studies carried out in the 1970s surprisingly demonstrated that aromatic side chains can undergo ring flips, *i.e.* 180° rotations around the χ_2 dihedral angle, despite their central role in maintaining the protein fold [1-3]. It was suggested that large-scale breathing motions of the surrounding protein environment are necessary to accommodate these ring flipping events. However, the structural details of these motions were previously unknown.

Here, we uncover the structural rearrangements accompanying a ring flipping event of a buried tyrosine residue in an SH3 domain. Using $^{1}H^{N}$ and ^{15}N relaxation dispersion experiments [4-5], we show that the tyrosine side chain flips to a low-populated (3%) excited state corresponding to a ring flipping intermediate. Based on a proteome-wide sequence analysis, we design single point mutations that invert the relative populations of the ground and excited state allowing us to capture the high-resolution structure of the ring flipping intermediate by X-ray crystallography. Our results reveal how hydrogen bonds and CH-π interactions are rearranged in the intermediate providing unprecedented structural insight into the protein breathing motions associated with aromatic ring flipping.

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DYNAMIC STRUCTURES AND STRESS INDUCED UNFOLDING OF SMALL ANTIMICROBIAL PROTEINS

Small antimicrobial disulfide proteins like AFP, PAF, PAFC, PAFB, NFAP, NFAP2 are famous for their antifungal activity and some of them exhibit anti-*Candida* or even anti-corona virus activity[1]. They are harmless for mammalian cells however, their diverse mode of action is not fully understood[2]. These miniproteins (50-60 aa) are produced by filamentous fungi and they form β-barrel tertiary structures. Folded forms are stabilised by 3-4 disulfide bridges and therefore believed to exist as rock hard entities in spite of many charged residues (e.g. lysines). We have shown[3] by cold & heat unfolding, ^{15}N-relaxation, ^{15}N chemical exchange saturation transfer (CEST) and ensemble molecular dynamics (MD) calculations that this is not the case. Even under the conditions of maximum protein stability nuclear magnetic resonance (NMR) invisible protein states may persist due to conformational and dynamical heterogenity. The hidden nature of these states for everyday NMR methods may be explained by the low populations and/or fading effects due to exchange between two or more states in the intermediate time scale (ms-us) regime. Chemical stress induced unfolding is also interesting since in pharmaceutical research as co-solvent, DMSO is often used. Here we present DMSO induced unfolding of PAF and variants as followed by NMR and differential scanning calorimetry (DSC), including thermodynamics parameters and structures. Partially unfolded states can be biologically relevant, e.g. connected to disulfide shuffling or S-S bond chirality switching. In general, less structured intermediates can be preferred for conformational-selection upon molecular recognition. In fact, there should not be a sharp boarder between the folded and intrinsically disordered proteins (IDPs) world and it is well known that many folded proteins have disordered regions. Practical consequences may have impact on the validation of MD simulations or protein concentration measurements.

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HEAVY WATER ENHANCES THE LIQUID-LIQUID PHASE SEPARATION OF A SUB-DOMAIN OF THE ANDROGEN RECEPTOR

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Phase equilibria of intrinsically disordered proteins (IDPs) are sensitive to changes in solution conditions, such as concentration of an IDP, ionic strength or temperature, and this can be used to study the driving forces in biomolecular condensation formation by liquid-liquid phase separation (LLPS) *in vitro*. Biomolecular condensates can have important roles in many cellular functions and dysregulation of their assembly and disassembly can be linked to various diseases, including cancer. In our work, we have studied the LLPS of the transcriptional activation unit (Tau-5*) of the activation domain (AD) of the androgen receptor (AR), a transcription factor that plays a role in the development of the male phenotype and is an atractive therapeutic target for prostate cancer and castration resistance prostate cancer, which currently is incurable [1]. In particular, we studied the effect that heavy water (D_2O), which is used in small amounts as a co-solvent in solution NMR to correct fluctuations in the magnetic field, has on the phase equilibrium of the protein.

Tau-5*, as well as the full length intrinsically disordered AD [2], undergoes lower critical solution temperature (LCST) LLPS, which is driven at least in part by hydrophobic interactions. In our study, we show that even small fractions of H_2O replaced with D_2O decrease the cloud point of LLPS of Tau-5*, likely reflecting a stabilization of the hydrophobic interactions that drive condensation. D_2O phenomenon to enhance hydrophobic interactions had already been known from protein folding and aggregation studies, where 100 % H_2O is replaced by D_2O. Here we show that even 10 % D_2O can reduce the cloud point of Tau-5* LLPS by 8 °C and 50 % by 20 °C, that represent a very profound alteration of the phase diagram of an IDP. Therefore, it is important to take this effect into consideration when studying phase separation phenomena with biophysical methods that require using D_2O as a co-solvent, such as solution NMR or EPR.

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PROTEIN PHOSPHORYLATION ASSIGNMENT BY FAST HIGH-RESOLUTION FOSY NMR.

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Phosphorylation is a prototypical example of post-translational modifications (PTMs) that dynamically modulate protein function, where dysregulation is often implicated in disease. NMR provides information on the exact location and time course of PTMs with atomic resolution and under nearly physiological conditions, including inside living cells, but requires unambiguous prior assignment of affected NMR signals to individual atoms. Yet, existing methods for this task base on a global, hence, costly and tedious NMR signal assignment that may often fail, especially for large intrinsically disordered proteins (IDPs). Here we introduce a suite of 2D Focused SpectroscopY (FOSY) experiments as a fast, sensitive and robust method to rapidly obtain only the relevant local NMR signal assignment. By employing the long-overlooked concept of selective polarisation transfer (SPT)[2], FOSY focusses onto one coupled nuclear spin system (i.e. a residue affected by PTM) at a time by selecting three to five known frequencies and, thus, provides the spectral dispersion equivalent to a 6D-7D experiment in only two dimensions and with an efficiency and versatility higher than achievable by traditional broadband experiments. We demonstrate the efficiency of FOSY by assigning, in just a few hours, two phosphorylation sites of proline-dependent glycogen synthase kinase 3 beta (GSK3β) in human Tau40, an IDP of 441 residues. The new approach will benefit NMR studies of protein hotspots, as sites involved in molecular interactions and conformational changes as well as assignment bottlenecks.

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IN OPERANDO MAGNETIC RESONANCE IMAGING FOR NEXT-GENERATION BATTERY CHEMISTRIES.

The demand for improved batteries is driving research towards the development of new electrode and electrolyte materials. However, the search for optimised materials can fall short in our aim of developing improved batteries. This is because the improvement of individual battery components cannot, on their own, provide the step-change in performance we are searching for. It is the collective performance of materials that is going to enable us to make high-performance batteries for the future. As battery components are typically a composite of materials, it is important to be able to distinguish the properties of the material from the assembled component. Also, as components work cooperatively, we need to develop analytical techniques that enable us to characterise the performance of the battery holistically. In the last 10 years, magnetic resonance imaging (MRI) has shown promise for non-invasively visualising the spatial distribution, speciation, and mobility of molecules and ions in batteries, across electrodes and electrolytes, integrating atomic information across mesoscopic and macroscopic length scales [1,2].

In this talk, I will present MRI of battery chemistry, under working conditions (in operando), and provide examples of 1H, 19F, 7Li and 23Na MRI for lithium-ion (LIBs), sodium-ion (NIBs) and Zn-air (ZABs) batteries [3,4]. In LIBs and NIBs, it is possible to image directly the electroactive species using 7Li or 23Na MRI. While 67Zn is NMR active, its properties do not lend themselves to MRI and so electroactive Zn species in ZABs cannot be observed directly. However, ZAB battery chemistry can be visualised using 1H MRI of the aqueous electrolyte, which is sensitive to the Zn-oxygen electrochemistry of the battery [3]. The use of 1H MRI is also valuable in LIBs and NIBs, enabling the visualisation of microstructural changes and dendrite formation with greater spatial and temporal resolution than by 7Li or 23Na MRI. Operando 1H, 7Li and 23Na MRI experiments are presented, which identify and map electroactive species across the electrode and electrolyte during charge cycling and galvanostatic plating. In the case of NIBs, the formation and evolution of dendrites are observed and mapped by 23Na MRI and their 3D microstructure visualised by 1H MRI of the electrolyte [4].

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NOT ONLY MICE AND RATS - ZTE MR IMAGING OF ROCKS AT 9.4T

Magnetic Resonance Imaging at the high magnetic field is successfully used for biomedical work, especially in *in-vivo* conditions, thanks to superior signal to noise ratio (SNR) and availability of a multitude of advanced imaging techniques. Most of these techniques are however useless or of limited use for the research of porous rocks, due to magnetic susceptibility-induced artifacts, compromising the observed NMR signal at high fields. Because of that the nuclear magnetic resonance and imaging performed at a low magnetic field are typically chosen as a useful tool for the laboratory-scale petrophysical characterization of oil-bearing rocks, as it assures the minimization of the influence of magnetic-susceptibility-induced internal gradients. The typical choice of the pulse sequence for MRI of such samples are Single Point Imaging (SPI), or its variants (e.g. SPRITE), which however have high demand on imaging gradient and/or limitations for the achievable 3D resolution in a reasonable experimental time [1-2].

Application of the zero echo time (ZTE) pulse sequence improves the possibility for time effective 3D imaging of samples with short T_2 [3-5]. Collection of the set of data points on FID during the TR period significantly accelerates measurements for 3D imaging, comparing to SPI. ZTE is a purely frequency-encoded method, and acquiring of the signal starts immediately after excitation using, *e.g.*, radial centre-out trajectories, without the need for preceding encoding gradients.

In the present lecture the application of 3D ZTE imaging for assessment of local hydration level in highly porous rocks (dolomite), as well as tight sandstone rocks are presented [4,5]. Correlation of the obtained results of the water content and spatial distribution in rock material, with the low field MR relaxometry as well as with gravimetric data provides interesting insight into the interpretation of the relaxation data in terms of assessment of sample's porosity. Correlation with high-resolution micro-CT images of the same rock samples allows for direct assessment of the role of the sample's composition and structure in the dynamics of water hydration into the porous rock's structure.

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UNRAVELLING MULTI-MODAL TRENDS IN QUANTITATIVE MRI DATA BY PHASOR ANALYSIS

Quantitative Magnetic Resonance Imaging (qMRI) methods are used extensively for non-invasive diagnostics in medicine and rely on obtaining high contrast images, which are suitable for analysis to recognize diseased tissues, based on either relaxation (T_1, T_2) or diffusion processes.

Often, these qMRI data sets show exponential or Gaussian decays, which need to be fitted, per image voxel, to extract the characteristic relaxation lifetimes or diffusion coefficients. When these signal decays are multi-modal, they become hard to analyse and, for every image voxel, an a priori assumption must be made regarding the number of parameters to be used for fitting.

To circumvent this issue, phasor analysis, a known non-fitting method for the study of lifetime fluorescence data, has recently been introduced for qMRI data processing by our group [1]. In phasor analysis, a plot is made that maps the lifetime information simultaneously for all pixels within the images. Creating this plot requires no assumptions on the type or multi-modal character of the decay underlying the data. By exploiting inter-pixel correlation in phasor plots, unique information can be obtained, for instance about partial volume effects, that are not supplied by per-voxel fitting analyses.

We demonstrate the use of phasor analysis for qMRI data, with applications ranging from medicine to porous media. Moreover, we introduce a method to greatly improve the accuracy of phasor processing, in a way that turns out to be unique for qMRI data over the original application of phasor in lifetime fluorescence [2].

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SUPRAMOLECULAR ADDUCTS BETWEEN MACROCYCLIC GD-COMPLEXES AND POLYAROMATIC SYSTEMS: A ROUTE TO ENHANCE THE RELAXIVITY THROUGH THE FORMATION OF HYDROPHOBIC INTERACTIONS.

MRI is the election imaging technique for the diagnosis and monitoring of numerous diseases. About 40–45% of MRI scans (ca. 38 million per year) are performed with the use of Gadolinium based contrast agents (GBCAs). The recent findings related to NSF and Gd-retention strongly required caution in the use of GBCAs.[1] Chemistry becomes central in looking for i) more stable and ii) more efficient GBCAs (i.e. enhanced relaxivity). Different routes to enhance relaxivity were exploited as i) the set-up of non-covalent binding interactions with macromolecules present in solution (e.g. albumin), ii) the increase of the number of coordinated or second sphere water molecules, iii) the increasing of prototropic exchange rates.[2,3]Herein, we describe the increase of relaxivity attainable through reversible binding interactions between the hydrophobic region of macrocyclic GBCAs and SO3-/OH containing pyrene derivatives.

Macrocyclic (ProHance, Gadovist, Dotarem) and linear (Magnevist, Omniscan, MultiHance) GBCAs were tested. The increase of relaxivity upon the addition of SO_3-/OH containing pyrene derivatives was assessed by ^{1}H-relaxometry and ^{1}H- / 17O-NMR. The binding parameters K_a (association constant) and R_b (relaxivity of the adduct) between GBCAs and the pyrene derivatives were calculated by using the PRE technique (0.5T). ^{1}H NMRD profiles were measured w or w/o of pyrene derivatives at variable B0 (0.00024 to 1.5 T). Insights into the formation of the adduct were obtained by high resolution 1H-NMR of YbHPDO3A complex w or w/o of pyrene derivatives (14 T). Finally, the in vivo proof of concept of the enhancement of contrast was obtained by MRI of tumor-bearing mice pre and post injection of GBCA (7T) upon injection of Gd-HPDO3A (0.15 mmol/Kg) or Gd-HPDO3A/HPTS adduct (0.15 and 0.45 mmol/Kg).

A high binding affinity of macrocyclic GBCAs toward pyrene derivatives was observed. The supramolecular adducts display a significant increase of relaxivity. No enhancement was observed for linear GBCAs. This is due to the increase of the molecular reorientation time (τR) and second sphere water molecules (for the presence of SO_3- and OH).

NMR spectra of the Yb-HPDO3A/ pyrene mixture support the formation of the supramolecular adduct. When HPTS/Gd-HPDO3A ratio is 3:1 (m/m), >90% of Gd-HPDO3A is in the associated adduct and there is a 40% relaxation enhancement in respect to the value observed for Gd-HPDO3A alone (i.e. 6.5 mM-1s-1 vs. 9.2 mM-1s-1 in serum). In T1w-MRI of tumor-bearing mice there is the increase of signal enhancement from 53% (upon i.v. of only Gd-HPDO3A) to 125% (upon i.v. of Gd-HPDO3A/HPTS adduct). By concluding, the reported results show a novel tool to enhance the relaxivity of GBCAs through the formation of supramolecular adducts at clinical doses.

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MAGNETIC RESONANCE IMAGING WITH NONLINEAR GRADIENT COILS

Gradient coils are usually designed such that they produce spatially linearly dependent magnetic fields along the direction of the static magnetic field. Specifically, three different gradient coils are used, each having a constant gradient of the magnetic field along one of the three orthogonal spatial coordinates [1]. This approach results in a simple relation between the frequency and the coordinate, where one is proportional to the other. This relation makes possible the introduction of the *k*-space, i.e., reciprocal space to the *r*-space, and also simple MR image reconstruction from the *k*-space data by multidimensional Fourier transformation. However, conventional linear gradient coils have also some drawbacks, such as complex design that usually leads to a higher inductance of the coils and therefore more difficult fast gradient pulse switching. In this study we examine the possibility of using nonlinear gradient coils for MRI.

We designed a simple set of two orthogonal nonlinear gradient coils. Each of the coils was made of a single 114 mm by 40 mm rectangular loop of which two longer segments were parallel with the static magnetic field (nonessential for imaging) and two shorter ones were perpendicular to it. One of the shorter segments lied in the imaging plane and was therefore producing considerable magnetic fields along the direction of the static magnetic field, while the opposite segment was too far from the sample to have any significant effect on imaging. Each of the loops was made of 50 turns of cooper wire and was tested with the maximum current of 3 A so that effective current along the segments was up to 150 A. The nonlinear gradient coils were connected to the gradient amplifiers of our system for magnetic resonance microscopy based on a 2.35 T superconducting magnet. Imaging with the nonlinear gradient coils was tested using the 2D spin-echo imaging sequence (without slice selection). One nonlinear gradient coil was used as the readout gradient and the other as the phase gradient. For the testing purposes was designed also a special checkerboard patterned sample in a form of a 26 mm diameter disc of which 2 mm deep square holes were filled with gel. Acquired signals were reconstructed in a standard manner using 2D Fourier transform. Since the signals were not *k*-space data, the obtained image was heavily distorted [2]. This was due to the nonlinear relation between the frequency and spatial coordinates; for the tested nonlinear gradient coils the frequency was inversely proportional to the spatial coordinate (measured as the distance from the effective loop segment). The obtained image can also be interpreted as the 2D spectrum and since the relation between the frequency and spatial coordinates was known, the spectrum was transformed to the coordinate system where axes were spatial coordinates. In this coordinate system the image of the sample was undistorted.

Results of the study confirmed that undistorted MR imaging with nonlinear gradient coils is feasible. Since such coils have considerably less constrains in their design, this approach also enables optimization of gradient coils for fast gradient pulse switching, which is essential for several MR imaging methods and other applications, e.g. diffusion measurements using PGSE, OGSE or MGSE sequences [3].

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INTEGRATION OF NMR, SAXS, AND COMPLEMENTARY TECHNIQUES FOR STRUCTURE DETERMINATION OF BIOMOLECULAR COMPLEXES

Structural analysis of biomolecules is a key challenge in current biology and a prerequisite for understanding the molecular basis of essential cellular processes. The use of solution techniques is important for characterizing structure, complex formation and dynamics of biomolecules and biomolecular complexes.

As experimental data for large biomolecules and biomolecular complexes are often sparse, it is advantageous to combine these data with additional information from other solution techniques. In my presentation I will show our recent achievements in integrating NMR data with complementary data from Small-Angle X-ray Scattering (SAXS), X-ray crystallography, electron microscopy, and mass spectrometry to study structure and dynamics of large proteins and biomolecular complexes [1-10]. By using our integrated approach we were able to provide a comprehensive and accurate description of protein complex structure and dynamics in a native-like environment. This underscores the central role of NMR for structure determination of biomolecular complexes and ensures its unique role and contributions in integrated structural biology approaches in the future.

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STRUCTURAL BASES OF HUNTINGTON'S DISEASE PATHOLOGICAL THRESHOLD. AN INTEGRATIVE STRUCTURAL BIOLOGY APPROACH

Huntington's disease (HD) is one of nine hereditary neurodegenerative disorders caused by an expansion of CAG triplet repeats beyond a pathological threshold. For HD, this expansion is located in the first exon of the huntingtin gene and results in an abnormally long poly-glutamine (poly-Q) tract within the N-terminus of the huntingtin protein (httex1). When the number of consecutive glutamines exceeds 35 (pathological threshold), the resulting mutant protein forms large cytoplasmic and nuclear aggregates, a hallmark of HD, and causes neuronal degeneration, especially affecting the neurons of the striatum. Aggregation, disease risk and age of onset correlate with the length of the poly-Q homo-repeat.

The origin of a pathological threshold in HD and the other poly-Q related diseases remains poorly understood and different toxicity models for httex1 have been proposed. The highly repetitive nature of httex1 sequence, with long tracts of glutamines and prolines, hampers high-resolution investigations in solution by Nuclear Magnetic Resonance (NMR). Our group has developed a novel chemical biology approach that allows for the first time to obtain atomic-resolution information of httex1 independently of the poly-Q length [1]. Using this strategy we have derived structural models of a pathogenic and a non-pathogenic versions of httex1 with 46 and 16 consecutive glutamines, respectively [2]. The comparison of these models shows that in both cases the protein consists of an equilibrium of helical conformations involving different fractions of the poly-Q. We also show that both poly-Q flanking regions define the structure of the homo-repeat. Using Small-Angle Scattering and computational approaches we have shown the presence of long α-helices, suggesting a mechanism of toxicity that involves the formation of oligomeric species through coiled-coil interactions. Taken together, these observations provide the structural bases to understand previous biophysical and functional data on httex1.

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INTEGRATION OF NMR, KINETIC ASSAYS AND MICROSCOPY TO INVESTIGATE INHIBITION OF AMYLOID FIBRIL FORMATION BY CO-CHAPERONIN PREFOLDIN

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Dysfunction of proteostasis leads to the accumulation of misfolded protein aggregates, as observed in many age-related amyloid diseases [1]. Among them is type II diabetes, caused by aggregation of Islet amyloid polypeptide (IAPP) in pancreatic beta cells [2]. Normally, the quality control machinery, notably the chaperone network, keeps aggregation in check, but in cases of misfolding diseases these systems are incapacitated, either due to age or overwhelm by more aggregation-prone protein species. So pharmacological targeting of the chaperone network is a promising avenue for finding disease altering therapeutics, requiring however a detailed understanding of chaperone action.

Here we report the mechanistic study of IAPP fibrillation inhibition by the cytosolic Hsp60 co-chaperonin prefoldin (PFD) [3], which has previously been shown to interact with other amyloidogenic substrates [4]. Our strategy, based on integration of kinetic data and structural studies by microscopy and NMR spectroscopy, allows to elucidate the different inhibition pathways and provides structural insight on the interaction.

We have established that PFD inhibits the formation of IAPP amyloid fibrils at sub-stoichiometric ratios. By using an integrative approach, we reveal the action mode of prefoldin during the fibril elongation process, responsible for this strong inhibitory effect. Analysis of ThioflavinT- fibrillation assays show that PFD inhibits the secondary nucleation and elongation of IAPP fibrils, which was further confirmed by an EM investigation of PFD fibril binding. To enable NMR studies, assignment of the heterohexameric chaperonin PFD (90kDa) was achieved with the aid of an in-house developed isotope-aided NMR method [5]. Liquid state NMR interaction studies with inovative PRE-labeling on IAPP were used to investigate binding of PFD to IAPP and extract kinetic parameters. We demonstrate that client binding takes place via two binding regions on IAPP, which bind in a highly dynamic fashion inside the PFD cavity.

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

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NMR-BASED STRUCTURAL INSIGHTS INTO PHOTOSYSTEM II ASSEMBLY

Biogenesis of photosystem II (PSII), nature's water-splitting catalyst, is assisted by auxiliary proteins that form transient complexes with PSII components to facilitate stepwise assembly events. Using cryo-electron microscopy, the structure of such a PSII assembly intermediate from Thermosynechococcus elongatus could be solved at 2.94 Å resolution [1]. It contains three assembly factors (Psb27, Psb28 and Psb34) and provides detailed insights into their molecular function [2]. Binding of Psb28 induces large conformational changes at the PSII acceptor side, which distort the binding pocket of the mobile quinone (QB) and replace the bicarbonate ligand of non-haem iron with glutamate, a structural motif found in reaction centres of non-oxygenic photosynthetic bacteria. NMR spectroscopy reveals that the carboxy-terminus of Psb28 is rigidified upon CP47 binding due to creation of an intermolecular β-sheet (Fig. 1). Together, these results bring to light mechanisms that protect PSII from damage during biogenesis until water splitting is activated.

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NMR INSIGHT INTO THE RECRUITMENT OF ATG9 BY ATG11 IN YEAST SELECTIVE AUTOPHAGY

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Autophagy is a conserved catabolic pathway that mediates the degradation of substances within lytic compartments. The degradation of these substances (from protein aggregates to damaged organelles), referred to as cargo, is achieved through their sequestration within de novo formed double membrane organelles called autophagosomes. In selective autophagy a specific cargo material is targeted for degradation. Selective autophagy starts with cargo recognition by a receptor and a scaffold protein (Atg11 in yeast, FIP200 for humans), which in turn triggers the recruitment of the autophagy machinery driving autophagosome formation around the cargo.

Vesicles containing the Atg9 protein are fascinating cogs of the autophagy machinery. These vesicles are 60 nm in diameter and contain 24 to 32 copies of the protein Atg9 (115 kDa) [1]. We have recently shown that Atg9 vesicles form platforms for the recruitment of the autophagy machinery as well as a membrane seed for phagophore expansion [2]. In yeast selective autophagy, the recruitment of Atg9 vesicles is mediated by the scaffold protein Atg11 (135 kDa) that interacts with the N-terminal domain of Atg9 [3].

In order to characterise the interaction between Atg11 and Atg9, we cloned, expressed and purified the N-terminal domain of Atg9 (residues 1 to 285). ITC titration showed that Atg9-NTD binds to Atg11 with a low micromolar affinity while the ^{1}H-^{15}N HSQC spectrum revealed that Atg9-NTD is largely disordered. Using ^{1}H-^{15}N HSQC based titration we were able to identify 2 Pro-Leu-Phe motifs in Atg9 that are mediating the binding to Atg11. Mutating these motifs to Pro-Ala-Ala completely abolished Atg11 binding in vitro as well as selective autophagy pathways in vivo.

Our data provide structural insights into the first steps of phagophore formation. Additionally, we illustrated how NMR data driven mutagenesis can lead to a better understanding of in vivo processes.

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DEVELOPMENT OF A HIGH-FREQUENCY RAPID SCAN ELECTRON SPIN RESONANCE SPECTROMETER

We report on the recent development of a high-frequency rapid scan electron spin resonance (FRASCAN) spectrometer at the Brno University of Technology. The basic principle of frequency rapid scan will be explained and compared to conventional methods. The FRASCAN operates in induction mode using quasi-optics with a super-heterodyne detection scheme. Fast frequency sweeps of the order of 1000 THz/s allows to access spin relaxation of the order of 1 ns [1], in a frequency range of 80 GHz to 1100 GHz [2], at temperatures from 1.8 K to 300 K, and at magnetic fields up to 16 T. We developed several sample holders for performing measurements on liquids, oriented single crystals, and air-sensitive samples, including the possibility of photo-excitation [3]. In addition, we developed a carousel sample holder for pressed powders that accommodates up to 6 samples, avoiding the time-consuming event of loading the probe into the cryostat and cooling down process. The carousel holder can be used for quantitative ESR. The FRASCAN is controlled by a home-written software in LabView, allowing to run experiments in an automatic mode controlled by scripts. Frequency rapid scan experiments on an oriented single crystal of LiPc will be presented along with simulation for calculation of the relaxation times. Furthermore, additional capabilities of FRASCAN are demonstrated using frequency-detected magnetic resonance spectra as a function of the orientation for a single-crystal of copper acetate and frequency-field ESR maps for Mn_{12} and TEMPO.

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TO BIND OR NOT TO BIND – THE SLINGS AND ARROWS OF BIOMOLECULAR BINDING STUDIES BY PULSE DIPOLAR EPR SPECTROSCOPY.

Pulse dipolar electron paramagnetic resonance spectroscopy (PDS) has become an increasingly popular tool to investigate topologies and conformational flexibilities of biomacromolecules. In addition to mere distance information quantitative PDS gives access to the number of interacting spins. Early model studies demonstrate that discrete multimeric states [1], dimerisation equilibria [2], metal ion binding site occupation [3], and cooperativities of binding [4] can be determined. We have recently exploited this to demonstrate the surprisingly efficient [5, 6] and robust [7] coordinative copper(II) based spin labelling of double histidine motifs in proteins offering highly precise distance measurements at nanomolar cooncentrations [8].

Two examples highlighting the level of detail that can be extracted from quantitative PDS will be presented. A single-stranded DNA binding protein [9] forming dimers and dimers of dimers, and a templated dimerisation of a model protein both allow extracting multiple parameters characterising the equilibria from PDS data.

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ACCESSING CALMODULIN BINDING NONEQUILIBRIUM KINETICS BY EPR DIPOLAR SPECTROSCOPY

As the cell's prototypical Ca^{2+} sensor, calmodulin (CaM) is responsible for amplifying and adapting this universal signal into specific downstream signals via interactions with numerous target proteins in a calcium-dependent manner. This biological adaptability correlates with the significant structural plasticity that has been observed in CaM. Here, the interaction of CaM and its 26-residue binding partner, M13, was probed on the millisecond time scale. The interaction mechanism is well established and, hence, serves as a model to evaluate the nonequilibrium kinetics by EPR dipolar spectroscopy. Our method uses a combination of selective protonation/deuteration [1], rapid mix and subsequent rapid freeze-quench [2] along the CaM and M13 reaction coordinates. Further, the acquisition of T_m-edited DEER [3] and subsequent processing by 2D SF-SVD enable detailed description of conformational components. The interaction between CaM and M13 gives rise to two intermediates originating from the initial interaction event (2 ms) and the coordination of the N- and C-terminal domains (8 ms) in addition to its halo- and fully bound state. The presented technique can be expanded to a wide range of molecular systems to answer questions of protein folding and domain coordination.

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DEER/PELDOR STUDY OF SUPRAMOLECULAR ASSEMBLIES OF HUMAN RIBOSOME AND RNAS

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Pulse dipolar EPR was used to investigated structural rearrangements in mRNA upon its binding to human 80S ribosomes [1,2,3]. The model mRNA (MR), 11-mer RNA containing two nitroxide spin labels at the 5 - and 3 -terminal nucleotides and prone to form a stable homodimer (MR)2, was used. Intramolecular spin-spin distances were measured by DEER/PELDOR spectroscopy in model complexes mimicking different states of the 80S ribosome during elongation and termination of translation. The formation of two different types of ribosomal complexes with MR was observed [1]. First, there were stable complexes where MR was fixed in the ribosomal mRNA-binding channel by the codon-anticodon interaction(s) with cognate tRNA(s). Second, we for the first time detected complexes assembled without tRNA due to the binding of MR most likely to an exposed peptide of ribosomal protein uS3 away from the mRNA channel. Our findings showed that a part of mRNA bound in the ribosome channel, which is not involved in codon-anticodon interactions, has more degrees of freedom than that interacting with tRNAs. The features of labile complexes of human 40S ribosomal subunits with RNAs, whose formation is manifested in the cross-linking of aldehyde derivatives of RNAs to the ribosomal protein uS3 through its peptide 55–64 located outside the mRNA channel was studied in details [2]. The measurements revealed that in all studied complexes, mRNA exists in two alternative conformations, whose ratios are different in post-translocation, pre-translocation and termination complexes. We found that the presence of aminoacyl-tRNA at the ribosomal A site decreases the relative share of the more extended mRNA conformation, whereas the binding of eRF1 (alone or in a complex with eRF3) results in the opposite effect. In the termination complexes, the ratios of mRNA conformations are practically the same, indicating that a part of mRNA bound in the ribosome channel does not undergo significant structural alterations in the course of completion of the translation. Our results contribute to the understanding of mRNA molecular dynamics in the mammalian ribosome channel during translation [3].

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STATISTICAL ANALYSIS OF ENDOR AT 263 GHZ REVEALS A CONFORMATIONAL DISTRIBUTION IN A PROTEIN TYROSYL RADICAL

Electron nuclear double resonance (ENDOR) spectroscopy probes the hyperfine (hf) interaction of unpaired electrons and magnetic nuclei in their environment, yielding structural information at atomic resolution. Technical advancements have enabled ENDOR spectroscopy at increasingly larger magnetic field strengths, recently extending to 9.4 T (263 GHz) [1]. The spectra recorded in this study on the $Y_{122}^{\bullet}$ radical in *E. Coli* ribonucleotide reductase suggested the presence of a hf interaction, which had previously been overlooked. The unusually broad appearance of this signal prompted us to develop a new approach for processing ENDOR data that allows for signals to be distinguished from baseline distortions [2]. The method is based on a mathematical modelling of the measurement signal considering variations between individual scan repetitions and a processing scheme to obtain the most-likely ENDOR signals. Furthermore, an estimation of confidence intervals for the spectrum becomes feasible. Application of this new method in combination with additional statistical tests confirms the presence of broad resonances in the $Y_{122}^{\bullet}$ ENDOR spectra in a quantitative way based on *p* values. We anticipate that our approach can be generally applied to ENDOR spectroscopy and will ultimately allow the quantification of uncertainties of spin density distributions and structural information that can be extracted from ENDOR data [2,3].

Isotopic labelling experiments were employed, which unambigously linked the newly recognized hf coupling to the β-methylene group of the radical. Finally, the unusually broad shape of these resonances could be explained by spectral simulations in conjunction with density functional theory by assuming a normal distribution of ring dihedral angles, which is known to strongly influence the hf coupling of β-methylene protons [4]. This study provides a new approach for analyzing structural inhomogeneity in tyrosyl radials, which are essential for a variety on biological systems.

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INVESTIGATION OF THE CONFORMATIONAL DYNAMICS OF DSRNA USING ORIENTATION-SELECTIVE PELDOR AND MD SIMULATIONS AS COMPLEMENTARY METHODS

DNA and RNA form the basis for storage and transmission of genetic information in living organisms. DNA carries the genetic information which is transcribed to RNA. Single- and double-stranded RNA (ssRNA and dsRNA) is needed for protein biosynthesis. Apart from their function as carriers of genetic information, nucleic acids also play a crucial role in the regulation of cellular processes. In all these tasks, the conformational dynamics of the nucleic acids can be ascribed as the root cause of their function.

The aim of this project was the investigation of the conformational dynamics of dsRNA. The system we chose for these studies was a set of 20-mer dsRNA molecules in A-helix form. Each dsRNA molecule was doubly labelled with the rigid **Çm** spin label [1] in varying positions. Pulsed ELectron Double Resonance (PELDOR) spectroscopy time traces are modulated by the electron-electron dipolar coupling. Due to the rigidity of the **Çm** spin label it is possible to obtain sets of orientation-dependent data using multi-frequency/multi-field orientation-selective PELDOR experiments. From such data insights about the mutual orientation and distances of the spin labels and with that insights into the dsRNA helix geometry and dynamics can be gained. This is, however, not a straightforward task and may yield ambiguous results. Here, we used conformers predicted by molecular dynamics (MD) simulations using different force fields to calculate orientation-selective PELDOR time traces.

Experimental orientation-selective PELDOR data were acquired at X- (9.5 GHz, 0.3 T) and G-band (180 GHz, 6.4 T) where differing degrees of orientation-selection are achievable. Using simulated data from MD predictions using the DESRES [2] force field we were able to achieve very good agreement between experimental and simulated data. This way we validated the accuracy of the DESRES force field for the dsRNA under investigation and can proceed with the analysis of the simulated data. It will be interesting to analyze the helix geometry and its conformational dynamics and compare our findings to previously reported findings for dsDNA [3] and dsRNA [4].

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UNDERSTANDING ONCOGENIC KRAS CYCLE THROUGH MULTIPLE TIME-SCALE DYNAMICS

KRas (Kirsten RAt Sarcoma virus) signaling protein is inactive when GDP occupies the binding site. Followed by the GDP to GTP exchange, a process catalyzed by GEF (Guanine nucleotide Exchange Factors) proteins, the spatial structure of the GTP binding protein changes and thus, becomes active in signal transduction. KRas GTP hydrolysis is accelerated by GAPs (GTPase Activating Proteins). Once GTP hydrolysis and there will be GDP again in the binding pocket, 3D-fold changes again and the protein will return to its off-state mode. kRas protein consists of two parts: the effector and the allosteric regions. As a result of our comprehensive Lipari-Szabo model-free and reduced spectral density mapping plus CPMG analysis on the wide type and 3 mutants, G12C, G12D and G12V, we found that both kRas.GTP.Mg^{2+}, kRas.GDP.Mg^{2+} has a major- and a minor-form. However, the latter on, crucial in understanding the details of GDP to GTP exchange, appears to be an on-pathway intermediate state of the transition. To characterize a minor form is a major challenge, but we found that the 3D-structure of the Mg^{2+}-free state of kRas shows high similarity to the minor-form.

1) We found that the relative distances of the P-loop, switch-I, and switch-II increase in the Mg^{2+}-free state, respectively, as they do in the X-ray determined (GDP.Mg^{2+})free KRas.GEF complex. 2) We observed that in the case of the wt, G12C and G12D mutants, the distance between selected residue pairs of the β2-β3 antiparallel strands increases in the Mg^{2+}-free-state. β-strands do separate and move away from each other. If we superimpose the wt, G12V and wt(GDP.Mg^{2+})free kRas.GEF 3D-structures, then will be clear that the bulge described above has functional significance. This bulge will host GEF protein when it forms a complex with kRas to facilitate the release of bound GDP. 3) We found that the strength of the H-bonds formed between HO2'- and HO3' of the furanose ring of GDP and adjacent C=Os (V29 and D30) changes with the Mg^{2+} ion release. We may ask how relevant all these data are for drug discovery? It would be good to design and then synthesize small molecules that are able to bind the herein described and characterized minor-form of kRas mutants and thus remove them selectively. The selectively elimination of harmful KRas mutants could stop abnormal signaling and thus, have the potential to cure tumor cells.

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NMR AND RATIONAL DRUG DESIGN

Nuclear Magnetic Resonance (NMR) spectroscopy is considered the most powerful analytical method as it finds plethora of applications in various fields including chemistry, biology, pharmacy and medicine. One of these applications that combines the use of the above mentioned fields is the "Rational Drug Design". Drugs now are now designed rationally using *in silico* techniques combined with analytical methods such as liquid and solid state NMR spectroscopy based on the existing molecular basis of information.

In our laboratory we are using this combined methodology for studying different aspects of rational drug design. In particular we:

(i) explore the conformational space of commericially available drug molecules in order to discover novel structures using a combination of NMR spectroscopy and computational chemistry and especially Molecular Dynamics calculations;

(ii) study the interactions of drugs with their vehicles such as cyclodextrins and calixarenes in an attempt to relate their biological properties and strength of their physical chemical interactions; Again, Molecular Docking, Molecular Dynamics and 3D Quantitative Structure Activity Relationships (3D QSAR) provide complementary information.

(iii) investigate the interactions of drug molecules in lipid bilayers in order to reveal the membrane role in their biological action;

(iv) examine the drug stability.

In the talk examples will be provided from our recent work which will illustrate that:

(i) NMR spectroscopy can provide a valuable information in the rational drug design;

(ii) It can be combined fruitfully with computational analysis to enhance the knowledge on drug action and understand aspects related to molecular basis of their action.

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TARGETING G-QUADRUPLEX AND I-MOTIF DNA STRUCTURES: A NEW OPPORTUNITY IN ANTI-CANCER THERAPY

The most widely recognized genomic DNA structure is the classical DNA double helix. However, DNA is structurally very dynamic and able to adopt several alternative secondary structures, such as cruciforms, G-quadruplexes, triplexes, and i-motifs [1]. G-quadruplex structures (G4s) are among the most extensively studied DNA secondary structures [2]. They can be unimolecular or intermolecular and can adopt a wide diversity of topologies depending on the combinations of strand orientation and length and composition of the loops. G4 structures are formed by guanine-rich sequences that are located mainly in telomeres, gene promoters and in the first intron of genes [3], where they are involved in the regulation of key biological processes, like, for example, telomere protection, regulation of transcription, and translation.

The G4-forming complementary strand is a single stranded C-rich sequence, potentially bound by single-stranded binding proteins [4]. Alternatively, the C-rich strand might fold in another four-stranded structure called i-motif (iM). This is composed of two head-to-tail, intercalated, parallel-stranded duplexes held together by hemi-protonated cytosine-cytosine+ (C·C+) base pairs. iM structures have also been found to be involved in telomere biology and in the regulation of gene-transcription [1]. Interestingly, the formation of iM structures is cell-cycle dependent, peaking at late G1 phase [5], whereas G4 formation is maximal during S phase [6]. This suggests that iMs and G4s might play different roles in regulating gene expression and transcription and potentially their presence could be mutually exclusive. Big efforts have been made by the international scientific community to find and develop small molecules that can selectively recognize G4 and iM structures, aiming at disentangling the individual biological processes regulated by these structures in the context of cancer cells. Many molecules display considerable selectivity for G4s over single-stranded and double-stranded DNA, and some of them are also capable to discriminate among G4 folding topologies, generally distinguishing parallel from anti-parallel quadruplexes. Very interestingly, many G4 ligands were found to exhibit intriguing anti-cancer activities. Differently from the well-documented examples of G4 ligands, the discovery of specific iM ligands lags far behind. In this communication examples of G4 and iM ligands will be shown.

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THE OUTER MEMBRANE INSERTASE AS AN ANTIBIOTIC TARGET

Proteins in the outer bacterial membrane are attractive antibiotic targets, because their location in the cellular periphery prevents many bacterial counteracting strategies. The outer membrane insertase BAM, one of two generally essential outer membrane proteins, is such a target. BAM receives its membrane protein substrates from a chain of periplasmic chaperones and then folds and inserts them into the membrane. BAM lacks a classical catalytic center and for long time it remained questionable whether BAM can be inhibited at all.

I will describe an integrated structural biology approach to resolve the functional mechanism of the BAM insertase in outer membrane protein biogenesis and to demonstrate that two recently discovered peptidic antibiotics, the natural compound darobactin and the engineered bicyclic OMPTA, inhibit BAM by direct interaction [1,2]. Solution NMR studies based on extensive sequence-specific resonance assignments of the BAM transmembrane barrel [3], combined with cryo-electron microscopy, X-ray crystallography, biophysics and molecular dynamics simulation show that darobactin inhibits the BAM complex by mimicking the cognate substrate recognition sequence [4].

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INVESTIGATING THE PROTEOME AND GENOME OF SARS-COV-2

SARS-CoV-2 contains a positive single-stranded RNA genome of approximately 30,000 nucleotides coding for ~ 27 different proteins. Within this genome, 15 RNA elements were identified as conserved between SARS-CoV and SARS-CoV-2. By nuclear magnetic resonance (NMR) spectroscopy, we determined that these elements fold independently and provided ^{1}H, ^{15}N chemical shift assignments for all these elements [1]. We optimized expression conditions for 24 proteins, conducted NMR experiments to establish folding conditions [2] and assigned more than 10 of these proteins within covid19-nmr [3]. We performed NMR-based screenings with a poised fragment library of 768 compounds for binding to these viral RNAs [4] and proteins [5] employing three different ^{1}H-based 1D NMR binding assays. Initial hits have been analyzed by cheminformatics approaches and follow-up strategies are being discussed for selected RNA and protein examples.

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

The glycan structures of the receptor binding domain of the SARS-CoV2 spike glycoprotein expressed in human HEK293F cells have been studied by using NMR. The different possible interacting epitopes have been deeply analysed and characterized, providing evidence of the presence of glycan structures not found in previous MS-based analyses. The interaction of the RBD 13C-labelled glycans with different human lectins, which are expressed in different organs and tissues that may be affected during the infection process, has also been evaluated by NMR. In particular, 15N-labelled galectins (galectins-3, -7 and -8 N-terminal), Siglecs (Siglec-8, Siglec-10), and C-type lectins (DC-SIGN, MGL) have been employed. Complementary experiments from the glycoprotein perspective or from the lectin's point of view have permitted to disentangle the specific interacting epitopes in each case. Based on these findings, 3D models of the interacting complexes have been proposed.

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R_{1R} RELAXATION DISPERSION UNDER FAST MAGIC-ANGLE SPINNING

Recent advances in instrumentations, isotope-labeling schemes, and the continued method developments for fast magic-angle-spinning (MAS) applications provide unprecedented insights into biologically relevant structural dynamics of large protein complexes or insoluble biomacromolecules. The importance of microsecond time-scale conformational exchange in protein-protein interaction, allostery, or signal transduction is widely recognized and extensively studied with solution-state NMR techniques; with solid-state NMR, such explorations are just about to unravel. In this talk, I briefly summarize the theoretical aspects and the practical considerations of solid-state magic-angle-spinning rotating-frame relaxation dispersion methods that are particularly sensitive to fast microsecond structural transitions. Unlike in solution state, in solid-state MAS measurementsrotating-frame ($R_{1\rho}$) relaxation dispersion can be monitored at two distinct effective radio-frequency field regimes (close to zero frequency and close to the rotary-resonance conditions) delivering complementary information about fast conformational exchange processes [1].

As an example, I will demonstrate the applicability of the method to study the structural and dynamic transitions of a de novodesigned soft polymer materialwhose amino acid sequence is based on the sequence of squid ring teeth proteins [2]. Hydration-inducedline narrowing in solid-state NMR spectra is a known phenomenon for soft biopolymer materials, such as elastin, collagen, silk. Enhanced motion on the nanosecond timescale can beprobed by ^{13}C longitudinal relaxation, while changes in microsecond timescale dynamics isbestassessed using ^{13}C rotating-frame relaxation dispersion techniques. In particular, I will demonstrate how ^{13}C off-resonance Near Rotary-Resonance Relaxation Dispersion (NERRD) method can be used to identify the origin and quantify the amplitude of the fast microsecond time-scale motion. The application of fast magic-angle spinning (55.55 kHz) and increasing spin-lock field strengths approaching the half-and full-rotary-resonance conditions enables the separatoin of the relaxation dispersion regime that reports on incoherent dynamics as opposed to coherent dephasing of the transverse magnetization due to the presence of dense proton network. These relaxation measurements revealed that the motion of proline side-chain carbons are the most affected by hydration giving rise to sharp signals, and hence to long coherence life times, in both dipolar-coupling-based and scalar-coupling-based 2D ^{1}H–^{13}C experiments.

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BASE-PAIR SWITCHING MODULATES MICRORNA ACTIVITY

Many functions of RNA depend on rearrangements in secondary structure that are triggered by external factors, such as protein or small molecule binding. These transitions can feature on one hand localized structural changes in base-pairs or can be presented by a change in chemical identity of *e.g.* a nucleobase tautomer. We use and develop $R_{1\rho}$-relaxation-dispersion NMR method [1] for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled on timescales spanning three orders of magnitude (µs to s).

The characterization of microRNA 34a targeting the p53 modulator mRNA of Sirt1. This microRNA – mRNA complex changes conformation to activate the RISC complex [2] (Nature 2020). I will furthermore give an outlook on recent efforts to measure in-cell NMR of nucleic acids in functional complexes [3].

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NARROWING THE GAP BETWEEN NMR RELAXATION AND MD SIMULATION STUDIES OF PROTEIN DYNAMICS

While NMR nuclear spin relaxation is arguably the most comprehensive experimental approach to measure protein dynamics at atomic resolution, it is only highly approximate due to the very sparse sampling of the spectral density function (SDF) it provides. MD simulation, on the other hand, describe molecular flexibility in amazing detail, but are constrained by limitations on the accuracy of the molecular mechanics force field models and conformational sampling. The two methods are therefore highly complementary for the study of picosecond to nanosecond time scale dynamics in proteins, and can act in a highly synergistic manner [1]. We find that the popular Lipari-Szabo 'model free' approximation of the internal time correlation functions is highly inadequate [2]. Instead, an objective comparison is feasible if we use the full MD trajectories to compute the SDF, including anisotropic molecular tumbling [2,3]. Examples of ^{13}C and ^{2}H NMR relaxation data will be provided and discussed [2-4]. The decreasing gap between prediction and experiment points to quantitative avenues for probing configurational entropy [5,6].

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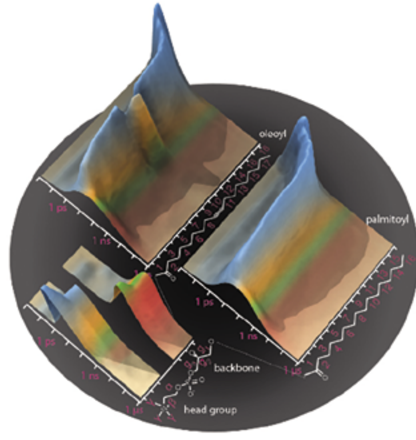
UNVEILING THE DYNAMIC LANDSCAPE WITH NMR RELAXATION AND MOLECULAR DYNAMICS SIMULATION

Biomolecular function is the result of a complex hierarchy of molecular motions. NMR relaxation is one of the premier experimental methods for characterization of dynamics, having an array of timescale-selective and bond-specific experiments available for the characterization of reorientational motion. Still, these experiments capture the aggregate influence of multiple motions that make up the total motion, where the shear complexity of the total motion prevents a complete parameterization based on experimental data only. On the other hand, the atomic detail provided by molecular dynamics simulation can, in principle, be used to separate the influence of these multiple motions on the reorientational dynamics of individual bonds. Therefore, we develop a *frame analysis*, which allows the careful separation of multiple motions based on the MD trajectory. The separated motions may be analyzed and described with a few parameters. Using detector analysis [1,2], we then compare results from the simulated frame analysis to experimental results, and refine parameters describing the separated motions using experimental results. We apply the proposed to method to POPC bilayers, a critical biological interface, resulting in a *dynamic landscape*: a comprehensive description of motion in POPC molecules, for which we obtain the generalized amplitude of reorientational motion as a function of molecular position and correlation time. While we have applied our method to POPC membranes, our approach is fully general and may be applied to any system for which experimental and simulated data is available.



Dynamic landscape of POPC. The landscape is derived based on a combination of NMR and MD data. Motional amplitude is plotted as a function of correlation time and position.

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ANTIBIOTIC RESISTANCE DRIVEN BY CHANGES IN CONFORMATIONAL DYNAMICS

Fusidic acid (FA) is one of few remaining antibiotics that can be used to treat MRSA but resistance has increased rapidly in recent years due to the expression of the FusB family of proteins. FA inhibits bacterial protein synthesis by binding to Elongation Factor G (EF-G) when it is bound to the ribosome and preventing its release. FusB binds to EF-G and promotes the dissociation of these stalled complexes. The solution structure of the EF-G_{C3}:FusB complex identified both conformational changes and a significant change in the conformational flexibility of EF-G in response to FusB binding [1]. In this study, methyl relaxation dispersion NMR experiments have been used to characterize the difference in EF-G conformational flexibility in the apo and FusB bound states showing a widespread change in the conformational flexibility of domain 3, reflecting a significant increase in a minor state. HD exchange mass spectrometry suggests this results from increased domain 3 disorder upon FusB binding. Mutations disrupting the effect of FusB binding on conformational flexibility but not affecting structural changes reduce the ability of FusB to confer FA resistance, showing the changes in dynamics are important in the resistance mechanism. These experiments show that FusB confers FA resistance though a significant change in the dynamics of domain 3 of EF-G, spreading throughout the domain and driving release of EF-G from the ribosome in a novel antibiotic resistance mechanism [2].

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MOLECULAR BASIS OF HOST-ADAPTATION INTERACTIONS BETWEEN INFLUENZA VIRUS POLYMERASE PB2 SUBUNIT AND ANP32A

Influenza A virus (IAV) is responsible for 3–5 million severe cases every year, resulting in 250-500,000 deaths. Most influenza strains evolve exclusively in the large reservoir of water birds, but some highly pathogenic avian strains (*e.g.*, H5N1, H5N8 and H7N9) can infect humans with lethal consequences (up to 60% mortality) and are potential pandemic threats for humanity if they develop human-to-human transmissibility [1].

Avian influenza polymerase undergoes host adaptation in order to efficiently replicate in human cells [2]. Adaptive mutants are localized on the C-terminal (627-NLS) domains of the PB2 subunit. In particular, mutation of PB2 residue 627 from E to K rescues polymerase activity in mammalian cells. A host transcription regulator ANP32A, comprising a long C-terminal intrinsically disordered domain (IDD), is responsible for this adaptation [3]. Human ANP32A IDD lacks a 33 residue insertion compared to avian ANP32A, and this deletion restricts avian influenza polymerase activity.

We used NMR to determine conformational ensembles of E627 and K627 forms of 627-NLS of PB2 in complex with avian and human ANP32A [4]. Human ANP32A IDD transiently binds to the 627 domain, exploiting multivalency to maximize affinity. E627 interrupts the polyvalency of the interaction, an effect compensated by an avian-unique motif in the IDD. The observed binding mode is maintained in the context of heterotrimeric influenza polymerase, placing ANP32A in the immediate vicinity of known host-adaptive PB2 mutants.

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INTERACTIONS OF MACROLIDE ANTIBIOTICS AND MACROZONES UNDER THE SCOPE OF NMR

Macrolide antibiotics, such as azithromycin have been in clinical use for over 60 years, mostly due to their high efficacy, safety and favourable pharmacokinetics [1]. Macrolides bind to the 23S rRNA of the 50S subunit, near or at the peptidyl transferase center (PTC), block the nascent peptides exit tunnel and thus inhibit the synthesis of bacterial proteins. Multi-drug resistant microbial pathogens compromise their use as effective antimicrobials and poses serious treats to human health worldwide which demand novel and more potent antimicrobial agents to be discovered. An effective approach to overcoming this problem is to understand the principles of how these drugs interact with their biological targets. Macrozones belong to a novel class of azithromycin-thiosemicarbazone conjugates that exhibit very good antibacterial activities against both susceptible and resistant bacterial strains [2].

In this talk results of interaction studies of macrolide antibiotics and some selected macrozones with their biological targets will be presented [2]. A combination of NMR experiments such as transferred nuclear Overhauser effect spectroscopy (trNOESY), saturation transfer difference (STD), diffusion and solvent paramagnetic relaxation experiments (PRE) and molecular modelling has been employed to characterize binding epitopes and asses bound conformations [4,5].

The data so obtained can serve as a basis for design of novel compounds with an improved biological profile.

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IMPROVING SENSITIVITY AND VERSATILITY IN NMR SUPERSEQUENCES

The structure characterisation of small molecules by NMR spectroscopy routinely employs well established 2D homonuclear and heteronuclear correlation experiments such as COSY, TOCSY, HSQC, HMBC, NOESY, ROESY and their many variants. In the arena of synthetic chemistry where many novel compounds require rapid structure verification, or in metabolomics where multiple biomarkers require identification, there is significant interest in developing experimental methods that allow more rapid data collection to enable such strucutral identification. NOAH (NMR by Ordered Acquisition using ^{1}H-detection) represents one approach to fast data collection that records multiple 2D data sets nested as specfically engineered aquisition modules within a single “supersequence”. [1,2] This approach requires only a single recovery delay for each series of nested modules, and so allows for significantly reduced data collection times.

In the work presented here, the sensitivity and versatility of NOAH supersequences are improved by modification of sensitivity-enhanced HSQC and HSQC-TOCSY experiments, adding further versatility for ^{13}C and ^{15}N modules. [3] Importantly, these heteronuclear modules have been specifically tailored to preserve the magnetisation required for subsequent acquisition of other heteronuclear or homonuclear modules in a supersequence. In addition, we present protocols for optimally combining HSQC and HSQC-TOCSY/COSY elements within the same supersequences, and further demonstrate that the associated time savings derived from NOAH can translate to increased detection sensitivity per unit time.

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DETECTION AND VERIFICATION OF A KEY INTERMEDIATE IN AN ENANTIOSELECTIVE PEPTIDE CATALYZED ACYLATION REACTION

The selective acyl transfer onto alcohols is done in nature by acyltransferases. They are used in many important signaling pathways, for example in plants for the synthesis of volatile esters while fruits are ripening, or in animals for the synthesis of the neurotransmitter acetylcholin. [1,2]

Trying to reproduce the selectivity of this important reaction and to make it usable by chemists, Miller et al. introduced smaller peptide catalysts. Bearing a π-methylhistidine moiety these peptides were able to transfer acyl groups from acetic anhydride onto racemic alcohols while producing enantiomeric excess in the acylated product. [3] Schreiner and coworkers were able to build upon this success by also incorporating a π-methylhistidine moiety into a less flexible tetrapeptide. This peptide is able to reach enantiomeric excess values of > 99% for the acylation of racemic trans-cycloalkane-1,2-diols in toluene. [4]

We herein report the detection of a key intermediate imidazolium-ion of the tetrapeptide, postulated to transfer the acyl-group onto the diols. Testing three different anhydrides in toluene and dichloromethane, we were able to identify imidazolium-ions for all of the anhydrides using ESI-HRMS. To further verify the existence of these ions in solution, HMBC- and selective NOESY-spectra were measured. Furthermore, a model reaction to test the prepared solution for the desired reactivity, was performed.

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LED-NMR INVESTIGATION OF SPIRO-γ-LACTAM FORMATION

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Spiro compounds are interesting building blocks in medicinal chemistry. These compounds have a three-dimensional structure that allows them to occupy the space in a pocket of a targeted protein of interest. They are also interresting moieties for a fragment based approach of drug design (FBDD).

In 2020, Ryder *et al.* [1] described a photocatalyzed synthesis route for γ-spirolactam compounds, starting from the corresponding primary amine. This synthetic approach was performed in the presence of 4CzIPN [2] as the photocatalyst (PC), tetrabutylammonium azide as the HAT catalyst, one equivalent of methyl acrylate (or 2-methoxyethyl acrylate) as the alkylating agent, without any protection of the amino group of the primary amine, at 425 nm in acetonitrile, and at room temperature. The corresponding γ-amino ester was formed as intermediate of synthesis, and, after an intramolecular reaction of elimination in presence of triethylamine in methanol solution under reflux, the ring closure formation of the γ-lactam was then observed.

LED-NMR [3] is a powerfull method to investigate photochemical reactions. Also, we followed the above described reaction using this approach, with an already described LED setup [4] on a Bruker AVIII spectrometer, equipped with an inverse Prodigy® cryoprobe. The LED setup was constituted of a 420 nm LED light source for microscope illumination (Mic-LED-420Z, Prizmatix), fixed on a beam collimator coupled to a PMMA optic fiber (1000 μm core, 6 m of length).

We investigated the photoredox catalysis reaction with three cyclic primary amines as starting materials, in CD_3CN. We followed, by 1D and 2D NMR experiments, the quantitative α-tertiary amine N-functionalisation, using methyl acrylate or 2-methoxyethyl acrylate as alkylating agent. Then, we determined the kinetic constants and the reactional rate of the reaction for these three different amines, and highlighted the spontaneous ring formation of the lactam in the reaction medium at room temperature, without additional triethylamine treatment.

LED-NMR has proven to be a powerful method for photochemical reactions monitoring, and can replace LC-MS as analytical tool in the absence of chromophores on chemical species.

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CHALLENGES IN THE DECOMPOSITION OF NMR SPECTRA OF SMALL MOLECULE MIXTURES

Despite the development of Nuclear Magnetic Resonance (NMR) methods to increase spectral resolution, the growing complexity of the samples leads to crowded spectra that compromise the analytical performances of this technique [1]. The association of mathematical methods for signal processing with the methodological developments in NMR is a promising alternative [2, 3]. In this context, this work is focused on the analysis of complex mixtures by NMR with two aspects: the development and optimization of NMR pulse sequences and the application of Blind Source Separation (BSS) algorithms to NMR data. This source separation technique, originally used for disciplines such as acoustics for audio signal processing, has shown its effectiveness for the demixing of 1D and 2D NMR spectra [4-7]. In this case, spectra decomposition is performed using correlations, essentially variations in concentrations, detected over a series of data sets, which allows the extraction of the pure spectra of the mixture constituents. Several types of samples such as synthetic mixtures of terpenes are used to evaluate the efficiency of algorithms for the extraction of the pure spectra.

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NANOSCALE MAGNETIC RESONANCE ENABLED BY DIAMOND QUBITS

A particularly interesting application of diamond based quantum sensing is the detection of nuclear magnetic resonance on nanometer scales, including the detection of individual nuclear spins or small ensembles of external nuclear spins. Single nitrogen vacancy (NV) color centers in diamond currently have sufficient sensitivity for detecting single external nuclear spins and resolve their position within a few angstroms. The ability to bring the sensor close to biomolecules by implantation of single NV centers and attachment of proteins to the surface of diamond enabled the first proof of principle demonstration of label-free detection of the signal from a single protein. Single-molecule nuclear magnetic resonance experiments open the way towards unraveling dynamics and structure of single biomolecules. However, for that purpose, NV magnetometers must reach spectral resolutions comparable to that of conventional solution state NMR. New techniques for this purpose will be discussed. We will also show first experiments towards hyperpolarisait of extrneal nuclear spins using shallow NV centers.

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PROBING MATERIALS WITH SINGLE SPIN QUANTUM SENSORS

Single spin probes are developing into versatile use to measure the properties of materials with high spatial resolution. Besides measuring diffusion through single spin detected NMR [1], specifically magnetic materials lend themselves as interesting objects to be investigated by single spin quantum probes. I will show how to measure the micro and nanomagnetic properties of two-dimensional material by measuring its local magnetic field [2]. To this end, a single spin system, like a single nitrogen vacancy centre, is scanned across a sample while at the same time measuring its electron spin resonance frequency. I will demonstrate that one can achieve a spatial resolution of a few 10 nm with a magnetic field sensitivity of below 1 μT.

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MAGNETIC RESONANCE FORCE MICROSCOPY: TOWARDS SINGLE ELECTRON SPIN DETECTION AND NANOMRI

In this presentation, we will introduce new methods and their challenges in MRFM, where magnetic resonance is generating forces that are detected using Atomic Force Microscopy. We will discuss particularly how the challenges of working at temperatures below 1 Kelvin might be addressed. We present data in which we show that nuclear magnetic resonance of copper allows nanoscale imaging, and discuss how this translates to the feasibility of using Boltzmann polarization of protons for nanoscale imaging, even at 1 MHz. Finally, we present the challenges of Force measurements on magnetic resonance of single electrons.

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HIGHLY COHERENT AND PROCESSABLE ORGANOMETALLIC SPIN QUBITS

Organometallic sandwich complexes of paramagnetic lanthanides ions are currently the focus of great interest because of the observation that dysprosium(III) derivatives present magnetic hysteresis of molecular origin, *i.e.* Single-Molecule Magnet behavior, above liquid nitrogen temperature [1].

Sandwich complexes of early transition metal ions such as Ti and V carrying one unpaired electron are also well known but their spin dynamics not equally investigated. In our earch for highly coherent and evaporable magnetic molecules as potential molecular spin qubits, we identified in [CpTi(cot)] and [CpV(cht)] where Cp= η^5-C_5H_5, cot= η^8-C_8H_8 , and cht= η^7-C_7H_7, promising candidates [2]. Pulsed EPR investigations have revealed that the coherence times reach ca.35 μs at low temperature in frozen deuterated toluene, despite the molecules being hydroge-rich. *Ab initio* calculations revealed that the low energy vibrational modes involving the rings rotation have a weak spin-phonon coupling, while hydrogen atoms are placed within the frozen sphere, thus not too detrimental for decoherence.

Moreover these small neutral molecules can be evaporated and deposited on different substrates with a moderate hybridization with the underlying metal, whose extent depends on the metal substrate and on the adsorption geometry [3]. They appears very promising candidates for single spin addressing via Scanning Tunnel Microscopy and for tip functionalization with a spin probe [4].

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OPTICAL CONTRASTS IN NMR USING NUCLEAR MAGNETO-OPTIC EFFECTS

In a typical NMR experiment, the information about the chemical structure of a molecule is encoded in the chemical shift, while the intensities of the NMR peaks carry quantitative information about the ratio of equivalent nuclei.

In addition to classical NMR, the nuclear magnetization can also be detected using polarized light [1]. The measured signal in these so-called nuclear magneto-optic (NMO) effects originates from a complex interplay of the nuclear magnetic moments, electron density of the molecule, and the light beam. In contrast to NMR, the intensity of NMO signal qualitatively depends on the local chemical environment around the nucleus. This effect is called optical chemical shift [2] and offers additional possibility for advanced resolving of complex molecular structures.

In this contribution I present our recent theoretical investigations on this topic [3]. In particular I discuss the NMO effect called nuclear spin-induced optical rotation (NSOR), which is a rotation of linearly polarized light by nuclear magnetization oriented parallel to the light beam. By investigating ^{1}H and ^{13}C NSOR spectra of a large set of hydrocarbons, patterns are predicted showing systematic relation between the intensity of NSOR and the local molecular structure. The signal intensities are grouped according to the chemical bonding near the nucleus and its neighbors, their positions within the molecule with respect to other nuclei, and local isomerism. This additional information can be used to distinguish the nuclei and help in resolving spectra of complex systems.

Further experimental development of NSOR technique and combining it with existing NMR methods could provide exciting opportunities for completely new kind of multidimensional experiments for higher resolution spectroscopy and measurements of new molecular properties.

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SINGLET-STATE ^{31}P NMR SPECTROSCOPY BETWEEN CHEMICALLY EQUIVALENT NUCLEI

The discovery of long-lived nuclear magnetic resonance (NMR) singlet states has been an exciting area of scientific inquiry over the past two decades. The attractive possibility to store phase information or population differences over long periods of time relative to other NMR timescales has garnered interest for using singlets to study slow molecular rearrangements, diffusion, and hyperpolarization storage [1]. NMR singlets have even been postulated to play a role in biological processes, particularly between ^{31}P nuclei in biomolecules [2]. We report the generation and lifetime measurement of a ^{31}P singlet for a chemically equivalent case in tetrabenzyl pyrophosphate (TBPP).

The parameters of the unusually complex ^{31}P and ^{1}H spectra, caused by magnetic inequivalence, were extracted by comparing the experimental results with simulations using the *Spinach* MATLAB package [3]. We modeled the compound as two ^{31}P nuclei, each J-coupling with 4 ^{1}H nuclei that are 3 bonds away. Excellent agreement with experimental results was further found by using the following J-coupling parameters: $^{2}J_{PP}$ = 16.4 Hz and $^{3}J_{PH}$ = 8.4 Hz. We also discovered by spectral simulation that the ^{1}H nuclei in each pair of methylene protons are chemically inequivalent, having a chemical shift difference of 0.0089 ppm (3.6 Hz at 9.4 T) and a J-coupling constant $^{2}J_{HH}$ of 11.6 Hz.The differential couplings between ^{1}H and ^{31}P nuclei enable access to a ^{31}P singlet state. We measured spin-lattice (R_1) and singlet (R_s) relaxation rates at 9.4 T at various temperatures using inversion-recovery and spin level induced crossing (SLIC) [4] sequences, respectively. Surprisingly, R_s exceeded R_1 considerably at all temperatures measured, contrary to expected results. In order to elucidate which mechanisms dominate singlet relaxation, we conducted molecular dynamics (MD) simulations with AMBER and calculated chemical shift anisotropy (CSA) tensors with Gaussian. We found that the unexpectedly short relaxation rates could be explained by the very large CSA present between ^{31}P nuclei in TBPP. The two CSA tensors for each phosphate are highly anticorrelated and at a relatively constant angle with one another, leading to enhanced relaxation.

In conclusion, we demonstrate the existence of a singlet state between magnetically inequivalent ^{31}P nuclei. We observe a very short ^{31}P singlet lifetime in TBPP dominated by large uncorrelated CSA interactions. It is likely that the singlet lifetimes are significantly longer at low magnetic fields. Future work will focus on ^{31}P singlets in other compounds, including biological species.

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RESONANCES IN ^{1}H MAS SPECTRA ARE NOT CENTERED AT THE ISOTROPIC CHEMICAL SHIFT

In solid-state NMR, the homonuclear dipolar coupling is the internal spin interaction that has the greatest contribution to the broadening of the ^{1}H spectral lines. Even at the fastest magic-angle spinning rates available today [100 kHz-150 kHz], the linewidths can extend over hundreds of Hertz, affecting ^{1}H resolution. Understanding and minimizing this contribution could lead to rich structural information for organic solids. In the past, systems of two and three spins were studied with Average Hamiltonian Theory [1] (AHT) to second order and Floquet Theory [2-3]. Here we study two and three inequivalent spin systems (I=½) with AHT, in the fast-spinning regime, and we develop analytical expressions of the average Hamiltonian to 3rd order.

The results show that the full expression of the 3rd order average Hamiltonian, without secular approximation or truncation to second-order, agrees the best with full numerical calculations.

We find that the effect on the NMR spectrum of the different Hamiltonian terms, is to produce both MAS rate dependent residual shifts and splittings in the three-spin case.

Powder lineshapes are also analyzed, and it is found that the anisotropic residual shift does not have zero average, indicating that the lineshape is broadened and shifted from the isotropic position, and we confirm this with experimental observations for ^{1}H MAS spectra in molecular solids [4-5].

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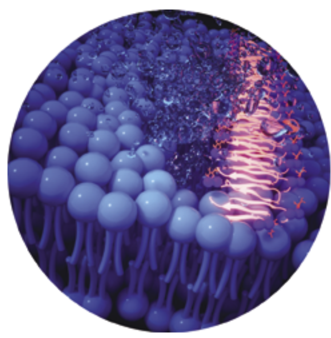
UNDERSTANDING ANTIBIOTICS WITH SOLID-STATE NMR

Antibiotics that use novel mechanisms are needed to resolve the antimicrobial resistance crisis. Promising templates could be antibiotics that target Lipid II, known as the Achilles' heel of bacteria, at a conserved and immutable pyrophosphate group. Such antibiotics, like plectasin, teixobactin, or malacidin [1-3] would kill the most refractory pathogens without causing resistance. However, due to the challenge of studying small antibiotic-target complexes in membranes, the structural correlates of the relevant binding modes are virtually unknown.

Here, using solid-state NMR in combination with microscopy, we report on the physiologically relevant binding modes of several Lipid II-binding antibiotics [4-6].



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COMBINING PHOTOCHEMISTRY AND SOLID-STATE NMR FOR MEMBRANE PROTEIN RESEARCH

The use of solid-state NMR for resolving the mode of action of photochemical tools in lipid bilayers and for mechanistic studies on photoreceptors is demonstrated and discussed.

First, it is shown by MAS-NOESY and R-PDLF experiments how photoswitchable lipids within lipid bilayers alter the general membrane properties [1]. The light-induced *trans-cis* isomerisation of these AzoPCs causes localized disorder in the membrane. The embedded integral *E. coli* membrane protein diacylglcerol kinase (DgkA) serves as a model to probe how these effects can influence protein structure and activity. The known resonance assignment of DgkA [2] enabled to record a series of 2D NCA spectra after illumination from which site-resolved, light-induced effects could be visualized. Protein activity has been assessed by real-time ^{31}P MAS NMR. Our data reveal in detail how photositchable lipids alter bilayer proerties and how these affect embedded membrane proteins. We also demonstrate how the enzymatic reaction catalyzed by DgkA can be triggeded by light under MAS NMR conditions [3].

While these experiments rely on high fields, extensive labelling and non-frozen samples, other experimental requirements apply when targetting photoreceptor intemediate states. Here, DNP-enhanced solid-state NMR in combination with light-induced cryo-trapping has been proven as an indispensable tool, which will be briefly illustrated by two recent examples. In case of channelrhodopsin-2 long-range double quantum recoupling sequences (SR26) and systematic cryo-trapping procedures enabled to differentiate between competing photocycle models [4], while in case of the sodium pump KR2, the chromophore configuration could be identified during the sodium transfer step [5].

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NECROSOME ASSEMBLY STUDIED BY SOLUTION AND SOLID-STATE NMR

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Receptor-Interacting Protein Kinase 3 (RIPK3) is the key effector that activates the mixed lineage kinase domain-like (MLKL) protein to execute necroptosis, a type of programmed cell death distinct from apoptosis [1]. Necroptosis activation requires the formation of intermediate signaling complexes named *necrosomes*. RIPK3 a common component in all distinct necrosome complexes, whose assembly is mediated by a conserved region named RIP Homotypic Interaction Motif (RHIM), with sequences I(V)QI(V/L/C)G. Of the different necrosomes, the RIPK1-RIPK3 complex is regarded as the canonical necrosome, and TRIF-RIPK3 and ZBP1-RIPK3 are other two non-canonical necrosomes, all leading to the activation of MLKL [2, 3]. All these complexes are amyloidal assemblies, and solid-state NMR studies on the RIPK1-RIPK3 canonical necrosome core revealed a 1:1 hetero-amyloid structure [4], changing our vision that amyloids are homo-polymers building on copies of a same type of protein. Density Functional Theory (DFT) calculations corroborated that 1:1 co-assembly is preferred over self-assembly. While ongoing solid-state NMR studies will reveal the key differences among distinct necrosomes, solution NMR has the unique potential to inform the conformational changes and interactions in free and amyloid-bound monomers to unveil the molecular determinants that drive homo- versus hetero-amyloid formation. Recent improvements in preparing, handling and stabilizing RHIM proteins are enabling for the first time characterizing of the monomeric form of RIPK3 and its oligomerization pathways in solution [5]. Analysis of chemical shifts and ^{15}N relaxation measurements illustrate a chiefly disordered domain with key residues around the RHIM region showing slowed motion in the μs-ms timescales, in agreement with the amyloid core mapped by both solid-state NMR and ^{15}N-DEST (Dark-state Exchange Saturation Transfer) experiments that we are using to characterize the interaction sites in the assembled state.

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FASTER MAS NMR DATA ACQUISITION AND FUNCTIONAL ANALYSIS OF THE LIGHT-DRIVEN SODIUM PUMP KR2

Solid-state NMR (ssNMR) is a powerful technique to study membrane protein function, dynamics and structure in a native-like environment. High sensitivity is required for a complete resonance assignment based on highly resolved 3D- and 4D- heteronuclear ^{13}C, ^{15}N correlation spectra recorded at high magnetic fields. Here we combine several techniques to improve sensitivity which enabled us to assign the first known light driven sodium pump *Krokinobacter eikastus* rhodopsin 2 (KR2) [1-3], a hepta-helical membrane embedded protein. Paramagnetic Gd^{3+}-doping [4] allowed a 2,5-times faster acquisition using an E-free MAS probe optimized for a higher duty cycle. Double-CP optimum control pulse sequences [5] enabled 4-fold faster data recording. Non-uniform sampling shortened experimental times 2-times [6]. BioSolids Cryoprobe additionally allowed a seven times faster data acquisition [7]. As a result, 3D and 4D spectra could be recorded in 5 and 12 days, respectively. The KR2 assignment covers more than 80% including numerous functional important residues which are mainly involved in the ion selectivity and pumping mechanism such as D116 and Q123 and allows the NMR analysis of functional KR2 mutants with a promising future application as optogenetic tools [8].

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HOW SOLID-STATE AND LOW-FIELD NMR CAN CONTRIBUTE TO THE SUPRAMOLECULAR ORGANIZATION ELUCIDATION OF THE LIGNOCELLULOSIC BIOMASS DURING DECONSTRUCTION?

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Over the last 15 years, research efforts have been dedicated to identifying the factors that influence lignocellulosic biomass (LB) enzymatic deconstruction to develop alternatives to fossil carbon resources. Accessibility of LB to enzymes is an important property involved in the recalcitrance in LB deconstruction related to the composition of the LB (hemicelluloses, lignin, hydroxycinnamic acids content...) and dependent on such structural factors as chemical structure and interactions of cell wall polymers, cell wall porosity... This study focused on the hot water pretreatment (HWP) of maize internodes (180°C applied during 20 or 40 min) that induces a reorganization of the 3D network.

The impact of HWP on cellulose supramolecular organization was investigated by ^{13}C CPMAS. An increase of cellulose crystallinity concomitant with an increase of agregate dimension was observed. Changes in the lignin structure, in relation to the decrease of β-O-4 bonds, were evidenced. To supplement these findings, polarization transfer kinetics was studied. The dynamical parameters $T_{1\rho}^{H}$ and T_{HH} were thus determined using a two proton-reservoir model [1]. HWP induced an increase of $T_{1\rho}^{H}$ and T_{HH} values associated with an increase of the molecular order and with the density of water molecules in the macromolecular assembly, respectively. In addition, the chemical characterization showed that HWP modified the cell wall composition with a loss of hemicelluloses and of ferulic acid cross-linking, associated with a lignin enrichment.

To characterize water dynamics, low-field T_2 measurement were carried out. An inverse Laplace transformation was applied to convert the relaxation signal into a continuous distribution of T_2. At the hydration level used in solid state experiments (~20% w/w), HWP induced a decrease of the T_2 peak width, indicating an homogenization of water environments. In the enzymatic hydrolysis humidity conditions (~80% w/w), a modulation of the structural domains due to HWP was observed, which can be associated with more water in pore sizes between 5 and 15 nm. This increase in meso-porosity should favour the accessibility of enzymes and their catalytic activity.

This study underlines that the loss and changes in polymers structural features induced by HWP leads to a reorganization of the cell walls. This results in a water redistribution improving the accessibility of cellulases and, consequently, the hydrolysis yield [2]. The results obtained by the combined use of solid-state and low-field NMR modalities, associated with those obtained by chemical analyzes, were synthesized in a schematic representation illustrating the effect of HWP on the macromolecular assembly structure.

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QUANTITATIVE METABOLOMICS METHODS DEVELOPMENT USING NMR

One of the ultimate goals of the field of metabolomics is a fully quantitative analysis of highly complex biological samples. Ideally, platforms that provide broad metabolome coverage allow the opportunity for deep insights into biological problems, while excellent quantitation ensures good data quality, opportunities for précising metabolic modeling, and allows an ability to compare across studies. However, these results can be difficult to achieve on a routine basis because the highly complex sample matrix often precludes reliable measurements of many metabolites and complicates quantitation efforts. NMR spectroscopy has much to offer the field of metabolomics due to its exquisite quantitative capabilities and reproducibility, especially in the area of new methods development.

Recently, we have investigated the use of new reference compounds for improved metabolite quantitation. The common NMR reference compounds that work well for simple solutions or mixtures produce inaccurate results when used to quantitate blood metabolites. Our approach takes advantage of a simple protein precipitation procedure that allows the absolute quantitation of over 80 metabolites using a single standard compound. These metabolites, including even some at sub-micromolar concentrations, span a broad range of classes and pathways, including organic and amino acids, as well as energy metabolites and co-enzymes. We show that this approach is more robust than current approaches. We have also shown that this quantitative NMR approach is also useful for calibrating quantitative mass spectrometry measurements, without the use of internal or external metabolite standards and has led to some unusual findings about the stability of well-known metabolites.

We are also very interested in understanding why metabolite levels can change from collection site to collection site. Some of these effects are likely caused by differences in sample treatment procedures and we are in the process of trying to model these with the goal of improving cross site validation studies. It may also be possible to develop methods to mitigate the effects of sample mistreatment using either experimental or modeling approaches. Recent progress in our work in this emerging area will be discussed.

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UNCOVERING METABOLIC FEATURES OF CELL PLASTICITY AND CARCINOGENESIS

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Nuclear Magnetic Resonance (NMR) is a powerful platform for molecular phenotyping of biological systems and its highly versatile nature opens up a range of analytical strategies for metabolomic phenotyping of biological systems. Here, we illustrate how NMR metabolic fingerprints of intra- and extra-cellular material provide a unique experimental platform for cellular biology. We show how solution and HR-MAS ^{1}H NMR investigations can provide complementary insights into the metabolism of pluripotent stem cells, and identify the early metabolic changes associated with cell differentiation.

In a second part, we present a metabolomics investigation of *TP53* mutations in primary human cell lines. *TP53* is the most mutated gene in cancer, with about 50% of cancers harboring a single missense mutation. Over the past 10 years, the p53 transcription factor that acts as a tumor suppressor has been identified as a key regulator of cell bioenergetics metabolism. Many mutations of p53 result in the stabilization of mutant forms of the protein exerting a wide range of gain-of-function effects but little in known on how these effects impact on cell metabolism. Here, we investigate a collection of non-transformed fibroblasts cell lines (non-tumor cells) from different carriers of germline *TP53* mutations, associated with Li-Fraumeni Syndrome (LFS), a dramatic familial predisposition to cancer. We then detail the impact of short hairpin RNA (shRNA)-mediated p53 knockdown in these cells and associated control fibroblasts expressing wild-type p53, to gain specific insight into gain-of-function features associated with p53 mutations.

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USING NMR SPECTROSCOPY TO INVESTIGATE THE LINK BETWEEN CIRCADIAN RHYTHMS AND METABOLISM

The circadian clock controls several biological processes and is in turn influenced by environmental cues and by metabolic signals. Studies have shown that disruption in circadian rhythms can cause growth retardation in plants and metabolic disorders in fruit flies. We used NMR-based metabolomics to profile the metabolites in *Drosophila melanogaster* that cycle with a daily rhythm. The plant circadian clock is intimately connected with its response to abiotic stress and we used NMR methods for metabolite fingerprinting of the leaves of *Bougainvillea spectabilis*. We used NMR fingerprinting to understand how the sunflower plant's circadian clock influences its solar tracking.

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NMR AEROSOLOMICS

Water-soluble organic compounds (WSOC) represent up to 80% of all organic compounds present in atmospheric aerosols [1]. Unlike composition of inorganic compounds or volatile organic compounds, which seems to be well explored, the knowledge about WSOC composition is still rather limited. The most frequently used method for WSOC analysis is GC-MS, which is a very sensitive technique [2]. However, the analysis of polar compounds via GC-MS requires derivatization and the quantification is extremely time consuming. The second widely used technique is ion chromatography (IC). Nowadays, IC is routinely used for analysis of specific groups of organic compounds such as carboxylic acids, amines or carbohydrates [3]. NMR spectroscopy was for the purpose of aerosol chemistry employed only recently [4]. So far, the use of NMR spectroscopy is mainly restricted to so-called Functional Group Analysis, of which main interest lies elsewhere than in identification of individual compounds.

NMR Aerosolomics offers a different approach to the analysis of complex aerosol mixtures inspired by compound profiling in metabolomics. For this purpose, an extensive library of compounds commonly present in aerosols was created. The method was evaluated on a series of 21 samples of PM2.5 and PM10 atmospheric aerosol collected in Prague suburban site in summer 2008 and winter 2009. In each sample, approximately 60 compounds were identified. Three compounds were identified for the first time in ambient aerosol and the presence of other four species – predicted by theory was confirmed. Altogether, the number of identified compounds climbed up to 79. The concentration levels found were subsequently used for statistical analysis. A clear discrimination between summer and winter samples was achieved while the separation according to particle size was indicated only in the group of summer samples. Univariate statistical analysis revealed substances responsible for the group separation and possible sources of these compounds were suggested [5].

Furthermore, the method was employed in WSOC analysis of size-resolved aerosol particulate matter obtained by 6-stage high-volume cascade impactor. Four series of samples collected in summer and winter were resolved into 6 fractions according to the particle size. In each sample, 31–45 compounds were identified, 73 compounds in total. The distribution profiles of individual compounds enabled identification of common sources or degradation pathways.

Contrary to other methods, NMR aerosolomics allows determination of several groups of WSOC simultaneously. The method offers a great potential for source apportionment, which is a resonating topic in contemporary aerosol chemistry.

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VARIABLE-TEMPERATURE NMR SPECTROSCOPY - A HANDY TOOL FOR METABOLITE IDENTIFICATION IN BIOLOGICAL MATERIALS

NMR spectroscopy is a beneficial method for identifying metabolites. Metabolic analysis is an essential part of the modern diagnosis of many disease entities [1]. However, ^{1}H-NMR spectra of metabolite mixtures (serum, plasma, urine ect.) usually used in medical screening are often very crowded and many peaks cannot be easily assigned to specific compounds. We can solve this problem by measuring 2D spectra but it is time-consuming [2], because of low concentrations and sampling requirements. In this work we exploit the fact, that chemical shift of metabolites change with temperature and that the rates of change ("temperature coefficients") are characteristic for compounds and consistent between the samples. To determine them efficiently, we use variable-temperature (VT) NMR. When combined with Radon transform [3], VT-NMR allows measuring full VT series in a time comparable with a single ^{1}H measurement. We show that certain metabolites (like alanine, lactate, L-valine, acetate, acetone, creatine, choline) have very specific and reproducible coefficients which can be used for their identification. We place hope in the fact, that VT-NMR can become an efficient tool for identifying chemical substances in biological materials and beyond.

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IN SITU ENANTIOSPECIFIC DETECTION OF MULTIPLE METABOLITES IN MIXTURES USING NMR SPECTROSCOPY

Chirality plays a fundamental role in nature, but its detection and quantification still face many limitations. To date, the enantiospecific analysis of mixtures necessarily requires prior separation of the individual components. The simultaneous enantiospecific detection of multiple chiral molecules in a mixture represents a major challenge, which would lead to a significantly better understanding of the underlying biological processes; e.g. via enantiospecifically analyzing metabolites in their native environment. Here, we report on the first in situ enantiospecific detection of a thirty-nine-component mixture. As a proof of concept, eighteen essential amino acids (AAs) at physiological concentrations were simultaneously enantiospecifically detected using NMR spectroscopy and a chiral solvating agent. This work represents a first step towards the simultaneous multicomponent enantiospecific analysis of complex mixtures, a capability that will have substantial impact on metabolism studies, metabolic phenotyping, chemical reaction monitoring, and many other fields where complex mixtures containing chiral molecules require efficient characterization [1].

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SPIN DIFFUSION AFTER INVERSION RECOVERY (SDAIR) FOR DETERMINATION OF 10 – 300 NM DOMAIN SIZES IN ORGANIC SOLIDS

^{1}H spin diffusion after inversion recovery (SDAIR) is a simple solid-state NMR experiment that can provide accurate estimates of domain sizes in organic solids in the range of 10 – 300 nm, in favorable cases. SDAIR can be described as a one-dimensional ^{1}H spin exchange experiment with ^{13}C detection; selective excitation of protons in component A is achieved by inversion recovery to the zero-crossing of component B. ^{1}H spin diffusion between the components during a mixing time of up to ~1500 ms results in distinctive exchange peaks of the initially nulled component B and an accelerated decrease of the retained signal A. While the intrinsic relaxation times need to differ by >30% for sufficient signal to be generated by SDAIR, the domain sizes can be obtained from peak intensities without knowledge of the intrinsic relaxation times, using versatile graphical analyses. The exchange peaks are largest for layer thicknesses of 40 – 80 nm, but can be detected for domains of up to 300 nm if the intrinsic relaxation times are sufficiently different. The experiment is demonstrated on PS-*b*-PMMA block copolymers.

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SOLID-STATE NMR VIEW OF LOCAL STRUCTURE IN ZEOLITE BETA DESIGNED FOR FUTURE APPLICATIONS

Zeolite Beta is one of the most important zeolites in the industry. It is widely used for the reduction of VOC, N_2O and NO_x, industrial gas purification, and automotive emission control. Its three-dimensional network of 12-ring pores makes it an ideal candidate for adsorption-based applications and also in catalysis for the production of various chemicals, especially if shape-selectivity is desired. To understand the favorable properties of zeolites such as adsorption affinity, total uptake, selectivity, catalytic conversion, an insight into the local structure of the framework is needed, and not many experimental techniques are capable to offer it. In this regard, solid-state nuclear magnetic resonance is one of the most powerful techniques.

We present zeolite Beta in its all-silica form, which shows outstanding capacity and affinity for the uptake of perfluorinated compounds (PFCs) such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid, even in the presence of other organic compounds. Relying mainly on ^{1}H and ^{19}F MAS NMR and ^{19}F–^{29}Si heteronuclear and ^{1}H–^{1}H DQ-SQ homonuclear correlation experiments we elucidated the way in which the PFOA molecules were adsorbed into the zeolite [1]. We showed that PFOA molecules were positioned in the straight a- and b-channels of the zeolite, with the hydrophobic chains in the channels and the carboxylic heads, forming hydrogen-bonded pairs, in the intersections. This resulted in a very favorable adsorption enthalpy. Additionally, the helical conformation of the perfluorinated chains made a very tight packing at maximal capacity sterically possible. This explained the exceptionally high affinity and selectivity of all-silica zeolite Beta for PFOA and similar perfluorinated compounds, which positions this zeolite as the lead candidate for combating the PFCs pollution. For utilization of zeolite Beta in catalysis, the number of Brønsted acid sites and their strength play an important role. A higher Si/Al ratio represents fewer but stronger acid sites and makes pores more hydrophobic. We showed that a lower Si/Al ratio resulted in a higher conversion of citric acid to tricarballylic acid, evidencing that in this reaction the number of acid sites is more important than the strength of these sites. The Si/Al ratio before/after the reaction was determined from ^{29}Si and ^{27}Al MAS NMR spectra [2]. We noticed a decent increase in the ratio due to dealumination by citric acid. After recycling the zeolite through realumination, a significant fraction of the initial activity of the catalytic system was regenerated. On the contrary, a high Si/Al ratio was preferential for the shape-selective conversion of simple aromatics to biarylic compounds using zeolite Beta loaded with ^{13}C-enriched $Pd(OAc)_2$. ^{13}C MAS NMR proved that one acetate out of the two react to form acetic acid, moving freely in the pores, while the second acetate remains immobilized at a Pd centre which is strongly bound to the framework aluminum [3].

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MOLECULAR MOTORS STUDIED BY SOLID-STATE NMR SPECTROSCOPY

Artificial molecular machines promise applications in many fields, including physics, information technologies, chemistry as well as medicine. The deposition of functional molecules in 2D or 3D assemblies in order to control their collective behavior and the structural characterization of these assemblies are challenging tasks. We exploit porous materials to form rigid matrix for mechanochemical preparation of bulk or surface host–guest inclusions with functional molecules, such as molecular rotors, molecular motors and molecular switches.

Unambiguous determination of the molecular structure and monitoring of the molecular function such as rotation of a molecular rotor or on/off switching of a molecular switch cannot be studied by X-ray analysis because the systems are typically heavily disordered fine powders. We use solid-state nuclear magnetic resonance (SS-NMR) spectroscopy to obtain atomic-level insights into the structure and dynamics of these functional materials. SS-NMR spectra provide valuable information about structure, interactions and dynamics in solids not available otherwise.

It will be demonstrated that SS-NMR experiments provide unequivocal evidence of the formation of the 2D and 3D assemblies and can also be used for the observation of such a molecular function as the photoisomerization of a molecular switch deposited on a surface. We have also developed a solid-state NMR method for investigation of two dimensional arrays of light-driven molecular motors [1-4].

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BAYESIAN PROBABILISTIC ASSIGNMENT OF ORGANIC SOLIDS

The starting point for any detailed NMR study is the assignment of the experimental NMR spectra. In organic solids at natural isotopic abundance, this is still a laborious and often challenging process. In solution, when required, probabilistic assignments can be obtained without prior knowledge of the three-dimensional structure through a statistical analysis of large experimental chemical shift databases [1]. However, no such database exists for organic solids.

Here, we obtain the chemical shifts of over 200 000 organic crystal structures obtained from the Cambridge structural database [2] using ShiftML, a machine learning model able to predict chemical shifts in molecular solids [3], 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 predicted chemical shifts, we propose a Bayesian probabilistic framework to determine possible assignments of experimental NMR spectra of organic crystals, along with their confidence, from the two-dimensional representation of these compounds only.

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SOLID STATE NMR PROBING POLYMER CHAIN MOBILITY AT DIFFUSED INTERPHASES FORMED IN MULTI-MICRO-/NANOLAYERED PVDF-PMMA FILMS

For multi-layered polymers, polymer–polymer interfaces play a crucial role in the final properties of these multiphase materials. Among all the techniques for producing multi-layered films, the coextrusion process is one of the most appealing and popular techniques for industrial scale processing. This process is widely used to form multi-layered sheets or films that are suitable for various products ranging from food packaging materials to dielectric capacitors. Using layer multipliers or layer-multiplying elements, products with thousands of layers can be produced, in which the layer thickness can be reduced to the nanometre scale (Figure 1a). Interfacial spatial confinement always dominates when layer thickness is decreased, which greatly alters the microstructure and dynamics of the multilayer polymers. Macroscopic proper ties, including mechanical, electric, and gas/liquid barrier properties, are also drastically altered. In addition, for the nanolayer coextrusion process, the laminar flow conditions combine polymers in the layer multipliers by producing a large number of interlayer interfaces without completed mixing. Interfacial behaviours involving interlayer diffusion and reactions are also critical in defining the structure and properties. The measurement of the interfacial properties is important for understanding the interdependence of processing, structure, and properties.

Solid-state nuclear magnetic resonance (NMR) is one of the most powerful techniques for elucidating details of segmental dynamics in solid materials. While fast dynamics can be characterized by techniques like line shape analyses or relaxation measurements [1], some specific dynamic information can only be obtained in exchange NMR experiments, where relatively slow segmental reorientations are observed in terms of changes of orientation-dependent NMR frequencies. The centerband-only detection of exchange (CODEX) NMR technique introduce by Schmidt-Rohr group is one of these exchange NMR technique, which is possible to observe and characterize very slow segmental reorientations with the highest available NMR sensitivity and site resolution, in sideband free magic-angle spinning (MAS) spectra. From short series of one-dimensional MAS spectra, the correlation function and correlation time can be determined. In this study, a series of multi-micro/nanolayered PVDF/PMMA films were analyzed by CODEX at 4 different temperatures. It is found that The α_a-relaxation of PVDF accelerated upon decreasing the layer thickness, while the α_c relaxation does not shift significantly. These results are further discussed in relation to DRS analyses and MD simulation [2]. The effects of the amount and orientation of crystalline phase on this suppression are also discussed via SAXS/WAXS and DSC analyses of these samples.

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AN NMR VIEW OF DYNAMIC MICROTUBULE-MAP INTERACTIONS

Microtubules (MT) play an essential role in cell migration, mitosis and polarization. Many of these functions critically rely on Microtubule-associated proteins (MAPs) and their association with microtubules. So far, little is known about the interaction of MAPs and their intrinsically disordered regions with the dynamic microtubule surface. In particular, the role of the unstructured C-terminal tails of tubulin, critical for many MAPs interactions, has remained elusive. Considering the importance of protein dynamics for MT function, and especially the role of the highly flexible tubulin tails in cellular processes and human disorders such as ciliopathies, cancer and neurodegeneration [1], we have employed nuclear magnetic resonance (NMR) to obtain structural and dynamical information about these interactions at atomic level [2,3,7].

Using a combination of solution- and solid-state NMR spectroscopy, we have examined the interaction of MT with different MAPs. In our studies, we made use of MT protofilaments, Tubulin-dimers and peptides including those representing the tubulin C-terminal tails. Specific experiments were designed to help probe interaction between the MAPs and the tubulin C-terminal tails. In our studies, we focused on three different members of the MAP family. [1] The CKK domain of the calmodulin-regulated spectrin-associated protein (CAMSAP), involved in the minus-end recognition of the MT [3,4]. MAP7 which is essential in regulating kinesin-based intracellular transport and competes with the MAP tau for MT-binding [3,6]. Lastly, we examined the binding mechanism of the Alzheimer's disease-related MAP Tau to MT [5].

In our contribution, we report on progress on combining our NMR results with information obtained by biochemical and biophysical methods to gain deeper insight into the binding behavior of these MAPs to MT.

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8-OXOGUANINES ARE ACCOMMODATED IN A G-QUADRUPLEX QUARTET

Reactive oxygen species (ROS) are a by-product of cellular metabolism in all living organisms. Cells posses (non)-enzymatic antioxidant mechanisms, which are able to maintain normal levels of endogenous ROS. Since guanine has the lowest redox potential of the four nucleobases, it is highly susceptible to oxidation damage. One such oxidation product is 8-oxoguanine [1]. Guanine rich regions are present in the promoter and telomere regions of genomes and are able to fold into noncanonical four-stranded structures called G-quadruplexes It is presumed that G-quadruplex forming sequences act as oxidation sinks in the genome. 8-Oxoguanine formation can lead to nucleic acid structural rearrangements and can have an effect on cellular mechanisms [2,3]. Insights into the effect of 8-oxoguanine incorporation on G-quadruplex structure will help expand the knowledge of ROS damage on nucleic acids and the structural changes that are a consequence of oxidative stress.

We have chosen a short model deoxyoligonucleotide TG_4T that is able to fold into a tetrameric G-quadruplex in solution. We have residue-specifically incorporated 8-oxo-deoxyguanosine into the model sequence. Using NMR spectroscopy, we determined the ability of the synthetized oligonucleotides to fold into G-quadruplexes and proved all four investigated oligonucleotides formed G-quadruplexes. We investigated the thermal stability of the G-quadruplexes with NMR melting experiments. Using two-dimensional through-bond and through-space NMR methods, we have confirmed the parallel topology of all formed G-quadruplexes. The accommodation of 8-oxoguanine moieties into a quartet was explored using simulated annealling molecular modelling. We confirmed a possibility of hydrogen bond formation between the amino group hydrogen and oxygen on position 8 between neighbouring 8-oxoguanine residues in a quartet.

With the attained data, we conclude that four 8-oxoguanine moieties are accommodated in an all-8-oxoguanine quartet, noticeably impacting the thermal stability of G-quadruplexes only when positioned inside the quadruplex core. The knowledge attained will guide further studies on effect of 8-oxoguanine formation on long guanine-rich sequences.

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CHARACTERIZATION OF THE LIQUID-LIQUID PHASE SEPARATION OF THE PROGESTERONE RECEPTOR ACTIVATION DOMAIN USING ITS SEQUENCE-SPECIFIC RESONANCE ASSIGNMENT

The uman progesterone receptor (PR) is a ligand-activated transcription factor member of the steroid receptor superfamily. The isoform B counts 933 residues and it is composed of a proline-rich intrinsically disordered (ID) activation domain (AD), followed by globular DNA- and ligand-binding domains. PR AD consists of 566 residues, and it is reported to play a crucial role for protein function [1]. It has recently been shown that transcription can be regulated by the liquid-liquid phase separation (LLPS) of the transcription machinery, stabilized by interactions between the ID regions of transcription factors [2].

In this work we have characterized the LLPS properties of PR AD by correlating the compaction degree of the monomer with the propensity to undergo LLPS. In order to get residue level information to identify the regions of sequence driving this process, we used NMR and carried out the sequence-specific resonance assignment of the disordered domain through the application of a mixed set of ^{1}H-detected and ^{13}C-detected NMR experiments [3]. With this approach, the N-terminal domain has been almost completely assigned, including the proline residues that represent the 15% of the sequence. With this information, we then assessed the chemical shift perturbations and intensity changes that different parameters that influence LLPS have on the PR AD spectra.

In conclusion, we have found out that PR AD undergoes LLPS in vitro and that the formed liquid droplets are stabilized by a mixture of electrostatic and hydrophobic interactions.

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ANALYSIS OF *NRAS* RNA G-QUADRUPLEXES

RNA guanine-rich sequences are able to form G-quartets through hydrogen bond interactions in the presence of monovalent cations such as potassium and sodium ion. G-quartets stack onto each other and assemble G-quadruplexes, which have broad diversity regarding loop lengths and arrangements, but they commonly adopt the parallel conformation in which all four strands are aligned in the same direction. RNA G-quadruplexes are dynamic, transient in cells, and are included in diverse biological processes, such as translation, regulation of alternative splicing, and the subcellular transport of mRNAs. They are used as therapeutic targets and agents.

RNA G-quadruplex forming sequence in the 5' untranslated region (UTR) of *neuroblastoma RAS viral oncogene homologue* (*NRAS*) proto-oncogene was studied here. *NRAS* proto-oncogene encodes for a protein called N-Ras, which is involved in regulating cell division through signal transduction. We will present our results on RNA G-quadruplex structures in the 5'-UTR of the *NRAS* mRNA utilizing NMR, CD, and UV spectroscopies. We also synthesized modified RNA G-quadruplexes with different G-tract and loop lengths in order to stabilize the most possible structures.

Our data show that modified sequences form one structure. *NRAS* RNA G-quadruplexes have parallel connectivity with propeller loops. Structure with G5 in the loop is the most probable. Results will help to develop new tools to use *NRAS* RNA G-quadruplexes as therapeutic targets.

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CAN MATURE LET-7 MICRORNAS FORM NONCANONICAL SECONDARY STRUCTURES?

The human genome contains thousands of non-coding (nc)RNAs that are transcribed from DNA but are not translated into proteins [1]. It has been shown that ncRNAs are involved in many normal cellular processes as well as disease states [1]. Herein, we focused on microRNAs (miRNAs), a group of ncRNAs, which play important roles in the regulation of gene expression through targeting messenger (m)RNAs for post-transcriptional gene silencing [2]. Specifically, we are studying letal-7 miRNA (let-7) family which has two major biological functions in cells, 1) as essential regulators of differentiation and 2) as fundamental tumor suppressors [3]. In humans, the let-7 family is composed of nine isoforms of mature RNAs (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, and miRNA-98). It was shown before that stable secondary structures, such as G-quadruplexes, in mature miRNAs can affect base pairing with mRNA and thus alter gene regulation [4]. Our NMR data revealed that all studied members of the let-7 family, except let-7a and miRNA-98, form stable secondary structures. Interestingly, three of them, let-7b, let-7c, and let-7e, adopt G-quadruplexes as indicated by characteristic imino signals in the region between 10.5 and 11.9 ppm observed in 1D ^{1}H NMR spectra. This observation was unexpected because although these RNAs are guanine (G) rich, they do not conform to a classical G-quadruplex motif composed of four runs of three(two) Gs. The secondary structures of the mature let-7 family were examined by CD spectroscopy which revealed that let-7 G-quadruplex structures adopt parallel topologies. The band migration on native polyacrylamide gels suggested the potential formation of multimeric G-quadruplexes, which was further explored by UV melting experiments. Interestingly, our preliminary NMR data demonstrate how mature let-7 miRNAs structures influence the base pairing with target mRNA.

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FORMATION PROPENSITY OF PSEUDOCIRCULAR DNA G-HAIRPINS

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As we have recently shown, *Saccharomyces cerevisiae* telomeric DNA can fold into an unprecedented pseudocircular G-hairpin (PGH) structure [1]. However, PGH formation in the context of extended sequences (a prerequisite for their function *in vivo* and their applications in biotechnology) has not been elucidated.

Herein, we show that the structure's circular nature tolerates single-stranded (ss) protrusions. The high-resolution NMR structure of prolonged sequence revealed a new member of the PGH family and showed atomistic details on a junction between ssDNA and structured PGH part. We also identified new sequences capable of folding into one of the two forms of PGH and defined minimal sequence requirements for their formation. The time-resolved NMR data revealed a possibility that PGHs fold via a complex kinetic partitioning mechanism. These data not only explain its cation-type-dependent formation but also explain the unusual hysteresis between PGH melting and annealing described in our previous study. Our findings have important implications for DNA biology and nanotechnology. Overrepresentation of sequences able to form PGHs in the evolutionary-conserved regions of the human genome implies their potential functionally important biological role(s) [2].

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NMR DIRECTED MUTATIONS MODULATE COILED-COIL INTERACTIONS OF CALCIUM CHANNEL PROTEINS

We investigate STIM1 the calcium sensor protein involved in the activation of the calcium released activated calcium (CRAC) channel. STIM1 spans from the endoplasmic reticulum into the cytosol [1]. It undergoes homo-oligomerization and spatial elongation once the Ca^{2+} store is depleted, resulting in what is known as "store operated" activation of the Orai Ca^{2+} channel. One long (CC1) and two short (CC2, CC3) coiled-coil segments are found in the cytosolic part of STIM1, and they are involved in various intra- and intermolecular interactions transitioning between the resting and active states [2].

In its monomeric form, the isolated STIM1-CC1 domain in isotropic solution was shown to form a three-helix bundle and stabilized mainly by two pairs of interhelical coiled-coil epitopes. In the Stormorken disease-related STIM1 R304W mutant these contacts are weakened, according to our new NMR-based solution structure model. Two interhelical sites inside the domain between CC1α1 and CC1α2 subdomain helices have been identified as critical for modulating channel activation [3]. Point mutations within interhelical interaction contact locations derived from NMR structure and dynamics data can restore the physiological, store-dependent activation behavior of injurious mutant in living cells. The NMR-directed design of mutations within coiled-coil domains where interhelical interactions α1-α2 of CC1 compete with CC1-CC3 molecular "clamp" to control the function of STIM1 and recover functionality lost through other pathogenic mutations is demonstrated.

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ORDER IN DISORDER: AUX/IAA PROTEIN AND ITS TIR1-AUX/IAA AUXIN CO-RECEPTOR SYSTEM

Auxin is a central signaling molecule in plant biology with roles in both the patterning of developmental events and the regulation of cellular growth. This is achieved via the TIR1/AFB-auxin-Aux/IAA co-receptor complex. Within this ternary complex, auxin acts as a molecular glue to promote binding of Aux/IAA transcriptional repressor proteins to $SCF^{TIR1/AFB}$ ubiquitin-ligase complexes, thereby catalyzing their ubiquitin-mediated proteolysis. A conspicuous feature of the crystal structure of the complex is a rare *cis* W-P bond within the binding site. This binding site is centered on a 13 amino acid motif called the degron. We have used NMR to determine the solution structure of the amino-terminal half of the Aux/IAA-protein AXR3/IAA17 and its binding in complex with TIR1 and auxin. We show that this is intrinsically disordered and yet the critical degron W-P bond occurs with an unusually high (1:1) ratio of *cis* to *trans* isomers. While the WPP sequence is one of the most strongly *cis* proline promoting elements. In peptides containing this motif, the population of the *cis* conformer is at most 36%. This demonstrates that there must be at least a transient structural element promoting the population of the *cis* conformer beyond this. Analysis of RDC's confirms a deviation of random coil structure both in the degron motif and near the N-terminus, where a transient helix is formed that provides a key interface for the recruitment of the co-repressor TOPLESS. We then show that assembly of the co-receptor complex involves both auxin-dependent and -independent interaction events. Further, using the synthetic auxin molecule cvxIAA we show that a subset of auxin-dependent binding events occur away from the base of the canonical auxin binding pocket in TIR1. Our results suggest the existence of an encounter complex before the formation of the fully docked ternary complex and the involvement of the region C-terminal to the degron in auxin independent interactions with TIR1.

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LEVERAGING DEEP LEARNING FOR AUTOMATED PROTEIN STRUCTURE DETERMINATION WITH NMR SPECTROSCOPY

Nuclear magnetic resonance spectroscopy (NMR) is one of the leading techniques for protein structure determination. Nonetheless, the full potential of NMR spectroscopy remains unfulfilled due to the tedious data analysis process. Nowadays, it takes weeks or months of manual work to deliver a structural model of the protein out of measured NMR spectra.

In this project, we have addressed the problem of fully automated protein structure determination with deep learning. Our method takes as input only the protein sequence and NMR spectra, producing as output: (a) peak lists for each spectrum, (b) a chemical shift list, (c) upper distance limit restraints, and (d) a protein structure in PDB format. The structure determination process does not require any human intervention and takes about 5 hours, making it possible to obtain a high-quality protein structure shortly after completing the NMR measurements.

A key factor for the successful delivery of deep learning solutions is the availability of large-scale training datasets. We have collected 1 314 NMR spectra (2D, 3D, and 4D experiments), allowing to reproduce 100 protein structures of 35–175 residues, which have been solved manually in the past. In addition, we have prepared a benchmark dataset, composed of 3 925 370 peak examples, including 650 675 manually labeled signals (true peak vs. artifact) and 13 831 signal deconvolution examples.

The above dataset has been used to train a deep residual neural network with 26 layers and over 3.5 million parameters, which performs automated peak picking and peak deconvolution. Signals identified by the model are passed to automated assignment with FLYA [1], yielding an initial chemical shift list. Subsequently, we use another module of our workflow, namely a deep graph neural network (GNN). This model has been trained on shift lists extracted from 2840 BMRB records. Its role is to capture short- and long-range patterns that are present in protein chemical shifts. The GNN model brings domain knowledge from previously solved proteins to our workflow, allowing to refine the outputs of FLYA and automated peak picking. The final step in our approach is automated NOESY assignment and structure calculation with CYANA [2].

Using our approach, we have managed to automatically solve 100 protein structures with a median backbone RMSD of 1.27 Å to the PDB reference structures. Moreover, the method correctly assigned 96.3% backbone and 85.5% side-chain chemical shifts (median accuracy), compared to BMRB depositions. Our method will be publicly available this year as a website, which allows for spectra upload and rapid protein structure determination.

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NMR STUDIES ON METAL-CHELATED POLYMER NANODISCS USED IN COMBINATION WITH G-QUADRUPLEXES

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There is considerable interest in the NMR community to speed up data acquisition in NMR structural studies on biomolecules. One way to achieve this is to significantly shorten the recycle delay in NMR experiments by speeding up the spin lattice relaxation time (T_1) by applying paramagnetic relaxation enhancement (PRE). We have focused on a modified poly-(styrene-co-maleic acid) polymer which forms nanodiscs with lipids and possesses the ability to chelate Cu^{2+} ions [1]. Using inverse recovery experiments, we were able to show that the T_1 rates of protons for both polymer and lipid-nanodisc components are significantly reduced, in some cases even 7-fold, when the nanodiscs are Cu^{2+}-chelated. This approach could potentially be very useful when structurally characterizing lipid soluble molecules such as transmembrane proteins with NMR spectroscopy. Additionally, we show that Cu^{2+}-chelated nanodiscs are also capable of decreasing the proton T_1 values for a water-soluble biomolecule, the human telomere (wtTel23) DNA G-quadruplex. When comparing G-quadruplexes to double stranded DNA we see that their cores are comprised out of stacked G-quartets, arrangements of four guanine residues and that they form four grooves instead of two. The guanines in G-quartets are connected by differently structured loop regions that further diversify G-quadruplex topologies. The T_1 data that we gathered for the wtTel23 G-quadruplex suggest that wtTel23 interacts with the nanodiscs by its groove or loop regions and not through stacking on the nanodiscs by the top or bottom G-quartet. Such a model of interactions is also supported by saturation transfer difference (STD) NMR experiments. We show that to utilize the PRE effect we had to remove excess KCl salt from the G-quadruplex and nanodisc solution. We believe that the KCl salt reduces the interactions between the G-quadruplex and the nanodisc due to the salt charge screening between the DNA and the lipid heads.

We believe that the described system could be especially valuable for NMR structural studies of large size RNA that exhibit very long T_1 values for protons and could enable multidimensional NMR experimental studies on membrane-associated peptides and proteins that may not be available in large quantity and/or are sensitive to heat for long data acquisition [2]. This study also creates potential avenues to use the paramagnetic nature of the chelated polymer nanodiscs for dynamic nuclear polarization (DNP) solid-state NMR experiments to overcome the sensitivity issues in studying membrane proteins [3].

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UNIQUE STRUCTURAL FEATURES OF PYRENE-MODIFIED G-QUADRUPLEX REVEALED BY NMR

G-quadruplexes are noncanonical secondary structures formed by guanine-rich nucleic acid strands that play roles in various cellular functions. As independent aptamer molecules, G-quadruplexes are able to bind specific target proteins and therefore represent potential drug candidates for the treatments of numerous diseases, most notably cancer. Small polyaromatic compounds can interact with solvent accessible DNA heterocyclic bases through aromatic stacking, which leads to the stabilization of G-quadruplexes and subsequently to the regulation of their functions. Alternatively, various chemical moieties and nucleotide analogs can be incorporated into G-quadruplex structure to fine-tune desirable properties of aptamers including structural stability, resistance against nuclease degradation, and binding of target proteins.

Thrombin-binding aptamer (TBA) is a 15-mer 5'-d[GGTTGGTGTGGTTGG]-3' DNA oligonucleotide, that folds into a chair-type G-quadruplex capable of binding and inhibiting thrombin protease in the presence of sodium and potassium cations [1,2]. In our study, TBA G-quadruplex served as a well-defined starting model which we modified by substitutions of individual thymines with fluorescent U^{py} (5-(pyrene-1-yl-ethynyl)-dUMP) nucleotides [3]. Our results show that in the presence of potassium ions the individual replacements of T4, T9, and T13 with U^{py} nucleotides stabilize G-quadruplex and greatly increase its resistance against nuclease degradation while retaining the TBA fold. In the case of T9 substitution, a dynamic equilibrium between the unimolecular and bimolecular G-quadruplex structure was observed, the latter being comprised of two distinct G-quadruplex units. We believe that our approach could potentially be used for the optimization of various therapeutically important G-quadruplex-based aptamers.

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NMR INSIGHT INTO G-QUADRUPLEX FORMATION IN THE REGULATORY REGION OF OSTEOPOROSIS-RELATED *RANKL* GENE

Pathogenic conditions that affect the skeleton, such as osteoporosis, disrupt balanced bone remodeling by accelerating the differentiation and maturation of osteoclasts. The pivotal regulator of osteoclast activity is the receptor activator of NF-κB (RANK) expressed by osteoclasts that interacts with RANK ligand (RANKL) formed by osteoblasts. The interaction initiates a cascade of intracellular signaling events and promotes bone resorption that in the case of excessive activity leads to osteoporosis [1]. One of the potential ways of gene regulation on transcriptional level is facilitation or inhibition of transcription by uncanonical secondary structures formation in the G-rich fragments of promoter regions [2,3]. A G-rich region in the *RANKL* gene proximal promoter sequence with the potential to adopt G-quadruplex structures was identified using bioinformatics.

A 20-nt long sequence RANwt, d(GGGGAGGGAGCGGGAGAGGG), folded into diverse structures, presumably due to four consecutive guanines in the first G-tract. Individual substitutions of G1 and G4 with thymine led to single, although completely different G-quadruplex conformations RAN1 and RAN4, respectively. High-resolution NMR structure showed that RAN1 folds into a parallel G-quadruplex with three G-quartets connected by 3 propeller loops, and a bulge. Residues from the G-A bulge adopt a *pseudo-loop* conformation, which is especially important for providing the optimized stacking between G-quartets [4]. On the other hand, RAN4 forms a two-quartet structure stabilized by two base triads. G/A-to-T substitutions of the residues from base triads uncovered the critical role of A5 for the formation of a distinct two-quartet topology; A5-to-T5 substitution switched the two-quartet structure into three-quartet (3+1) hybrid G-quadruplex [5].

To test whether the presence of a G-rich sequence able to form G-quadruplexes can affect the gene expression levels, a fragment of the human *RANKL* proximal promoter region was cloned into the luciferase report vector (collaboration with Faculty of Pharmacy, University of Ljubljana, Slovenia). Different mutations in the G-rich region were analyzed, including RAN1, RAN4, and deletion of the entire G-rich sequence. The latter resulted in increased expression levels in HeLa and A549 cell lines, suggesting that the presence of the G-rich sequence causes repression of *RANKL* promoter activity. Our results, to the best of our knowledge, are the first to suggest possible regulation mechanisms of the RANK/RANKL pathway by the formation of noncanonical DNA structures in the regulatory region of the *RANKL* gene.

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STUDY OF STRUCTURAL MOTIFS ADOPTED BY RNA SEQUENCES WITH ADENINE, GUANINE, AND CYTOSINE REPEATS

Recently, a new DNA tetrahelical structure was discovered in our department, formed by sequences with AGCGA repeats, which consists of mixed GAGA and GCGC quartets [1]. This type of motifs has never been described for RNA, therefore the study of structures adopted by a family of RNA oligonucleotide sequences containing mixed adenine, guanine, and cytosine repeats is important due to the fact that RNA secondary structures are often involved in various regulatory processes including protein expression. Also, such sequences are relatively common in vertebrate (including human) transcriptomes which indicates their biological relevance.

We initiated a study on RNA oligonucleotides with tandem AGCGA repeats. RNA oligonucleotides demonstrated drastically different behavior in comparison to DNA counterparts. They showed a preference for G-quadruplex formation rather than AGCGA-quadruplex. Not too surprisingly, as the 2′-OH hydroxyl groups in the RNA quadruplex play a significant role in redefining hydration structure in the grooves and the hydrogen-bonding networks. This structural change for example increases the stability of RNA telomeric quadruplexes over DNA ones [2].

In the following steps, we studied sequences without G-tracts designed in such a way that would enable the formation of tetrahelical secondary structures involving mixed quartets of adenine, guanine, and cytosine. Initially different types of sequences were tested, however, our efforts were mostly focused on sequences with the propensity of folding into parallel type tetrahelical structures.

The dynamic nature of studied oligonucleotides manifested as an equilibrium between multiple species including hairpin structures made the investigation challenging. Nevertheless, a few intriguing trends were found. Namely, some of the designed sequences lead to the formation of different structures depending on solution conditions. We also observed folding topologies which are characterized by stabilization of non-canonical base pairs in addition to Watson-Crick pairs, which is unlikely for the hairpin. NMR signals of studied RNA oligonucleotides allowed us to propose a partially symmetrical tetrahelical structure.

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TEMPERATURE, CATION, AND STRAND CONCENTRATION CONTROLLABLE DNA SWITCH

Nucleic acids are carriers of molecular information in biology. But they are more than that - as biopolymers, they have exciting physicochemical properties, which can be rationally influenced by the base sequence. Additionally, some non-double-stranded structural variations adopted by oligonucleotides with various repetitive sequences expand the potential of nucleic acids to be used for building blocks for nanotechnology, to self-assemble into molecular nanostructures, and to be used as a material for machine-like nanodevices. To be assembled and to change in a controllable manner, nucleic acids were designed to respond with structural change to external stimuli, such as photons, temperature, pressure, magnetic or electric fields, or altered chemical environments. Nucleic acid systems that respond to several stimuli in a different way enable the design of multifunctional devices [1].

We describe a short, 17 nucleotides long DNA switch that is capable of adopting six different structures in response to temperature, cation, and DNA strand concentration. NMR analysis identified the type of structures present at a certain combination of external stimuli and conditions at which they interconvert. Change in distance between oligonucleotide ends for each of the structures is the basis for the design of a reporter signal and the development of multifunctional nanodevices.

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METHYLATION OF CYTOSINE IN THE PROMOTER REGION OF BCL2 DOES NOT IMPACT STRUCTURE

Methylation of cytosine in the CpG dinucleotide step by the enzyme DNA methyltransferase (DNMT) occurs at the C5 position of the cytosine to form 5-methylcytosine (mC). The methyl group on cytosine alters both stereoelectronic and hydrogen bond properties. However, mC can still base pair with a guanine residue on the complementary strand, leaving the DNA coding capacity unaltered. But the introduction of epigenetic modification into the gene promoter region could affect the DNA local structure and its stability [1].

We studied the impact of mC on the G-quadruplex structure using a model sequence (5'-GGGCGCGGGAGGAATTGGGCGGG-3') from the Bcl2Mid promoter region. Bcl2Mid oligonucleotide has been shown to form an intramolecular (3+1) G-quadruplex structure in the presence of K^+ ions [2]. Special attention was paid to the first loop region of Bcl2Mid (C4-G5-C6) which defines and stabilizes the Bcl2Mid structure due to its specific way of interaction with the core G-quartets.

We introduced mC at positions 4, 6, and 20 of the parent Bcl2Mid oligonucleotide. In the respective 1D ^1H NMR spectra we observed twelve well-resolved imino proton resonances located between 10.5 and 12.0 ppm that clearly indicate the formation of a G-quadruplex structure with three G-quartets.

C4 adopts an unusual orientation in the parent Bcl2Mid structure. It isn't stacked over the G3•G23•G19•G7 quartet but is positioned into the groove that is close to the H1'/H4' side of the G3 sugar. Since the NOEs that position mC4 in the groove are weaker compared to the parent Bcl2Mid, we consider the positioning of mC4 inside the groove as less favorable.

In the case of mC6 substituted oligonucleotide, we observed small differences in distances between the mC6 and adjacent guanines in the upper G-quartet.

No detectable differences compared to the parent Bcl2Mid were observed in the NOESY spectrum in the case of mC at position C20.

Thermal stabilities of the parent G-quadruplex and mC analogs were comparable (±1°C).

CD spectra of Bcl2Mid G-quadruplex and analogs with mC in the presence of K^+ ions were very similar, with a negative peak near 240 nm and two positive peaks at 270 and 295 nm, which is in agreement with the (3+1) hybrid G-quadruplex structure.

The structural changes caused by the presence of mC at positions 4, 6, and 20 are small and limited to local perturbations. Based on 2D NOESY structural data we can infer that the structural impact of methylation of cytosine in the CpG dinucleotide steps of Bcl2Mid oligonucleotide is minimal and causes no significant structure rearrangements.

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MAPPING MOLECULAR INTERACTIONS THAT DRIVE G-QUADRUPLEX CONFORMATION AND STABILITY

G-Quadruplexes (G4s) are non-canonical secondary structures of nucleic acid formed by guanine rich sequences. G4s are the four stranded DNA structures comprising four guanines in one plane known as G-quartet, for three planes G4s each G strand contains at least three G-residue separated by loops. G-quartet which is formed by the interaction of guanine from each strand is stabilized by Hoogsteen hydrogen bonds, stacking of quartet and central counter ion typically monovalent cations. Interestingly, G4 structures are polymorphic in nature and depending upon the cation present and the nucleotides in the loops are evidently found in different topologies like parallel, hybrid (3+1), antiparallel (2+2) and antiparallel. G4s are known to be present in various regions of the human genome such as telomere, promoter and 5'-UTRs [1]. They have gained enormous attention due to their implication in several crucial biological processes like replication, transcription, translation and telomere maintenance [2] which makes G4s a potential drug target [3].

This study focuses on mapping molecular interactions that drive G4s conformations and stability. To decipher the conformational complexity and the thermodynamic stability of G4s we have chosen the model system TTGGGQ: 5'-AAGGG(TTGGG)3AA-3' which forms parallel conformation in K^+ and Hybrid (3+1) in Na^+ conditions [4]. The TTGGGQ sequence has been modified by substituting each guanine with inosine to reduce one hydrogen bond at a time to check the role each guanine plays in the stability. CD spectroscopy has been used to characterize different topologies of G4s [5], herein we observed the biphasic behavior for TTGGGQ and inosine modified sequences in K^+ condition with a reduction in thermal stability(T_m) for the latter case. With NMR spectroscopy, we observed that in K^+ condition inosine modification favors a single as well as multiple conformations (in few cases), giving the validation of some crucial molecular interactions which are responsible to drive a particular G4 conformation.

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STRUCTURAL STUDY OF CGAG-RICH SEQUENCE FROM THE PROMOTER OF AUTS2 GENE INVOLVED IN NEURODEVELOPMENT

The human AUTS2 gene has been shown to importantly influence brain development by controlling the number of neurons, affecting the structure of neurons by promoting the growth of axons and dendrites as well as regulating neuronal migration [1]. AUTS2 is also a key regulator of transcription of multiple genes crucial for brain development and misregulations in AUTS2 expression have been correlated with the occurrence of autism spectrum disorders [2,3].

A CGAG-rich sequence (A38) capable of adopting a non-canonical secondary structure was found in the promoter region of AUTS2 approximately 150 bases upstream of the transcription start site. Using NMR complemented by UV/Vis spectroscopy and gel electrophoresis we show that A38 adopts a stable non-canonical hairpin structure composed of six GC, four GA base pairs, and a 14-nucleotide loop.

Furthermore, the A38 sequence is rich in CpG dinucleotides which are known hotspots for epigenetic modifications. The most abundant epigenetic modification in the human genome is 5-methylcytosine that is involved in gene silencing [4]. The demethylation process can start by deamination of 5-methylcytosine which yields thymine now associated in a G:T mismatch. This mismatch is readily recognized by thymine DNA glycosylase and converted to cytosine via a base excision repair pathway [5]. We show that the incorporation of a single C-to-T mutation in certain positions of the A38 sequence alters the structure of the hairpin. This change significantly affects the structure of the loop region and can be understood as four-nucleotide slippage compared to the native sequence. The formation of different structures based on epigenetic modifications, or their repair pathway intermediates, may play important role in the regulation of AUTS2 expression. Additionally, the high-resolution structures will help expand our knowledge of structures formed by repetitive sequence motifs.

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ROLE OF CATION NATURE AND GC ENDS IN THE ASSEMBLY OF DNA G-QUADRUPLEXES

Guanine-rich DNA oligonucleotides can adopt non-canonical, four-stranded secondary structures, termed G-quadruplexes. Their main building block is a G-quartet, which is formed by four guanine residues in planar arrangement held together by eight Hoogsteen-type hydrogen bonds. The formation of G-quadruplexes requires the presence of cations. In fact, cation nature is one of the major factors contributing to the structural diversity of G-quadruplexes. Their self-assembling ability as well as programmable control of their shape and size make them attractive candidates for nanotechnological applications. One of the proposed ways for programming self-assembly is by designing G-quadruplex forming DNA sequences with complementary GC ends, which would act as 'sticky ends' and form linkages between two successive G-quadruplexes via inter quadruplex GCGC-quartet formation [1, 2].

In the present NMR study, we analyzed the influence of GC ends within oligonucleotides *GCn* and *GCnCG*, where *n* = $G_2AG_4AG_2$ on resulting G-quadruplex structures. Additionally, we evaluated the effect of different monovalent cations on the folding of *GCn* and *GCnCG* G-quadruplexes. Na^+, $^{15}NH_4^+$ and K^+ ions promote the formation of symmetric, dimeric G-quadruplexes, which can be considered, as structures composed of two blocks the 5'-antiparallel and the 3'-parallel block. Although the global fold of G-quadruplexes, which includes A(GGGG)A hexad sandwiched between two G-quartets is preserved, some structural elements were cation dependent. Furthermore, $^{15}NH_4^+$ and K^+ ions promote dimerization of *GCn* G-quadruplexes through 3'-3' stacking interactions of terminal G-quartets. Such stacking is precluded by 3'-GC ends in the case of *GCnCG* G-quadruplexes [3].

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c-kit2 G-QUADRUPLEX STABILIZED VIA A COVALENT PROBE: EXPLORING G-QUARTET ASYMMETRY

The KIT receptor is a transmembrane protein that participates in a variety of physiological processes [1]. Due to its role in the pathogenesis of cancer, KIT is an attractive target for anti-cancer treatment [2]. The human KIT proto-oncogene promoter contains three G-rich regions, c-kit1, kit*, and c-kit2, which are capable of folding into G-quadruplexes. Importantly, the promoter segment comprising kit* and c-kit2 contains a putative binding site for the Sp1 transcription factor, which can (apart from binding to a double-stranded consensus motif) bind to a G-quadruplex [3]. Considering that Sp1 binding is critical for the activity of the human KIT promoter [4], highly stable G-rich oligonucleotides mimicking G-quadruplexes from KIT could be used as decoys to sequester these proteins and modulate KIT expression.

Polyaromatic moieties can be employed for modulating G-quadruplex properties via their stacking with G-quartets. In this study, we focused on G12T/G21T mutant of the genomic c-kit2 sequence forming a monomeric three-quartet G-quadruplex, as a representative of parallel G-quadruplex structures found in human promoter regions. We showed that individual incorporation of U^{py} (5-(1-pyrenylethynyl)-2'-deoxyuridine) in the pentaloop of c-kit2 caused structural polymorphism and in some cases also destabilization. On the other hand, the introduction of pyrene moieties to an individual or both termini of the c-kit2 sequence resulted in highly stable G-quadruplex structures. Although the parent parallel fold remained unchanged despite the terminal substitutions, a detailed analysis revealed major differences in structural dynamics of U^{py} between the two terminal analogues. We believe that the contrast between structural dynamics of $U^{py}1$ and $U^{py}21$ might stem from an intrinsic asymmetry of c-kit2 G-quartets. This way U^{py} acts as a probe for local G-quadruplex dynamics, which is true especially for c-kit2, where outer G-quartets are exposed and U^{py} interactions with propeller loops are minimized. This is a vice-versa effect to the binding of ligands comprised of unfused aromatic rings to G-quadruplexes, where ligand planarity is key for efficient stacking.

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THE EFFECT OF 8-OXOGUANINE ON G-QUADRUPLEX STRUCTURE

Reactive oxygen species (ROS) are a byproduct of aerobic cellular metabolism in all living organisms. The inability to neutralize excessive ROS results in oxidative stress causing many types of DNA lesions, to which cells respond by activating relevant DNA repair pathways or apoptosis if repair is unsuccessful. Among the four DNA nucleobases, guanine (G) has the lowest redox potential and is therefore most prone to oxidation. Furthermore, guanines in tracts are more susceptible to oxidation than isolated guanines.

G-rich regions are found at telomeric ends of chromosomes and fold into G-quadruplex structures. Four repeats of the human telomeric sequence (hTel) form three stacked G-quartet planes with mixed parallel/antiparallel G-tract directionalities. We have individually probed all guanine positions in hTel by substituting them with 8-oxo-7,8-dihydroguanine (^{oxo}G), a common oxidation product of guanine. Due to ^{oxo}G's distinct hydrogen bonding properties, a loss of G-quadruplex structure was observed for most oligonucleotides containing oxidative lesions. However, some positions in the hTel sequence can tolerate substitutions with ^{oxo}G. Two positions, which adopt *anti* and *syn* glycosidic conformations in the parent hTel G-quadruplex, respectively, were selected for the further analysis and determination of high-resolution structures by solution-state NMR (PDB IDs: 6IA0 and 6IA4). However, due to ^{oxo}G's preference for the *syn* conformation, distinct responses were observed upon replacing guanines with different glycosidic conformations. Accommodation of ^{oxo}G at sites in *syn* or *anti* in non-substituted hTel G-quadruplex requires a minor structural rearrangement or a major conformation shift, respectively. The system responds by retaining or switching to a fold where ^{oxo}G is in *syn* conformation. Importantly, the two G-quadruplex structures containing ^{oxo}G are still stable at physiological temperatures and should be considered detrimental in higher-order telomere structures [1].

Human promoters are also enriched in G-quadruplex forming sequences. Misregulation of BCL2 expression has been observed in many diseases and is associated with cellular exposure to reactive oxygen species. A region upstream of the P1 promoter in the human BCL2 gene plays a major role in regulating transcription. A similar strategy was used to simulate oxidative events within a long G-tract by ^{oxo}G substitutions. Surprisingly, ^{oxo}G at a specific position within a 25-nt construct boosts thermal stability of the resulting G-quadruplex. This is achieved by distinct hydrogen bonding properties of ^{oxo}G, which facilitate the formation of an antiparallel basket-type G-quadruplex with a three G-quartet core and a G·^{oxo}G·C base triad (PDB ID: 6ZX6). While ^{oxo}G has previously been considered detrimental for G-quadruplex formation, its stabilizing effect within a promoter suggests a potential novel regulatory role of oxidative stress in general and specifically in BCL2 gene transcription [2].

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LOCAL THERMODYNAMICAL INVESTIGATION OF Pin1 WW-DOMAIN USING eNOEs

The traditional way of determining the structure of bio-molecules using solution-state NMR involves using a large set of NOE rates derived from NOESY spectra and converting them to inter-proton distances. The biggest limitation of this method is that we are only able to semi-quantitatively determine the cross-relaxation rates from the spectra and consequently the distances that we obtain only work as upper-limits of the actual distances with huge tolerances. The main reason behind this is **spin diffusion**, the higher-order magnetization transfer to the spins outside of the two spins being studied.

One of the methods to provide accurate values of rates and distances and in turn more precise structures is exact NOEs (**eNOEs**) developed by Vogeli et al. [1]. This method utilizes a hybrid approach where we use an X-ray or a preliminary NMR structure and subject it to **full-matrix formalism** to determine the ratio of magnetization transfer between the two spins in question with and without the influence of the neighboring spins. This ratio, called the correction factor is then applied to all experimentally determined NOE build-ups to gain precise and unambiguous values of relaxation rates and distances, with both upper and lower limits.

In our work, we use the eNOE rates obtained through this method to study thermodynamics of protein (S18N/W34F mutant of Pin1-WW domain) at **local level** and expand the utility of eNOE toolkit. The 34 residue WW domain used in this study is the N-terminal part of Pin1 and folds into the three-stranded anti-parallel beta-sheets, typical of WW-domains. Over the years, multiple kinetic and thermodynamic studies of WW-domain have shown that it displays a strict **two-state behavior** [2].

We extracted the structural information of the protein for a wide temperature range **(278K-303K)** and tried to model the evolution of the obtained rates for every spin pair with increasing temperature under a two-state thermodynamics paradigm. This approach enabled us to extract parameters like enthalpy, Δ**H**, heat capacity, $\Delta\mathbf{C}_p$, and temperature, $\mathbf{T}_t$ (where ΔG = 0), for proton pairs spread across the breadth of protein which were inaccessible by other approaches, as they only gave us information at the global level.

The mean value of parameters T_t, ΔH_t and $\Delta C_{p,t}$ is 289.03 K, 5.71*10^4 J/mol and -2.14*10^3 J/Kmol, respectively and the standard deviation is 9.83 K, 1.51*10^4 J/mol and 4.80*10^3 J/Kmol, respectively. The values of T_t and ΔH_t seem to be constrained within a narrow range and have relatively low variance. This hints towards some kind of **correlated transition state** at ~ 290K, much below the melting temperature (~ 320K). This is in line with the two-state structure we obtained with our protein allostery calculations [3].

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ZOOMING ON THE INTERACTION BETWEEN α-SYNUCLEIN AND CALCIUM IONS APPROACHING PHYSIOLOGICAL CONDITIONS

Intrinsically disordered proteins (IDPs) and protein regions (IDRs) lack a stable 3D structure but are nevertheless functional thanks to their dynamic properties [1]. Their fine-tuned features are expected to be modulated by side-chains as well as local solvent exposure [2] so it is crucial to characterize them with atomic-resolution approaches. Here we propose a ^{13}C based strategy to provide a unique tool to investigate IDPs behavior in different experimental conditions relevant for their physiological function. A set of carbonyl carbon direct detected NMR experiments was implemented to monitor the IDPs/IDRs both from the backbone and side chains point of view. The latter is seldom studied in highly flexible and disordered proteins because of extensive signal overlap [3]. In addition, a novel pulse sequence based on the CON experiment was designed to achieve information of amide proton exchange with the solvent (DeCON). This set of experiments was used to obtain a fingerprint of α-synuclein that is fully disordered in its native conditions and to study the interplay between this IDP and Ca^{2+} ions [4], zooming into the metal ion coordination sphere and revealing the motifs involved in the interaction [5].

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BUSY BAZAAR: WHAT HAS NMR TOLD US ABOUT EXTRINSIC PROTEINS OF PHOTOSYSTEM II?

Psb proteins of Photosystem II are a class of extrinsic proteins that adjoin the oxygen evolving center from the cytosolic side. They act as gatekeepers who maintain proper conditions for the water splitting reaction which ultimately leads to the release of molecular oxygen in higher plants [1]. This is achieved by fine tuning the concentrations of ions present in the photosynthetic machinery via protein-protein and protein-ion interactions. The exact functional mechanisms of this protein cohort have not yet been described in detail since all of these proteins contain a large degree of disordered regions which so far impeded the resolution of complete proteins by means of X-ray crystallography or cryo-electron microscopy [1, 2].

We have studied 3 main extrinsic proteins – PsbP (23 kDa), PsbQ (16 kDa) and PsbO (33 kDa) as well as the extrinsic domain of the larger membrane protein, CP43 (CP43ext., 12 kDa). Using solution NMR we have been able to determine three dimensional structures of PsbP and PsbQ in solution including their highly flexible regions. We have obtained partial resonance assignment and relaxation data of PsbO. Recently, we have initiated the NMR characterization of the extrinsic domain of CP43 which is in spatial proximity to all three extrinsic proteins. The most interesting information about detailed interplay of those proteins and their dependence on the presence of metal cations has been assessed using a combination of techniques, such as NMR titration and chemical exchange saturation transfer (CEST), biolayer interferometry (BLI) and microscale thermophoresis (MST).

The metal binding sites we found in the solution differ substantially from the ones previously reported in crystallographic structures of isolated higher plants of PsbP and PsbQ [3, 4]. All of the protein interaction sites (with other proteins as well as the cations) included dynamic parts which suggest the so far unrecognized functional importance of these regions.

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CHEMICAL SHIFTS AND MACHINE LEARNING BASED METHODOLOGY TOWARDS CHARACTERIZATION OF DNA G-QUADRUPLEXES

NMR based structural genomics has immensely contributed to the field of structural biology. Tertiary structure modelling has seen a paradigmatic shift with the development of chemical shifts (CS) based methodology. Similar protocols for nucleic acids are in their infancy, with ^{1}H CS based structural characterization for RNA being the most recent. We envisioned that similar protocols for DNA G-quadruplexes (G4) would be immensely helpful, given their diverse roles performed within the cell and also their applications across the fields of Chemistry and Biology.

G4s are widely observed in guanosine (G-)rich DNA and RNA sequences resulting from the stacking of two/three G-tetrad pseudo-planes that are in turn formed by Hoogsteen H-bonds between the G-nucleobases. The relative orientation of the four backbone strands defines their classification as parallel, hybrid (3+1), (2+2) antiparallel and antiparallel structures. Additionally, the loops that connect the adjacent backbone strands are also commonly found in propeller, lateral and diagonal conformations, resulting in range of tertiary structural topologies. Conventional NMR characterization methods are either expensive and/or time-intensive. Our aim was to develop a CS-based methodology that overcomes these limitations.

Folding of G4s was known to be driven by the formation of backbone grooves, which upon closer inspection unravelled that each backbone topology is associated with a specific distribution of the glycosyl dihedral angles (*viz.* “syn” and “anti”). From BMRB search, we observed that ^{1}H-H8 CS aids in the dihedral angle distribution, albeit with a lower level of accuracy due to significant overlap of chemical shift distributions. Due to the dearth of ^{13}C/^{15}N data, 18 different sequences were prepared (natural isotopic abundance) for which PDB structural models are available.[1] 2D ^{13}C-^{1}H and ^{15}N-^{1}H correlation data were obtained for samples (concentrations 0.8-3 mM) within a short period (8-30 hours/sample) to build the chemical shift database for the various topologies. ^{13}C chemical shifts C8 and C1′ provided an unequivocal way of determining the distribution, thus leading to reliable and rapid analysis of topology.[1] Additionally, C1′ CS are found to report on subtle structural features present within a given topology, aiding in detailed characterization.[1] We further optimized a machine learning based methodology using ^{1}H CS to determine structural features of the molecule that provides an accuracy of ~99% for parallel and ~80% for hybrid (3+1) topologies.[1] The fidelity of the methodology is demonstrated by its application to a system that folds into two dissimilar topologies in different ionic conditions, providing its first atomic level characterization.[1] This methodology forms the first step towards creating a database of ^{13}C and ^{15}N chemical shifts for DNA G4s. The insights obtained from this study immediately opens up avenues of extending the methodology to RNA, map their *in vivo* structure and monitor refolding of G4s real-time.

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NMR STUDY OF PURINE RICH TANDEM REPEATS LINKED TO REPLICATION FORK COLLAPSE

Purine rich tandem repeats form unusual secondary structures linked to replication fork collapse [1]. This phenomenon was demonstrated for different DNA segments, including tandem repeats of the d(CAGAGG) sequence, of which three or more tandem repeats are present in more than half of all human chromosomes, some even inside important coding sequences like ADAM metallopeptidase, transcription factors and proteins with zinc finger protein motif.

We prepared RS4 and SLL1 oligonucleotides comprising three and one repeats of the d(CAGAGG) motif, respectively, where extensions at the 5'- and 3'-ends were introduced to promote thermodynamic stability. With an extensive NMR study, we have discovered that RS4 forms structures with stable base-paired stem and AGAG tracts in peculiar conformation, which thus far have not been characterized with high-resolution techniques. We proved that RS4 in the presence of potassium ions folds into a structure that contains two symmetric elements and exhibit two GC and two TA base pairs in Watson-Crick geometry. It is interesting that several ^{1}H NMR signals are extremely broad or could even not be observed, indicating structural equilibrium. Nevertheless, we assigned most of the ^{1}H NMR signals by using isotope-labelled olionucleotides along with constructs specifically designed to carry single-residue substitutions. Unfortunately, for two out of three AGAG segments there is a lack of NOE correlations in NMR spectra of RS4, which is related to extreme broadening of ^{1}H NMR signals. The limited NMR-based insights in structures adopted by RS4, especially the lack of data for the AGAG segments, motivated us to focus on the smaller oligonucleotide SLL1. We found that salt concentration has a great effect on structuring of SLL1, as the higher potassium ion concentrations result in dimer structure formation. With the use of ^{13}C- and ^{15}N-isotopic residue-specific labelling and with the use of 2D NMR methods we assigned most of the ^{1}H NMR signals of SLL1 which allowed further analysis of NOE correlations. While the structural data is consistent with SLL1 adopting a hairpin structure with stable stem, details of the AGAG loop remain to be elucidated further in order to explain the evident hydrogen-bonding that involves imino protons of both guanine residues.

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PRE-FOLDED STRUCTURES: FOLDING PATHWAYS OF HUMAN TELOMERIC G-QUADRUPLEXES

Guanine-rich (G-rich) DNA regions exhibit potential to form G-quadruplexes in the presence of stabilizing cations, and demonstrate vast structural diversity and polymorphism. G-rich DNA sequences are present throughout the human genome including regulatory regions of (onco)genes and telomeric repeats. Consequently, G-quadruplexes are attractive targets for anticancer and antiviral drug development.

Understanding the mechanism by which biological macromolecules fold into their functional native conformations represents a problem of fundamental interest. DNA oligonucleotides derived from human telomeric repeat d[TAGGG(TTAGGG)$_3$] and d[TAGGG(TTAGGG)$_3$TT] fold into G-quadruplexes through diverse steps. NMR and other data acquired at different experimental conditions undoubtedly revealed that both oligonucleotides exhibited defined pre-folded structure(s) already in the absence of cations that are believed to be a starting point of folding process [1]. All determined structures (with base pairs and base triples, with antiparallel chair-like topology as well as hairpin) showed that the first and the second G-tracts are connected in antiparallel orientation. This structural feature could be the main reason for different folding of d[TAGGG(TTAGGG)$_3$] and d[TAGGG(TTAGGG)$_3$TT] into their final hybrid-1 and hybrid-2 G-quadruplexes, respectively. While formation of hybrid-2 G-quadruplex can proceed directly from antiparallel pre-folded structure, reorientation of the first and the second G-tracts into parallel alignment is required for formation of hybrid-1 G-quadruplex. Therefore, immediately after the addition of K^+ ions into solution of d[TAGGG(TTAGGG)$_3$], G-quadruplex structure with antiparallel orientation of the first and second G-tracts is likely formed before the final hybrid-1.

Studies where we can experimentally characterize structures of pre-folded forms are essential to establish mechanisms of G-quadruplex folding, where pre-folded forms can be on- or off-pathway intermediates and can lead to development of novel type of selective ligands that will target peculiar structural elements.

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NMR STRUCTURAL DETERMINATION OF OxyS sRNA

Small non-coding RNAs (sRNAs) are an important class of RNAs in bacteria with regulatory roles in stress response and adaptation to environmental changes [1]. OxyS is a 109 nucleotide long, stable, trans-encoded sRNA found in *Escherichia coli* [2]. It is regulated by OxyR and is induced in high concentration in response to oxidative stress caused by an elevated concentration of hydrogen peroxide (H_2O_2). OxyS is a global regulator affecting the expression of multiple genes, mainly through direct base-pairing with relevant mRNAs [3]. OxyS is predicted to adopt a secondary structure containing three stem-loops, which was experimentally verified by chemical probing [2]. To understand and study base-pairing interactions between target mRNAs and OxyS, more detailed knowledge about its structure is needed.

We adopted the *divide and conquer* approach to separately study the parts of OxyS sequence that are predicted to fold into hairpins. We recorded a series of homonuclear and heteronuclear NMR experiments to determine the solution structures of the isolated stem-loops SL1, SL2 and SL3. Later we in vitro transcribed the full-length OxyS, to study its structure with NMR. We confirmed the presence of stem-loops SL1, SL2 and SL3 in OxyS. Unexpectedly, we identified an additional short hairpin SL4 located in the predicted linker region.

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STRUCTURAL INSIGHTS INTO NAPHTHALENE DIIMIDE ANALOGUE INTERACTIONS WITH G-QUADRUPLEXES

G-quadruplexes are four-stranded DNA structures, which intriguing biological roles relate to their distinguished features with respect to the canonical double-stranded helix along with their prevalence in regulatory regions, *e.g.* promoters of oncogenes and telomeres [1]. G-quadruplex core comprises stacked G-quartets, each formed by four Hoogsteen hydrogen-bonded guanine residues. The loops connecting the residues in the core as well as the four stems can be diverse in terms of length, composition and progression, altogether making the G-quadruplexes family extremely polymorphic. Such broad variety of structures adopted by guanine-rich DNA is important in context of the evolving therapeutic strategies that rely on drugability of specific G-quadruplex forming regions [2].

Motivated by naphthalene diimide analogue NDI-5 anticancer activity that relates to G-quadruplex binding [3] we explored the underlying structural details. We investigated NDI-5 interactions with two G-quadruplex-forming DNA, namely M2 and m-tel24, characteristically adopting parallel and hybrid topology, respectively. For m-tel24 we observed stacking of NDI-5 only at the 5'-end. On the other hand, NMR results revealed ligand interactions at both, the 5'- and 3'-end G-quartets of the parallel M2 G-quadruplex. The ^{1}H NMR spectral features in case of NDI-5–M2 G-quadruplex interactions rendered more detailed investigation with the assignment of ^{1}H NMR signals accomplished with the use of site-specifically ^{13}C and ^{15}N-labelled oligonucleotide synthesis and multi-dimensional NMR experiments. The determination of NDI5–M2 G-quadruplex binding stoichiometry afforded the value of 1:3, while at higher ratios, i.e. at excess of NDI-5, a complex with 'ligand shell' features is formed, whereby ligand molecules interact with M2 G-quadruplex and/or pre-bound ligand. To evaluate the roles of the residues connecting the guanines in G-quartets, we utilized natural-to-abasic residue substitutions at the loop regions that preserved the parallel stranded topology of M2 G-quadruplex. The NMR-based assessment showed that loops modulate the secondary binding of NDI-5, but are not decisive for the predominant interactions [4].

The present results will help in optimizing properties of G-quadruplex-binding naphthalene diimide analogues aiming to fulfill the premises of the novel approaches for treatment of different cancers.

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A STATISTICS-DRIVEN NMR APPROACH TO SITE-SPECIFIC ANALYSIS OF STATIC PROTEIN DISORDER

Protein conformational disorder is vital for functionality of a large part of cellular processes and can translate into heterogeneity accessible by solid-state NMR.[1] Understanding the conformational distribution of protein (mis) folding or reaction intermediates on a single-residue level can provide insights useful both from a biological as well as a pharmacological perspective.

Disorder in solid-state protein samples has been tackled by various NMR techniques [1]. Here we present two approaches to reconstruction of residue-specific dihedral angle distribution based on analysis of 4D hCBCANH correlations. The first approach is based on a comparative analysis of chemical-shift distributions with the relational database PACSY [2]. The second approach involves dihedral angle predictions for the entire 4D peak using TALOS-N [3]. We test both methods on a highly heterogeneous sample of GGAGG pentapeptide.

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PROTON-DETECTED SOLID-STATE NMR SPECTROSCOPY AT HIGHEST FIELD: THE GAIN IN RESOLUTION AT 1200 MHZ

Progress in biomolecular NMR spectroscopy is driven by increasing magnetic-field strengths leading to improved resolution and sensitivity of the NMR spectra. We here investigate proton-detected solid-state NMR spectra of a variety of biomolecular systems recorded with the recently available superconducting magnet operating at 1200 MHz proton resonance frequency using magic-angle spinning (MAS) frequencies of 100 kHz. Among them are the deuterated Hepatitis B virus core protein [1], the protonated Rpo4/7 protein complex of two subunits of archaeal RNA polymerase II [2] and the protonated amyloid fibrils of the fungal prion HET-s (218-289) [3,4].

The spectra reveal a significant gain in resolution compared to spectra recorded at 850 MHz. The linear improvement of the line width expressed in ppm with increasing B_0 field by a factor of 0.7 (ratio of the two magnetic fields) was shown to be achieved for all the samples. For side-chain protons (in particular CH_2 and CH_3 protons) an even stronger narrowing was observed as the strong couplings are partially suppressed by the increased chemical-shift separation. This gives access to structure and dynamics studies of aliphatic protons in fully protonated systems. The longer T_2' times, measured for the bulk amide protons at the highest field further allow, in combination with faster MAS, to use *J*-coupling based sequences to observe dynamic domains of biomolecules that currently escape detection using CP-based magnetization transfer experiments.

We recorded as well carbon-13 experiments, and show that in ^{13}C spectra substantially higher numbers of peaks can be resolved compared to 850 MHz.

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THE MODE OF ACTION OF PLECTASIN & VARIANTS IN MEMBRANES

Plectasin is an antimicrobial peptide (AMP), belonging to the cysteine stabilized alpha-beta (CSαß) defensins first discovered back in 2005 in the fungus *Pseudoplectania nigrella*. [1] Plectasin and its variants (e.g. NZ2114, MP1102) have excellent bactericidal activity against a variety of gram positive bacteria. This includes some clinically relevant strains, among which MRSA, infamous in hospitals for causing difficult to treat infections. Binding of peptidoglycan-precursor Lipid II (LII) is thought to be the mechanism of action of these peptides [2] but much remains unclear about the structural details.

Unfortunately, studying the atomic structure of peptide-Lipid II complexes in their native environment, a membrane, by conventional techniques -such as X-ray diffraction- is challenging. Furthermore, without these data the development of these drugs is like stumbling in the dark, hampering the transition to the clinic of these high-potential candidates. By studying the Plectasin-LII complex in membranes with solid-state NMR we show a markedly different binding mode than previously found in micelles. In particular, we find that the αß-loop is critical for LII binding and forms a rigid 'clamp'. ^{13}C spin diffusion experiments with universally ^{13}C labeled Plectasin and LII identify different residues in direct contact with LII. Furthermore, Plectasin-LII complexes aggregate and form micrometer-sized clusters that are clearly visible even with microscopy, potentially pointing to an additional mode of action. Variants of Plectasin with altered microbial scope such as NZ2114 are modified not near the proposed binding site, but around a negatively charged on the opposite side of the peptide. We, therefore, hypothesize that these modifications change the aggregation kinetics and thereby influence antimicrobial activity. This is supported by the observation that the NZ2114-LII complex is more dynamic compared to Plectasin.

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GM3 LIPIDS STIMULATE CO-ASSEMBLY OF VESICLES AND ALPHA-SYNUCLEIN

Parkinson's disease is characterized by co-aggregates of the presynaptic protein alpha-synuclein and lipids in the brains of patients [1]. This process could cause disease by alpha-synuclein fibril- or oligomer toxicity, depletion of functional alpha-synuclein or by damaging lipid membranes [2]. We used NMR spectroscopy together with cryo-transmission electron microscopy to investigate co-assemblies formed when monomeric alpha-synuclein is aggregating in the presence of POPC vesicles containing ganglioside GM3, a bio-synthetic precursor for brain gangliosides [3].

Our results show that lipids co-assemble with alpha-synuclein to form μm-sized amorphous aggregates, whose properties depend on the lipid-protein ratio in the incubation mixture and the fraction of ganglioside GM3 in vesicles. Solid state ^{13}C MAS NMR analysis of the aggregates together with quantitative solution ^{1}H NMR of supernatants show that GM3-rich vesicles stimulate more lipids to co-assemble with alpha-synuclein, in a manner that is selective for GM3 over POPC. From measurements of ^{1}H-^{13}C residual dipolar couplings, ^{31}P chemical shift anisotropy, and ^{13}C relaxation rates – reporting on molecular segment reorientation on time-scales from nanoseconds to milliseconds – we deduce that co-assembly with alpha-synuclein induces curvature of the lipid bilayers and slows down reorientation about the molecular long-axis and transitions between conformational isomers.

The present findings add insight into the structure and dynamics of lipid/alpha-synuclein co-assemblies and may also hint at how ganglioside/alpha-synuclein interactions could have an effect on the structure-function-toxicity relationship related to Parkinson's disease.

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INSIGHTS INTO SITE-SPECIFIC DYNAMICS OF RNA BY SOLID-STATE NMR SPECTROSCOPY

The study of biomolecular dynamics provides information about a multitude of cellular processes including folding, binding affinity, allosteric regulation and enzymatic catalysis. Magic-angle spinning solid-state NMR (MAS ssNMR) is able to characterize motions on the pico- second-to-millisecond timescale and thus gives access to important biological dynamic processes ranging from bond fluctuations to folding events [1]. Despite significant progress in the research of protein structure and dynamics by ssNMR, corresponding studies of RNA remain at a relatively early stage due to a lack of appropriate experimental protocols and the strong chemical similarity of the four-nucleotide building-blocks, which inevitably leads to spectral crowding [2]. In recent years we have developed the methods that allowed us to obtain the first structures of an RNA and a protein–RNA complex solely by ssNMR [3,4]. Here we report for the first-time site-specific relaxation data for RNA measured by ssNMR spectroscopy on a uniformly ^{13}C,^{15}N labeled sample. We have measured the ^{15}N longitudinal relaxation (T_1) of amino and imino nitrogens of all nucleotides in the structured regions of a 26mer box C/D RNA in complex with L7Ae protein [3] at different MAS rates. On average, the ^{15}N T_1 values of the amino nitrogens are significantly shorter than those of the imino nitrogens, indicative of faster relaxation of the former due to a stronger overall ^{15}N-^{1}H dipolar interaction. On a nucleotide-specific basis, significantly shorter T_1 values are reported for the non-canonical region of the RNA (loop), whereas those for nucleotides in the canonical elements (stem) and the protein-binding region (kink-turn) are longer, implying reduced flexibility and increased stability in the canonical stem-loop and kink-turn regions. Experimental challenges in measuring ^{15}N T_1 relaxation of RNA by ssNMR are discussed and the contribution of spin-diffusion to the relaxation rates is assessed.

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STRUCTURAL STUDY OF WILD TYPE FAT10 BY MAS NMR SPECTROSCOPY

Human leukocyte antigen (HLA)-F adjacent transcript 10 (FAT10) is a protein exclusively found in mammals [1]. FAT10 is restricted to organs and cells of the immune system [2,3]. However, FAT10 expression gets induced in other types of tissue by the synergistic action of tumor necrosis factor alpha (TNF-α) and interferon (IFN)γ under inflammatory conditions [4,5,6].

The protein belongs to the ubiquitin-like modifier (ULM) family which is characterized by a common three-dimensional structure known as the β-grasp fold [7]. FAT10 itself consists of two ubiquitin-like domains which are connected by a flexible linker [8]. The free C-terminal diglycine motif is used for covalent attachment to substrate proteins. Beside ubiquitin, FAT10 is the only ULM that targets its substrate proteins for fast and direct degradation by the 26S proteasome. Mono-FATylation is sufficient for degradation and FAT10 is degraded along with its substrates [9].

The extremely instable FAT10 tends to precipitate and for this reason, the cysteine residues were replaced in a structural study of the protein [10]. High-resolution structures determined by X-ray diffraction and solution NMR of the individual stabilized, cysteine-free domains reveal that both domains fold similarly to ubiquitin, but exhibit completely different electrostatic surface potentials to each other as well as to ubiquitin translating into diverse non-covalent interactions. As the stabilized version of FAT10 and its conjugates are degraded at slower rates [10], a structural study of wild type FAT10 will likely provide new, biologically relevant information. MAS NMR spectroscopy is ideally suited for this purpose.

Since FAT10 has not been studied by MAS NMR before, we have started our structural studies at the N-domain. We successfully expressed and purified the uniformly ^{13}C-^{15}N-labelled, cysteine-free N-domain of FAT10. A protocol for growing microcrystals was developed and resulted in high-resolution ^{1}H-^{13}C CP spectra of the microcrystalline FAT10 N-domain in natural abundance at 400 MHz. At 800 MHz, good quality ^{13}C-^{13}C correlation spectra of the uniformly ^{13}C-^{15}N-labelled, cysteine-free N-domain of FAT10 were obtained. ^{13}C-^{15}N ZF TEDOR spectra at 800 MHz will follow in the near future.

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STRUCTURE AND DYNAMICS OF LONG N-ALKANES IN LIPID BILAYERS DETERMINED BY SOLID-STATE NMR

Understanding the fate of hydrophobic polymers in cells is important due to increasing environmental concerns, such as the accumulation of nanoplastics in our oceans. Surprisingly, little is known about the potential incorporation of long hydrophobic chains into the hydrophobic core of cellular membranes [1].

Due to their simplicity, n-alkanes are thought a convenient starting point for investigating combined systems of lipids and hydrophobic polymers. However, contrary to shorter n-alkanes, the mixing of long alkanes (20 carbons and longer) with lipid bilayers has not been studied in detail [2,3].

Solid-state NMR is well suited to measuring the dynamics of lipid bilayers and incorporated molecules. We are using ^{1}H-^{13}C dipolar recoupling solid-state NMR [4] and static ^{2}H/^{31}P NMR, combined with x-ray scattering and MD simulations to characterize the structure and dynamics of various n-alkane/phospholipid membranes, and to determine how hydrophobic chain length (up to 30 carbons) and hydration affect the membrane molecular properties. The combination of experiments and simulations used enables to describe the systems with unprecedented atomistic detail.

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A LITMUS TEST USING HIGH-POWER RELAXATION DISPERSION FOR CLASSIFICATION OF RECOGNITION MECHANISMS OF TRANSIENTLY BINDING PROTEINS

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Molecular recognition in proteins is critical for all biological functions, and yet, delineating its mechanism is challenging, especially when recognition happens within microseconds [1]. We present a novel theoretical and experimental framework to distinguish between two-state vs three-state binding, including conformational selection and induced fit [2], based on straightforward kinetic experiments using NMR high-power relaxation dispersion [3], sensitive to single-digit microseconds. The novel framework predicts that conformational selection prevails on ubiquitin's paradigmatic interaction with the SH3c domain from an adapter protein [4]. We then reveal the residues that engage in the conformational selection mechanism using molecular dynamics simulations and Markov state modeling [5, 6]. The novel framework is robust and expandable for implementation in other binding scenarios with the potential to show that conformational selection might be the design principle of the hubs of interaction networks.

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STRUCTURAL ANALYSIS OF 3' SPLICE SITE RNA RECOGNITION BY U2AF2-SF1 COMPLEX IN SOLUTION

Recognition of non-coding introns from the pre-mRNA by splicing assembly is a crucial step of gene regulation in eukaryotes. Several multi-domain complex proteins participate in intron recognition at the early stage of complex E. In this assembly, 3' splice site (SS) of the intron is recognized by the U2 auxilliary factor heterodimer (U2AF), comprised of a small (35kDa, U2AF1), and large (65 kDa, U2AF2) subunit and splicing factor 1 (SF1), which bind to the conserved yAG splice site (3' SS), the poly pyrimidine tract (PY-tract), and the branch point site (BPS), respectively. Multiple protein-protein interactions also stabilize the assembly. For example, the U2AF1 UHM (U2AF homology motif) domain binds to the U2AF2 ULM (UHM ligand motif), while the U2AF2 UHM domain binds to the SF1 ULM motif[1,3]. Additionally, SF1 has a helix-helix domain preceding the KH-QUA2 RNA binding region with a conserved RSPSP motif, which undergoes post-translational modification[2]. Altogether, the multi-domain complex assembly of U2AF2-SF1 is around 120kDa in size and the structures of individual domains are solved by NMR and X-ray crystallography. However, the overall architecture of the U2AF2-SF1-RNA complex structure is not known.

Here, we characterize the architecture of the SF1-U2AF2-RNA complex by combining biochemical and NMR experiments with SAXS data. We studied binding of a range of RNA motifs to the U2AF2-SF1 complex representing variable strength of cis regulatory RNA motifs. Depending on the RNA binding affinities the overall architecture of the U2AF2-SF1 RNA complex adopts compact, semi-compact to open conformation in solution. We use the paramagnetic relaxation enhancement (PRE), pseudo-contact shifts (PCS) and residual dipolar coupling (RDC) data to determine inter-domain distances and orientation restraints, which are used for rigid body modeling to calculate the ensembles of U2F2-SF1-RNA ternary complex. Analysis of ^{31}P NMR spectra of phosphorylated SF1 shows differential line broadening of two ^{31}P signals upon complex formation with U2AF2 and RNA, suggesting a long range effects, that are mirrored by corresponding NMR chemical shift perturbations(CSPs) in U2AF2. In summary, our results provide the structural insight of RNA recognition by U2AF2-SF1 complex and effect of phosphorylation.

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EXTENDING THE SENSITIVITY OF CEST NMR SPECTROSCOPY TO MICRO-TO-MILLISECOND DYNAMICS IN NUCLEIC ACIDS USING HIGH-POWER RADIO-FREQUENCY FIELDS

Biomolecules undergo motions on the micro-to-millisecond timescale to adopt low-populated transient states that play important roles in folding, recognition, and catalysis. NMR techniques, such as Carr–Purcell–Meiboom–Gill (CPMG), chemical exchange saturation transfer (CEST), and $R_{1\rho}$ are the most commonly used methods for characterizing such transitions at atomic resolution under solution conditions. CPMG and CEST are most effective at characterizing motions on the millisecond timescale. While some implementations of the $R_{1\rho}$ experiment are more broadly sensitive to motions on the micro-to-millisecond timescale, they entail the use of selective irradiation schemes and inefficient 1D data acquisition methods. Herein, we show that high-power radio-frequency fields can be used in CEST experiments to extend the sensitivity to faster motions on the micro-to-millisecond timescale. Given the ease of implementing high-power fields in CEST, this should make it easier to characterize micro-to-millisecond dynamics in biomolecules.

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DOES DUPLEX IMPACT QUADRUPLEX: CHARACTERIZATION USING NMR AND MD SIMULATIONS

In genome, nucleic acids form duplex and non-duplex structures, where they coexist. G-quadruplexes (G4s) are well studied non-duplex structures formed by guanine rich sequences. They play pivotal role in genome stability, telomere maintenance, transcription regulation, protein chaperoning and liquid-liquid phase separation.[1] G4s are considered to be potential drug target for cancer and viral infections.[2] So far G4s were studied individually, structural characteristics of G4s in presence of duplex has not been well characterized. We designed quadruplex-duplex (QD) systems to mimic QDs formed at promoters and telomeres, where duplex exist on 5'/3' end G4 forming sequences. 3-plane parallel G4 and dodecamer duplex were attached with varying linker lengths. 5'QD (or 3'QD) represents duplex at 5'-end (or 3'-end) of G4 sequence. Here we present atomistic characterization G4 and impact of adjacent duplex using NMR and molecular dynamic (MD) simulations.

Structural and dynamic analysis of G4 pointed out increased flexibility of 5'-tetrad guanines deoxyribose sugars. Interestingly same 5'-tetrad residues form most planar rigid quartet. Trends indicate delicate 3'-tetrad, shows least planar structure. QD systems showed similar trends, yet with chemical shift perturbations (CSP) and varied dynamics. 3'QD show minimal CSP and increasing linker length further decreased CSP. Such changes were rationalized with stacking interactions between tetrads and flanking residues. We observed decreased flexibility of junction residues, coaxial stacking between G4 and duplex. Further insights on ligand binding ability to QD systems are provided.

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SUBSTRATE RECRUITMENT OF THE HEXAMERIC MecA-ClpC COMPLEX

Regulated proteolysis by ATP-dependent AAA+ proteins is a critical component of the protein quality-control system that has evolved in all domains of life to maintain cellular homeostasis and to respond to external stimuli and environmental changes. In bacteria, the unfoldase ClpC, which is a member of the Hsp100/Clp family of AAA+ proteins, interacts with its adaptor protein MecA to form a hexameric molecular machine that is responsible for controlled unfolding of protein substrates. Hexameric ClpC can further associate with the protease ClpP to form a complete protein degradation complex (ClpCP); proteins unfolded by ClpC are translocated to ClpP for proteolytic degradation [1].

As a specific adaptor protein of ClpC, the two-domain protein MecA performs a dual role in the unfolding process. It binds substrate proteins by binding to them via its N-terminal domain (NTD), while its C-terminal domain (CTD) interacts with ClpC, promoting its assembly into the functional hexameric state. ClpC itself is comprised of two nucleotide-binding domains, D1 and D2. The D1 domain interacts with MecA, while the D2 domain interacts with ClpP in the ClpCP complex.

One of the most important substrates for the ClpCP system is the transcription factor ComK, which is responsible for competence development (uptake of exogenous DNA). Under favourable growth conditions, the degradation of ComK via MecA-mediated targeting to the ClpCP system acts to maintain the cellular concentration of ComK at a low-level. However, when cell-density is high, the degradation of the ComK is blocked by synthesis of an anti-adaptor protein, ComS, which competes with ComK for binding to MecA and allows development of competence by rescuing ComK from degradation [2].

Despite a number of mechanistic studies on Hsp100/Clp proteins, we lack a clear picture of the structural changes occurring at the various stages of the unfolding cycle. For example, the available crystal structure of the MecA-ClpC complex [3] lacks all the mobile elements involved in ATP-binding, hydrolysis and substrate recognition, leaving open critical questions regarding the mechanisms of substrate selection, recognition and unfolding and the coupling of these steps to the ATPase activity.

Here, we report for the first time the *in-vitro* assembly of the ComK–MecA–ClpC ternary complex for biochemical and structural analyses. As a step towards obtaining additional high-resolution structural information, we have performed crystallization of the ternary complex with a ΔD2 variant of ClpC. In addition, we have undertaken preliminary investigations of both the MecA–ClpC and ComK–MecA–ClpC complexes by solution NMR, acquiring methyl-TROSY spectra of the MecA component in these ~650kDa and ~700kDa species, respectively. These spectra represent a starting point for further NMR-based structural and functional studies of this important system.

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IN SITU LABELING AND DISTANCE MEASUREMENTS OF MEMBRANE PROTEINS IN *E. COLI* USING FINLAND AND OX063 TRITYL LABELS

In situ structural and dynamical investigations are essential as the cellular environment can critically impact the conformation and function of a protein. Yet such studies represent a challenging task. Electron spin resonance (ESR) spectroscopy combined with site-directed spin labeling (SDSL) is emerging as a powerful technique for this purpose.

Over the past years, we demonstrated a pulsed electron-electron double resonance (PELDOR or DEER)-based approach to observe the structure and conformational changes of outer membrane proteins (OMPs) in intact *E. coli* and the isolated native outer membrane (OM) [1]. The cobalamin transporter BtuB was labeled with MTSL and distance measurements were performed to a spin labeled cobalamin in *E. coli* and isolated native outer membranes. We also reported distance measurements on doubly labeled BtuB to observe the ligand-induced conformational changes in *E. coli* [2]. The nitroxide label (MTSL) suffers from a short lifetime in the reducing cellular environment and a high background labeling diminishing the overall sensitivity.

The Finland trityl (FTAM) based labels have emerged as promising tags for *in situ* ESR spectroscopy. Their narrow spectrum, long phase memory time (T_M), and high stability under reducing environments are very favorable for *in situ* applications. On the other side, their hydrophobicity may lead to poor labeling, reduced T_M, or aggregation. Replacing the FTAM core with the OX063 core increased the water solubility. Here we spin labeled BtuB with a FTAM and OX063 based labels, all of which bind to the protein with varying linkers through a methanethiosulfonate group. We show that both the Finland and OX063 based trityl labels possess an increased redox stability and enable specific labeling of BtuB in *E. coli* with high efficiency. The OX063 labels revealed a long phase memory time close to 5 µs (vs. ~3 µs for the FTAM label) at 100 K when attached to the protein. All trityl labels enabled in-cell distance measurements between BtuB and an orthogonally nitroxide labeled substrate (TEMPO-CNCbl) with high selectivity and sensitivity down to a few micromolar of concentration. Overall, all trityls gave relatively narrow distance distributions and did not interfere with ligand binding. The orthogonal labeling and PELDOR we demonstrated here would facilitate the investigation of conformational dynamics and or inter-subunit interactions of key membrane protein complexes in their native environments [3].

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PROBING THE G-QUADRUPLEX-LIGAND INTERACTIONS IN THE INTRACELLULAR SPACE BY IN-CELL NMR SPECTROSCOPY

The ^{1}H-detected in-cell NMR spectroscopy is a powerful technique used to characterize the structural behavior of nucleic acids in living cells [1]. The applications of in-cell NMR studies in human cells have involved double-stranded and i-motif DNA and hairpin and aptamer RNA.

This study explores the potential of applying ^{1}H and ^{19}F-detected in-cell NMR spectroscopy to profile targeting DNA G-quadruplexes by ligands. We show that the extension of the original in-cell NMR approach to polymorphic G-quadruplex-based targets is not straightforward. The severe signal broadening and overlap in ^{1}H in-cell NMR spectra of polymorphic G-quadruplexes and their complexes complicate their quantitative interpretation. Nevertheless, the ^{1}H in-cell NMR can be used to assess the capability of ligand to bind G-quadruplexes in the native environment. In addition, we tested a recently developed 3,5-bis(trifluoromethyl)phenyl probe to monitor the intracellular G-quadruplex-ligand interactions via ^{19}F-detected in-cell NMR [2]. The ^{19}F-signal from the probe allows to discriminate different G-quadruplex topologies and reveals their number and relative populations. Notably, it also reveals information on G-quadruplex-ligand interaction both *in vitro* and *in cellulo*.

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A THERMO-SENSITIVE GEL MATRIX FOR CELL ENCAPSULATION IN BIOREACTORS

Studying biomolecules directly in living cells using the in-cell NMR approach raises the issue of keeping the cells at favorable culture conditions inside the NMR cuvette during the experiment. Recently, various types of bioreactors have been employed to provide continuous nutrient supply to the sample. To ensure the even distribution of the fresh cultivation media, cells in the cuvette are usually encapsulated in a porous matrix. Commonly used gels, such as alginate or agarose, don't allow gentle recovery of intact single cells for further assays, e.g., flow cytometry analysis. Here, we show an alternative type of matrix with a temperature-sensitive sol-gel transition: liquid at low temperatures and gel when heated. It is non-toxic to the cells, suitable for in-cell NMR experiments using a bioreactor, and allows recovery of single cells without impairing their viability.

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SOLID-STATE NMR STUDIES OF ARTIFICIAL METALLOENZYMES IN WHOLE *E. COLI* CELLS

NMR has become a powerful spectroscopic tool to elucidate enzyme structure and dynamics in solution and solid-state NMR. Thus far, such studies have focused on characterizing proteins under *in vitro* conditions.

Recently, we could show that in-cell solid-state NMR (see, e.g., Ref. [1]) can be used to probe an artificial metalloenzyme (ArM) at atomic level in whole *Escherichia coli* cells by employing magic-angle spinning (MAS) solid-state (ss) NMR spectroscopy. Our studies focused on an ArM comprised of copper(II) phenanthroline bound to the homodimer of lactococcal multidrug resistance regulator (LmrR) that is capable of catalyzing Friedel-Crafts alkylation of indoles with high yields and exceptional enantioselectivity[2].

So far, our in-cell ssNMR studies were restricted to using dynamic nuclear polarization (DNP) MAS-ssNMR at comparably low magnetic field (400 MHz/263 GHz) due to the unfavorable magnetic field-dependence of bi-nitroxide radicals [3]. Here we present experimental results of LmrR expressing cells obtained at 800 MHz/527 GHz DNP conditions, making use of recent advancements in DNP radical design (see, e.g., Ref. [4]) and compare our spectroscopic results to our earlier work [2].

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CELL CYCLE RESOLVED NUCLEIC ACID IN-CELL NMR SPECTROSCOPY

In-cell NMR spectroscopy is a powerful tool for investigating the behavior of nucleic acids inside living cells in a close-to-physiological environment [1]. However, this method has only been limited to studies of nucleic acid structure and interactions in the asynchronous cell suspensions. Studying the processes and parameters that are changing in the course of cell cycle progression has become essential for understanding the regulation of many cellular functions and human pathologies, including cancer [2,3].

This study focused on adapting the in-cell NMR approach for monitoring nucleic acid structure and interaction in separate cell cycle phases. Using a model example of a double-stranded DNA, we show that the in-cell NMR spectroscopy method can be applied to evaluate the nucleic acid behavior in cells synchronized in mitosis and early S phase.

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EVALUATION OF THE BENEFIT AND INFORMING CAPABILITY OF 2D NMR EXPERIMENTS FOR STRUCTURE ELUCIDATION USING CASE SOFTWARE

Computer Assisted Structure Elucidation (CASE) has been around for more than 50 years [1,2]. It has experienced a significant boost after the introduction of routine 2D NMR experiments in the 1990's, as the increased information content offered allowed much more complex problems to be addressed. The evolution of computers and the processing power of modern CPUs allowed CASE to be a tool of reference for resolving the unique and unprecedented structures of natural products[3]. Some common questions encountered when spectroscopists are introduced to CASE are regarding the minimum set of experiments required to solve a problem by CASE and what is the value and informing capability of advanced NMR experiments. Although the answers vary depending on the problem complexity, in this poster we will try to tackle both questions, by demonstrating a few cases.

We will look at three example structures to answer these questions more efficiently: 2-Ethylindanone, Spirodactylone [4] and a xanthone-class natural product [5-6]. In the first example, which is a very simple molecule, we will examine the benefits of basic NMR experiments and how they affect the calculation time. The second example is a challenging natural product, and we will examine the benefits and potential problems that are encountered when using modern experiments like LR-HSQMBC, in addition to the traditional HSQC and HMBC. Here, we will also study the influence of manually adjusting atom properties (such as hybridization and connection to heteroatoms). The final example serves for considering the inter-relation between HMBC and INADEQUATE when a CASE system is used.

We see that even though the first two structures can be solved with only the most basic NMR experiments, addition of specialised correlation experiments can decrease the elucidation time dramatically. In the third example we see that even though INADEQUATE can speed up the elucidation time, this is not of too much benefit; the problem can be solved with only HMBC data in a reasonable time, much less than the time required to setup and record the INADEQUATE.

Exact details of the type of spectra used, and the calculations performed will be shown, as well as comparative tables on the achieved improvements in performance.

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NMR ELUCIDATION OF THE DYNAMIC FORMATION AND REORIENTATION OF FLUORESCENT CALIX[6] ARENE BASED ISOMERIC PSEUDOROTAXANES

The stimuli-triggered dynamic equilibrium of supramolecular complexes has a crucial role in the growing interest for supramolecular chemistry and is the base for most potential applications. In the case of the encapsulation of a fluorescent dye, the displacement of the relative complexation equilibrium can lead to interesting optical properties. For example, the optical properties of the dye can be tuned as a function of its geometrical arrangement inside the host molecule [1], and the dye spatial confinement in the complex may prevents/reduces quenching phenomena [2]. However, the elucidation of dynamic equilibria is not often trivial. Mixing data obtained from multiple analytical techniques is needed, and among these NMR experiments are crucial for structural elucidation, even though these systems often lead to complicated spectra with significant overlap and broad signals.

Here we exploit a thorough NMR characterization to investigate the encapsulation of a stilbazionium salt into a non-palindromic tris-(N)phenylureido calix[6]arene [3]. The mixing of the two components in a low polar solvent leads to the formation of two isomeric complexes endowed with different optical properties and in a temperature triggered dynamic equilibrium between each other. The NMR spectra taken on a 1:1 host/guest mixture were all characterized by complex signals patterns due both to a high conformational mobility of the complexed species and to exchange processes occurring on the NMR time scale. A plethora of NMR experiments, including DOSY, ROESY and VT experiments, were exploited to overcome the difficulties related to peak overlap and broad lineshapes. These experiments made it possible to assess that the two complexes differ for the orientation of the guest inside the host, the DOWN isomer shows the diethylamino group in proximity of the lower rim of the host, while in the UP isomer this group is oriented at the upper rim of the host. The spectroscopic optical measures on the mixture allowed to assess the two spectroscopic behaviors present in solution but are blind on their assignment to a specific geometry. A kinetic follow up of the temperature triggered equilibration process performed through both UV-vis and fluorescence and NMR experiments allowed to assess which optical properties belong to which isomer.

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DIFFERENTIATION AND IDENTIFICATION OF ENANTIOMERS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY WITH SUPPORT OF QUANTUM MECHANICAL COMPUTATIONS

Differentiation and identification of the chiral molecules are crucial parts of the characterization of active pharmaceutical ingredients (API). Therefore, the development of methods of the characterization of optical isomers impacts the pharmaceutical industry, and NMR spectroscopy is one of the tools used for this purpose.

Discrimination of chiral molecules by NMR is based on indirect methods since, in normal conditions, the NMR spectra of both enantiomers are indistinguishable. These methods utilize the formation of diastereomers whose spectra manifest chirality-induced shifts of NMR lines. For instance, one can use chemical solvating agents (CSAs), chemical derivatizing agents (CDAs), complexes with metals, and chiral liquid crystals to differentiate enantiomers [1–3]. The observed peak shifts may be attributed to the presence of a given enantiomer using empirical rules; however, a much more effective and general method would apply results of quantum mechanical computations.

We tested the latter approach using 1-phenyl-1,2,3,4-tetrahydroisoquinoline (**1**) and 1-azabicyclo[2.2.2]octan-3-ol (**2**). The compounds are chiral building blocks of solifenacin succinate, the API used to treat overactive bladder. W used two CDAs: Mosher's acid (**3**) and 1-(9-anthryl)-2,2,2-trifluoroethanol (**4**). In the racemic mixtures of **1**, the shifts of ^{13}C NMR peaks due to the presence of the CDAs were 0.05 ppm using (*R*)-**3** and 0.10 ppm after application of (*R*)-**4**. For compound (3*R*)-**2**, ^{1}H NMR shifts induced by CDA were of the order for 0.06 ppm.

Quantum chemical computations were performed in the Dalton computer program for the same pairs of compounds as measured experimentally using the density functional theory. Then, obtained in computations, nuclear magnetic shielding was converted to chemical shifts using data from Ref. [4]. Our final results show that the small amplitude of the induced chiral shifts makes it necessary to model the dynamic interaction between studied compounds and CDA very accurately since intermolecular interactions may affect the observed shifts in a degree comparable with that induced by CDA.

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RAPID CHARACTERIZATION OF TETRAHYDROFURFURYL 3-MACROZONE DERIVATIVE REACTION MIXTURE COMPONENTS USING LC-SPE/NMR APPROACH

Macrozones are novel bioactive conjugates of azithromycin and thiosemicarbazones that possess very good *in vitro* antibacterial activity against selected Gram-positive and Gram-negative bacteria [1]. Due to a global problem of growing bacterial resistance, discovery of new antibiotics is of an outmost importance and various studies are in progress to overcome the resistance mechanisms [2-3].

Efficient and rapid isolation, as well as structural characterization, of newly synthesized compounds are crucial steps prior to biological evaluation. Furthermore, impurity profiling is very important procedure during the drug development in pharmaceutical industry. Classical purification methods, such as preparative and semi-preparative liquid chromatography, can be time and solvent consuming. Using hyphenated systems enables shorter analysis time and reduces solvent consumption [4-5].

Hyphenated LC-SPE/NMR system enables efficient one step separation of reaction mixture components, extraction of each individual component on SPE-cartridge and structural characterization of isolated compounds by NMR spectroscopy. In this study we have successfully isolated and structurally characterized the newly synthesized tetrahydrofurfuryl 3-macrozone derivative and main reaction mixture components using the LC-SPE/NMR methodology. Structures of the isolated compounds were readily elucidated on the basis of one- and two-dimensional NMR spectra and MS spectra. Some of the isolated components were diastereomers which were additionaly analysed by NOESY spectroscopy.

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ILLUMINATED-NMR SPECTROSCOPY FOR THE MONITORING OF THE PHOTODIMERIZATION PROCESSES OF EXEMPLARY ANTHRACENE DERIVATIVES

It has been recently demonstrated that Illuminated Nuclear Magnetic Resonance (INMR) spectroscopy can be a very powerful tool for investigation of photo-driven systems, as this method offers in-depth insight into molecular structures, as well as different chemical processes at the molecular level [1]. Photochemical reactions of anthracenes have long provoked interest due to their numerous applications. This class of aromatic organic compounds plays an extremely important role as multi-faceted organic building blocks in nearly all aspects of chemical and industrial research [2], especially in the hi-tech and green energy sectors. Therefore, the goal of this work was to implement the INMR for the monitoring of their photodimerization.

Anthracene and its derivative 9-bromoanthracene were chosen as the model compounds. Unless stated otherwise, NMR experiments were performed at 9.4 T magnetic field after illuminating the samples with a 365 nm wavelength LED. Dichloromethane was chosen as a solvent. Different experimental conditions, such as the oxygenation level, the method of sample preparation, the concentration, as well as the volume of the illuminated sample were investigated, and the effect of these conditions on photoreactivity was evaluated.

The analysis of reaction curves derived from ^{1}H-NMR spectra revealed that the photodimerization of anthracene was about 10 times faster compared to 9-bromoanthracene. However, the experimental results showed that the reaction rate of the photodimerization strongly depends on the experimental set-up. Therefore, a mixture of anthracene and 9-bromoanthracene was also investigated. Thus, in the one sample with two compounds the photoreactions were monitored under the same conditions. Estimated reaction rates showed that the this time photodimerization of anthracene was about 2 times faster compared to 9-bromoanthracene. Moreover, in the system with two substrates, the formation of three possible products was demonstrated.

The presented approach helped us to evaluate the experimental conditions and their influence on the photochemical reactions of anthracenes, and suggested that the relative reactivity obtained from the system with two substrates is more informative when the comparison of several compounds is a goal. This will result in a better understanding of the photochemical processes, which is essential for the practical utilization of the photodimerization of acenes. Moreover, the approach can be easily extended to different photoactive reactions and processes. The obtained knowledge opens up an easy and reliable route for further development of the in-situ illuminated-NMR monitoring system.

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NMR CHARACTERIZATION OF POLYSACCHARIDE DERIVATIVE SCAFFOLDS FOR REGENERATIVE MEDICINE

Nature itself can built multicomplex but still spatially resolved stable structures as shown on this image of bacterial cell wall and knee cartilage. Idea of our colleagues from Maribor and Graz that design new biomaterials is to build structures close to nature, using bioprinting and materials which are already present in living organism. Such biomaterials can then be used for bone tissue regeneration.

In this study we characterized several polysaccharide-amino acid conjugates. Two different types of amino acid esters: glycine- and tryptophan- were conjugated to the polysaccharide carboxymethylcellulose (CMC) in water using carbodiimide at ambient conditions.

Characterization of both CMC-amino acid conjugates is based on NMR spectra, which includes ^{1}H, ^{13}C-DEPT 135 as well as two-dimensional ^{1}H-^{13}C HSQC/HMBC correlation spectroscopy. Besides structure characterization there were two questions that we tried to answer: can we provide solid and direct evidence for the successful conjugation of the amino acid esters to the CMC backbone via an amide bond?

Long-range proton-carbon correlation signals in the HMBC of the CMC-Gly-OMe between allowed unambiguous identification of the carboxyl ester group. Furthermore, the methylene group of the glycine moiety showed correlation signals to both carboxyl ester and carboxamide carbons. The second goal was to determine the degree of substitution, which means the amount of Trp-OMe that was found in Trp-OMe conjugates. The example here shows quantification of all functional groups, which were unambiguously assigned. Calculation confirms that the ratio between tryptophan and hyaluronic acid scaffold is approximately 1:3.

NMR characaterization of novel polysaccharide derivatives is an important step in further design and development of materials used for regenerative medicine.

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DETERMINATION OF SULPHUR-33 NUCLEAR MAGNETIC MOMENT FROM GAS-PHASE NMR STUDIES OF ^{3}HE AND SF_6

Gas-phase NMR spectroscopy can provide highly accurate and precise values of nuclear magnetic dipole moments. Results of measurements conducted in condensed phases require corrections for nuclear magnetic shielding, bulk magnetic susceptibility, and intermolecular interactions. The latter factor may be omitted in the gas-phase studies since extrapolating the resonance frequency to the zero-pressure limit removes the intermolecular effects [1].

We examined the density-dependences of ^{19}F, ^{33}S, and ^{3}He resonance frequencies in gaseous mixtures of helium and sulfur hexafluoride. The gaseous samples contained small amounts of ^{3}He in excess of SF_6 used as a buffer gas. As the reference nucleus, which NMR parameters are known with high precision, helium-3 was used in this study [2]. The total densities of the samples were from 0.22 to 1.00 mol/L. The ^{19}F, ^{33}S, and ^{3}He NMR resonance frequencies were measured using the Varian INOVA 500 NMR spectrometer at T = 300 K. They are collected in the table below (for shielding constants, see ref. [3]).

nucleus	spin	extrapolated NMR frequency (MHz)	shielding constant (ppm)	nuclear magnetic moment (nuclear magneton)
^{33}S	3/2	38.4197875(6)	392.6 (rel.)	0.6432555(10)
			350.0 (non rel.)	0.6432281(10)
^{3}He	1/2	381.3575177(5)	59.96743(10)	2.127625308(10)
^{19}F	1/2	471.0653234(5)	158.3 (rel.)	2.628321(13)
			157.8 (non rel.)	

At the constant external magnetic field B_0 = 11.75 T, the measured resonance frequencies are linearly dependent on SF_6 densities. New experimental value $\mu(^{33}S)$ calculated against that of ^{3}He is +0.6430416(10) μ_N without shielding corrections and +0.6432555(10) μ_N with these corrections calculated in relativistic approach. This more accurate result generally agrees with previously reported results [1]. Recalculated from these data isotropic ^{19}F shielding in SF_6 molecule is $\sigma_0(^{19}F)$ = 406.2 ppm. It is significantly higher than the absolute shielding constant obtained from the relativistic theoretical approach. It means the necessity of new measurements ^{19}F nuclear moment corrected by relativistic factors.

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A SIMPLE FLOW CHART DIAGRAM BASED ON ^{1}H–^{15}N HMBC NMR SPECTROSCOPY FOR DISTINGUISHING BETWEEN ISOMERIC AZAINDOLES IN NEW ILLEGAL DRUGS

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Recently, the development of new synthetic cannabinoid receptor agonists (SCARs), which are classified as psychoactive substances, has greatly progressed. However, their evolvement represents new challenges in terms of their detection and identification. Due to the lack of data, distinguishing between SCARs of isomeric nitrogen containing heterocyclic structure is difficult and represents one of the main obstacles [1]. The use of NMR spectroscopy, which is a corner-stone method for the identification and characterization of organic compounds, is of great help in solving this problem [2]. In particular, for the nitrogen containing compound, ^{1}H– ^{15}N HMBC spectroscopy plays an important role in structural identification [3].

Herein, a simple and rapid tool, based on ^{1}H and ^{1}H– ^{15}N HMBC NMR spectroscopy for identification of different isomeric azaindole type of SCARs, is presented. The constructed flow chart diagram was also tested on seized sample, 5F-MDMB-P7AICA.

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A RAPID DETERMINATION OF AZAINDOLE CORE IN NEW ILLEGAL SUBSTANCES BY ^{1}H–^{15}N HMBC NMR CORRELATION SPECTROSCOPY

One of the most important techniques for the analysis of the natural occurring and synthetic compounds is NMR (nuclear magnetic resonance) spectroscopy [1]. Various experiments using 2D NMR techniques such as COSY, HSQC and HMBC enable us to identify unknown complex compounds and determine their structure. 2D NMR techniques in addition to proton–carbon correlations, also allow us to determine the proton–nitrogen framework [2].

A rapid growth in the number of new synthetic cannabinoid receptor agonists (SCARs) renders this group of new psychoactive substances particularly demanding in terms of detection, identification, and responding. With no reference data available, differentiation and structural elucidation of constitutional isomers represents one of the major challenges. Since nitrogen is a common element in biological organic compounds, ^{1}H–^{15}N HMBC plays an important role in structural identification [3].

In determining the structure of nitrogen containing compounds, the use of ^{1}H and ^{1}H–^{15}N HMBC NMR is inevitable, but sometimes interpretation of results can be time consuming. For this purpose, we constructed a simple flow chart diagram based on ^{1}H and ^{1}H–^{15}N HMBC NMR spectroscopy for rapid and efficient determination of azaindole core.

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ASSURE ACCURACY OF YOUR QUANTITATIVE NMR RESULTS BY qNMR STANDARDS, PERFORMANCE QUALIFICATION AND PROFICIENCY TESTING

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Since quantitative NMR (qNMR) spectroscopy is considered a relative primary method [1-3], the technology is becoming increasingly popular for the characterization and potency determination of organic compounds. In comparison to LC or GC applications the method has a variety of advantages as no substance specific calibration is needed. However, in order to achieve accurate results three factors are of highest importance, which will be described in detail in this presentation:

First, the method depends on the availability of suitable standards e.g. Certified Reference Material (CRM). CRM are characterized by specific requirements such as traceability to the SI unit, measurement uncertainty, homogeneity assessment, stability testing and a hard expiry date. All these attributes are defined in ISO/IEC 17025 and ISO 17034 [4,5]. Through the last years, Merck has been working on the development of neat CRM for use in ^{1}H, ^{31}P and ^{19}F-qNMR as well as pre-dissolved ready-to-use standards [6,7].

Second, it has to be demonstrated especially in regulated environments that the NMR instrument itself performs according to requirements given by the authorities or guidelines. Through a collaboration between Bruker and Merck a two-component mixture has been designed for its use as quantitative performance qualification (qPQ) sample. The mixture consists of two CRM for qNMR in DMSO-d6 delivered in ampules and can be applied in combination with Bruker's Assure software [8].

The last important factor is the proficiency of the personal operating NMR instruments and performing experiments. To assure high quality and accuracy of measurement results these individuals have to demonstrate their expertise in regular intervals. For this purpose, we developed a qNMR proficiency testing scheme, through which participants can compare their skills by reporting back their results against a validated mean value.

The implementation of these three pillars – qNMR CRM standards, performance qualification and proficiency testing – assures precision and accuracy of quantitative NMR measurements.

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HYDROGEN BONDING OF METHYLATED NUCLEOBASE ANALOGUES STUDIED BY NMR SPECTROSCOPY

Hydrogen bonding (H-bonding) is a type of weak noncovalent interaction with enormous importance for the life on Earth. They determine, for example, the structure and proper function of proteins and nucleic acids, however, they are an order of magnitude weaker than covalent bonds. According to Grabowski there are three types of hydrogen bonds – weak (bond energy between 0.5–4 kcal/mol), moderate (4–15 kcal/mol) and strong (15–60 kcal/mol) [1].

The intermolecular binding in nucleic acids (NAs) was described by Watson and Crick in 1953 [2] and further extended by Karst Hoogsteen few years later [3]. In Hoogsteen base pairing is purine base flipped by 180°, and the nitrogen atom in position 7 is involved in hydrogen bond. This alternative pairing was found e.g. in DNA triplexes [4] and quadruplexes [5].

The methylation of nucleobases is one of the most important epigenetic mechanism associated with aging, genomic imprinting and tumorigenesis [6]. Adenine methylation in position 6 has been considered absent in human genome until 2018 [7]. Nowaday, this modification is related to development of obesity and Alzheimer's disease. The methyl group in position 6 can adopt two orientations because the bond between (methyl)amino group and purine ring is of an order higher than one, and the rotation around this bond is restricted. Each of the conformation isomers (rotamers) has a different hydrogen bonding pattern, and the steric hindrance of methyl group discriminates Watson-Crick or Hoogsteen base-pairing site.

In this work we present the NMR and DFT study of intermolecular hydrogen bonds of methylated adenine analogues with their complemental partner, thymine. We studied the binding interactions of adenine derivatives by low-temperature NMR in different solvents. We found out that the *N*-methylation stabilizes Hoogsteen base pairing. Hoogsteen base pairing is also preferred for those adenine derivatives which are able to form both (Watson-Crick and Hoogsteen) complex types via two H-bonds.

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TENSORVIEW FOR MATLAB

TensorView for Matlab[1] is a molecular visualization tool with a focus on NMR tensors, which is a transformative work from professor Leonard Mueller's TensorView, which is made for Mathematica.[2] Good visualization tools are needed for researchers and within undergraduate education to mediate some of the more spatial concepts within the field of NMR. In particular for tensor rotations, the lack of conventions can cause great confusion for the uninitiated. For that reason, a toolbox is included with a few common rotation schemes used within the scientific community. The graphical user interface version of TensorView for Matlab can be run without a Matlab license using Matlab runtime, and is available for free through github at:

https://github.com/LeoSvenningsson/TensorViewforMatlab. A script version of TensorView for Matlab with all source files are also available that can easily be glued together with other software.

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INVESTIGATING THE ELUSIVE OXYGEN DONOR COPPER SITE WITHIN A PROTEIN TYPE SCAFFOLD

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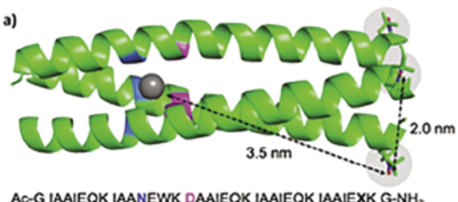

Ac-G IAAIEQK IAANEWK DAAIEQK IAAIEQK IAAIEXK G-NH_2
MB1-2, X is Q. MB1-2_{TOAC}, X is TOAC (shown in figure)

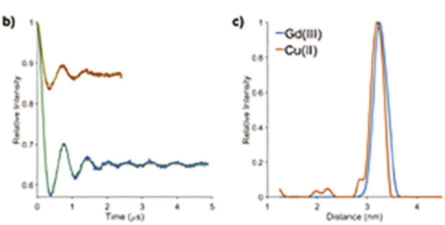

Figure: (a) Cartoon representation, rendered using ALLNOX, of models of the peptides studied in this work. The grey sphere is the proposed metal binding site. The predicted distances between a metal in the engineered binding site and the nitroxide radical of the TOAC spin label, and the inter TOAC distances, are presented. Four-pulse DEER experimental data for Gd(MB1-2_{TOAC})$_3$ (blue) and Cu(MB1-2_{TOAC})$_3$ (orange) showing (b) the time domain data after background correction and (c) the corresponding distance distributions. DEER experiments were performed at 10 K at Q-band and 15 K at X-band. Data were processed using DEERAnalysis2019.

Here we report on the preparation and characterisation of a highly elusive Cu(II) binding site bound exclusively to oxygen donor atoms within a miniature artificial protein scaffold, a parallel three-stranded coiled coil, through an oxygen-rich binding site. Though originally designed for binding of lanthanides[1] (e.g. Gd(III)), the copper binding has been characterised by a range of spectroscopic techniques, and in particular EPR. EPR experiments with MB1-2 (Figure 1a) were able to unambiguously rule out nitrogen coordination as they found no evidence of either weakly coupled or directly bound nitrogen. The results were consistent with Cu(II) bound exclusively to oxygen donors, with evidence of directly bound water. EPR distance measurements using DEER and RIDME between bound Cu(II) and nitroxide radical spin label MB1-2TOAC (Figure 1) further verified this assignment as comparison of the distance distributions reveal that both Gd(III) and Cu(II) appear to be binding at the same site. Despite coppers prevalence in biology and its affinity for oxygen donors, proteinaceous CuOx binding sites remain elusive. However, this technique demonstrates the power of a protein design approach to access chemistry not, to the best of our knowledge, exploited by biology.

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METABOLIC PROFILE OF PATIENTS RECOVERED FROM COVID-19 USING 1H-NMR METABOLOMICS

SARS-CoV-2 is a highly transmissible virus described for the first time in Wuhan (China) in December 2019. COVID-19 dissease has an incubation period from 1 to 14 days resulting in a plethora of symptoms and can cause even death in the most severe cases. On the metabolomic aspect of COVID-19, although there are several studies which describe that this is a systemic infection whith a significant impact on the metabolism [1], the question remains of to what extent the metabolism of patients recovered from this disease returns to normal values. NMR-based metabolomics, used in this study, is able to quantify and identify metabolites and lipoproteins at the same time [2]. The aim of this work was to determine whether there is a recovery of the metabolic and/or lipoprotein profile associated with patients diagnosed and recovered from COVID-19.

We analyzed plasma samples from healthy donors (n=73) and patients that had been diagnosed with COVID-19 by a RT-PCR assay and have recovered with at least 3 months of delay after the acute phase of the disease (n=70). The samples were measured in a 600 MHz IVDr spectrometer (Bruker Biospin, Germany), daily calibrated by following all the SOPs to ensure reproducibility and the highest spectral quality. Three different ^{1}H NMR experiments were recorded per sample at 310 K: a (1D) ^{1}H NOESY, a (1D) ^{1}H CPMG and a (2D) JResolved experiment. Individual metabolite or lipoprotein subclasses were quantified with Bruker's B.I.Quant-PS and B.I.LISA methods and the effect size and statistical difference of recovered vs. negatives were summarized in forest plots, while data distribution and the probability density were represented by violin plots.

Our preliminary results showed a decreased of ketone bodies acetoacetic acid, 3-hydroxybutyric acid and acetone, which are produced predominantly in the liver from fatty acid oxidation-derived acetyl-CoA and are accumulated during acute phase of COVID-19 [1]. Also the synthesis of 2-hydroxybutyric acid, marker of oxidative stress, was restored to normal values in recovered patients. Moreover, we observed a similar level of TG-VLDL and Apo-B/Apo-A1 ratio in recovered patients vs. healthy donors that could be due to a reestablished hepatic capacity to oxidize acetyl-CoA in the mitochondria. However, the level of Glyc A, a maker related to a general inflammation, is elevated as well as COVID-19 positive patients in the acute phase of the disease [3].

These results suggest that some of the changes that COVID-19 induces in the fatty acid metabolism of the SARS-CoV-2 positive patients can be reversed to healthy values after their recovery. However, Glyc A inflammation marker is still increased after 3 months of the SARS-COV-2 infection. Further studies are necessary using a larger cohort of recovered individuals from COVID-19.

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COMBINED NMR SPECTROSCOPY AND COMPUTATIONAL STUDIES REVEAL NOVEL LOX INHIBITOR

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Lipoxygenase (LOX) is an enzyme playing a key-role in the inflammation pathway. LOX's metabolites are linked not only to inflammation but also to a variety of different diseases such as asthma, atherosclerosis, rheumatoid arthritis, psoriasis, brain disorders and cancer [1]. Recently, the concept of "drug repurposing" has gained ground since it promotes the discovery of novel uses for approved drugs as a mean to deliver the quickest possible transition from bench to bedside [2] . Food supplements have been also explored for their potent pharmaceutical properties [3]. The discovery of special properties to such compounds may accelerate their commercial circulation, since they have already been assessed for their safety and toxicity. In this investigation, NMR experiments in combination with *in silico* studies have been performed in order to explore the potency of aspartame to serve as an anti-inflammatory drug targeting LOX enzyme. Molecular docking studies indicated that aspartame presents strong binding to LOX's active site, while the stability of "LOX-aspartame" complex was further evaluated by molecular dynamics simulations. The molecular profile of aspartame inside the enzyme's cavity was evaluated by Saturation Transfer Difference (STD) NMR experiments, where all the interactions that aspartame forms with the residues of LOX's cavity have been discovered. These critical interactions unveiled by STD NMR have been confirmed by Quantum Mechanics calculations. Furthermore, in *vitro* biological assays indicated the compound's inhibitory activity against LOX and pinpoint aspartame as a significant compound that could lead the anti-LOX drug design process.

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INTERACTIONS OF THE SARS-CoV-2 Nsp3 SUD M DOMAIN WITH 3'-UTR G-QUADRUPLEXES OF HOST mRNA

The present work focuses on the discovery of potential interactions of the non-structural protein 3 (Nsp3) of the SARS-CoV-2 with four-stranded DNA structures – G-quadruplexes –found in human mRNA 3'-untranslated regions (3'-UTRs). Nsp3 consists of 1946 amino acid residues, which makes it the largest viral non-structural protein in SARS-CoV-2. It is comprised of 8 domains, including three SARS unique domains (SUDs) [1,2]. The roles of several domains, including SUD M, remain unknown, but there is evidence that Nsp3 associates with Nsp4 andNsp6 to form a molecular pore complex in double membrane vesicles that function as replication factories for the virus in the host cell [3]. It has also been shown that the absence of SUD M abolishes viral replication in vivo. It has been previously reported that SARS-CoV SUD M binds G-quadruplexes. Comparison of the genomic RNA sequences of SARS-CoV and SARS-CoV-2 show sequence homology in the putative binding region of SUD M [4,5]. Therefore, we hypothesize that the same domain inSARS-CoV-2 will also bind G-quadruplexes. Because the SARS-CoV-2 genomic RNA contains no classical G-quadruplex-forming sequences, we searched for interactions with host RNA instead, focusing on potential G-quadruplex-forming sequences found in the 3'-UTR mRNA transcripts of human genes coding for the mitogen MAPK 1, TAB 3 protein involved in NF-κB signalling, the pro-apoptotic protein Bbc3, and the RAS oncogene family RAB6B. Folding of the selected G-rich sequences was evaluated in the presence of potassium cations alone, and in the presence of a peptide originating from the SUD M domain of Nsp3. We showed that the peptide interacts with several of G-rich oligonucleotides and induces the formation of distinct G-quadruplex structures.

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GUANINE RICH SEQUENCES IN GENOME OF SARS-CoV-2

As the world has been shaken by the COVID-19 pandemic, the scientific world has seemingly never concentrated on one single subject more than on the SARS-CoV-2 coronavirus. Regulatory regions of viral genomes often contain guanine rich sequences that form structures called G-quadruplexes (G4s), with G4 ligands displaying antiviral properties. G4s are four-stranded nucleic acid secondary structures that form through self-recognition of guanine bases. G4s are involved in important cellular processes such as gene expression, telomere stabilization, transcription and translation [1]. Guanine rich sequences of SARS-CoV-2 genome are therefore considered a promising therapeutic target [2].

The typical guanine rich sequences in the form of $G_{2-5}N_{0-7}G_{2-5}N_{0-7}G_{2-5}N_{0-7}G_{2-5}$, where N is any nucleotide, occur less frequently in the SARS-Cov-2 genome compared to SARS-CoV [3]. This presents an opportunity to investigate atypical G-rich sequences with bulges and hairpin stem loops, which simultaneously represent additional sites for ligand binding. Moreover, hairpin stem loops were shown to stabilize the G4s and promote their folding [4,5]. Dual ligands that bind both duplex and G4 structures could also resolve common G4 ligands problems of non-selectivity and low specificity [6]. Many algorithms that find putative typical G4s exist, and our goal is to re-formulate chosen algorithms for atypical G4 detection to include both bulges and hairpin stem loops and characterize them *in vitro* with NMR spectroscopy and other biophysical techniques.

We are re-formulating two chosen existing algorithms G4Hunter and QPARSE [7,8]. G4Hunter is a scoring algorithm that finds typical putative G4s and has an advantage of negatively scoring cytosine residues that would form duplexes with guanine residues, whereas QPARSE searches for degenerate potential G4s. It is a novel graph-based algorithm with a dynamic programming approach that includes search for hairpins and bulges, but only in the G4 loop regions (N tracts), not in between G tracts. Reformulating these two algorithms with distinct advantages is thus our current approach on the way to find atypical G-quadruplexes in the genome of SARS-CoV-2.

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INTERACTIONS OF AZITHROMYCIN AMINOPROPYL DERIVATIVES WITH *E. COLI* RIBOSOME STUDIED BY NMR SPECTROSCOPY

Azithromycin is a semisynthetic macrolide antibiotic which possess excellent pharmacokinetic properties and demonstrates satisfactory biological effect. Macrolides are bacteriostatic antibiotics. They exert their biological activity by binding to the 50S subunit of the ribosome. Macrolides are effective against Gram-positive and some Gram-negative bacterial strains [1,2].

Due to the frequent and improper use of drugs bacterial resistance has emerged, so there is a need to discover more effective antibiotics. Azithromycin aminopropyl derivatives are precursors in synthesis of macrozones. Macrozones are novel bioactive macrolide derivatives and conjugates of azithromycin and thiosemicarbazones [3].

In order to fully understand the mechanism of macrozone bioactivity it is important to determine their bound conformation and interactions with biological targets such as ribosome [4].

In our study, we used NMR spectroscopy to analyse binding of azithromycin derivatives with ribosome isolated from *Escherichia coli*. Conformations in free and bound state were studied by NOESY (nuclear Overhauser effect spectroscopy) and trNOESY (transferred nuclear Overhauser effect spectroscopy) experiments. STD NMR (Saturation transfer difference Spectroscopy) was used to determine the binding epitopes of azithromycin derivatives. By these techniques relationship between structure and activity will be explored.

These results should contribute to the discovery and design of new, more effective macrolide antibiotics.

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SEMAPHORIN3A - GLYCOSAMINOGLYCANS INTERACTION AS TARGET FOR AXONAL REGENERATION

Semaphorin 3A (Sema3A) is a cell secreted protein that participates in the axonal guidance pathways. In the adult central nervous system (CNS), Sema3A acts as a canonical repulsive axon guidance molecule, inhibiting CNS regenerative axonal growth and sprouting. Therefore, interfering with Sema3A signaling is a therapeutic target for achieving functional recovery after CNS injuries. Moreover, several studies have suggested a role for proteoglycans (PGs) in Sema3A and its Nrp1 receptor function. Some studies showed that Sema3A adheres to the PG component of the extracellular matrix (ECM) at perineuronal nets or cortical neurons, and selectively binds to heparin and chondroitin sulfate-E (CS-E) glycosaminoglycan (GAG) [1]. In our previous work, we identified a peptoid, termed SICHI (semaphorin-induced chemorepulsion inhibitor), that blocks Sema3A chemorepulsion and growth-cone collapse in axons at the extracellular level [2]. Later, we proposed that SICHI blocks the biologically relevant interaction between Sema3A and GAGs competing with Sema3A C-terminal polybasic region for binding to GAGs [3]. For these earlier studies, we used basic peptides derived for Sema3A basic tail.

We present our recent work, in which the main puporse was to characterize the interaction of the whole Sema3A C-terminal polybasic region (Sema3A 725-771) with GAGs and its inhibition. First, we produced, purified ^{15}N,^{13}C-labeled basic domain and performed the backbone assignment by acquiring 3D ^{1}H and ^{13}C direct detected NMR experiments. The limited spectral dispersion, and the lack of defined secondary structure elements, predicted based on chemical shifts, categorizes human Sema3A C-terminal polybasic region as an intrinsically disordered region. Next, we used a combination of biophysical techniques (NMR, SPR, Fluorescence and Heparin Affinity Chromatography) to gain insight into the interaction of the Sema3A C-terminal domain with GAGs. These analyses confirmed that Sema3A C-terminal polybasic region binds to GAGs, preferably to heparin, and allowed us to identify the specific residues involved in the interaction. Last, we studied the effect of a new peptoid molecule (CSIC002) in the ineraction between Sema3A and heparin (dp14 oligosaccharide). We observed a displacement of Sema3A basic tail from heparin by CSIC002 using 2D NMR ^{1}H,^{15}N-HSQC spectra chemical shift pertubation. Our structural study paves the way toward the design of new molecules targeting these protein-GAG interactions with potential therapeutic applications.

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BACKBONE ASSIGNMENT OF Cov-Y DOMAIN FROM NONSTRUCTURAL PROTEIN 3 OF SARS-Cov-2

Like other (+) RNA viruses severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2 or covid-19) forms specialized membranous organelles in infected cells. These double membrane vesicles (DMVs) made from ER membrane serve the purpose of creating favorable microenvironment for viral replication machinery by concentrating viral components and at the same time shielding them from the host immune defenses. Nonstructural protein 3 (nsp3) of SARS-Cov-2 together with nsp4 and nsp6 are involved in DMV formation. Nsp3 has 1945 residues that comprise more than a dozen domains. The C-terminal part of SARS-Cov-1 nsp3, including two transmembrane and three extramembrane domains, is necessary for DMV formation [1]. This region is highly conserved among all coronaviruses but there has been no structural information on any C-terminal domains previously reported. Here we present an NMR study of the most C-terminal domain of nsp3, Cov-Y.

We reported protein expression and purification protocol for nsp3 Cov-Y of SARS-Cov-2 in a collaborative article with the international covid-19 research consortium [2]. Here we present an optimized construct with the first 22 flexible residues deleted. The final protein sequence contains the most C-terminal 286 residues of nsp3 (1660-1945). It was expressed and purified as described [1] at a yield of 20mg of the protein from 1L deuterated M9 media with ^{15}N-labeled ammonium chloride and ^{13}C-labeled glucose. All NMR experiments were done with a sample of 0.35 mM ^{2}H,^{13}C,^{15}N-labeled nsp3 Cov-Y in 25 mM MOPS buffer pH 6.4 with 100 mM LiBr and 2 mM DTT.

Backbone assignment of nsp3 Cov-Y from SARS-Cov-2 were performed with TROSY-based triple resonance experiments: HNCO, HNCA, HNCACB, HNCACO and ^{15}N-NOESY. All NMR experiments were acquired at 25°C using an Agilent 800MHz spectrometer equipped with a cold probe.

Nsp3 Cov-Y is a fully folded protein domain with small flexible regions at the termini. Nearly full ^{1}H, ^{13}C and ^{15}N backbone assignment was obtained (96% H^N, 94% N, 96% C, 96% Cα, 90% Cβ). Secondary structure elements were predicted by Talos+ based on measured backbone chemical shifts. Nsp3 Cov-Y consists of 9 long (more than 5 residues) α-helixes and 11 β-strands. The excellent NMR data quality provides a firm basis for structure determination.

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THE DISORDERED REGIONS OF THE SARS-CoV2 NUCLEOCAPSID PROTEIN STUDIED BY CARBON-13 NMR EXPERIMENTS WITHIN THE 1-248 CONSTRUCT

The SARS-CoV2 nucleocapsid protein (N-protein) is a multifunctional and multidomain protein characterized by three intrinsically disordered regions (IDRs), which cover about 35% of the primary sequence, separated by two globular domains (NTD and CTD). Previous studies performed on the homologous protein from SARS-CoV demonstrated that the three IDRs are involved in different processes, ranging from binding with RNA to interaction with other viral proteins [1]. High-resolution data that could describe the role of these IDRs are fundamental to access atomic information about the whole N-protein both in its native conditions and upon interactions with partners and targets. We started focusing on the NMR characterization of the first two IDRs of N-protein, the N-terminal linker (IDR1, 1-44) and the central serine-arginine rich region (IDR2, 181-248) in the 1-248 construct (IDR1-NTD-IDR2). A set of ^{13}C detected experiments were acquired to assign the resonances of the disordered signals and to access atomic resolution for these two IDRs thanks to the high resolution that could be achieved with the carbon detection approach [2-3].

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NUCLEAR MAGNETIC RESONANCE AND *AB INITIO* STUDIES OF ORGANOTIN METALLOTHERAPEUTICS

This study involves the structure elucidation and conformational analysis of three metallotherapeutics organotin (IV) derivatives of cholic acid (CAH) with the formulae $R_3Sn(CA)$ (R= Ph- (**1**), *n*-Bu- (**2**)) and $R_2Sn(CA)_2$ (R= Me- (**3**). The structures of compounds (**1**-**3**) were determined using a combination of homonuclear and heteronuclear 2D NMR spectroscopy. Subsequently, ^{119}Sn NMR experiments and some semi-empirical quantum mechanics computations (method PM6) were performed, in order obtain the conformational properties and the possible geometry of complexes under study. Using ^{119}Sn chemical shifts of compounds (1-3) and comparing them to the reference compound $SnCl_4$, it was found that the geometry is triangular dipyramid for (**1**) and (**2**), and octahedral geometry for (**3**). Semi-empirical quantum mechanics computations PM6 [1], based on the Neglect of Diatomic Differential Overlap (NDDO), contributed to the estimate of an optimized conformation and the geometry of compounds too. According to the obtained results, their optimized geometry is distorted tetrahedral. Biological activity of these compounds is related with the conformation of atoms which surround the metal of Sn. Having received all *in vitro* results and obtaining the necessary information about the structure and the conformation of molecules, it was deemed necessary to perform *in silico* studies. This study was about the binding of compounds to the receptor 1A52 of ER-α (Estrogen receptor alpha ligand) hormone, to provide a plausible explanation and proceed to a rational design of new molecules.

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TUMOR LIPID SIGNATURES DESCRIPTIVE OF ACQUISITION OF RESISTANCE TO ENDOCRINE THERAPY IN AN ENDOCRINE-RELATED BREAST CANCER MOUSE MODEL

Breast cancer (BC) is the most common type of cancer in women and, in most cases, it is hormone-dependent (HD), thus relying on steroid hormones (estrogens/progestins) to activate intracellular receptors and stimulate tumor growth [1]. Endocrine therapy (ET) aiming at hormone receptors is the primary treatment strategy, however, about half of the subjects, develop resistance in time [2]. This involves the development of hormone independent (HI) tumors, initially therapy-responsive and which subsequently become resistant (HIR) to ET. The mechanisms that promote HI to HIR conversion are varied, and not completely understood. Lipid metabolism is affected by tumorigenesis and changes in lipid metabolism are associated with anti-cancer drug resistance [3]. Lipids can function as lipid signalling molecules, influence the metastatic potential of cancer cells, protect or enhance oxidative stress and are important mediators of the immune response [3]. Therefore, the aim of this work was to characterize the specific lipid signatures associated to the acquisiton of hormonal independence and of ET-resistance, using NMR metabolomics. Tumor tissue and non-compromissed mammary gland obtained from mice implanted subcutaneously with HD, HI and HIR tumors from the medroxyprogesterone acetate (MPA)-induced BC mouse model [4] were analyzed. Compared to healthy tissue, the tumors exhibited global increases in total/free cholesterol, phosphatitylcholine (PtC), phosphatidylethanolamine (PtE), sphingomyelin (SM), arachidonic/hexadecanoic acids (ARA/DHA), and decreases in monounsaturated and polyunsaturated fatty acids (MUFAs/PUFAs) and triacylglycerides (TAG). The HD to HI transition was characterised by increases in ARA/DHA and PtE and decreases in MUFAs/PUFAs and TAG. HI transition to HIR involved increases in ARA/DHA, free/total cholesterol, PtC, PtE and SM. Putative interpretation of these results is discussed and possible markers of resistance are suggested.

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METABOLIC MARKERS OF OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS THROUGH NMR METABOLOMICS

Mesenchymal stem cells (MSCs - multipotent cells) can differentiate into a variety of cell lineages, namely osteogenic, thus having great potential in bone regenerative medicine. Metabolomics offers exquisite insight into the metabolism of living organisms. However, only a few reports have monitored osteogenesis, with mass spectrometry approaches predominating [1-3] compared to nuclear magnetic resonance (NMR) spectroscopy [4]. NMR fingerprinting and preliminary results on footprinting of human adipose tissue MSCs (hAMSCs) during osteogenesis are presented, throughout the 21 days of osteogenesis in 2D cultures. Endometabolome results showed significant dynamic differences in several amino acids, creatine/phosphocreatine, choline compounds, glycerolipids, phospholipids, among others. Moreover, preliminary results indicate alanine, glycerol and citrate as important secretome components. A clear metabolic separation was observed before and after 7 days of culture, although metabolic changes are observed from early on until day 21. This work paves the way to characterize the dynamic metabolism of stem cells during osteogenesis, ultimately enabling its monitoring through metabolic biomarkers, eventually translatable to *in vivo* clinical tissue regeneration strategies.

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A METABOLIC SYNDROME DEFINITION BY URINE NMR-METABOLOMICS

Metabolic syndrome (MetS) is a complex health condition that arise as a result of the presence of various risk factors: problems with the metabolism of the glucose, obesity, elevated levels of triglycerides, low HDL cholesterol and hypertension. Due to the increasing number of people affected by this disorder, MetS constitutes a huge worlwide problem [1] but unfortunately, the molecular basis of MetS are not yet well understood. Several associations have tried to determine a medical definition for this syndrome but these diagnostic criteria are only based on the compatible symptomatology. In this study we have analised by NMR-metabolomics a large cohort of urine samples (from 11,754 individuals, 18-75 years old) to examine the molecular signature of MetS. Volounteers were classified into 16 different profiles to populate all possible intermediate conditions from the total absence of any risk factor up to the presence of individuals with MetS (4-5%, depending on the definition). NMR metabolomics has shown to be sensitive to MetS, with each one of the contributing risk factors represented by at least one of the identified metabolite that have shown to be up- or down-regulated in this disease. This led to the design of a metabolic model of the syndrome that can discriminate between individuals with and without MetS with statistical significance. Aging and non-alcoholic fatty liver disease (NAFLD) are also risk factors that have been previously associated with MetS [2,3], but our results evidenced that they do not directly interfere with the metabolic discrimination of the syndrome. In conclusion, thanks to the obtained results our model add an unprecedented diagnostic molecular dimension and may potentially improve clinical decision guiding early interventions in this syndrome.

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NMR METABOLIC PROFILE OF THE *IN VIVO* IMPACT OF A POTENTIAL ANTICANCER DRUG - PD_2(SPERMINE)

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Platinum (Pt(II))-containing drugs are currently some of the most used antineoplastic agents in the treatment of several types of solid tumors [1]. In particular, cisplatin (cDDP) is used in lung, testicular, breast, and ovarian cancer therapies, although tumor acquired-resistance and high toxicity are limiting factors for its clinical use [1]. Therefore, palladium (Pd(II))-complexes are increasingly emerging as alternatives to Pt(II)-based drugs, mostly due to chemical similarities to Pt(II) and initial promising anti-tumor properties [2]. In particular, Pd(II)-complexes with biogenic polyamines, such as spermine (Pd_2Spm), have exhibited beneficial cytotoxic properties [3,4]. Metabolomics can provide a holistic view of the metabolic profile of drug-exposed tissues, giving useful insights into drug mechanisms and potentially revealing new markers of drug efficacy and toxicity [5].

This work reports a ^{1}H NMR metabolomics characterization of the Pd_2Spm impact, compared to cDDP, on the metabolism of healthy mice. Polar and lipophilic extracts were obtained from kidney, liver, and breast tissue of female BALB/c mice, excised at 1, 12 and 48 h after a single-dose injection of 2.7 mg/kg Pd_2Spm, 3.5 mg/kg cDDP, and 200 μL of phosphate-buffered saline for the controls.

The magnitude and dynamics of metabolome response to both drugs are tissue-dependent, as expected, with kidney as the most affected organ, followed by liver and breast tissue. Polar metabolites tend to respond more strongly and rapidly (1 h) to Pd_2Spm than to cDDP in all tissues, with main variations in levels of amino acids, nucleotides, Krebs cycle intermediates (*e.g.* succinate and fumarate), and other metabolites such as dimethylamine and dimethyl sulfone. On the other hand, lipid metabolism exhibits a delayed (48 h) or weak response in kidney and liver/ breast tissue, respectively. In brief, Pd_2Spm triggers faster responses and recovery to control levels for all organs tested, except for kidney lipophilic metabolism. These results are suggestive of a potential lower adverse effects/ toxicity of Pd_2Spm exposure, encouraging its potential use as an anticancer drug for clinical trials.

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^{1}H NMR-BASED METABOLOMICS TO INVESTIGATE THE EFFECT OF PROBIOTICS ON HUMAN PHENOTYPE

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The human gut hosts around one-thousand of different species of commensal microorganisms that play a crucial role in health promotion, implementing host's physiology and metabolism [1]. In this perspective, probiotics are increasingly used, with the final aim of manipulating the composition of the gut microbiota, and with the aim of improving balanced microbial communities [2,3]. In this context, the challenge is to characterize and to understand potential metabolic changes that could determine and affet the dynamic relationship between host and microbiome.

Nuclear Magnetic Resonance (NMR)-based metabolomics offers the possibility to investigate hundreds of various metabolites and lipids detectable in biological fluids (*i.e.* serum, urine, etc.), providing a global representation of the molecular mechanisms and potential effects of the dynamic and evolving interactions between the microflora and host, and of the response to probiotic assumption [4,5].

In this study, using a NMR-based metabolomic approach, we highlighted the molecular effects obtained by microorganisms modulation through probiotic treatment, on human urine and serum metabolome. Twenty-one healthy volunteers were enrolled in the study and administered with two different dosage of probiotic (high and low) for a total of 8 weeks. 20 urine samples per subject and 1 serum sample per subject were collected before and during the probiotic assumpion. Univariate and multivariate statistical analyses were used to evaluate the ^{1}H-NMR urine and serum spectra acquired, and to characterize the individual effects of the treatment. After the treatment, probiotics influence the urinary metabolic profiles of the volunteers, without altering their subject-specific phenotypes. In particular we observed a cumulative effect of probiotics during the time of the administration. Modifications in metabolite levels, especially in glucose, isoleucine, valine, 3-hydroxyisobutyricacid, 4-hydroxyphenylacetate, and acetoaceticacid levels, were monitored. We observed, also, that probiotics influence the serum metabolic profiles, in particular they induce fluctuations in acetone, ascorbate, and citrate levels.

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SALIVA NMR METABOLOMICS IN THE SEARCH FOR GESTATIONAL DIABETES MELLITUS BIOMARKERS

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance with onset or first recognition during pregnancy [1]. Although diagnostic methods and therapies are available already, methods for the prediction of the condition and individual therapy response are still lacking. Hence, metabolomics of maternal urine and blood has searched for new GDM biomarkers [2] but little is known about the usefulness of saliva, a non-invasive and easy to collect biofluid, which has emerged more recently as a valuable resource for disease research [3].

This work reports a ^{1}H NMR metabolomics study of saliva to characterize its stability under typical handling/analytical conditions of sampling in a clinical setting. Results show that saliva is stable up to 6h at room temperature and at 4ºC. Applied to pregnant women, the effects of different GDM treatments (diet, insulin and metformin) were explored through the saliva metabolome of the subjects. Multi- and univariate analysis suggested that the saliva metabolic profile of GDM patients stabilizes upon diet treatment, while insulin or metformin therapies induce several more enduring changes, reflecting mostly the impact of diet and/or oral bacterial metabolism. The potential and challenges of the use of saliva as a mean to aid in GDM diagnosis and management are discussed.

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NMR METABOLOMICS IN TOXICOLOGY: EFFECT OF NANOPARTICLES INHALATION ON BLOOD PLASMA AND EXHALED BREATH CONDENSATE PROFILES

Although the application of nanoparticles (NPs) in different fields (electronics, optoelectronics, drug delivery and medical diagnostics) has been growing extremely, concerns about adverse effects on public health, consumer safety and occupational safety are not properly described and understood [1]. Since NPs are able to cross cell barriers, enter cells and interact with subcellular structures, a common response to NPs exposure is the induction of oxidative stress and inflammation [2]. Potential risks of NPs exposure need to be assessed and biomarkers need to be identified to enable early diagnosis and prevention of occupational diseases. Surprisingly, the standard operating procedures for monitoring NPs exposure at the work place are still insufficient.

Metabolomics offers a suitable strategy for analysis of individual subjects before and after NPs exposition, which allows to study perturbations in levels of metabolites and consequent alterations in metabolic pathways induced by acute toxicological effect. Moreover, comparison of subjects under long-term NPs exposition with healthy controls enables to assess chronic (subacute) toxicological effect [3]. In our study, ^{1}H NMR metabolomics was used to analyse samples of exhaled breath condensate and blood plasma of pre- and post-shift researchers perfimng various tasks related to the processing of nanocomposite materials and healthy individuals, who do not encounter NPs in their occupation. The main aim of this work is to evaluate the effect of NPs inhalation. The altered metabolite levels were identified in both biofluids. Thus, the metabolic pathways affected by NPs exposure were determined and differences in acute and chronic effects were observed. The achieved results confirm the applicability of NMR metabolomics in toxicological studies in the evaluation of the extent of toxic insult and understanding the molecular mechanism of nanoparticle-organism interaction.

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IDENTIFICATION OF METABOLIC PROFILES SPECIFIC FOR NORMAL AND TUMOR CELL LINES WITH THE GENERAL EXPLANATION METHOD AND NMR SPECTROSCOPY

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Potential of machine learning is recognized rapidly in the field of systems biology, where valuable knowledge is hidden in large amounts of data produced by various analytical techniques including NMR. Machine learning models in metabolomics, despite their excellent prediction accuracy are still not adopted widely due to the lack of efficient explanation of their predictions. In ourstudy we tested the use of the general explanation method [1] that enables intuitive interpretation of arbitrary machine learning model's predictions. The method was tested on a dataset of 1D ^{1}H NMR spectra acquired on normal and tumor cell lines. The random forests and artificial neural network models were applied to a dataset and showed excellent prediction accuracy. Our results demonstrate that by using the general explanation method to explain models' predictions, a concentration profiles of metabolites can be identified that discriminate individual normal and tumor cell line types [2]. We believe that our results will encourage scientists to use the general explanation method and give rise to further in-depth exploration toward metabolic phenotyping, biomarker identification and therapeutic target detection of complex diseases through employment of machine learning in datasets that include clinical and biological information.

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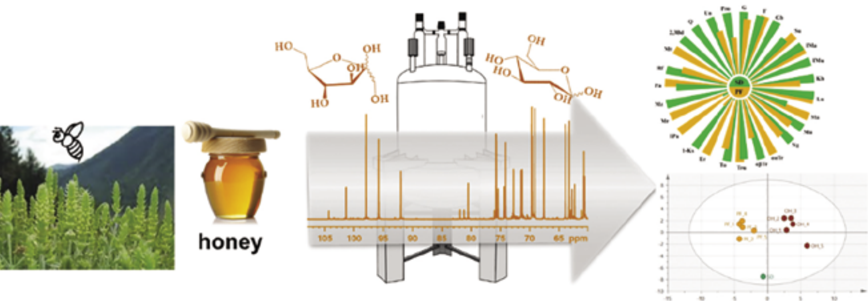
WHAT SPINS DIFFERENTLY IN PURE HONEY?

The variety of new honey types connected with the exploitation of the plant biodiversity, the arising environmental and ecological issues as well as the traditional question for food authentication constantly require introduction of appropriate methods of analysis. Numerous challenges arise in trying to keep the natural and administrative regulations for ensuring the consumer protection. The content, the labeling and even the nature of many components within honey are subject of growing interest and investigations. In recent years a number of scandals in different counties show the utility of NMR spectroscopy as an important method for complete analysis, covering numerous aspects related to authenticity, quality control and quantification.



Here, we present several recent investigations using the approuch of combined ^{13}C NMR profiling combined with different chemometric approaches to differentiate botanical and geographical origin of honey, to find biomarkers and to detect adulteration on the example of honeys from the region (Bulgaria, North Macedonia) and from stingless bees (Tanzania) [1,2,3].

The importance of honey as an effective natural therapy to attenuate acute inflammation is discussed in a recent study describing the potential therapeutic effects of honey in the context of the COVID-19 pandemic [4].

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NMR STUDIES OF SLOVENIAN HONEY

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Beekeeping is an important agricultural activity in Slovenia with a long and rich tradition. Slovenians are a beekeeping nation as more than 10,000 inhabitants are engaged in this activity. Honey is an important and widely used food product for nutrient as well as in cosmetic and medicinal purposes. It is one of the most adulterated foods worldwide[1] so the analysis and determination of chemical composition, especially carbohydrate composition, of Slovenian honey are needed to protect this valuable product. NMR spectroscopy is powerful tool in food analysis as it provides important structural and chemical characterization. Honey is mainly composed of a rich repertoire of sugars, fructose and glucose being predominant. The composition of sugars in honey depends on its geographical, botanical origin and other factors. Identification of carbohydrates is due to multiple isomeric forms and structural similarity very difficult. In our preliminary study we examined 25 samples of different types of Slovenian honey (forest, fir, linden, buckwheat, chestnut and acacia) with NMR in order to characterize chemical profiles specific for each group. Using 2D NMR homonuclear and heteronuclear techniques we achived assignment of carbohydrate peaks in 1D ^{1}H spectrum of honey samples.[2] With PCA analysis we established key features for each group. Melezitose is the main metabolite found in forest and fir honey. Higher amounts of glucose can be found in buckwheat and linden honey while higher amounts of fructose are specific for chestnut and acacia honey.

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CELIAC DISEASE AND NON-CELIAC GLUTEN SENSITIVITY TWO DIFFERENT GLUTEN-RELATED DISORDERS: A SERUM METABOLOMICS AND LIPIDOMICS STUDY

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Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible subjects. Under the inclusive definition of celiac disease, potential celiac disease (pCD) is defined by the presence of positive serum antibodies, HLA-DQ2/DQ8 haplotypes, and a normal small intestinal mucosa (Marsh grade 0-1)[1]. This condition occurs in one-fifth of CD patients and usually represents a clinical challenge. In the setting of gluten-related disorders is also present the non-celiac gluten sensitivity (GS), which has been defined as the development of intestinal and/or extraintestinal symptoms related to the ingestion of gluten in patients without celiac disease[2]. In GS neither allergic nor autoimmune mechanisms are involved, and is estimated that affected 6% of the European population[3]. This disease and its pathogenesis are yet poorly understood and relationships between GS and CD need to be further investigated. There are no diagnostic biomarkers available for GS, therefore the diagnosis is complex as symptoms overlap with those of CD, irritable bowel syndrome and inflammatory bowel disease. Metabolomics has already proved to be a valuable instrument for the study of the systemic metabolic alterations induced by CD with respect to controls[4–7]. The present study aims at providing further insights into the metabolic processes underpinning these three gluten-related disorders (overt CD, pCD, GS) via NMR-based serum metabolomics and lipidomics.

Multivariate analysis on NMR data allowed the selective discrimination between CD (overt and potential) and GS with high discrimination accuracies. In these discriminations both low molecular weight metabolites as well as lipoproteins significantly contribute. The most relevant pathways identified are Glyoxylate and dicarboxylate metabolism and Glycolysis/ Gluconeogenesis. Whereas oCD and pCD show only slight differences (accuracy ~65%).

In conclusion, NMR-based metabolomics seems to be effective in discriminating gluten-related disorders, this is particularly relavant for GS because no diagnostic tools are currently available.

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TOWARDS PH_2 HYPERPOLARIZED ANALYSIS OF URINE WITH MINIMAL SAMPLE MANIPULATION

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Urine is an easily obtainable highly complex biofluid containing thousands of compounds, with various chemical/physical properties and concentrations [1]. Ability to analyze these compounds is of great diagnostic and scientific interest. Nuclear magnetic resonance (NMR) is one of the principle analytical techniques used for the purpose, but many urinary metabolites occur below the NMR limit of detection (LOD). Sensitivity can be improved by Iridium catalyzed pH_2 hyperpolarized chemosensing (pH_2-HP) [2], where additional spectral resolution can be derived from 2D zero-quantum (ZQ) spectroscopy [3].

This method can detect nanomolar concentrations of nitrogen containing compounds [3]. However, direct analysis of urine is challenging, because of high quantity of ammonia in urine. Previously solid phase extraction (SPE) methods have been used to remove ammonia and other pH_2-HP interfering components like water, salts, protein and urea from urine. The SPE organic solvent provides optimal working conditions for the iridium catalyst [4]. The drawback of SPE based methods is the tendency to decrease overall metabolite coverage and, hence, SPE is often avoided in global analysis of compound in biofluids [5].

Herein we present the results of our current work, where ammonia, urea and water are removed from urine sample by raising the pH and freeze drying. Resulting solids are reconstituted with water and salts are precipitated with methanol. The resulting methanol-water mixture is compatible with the usual Iridium based pH_2-HP chemosensing catalyst and results in a reproducible hyperpolarized spectrum allowing to detect several endogenous metabolites that occur below the LOD of regular NMR.

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PROBING LIGAND COORDINATION DRIVEN SHAPE-SELECTIVE GROWTH OF NANOPARTICLES BY DNP NMR SPECTROSCOPY

Zinc oxide nanoparticles (NPs) find applications in numerous fields such as solar cells, sensors, medicine, paint, and rubber industries [1]. This has motivated researchers to optimize synthetic procedures to produce NPs with desired applications. The shape, such as spherical, rod-like or platelet, is among the key features that can be modulated to obtain specific function. Thus, there is a need to investigate the interface of these materials in order to control their morphology which is largely dependent on the nanocrystal-ligand interactions at this interface [2]. Among contemporary analytic techniques, DNP-enhanced solid-state NMR spectroscopy is highly suitable for characterizing these interfaces due to its site-specific detection ability coupled with significant gain in NMR sensitivity. In this presentation, it will be discussed how MAS-DNP NMR in combination with complementary techniques such as density functional theory (DFT) calculations and HRTEM, can be used to determine ligand coordination modes and atomic-scale arrangements on bi-faceted hexagonal ZnO nanoplatelets, that are typically used to produce highly-ordered ultra-thin (< 5nm) films which are considered as prime candidates for new generation optoelectronic devices [3]. Moreover, insights into the growth of these NPs will be provided. This work relating ligand binding modes and arrangement with particle morphology will allow controlled production of rationally-designed nanocrystals with specific functions.

More specifically, DNP enhanced ^{13}C homonuclear dipolar correlation spectra for carbon peaks from surface ligands at ^{13}C natural isotopic abundance will be presented. These were used to study ligand proximity and arrangement of ligands on the ZnO surface. Also, DNP-enhanced ^{13}C and ^{15}N MAS NMR spectra and ^{1}H-^{15}N heteronuclear correlations, recorded at natural isotopic abundance will be discussed. These spectra evidence the presence of various ligand-binding sites at the inorganic interface and these will be associated with different facets of the ZnO nanoplatelets. The rich structural information obtained from the NMR experiments is complemented with DFT chemical shift calculations of various ZnO surface models to help provide a full picture of the nanoplatelet interfaces.

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THE ROLE OF METHYL DYNAMICS IN DNP

Methyl NMR studies have become very popular during the last decades. The fast three-fold reorientation of the methyl group around its symmetry axis (H_3C-C) yields advantageous relaxation properties and thus well-resolved NMR spectra [1]. For instance, these properties are utilized in methyl TROSY HMQC which is commonly used for structure determination of large biomolecules. Moreover, it was recently shown that the three-fold reorientation is still active under DNP conditions [2]. The observed effect is exploited in SCREAM-DNP (Specific Cross Relaxation Enhancement by Active Motions under DNP). Here, polarization transfer from the hyperpolarized ^{1}H spins to ^{13}C is driven by the cross-relaxation-promoting methyl dynamics. The specifically hyperpolarized methyl-^{13}C can then be used, for example, for either measuring the binding of a ligand to an RNA aptamer [3] or to membrane proteins such as the green absorbing proteorhodopsin [4].

For a more detailed molecular understanding of the mechanism, the enhancement factors of SCREAM-DNP of all methyl-bearing amino acids were determined in a temperature range from 110 K to 165 K [5]. Therein, a clear correlation between the DNP enhancement and the expected methyl dynamics has been shown, providing the prospect of using SCREAM-DNP as a quantitative measure for local dynamics/crowding [4]. However, until now, no studies of methyl dynamics particularly under DNP conditions were performed. Thus, the aim of this project is the measurement of the three-fold reorientation dynamic under DNP conditions in various methyl-bearing molecules. Therefore, we studied three different methyl-bearing amino acids in which the methyl protons were selectively deuterated in polycrystalline powder. The selective deuteration enabled us to determine the correlation time of the three-fold reorientation dynamics by T_1 relaxation measurement in polycrystalline samples. The activation energy of the reorientation was further calculated by applying the Arrhenius equation yielding activation energies between 8 and 21 kJ/mol. The calculated activation energies show a large difference for Ala-$C_\beta D_3$ and Met-$C_\varepsilon D_3$, which explains the lower observed enhancement factor for Ala due to the higher energy barrier for the cross relaxation-promoting three-fold reorientation.

Experiments under DNP conditions of selectively deuterated molecules are currently underway. The preliminary results suggest a distribution of T_1 relaxation rates represented by a stretched exponential function as it was also shown in wetted protein powder [6]. However, these experiments have to be conducted in more detail.

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TRIPLE RESONANCE (E, ^{1}H, ^{13}C) PROBEHEAD FOR LIQUID-STATE DNP EXPERIMENTS AT 9.4 TESLA

In DNP experiments, nuclear spin polarization is enhanced by transferring the relatively larger electron polarization to target nuclei via microwave irradiation. Here, we describe the design and performance of a probehead for Overhauser DNP experiments on protons and ^{13}C in liquid samples of about 100 nanoliters. Proton decoupling under DNP conditions as a new tool is possible with the developed probehead. Besides, heat dissipation was improved with respect to the DNP probeheads described before [1, 2] that helped to keep aqueous samples at constant temperature under irradiation by microwave power up to 5 Watts. This feature opens opportunity to disentangle temperature and microwave saturation effects on DNP enhancement in liquids. Performance of the probe was tested by DNP experiments on aqueous 3-^{13}C-sodium pyruvate solutions.

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A 3D-PRINTED ^{1}H-^{13}C BACKGROUND-FREE RADIOFREQUENCY COIL ENABLES SIMPLE IMPLEMENTATION OF CROSS-POLARIZATION AT LOW TEMPERATURE UNDER DISSOLUTION-DYNAMIC NUCLEAR POLARIZATION CONDITIONS

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 using 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.

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PHOTO-CIDNP NMR AS A TOOL TO INVESTIGATE CONFORMATIONAL TRANSITIONS IN SWITCH PEPTIDES

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Photo-Chemically Induced Dynamic Nuclear Polarization (Photo-CIDNP) is an excellent probe to scrutinize the solvent exposure of individual tryptophan, tyrosine or histidine amino acids in a sequence, i.e. which of those aromatic side chains are participating in an interaction (hindered from the solvent) and which are freely exposed. This approach involves a spin-selective photochemical process between the target molecule and a laser-excited photosensitizer dye and can lead to positive and negative enhancements of NMR signals. Interestingly, signal enhancements produced by Photo-CIDNP can be transferred between nearby nuclei in a mechanism similar to the NOE effect, namely cross polarization, which can be interpreted to give an idea about the mobility of a molecule [1].

These interesting features encouraged us to use photo-CIDNP to gain insights into the micelle-triggered conformational transition observed in $LytA_{239-252}$, a surface protein fragment present in pneumococcus, the most common pathogen of the respiratory tract that causes pneumonia. $LyTA_{239-252}$ is a fragment of a choline-biding protein that are essential for bacterial colonization and virulence, with the involvement of conformation transition [2].

Herein, we report valuable residue-level information by Photo-CIDNP experiments that affords a clear picture of the conformational transition of $LytA_{239-252}$ peptide from the native β-hairpin into the α-helix triggered by the presence of DPC micelles. We show that aromatic stacking and disruption encodes the key for the conformational switching. Furthermore, considering that when executing Photo-CIDNP experiments, the uniform illumination of the whole sample volume faces several problems, we describe the whole optimization process for the NMR experimental conditions, illustrating the advantages of using low NMR active volumes to gain all information from the spectra. In this sense, our previous experience [3] in the development of *in-situ* NMR illumination devices for pushing the NMR sensitivity for small sample volumes, supports the importance of the optimization of the photon flux in the NMR active volume.

Our results herein, demonstrate the great potential of using Photo-CIDNP experimental data in an optimized set of experimental conditions to determine conformational transitions of switch peptides.

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NUMERICAL SIMULATIONS OF TRIPLET STATE GENERATION IN PHOTO-DNP SAMPLES

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In photo-DNP, light-excitation of a suitable molecular system is used to create a high, non-Boltzmann polarization, which is then transferred to nuclei to enhance the sensitivity of an NMR measurement. Perhaps the best-known example is triplet-DNP, introduced by Wenckebach *et al.*, in which the polarization of the excited triplet state of pentacene is transferred to ^1H in the naphthalene matrix by the integrated solid-effect.[1] To obtain an optimized and uniform enhancement of the ^1H spin polarization throughout the entire sample in these and other photo-DNP experiments, the excitation light source must be appropriately chosen and match with the concentration as well as the photo-physical properties of the photo-sensitive molecules. To this end, numerical simulations are indispensible.

With the help of MATLAB, we wrote a script to simulate how the triplet state population changes with time (**t**) and penetration depth (**z**) of a laser beam into the sample. The script is based on the rate equations provided by Takeda *et al.*,[2] given here in the notation of Wenckebach.[3]

$$\frac{\partial N_0}{\partial t} = -B\hbar\omega\rho(\omega)N_p(N_0 - N_1) + \left(\frac{1-\Phi_{ISC}}{\tau_F}\right)N_1 + (k_{Ph} + k_{ICT})N_T \quad ...(1)$$

$$\frac{\partial N_1}{\partial t} = B\hbar\omega\rho(\omega)N_p(N_0 - N_1) - \left(\frac{1}{\tau_F}\right)N_1 \quad ...(2)$$

$$\frac{\partial N_T}{\partial t} = \left(\frac{\Phi_{ISC}}{\tau_F}\right)N_1 - (k_{Ph} + k_{ISC}^T)N_T \quad ...(3)$$

$$\frac{\partial N_p}{\partial t} = -B\hbar\omega\rho(\omega)N_p(N_0 - N_1) - \frac{c}{n}\left(\frac{\partial N_p}{\partial z}\right) \quad ...(4)$$

N_0, N_1, N_T are the population densities of the S_0, S_1 and T_1 states, and N_p is the photon density. With the script, we calculated how the triplet states become populated, even after the excitation source is switched off, and how the triplet population decays over time, depending on the phosphorescence lifetime ($\tau_{Ph} = 1/(k_{Ph}+k_{ICT})$). It allowed us to optimize parameters such as laser pulse length and energy to generate ~100% triplet state population, given the photophysical properties such as fluorescence lifetime (τ_F) and ISC quantum yield (Φ_{ISC}).

We used the script to investigate the triplet state generation in photo-DNP experiments with quinone-TEMPO molecular complexes in frozen toluene [4], which were recently performed in our laboratory. In these experiments, we used an excimer laser (pulse length of 5 ns, pulse energy 2 mJ) as the light source. We measured the photophysical properties for the quinones and quinones/TEMPO complexes in frozen toluene and methylcyclohexane to provide the script with realistic parameters. According to our calculations, the triplet state population was only a few percent, explaining the very modest enhancements we observed. Specifically, the calculations suggested that the photo-DNP efficiency in our experiments can be strongly improved by choosing a more suitable excitation source.

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SPIN DENSITY ACCESSIBILITY AND LOCAL DYNAMICAL FLUCTUATIONS OF ORGANIC RADICALS AFFECT LIQUID-STATE DNP EFFICIENCY

Within the last decade, the polarization transfer mechanisms of Overhauser dynamic nuclear polarization (ODNP) in the liquid state have been extensively studied for nuclei such as ^{1}H and ^{13}C. For large signal enhancements (10^2 – 10^3) at high magnetic fields, room temperature and ambient pressure, a scalar polarization transfer (Fermi contact interaction) is beneficial. Scalar polarization transfer is mediated by molecular collision, whose frequency and duration are usually short enough (ps or sub-ps) to still contribute to the cross-relaxation rates at magnetic fields higher than 9.4 T. This is particularly true for ^{13}C nuclei [1, 2].

To highlight how the scalar mechanisms critically depend on the choice of the polarizing agent (PA)/ target molecule system, we performed a ^{13}C ODNP study on six polarizing agents, five nitroxide derivatives (NODs) and α,γ-bisdiphenylene-phenylallyl (BDPA) in model systems at 1.2 T. As a measure for the efficiency of each PA, we use the coupling factor ξ extracted from the Overhauser equation. We identified features that affect the DNP efficiency by approximately a factor of five and observed a clear trend in DNP efficiency going from the least efficient PA (BDPA), over small NODs, and up to the most efficient FN2a (a nitroxide linked to a fullerene). Interestingly, this behavior is unique for the scalar mechanism, and has not been observed in comparative ^{1}H-DNP measurements [3, 4]. Supported by a DFT analysis, we recognized that the electron spin density on the PA needs to be accessible and highly localized. In particular, the solvent accessible surface (SAS), calculated for the positions with highest spin density, correlates well with our results of the DNP efficiency. Indeed, a reduced accessibility of the radical site should change the likelihood of an encounter between PA and a target, and therefore influence the frequency of such collisions [3].

Additionally, we closely investigated the case of FN2a, whose high signal enhancement at low magnetic fields (1.2 T) rapidly decays at high fields (9.4 T) and there is outperformed by smaller NODs by almost a factor of 40 [2]. Field dependent DNP measurements suggested an additional contribution to the scalar relaxation with a longer correlation time (~10 ps). This contribution is absent for smaller NODs. Complementary molecular dynamic simulations (MD) of FN2a show a rapid reorientation of the methyl groups on a timescale that correlates well with the one extracted from the field-dependent DNP data [4].

In summary, we identified a field independent (a localized and accessible spin density) as well as a field dependent (rapid local structural fluctuations) feature of efficient PAs for DNP in the liquid state at low and high magnetic field. Similar to solid-state DNP-NMR, where the new design of PAs is a thriving area for DNP optimization, our results should help to significantly boost ODNP in the liquid state.

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SIMULATION OF NITROGEN NUCLEAR SPIN MAGNETIZATION OF LIQUID SOLVED NITROXIDES

Nitroxide radicals are widely used in Electron Paramagnetic Resonance (EPR) applications. Nitroxides are stable organic radicals containing N-O group with hyperfine coupled unpaired electron and nitrogen nuclear spins. In the past, much attention was devoted to studying nitroxide EPR spectra and electron spin magnetization evolution under various experimental conditions. However, the dynamics of nitrogen nuclear spin was not investigated in detail so far. In this work, we performed quantitative prediction and simulation of nitrogen nuclear spin magnetization evolution in several magnetic resonance experiments. Our research was focused on fast rotating nitroxide radicals in liquid solutions. We used a general approach allowing us to compute electron and nitrogen nuclear spin magnetization from the same time-dependent spin density matrix obtained by solving the Liouville/von Neumann equation [1]. Especially we want to emphasize our prediction of a large dynamic nuclear polarization of nitrogen upon nitroxide irradiation with microwaves and influence of nitrogen nuclear spin dynamics on the nitroxide EPR saturation factor [2,3].

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DEVELOPING ANALYTICAL APPLICATIONS FOR PARAHYDROGEN HYPERPOLARIZATION: URINARY ELIMINATION PHARMACOKINETICS OF NICOTINE

Pharmacokinetics is essential in modern drug development in order to understand the (bio)chemical processes that a drug undergoes in the body from its intake to excretion. To follow minute concnetrations of analytes in complex mixtures like urine or blood, exceptional resolution and sensitivity is desired. Nuclear magnetic resonance spectroscopy (NMR) is an attractive choice for PK for it offers straightforward data interpretation and quantitative analysis. However, pharmacologically active compounds and their metabolites in biofluids often appear in minute concentrations, well below the detection limit of NMR.

To improve the NMR sensitivity parahydrogen (pH_2) hyperpolarization (HP) has been adapted for measuring biofluids [1]. Recent progress in pH_2 HP techniques allows multiscan experiments [2], [3] and to resolve complex spectra of urine with 2D spectroscopy [4]. By combining pH_2 HP and 2D spectroscopy, it is possible to overcome the NMR sensitivity barrier and detect midnanomolar concentrations of a drug and a drug metabolite in a biofluid matrix [5].

Herein, we demonstrate how pH_2 HP can be applied in pharmacokinetics. As a proof of concept, we measured urine of humans exposed to nicotine and compared two common intake methods – smoking and absorption through skin by using a transdermal patch. We followed urinary elimination of nicotine, and its main metabolite cotinine, and determined their concentrations in urine during the onset and withdrawal from nicotine consumption. An NMR limit of detection of 0.1 μM and a limit of quantitation of 0.7 μM was achieved in a practical pharmacokinetics scenario where precise quantitative and qualitative analysis is desired.

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ELECTRON DRIVEN SPIN DIFFUSION UNDER DYNAMIC NUCLEAR POLARIZATION AT LOW TEMPERATURE

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Dynamic nuclear polarization (DNP) is a widely used tool for overcoming the low intrinsic sensitivity of magnetic resonance spectroscopy and imaging. DNP relies on transferring the high polarization of unpaired electrons to nuclear spins via microwave irradiation. Nuclei which are too far from the electron to interact directly with it may still be polarized indirectly by nuclear spin diffusion from the nuclei closer to the electron. However, the closest nuclear spins, even if they are potentially most efficiently polarized by the electron, have their Larmor frequencies so strongly shifted by the interaction with the electron that they cannot exchange polarization with the bulk spins. These nuclei are said to be within the so-called 'spin diffusion barrier' [1].

A quantitative assessment of this barrier used to be hindered by the lack of general methods for studying nuclear polarization flow in the vicinity of paramagnetic centers. We have recently filled this gap by introducing a general set of experiments based on microwave gating that can be readily implemented [2]. The hyperpolarization resurgence experiments (HypRes) consist of generating a large polarization gradient between the core and bulk spins and observing the flow of polarization between them. We have demonstrated the versatility of our approach in ^{1}H DNP experiments with TEMPOL radicals conducted between 1.2 and 4.2 K in static mode and at 100 K under magic angle spinning (MAS) - conditions typical for dissolution-DNP and MAS-DNP - and directly observed the dramatic dependence of polarization flow on temperature. Using broadband adiabatic inversion pulses to manipulate the non-observable spins near the electron, we further showed that nuclear spins as close as 3 Å to the electron could still exchange polarization with bulk nuclei [3-4].

We now show similar findings for ^{13}C DNP with trityl radicals between 1.2 and 4.2 K and investigate possible mechanisms for these spin diffusion processes. We find that the fluctuation of the electron spin state could be responsible for compensating the energy mismatch between $|\alpha\beta\rangle$ and $|\beta\alpha\rangle$ nuclear spin states and therefore allow nuclear spin diffusion [5]. These fluctuations are quenched when the electron polarization reaches 100%, which explains why diffusion is slower at 1.2 K.

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DYNAMIC NUCLEAR POLARIZATION ENABLES THROUGH-BOND 195PT-13C SOLID-STATE NMR CORRELATION SPECTROSCOPY OF SURFACE-SUPPORTED PT COMPLEXES AT NATURAL ABUNDANCE

Supported single-site Pt species have recently attracted much interest as highly efficient heterogeneous catalysts by themselves or as precursors to generate metallic nanoparticles. In that context, well-defined Pt complexes have been grafted on silica, whose coordination environment needs to be understood at the molecular level. Platinum-195 NMR would in principle be a method of choice to characterize the local chemical environment and electronic structure of the metal center, providing invaluable structural information to rationalize the design of the catalytic materials. ^{195}Pt is however a low-sensitivity NMR probe as it usually experiences an extremely broad chemical shift anisotropy (CSA) that greatly reduces the experiment sensitivity. For surface complexes, this limitation adds to the low concentration of Pt sites, making any investigation by ^{195}Pt NMR extremely challenging.

We have recently demonstrated that the ^{195}Pt CSA parameters and the Pt–H distances of Pt surfaces sites could be determined by combining room temperature proton-detected NMR spectroscopy with dynamic nuclear polarization surface enhanced NMR spectroscopy (DNP-SENS) [1]. Measuring correlations between the Pt center and other neighboring NMR-active nuclei would be highly desirable to increase structural content.

Here, we demonstrate that the sensitivity boost provided by DNP allows one to record ^{195}Pt-^{13}C correlations on diluted platinum surface species. Our approach exploits $^{1}J(^{195}$Pt-^{13}C) couplings in Pt complex having Pt-C bonds, as these scalar interactions are commonly large while being extremely sensitive to the local structural environment. We demonstrate this approach on two silica-anchored Pt complexes: in the first sample (**Pt-NHC@SiO_2**) the coordination carbon has been isotopically enriched while the second sample (**PtMe(COD)@SiO_2**) was synthesized by grafting natural abundance precursors. In the two cases, two-dimensional (2D) ^{13}C{^{195}Pt} *J*-HMQC spectra were successfully acquired in short experimental times. For **Pt-NHC@SiO_2**, the 2D ^{13}C{^{195}Pt} *J*-HMQC spectrum unexpectedly reveals two kinds of Pt surface sites. Both the corresponding isotopic ^{195}Pt and ^{13}C chemical shifts as well as the $^{1}J(^{195}$Pt-^{13}C) values for each site were determined. These two distinct sites could not be detected by previous NMR investigations [2] nor by static ^{195}Pt DNP SENS measurements. DFT calculations are currently under way to propose structural models for the two sites. A correlation between the Pt center and methyl carbon is observed in the 2D ^{13}C{^{195}Pt} *J*-HMQC spectrum of **PtMe(COD)@SiO_2**, providing direct evidence that the methyl ligand persists on the surface and is bounded to Pt. The $^{1}J(^{195}$Pt-^{13}C) value measured from this spectrum is in agreement with that observed for the molecular precursor [3]. The methodology described here represents a new step forward in the atomistic description of catalytically relevant surface metal complexes.

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STUDY OF HEAVY METAL LOADED RESINS BY BENCHTOP NMR

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Heavy metals discharged by industrial wastewater to the environment has become a major public health and environmental concern [1]. For example, heavy metals ions such as Ni (II) and Cu (II) are known to be toxic and must be removed from wastewater. However, Ni (II) and Cu (II) ions also have paramagnetic properties which previously allowed the use of Magnetic Resonance Imaging (MRI) and Nuclear Magnetic Resonance (NMR) relaxometry to follow their migration and their adsorption on different media [2-4]. The purpose of this study is to monitor the removal of Ni (II) and Cu (II) from water by amberlite IR120 with T1 and T_2 relaxometry. In order to obtain the adsorption isotherms, different samples containing the same amount of Amberlite IR120 resin was put in contact with aqueous solutions containing Ni (II) or Cu (II) ions at different concentrations, before being shaken by a vortex mixer. Once the equilibrium reached, the longitudinal and transversal relaxation time (T_1, T_2) of the solution was measured which allowed the determination of the amount of adsorbed metal. Study of the loaded resin was afterwards carried out using a larger amount of resin which was dried and rehydrated before being analyzed. The equilibrium adsorption behavior of Ni (II) or Cu (II) can be satisfactorily described by the Langmuir model, with maximum adsorption capacity of 81.5 mg g^{-1} and 78.3 mg g^{-1} for Cu (II) and Ni (II) respectively whereas the sorption equilibrium constants are 0.98 L mg^{-1} (Cu (II)) and 1.8 L mg^{-1} (Ni (II)). The longitudinal and transverse relaxation of the wet resin are shown to be biexponential. The relaxation rate of the fast relaxing water fraction of the wet resin can be correlated with metal contents obtained by Atomic Emission Spectroscopy (ICP-AES). The next step will be to reproduce these experiments for other adsorbents and paramagnetic ions at different magnetic fields. With this methodology, the adsorption could be followed with low-cost portable NMR device. In the future, it will also be interesting to carry out a so-called NMR column experiment in order to investigate the adsorption within the resin in real-time.

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A NOVEL FAST FIELD CYCLING APPROACH TO OBTAIN FIELD DEPENDENT T_1-T_2 2D MAPS

Fast field cycling (FFC) NMR relaxometry [1] is a very valuable tool for studying molecular dynamics of many different chemical systems such as soils, food, environment [2], organic systems [3] and a number of different porous materials [3,4]. Up to now FFC NMR relaxometry has been used to describe the dependence of longitudinal relaxation time T_1 from the relaxation field over a wide range of values spanning from ultra-low magnetic field up to a few Tesla. This unique capability offered by FFC NMR relaxometry is exploited to obtain both nuclear magnetic resonance relaxation dispersion (NMRD) profiles reporting $1/T_1$-vs-n_L, and distributions of T_1 components at different fields.

So far, no information has never been obtained on the behavior of the transversal relaxation time T_2 as affected by the modulation of T_1 when the proton Larmor frequency of the applied magnetic field is changed. In the past, the main reason for such a lack of information was due to some hardware limitations. However, some recent developments of the electronics, allow us to produce FFC NMR instruments which can be used also to measure T_2 relaxation values.

In the present study, an attempt to monitor the behaviour of T_2 as affected by T_1 modulation at different magnetic fields was given by computing the experimental data with a new robust 2D NMR inversion algorithm that uses a locally adapted multi-penalty regolarization approach [5]. A cheese sample was analysed in order to obtain FFC 2D maps [6] from which more information on the different motion components can be retrieved. According to a previous study [7], at least four different components could be identified (i.e. bulk water, bound water interacting with the polar groups in cheese, fats, proteins).

This study highlights a novel method for obtaining 2D maps at different magnetic fields and shows the usefulness of these maps for discriminating between different motion components in only a few shots.

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MOLECULAR DYNAMICS IN *CLOSO*-BORATE SYSTEMS STUDIED BY MEANS OF NMR

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Metal *closo*-borates are an emerging class of solid electrolytes, showing high thermal, chemical, and electrochemical stability, as well as superionic conductivity in their disordered state. Generally, the compounds exist with an ordered crystal structure at room temperature (RT) and undergo a thermally induced order–disorder transition, in which both the anion and cation sublattices become disordered as the large anion cages are distributed over two crystallographic positions. This liquid-like environment allows the cations to easily move in the structure, resulting in a sharp, order of magnitude increase in the ionic conductivity. The conductivity is assisted by the rotations of the boron cages.

Here, we present a study of molecular dynamics in three *closo*-borate systems, $(NH_4)_2B_{12}H_{12}$, $(NH_4)_2B_{10}H_{10}$, and $Ag_2B_{12}H_{12}$, the first two being weak ionic conductors because of the large mobile ion NH_4^+, while the third one is an excellent conductor [1,2]. We determined the activation energies for the reorientations of boron cages by means of temperature-dependent NMR spin-lattice relaxation measurements on ^{1}H and ^{11}B and complemented them by ionic conductivity measurements.

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PROBING MOLECULAR DYNAMICS IN THE N AND NTB PHASES OF THE TWIST-BEND NEMATOGEN DTC5C7 THROUGH MAGNETIC RESONANCE RELAXOMETRY

The liquid crystal (LC) materials forming the Ntb phase are interesting not just owing to their unique phase structures and properties but also their exploitation in many new applications [1]. Despite a decade of discovery [2], the Ntb phase remains extensively debated over its structure and properties [3,4]. Alternative to the usual twist-bend model [2,3], the polar-twisted (Npt) geometry has also been endorsed for the structure of the Ntb phase [4]. Thus, neither of these geometries has been fully established experimentally. Also, despite a large body of studies devoted to the Ntb materials, many of their physical properties and molecular features influencing the onset of the Ntb phase have not been fully conclusive.

In this work, by means of proton nuclear magnetic resonance (1H NMR) relaxometry, we present a molecular dynamics study of the liquid crystal dimer DTC5C7. This material remains one of the rare examples of LC materials that in addition to exhibiting nematic and twist-bend nematic phases also possesses a lamellar smectic phase.

To this aim, dispersions of relaxation rates were measured over a very broad frequency range and extensively analyzed in the nematic, Ntb, and smectic phases. With this investigation, we expand the current knowledge on these systems as the proton NMR relaxation techniques give a detailed insight into physical properties such as correlation times, viscoelastic parameters and correlation lengths that are signature characteristics of the structural properties of the studied mesophases. Our results indicate that the molecular organization in the Ntb phase has a substantial impact on the dynamic behavior of the studied system. Significant differences were observed between the structures of the N and Ntb phases through distinct signatures of temperature- and frequency-dependent collective mode, particularly in the low-frequency domain. Similar dispersion characteristics of the Ntb and smectic phases suggest smectic-like clustering in the Ntb phase.

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FAST FIELD-CYCLING NMR RELAXOMETRY AND LIQUID CRYSTAL BIAXIALITY

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In the search of the predicted biaxial nematic phase, a series of shape-persistent board-shaped mesogens with maximum molecular biaxiality and a dipole along the minor molecular axis were designed to form nematic (N) mesophases. One compound exhibits a wide nematic temperature range, which can be supercooled to room temperature. A comprehensive variable temperature X-ray study on aligned samples reveals patterns being dominated by the form factor of very small aggregates, from which the aspect ratio of the lead compound with length (L):breadth (B):width (W) of 10.73:3.16:1.23 could be obtained. Variable temperature proton relaxation studies on this mesogen were carried out over a wide frequency range.

The global fit of the frequency dispersions at five temperatures with a motional model requires in addition to the usual rotation/reorientation contribution, two independent director fluctuations contributions: one for the conventional nematic order director (**n**) fluctuations and the other for the minor director (**m**) fluctuations (normal to **n**). The correlation length of the minor directors determined by NMR could extend to 5–8 molecules in the W direction, but only to the nearest neighbour in the B direction, as found by X-ray diffraction. Both X-ray and NMR studies indicate that these new types of lead structure are extremely promising to find the long sought-after biaxial N mesophase [1].

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ZN INCORPORATION IN SYNTHETIC C-S-H AND ITS EFFECT ON CEMENT HYDRATION THROUGH DNP ENHANCED MAS NMR

The contribution of CO_2 emissions from cement production account for 8-10% of the total emissions we produce. One possible solution 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. However, it has recently been demonstrated that the addition of Zn can enhance the mechanical strength of a clinker.

The goal of this project is to understand the role of Zn in synthetic single-phase C-S-H [1] by means of solid state nuclear magnetic resonance (NMR). Results show evidence of Zn incorporation into the C-S-H structure and insight about the exact position of Zn at the atomic level. DNP enhanced Solid state 29Si NMR CP experiments show two clear trends. Firstly, the intensity of the peak which corresponds to the Q2 sites increases as the Zn content is increased; and secondly, a new peak around -70ppm becomes more evident as more Zn is added to the sample too. These two facts verify the hypothesis that Zn is inside the C-S-H structure since it is changing the chemical environment of some Si sites and changes the ratio between Q1 sites (which correspond to Si at the end of a silicate chain) and Q2p (which correspond to Si which are adjacent to a Q1 Si and a bridging Si).

Incredible Natural Abundance Double-Quantum Frequency Transfer Experiments (INADEQUATE) have also been done in order to determine how the relation between Q species populations evolve as more Zn is incorporated into the system. Results show that the addition of this element promotes longer chains at the atomic scale.

Understanding the mechanisms by which Zn is incorporated into the C-S-H structure will be vital in order to be able to add this element into the industrial process with the ultimate goal of reducing the CO2 emissions of the cement industry.

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SUPRAMOLECULAR STRUCTURE OF LAYERED HYBRID PEROVSKITES DETERMINED USING NMR CRYSTALLOGRAPHY

Here, we show how multinuclear (^{1}H, ^{19}F and ^{13}C) solid-state NMR in combination with molecular dynamics and chemical shift calculations can be employed to determine the complete atomic-scale molecular structure of the organic spacer cations in layered hybrid perovskites [1]. Solid-state NMR has recently emerged as a powerful technique to study the structure and dynamics of hybrid organic–inorganic perovskites. NMR is exquisitely sensitive to chemical information such as cation incorporation, halide mixing, phase segregation and dynamics [2,3]. Layered perovskites, which comprise slabs of the perovskite structure separated by a hydrophobic organic spacer layer [4], have shown potential to improve the stability of perovskite solar cells, but they suffer from poor crystallinity and conductivity which impair photovoltaic efficiency. Supramolecular engineering [2,5] is a promising approach to improve the electronic property and crystallinity by harnessing non-covalent interactions to direct the structure of the organic spacer layer. However, to assess this structure directing effect the complete atomic-level structure must be determined, which was not previously possible.

NMR is ideally suited to study hybrid materials. Here, chemical shift-based NMR crystallography was used to determine the structure of the spacer layer in Ruddlesden–Popper perovskites featuring a bilayer of monovalent organic spacer cations, in this case phenylethylammonium (PEA^+), pentafluorophenylethylammonium (FEA^+), or a 1:1 mixture of the two. Double resonance NMR experiments demonstrate the proximity of the two aromatic systems; however, comparison of chemical shifts obtained from candidate structures with experiment indicates that a phase segregated structure most closely matches the experimental NMR parameters. Taken together, this shows the presence of a nano-scale phase segregated structure.

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LOCAL STRUCTURAL TRENDS AFTER DIFFERENT TEMPERATURE CALCINATION OF AL_2O_3 AND CU-AL_2O_3 CATALYST SUPPORTS: INSIGHT BY SOLID STATE NMR

In the field of catalysis aluminium oxide, Al_2O_3, is often used as catalyst support, which can be modified by distinct preparation methods such as different synthesis routes, additives and calcination temperatures. Such variation in preparation may lead to controlled manipulation of coordination number and structural order around aluminium atoms, and consequently enhanced catalytic properties for specific degradation processes of volatile organic compounds [1].

Synthesis of catalyst supports presented here was based on alumina pseudoboehmite powder, which was calcined in oxidative atmosphere, ensuring highest oxidation state of the material. In this preliminary study we investigated the local structure of aluminium atoms by using solid state nuclear magnetic resonance spectroscopy (ssNMR). From a series of Al_2O_3 catalyst supports calcined at different temperatures, ranging from 800 to 1400 °C, we reveal some of the temperature dependent trends regarding the local surrounding of Al atoms. In parallel we also looked at the influence of copper additive on Al coordination at various calcination temperatures. In all these samples 8 weight % of Cu relative to the Al_2O_3 support was used.

By studying ^{27}Al ssNMR spectra we observed that by increasing calcination temperature the local structure around Al atoms gets more ordered – peaks get narrower and take more distinct quadrupolar shape. A parallel series of supports with Cu as dopant have the same general temperature trend. We also find that supports with Cu additive, calcined at temperatures up to 1000 °C, have slightly more tetrahedral coordinated Al compared to those without added Cu. Interestingly, calcination at 1200 °C is an exception, where addition of Cu significantly changes the local structure of Al atoms. Addition of Cu at highest calcination temperature of 1400 °C has no influence on Al environment, where we are left with only one type of tetrahedral coordinated Al sites. To investigate if the operation of the catalyst support influences the Al local structure, we performed a degradation test of toluene at 200 °C, and found out that the Al_2O_3 support is stable, meaning we did not observe any difference in ^{27}Al ssNMR spectra before and after use of said catalyst support.

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INSIGHT INTO TRANS-[Ru(NO)(py)$_4$F](ClO$_4$)^2 PHOTO-COMMUTATION PROPERTIES BY SOLID STATE NMR AND DFT CALCULATIONS

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Photoinduced isomers can be generated in a variety of compounds, each having its specific structural response and wavelength sensitivity. This gives rise to interesting properties such as photochromism and photorefractivity for holographic data storage and optical switches. Fundamental questions in this context are to what extent the electron density distribution in the photo-generated isomer has changed with respect to the ground state configuration, as well as how the structural and electronic characteristics of the photoswitches are linked to the thermal stability and optical properties of the photoproducts.

In this communication we present solid-state NMR results obtained on the complex trans-[Ru(py)$_4$(^{15}NO)F](ClO$_4$)$_2$, where the quasi-fourfold axis F-Ru-^{15}N-O is nearly linear in the ground state and the unit cell contains two crystallographic independent molecular units. Upon light irradiation (420nm), two metastable linkage isomers can be generated, one by rotating NO by about 90° (MS2) and one by an inversion of the NO ligand by 180° (MS1) [1]. We show here the successful low temperature trapping and SSNMR observation of the diamagnetic light induced metastable state MS1. In order to gain insight into the bonding properties and changes of the electron density upon photo-isomerization, we compare the ^{15}N and ^{19}F solid-state NMR results with those obtained from DFT calculations based on our recent photo-crystallographic X-ray diffraction study [2]. These results are then interpreted based on local changes of the electron density of the atoms along the F-Ru-NO axis.

Interestingly, we observe different commutation properties within the same crystal for the two inequivalent cations and supply an explanation based the interaction of the cation with it's counterion.

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SOLID-STATE NMR AS AN INNOVATIVE NON-DESTRUCTIVE EVALUATION METHODOLOGY IN COMPOSITE SELF-HEALING SYSTEMS FOR AEROSPACE ENGINEERING APPLICATIONS.

The self-healing polymer composites have gained increasing research interest over the past several decades and the technological interest of the most demanding industries such as aerospace, automotive and ship building industries [1]. The researches aim to find an efficient healing mechanism that can lead to an extended life cycle of the structure [2][3]. This study focused on the development of an innovative non-destructing evaluation methodology by taking the advantage of a powerful spectroscopic technic such as Nuclear Magnetic Resonance (NMR) and transform it into a non-destructing evaluation tool. In this study we examine the rheological behavior and the polymerization mechanisms of the self-healing agent after the damage occurs. The examined structure of this scenario is a protective coating (Aerowave 3003 epoxy primer) with dispersed self-healing microcapsules and catalyst Curing Solution 6007. The enclosure of the self-healing microcapsules is a mixture of an epoxy part, a diluent and a chromophore (DGEBA+ BDE+ Solvent Red 242). Our approach is to mapping the polymerization reactions during the self-healing process in real time by monitoring the T2 relaxation time and visualizing the results in polymerization maps.

The self-healing system was tested in different conditions in order to understand the behavior of the self-healing process, we examine the polymerization progress at three different temperatures (25°C, 40 °C, 60 °C) those temperatures were chosen due to operation environment of aerospace applications and we examine two different epoxy-diluent mixtures (10%,25%) in order to observe how the presence of diluent effect the polymerization progress. 2D ^{1}H NMR diffusion–relaxation D–T2 measurements were performed to monitor the diffusion properties of the polymerization reactions of the self-healing agent during the self-healing progress of the damaged structure. The final step of this study was to simulate the final self-healing system, the coating with the dispersion of micro-capsules and hardener then we damaged the system and we monitored the diffusion properties of the system during a thermal scanning (25°C, 40 °C, 60 °C) and we achieved to monitor the consumption of the hardener after he damage occurs. All the above experimental procedures were performed in the stray field of a 4,7 T Bruker superconductive magnet providing a 34,7 T/m constant magnetic field gradient at ^{1}H NMR frequency of 101,324 MHz.

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CONFORMATION AND PACKING OF POLYANIONS IN POLELECTROLYTE COMPLEX COACERVATES – A COMBINED PFG AND SOLID-STATE NMR STUDY

Polyelectrolyte complex coacervates are formed mixing charged polymer, polycation and polyanion in solution. Such complexes find wide application in water treatment for flocculation or for controlled drug release. The conformation of polyelectrolytes in solution depends on the repelling force from the charges along the polymer chain. For a weak polyanion poly(maleic anhydrite-co-ethylene) the depends on pH and ionic strength. PFG NMR offers measures for both conformation and charge of polyelectrolytes in solution. The effective charge is inferred from electrophoresis NMR while diffusion NMR yields the hydrodynamic size as a measure for the conformation. With increasing pH the weak polyacid dissociates generating more charges and thus a more stretched conformation.

The degree of dissociation of the solid materials is determined from the relative acid proton signal intensity in ^{1}H MAS spectra. Separating ^{1}H spectra in two-dimensional single-quantum-double-quantum correlation spectra distinguishes between acid protons hydrogen bonded to other acid protons from others and thus identifies polyanion-rich regions in solid polymers or complexes. Two-dimensional integration of the on-diagonal and off-diagonal signals of the acid protons enables quantification. Apparently the conformation of the polyelectrolytes in the parent solutions from which the complexes are formed is partially retained in the complexes. At low pH (weak charge) this are reduced by a factor of three in the complexes at higher pH (high nominal charge) with a more stretched conformation almost none acid-acid contacts are found in the complexes. As the pH is adjusted by NaOH, ^{22}Na proides additional insight. In the complexes exhibiting ion pairs between polyanion and polycation signals from NaCl are found while the rest shows signals from maleate.

Partially dissociated poly(maleic anhydrite-co-ethylene) exhibits strong down-field shifts for the reamining acid protons up to 20 ppm. This is confirmed for maleic acid at various pH values. The strong hydrogen bonds are seen in DFT calculations using CASTEP.

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AMMONIUM QUANTIFICATION (AQUA) IN HUMAN PLASMA BY NMR FOR CLINICAL STAGING OF FATTY LIVER

Fatty liver disease (FLD) is a common disease with identifiable markers. However, there is a lack of tools powerful enough to provide a reliable diagnosis for FLD without requiring a biopsy [1]. It has been demonstrated that there is a direct relationship between FLD and hyperammonemia in plasma because of the downregulated activity of the urea cycle enzymes [2]. The physiological ammonia level in blood is constantly maintained around 40 µM and, at physiological pH (pH ≈ 7.4), around 98% of ammonia is in ionic form (NH_4^+). Pathological conditions such as liver dys-function may disrupt the metabolism of NH_3 and lead to a life-threatening increase in blood [3].

Here we show how proton nuclear magnetic resonance (^{1}H-NMR) spectra of human plasma can correctly diagnose the presence of FLD with the proper ammonia quantification. The procedure has been optimized testing different deuterated solvents and pHs in order to achieve an accurate and realible quantification. The study has been performed with alcoholic fatty liver disease (AFLD) and non-alcoholic steatohepatitis patients (NASH). Our studies show for the first time a technique capable of providing an accurate and rapid diagnosis of FLD without the need for an invasive biopsy. This technique can be used in a clinical setting either in population screening or to allow effective targeting of treatment.

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INDIRECT NMR DETECTION OF NUCLEI SUBJECT TO LARGE ANISOTROPIC INTERACTIONS VIA PROTONS USING T-HMQC SEQUENCES

Solid-state NMR is a unique technique for the characterization of the atomic-level structure and dynamics of materials. Nevertheless, this technique can often be challenging when the investigated materials contain *S* nuclei subject to large anisotropic interactions, such as chemical shift anisotropy (CSA) or quadrupolar coupling for $S \geq 1$. Recently, a through-space HMQC variant using the TRAPDOR (TRAnsfer of Populations in DOuble-Resonance) recoupling, called T-HMQC, has been successfully applied for the indirect observation via protons of quadrupolar isotopes such as ^{14}N ($S = 1$) or ^{35}Cl ($S = 3/2$) [1,2]. This simple sequence is made of only four rectangular pulses: one $\pi/2$ and one π pulses forming a spin-echo on the ^{1}H channel and two symmetrical long pulses applied to the *S* spin, which reintroduce the ^{1}H-*S* dipolar couplings and transfer coherences between ^{1}H and *S* isotopes. Due to the absence of any recoupling scheme on the ^{1}H channel, the T-HMQC sequence is robust to spinning speed fluctuations, and hence eliminates the t_1-noise. Additionaly, its sensitivity is higher than the conventional D-HMQC.

In the present work, we have analyzed with spin dynamics simulations and experiments the efficiency and robustness of the T-HMQC sequence at fast MAS for the indirect detection of ^{195}Pt, ^{14}N and ^{35}Cl isotopes [3]. For ^{195}Pt isotope, the method is robust to offset, and its efficiency increases for increasing CSA values and is still significant for values of 3 MHz. For quadrupolar nuclei, we demonstrate that the maximum sensitivity is achieved for a moderate rf-field and an offset equal to the MAS frequency. In the case of ^{14}N nuclei, the T-HMQC methods can select the 1Q or 2Q coherences during t_1 period. The selection of 2Q coherences enhances the resolution. For ^{35}Cl isotope, the selection of 2Q or 3Q coherences during t_1 period improves the resolution by a factor of 18 or 3.9 with the respect to *D*-HMQC sequence, respectively.

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MULTIQUANTUM COUNTING OF TRITYL RADICALS

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Multi-Quantum coherence (MQC) has been used in NMR [1, 2] to count coupled spin in clusters. In EPR DQC experiments, first introduced by the Freed group [3], are used to selectively detect the dipolar interaction within a coupled electron spin pair. Here we show that the number of coupled electron spins can also be obtained for larger spin clusters using this method [4]. This approach has the potential to determine the oligomeric state of spin-labeled proteins complexes in their native environment.

To count the number *n* of dipolar coupled spins, experiments which selectively filter the *n*-quantum coherences are proposed. For this experiment a high accuracy in the phases of the microwave pulses and a sufficient broad bandwidth to homogeneously excite the electron spins is mandatory. This is achieved using a home built X-band EPR setup based on an arbitrary wave form generator. The trityl radicals have narrow spectral width so that quantitative and precise excitation of all spins by the pulses is possible. 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. More common spin label for proteins are nitroxides. This spin labels have much broader EPR spectral width in comparison to trityl radicals, exceeding the excitation bandwidth of rectangular microwave pulses. Our first experimental results using broadband microwave excitation pulses on multi-nitroxide model compounds will be shown.

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A GENERALIZED FLOQUET TREATMENT FOR PULSE SEQUENCE OPTIMIZATION

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Floquet theory is one of the main methods to analyze time-dependent Hamiltonians. In the standard form, Floquet theory requires a periodic Hamiltonian and does not take into account the finite length of typical rf-irradiation schemes [1]. We have developed a generalized Floquet treatment for magnetic resonance that is based on a frequency-domain representation of the Hamiltonian. The new treatment does not require a periodic time-dependent Hamiltonian and takes the finite length of pulse sequences into account. In contrast to the standard Floquet treatment that is described by Fourier coefficients at integer multiples of the basic frequency, the new treatment is based on a Hamiltonian that is defined on a continuous frequency axis.

Homonuclear recoupling schemes under MAS received a great deal of attention since they can provide data for distance restrains in biomolecules. Proton-drive spin diffusion (PDSD) is one of the most important sequences to obtain distance restraints [2]. MIRROR and AM-MIRROR recoupling are tunable PDSD-based sequences that allow broadband and band-selective recoupling even at high MAS frequencies [3-5]. We present a refinement of the band-selective second-order MIRROR recoupling scheme, which allows targeted distribution of the polarization transfer, by creating side-bands in the zero-quantum spectrum. This development was enabled by the generalization of the Floquet theory described above, which provides a bijective mapping between the side-band pattern and the pulse scheme. This formalism does not require periodicity of the Hamiltonian and offers a simple route to gain analytical insight into finite-length arbitrary pulse irradiation schemes.

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FAST ACQUISITION OF TOCSY TRANSFER CURVES TO ASSIST THE ANALYSIS OF UNLABELED PEPTIDES

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The unlabeled peptides at low concentrations are usually studied by employing homonuclear ^{1}H experiments. One of the routine experiments performed in such studies is 2D ^{1}H TOCSY. The TOCSY experiment carries crucial information on the residue-type based on correlations observed in the residue-specific spin-system. The cross-peaks for individual residues usually appear as an easily recognizable pattern with typical chemical shifts. Sometimes, however, the cross-peaks pattern is difficult to recognize, e.g. due to the peak overlap. We present that TOCSY transfer curves may serve as an extra dimension to resolve potential ambiguities in the peak assignment process.

The acquisition of a series of 2D TOCSY experiments to obtain the TOCSY pseudo-dimension is very lengthy. In our study, we recorded a series of twenty-one 2D ^{1}H TOCSY experiments with different settings of spinlock duration for each of two unlabeled peptides (Repeat-3 and Repeat-4 domains of TAU). Using this data, we evaluated if applying non-uniform sampling allows us to reduce experiment time without significantly affecting the quality of TOCSY transfer curves. To improve the analysis of the TOCSY dimension, we simulated thousands of TOCSY transfer curves for protons in peptides and proteins employing Spinach [1] and data from Protein Data Bank [2] and used them for reference if necessary.

Combining the experimental and simulated data, we present that measuring the TOCSY-transfer dimension improves the analysis of unlabeled peptides. Moreover, the data can be required in a reasonable time thanks to the NUS. The approach provides increased reliability of the peak assignment and seems to be especially suited for studies carried at the lower magnetic fields, where resolution may be insufficient.

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PROBING INTERLAYER ANIONIC STRUCTURES BY SOLID-STATE NMR IN NI/AL PARAMAGNETIC LAYERED DOUBLE HYDROXYDES

Many recent solid-state NMR studies have presented various results on structural and dynamic aspects in the chemistry of Layered Double Hydroxide (LDH) materials. Some of the most recent investigations, based on recent innovations, demonstrated deep insights into the structure of the cationic part of Mg/Al-based LDH materials, as well as the layer/interlayer interface[1,2,6]. The influence of synthesis conditions on the interlayer anion composition has been demonstrated as well as dynamic information inferred from relaxation studies on anionic species [3-5].

In this presentation, we propose to show how we used a different set of metal centres, including paramagnetic transition metals in the hydroxide layers in order to take advantage of the paramagnetic interactions between electrons and nuclei and probe some intriguing structural features in nitrate LDH that show X-Ray characteristics as well as dynamical behaviour much different than that of their carbonated equivalent[7].

We implemented a model for fast-prediction of NMR spectra in paramagnetic species. Since anions in the interlayer space are not bound to cations in the hydroxide layers, we reduce the calculation of the hyperfine interaction in paramagnetic species to its dipolar component. By calculating this dipolar interaction and superimposing it on the chemical shift tensor measured on diamagnetic equivalents of carbonate and nitrate LDH, we can predict the spectra and measure the relative orientation of the diamagnetic and paramagnetic chemical shift tensors.

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SURFACE NMR USING QUANTUM SENSORS IN DIAMOND

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This work reports the experimental demonstration of NMR-spectroscopy at functional planar surfaces using a new technique called "surface NV-NMR". With this approach, we successfully realize a longstanding goal in surface and interface science, with immediate relevance to catalysis, material, and bioanalytical research [1]. Characterizing the molecular properties of surfaces under ambient and reactive conditions with non-invasive probes is a fundamental scientific challenge. NMR is a non-invasive, molecular level spectroscopic technique and is ideally suited for this purpose but lacks the sensitivity to probe the small number of spins at planar surfaces [2]. Here, **we demonstrate a novel method based on diamond quantum technology** [3] **that is applied to overcome this long-standing fundamental limitation of NMR, thereby providing a new tool for chemical analysis at surfaces.** This technique is based on the utility of quantum sensors in diamond and demonstrates their capability for performing NMR at surfaces and interfaces under ambient conditions. We use atomic layer deposition to prepare thin Al_2O_3 films on the diamond. This material is a common support in catalysis and materials science and thus represents a scientifically and industrially relevant functional interface for demonstrating our method [4]. This surface is then functionalized by means of phosphonate chemistry to form self-assembled monolayers. Surface NV-NMR is not only able to detect these organic monolayers but also to monitor the surface chemistry in real time during molecular self-assembly at the solid-liquid interface. The use of a diamond-based sensor that is chemically inert and can withstand high temperatures and high pressures will not only bridge the "pressure gap" in surface science but has the resilience to probe chemical reactions even under harsh conditions *in-situ* [5]. The functionality of surface NV-NMR under chemically relevant conditions and with low technical complexity makes it an advantageous technique for surface science (i.e. not just under vacuum, but also at active solid/gas and solid/liquid interfaces). Our technique introduces NMR at surfaces and is a key step towards wide-ranging applications for *in-situ* analysis of catalysis, materials, biological, and 2D materials research.

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^{13}C NMR OF PARAMAGNETIC LAYERED DOUBLE HYDROXIDES (LDH)

Layered double hydroxides (LDH) find widespread application as catalysts and sorbents. They are layered inorganic materials consisting of positively charged metal cation layers separated by an interlayer, which contain anions for charge neutrality and water [1]. LDH are notorious for structural disorder and lower crystallinity, which renders their characterization challenging. A series Mg_2Al-LDH doped with 0, 18%, 34%, 68% and 84% Ni(II) [2] with ^{13}C labelled carbonate in the interlayer has been synthesized. ^{13}C MAS NMR spectra of these materials has been recorded. It was observed that the ^{13}C NMR shielding tensor has three distinct eigenvalues, i.e, it possesses both anisotropy and rhombicity (asymmetry) for all samples, but both the anisotropy and asymmetry are strongly dependent on the concentration of the paramagnetic Ni ion doping. In contrast, axial symmetry is prediced based on the commonly used hydrotalcite crystal structure. Thus, we use computational approaches to explain the experimental observations and gain insight into the atomic level structure of LDH.

The contributions to the total ^{13}C shielding tensor of the intercalated CO_3^{2-} can be divided into diamagnetic contribution within the CO_3^{2-} ion and long-range paramagnetic contribution of the metal-oxygen layers. Since the anisotropy and asymmetry are strongly dependent on the concentration of the Ni ions, we concentrate mainly on the long-range paramagnetic contribution due to dipolar part of the hyperfine coupling, modelled as a lattice sum of the electron-spin susceptibility a localized point dipole of the Ni centre [3]. This effect constitutes an important contribution to both the anisotropy and asymmetry, whereas the isotropic shift hardly changes as observed experimentally. Still, the experimental shift asymmetry is not quantitatively reproduced by the most precise single crystal structure (1M quintinite). On the other hand, other contributions to the anisotropy and asymmetry may arise for admixture of further structural polytypes (stacking disorder) and consideration of the hydrogen bonding situation of the carbonate anion.

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MISSTEC SEQUENCE, A NEW APPROACH FOR RF PULSES CALIBRATION

NMR sequences are composed of multiple radio-frequency pulses. The probe adjustment, the sample concentration and the deuterated solvent influence the duration and the power of these pulses, impacting the applied angle. Therefore, it is important to calibrate them to reduce imperfections, loss of SNR, and to keep coherence selection. The commonly used method is to measure the nutation curve, variating the pulse duration or the pulse power to know the 90° angle. However, this method is impacted by the off-resonance effects, radiation damping, B_1 and B_0 inhomogeneities (shims) [1] even if an optimised model is used to fit this curve [2]. Furthermore, taking into account relaxation implies to know each T_1 value. Most of the time these values cause a long acquisition time (up to 30 min to obtain a complete and well digitized nutation curve).

The MISSTEC sequence (alpha-(TE/2)-2*alpha-(TE/2)-acq-TM-alpha-TE/2-acq) was first proposed for mapping B_1 inhomogeneity in MRI [3], and later it was applied on high resolution spectrometers for the same purpose [4]. The flip angle alpha is obtained from the ratio between the stimulated and the spin echo which are sampled in presence of field gradient. Therefore, the results are not impacted by the B_1 and B_0 inhomogeneities or the relaxation times (T_1 and T_2). Measurements performed with the MISSTEC sequence are comparable to nutation results for ^{1}H. We show that with this sequence it can be possible to have 0.3% of precision in 8 seconds for ^{1}H.

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THE MAGNETIC SPY: PROBING LOCAL MAGNETIC PROPERTIES WITH SOLID-STATE NMR

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Acquisition of information on the magnetic properties at the molecular scale — namely the local magnetic susceptibility — is important because it will lead to faster developments of advanced technological applications based on local magnetic properties such as new methods for information storage [1], contrast agents for MRI [2], spintronics [3], etc...

Polarized Neutron Diffraction [4], SQUID-based magnetometry [5], muon spin rotation [6], Electron Paramagnetic Resonance [7] give a precise insight on local magnetic susceptibility. These methods however require heavy equipment as well as relatively large crystalline samples which may be difficult to obtain.

We implemented a model that has been tested and proven efficient in predicting paramagnetic SS-NMR spectra of microcrystalline powders [8]. This model calculates the effect of the hyperfine interaction on the NMR spectra. To this end, we need the crystallographic structure of the molecule. With the structure, the program will generate the position of all paramagnetic atoms on a given radius (50 Å) and set an initial rank-2 magnetic susceptibility tensor. This tensor is the only free parameter of this model and it affects the shape of the theoretical spectrum. From there, the program calculates the hyperfine interaction between all the paramagnetic atoms and one NMR observable nucleus and repeats this for each observable nucleus in the asymmetric unit cell. In the end, the program uses an optimization function to find the best agreement between the experimental and theoretical spectra, giving us an orientation of the local magnetic susceptibility tensor.

This model was compared to experimental data on a series of isostructural lanthanide oxalate metal-organic frameworks. We will present and discuss our results on lanthanum, praseodymium and cerium oxalates.

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ACQUISITION DURING TEMPERATURE CHANGES: SIGNAL PROCESSING METHODS AND A PROTEIN NMR EXAMPLE

In protein NMR, temperature coefficients (TC), i.e., the dependencies of chemical shifts on temperature, reveal valuable information. For instance, non-linearities in TC indicate the presence of a protein compact state.

The acquisition for TC experiments, however, is hampered. One would need a series of at least 3-dimensional spectra. The acquisition of a single spectrum alone takes hours. The series should contain a number of spectra with temperature increment. This number should be big enough to provide good resolution. Such acquisition will take too long to be practically feasible.

Here, we present two methods to overcome this obstacle. Both are based on non-uniform sampling (NUS): some points of the indirect dimensions are skipped and later reconstructed based on definite mathematical assumptions about the spectrum. One and the same NUS data-set can then be treated by both methods.

1) time-resolved NUS [1]: the data is divided into overlapping subsets and reconstructed. This gives a boost in resolution/acquisition time balance. The reconstruction we use is based on compressed sensing principle: out of all possible spectra, the sparsest one is iteratively selected.
2) a variant of the Radon transform [2], where temperature is treated as an additional dimension.

We applied the aforementioned approaches to temperature-swept 3-dimensional HNCO spectra of two intrinsically disordered proteins - osteopontin and CD44 cytoplasmic tail [3]. Then, we established the non-linearities in their TC. The results were in line with general biochemical considerations.

During my presentation, I will focus on the signal processing aspects of the two methods and show how they are complementary in terms of sensitivity and resolution.

The software developed in our group for both processing methods is available at www.nmr.cent.uw.edu.pl → Downloads.

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ROTATIONAL DIFFUSION OF CHIRAL MOLECULES IN THE ELECTRIC FIELD STUDIED BY MOLECULAR DYNAMICS

The electric field ***E*** perturbs the rotational diffusion of a molecule that bears the permanent electric dipole moment. Studies of this perturbation are especially pronounced if one considers a chiral molecule. In this case, it has been predicted that interaction involving the so-called antisymmetric part of the indirect spin-spin coupling (***J****) generates signals which allow direct discrimination between enantiomers [1, 2], *i.e.*, without the usage of chiral derivatizing agents and solvents. This effect may be analyzed using tensor calculus, but in order to include the fact that the molecule is subjected to friction in the solution, *i.e.*, it cannot follow the time-varying ***E*** field instantly, one needs to use more advanced models of the rotation diffusion such as those based on the molecular dynamics.

We chose two chiral compounds – L-alanine and (*R*)-1,1,1-trifluoropropan-2-ol. In the former molecule, the spin system was ^{19}F-^{1}H, *i.e.*, C$^{19}\mathbf{F}_3$-HC(O$^{1}\mathbf{H}$)-CH_3), and in the latter ^{15}N-$^{13}C_\alpha$. In order to provide the most reliable data for our simulations of the predicted effect in trifluoropropanol, we used the spin-spin coupling determined from the gas-phase NMR studies. We found that extrapolated to the zero-density limit spin-spin coupling $^{3}J(^{19}\mathrm{F}, {}^{1}\mathrm{H})$ is (6.5 ± 0.5) Hz. This value agrees with the results of quantum chemical computations performed in Dalton. Moreover, the computations indicate that the antisymmetric parts of the studies couplings are: $^{1}J^*(^{15}\mathrm{N}, {}^{13}\mathrm{C}) = -0.332$ Hz and $^{3}J^*(^{19}\mathrm{F}, {}^{1}\mathrm{H}) = 1.25$ Hz.

Molecular dynamics calculations have been carried out with the GROMACS program for the alanine zwitterion in water and trifluoropropanol dissolved in ethanol (simulation length > 10 ns, box size ~6×6×6 nm). We considered two cases: (*i*) there is no electric field; thus, isotropic rotational diffusion occurs, and the effect is not present (estimation of the uncertainty of the simulation), and (*ii*) application of the ***E*** field of the amplitude 1 V/nm and frequency 3 GHz (computation of the amplitude of the effect). We quantified the influence of the electric field on the rotational diffusion by evaluation of the ensemble-averaged products of Wigner functions.

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ELECTRICALLY DETECTED MAGNETIC RESONANCE SETUP BASED ON A NOVEL THz EPR SPECTROMETER

Electrically detected magnetic resonance (EDMR) is a sensitive and powerful technique for the determination of fundamental intrinsic properties of semiconductive solid-state materials. We develop an EDMR setup based on the terahertz EPR spectrometer located in CEITEC BUT, which will operate at frequencies up to 1.1 THz and external magnetic fields up to 16 T. In this way, our main THz EPR setup will allow scanning both the magnetic field and frequency. Thus, compared with the conventional CW EDMR, this will allow obtaining not only the dependence of the voltage change on the external magnetic field at a certain frequency, but also to take a frequency-field map [1].

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CORE SCIENTIFIC DATASET MODEL: A LIGHTWEIGHT AND PORTABLE MODEL AND FILE FORMAT FOR MULTI-DIMENSIONAL SCIENTIFIC DATA

With increasing pressure from the funding agencies and scientific journals to archive and share primary and processed data, there is a growing sense of urgency for a stable, resourceful, and future-proof file format for the exchange of scientific datasets. We address this problem by proposing a Core Scientific Dataset (CSD) Model[1] that can encode a wide variety of multi-dimensional and correlated datasets. The CSD model with JavaScript Object Notation (JSON) serialization is presented as a lightweight, portable, and versatile standard for intra- and interdisciplinary scientific data exchange. This model supports datasets with a p-component dependent variable, $\{U_0,\ldots,U_q,\ldots,U_{p-1}\}$, discretely sampled at M unique points in a d-dimensional independent variable $(X_0,\ldots X_k,\ldots X_{d-1})$ space. Moreover, this sampling is over an orthogonal grid, regular or rectilinear, where the principal coordinate axes of the grid are the independent variables. It can also hold correlated datasets assuming the different physical quantities (dependent variables) are sampled on the same orthogonal grid of independent variables. The model encapsulates the dependent variables' sampled data values and the minimum metadata needed to accurately represent this data in an appropriate coordinate system of independent variables. The CSD model can serve as a reusable building block in the development of more sophisticated portable scientific dataset file standards. It is currently supported by the csdmpy Python package, SIMPSON, EasyNMR, DMFit, and RMN.

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SOFTWARE FOR VERSATILE HFEPR SPECTROMETER

Even though nowadays some High Frequency Electron Paramagnetic Resonance (HFEPR) spectrometers are commercially available, most of them are still custom-built machines. A versatile HFEPR spectrometer with a Frequency Rapid Scanning (FRaScan) feature was built at CEITEC BUT [1]. A specific software solution for control of spectrometer was developed in LabVIEW [2] because it simplifies memory management, parallel execution, and communication with instruments via standard interfaces. The software is composed of individual modules for each instrumental part and main controller, which are connected via messaging service. This allows modules to run independently on each other, distribute tasks in threads and decrease average CPU load. The main controller is the top software component that provides an event driven user interface, as well as execution of a measurement routine and distribution of messages to modules.

Currently, there are 3 types of user interface for different tasks. One for Continuous Wave (CW) measurements, second for Frequency Domain Magnetic Resonance (FDMR) measurements and third for Zeeman diagram measurements [3]. All of them have a similar design and share most of the back-end components. In case of CW and FDMR measurements it is possible to use a scripting feature to automatically execute a sequence of measurements. An integrated script editor utility can generate a batch of measurement sequence with sweep of desired parameter such as microwave frequency or magnetic field, orientation and temperature. Because FDMR measurements are much faster to perform and can yield a large data, the TDMS file format was used to organise datasets obtained from whole measurement sequence. The Zeeman diagram measurements are done by performing an FDMR measurements during continuous sweep of a magnetic field

To demonstrate capabilities of spectrometer and its automated control we performed a measurements on single crystal of copper acetate as a model system for molecular magnetism. Measurements in a frequency range 205-245 GHz, at temperature 200 K and magnetic field 7.5 T were done in less than 2 hours for 280 orientations with step 1 degree. For comparison, alike measurements in a CW mode would take approximately 30 hours. Obtained data were post processed in orientation vs frequency map. Additionally, a Zeeman diagram was measured in a field range 4.5 to 9 T, and a frequency range 180 to 240 GHz.

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COMPACT HIGH-PRESSURE GAS-PHASE NMR SPECTROSCOPY

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A new, low-cost, and highly versatile setup for benchtop NMR spectroscopy and relaxation measurements of gases at high-pressure is introduced [1]. It utilizes mostly commercial parts, which can be easily exchanged, and it includes multiple safety features. Except for the gas bottles, the novel high-pressure (HP) setup is small enough to fit into a 1.2 m wide fume hood. It enables pressures up to 200 bar on single gas components and mixtures of multiples gases. A chosen pressure can be set with the help of a mixing chamber within a couple of seconds.

The versatility of the setup is demonstrated in the first step by quantitatively analyzing the pressure dependence of the pure methane and the composition of a gas mixture containing two hydrocarbons and hydrogen at 200 bar total pressure with the help of ^{1}H NMR spectra. The sensitivity of modern benchtop NMR spectrometers is so good that a signal-to-noise-ratio above 10^4 can be achieved, although densites of hydrocarbon gas mixtures are about 6 times lower compared to water. Moreover, the spectral resolution of modern benchtop NMR spectrometers is high enough to quantify the gas composition of this particular self-made 3-component mixture simply by integration of the signals of interest without the need of a spectral deconvolution software.

Furthermore, the newly designed HP setup was applied to investigate a gas-solid interaction by exposing solid polyvinyl chloride (PVC) to CO_2 gas. Although CO_2 is invisible in proton NMR, the ingress of gaseous and eventually supercritical CO_2 into the polymer matrix can be observed by changes in the PVC spectrum in terms of ^{1}H linewidth and peak integral. In addition the gas-solid interaction, the gas-liquid interaction between benzene and methane is observed. As methane pressure increases, the mole fraction of dissolved gas rises accordingly.

The presented results demonstrate that the proposed HP-setup bears great promises in various gas applications. We expect that this novel setup will open up new opportunities also for investigations which currenly largely rely on other analytical methods, such as gas chromatography.

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LOW FIELD NMR CHARACTERIZATION OF TISSUE MIMICKING GELS FOR MRI PHANTOM APPLICATIONS

Magnetic Resonance Imaging (MRI) phantoms are calibration objects which provide tissue-equivalent properties (e.g. relaxation times, electrical conductivity, etc), used to evaluate the effectiveness and performance of MRI scanners and quantitative MR algorithms. An MRI phantom, as a standard system, should exhibit anatomical, physiological, or bio/chemical characteristics that are do not change over time, and result to be comparable between scanners, manufacturers, and measurement protocols. Therefore, an MRI phantom should have traceable, validated, and monitored components. In this sense, the characterization of phantom materials with respect to physical Nuclear Magnetic Resonance (NMR) properties and stability is essential [1]. Especially, low field NMR systems, with their magnetic field strengths corresponding to those of MRI scanners (1.5 T, 3 T) are valuable reference platforms to characterize the relaxation times of phantom materials and to check their long-term stability.

MRI phantoms, typically composed of paramagnetic ions such as $CuSO_4$, $NiCl_2$, $MnCl_2$, or $GdCl_3$ in order to have comparable relaxation times to those of human tissues, are prepared either as aqueous solutions or as gels. Upon two, gels give the advantage of making large and strong phantoms. Moreover, being robustly processable, they can be shaped in more more complex geometries, serving the production of heterogeneous phantoms in the shapes and sizes of human organs, without the need for rigid containers and septa. Therefore, gels are recognized as interesting materials in the development of MRI phantoms. Especially, polysaccharides such as agarose, agar, gelatin, carrageenan, and synthetic polymers such as polyvinyl alcohol, polyacrylamide are well-known materials to prepare the MRI phantoms because of their physically crosslinkable feature [2, 3]. These materials dissolve in hot water and upon cooling turn out to a semi-solid gel, giving the advantage of easy preparation.

In this work, gellan gum, and agar based polysaccharide gels have been studied in order to mimic the relaxation properties of brain tissues (white matter, grey matter) and cardiac muscle. Gellan gum has been chosen as the gel matrix to increase the strength and the mechanical stability of the gel while different amounts of $GdCl_3$ and Agar have been used in the formulation to tune T_1 (spin-lattice), T_2 (spin-spin) relaxation times, respectively. NaN_3 has also been added as a preservative to retard mold formation over time. 1.4 T (60 MHz) desktop NMR machine has been used in the characterization of T_1, T_2 relaxation times of the gels as well as for monitoring their long-term stability.

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NOVEL LOW-COST NMR METHOD FOR IDENTIFICATION AND QUANTIFICATION OF PVC PLASTICIZERS

Polyvinyl chloride (PVC), the third most produced polymer world-wide and has a large variety of applications ranging from medical to industrial products. To meet the largely different property requirements, various plasticizers are added in different amounts to the PVC product. Yet, in most cases, the plasticizers are only mixed with the PVC matrix and consequently they tend to migrate out of the PVC product with time. This leads to a deterioration of the PVC product properties as the amount and type of plasticizer have a critical impact on them. Moreover, legal regulations exist about the use of particular plasticizers in contact with human skin and groceries. Thus, it is of key importance to identify high throughput analytical methods which are able to identify the used plasticizers and quantify their amounts. To meet these current challenges, our work introduces a new, simple, and low-cost experimental methodology for the identification and quantification of PVC plasticizers in solution or in a product [1]. It is based on low-field ^{1}H spectra acquired with a low-cost and easy to operate NMR benchtop device and the usage of appropriate non-deuterated solvents.

Despite being rather complex molecules with molar masses around 200 g mol^{-1}- 500 g mol^{-1}, the ^{1}H spectra of all investigated plasticizers show well separated peak-clusters for aromatic (~7 ppm), α-CH_2 (~4 ppm) and aliphatic groups (1-2 ppm). Since the aliphatic peak regions of various plasticizers are rather similar, the discrimination and quantification of the various plasticizers can be easily done using characteristic signals in a spectral range above 2.5 ppm and in the presence of non-deuterated solvents which have signals outside the spectral range of interest. For this purpose, non-deuterated n-hexane, a suitable solvent for PVC, was chosen as an alternative to more costly deuterated solvents. The use of appropriate non-deuterated solvents, shown here for the first time, is very appealing due to their much lower costs compared to the costs of the deuterated solvents, which are the standard solvents used for liquid-state NMR spectroscopy. The reliability of the proposed method using non-deuterated solvents is demonstrated by comparisons with the spectra recorded on the same plasticizer dissolved in deuterated chloroform solvent.

Given that low-field NMR equipment is, by far, more affordable then the high-field NMR and the costs for maintenance, extra personnel, and facilities are negligible, the shown results indicate that the proposed low-field NMR analysis is a low-cost alternative for the study of PVC plasticizers. Plasticizer concentrations below 2 mg mL^{-1} in solution, corresponding to 3 wt% in a PVC product, can be quantified within only 1 minute. Due to its simplicity, the proposed methodology is appealing even to non-NMR experts and can be applied to identify and quantify plasticizers of large quantities of polymer samples in a short experimental time.

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FOLLOWING CHROMIUM (IV) REDUCTION BY LOW RESOLUTION NUCLEAR MAGNETIC RELAXOMETRY

Chromium is released in the effluents of several industries. It is for example used for stainless steel production, leather tanning and electroplating. Cr(VI), mostly of anthropogenic origin, is very soluble and toxic for the flora and fauna, even at low concentration in water (0.1 mg/L) while Cr(III) is an essential nutrient (daily intake of 25 - 35 µg for an adult) that is only toxic at high concentrations[1]. One strategy to reduce the toxicity of chromium in water is thus the reduction of noxious Cr(VI) to Cr(III). Several compounds can be used to achieve this reduction such as hydrogen peroxide[2], Fe(II) ions[3], zerovalent aluminium[4] and plant extracts[5]. The monitoring of the reduction is often carried out by UV-Visible spectrometry after the addition of diphenylcarbazide to the solution[6], the so-called *"carbazide test"*. This technique is really sensitive since it allows the detection of Cr(VI) in water even at very low concentrations (0.002 mg/L). However, it is destructive for the sample and can only be used for clear solutions which is a limitation when using reductants as powders of zerovalent aluminium or iron, and when working directly on wastewater. Moreover, the quantification with carbazide must be achieved in a given concentration range in order to remain linear and cannot be used at Cr(VI) concentrations larger than 2 mg/L.

At higher concentrations of chromium and in complex systems, nuclear magnetic resonance relaxometry, based on the magnetic properties of chromium, could be applied. Indeed, all the orbitals of Cr(VI) are full which results in a null magnetic moment. On the contrary, the electronic configuration of Cr(III) ($3s^2 3p^6 3d^3$) results in a non-null magnetic moment of 3.97 μ_B. Cr(III) is thus paramagnetic and will shorten the water proton relaxation times T_1 and T_2 in aqueous solutions[7]. In this study we show that the reduction of Cr(VI) to Cr(III) by hydrogen peroxide in acidic conditions can be followed through the shortening of water T_1 and T_2. The relaxation rates $1/T_1$ and $1/T_2$ of partially reduced Cr(VI) solutions are linearly depending on the actual Cr(III) content of the mixture, which was determined using direct UV-visible spectroscopy, without the use of carbazide[8]. In order to determine the best experimental conditions, the field, temperature and pH dependences of the relaxation rates of completely reduced solutions have been studied. The use of T_2 seems more suited to follow the chromium reduction, since it is faster to measure than T_1 and also because $1/T_2$ increases with the field while $1/T_1$ decreases.

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EPR STUDY OF HIGHLY STABLE BIRADICALS PERSPECTIVE FOR DNP IN CELL

Dynamic Nuclear Polarization (DNP) is a powerful technique to improve the sensitivity of solid state NMR experiments on biological materials, which allows high-resolution spectra to be obtained. Biradicals are often used as polarizing agents due to the relatively strong exchange interaction between the paramagnetic moieties[1]. But recent studies showed the crucial factor for CE-DNP is not the large sum, spin exchange interaction (*J*) and dipolar coupling (*D*), but rather the relative magnitude of *J* and *D*, expressed as the *J*/*D* ratio [2]. Therefore, the problem arises of obtaining biradicals with a certain value of spin exchange. Also when working with polarizing agents, there are problems with their solubility in water and their stability in reducing media which is very important at the biological sample preparation. If the problem with water solubility in some biradicals is solved, but their stability is uncertain. According to previous studies the stability of nitroxides depends on ring size and substituents adjacent to the paramagnetic center [3].

In this work we studied the biradicals based on five-membered ring nitroxides with tetraethyl substituents, which will provide additional stability. Also, the use of trityl as one of the paramagnetic centers makes it possible to increase the resistance to reduce and attenuate the effect of depolarization at high magnetic field [4]. A series highly stable nitroxyl-nitroxyl and trityl-nitroxyl with different *J* values were synthesized and their magnetic resonance parameters as well as stability against reduction by ascorbic acid in buffer solution at pH 7.4 were studied. The stability of obtained DNP agents is very high and can allows to use them in the cell. One biradical based on low toxic OX063 trityl and tetraethyl pyrrolidine radical reveal the highest stability and water solubility.

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X-BAND EPR STUDY OF A POTENTIAL CHEMICAL SYSTEM FOR INDIRECT PHOTO-DNP

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In certain organic molecules, triplet states are generated by pulsed laser excitation of the sample. The excited triplet state is populated via intersystem crossing (ISC) from the excited singlet and often exhibits a non-Boltzmann polarization. It is possible to exploit this high polarization and directly transfer it to nuclei to enhance the NMR signal in a direct photo-DNP, or triplet DNP, experiment. In indirect photo-DNP, one transfers the polarization from triplet electrons first to a doublet species. The surrounding nuclei obtain the polarization from the doublet species in a consecutive step. The large majority of the photo-DNP experiments has been done with pentacene, and the polarization transfer was direct. But there are disadvantages to this molecule such as the poor photo-stability. Also, so far for all photo-DNP has been done at low field (X-band). Therefore, we are exploring a new chemical system for indirect photo-DNP, which could possibly also be used for high-field photo-DNP.

In 2002, Tarasov *et al.* reported non-equilibrium spin polarization of nitroxide radicals upon photo-excitation of molecular complexes of a nitroxide radical and a quinone, which form in frozen toluene [1]. They measured the transient X-band (9.7 GHz) EPR spectra of a series of para-quinones with nitroxide radical (TEMPO) in frozen solutions of toluene. They observed the transient triplet signal from the quinones as well as a strong emissive transient signal from the nitroxides. The latter suggests that the nitroxides obtain a strong, non-thermal polarization. In our laboratory, we reproduced the results of Tarasov *et al.* using an excimer laser as the photo-excitation source. We expanded the transient EPR data set and simulated the triplet spectra of the quinones using EasySpin.[2]. We found that all three quinones show two triplet species with distinct ZFS parameters. Furthermore, to confirm the enhanced nitroxide polarization, we recorded echo-detected EPR spectra at X band. Upon photo-excitation these spectra showed a decrease of the absorptive TEMPO signal, which could result from emissively polarized nitroxide radicals, but also from sample heating by the laser. However, when performing an inversion recovery experiment, we observed an increase of the inverted echo signal when the laser flash precedes the π-pulse. This suggests that at least some of the nitroxide radicals in the sample have obtained an enhanced polarization. To improve our experiment, we are currently assembling a laser set up, which will enable us to generate triplets in our samples more efficiently.

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NEW, HIGHLY SENSITIVE OFF/ON EPR PROBES TO MONITOR ENZYMATIC ACTIVITY

Many pathologic conditions are often associated with unregulated level of enzyme activity. Therefore, the detection and quantification of the enzymatic activity is extremely important for a diagnostic purpose. [1] In this contest, a particularly interesting class of enzymes is represented by carboxylesterases (CEs). These enzymes belong to the serine hydrolase superfamily and are involved in the hydrolysis of endogenous ester-containing substrates as well as ester-containing drugs, thus playing a crucial role in a variety of metabolic processes. [2] CEs are upregulated in many tumors and the assessment of their activity may be of diagnostic interest as well it may provide relevant information regarding chemotherapeutic effects of antitumor ester-containing drugs and pro-drugs. Currently CE activity is assessed by means of fluorescence and UV-based methods. [3] In this study, we propose the use of electron paramagnetic resonance (EPR) as an easy method to probe CE enzymatic activity *in vitro*. EPR has the advantage to be highly sensitive and with limited interferences from the matrix, also in the presence of turbid samples.

For this application, TEMPO derivative nitroxide radicals were conjugated to a fatty acid (Dodecanoic acid) *via* the formation of an ester bond to yield **Tempo-C12** (TC12) and **Tempo-2-C12** (T2C12). Both compounds exhibit a low solubility in water and aggregate to form stable micelles with the lipophilic tail in the core and the nitroxide radical exposed to water. The radicals in the micellar aggregates are practically EPR silent showing a low and broad EPR signal. The hydrolysis of the ester bond catalyzed by CEs release two different nitroxide radicals: 4-Hydroxy-TEMPO and 4-Oxo-TEMPO. The first one is released from the TC12 micelles while the second from T2C12 micelles and they generate an intense and narrow EPR signal, that is proportional to the enzymatic activity. [4,5]

CEs1, CEs2 and esterase from porcine liver (PLE) were tested. The result obtained show that the micelles of TC12 and T2C12 have a much higher selectivity toward the CEs2, and a Limit of Detection of the same order of those ones obtained with optical methods. In conclusion, this is a new promising tool to quantitatively detect the CEs2 activity showing an interesting off/on EPR signal after the enzymatic activity. The method can be applied for monitoring the enzymatic activity *in vivo*, eventually also through the detection of the Overhauser MRI response.

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LONG RANGE DISTANCE DETERMINATION ON FULLY DEUTERATED RNA

PELDOR (pulsed electron electron double resonance) also called DEER (double electron electron resonance) is a magnetic resonance method for to determine the distance and the distance distribution in double spin-labeled macromolecules like proteins, RNA, or DNA as well as polymers [1,2]. The maximum accessible distance r_{max} is limited by the dipolar observation time window t_{dip} spanned by the pulse settings $r_{max}[nm] \approx 4\sqrt[3]{t_{\mathrm{dip}}[2\mu s]}$ [3]. This time window depends strongly on the phase memory time of the nitroxide spin labels. The phase memory time at the experimental temperature of 50 K is manly determined by the coupling to other spins in the sample and depends on the gyromagnetic ratio of such spins. Therfore protons and other electrons are the main contributors. Basic techniques to get rid of such spins is substituting protons by deuterons and reducing the radical (and therefore sample) concentration [4,5].

In this contribution we demonstrate that PELDOR experiments on fully deutereated RNA molecules allow to adress distances up to 10 nm quantitatively and to analyse conformational flexibility of long RNAs. We demonstrate this on a fully deuterated dengue virus (DENV) 3'SL RNA construct [6,7], using an unnatural base pair in a modified genetic code for posttranscriptional spin labelling [8].

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THE OUTER MEMBRANE INSERTASE BAMA EXCURSES OVER A BROAD CONFORMATIONAL SPACE IN NATIVE ENVIRONMENT

Outer membrane proteins (OMPs) are involved in several cellular processes vital for the survival of Gram-negative bacteria. The proper folding and insertion of the unfolded OMPs into the outer membrane is a crucially regulated process. In *E. coli*, this process is catalysed by the β-barrel assembly machinery (BAM) complex. BAM is a heterooligomeric complex composed of the central β-barrel protein BamA and its associated lipoproteins BamB-E. BamA is highly conserved and essential, making it an attractive target for novel antibiotics. In BamA, the C-terminal transmembrane domain is connected to five N-terminal polypeptide transport associated (POTRA) domains located in the periplasm. The first and last strands of BamA (β1and β16) creates a lateral gate, which acts as the functional hotspot for protein folding. Structural studies have revealed three major conformations of the BamA barrel namely inward open (IO), lateral open (LO), and lateral open substrate-bound states [1,2]. In the IO state, the β16 strand exists in a fully zipped conformation, whereas in the LO, it is in a kinked state. The lateral opening further increases when the substrate is bound. Only BamA and BamD are essential and the role of other lipoproteins or how the conformational dynamics of the complex is regulated remains unknown. The outer membrane is an asymmetric bilayer consisting of phospholipids and lipopolysaccharides. It has been impossible to replicate the unique properties of the outer membrane *in vitro*, which largely hindered a thorough understanding of the BAM function.

Here we used *in situ* pulsed electron-electron double resonance spectroscopy (PELDOR or DEER [3]) to investigate the conformational states of BamA in the native membrane or in the LDAO detergent micelles. Cysteine pairs were introduced at the extracellular and periplasmic areas of BamA near the lateral gate. These cysteine pairs were labeled with MTSL either in the LDAO micelles or directly in the isolated native outer membranes. We experimentally determined the distances between the extracellular loops L1-L8, L3-L8, and the periplasmic turns T1-T6. In detergent micelles, BamA is observed mostly in the inward-open conformation. The native membrane considerably modulated the conformational space. BamA exists as a monomer and excurses over a broad range of conformations including the inward open, lateral open, and lateral open substrate-bound states. Our results validate the three major conformations of BamA in the cellular membrane. In summary, BamA alone can transverse through multiple conformations without any of the lipoproteins, which might be the crucial aspect underlying its divergent functions *in vivo*.

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RESOLUTION OF CHEMICAL SHIFT ANISOTROPY IN ^{19}F ENDOR SPECTROSCOPY AT 263 GHZ/9.4 TESLA

Fluorine spin labels have found many applications in magnetic resonance due to their favorable properties. Particularly, the nuclear spin of 1/2, 100% natural abundance, a high gyromagnetic ratio and a large range of chemical shifts make them valuable in NMR and high field ENDOR.[1,2] Our quasi-optical EPR spectrometer enables ENDOR measurements at 263 GHz with field strengths comparable to NMR (9.4 T), which increase spectral resolution and orientation selectivity.[3]

In this contribution ^{19}F ENDOR spectra at 263 GHz and 94 GHz are compared for five nitroxide radical model systems consisting of a phenyl group substituted with fluorine or a CF_3-group. The spectra at 263 GHz display asymmetry, which is not resolved in the spectra at 94 GHz. It indicates that the chemical shift anisotropy (CSA) is resolved, which so far has been neglected in EPR and ENDOR spectroscopy.

Analysis of the spectra both at 263 GHz and 94 GHz is presented. For the simulations, the orientation of the chemical shift tensor towards the g-frame is described by Euler angles and can provide further structural information on the sample. The CSA can be resolved also for difluorinated samples as long as the hyperfine coupling tensors are resolved. For the CF_3 substituted system the resolution of chemical shift is possible in case free rotation of the group is hindered, however, analysis relys on DFT calculations due to the many interdependent parameters.

In comparison to 94 GHz, ^{19}F ENDOR at 263 GHz can display high resolution spectra and can improve spectral interpretation by providing information on the chemical shift tensor.

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TOWARD TIME-RESOLVED GD-GD DIPOLAR EPR FOR TRIGGERED FUNCTIONAL DYNAMICS IN PROTEINS

Protein based molecular machines are central to every biological process and enable living cells to perform basic functions. Our understanding of these machines is primarily based on their 3D structure -- a static picture. In order to understand their operation as machines it is necessary to investigate the evolution of their conformational changes in time, triggered by an external stimulus. The combination of site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) is a well-established biophysical tool that has been used to study light activated conformational changes in proteins in real time at room temperature, which can also be correlated with chromophore-sensitive photocycle kinetics [1]. We are developing time-resolved (μs-s timescales) Gd-Gd dipolar-broadening EPR (TiGGER) for tracking protein functional dynamics in a close-to-native environment.

We will present our work toward TiGGER, which includes progress in high efficiency double-spin labeling of a light-sensitive protein, as well as hardware design and testing. TiGGER makes use of the ability of 240 GHz Gd(III) cwEPR to extract inter-spin distances exceeding 2 nm at room temperature [2] and will utilize rapid-scan transient EPR to track changes in residue local environment in real time [3]. Taking advantage of the well-known effects of dipolar coupling on EPR linewidth, TiGGER will collect linewidth information of the protein sample every 10μs that can be used to determine inter-spin (and therefore, residue-residue) distance as a function of time. Further, due to the exceptionally narrow line of Gd(III) labels at high field (~8.57 T) [4], a low rapid-scan sweep rate will avoid rapid passage effects while still collecting the full lineshape in a matter of microseconds [5].

We chose to focus on a light-sensitive protein called AsLOV2 which has gained great popularity in the bioengineering field as mechanical actuators, because they offer control of cellular activities with spatiotemporal specificity. The widespread application of these proteins has motivated efforts to engineer improved variants [6,7], making it necessary to understand the long-range non-equilibrium structural changes [8] that accompany AsLOV2 on light activation. We will apply TiGGER to elucidate important time-dependent structural information on AsLOV2, as well as other optogenetic mechanosensitive proteins in the future.

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DEER AND RIDME MEASUREMENTS OF THE NITROXIDE-SPIN LABELLED COPPER-BOUND AMINE OXIDASE HOMODIMER FROM ARTHROBACTER GLOBIFORMIS

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In the study of biological systems, pulse dipolar spectroscopy (PDS) methods are known to be invaluable techniques capable of measuring dipolar-dipole interactions between paramagnetic centres which, in turn, aid the elucidation of nanometre-scale distances. In this work, Double Electron Electron Resonance (DEER) and Relaxation Induced Dipolar Modulation Enhancement (RIDME) experiments are carried out on the copper amine oxidase from *Arthrobacter globiformis* (AGAO), which contains multiple measurable nanometre-scale distances between nitroxide spin labels and bound Cu(II) paramagnetic centres. To aid in the identification of the distances measured within the AGAO homodimer, protein modelling techniques are employed. Using these models for comparison to experimental data, we determine that the distances measured within the homodimer indicate that the active Cu(II) binding sites are unoccupied, and instead, the Cu(II) is binding to surface sites. We show that two distinct distances can be measured, with the longer of the two providing a measurement that exceeds 5 nm, through optimisation of experimental parameters and careful data analysis, and using these results, we also discuss that the use of RIDME experiments may hold advantages over DEER for measurement of these long distances [1].

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INVESTIGATIONS ON THE RELAXATION BEHAVIOUR OF NITROXIDES AT HIGH FIELDS AND PREDICTIONS OF THE RESULTING RIDME BEHAVIOUR

Pulsed dipolar spectroscopy (PDS) includes multiple methods in electron paramagnetic resonance (EPR) that allow to extract distance information from the dipolar interaction between paramagnetic spin labels in a 2-8 nm range. By far the most popular PDS technique is pulsed electron-electron double resonance (PELDOR also called DEER). But particularly at higher fields, where the *g*-anisotropy causes spectra to be extremely broad, the efficiency of PELDOR is severely limited by the small available excitation bandwidth of high frequency pulses.

A PDS technique which is less affected by the small microwave pulse excitation bandwidth is Relaxation-induced dipolar modulation enhancement (RIDME). In contrast to PELDOR, RIDME does not use a microwave pulse for the inversion of the dipolar coupled spin, but relies on relaxation processes and is therefore independent of the available excitation bandwidth. This should make RIDME particularly suited for high field measurements.

In the past we have shown that RIDME with high spin Mn^{2+} centers gives both higher modulation depths and higher signal-to-noise ratio compared to PELDOR at 260 GHz microwave frequency.[1] In the study presented here, we were interested in how efficient RIDME with bis-nitroxides at high magnetic fields could be. Therefore we investigated the relaxation behavior of nitroxides in dependence of different parameters, such as magnetic field strength B_0, sample temperature T, radical concentration c and mixing time T_{mix} in a RIDME experiment.

Very long phase-memory times T_m between 20-30 µs where measured at 263 GHz for two different nitroxides at a concentration of 0.5 mmol/L in a deuterated solvent matrix. Even higher phase-memory times can be expected by further reducing the concentration, since an increase of 10 µs was observed with halve of the concentration. While these values predict a high potential of the RIDME method for nitroxide spin-labels, we also realized that a possible limitation for the RIDME efficiency at high fields might be an enlarged spectral diffusion contribution to the relaxation times.

Inspired by the paper from Ritsch *et al.* [2] we propose a method for estimating optimal temperatures and mixing times in a RIDME experiment for bis-nitroxides. We also consider the high spectral anisotropy of T_1 and an effective memory time T_m as a function of the mixing time T_{mix} for the calculation of the RIDME experiment efficiency.

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THROUGH THE LOOKING-GLASS: A GLIMPSE AT HOW SMALL LIGANDS MODULATE THE MAGNETIC PROPERTIES OF LOW-SPIN HEMOGLOBIN ADDUCTS

In the beginning there was the protein, and the protein was hemoglobin, but not the ferric type. Even though it is probably the best known protein, hemoglobin still continues to unfold new roles and properties, ranging from physiological implications and moonlighting to nanotechnological applications. In the early days of biochemistry, the Fe^{3+} form of hemoglobin was deemed a useless byproduct since (by contrast to the Fe^{2+} form) it has no ability to bind molecular oxygen. It was later understood that a large number of heme-containing enzymes in fact rely primarily on their ferric forms to perform catalysis (e.g., catalase, ascorbate peroxidase, manganese peroxidase, cytochrome P450NOR). Ferric heme proteins additionnally have an increased ability (compared to their ferrous counterparts) to bind probe ligands - from very small ones such as cyanide, to the larger molecules such as anthracene as seen in cytochromes P450.

Starting with the pioneering work of Peisach and Blumberg, through Taylor´s formulae for calculation of ligand field parameters [1], interest has been paid in understanding how various small ligands affect the magnetic properties of hemoglobin in EPR spectroscopy. Even at low temperatures such as 70 K, an unusually large broadening is observed for low-spin complexes [2], where normally spin-lattice relaxation has no effects whatsoever - a phenomenon known as g strain: the linewidth broadens as a result of random distribution of protein conformations.

The present work explores the behaviour of various small strong-field ligands in their binding to ferric hemoglobin from the perspective of the broadening effect in CW-EPR. From this ligand series, some structurally-related small moieties stand out as imposing a longer relaxation time, thus rendering a violation from the g strain rule. Among these, one small molecule, used in the past to treat alcohol poisoning, goes beyond all expectations and gives rise to a very rare methemoglobin EPR signal, where the expected broadening is almost absent. The observed paramagnetic behaviours are explored by use of molecular modeling approaches in trying to understand which factors are responsible for such a g-strain-ignorant EPR manner. If you are interested to know why some low-spin heme adducts can become so impolite as to refuse to abide the rules, stay tuned and check out the poster.

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ELECTRON DECOUPLING ON ENDOFULLERENES AND MAS ELECTRON SATURATION RECOVERY

Presented here is a demonstration of control over electron spins in two different systems: 40 mM Trityl and nitrogen-endofullerene (N@C60). The electron saturation recovery of the unpaired electron spins in 40 mM Trityl is observed through the enhanced nuclear magnetic resonance (NMR) signal of proline, using a rapid-acquisition scheme involving electron decoupling and direct dynamic nuclear polarization (DNP), while magic-angle spinning (MAS) at temeratures below 5 K. Additionally, electron decoupling is extended to a dilute electron-spin system, polycrystalline N@C60: C60-fullerene (160 parts-per-million (ppm) endo-species), to improve signal intensity.

DNP is a process whereby the relatively high polarization of electron spins is transferred to nuclear spin; here, the transfer is accomplished with microwaves from a custom frequency-agile gyrotron. [1-2] MAS NMR is a technique used to improve the senstivity and resolution of solid state NMR, making possible accurate determination of structrue and dynamics in a wide range of low-mobility systems, from inorganic surfaces to in-cell proteins. DNP drastically decreases the time required for these information-rich experiments, but unfortunately the electron-nuclear dipolar contact utilized for signal enhancement also broadens the NMR signal, resulting in spectra with poor resolution. Electron decoupling attenuates this electron-nuclear interaction and recovers the intensity and resolution of DNP-enhanced nuclear spins.[3] The longer electron spin relaxation at lower temperatures (90 K, and especially below 5 K) improves our ability to control electron spins with frequency-modulated microwaves.

Here, electron decoupling provides electron saturation for “direct” DNP experiments, where the polarization period varies from milliseconds to tens-of-milliseconds, after which the longitudinal recovery of the electron spin (40 ms) is observed through the enhanced nuclear spin signal.[4] To successfully encode this electron spin information in the nuclear signal, the longitudinal relaxation of the 40 mM Trityl electron spins must be longer than the DNP transfer period, thus MAS is performed at 5 K using liquid helium for cooling. A rapid-acquisition scheme optimizes the signal-per-unit-time of the spectrometer, allowing for the necessary signal acquisition at these short polarization periods.

In contrast to 40 mM trityl, which features some radical-clusters, 160 ppm N@C60 provides an environment with essentially no electron-electron interaction. In this electron-spin-dilute system we successfully perform electron decoupling, observing a 12% increase in signal intensity at 7 s DNP period and a 5% increase in signal intensity at 30 s DNP period (for the isotropic peak).[8]

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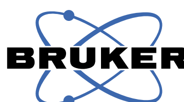

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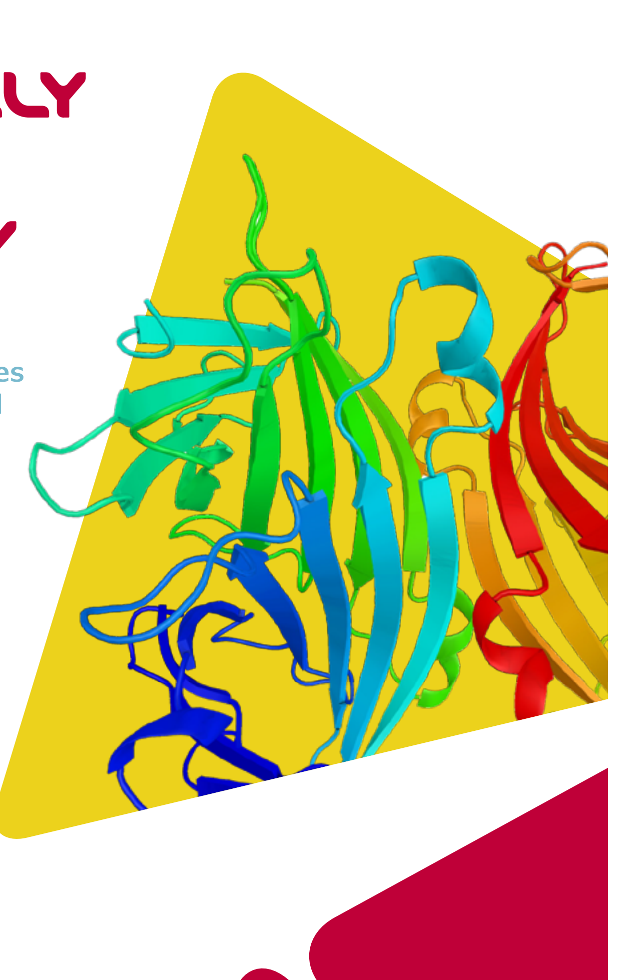

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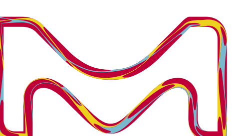

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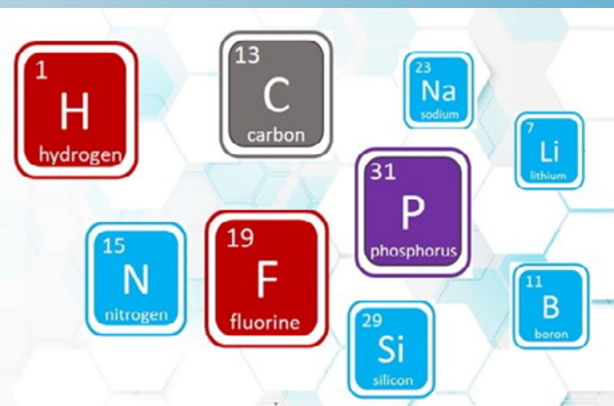

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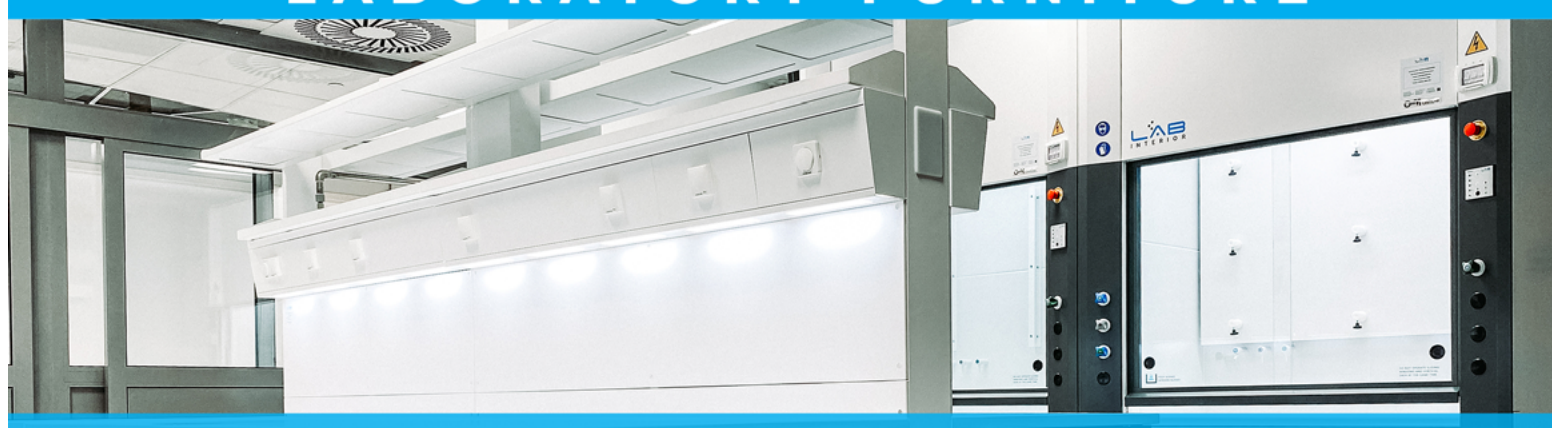

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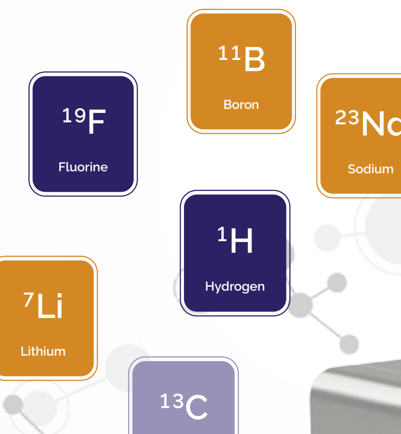

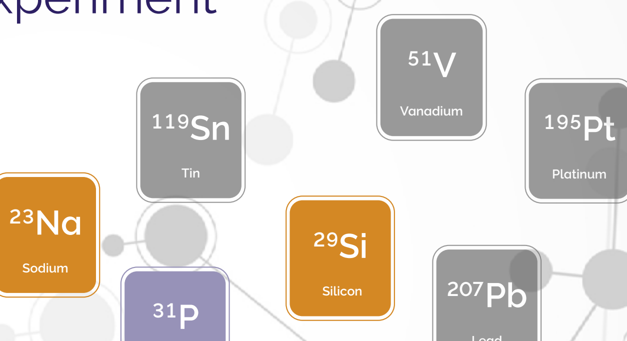

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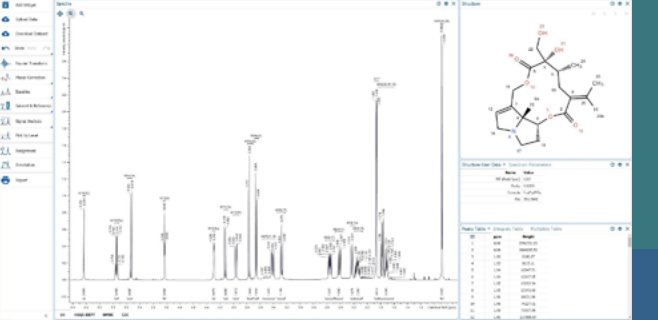

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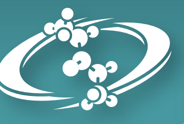

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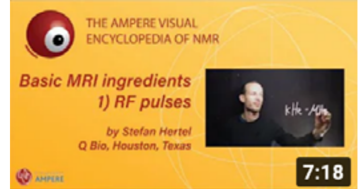

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