

PROGRAMME

BOOK OF ABSTRACTS

FEB 27TH - MAR 3RD 2019

PRAGUE, CZECH REPUBLIC

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MISSION STATEMENT

The conference *Magnetic Moments in Central Europe* (MMCE) was conceived in 2007 with the vision of providing a unique knowledge-sharing event for NMR scientists and students in the region. The philosophy behind MMCE is centered on the following main goals:

- The conference is didactic in its fundamental spirit. Rather than expecting scientists to communicate their latest results aimed to be published in a scientific journal, the conference wants to offer, in the form of so-called Tutorial Talks, scientists an opportunity to speak about their research, their intellectual and emotional struggles, their aha!-moments, their provocative thoughts and their acquired wisdom in relation to their chosen topic in such an informal, conceptual, personal and edifying manner that would not normally be possible in the context of a “regular” scientific presentation. Tutorial Talks are expected to project a kind of distilled wisdom on a topic that can potentially be more captivating and instructive to both students and seasoned NMR scientists than a new-result-centered “regular” lecture.
- In addition to being beneficial for specialists in a given field, Tutorial Talks should foster communication and understanding between various branches of NMR.
- MMCE aims to address all walks of theoretical and applied NMR spectroscopy, ranging from fundamental theory through small-molecule structure determination, new pulse sequences, software and hardware development, etc. to biological molecules.
- The talks offered by a speaker at MMCE need not necessarily be “trendy”; old concepts addressed from a new, exciting, or eye-opening perspective are welcome.
- MMCE also accommodates the presentation of cutting-edge new results, with the speakers being encouraged to add some didactic flavor to their talks.
- MMCE wishes to act as a dynamic NMR discussion forum in both an intellectual and social sense. Discussions are encouraged after each presentation, and to that end sufficient room is provided in the program.
- It aims to become a well-consolidated platform for building social capital in the NMR community of Central Europe and beyond.
- MMCE is dedicated to involving many young scientists and university students as participants.

MMCE is held every second year in varying locations within Central Europe. It usually lasts for five days, starting Wednesday evening and ending Sunday noon. Topics are divided into sessions. There are no parallel sessions. As a general rule of thumb, each session starts with a Tutorial Talk lasting about 40 min, followed by a few Invited Talks which are 30 min long. Tutorial Speakers and Invited Speakers are invited by the scientific board of MMCE. Generally, all travel expenses are covered for the Tutorial Speakers and local transportation fees are covered for the Invited Speakers. For both the Tutorial and Invited Speakers all other conference expenses (registration, accommodation, meals and social events) are covered for the whole duration of the conference with the understanding that they take part in the discussions prior to and after their own presentations. Each session also provides space for a few 20 min talks delivered by the regular participants of the conference. There are also poster sessions and student presentation sessions.



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LIST OF PREVIOUS MEETINGS

2009 – Otočec (Slovenia)

2011 – Tatranská Lomnica (Slovakia)

2013 – Semmering (Austria)

2015 – Krynica-Zdrój (Poland)

2017 – Budapest (Hungary)

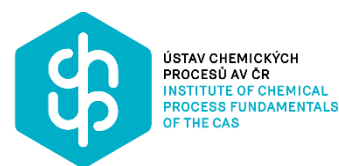


MAGNETIC MOMENTS IN CENTRAL EUROPE 2019
PRAGUE, CZECH REPUBLIC, FEB 27TH - MAR 3RD



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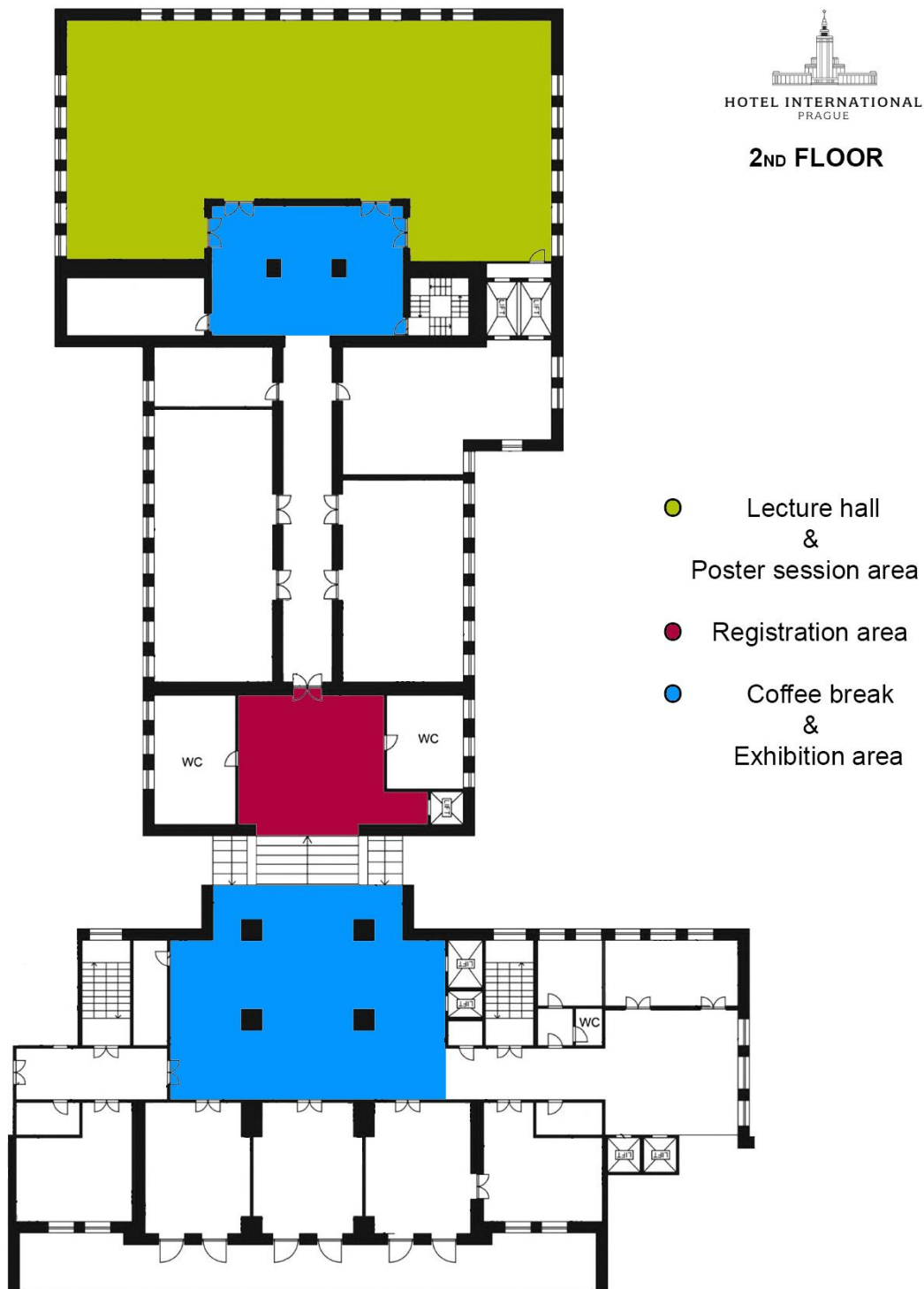


OTHER SPONSORS





CONFERENCE VENUE FLOORPLAN





SOCIAL EVENTS

WEDNESDAY

February 27th, 2019

Welcome Mixer

Hotel Tower, access by elevator at 2nd floor only

18:00 – 21:00



FRIDAY

March 1st, 2019

Excursion to Únětice Brewery

Departure by bus, 17:15 from the hotel

18:00 – 21:00



SATURDAY

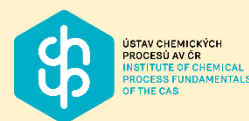
March 2nd, 2019

Organ Recital by Jan Rotrekl

St. Bartholomew's Church, Bartolomějská Street, Prague I

*Departure at 17:15 by tram no. **18** from the hotel to Národní divadlo station (tram tickets available at registration)*

18:00 – 18:45





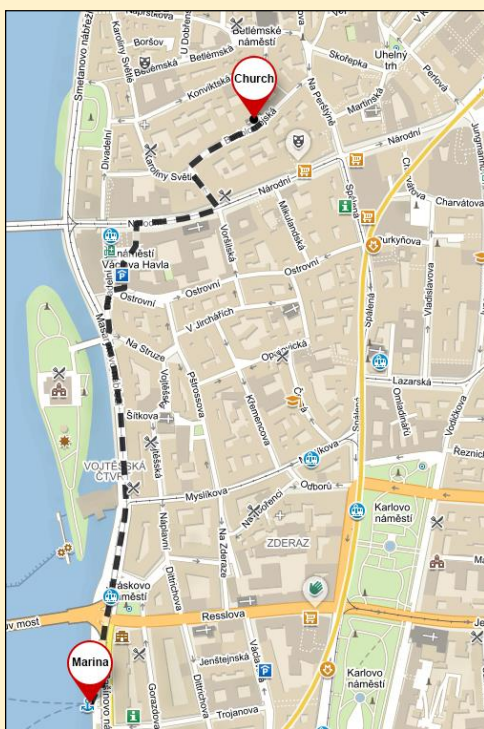
SATURDAY

March 2nd, 2019

Conference Dinner, Cruise on the Vltava River

Marina at Rašínovo nábřeží, walking distance

19:00 – 22:00



Way from Church to Marina



Way back to hotel



PROGRAMME AT A GLANCE

WEDNESDAY, 27 FEB.	THURSDAY, 28 FEB.	FRIDAY, 1 MARCH	SATURDAY, 2 MARCH	SUNDAY, 3 MARCH
	8:30 – 10:35 Session 1: BASICS <i>Csaba Szántay</i>	8:30 – 10:35 Session 4: PHARMACEUTICALS <i>Predrag Novak</i>	8:40 – 10:35 Session 7: BIOMOLECULES I <i>Václav Veverka</i>	9:00 – 11:05 Session 9: CALCULATIONS <i>Ján Tarábek</i>
	coffee break	coffee break	coffee break	coffee break
	11:00 – 12:55 Session 2: SMALL MOLECULES <i>Radek Pohl</i>	11:00 – 12:55 Session 5: METABOLOMICS <i>Jan Sýkora</i>	11:00 – 12:55 Session 8: BIOMOLECULES II <i>Georg Kontaxis</i>	11:30 – 11:50 Closing Remarks
	lunch	lunch	lunch	lunch
14:30 – 17:00 Arrival & Registration	14:30 – 16:25 Session 3: SOFTWARE & HARDWARE NEWS <i>Krz. Kazimierczuk</i>	14:30 – 16:45 Session 6: SOLIDS <i>Martin Dračinský</i>	14:30 – 17:00 MestreLab users' meeting <i>Manuel Perez</i>	
	coffee break	coffee break	coffee break	
17:00 – 17:15 Welcome words 17:15 – 18:00 Opening lecture	17:00 – 18:00 Come to see my poster 2 min presentations <i>Wiktor Koźmiński</i>	17:15 Departure by bus	17:15 Departure by tram	
18:00 – 18:30 Welcome drink 18:30 – 21:00 Dinner	18:00 – 19:00 Dinner 19:00 – 21:00 Poster Session	18:00 – 22:00 Excursion to Únětice brewery & Dinner	18:00 – 18:45 Organ Resonances 19:00 – 22:00 Conference dinner	



DETAILED PROGRAMME

WEDNESDAY, FEBRUARY 27

14.00	Arrival & Registration
17.00 – 17.15	Welcome words <i>Jan Sýkora</i>
17.15 – 18.00	<i>Petr Slavíček (I)</i> Science of Science
18.00 – 18.30	Welcome drink
18.30 – 21.00	Dinner

THURSDAY, FEBRUARY 28

Session 1: BASICS, Chair: Csaba Szántay

08.30 – 09.15	<i>Stan Sykora (T)</i> Tutorial on Spin Systems in Magnetic Resonance
09.15 – 09.45	TBA
09.45 – 10.15	<i>Klaus Zangger (I)</i> Enhancing Time and Frequency Resolution by <i>Restricted Acquisition</i>
10.15 – 10.35	<i>Małgorzata Rytel</i> Accelerated pseudo-2D pure-shift acquisition for serial NMR experiments
10.35 – 11.00	Coffee break with ExtraByte

Session 2: SMALL MOLECULES, Chair: Radek Pohl

11.00 – 11.45	<i>Christina M. Thiele (T)</i> Opportunities and challenges of using Residual Dipolar Couplings for the Structure Determination of Catalytically Active Species
11.45 – 12.15	<i>Hélène Freichels (I)</i> NMR on your Bench – Possibilities for Structure Elucidation / Confirmation and Reaction Monitoring
12.15 – 12.35	<i>Dariusz Gołowicz</i> Monitoring Hydrogenation Reaction with Enhanced Sensitivity and Resolution using Benchtop NMR
12.35 – 12.55	<i>Petra Cuřínová</i> Dialytic Separation of Anions from DMSO Solution Facilitated by Dendritic Receptors
12.55 – 14.30	Lunch

Session 3: SOFTWARE & HARDWARE NEWS, Chair: Krzysztof Kazimierczuk

14.30 – 15.15	<i>Michal Maloň</i> JEOL
15.15 – 15.45	<i>Dimitris Argyropoulos (I)</i> Computer Assisted Structure Elucidation (CASE) Workflows, Problems and Capabilities
15.45 – 16.05	<i>Alexandra Shchukina</i> Burst sampling for interferogram pure-shift NMR acquisition
16.05 – 16.25	<i>Nicholle G.A. Bell</i> Reduced dimensionality NMR experiments: Reducing overlap in spectra of complex mixtures
16.25 – 17.00	Coffee break with ACD/Labs



COME TO SEE MY POSTER session, Chair: Wiktor Koźmiński

17.00 – 18.00 2 min / poster presentations

18.00 – 19.00 **Dinner**

19.00 – 21.00 **Poster Session**

FRIDAY, MARCH 1

Session 4: PHARMACEUTICALS, Chair: Predrag Novak

08.30 – 09.15 *Petr Váchal (T)*

The Invention of MK-8262, a CETP Inhibitor Backup to Anacetrapib (with best in class properties)

09.15 – 09.45 *Marek J. Potrzebowski (I)*

Solid State NMR Spectroscopy as a Tool Supporting the Mechanochemistry Development

09.45 – 10.15 *Manuel Perez*

Determination of Conformation in Solution: A survival guide for Medicinal and Analytical Chemists

10.15 – 10.35 *Václav Veverka*

Disentangling the LEDGF/p75 Interactome

10.35 – 11.00 **Coffee break with Magritek**

Session 5: METABOLOMICS, Chair: Jan Sýkora

11.00 – 11.45 *Leonardo Tenori (T)*

NMR-based metabolomics in biomedicine

11.45 – 12.15 *Ana Gil (I)*

Human and *in vitro* NMR Metabolomics Impacting on Health Research

12.15 – 12.35 *Michal Kaliňák*

An Investigation of Cerebral Metabolic Profile in the Rat Model of Age-Related Dementia

12.35 – 12.55 *Lenka Michálková*

Metabolomic Study of the Association between Pancreatic Cancer and Diabetes Mellitus

12.55 – 14.30 **Lunch**

Session 6: SOLIDS, Chair: Martin Dračinský

14.30 – 15.15 *Beat Meier (T)*

Protein dynamics: from local motions to molecular machines

15.15 – 15.45 *Yusuke Nishiyama (I)*

Internuclear distance measurements in rigid solids at natural abundance

15.45 – 16.05 *Jan Stanek*

Hyperdimensionality and shared-time acquisition – new hope for protein resonance assignment in solids?

16.05 – 16.25 *Marta K. Dudek*

Solid-state NMR and crystal structure prediction: a perfect alliance? A difficult case of solvate-hydrate of catechin

16.25 – 16.45 *Eliška Procházková*

Polymorphic Transformation of Drugs Loaded into Glycopolymetric Micelles Probed by Solid-State NMR

16.45 – 17.15 **Coffee break with PharmaCan**

17.15 **Departure by bus**

18.00 – 22.00 **Excursion to Uněťice brewery / Dinner**



SATURDAY, MARCH 2

Session 7: BIOMOLECULES I, Chair: Václav Veverka

- 08.40 – 09.25 *Lukáš Žídek (T)*
NMR as a window to the dynamic microcosmos of (disordered) proteins
- 09.25 – 09.55 *Paul Schanda (I)*
Solid-state NMR combined with cryo-EM to determine the structure and functional dynamics of a large enzyme
- 09.55 – 10.15 *Katarína Pšenáková*
Structural characterization of protein kinase ASK1 and its interaction with thioredoxin
- 10.15 – 10.35 *Bertalan Kovács*
Interdomain mobility in PDZ tandem captured by externally restrained MD simulation
- 10.35 – 11.00 **Coffee break with Jeol**

Session 8: BIOMOLECULES II, Chair: Georg Kontaxis

- 11.00 – 11.45 *Katja Petzold (T)*
When Artifacts turn out to be Real Data: Discoveries of RNA Dynamics
- 11.45 – 12.15 *Hana Macíčková Cahová (I)*
How to find needle in a haystack? (New RNA modifications)
- 12.15 – 12.35 *Martina Lenarčič Živković*
Adenine-Dependent G-Quadruplex Structural Switch
- 12.35 – 12.55 *Witold Andraś*
Unravelling the structural basis for the exceptional stability of RNA G-quadruplexes capped by a uridine tetrad at the 3' terminus
- 12.55 – 14.30 **Lunch**

MESTRELAB USERS' MEETING, Chair: Manuel Perez

- 14.30 – 16.45 Mestrelab Users' Meeting
- 16.45 – 17.15 **Coffee break with Mestrelab**
- 17.15 **Departure by tram**
- 18.00 – 18.45 **Organ resonances**
- 19.00 – 22.00 **Conference Dinner**

SUNDAY, MARCH 3

Session 9: CALCULATIONS, Chair: Ján Tarábek

- 09.00 – 09.45 *Trygve Helgaker (T)*
Quantum-Chemical Calculations of NMR parameters
- 09.45 – 10.15 *Martin Dračínský (I)*
Towards Accurate Predictions of NMR Parameters
- 10.15 – 10.45 *Torsten Herrmann (I)*
Bioinformatics-driven NMR structure determination
- 10.45 – 11.05 *Krzysztof Kazimierzczuk*
How to Collect and Process Data in Serial NMR Experiments?
- 11.05 – 11.30 **Coffee break with Spin-Doc**
- 11.30 – 11.50 **Closing Remarks**
- 11.50 – 13.00 **Lunch**



MAGNETIC MOMENTS IN CENTRAL EUROPE 2019
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TUTORIALS (T)

INVITED LECTURES (I)

ORAL PRESENTATIONS (O)

POSTERS (P)



T1 - TUTORIAL ON SPIN SYSTEMS IN MAGNETIC RESONANCE

Stanislav Sykora

Castano Primo (Milano), Italy

This tutorial has four Sections dedicated to spectra of coupled spin systems, all of which are little more than reminders of what the participants should be aware of. The slides will be all taken from an accompanying booklet with more detailed descriptions, to be made available online by February 20.

Section I: Generalities.

A brief review of the very basic concepts regarding magnetic particles, including atomic nuclei. This means the angular momentum (spin), magnetic dipole moment, quadrupole electric moment, chemical screening, list of interactions in which magnetic particles can participate, the approximation of Weston Anderson's spin-system Hamiltonian and its parameters (chemical shifts and coupling constants).

Section II: From spin Hamiltonian to spectra.

Generic properties of the Hamiltonian and their observable consequences. Special cases for various types of systems (liquids/solids, isotropic/axial). Experimental ways to affect the Hamiltonian (decoupling, MAS). Spectral invariants, equivalent groups and sub-spectra. Mathematical definition of NMR spectra and their invariants (spectral moments). Various types of symmetries, equivalent groups and sub-spectra. Forward simulation of NMR spectra and its combinatorial complexity: static and dynamic cases. A mentions of the inverse problem (parameters "fitting").

Section III. Molecular NMR spectra in isotropic solutions.

Given the audience, this is the central part of the tutorial. Revisiting equivalent groups, sub-spectra and symmetry effects. Multiplets in proton and other 1D spectra. Hetero couplings, isotopomers, symmetry breaking. Weak and strong couplings of first and second type, roof effects, combination transitions, indirect coupling effects. A mention of the obstacles that arise when "interpreting" real NMR spectra (solvent signals, rotamers, impurities, residual reaction solvents, fingerprints, spikes and other artifacts).

Section IV: Special effects and systems

Relaxation effect on HR-NMR spectra; relaxation in coupled spin systems. Through-space couplings. Spectra of non-isotropic systems (oriented, RDC's) and spectra of solid samples.



I1 - ENHANCING TIME AND FREQUENCY RESOLUTION BY *RESTRICTED ACQUISITION*

Simon Glanzer^a, N. Helge Meyer^a, Gabriel Wagner^a, Nina Gubensäk^a, Sebastian Tasotti^a, Predrag Novak^b and Klaus Zangger^a

^a *Institute of Chemistry, University of Graz, Heinrichstraße 28, Graz, Austria*

^b *Department of Chemistry, University of Zagreb, Horvatovac 102, Zagreb, Croatia*

Compared to other NMR detectable nuclei, ¹H spectra typically suffer from low resolution and severe signal overlap, mainly due to extensive scalar coupling between protons. Pure shift NMR, which leads to a collapse of ¹H signals into singlets vastly increases the resolution, which in some cases corresponds to a theoretical signal dispersion of NMR spectrometers at several GHz [1]. We reported a volume restricted NMR approach to record fully homonuclear decoupled NMR spectra [2]. The elimination of homonuclear scalar coupling is achieved by selective decoupling of individual signals in different slices of the NMR sample tube. Slice-selective pure shift NMR can be achieved during the acquisition by interruption of the FID after individual chunks of ~20 ms and reversing scalar coupling evolution. Scalar coupling information, which is often key in analyzing chemical structures, is of course completely lost in such experiments. In contrast to pure shift NMR spectra it is possible to selectively enhance the scalar coupling to make its extraction more accurate [3]. By this real-time J-upscaling experiment the acquisition is restricted to individual time blocks to allow for additional scalar coupling, but not chemical shift evolution. Enhanced resolution in the time domain of series of 1D NMR spectra can be achieved by restricting the acquired sample volume to narrow slices of the NMR tube [4].

1. Aguilar, J. A.; Faulkner, S.; Nilsson, M.; Morris, G. A. *Angew.Chem.* **49**, 3901-3903, 2010.
2. Meyer, N. H.; Zangger, K. *Angew.Chem.* **52**, 7143-7146, 2013.
3. Glanzer, S. and Zangger, *J.Am.Chem. Soc.* **137(15)**, 5163-5169, 2015.
4. Wagner, G. Sakhaii, P. Bermel, W. and Zangger, K. *Chem.Comm.* **49(30)**, 3155-3157, 2013.



O1 - ACCELERATED PSEUDO-2D PURE-SHIFT ACQUISITION FOR SERIAL NMR EXPERIMENTS

M. Rytel¹, P. Kasprzak², K. Kazimierczuk³

¹ Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw, Poland

² Department of Mathematical Methods in Physics, Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw, Poland

³ Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

There exist a plethora of experimental techniques providing homodecoupled (“pure-shift”) ¹H spectra. Most of them are based on the pseudo-2D acquisition of consecutive chunks of FID, which causes them to be highly time-consuming [1]. One of the most effective techniques is TSE-PSYCHE [2], which provides relatively clean (artifact-free) spectra. Still, however, it is based on lengthy data collection, which is particularly problematic when long series of experiments is to be performed. A common example is the experiment repeated several times for the same sample at different temperatures.

Here we present a new technique which allows to obtain serial pseudo-2D pure-shift spectra in a short time and/or with improved sensitivity. This is achieved by avoiding to record the whole pseudo-2D data set in each experiment in a series. Instead, only one chunk of data is collected per experiment and a modified Radon transform is used to process the data [3][4]. A new technique provides good pure-shift spectra and allows to decode rates of linear changes of peaks positions caused by temperature variations in the sample.

References

1. K. Zangger, *Prog. Nucl. Magn. Resonance*, 86-87 (2014), pp. 1-2015
2. M. Foroozandeh , R. W. Adams , P. Kiraly , M. Nilsson and G. A. Morris , *Chem. Commun.*, **2015**, 51 , 15410 -15413.
3. R. Dass, P. Kasprzak, K. Kazimierczuk, *J. Magn. Reson.* **2017**, 282, 114
4. E. Kupče, R. Freeman, *Concepts Magn. Reson.*, 22A (2004), pp. 4-11



T2 - OPPORTUNITIES AND CHALLENGES OF USING RESIDUAL DIPOLAR COUPLINGS FOR THE STRUCTURE DETERMINATION OF CATALYTICALLY ACTIVE SPECIES

Christina M. Thiele¹

¹ Technische Universität Darmstadt, Clemens Schöpf Institut für Organische Chemie und Biochemie, Alarich-Weiss-Strasse 16, 64287 Darmstadt, Germany

Information about the three dimensional structure of organic or organometallic compounds can improve our understanding of their function. Thus the determination of their 3D-structure in as native an environment as possible is necessary. This is also true if catalytically active species are to be investigated. Together with ³J couplings providing angular information and NOE parameters providing distance information residual dipolar couplings (RDCs)¹ can be used for this purpose. This poses additional challenges. These will be described together with the opportunities and benefits.

First a short overview of the use of RDCs for organic structure determination will be given, highlighting also less well-known approaches as the use of local alignment tensors.² Their complementarity with other NMR parameters will be discussed on one selected example, in which only making use of many different methods of data interpretation allowed for the determination of the structure of an enantioselective catalyst³.

References:

1. Reviews: C. M. Thiele, *Eur. J. Org. Chem.* **2008**, 5673-5685; V. Schmidts, *Magn. Reson. Chem.* **2017**, 55, 54-60.
2. C. M. Thiele, A. Maliniak, B. Stevensson, *J. Am. Chem. Soc.* **2009**, 131, 12878-12879.
3. B. Böttcher, V. Schmidts, J. A. Raskatov, C. M. Thiele, *Angew. Chem.* **2010**, 122, 210-214, *Angew. Chem. Int. Ed.* **2010**, 49, 205-209.



12 - NMR ON YOUR BENCH – POSSIBILITIES FOR STRUCTURE ELUCIDATION/CONFIRMATION AND REACTION MONITORING

Hélène Freichels¹

¹ Magritek GmbH, Aachen, Germany

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and information rich analytical technique. Nevertheless, due to the size and complexity of high field NMR instruments, this method is limited in its use. During the last years benchtop NMR has overcome some limitations of HF instruments. The systems are cryogen free, maintenance-free, easy to use, robust and can be easily moved between labs. Thanks to the large versatility of the systems a wide range of applications like identification of adulteration, forensics [1], education [2], process automation [3], and inline monitoring of reactions are possible. In the frame of this talk, an overview of recent hardware developments will be given and several technical solutions combined with application examples will be presented. It will be shown that with the help of advanced 1D and 2D methods combined with the power of the Spinsolve 80 MHz system, structures of complex molecules like phytochemicals can be easily confirmed. In the second part the benefits of the Spinsolve Ultra systems will be introduced, which provide ultra-high field homogeneity and therefore enabling the use of solvent suppression techniques. The application of the system will be discussed on the example of urine samples. The last part will be dedicated to the online monitoring of reactions with (simultaneous) detection of the nuclei of ¹H and ¹⁹F. Figure 1 shows a typical reaction monitoring setup (right) and results obtained on a Spinsolve 60 MHz for the acetylation of L-phenylalanine (left).

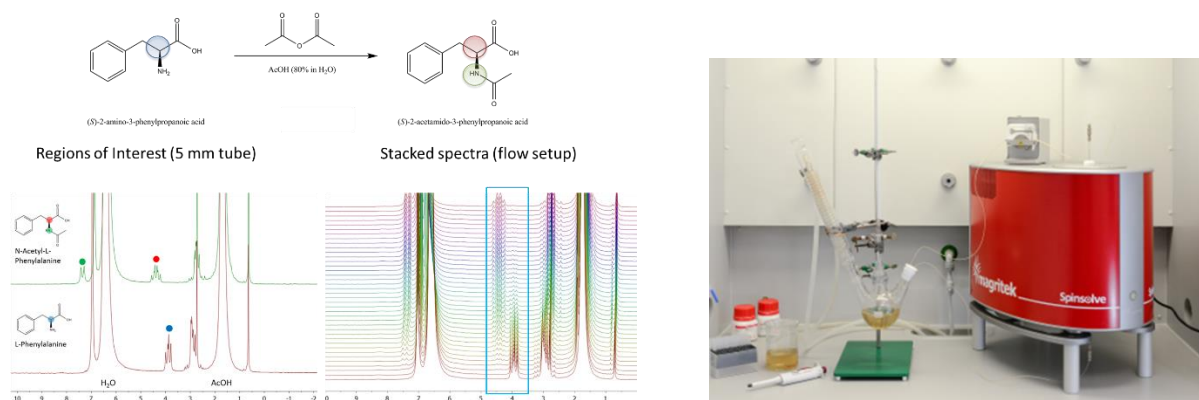


Figure 1: left: NMR spectra of the N-acetylation of L-phenylalanine; right: typical reaction monitoring setup

References

1. D. Picard et al., *Angew. Chem. Int. Ed.* **2017**, 56 (26).
2. A. Zivkovic et al., *Chem. Ed.* **2017**, 94 (1), 115–120.
3. S. Kern et al., *Anal. Bioanal. Chem.* **2018**, 410 (14), 3349–3360.



O2 - MONITORING HYDROGENATION REACTIONS WITH ENHANCED SENSITIVITY AND RESOLUTION USING BENCHTOP NMR

D. Gołowicz^{1,2}, K. Kazimierczuk², M. Urbańczyk^{2,3}, T. Ratajczyk⁴

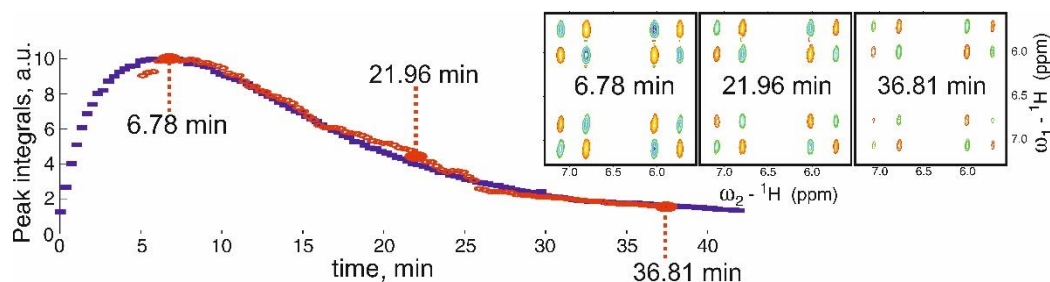
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³ NMR Research Unit, University of Oulu, Oulu, Finland

⁴ Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

Benchtop NMR spectrometers (BT-NMR) are attracting growing interest from chemical laboratories because of their moderate price, low handling cost, and mobility. Despite many advantages, the relatively low magnetic field strength provided by permanent magnets strongly limits their applications. This is due to the insufficient resolution and sensitivity for many analytical/bioanalytical



tasks.

Figure 1: Integrals of monitored peak from interleaved TR-NUS experiment. Red points - 2D peak integrals, blue points - 1D peak integrals.

In this work, we present a method suitable for the real-time monitoring of hydrogenation reactions using benchtop NMR with 2D resolution and greatly enhanced sensitivity [1]. To achieve our goal, we combined two techniques: parahydrogen induced polarization (PHIP) [2] and time-resolved non-uniform sampling (TR-NUS) [3]. Moreover, we performed interleaved acquisition of 1D NMR with 2D NUS NMR in parallel to occurring reaction. Our measurements were conducted on Magritek Carbon spectrometer operating at 43 MHz ¹H frequency and working in a continuous flow system. The interleaved spectra were ¹H NMR and NUS DQF-COSY. We demonstrate the proposed method using two examples: the hydrogenation of ethylphenyl propiolate, and the hydrogenation of a mixture of two substrates – ethylphenyl propiolate and ethyl 2-butynoate. We also show that interleaved acquisition of 1D spectra (with 2D TR-NUS) may be beneficial for post-processing reduction of t_1 -noise artifacts present in 2D NUS spectra.

References

1. D. Gołowicz et al., *ChemistryOpen* **2019**, accepted.
2. C. R. Bowers, D. P. Weitekamp, *Phys. Rev. Lett.* **1986**, 57, 2645-2648.
3. M. Mayzel et al., *J. Biomol. NMR* **2014**, 58 (2), 129-139.



O3 - DIALYTIC SEPARATION OF ANIONS FROM DMSO SOLUTION FACILITATED BY DENDRITIC RECEPTORS

Petra Cuřínová^{1,2}, Maximilian Winkler², Alena Krupková^{1,2}, Jan Budka³, Chang Nga Wun³, Vratislav Blechta², Lucie Červenková Šťastná^{1,2}, Jan Sýkora² and Tomáš Strašák^{1,2}

¹ Jan Evangelista Purkyně University, Ústí nad Labem, Czech republic

² Institute of Chemical Process Fundamentals, Czech Academy of Sciences, Prague, Czech republic

³ University of Chemistry and Technology, Prague, Czech republic

For complexation of anions via hydrogen bonding, a variety of receptors was proposed, synthesized and tested so far[1]. Attachment of a well explored anion sensing moiety, an isophthalamidic group[2], to dendritic structures of high molecular weight[3] leads to a new class of receptors. These compounds possess the advantage of multiple complexation sites with high affinity towards anions. Moreover, they offer the possibility of separation of the formed complex from the solution and subsequent recycling of the receptor.

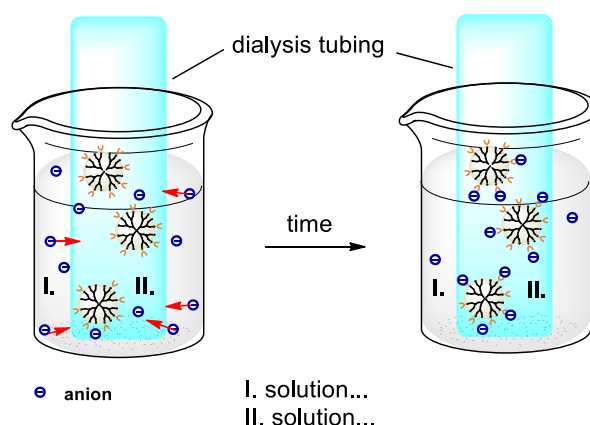


Figure 1: schematic facilitated dialytic separation of anions from solution

As the dialytic tubing is impermeable for big molecules of receptor, the anions crossing the barrier of dialytic tubing to form complex with the receptor stay entrapped inside and can be removed from the solution. NMR methods were used to study the complexation properties of given receptors as well as to determine the concentration changes during dialytic experiments.

References

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Acknowledgement

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I3 - COMPUTER ASSISTED STRUCTURE ELUCIDATION (CASE) WORKFLOWS, PROBLEMS AND CAPABILITIES

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Computer Assisted Structure Elucidation (CASE) was initially proposed as a technique in 1968 with the intention of using IR data and computing power to determine the structure of an unknown compound. It rapidly evolved into a method that relied on NMR spectra, mass spectra and other information. The aim of CASE is to always find the chemical structure that is the best fit to the spectral data.

In this presentation we will cover the basic workflow for a typical computer assisted structure elucidation. This includes analyzing the available spectra and generating a Molecular Connectivity Diagram (MCD). We will also explore the options for structure generation before it is executed and the results are ranked. The three most common problems of this approach (unexpected correlations, spectral ambiguity and symmetry) will be discussed together with the possible solutions. Finally we will present the different options for ranking the generated structures and how to increase the reliability of the ranking.

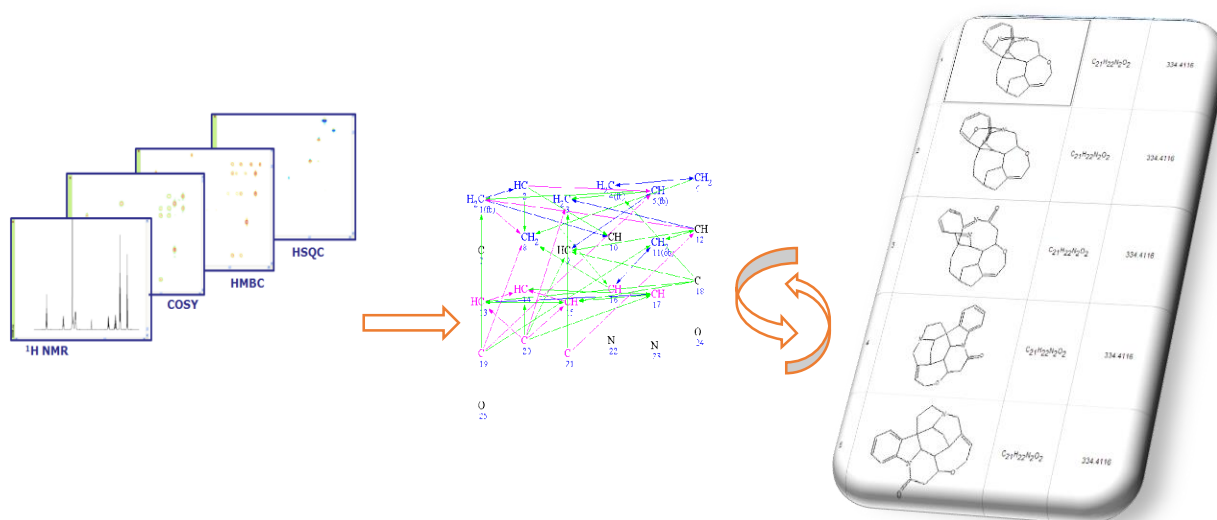


Figure 1: Schematic workflow of CASE from spectra to best matching structure

The last part of the talk will focus on what lies ahead for CASE, what challenges are still there to be addressed and whether and how relevant will CASE and the human chemist be in the future.

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O4 - BURST SAMPLING FOR INTERFEROGRAM PURE-SHIFT NMR ACQUISITION

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Pure-shift NMR experiments were designed to eliminate the manifestation of J-couplings in a spectrum. J-couplings are suppressed with echo-type pulse sequences with selective pulses, and then a chunk of FID is acquired [1]. This chunk should be short enough for the couplings not to arise again. The whole FID is then constructed of these chunks.

Pure-shift techniques fall into two types. The first one, real-time experiment, is designed so that FID chunks alternate with gaps during which the receiver is off and couplings are being refocused. In the second type, interferogram experiment, a pulse sequence block for decoupling is implemented so that measured FID chunks follow each other end-to-end (see Fig.2 in [2]). Interferogram acquisition is much more time consuming, but, in contrast to real-time acquisition, does not lead to undesirable peak broadening: in real-time experiments, acquired chunks are concatenated, but the relaxation during the omitted gaps leads to this apparent broadening.

Thus, interferogram experiments, such as e.g. PSYCHE pulse sequence, tend to yield spectra of better resolution, but are time-consuming. This is what leads to an idea to accelerate interferogram pure-shift experiments with non-uniform sampling (NUS). This type of NUS is, however, crucially different from the one applied in NMR in other cases: here, we can only omit whole chunks, not separate points of the FID. We call this type of sampling “burst sampling”. This is potentially a more problematic situation in comparison to conventional NUS, as any regularity can increase undersampling artefacts and hamper the consequent reconstruction of the omitted points. However, this procedure still works, as was demonstrated in [2].

On the other hand, we have prior information on the spectrum at our disposal: the conventional ¹H spectrum can tell us, within some tolerance, where to anticipate peaks in a pure-shift spectrum. In this work, we show how to optimize the burst sampling scheme taking the advantage of this prior information. Our aim is to minimize the risk of high undersampling artefacts and thus assist the reconstruction.

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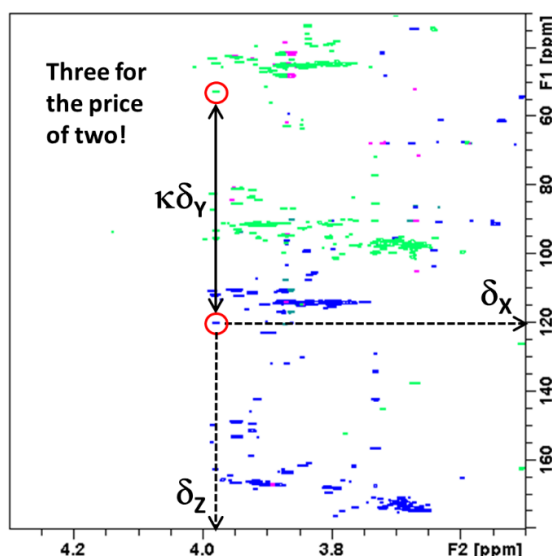


O5 - REDUCED DIMENSIONALITY NMR EXPERIMENTS: REDUCING OVERLAP IN SPECTRA OF COMPLEX MIXTURES

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Reduced-dimensionality experiments (1-3) provide the equivalent information to the corresponding higher dimensionality techniques. They correlate n chemical shifts, but achieve this in $n-x$ chemical shift space by simultaneous sampling of several chemical shifts during each or some of the incrementable periods of $(n, n-x)$ D experiments. In the case of $(3,2)$ D X,Y,Z correlated experiments X and Y chemical shifts are sampled simultaneously to record $\Omega_x \pm \kappa\Omega_y$ offset frequencies, where κ is a scaling factor between the X and Y nuclei, while the chemical shift of Z is recorded in the directly detected dimension. Cosine and sine modulated spectra with respect to the Y chemical shift are processed to produce two data sets containing only one set of cross peaks each. The separation of these cross peaks codes for the chemical shift of Y.



In this work, existing 3D and 4D NMR experiments (4-5) designed for the analysis of ¹³C-methylated organic matter extracted from peat soils were modified to produce their reduced dimensionality (4, 3) and (3, 2)D versions. This approach leads to increased digital resolution of spectra that is essential for the analysis of complex mixtures.

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T3 - THE INVENTION OF MK-8262, A CETP INHIBITOR BACKUP TO ANACETRAPIB (WITH BEST IN CLASS PROPERTIES)

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Heart disease represents a significant global health risk, accounting for approximately 25% of deaths worldwide despite of significant progress to negate this statistics made in the last two decades, including pharmaceuticals such as statin therapy, surgery options, and life choices related to increased health awareness. New options on the top of the currently available choices are paramount to significantly reduce the risk of coronary heart disease (CHD). One such mechanism of possible intervention is inhibition of cholesteryl esterase transfer protein (CETP) which is one of the key regulators of the homeostasis of lipid particles including the HDL and the LDL particles. We will discuss the invention of MK8262, the best CETP inhibitor known to date, in context of the entire class. We will show preclinical and clinical evidence of its efficacy by means of HDL increase and LDL reduction, the key indicators for reduced CHD risk.



14 - SOLID STATE NMR SPECTROSCOPY AS A TOOL SUPPORTING THE MECHANOCHEMISTRY DEVELOPMENT.

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Mechanochemistry is one of the most rapidly growing research directions, which opens interesting possibilities in new materials synthesis. In many cases it simplifies standard procedures, as well as changes the course and yield of chemical processes. It is also a strategy, which fulfills economic and ecologic requirements and expectations. In 'green chemistry' one of the most crucial assumptions, and in a long perspective an important achievement, is that the solvents used in standard synthetic procedures will be totally eliminated or at least reduced to minimal amounts. In mechanochemistry, simply saying, two types of processes can be distinguished: the first one is associated with a new covalent bond formation (this process is often called mechanosynthesis), whereas as a result of the second one a new product is formed due to non-covalent interactions (aromatic-aromatic, Van der Waals interactions, formation of hydrogen bonds, etc.). In the talk both types of processes will be discussed.

The role of Solid State NMR spectroscopy as very diagnostic technique in analysis of intermediates and final products of mechanosynthesis will be shown.¹ The power of relatively new solid state NMR methodology, called Very Fast MAS in controlling of preparation and structure elucidation of inclusion complexes containing C60 and C70 as guest molecules embedded into molecular capsules will be presented. The applicability of ¹H-¹³C invHETCOR, ¹H-¹⁵N invHETCOR as well ¹H-¹H RFDR MAS and ¹H-¹H SQ-DQ BaBA correlations recorded with spinning rate 60 kHz in structural analysis of pharmaceutical co-crystals will be revealed.²

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O6 - Determination of Conformation in Solution: A survival guide for Medicinal and Analytical Chemists

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Scientists have always pursued information about the behaviour of molecules in solution. For the last 50 years this has been pursued using many different techniques, including NMR with varied results. It has been over the last 20 years that significant advances have been made in this area. It was initially with approaches like NAMFIS¹ that it was possible to gain an insight in the conformation population of molecules in solution.

It will be shown the efforts in the direction of automation of the analysis, considerations on data preparation as well as the different approaches taken to minimise the overfitting of results.² The usefulness and applicability of the technique will be discussed as well as its applications in Medicinal Chemistry.



O7 - DISENTANGLING THE LEDGF/p75 INTERACTOME

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LEDGF/p75, or “lens epithelium-derived growth factor,” is a protein that contributes to the regulation of gene expression. It does so by tethering other proteins to a specific epigenetic mark on chromatin. This chromatin tethering activity is hijacked in two important disease settings: HIV and mixed lineage leukemia. The lack of information about biological regulation of LEDGF/p75’s interaction to binding partners limits the development of therapeutic targeting of LEDGF/p75 in human disease.

Here, we present the work that led to better understanding of the regulation of the LEDGF/p75 interaction network. We obtained NMR structures of the complete interfaces between the LEDGF/p75 and its cellular binding partners and revealed that structurally conserved interaction motifs on known LEDGF/p75 binding partners can be regulated by phosphorylation, permitting switching between low- and high-affinity states.

The structure-guided elimination of phosphorylation sites in MLL1, one of the LEDGF/p75 interaction partners, reduces the ability of leukemic cells to remain in a cancer-like state. Interestingly, this interaction is only necessary for cancer and does not appear to be needed for normal function of blood cells, which may open new therapeutic opportunities.

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T4 - NMR-BASED METABOLOMICS IN BIOMEDICINE

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Metabolomics is the comprehensive analysis of the metabolome, which is the complete set of metabolites in a biofluid or cell. Nuclear Magnetic Resonance (NMR) is an extremely powerful and highly reproducible technique able to provide the metabolic profile of a subject through the acquisition of spectra that require relatively short acquisition times and little sample handling. Biofluids like serum, saliva and urine can be collected non-invasively and are rich of metabolic information at the whole-body level, while tissues can provide information on specific compartments. NMR-based metabolomics has been successfully exploited in different pathological contexts, providing significant information on a wide range of pathologies.

In this talk, after a general introduction to the topic, examples of applications of NMR-metabolomics in biomedicine involving different kind of biological samples will be provided.



15 - HUMAN AND *IN VITRO* NMR METABOLOMICS IMPACTING ON HEALTH RESEARCH

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This presentation is composed of two parts, one regarding the more common use of metabolomics to search for disease biomarkers in biofluids, and a second part relating to selected *in vitro* applications that may, in time, also impact positively on human health.

Increasingly, biofluid metabolomics has been developing towards non-invasive biological samples, such as urine and saliva. The former biofluid is particularly interesting due to its exquisite complexity and sensitivity to general health status of the human organism. However, these same features also lead to added challenges related to the contribution of individual and population phenotype (including the effects of diet, lifestyle, geographic characteristics, etc) to the sought disease signature or biomarker. In this talk, urine metabolomic studies focusing premature birth, exposome effects on pregnancy or age-related diseases will be presented, while considering the effects of phenotype variability, either within single cohorts, or between different national or trans-national cohorts.

Following a bottom-up approach, *in vitro* metabolomic strategies may provide valuable levels of biological information (e.g. regarding drugs or environmental contaminants), which may eventually translate into tissue or biofluid biomarkers of potential clinical use (to gauge drug performance or biotoxicity). This will be exemplified by metabolomic studies of anti-cancer drugs and of silver nanoparticles as an ubiquitous contaminant. Ours results show that cell metabolomics may provide new and detailed information on the response of cells and tissues to drugs and contaminants.



O8 - AN INVESTIGATION OF CEREBRAL METABOLIC PROFILE IN THE RAT MODEL OF AGE-RELATED DEMENTIA

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Aging is a phenomenon that all living organisms inevitably face. Every year 9.9 million people globally suffer from dementia, an indicator of the aging brain [1]. In the quest for biomarkers of the onset and progression of dementia *in vivo* ¹H MRS and NMR-based metabolomic study was performed on the animal model of brain aging induced by D-galactose. D-galactose accelerates brain-aging in animal and causes neurodegeneration, inflammatory response and disordered neurotransmitter metabolism [2].

We performed a comparative study of rat brain metabolites by *in vivo* as well as *in vitro* MRS. The aim was to quantify and interpret changes in the neurochemical profile of important cerebral regions which are responsible for cognitive activity in order to predict the progression in the early stages of neurodegeneration. The selected regions from the left hippocampus and the cortex were first measured at 4.7 T by *in vivo* localised ¹H MRS. We also wanted to see if higher magnetic fields could detect more metabolites, so we used quantitative ¹H NMR spectroscopy at 14.1 T on tissue extracts from the same regions of the brain. We simultaneously tested the effect of a neuroprotective drug Huperzin A (a centrally active acetylcholinesterase inhibitor). The experiment used 3 rat groups: control group treated with saline (N = 9), D-galactose-induced neurodegeneration group (N = 10) and a similar group treated with Huperzin A (N = 10). We were able to quantify 13 cerebral metabolites that were statistically analysed for both brain regions separately.

No significant, only marginal differences in brain metabolites were seen after 8 weeks of D-galactose administration. However, after one month of simultaneous Huperzin A treatment a significant decrease in some important cerebral metabolites (NAA, glutamate, myo-inositol) has been found. The same results were obtained by *in vitro* NMR but in addition we found a significant decrease in GABA levels that indicate the progression of neurodegeneration. In our comparative analysis we found that both significant and marginal changes of metabolites mapped by *in vitro* and *in vivo* ¹H MRS methods correlated very well. The most important finding of our study was that a systemic treatment with Huperzin A in rats is not an effective therapy, when the drug after 4 weeks of D-galactose administration have been given (the brain degeneration already started) for age-related neurodegeneration model.

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Acknowledgement

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O9 - METABOLOMICS STUDY OF THE ASSOCIATION BETWEEN PANCREATIC CANCER AND DIABETES MELLITUS

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Pancreatic cancer (PC) is considered one of five most lethal cancer types, with a globally reported incidence increase.¹ Local symptoms, such as pain, jaundice, cachexia or cholangitis, appear late. Early symptomatology, including weakness, nausea, abdominal pain or unexplained weight loss, is not specific and may have many other causes.² Therefore, there is currently no reliable early-stage diagnosis. Unfortunately, the prognosis is highly unfavourable, 95–97% patients would not survive more than 5-years. Consequently, the search for early symptoms and specific biomarkers of PC remains a subject of intense research. A proper biomarker would open the possibility to suggest a screening program for early PC diagnosis, before late symptoms occur.³ At least 3 groups of patients can benefit from such screening program, namely individuals with specific risk factors, hereditary factors and pancreatogenic type 3 diabetes (T3cDM). These patients can exhibit early PC symptoms 2–3 years before any local symptoms occur, which represents a large diagnostic window.⁴

In our study, ¹H NMR metabolomics was employed to plasma samples of pancreatic cancer patients, individuals with long-term diabetes mellitus type 2 (lasting more than 5 years) and healthy controls. The NMR analyses were followed by establishing a statistical model based on principal component analysis and discriminant analysis. The aim was to discover differences between these groups and to define a potential biomarker panel. The statistical evaluation of metabolomics-based profiles provided high values of sensitivity and specificity. Subsequently, plasma samples of the risk group, specifically patients with recently diagnosed diabetes mellitus with a duration of <3 years (possible T3cDM), were analysed and the possibility of PC development was predicted. The achieved results showed strong potential of ¹H NMR metabolomics to establish a biomarker panel that would facilitate the early diagnosis of PC and the possibility to identify diabetic individuals, who are at risk of developing PC.

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T5 - PROTEIN DYNAMICS: FROM LOCAL MOTIONS TO MOLECULAR MACHINES

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The driven motion of motor protein e.g. like the helicase DnaB, a bacterial, ATP-driven enzyme that unwinds double-stranded DNA during DNA replication will be characterized by solid-state NMR. Conformations mimicking the pre-hydrolytic state, the transition state and a post-hydrolytic state are arrested and then investigated by 3D NMR spectroscopy. DNA binding as well as translocation along the DNA are studied. The processes are fueled by ATP and the consequences of ATP binding for structure and dynamics will be discussed.

Fast magic-angle spinning allows for highly resolved protein spectra and gives access to site-specific relaxation data in solid proteins. We discuss the ways such data can be interpreted and also discuss the different contributions to the proton linewidth under fast MAS.



I6 - INTERNUCLEAR DISTANCE MEASUREMENTS IN RIGID SOLIDS AT NATURAL ABUNDANCE

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Quantitative distance information between two nuclei is of critical information to reveal molecular structures at an atomic scale. Solid-state NMR is capable to measure internuclear distances in a great precision as the size of dipolar coupling is inversely proportional to cube of internuclear distances. While the distance measurements have typically been performed with isotopic labeling including ^{13}C , ^{15}N etc., measurements at natural abundance is more favorable. The use of ^1H is one solution as ^1H is highly abundant nuclei. Especially, ^1H -X provides unique information as proton position is poorly located by XRD [1]. However, the large gyromagnetic ratio and high abundance ironically prevent ^1H NMR measurements of rigid solids due to intense homonuclear dipolar coupled network. Fortunately, the dipolar networks among ^1H s are simplified at very fast MAS regime > 70 kHz. Here, we will present distance measurements between ^1H - ^{15}N [2], ^1H - ^{14}N [3] and ^1H - ^1H [4].

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O10 - HYPERDIMENSIONALITY AND SHARED-TIME ACQUISITION – NEW HOPE FOR PROTEIN RESONANCE ASSIGNMENT IN SOLIDS?

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In solution NMR, experiments for sequential backbone resonance assignment generally fail due to low sensitivity for slowly tumbling proteins (approximately for MW > 25 kDa), unless extensive deuteration is employed. In contrast, high molecular weight does not impact experiments in the solid-state, but – to date – very few proteins of > 200 aa have been assigned by MAS (magic-angle spinning) NMR due to prohibitive complexity of spectra. Here we show that MAS with ¹H-detection at $\nu_R > 100$ kHz^{1,2} represents a new route to assign large proteins, with similar requirements on sample amount (< 1 mg of uniformly ¹³C,¹⁵N-labelled material) but without deuteration.

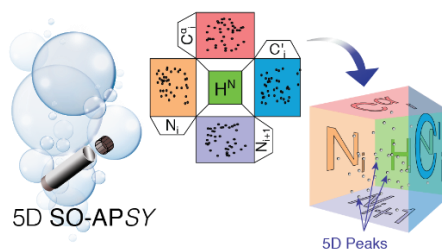


Figure 1: A schematics illustrating a 5D hypercube correlating protein backbone shifts

We developed a strategy for protein backbone resonance assignment, which requires limited acquisition time and is compatible with automatic data analysis. A key element of the approach are simultaneous experiments which (a) take an advantage of full protonation to independently polarize ¹³C α /C' and ¹⁵N nuclei, and (b) allow multiple coherence pathways that are (c) acquired separately on amide³ and α -protons¹. Shared-time, single-receiver acquisition of up to 8 experiments ensures maximum consistency of the observed chemical shifts. Narrow line-widths of ¹H resonances due to fast MAS and of heteronuclei under low-power ¹H-decoupling provide high resolution to 3D spectra and allow to link resonances with low ambiguity even for proteins as large as a 371 aa maltose binding protein. We prove the data quality, redundancy and power of the recorded resonances for the automated sequential assignment using FLYA.⁴

We also show that hyperdimensional (>3D) automated projection spectroscopy is now feasible in protein solid-state NMR. We present a first implementation of this approach where 5D peak lists are reconstructed from a number of 2D projections for protein samples of different molecular size and aggregation state, featuring limited dispersion of chemical shifts or inhomogeneous broadenings. The resulting datasets are particularly suitable to automated analysis, yielding rapid and unbiased backbone resonance assignments.

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O11 - SOLID-STATE NMR AND CRYSTAL STRUCTURE PREDICTION: A PERFECT ALLIANCE? A DIFFICULT CASE OF SOLVATE-HYDRATE OF CATECHIN

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Crystal Structure Prediction (CSP) is one of the most rapidly developing areas of computational chemistry, as it aims at offering the possibility of predicting a range of energetically plausible crystal structures, without any prior knowledge on their crystallization preferences. It is often used in conjunction with solid-state NMR experiments to determine crystal structures of new polymorphic forms, which are otherwise elusive for classic analytical methods. In this work we present the limitations and applicability of a joint CSP-NMR approach to elucidate crystal structures of polyphenols epicatechin, catechin and procyanidin A-2, including a new, yet undescribed form, methanol solvate-hydrate of catechin. The idea of this approach assumes, that there will be a noticeable difference in the agreement between experimental and theoretical NMR data for the correct and incorrect structures. Indeed there are several examples in the literature demonstrating that this may be the case [1, 2]. On the other hand, if we are dealing with multicomponent systems, this difference in terms of RMS values for predicted ¹H solid-state NMR chemical shifts may not be that distinctive. Figure 1 presents the RMS values obtained after plotting ¹H experimental chemical shifts against the ones calculated for theoretical structures generated in CSP calculations. Clearly, for epicatechin only one structure gives the best agreement with the experiment, but for procyanidin A-2 there are a number of structures with similar agreement. In our work a question of how good agreement is good enough is addressed.

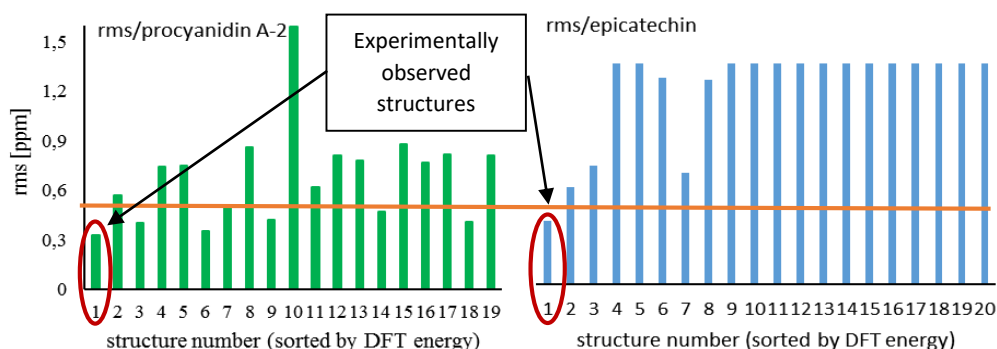


Figure 1: RMS values obtained after comparison of the experimental and theoretical ¹H chemical shifts for the CSP generated structures of epicatechin and procyanidin A-2 dimer. Orange line indicate a 0.5 ppm cut-off.

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O12 - POLYMORPHIC TRANSFORMATIONS OF DRUGS LOADED INTO GLYCOPOLYMERIC MICELLES PROBED BY SOLID-STATE NMR

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Polymeric micelles are nowadays widely used nanocarriers in drug delivery due to their tunable properties such as size, shape, surface functionalization or circulating properties. Understanding the nature of the drug-polymer interactions is essential for the rational design of polymeric matrices as suitable carriers for a particular drug. Solid-state NMR spectroscopy is a powerful tool to gain structural insight into the polymeric systems, to provide evidence for drug localization or control quality of the starting polymers.

In this work, we prepared glycopolymeric micelles poly(1-*O*-methacryloyl- β -D-fructopyranose)₃₆-*b*-poly(methyl methacrylate)₁₆₀, designed to target tumor cells and we loaded them separately with two drugs, ellipticine and curcumin. After drug loading, we found an intermolecular drug-polymer interaction indicating that both drugs are localized in the core. The structure, size or dynamics of the polymeric micelles did not change significantly, ascribed to the low drug loading (ca. 5%). On the other hand, the polymorphic form of the drugs changed significantly after loading. Both drugs recrystallized as different polymorphs than that found in the bulk material. As an example, the curcumin case is shown in Fig. 1. While purified curcumin occurs in bulk as thermodynamically stable monoclinic Form I, inside the micelles, it recrystallized as the metastable orthorhombic Form II. Ellipticine displayed similar behavior, however, no solid state NMR data of ellipticine have been reported so far. Therefore, we extracted NMR parameters from the reported crystal structures using CASTEP and compared to the experimental data supporting our hypothesis that we discovered a new polymorph of ellipticine (Form III). However, inside the micelles, ellipticine form is different.

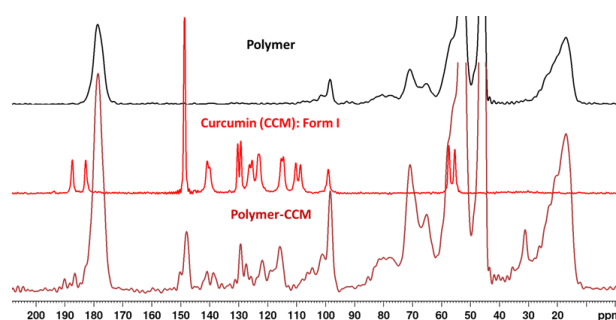


Figure 1: Solid-state ¹³C CP-MAS-TOSS spectra of both components separately (the polymer and curcumin) and their polymer-curcumin conjugate measured at 6.5 kHz spinning speed.

Our results are crucial for the whole drug delivery concept. The glycopolymeric micelles can induce polymorphic transformations of the drug leading to potential lack of its bioavailability, which should be considered in polymer-drug design and in further biochemical screening. Moreover, we discovered new polymorph of ellipticine, Form III.

Acknowledgement to the Czech Academy of Sciences (grant No. MSM200551801).



T6 - NMR AS A WINDOW TO THE DYNAMIC MICROCOSMOS OF (DISORDERED) PROTEINS

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In this tutorial, NMR spectroscopy of proteins will be reviewed, especially for NMR spectroscopists working with other molecules. First, the features of protein backbone spin systems that greatly facilitate protein NMR spectroscopy will be emphasized, and limitation of NMR by the size of investigated molecule will be mentioned. Then, the attention will be paid to so-called intrinsically disordered proteins (IDPs). Dynamics of IDPs will be discussed, showing how it makes NMR spectra of IDPs easy to measure but relaxation rates of IDPs more difficult to interpret. The problem of spectral resolution and its solutions will be mentioned. As the main topic, extraction of structural information from the spectra of IDPs will be discussed. Chemical shift analysis will be described as the major tool to identify transient local structures and to determine their populations in the structure ensembles. Paramagnetic relaxation techniques will be presented as approaches probing long-range structural features and allowing us to specifically reveal minor compacted states of proteins important for their biological function. Complementary sources of structural information will be also mentioned. Methods to study covalent modifications and non-covalent intermolecular interactions of proteins will be presented. Application of the discussed methods to real proteins will be documented using NMR spectra of microtubule associated proteins, mostly obtained in the speaker's laboratory [1].

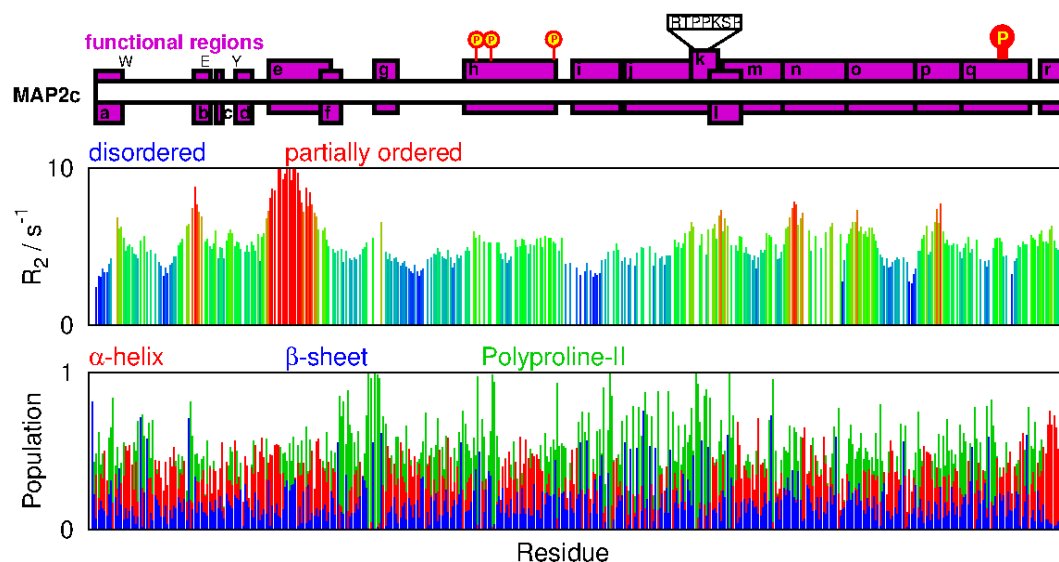


Figure 1: Dynamics and transient secondary structures of microtubule associated protein 2c

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17 - SOLID-STATE NMR COMBINED WITH CRYO-EM TO DETERMINE THE STRUCTURE AND FUNCTIONAL DYNAMICS OF A LARGE ENZYME

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Atomic-resolution structure determination is the key requirement for understanding protein function. Cryo-EM and NMR spectroscopy both provide structural information, but currently cryo-EM does not routinely give access to atomic-level structural data, and, generally, NMR structure determination is restricted to small (<30 kDa) proteins. We introduce an integrated structure determination approach that simultaneously uses NMR and EM data to overcome the limits of each of these methods. The approach enabled determination of the high-resolution structure of the 468 kDa large dodecameric aminopeptidase TET2 to a precision and accuracy below 1 Angstrom by combining secondary-structure information obtained from near-complete magic-angle-spinning NMR assignments of the 39 kDa-large subunits, distance restraints from backbone amides and specifically labelled methyl groups, and a 4.1 Angstrom resolution EM map. The resulting structure exceeds current standards of NMR and EM structure determination in terms of molecular weight and precision. Importantly, the approach is successful even in cases where only medium-resolution (up to 8 Angstrom) cryo-EM data are available, thus paving avenues for the structure determination of challenging biological assemblies.

We will furthermore show studies of functional dynamics of the intact TET particle.



O13 - STRUCTURAL CHARACTERIZATION OF PROTEIN KINASE ASK1 AND ITS INTERACTION WITH THIOREDOXIN

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family in cell defence system against stressors. ASK1 activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis in various cells, the regulation of ASK1 depends on several stimuli, including oxidative stress (presence of ROS) and therefore, ASK1 plays a key role in the pathogenesis of many diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin 1 (TRX1) and the 14-3-3 protein that both function as inhibitors of ASK1 [1]. Under normal condition, ASK1 with bound TRX and 14-3-3 is in inactive state. As a response to oxidative stress, TRX and 14-3-3 dissociate and ASK1 becomes an active kinase. However, the molecular mechanism of the ASK1 activation is unknown, as there are almost no structural data available to date. Therefore, the aim of this study was to study the impact of the structural changes of the TRX-binding domain of ASK1 (ASK1-TBD) and ASK1-TBD:TRX complex formation on the molecular mechanism of the ASK1 activation.

We have previously shown that ASK1-TBD forms with TRX well defined and stable complex under reducing conditions. ASK1-TBD contains seven cysteine residues with the residue Cys250 being the only cysteine which is both solvent exposed and essential for TRX binding in reducing conditions. The oxidative stress also induces intramolecular disulfide bonds formation within ASK1-TBD and affects its structure in regions important for TRX1 binding [2,3].

In this study we present structural characterization of the regulation of ASK1 via structural model of ASK1-TBD in both reduced and oxidized conditions and ASK1-TBD:TRX complex based on sparse NMR data, crosslinking mass spectrometry and small-angle x-ray scattering (SAXS) data. Introducing the Cys250 mutation causes structural changes that prevent the TRX binding, rather than being directly involved in the complex formation. Oxidative stress induces structural changes in the ASK1 molecule in regions involved in TRX binding, suggesting that the ASK1 oxidation is also involved in the complex dissociation.

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O14 - INTERDOMAIN MOBILITY IN PDZ TANDEM CAPTURED BY EXTERNALLY RESTRAINED MD SIMULATION

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PSD-95 (DLG4) is one of the most studied proteins in the post-synaptic density of nerve cells. [1] It is involved in organizing and regulating the distribution of membrane receptors at the post-synaptic site. NMDA receptor, voltage-gated potassium-channel Kv1.4 and many other membrane proteins were previously identified as its binding partners, however the exact structure and mechanism of the post-synaptic protein network remains poorly understood.

The PDZ1-2 tandem of the PSD-95 protein was shown to possess a rigid structure in the apo-state, however ligand binding induces considerable interdomain mobility. [2] In the tandem arrangement, PDZ domains usually fold and function in an interdependent way. We presume that regulation of the relative orientation and dynamics of the two PDZ domains might be a key feature in organizing the distribution of the binding partners.

In order to elucidate the atomic-level mechanism of the inner motions in the PDZ tandem, we performed all-atom molecular dynamics (MD) simulations, including NMR-derived experimental dynamic parameters (NOEs and S2 order parameters) as external restraints. As a result, we obtain a number of dynamic structural ensembles of the modeled structure that properly describe its dynamics on the fast (ps-ns) timescale while maintaining good correspondence with the observable dynamic parameters.

Analysis of the structural ensembles proved an interdependence between inter- and intra-domain motions in the PDZ1-2 tandem. The intradomain motions reveal the β 2- β 3 loop, near the ligand binding groove, as a region that changes dynamic behavior upon ligand binding.. This region was earlier reported to have a role in ligand binding specificity of PDZ domains. Furthermore, the displacement of the two domains in the PDZ1-2 tandem relative to each other is exhaustively sampled in our simulations, which allows for clustering the resulting ensembles according to the mode of interdomain interactions.

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T7 - WHEN ARTIFACTS TURN OUT TO BE REAL DATA: DISCOVERIES OF RNA DYNAMICS

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RNAs are dynamic: But what does it mean, and how can we study and describe the involved structures? These RNA dynamics has been measured for decades, but only recently it became feasible to actually detect and characterize higher energy states. Fast dynamics (faster than τ_c) has been recorded and molecular dynamics had been used to interpret data, as direct characterization of the involved states, was, and still is, not possible. However, when new phenomena appear, one first thinks, that can't be right – artifacts come to mind.

Years later, with new methodology developed, it becomes feasible to study these and other dynamics/structures in detail. I will therefore talk about my story of how I discovered RNA dynamics and learned about the limitations of current methodology and to keep an open mind.

Latest results will, additionally, be presented on secondary structural switches that require the breaking and reconfiguration of base-pairs and occur at second or faster timescales. These transitions can feature on one hand localized changes in base-pair alignments in and around non-canonical base-pairs¹, such as internal loop and bulges, or can be presented by a complete change in chemical identity of a nucleobase². We describe $R_{1\rho}$ -relaxation-dispersion NMR methods {Schlagnitweit:2018gg} for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled at three orders of magnitude faster timescales. The characterization of different types of transient structures is presented. 1) The HIV-1 dimerization initiation site (DIS) undergoes large secondary structure rearrangements, that provide the basis for a molecular zipper.¹ 2) The GU wobble base-pair undergoes a change from standard wobble geometry to appear like a Watson-Crick base-pair stabilized by Keto-Enol tautomerization.² 3) the microRNA that switches base-pairing and steers so activity in the RISC complex (unpublished).

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I8 - HOW TO FIND NEEDLE IN A HAYSTACK? (NEW RNA MODIFICATIONS)

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Nowadays, there are more than 140 RNA modifications known in eubacteria, archaea and eukaryotes. The majority of chemical RNA modifications have been discovered in highly concentrated RNA such as rRNA and tRNA. Nevertheless, the number of modifications mapped in messenger RNA or regulatory RNA are expanding. Beside the classical methylations of nucleobase and ribose moieties, the more exotic RNA modification such as Nicotinamide adenine dinucleotide (NAD) has been identified as a new cap in prokaryotes^[1] and eukaryotes.^[2] The exact role of NAD has not been elucidated yet.

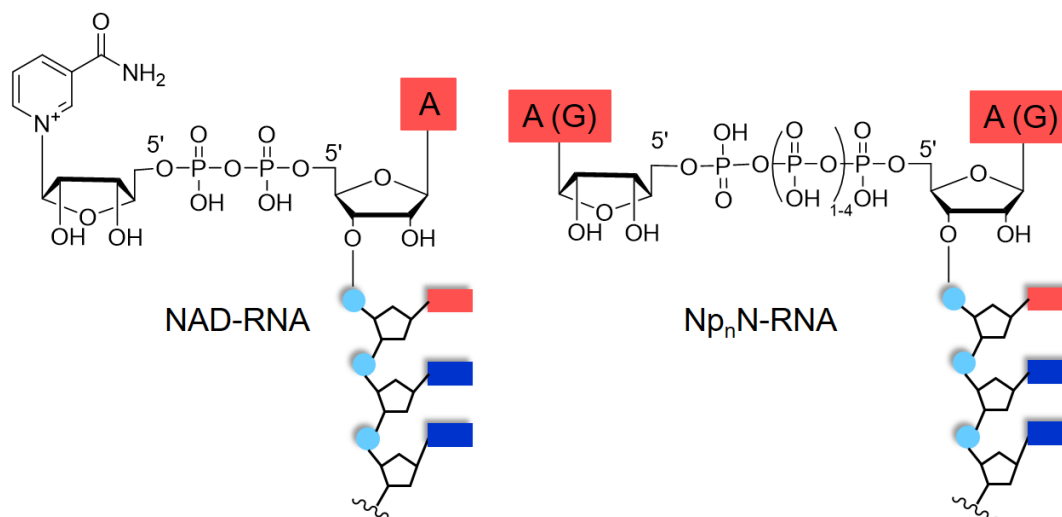


Figure 1: Structure of recently discovered RNA caps.

In our search for new RNA modifications, we hypothesized that beside NAD other RNA caps may exist. Dinucleoside polyphosphates (Np_nN) are signaling molecules that are present in all cells and they have similar structure to NAD or canonical eukaryotic cap. We found that they were excellent substrates for RNA polymerases and initiated transcription. Np_nN -RNAs were efficiently cleaved by *Escherichia coli* decapping enzyme. Furthermore, LC-MS analysis of short RNA from *E. coli* confirmed presence of nine new RNA caps with Np_nN structure.

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O15 - ADENINE-DEPENDENT G-QUADRUPLEX STRUCTURAL SWITCH

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Excessive activity of the *RANKL* gene leads to unbalanced bone remodeling and can influence the incidence of osteoporosis [1]. A 20 nucleotides long G-rich sequence, d(G₄TAGG₃AGCGG₃AGAGG₃), has been found in the regulatory region of the *RANKL* gene, suggesting that the expression of the *RANKL* may be regulated by putative folding of its G-rich region into unusual DNA structures, such as G-quadruplexes.

Wild type sequence folds into diverse G-quadruplex conformations. Simple G4-to-T4 modification results in a G-rich sequence with four GGG-tracts (RAN4) and forms a two-quartet G-quadruplex stabilized by A5•G3•A17 and G20•G8•G12 base-triads. Detailed analysis of high-resolution NMR structure of RAN4 (PDB id 6GZN) together with G/A-to-T modifications of residues from both base-triads uncovered the critical role of a single loop adenine A5 for the formation of distinct two-quartet topology. Comparison of RAN4 and RAN4A5T topologies (Figure 1) reveals significant structural changes induced by A5-to-T5 modification including a switch from two- to three-quartet G-quadruplex [2].

Our results indicate that specific loop interactions involving an adenine residue can critically influence the structure of a G-quadruplex and provide insights into the complexity of intricate interactions that influence the folding process of G-rich DNA sequences. Additionally, our study, to the best of our knowledge, is the first to suggest that the expression of the *RANKL* gene may be regulated by G-quadruplex(es) formation in its regulatory region.

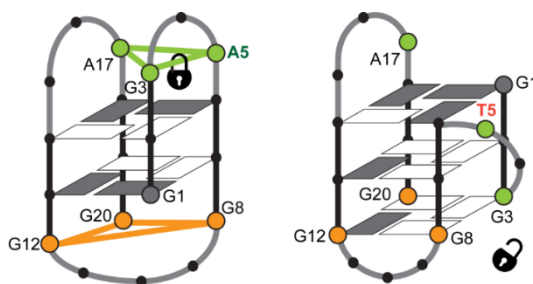


Figure 1: Switch between two-quartet (left, RAN4) and three-quartet (right, RAN4A5T) G-quadruplex depends on interactions of adenine A5 from A•G•A base-triad, where it forms hydrogen bonds with G3.

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Acknowledgements

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O16 - UNRAVELLING THE STRUCTURAL BASIS FOR THE EXCEPTIONAL STABILITY OF RNA G-QUADRUPLLEXES CAPPED BY A URIDINE TETRAD AT THE 3' TERMINUS

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The presence of a 3' terminal U-tetrad in parallel, tetramolecular RNA G-quadruplexes is associated with a pronounced increase of the thermodynamic stability of the folded structure (1,2). This effect was also observed in the biologically relevant context of human telomeric RNAs, for which it was demonstrated that the displacement of the 3' terminal U-tetrad to the 5' end of the molecule leads to a 30 °C drop in the melting temperature of the G-quadruplex (2). Understanding the structural features underlying the context dependent differences in the stability of the U-tetrad motif thus appears a relevant problem.

In order to shed some light onto this question, we have solved the solution structure of the G-quadruplex formed by the r(UGGUGGU) sequence. We show that the molecule adapts a parallel, tetramolecular fold in which all three uridines (the 5' terminal, central and the 3' terminal) are indeed involved in the formation of U-tetrads. However, the 3' terminal U-tetrad turned out to be embedded into a highly unusual structural context. Namely, our atomic resolution NMR structure features an almost 180° turn of the phosphate backbone between G6 and U7, leading to the directionality of the final U-tetrad being opposite to that all the other tetrads. Moreover, when coupled with extensive explicit solvent MD simulations, our structure reveals important features of such a conformation, likely responsible for its stabilizing effect. These include the presence of a very stable 2'OH to phosphate hydrogen bond, as well as, the formation of an additional K⁺ binding pocket in the quadruplex groove. Interestingly, a similar fold was already observed in some crystal structures of G-quadruplexes capped by a 3' terminal U-tetrad (3), but it was never reported in solution, nor connected to the thermodynamic stability of the quadruplex. The exact structural requirements for the formation of such a 'reversed U-tetrad' motif, as well as, the origins of its stabilizing effect were further studied through NMR and UV analysis of a series of cognate sequences containing different modifications on the U7 site (dU, 5MeU, C, 2'OMe-U etc.).

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Acknowledgment

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T8 - QUANTUM-CHEMICAL CALCULATIONS OF NMR PARAMETERS

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The calculation of NMR parameters by quantum chemistry goes back to 1950, when Hylleraas and Skavlem calculated the nuclear magnetic shielding constants of H₂ [1] and to 1974, when Kowalewski *et al.* calculated the indirect spin–spin coupling constant of HD [2]. Since then, the calculation of NMR shielding constants and indirect nuclear spin–spin coupling constants has become a routine tool of chemistry, with hundreds of publications appearing every year [3,4]. Nearly all such calculations are based on the nonrelativistic perturbation theory developed by Ramsey in the early 1950s [5,6], although relativistic treatments are becoming more common. Most studies are performed using density-functional theory (DFT), but for high accuracy methods such as coupled-cluster theory and full configuration-interaction theory are also used.

This talk gives an overview of methods of calculation of NMR parameters and NMR spectra, from highly accurate studies small molecular systems to qualitative calculations on very large systems, containing hundreds of atoms. The principles behind such calculations are discussed, as well as important practical considerations such as the choice of basis set, electronic-structure method, and the need to include relativistic and vibrational corrections. Recent benchmark studies are presented.

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I9 - TOWARDS ACCURATE PREDICTIONS OF NMR PARAMETERS

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Predictions of NMR parameters are required in many NMR studies to complement experimental data and to provide a deeper insight into the structure and dynamics of molecules that cannot be obtained by experiment alone. Quantum chemical calculations and computers made a tremendous progress in the past 50 years. Predictions of molecular properties of larger molecules (hundreds to thousands atoms) can nowadays be routinely done.

Typical NMR calculation that is very often performed to support or help to interpret experimental data consists of geometry optimization of an isolated molecule and NMR calculation, typically at the density functional theory (DFT) level. However, this simple model is not sufficiently accurate in many cases and fails completely for dynamic systems or systems with significant intermolecular interactions, such as in the solid state.

When highly accurate predictions of NMR parameters are desired, several factors have to be considered:

- 1) The computational method: For example, the choice of the functional in a DFT calculation may be crucial for the accuracy. DFT calculations fail in some cases completely (an example is prediction of fluorine chemical shifts). The inclusion of relativistic effects is crucial for heavy atoms.
- 2) The molecular environment: Solute-solvent interactions and intermolecular interactions in solids can significantly influence NMR parameters. The intermolecular calculations can be modelled by molecular clusters or by calculations with periodic boundary conditions.
- 3) The molecular dynamics (MD): Dynamic processes may range from fast vibrational and librational motions to larger scale conformational changes in solutions and disordered materials. Various MD simulation techniques have to be used to simulate motions with different timescales (classical MD, DFT-MD, metadynamics).

Several examples of recent applications of accurate predictions of NMR parameters will be discussed. A special emphasis will be given to the influence of fast molecular motion on NMR observables. For example, we have been developing methods for including vibrational averaging, nuclear delocalisation, isotope effects and temperature effects in NMR calculations based on convoluting DFT-calculated shielding and coupling surfaces with probability distributions of relevant bond distances and valence angles obtained from DFT path-integral molecular dynamics (DFT-PIMD) simulations [1–3].

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I10 - BIOINFORMATICS-DRIVEN NMR STRUCTURE DETERMINATION

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Structural biology studies provide key insights for life sciences, ranging from drug discovery to bio-fuel engineering, and from understanding metabolic disease mechanisms to improve human and animal health. Nuclear Magnetic Resonance (NMR) spectroscopy enables high-resolution three-dimensional studies of biomolecules and provides unique information on molecular dynamics and interactions.

The downside of protein structure determination by NMR is however the elaborated data analysis workflow, involving many imperfect experimental and computational steps. In fact, NMR-only driven structure determination projects will always be limited by the intrinsic experimental imperfection of the technique, therefore truly robust, objective and highly accurate data analysis of large protein complexes (> 30kDa) is hard to achieve using NMR data only.

The current limitations of NMR studies can however be bridged by simultaneous consideration of multiple types of information. A promising strategy consists in supplementing the conventional structure determination process with *a priori* knowledge either extracted from chemical shift and structural databases, or from co-evolution sequence analysis of protein families. Especially the later in form of evolutionary couplings can be profitably used for structural characterization of large and complex biological systems.

Here we will describe such an integrated, hybrid approach for NMR structure elucidation. We will present application examples for rapid and accurate structure determination of small to medium-sized soluble proteins and large protein assembly by solid-state NMR.



O17 - HOW TO COLLECT AND PROCESS DATA IN SERIAL NMR EXPERIMENTS?

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Serial NMR measurements are common in the analysis of chemical and physical changes. Experiments in a series can differ e.g. in sample concentration, temperature, pressure, pulsed field gradient value (diffusion-ordered spectroscopy) or simply can be performed at different moments of time (studying chemical reactions).

For complex samples, the multidimensional correlation spectra are usually necessary. However, serial acquisition of multidimensional spectra is time-consuming, due to costly sampling of indirect time domain. The non-uniform sampling (NUS) can accelerate the experiment, but so far was limited to frequency dimensions. However, one can imagine a series of spectra forming an additional “non-frequency dimension”. Then, novel processing methods can be proposed and provide numerous benefits in terms of sensitivity, speed, data analysis etc.

In this talk I will discuss the perspectives of the use of various methods to process „non-Fourier” dimensions and provide examples from NMR of proteins and small molecules.

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P1 - SPINAL CORD INJURY EVALUATED BY SERIAL DIFFUSION TENSOR IMAGING IN RAT

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Identification of injury range and evaluation of spinal cord parenchyma over time is crucial for spinal cord injury (SCI) treatment. Diffusion tensor imaging (DTI) seems to be an effective method to address these objectives *in vivo*.

In the following study, we used the DTI technique to evaluate the spinal cord lesion in the rat contusion model [1] over two months after the lesion onset. We tested four DTI parameters: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) in a 2cm spine cord segment over the lesion epicenter. Moreover, fiber tractography was used to visualize spared fibers and the deformities located mainly in dorsal column. Finally the *in vivo* MR parameters were correlated with histological and locomotor outcome to check for their prognostic value.

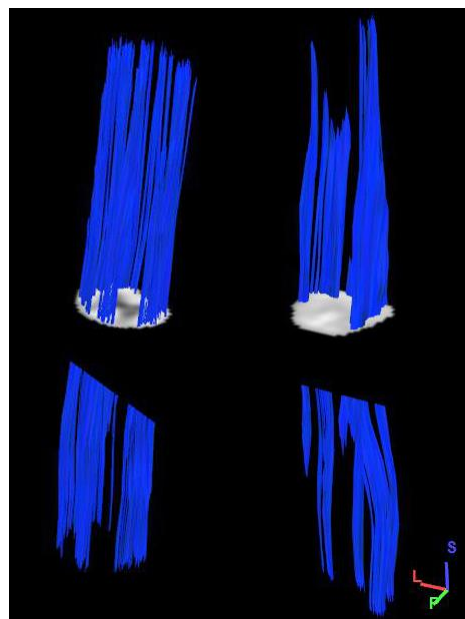


Figure 1 Fiber tractography of rat spinal cord. Control (left) and injured (right).

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P2 - N-ISOPROPYLACRYLAMIDE BASED CROSS-LINKED HYDROGEL STIMULI-RESPONSE BEHAVIOR OBSERVED BY MRI

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Stimuli-responsive hydrogels are interesting systems with broad use in medicine and engineering [1]. *N*-isopropylacrylamide (NIPAM) has been extensively studied as a model system [2]. Hydrogels based on NIPAM have a reversible phase transition, collapse and swelling are started by increasing or decreasing the temperature, respectively.

Cross-linked macroscopic hydrogels only from NIPAM (cross-linker ration 40:1) have very long time to get to equilibrium due to skin effect, water is trapped inside a hydrogel. One way to make collapse and swelling much faster is to create semi-interpenetrating network from NIPAM and linear poly(acrylamide) [3].

MRI provide a non-destructive view inside hydrogel during collapse and swelling. Micro 5 gradient system and ParaVision 6.0 were used to acquire proton density and diffusion weighted images [4]. Time and spatially resolved measurement enable characterization of collapse and swelling by observation of solvent signal.

Proton density and diffusion weighted images provide characterization of collapse and swelling of macroscopic cross-linked hydrogel based on NIPAM.

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Acknowledgement:

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P3 - EASILY MODULATED PHOTOCHROMIC HYDRAZONES

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In the last three years, hydrazones found important application as molecular switches [1-3]. This study focuses on the comprehensive investigation of photoswitching behaviour of novel pyridyl-based hydrazone derivatives synthesized from commercially available aromatic ketones. Our results showed that designed skeleton exhibits desired photochromic behaviour with very good addressability and fast photochemical *E-Z* and also back photochemical *Z-E* isomerization around the $-C=N$ double bond (Figure 1) - contrary to the locked *Z* isomers of pyridyl-2-aldehyde phenylhydrazones and acyl hydrazones prepared by prof. Lehn et al., which are highly resistant to *Z-E* isomerization because of the intramolecular H-bond [2].

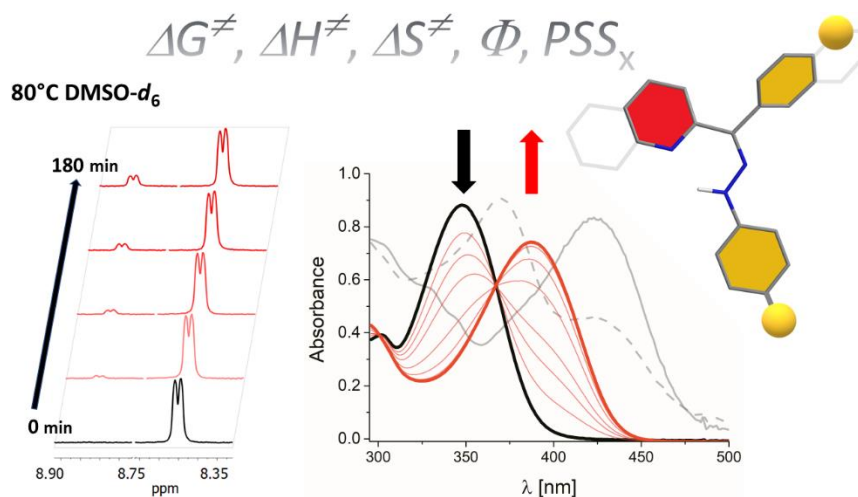


Figure 1: Photoswitching behaviour of pyridyl-based hydrazones

Our work was focused on the optimization of photochromic characteristics by small structure modification. Contrary to preparation of the known hydrazone photoswitches by diazo-coupling reaction published by prof. Aprahamian et al., we would like to demonstrate that potent hydrazone photoswitches can be prepared by simple condensation of corresponding hydrazines and keto-compounds.

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Acknowledgements

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P4 - DYNAMIC BEHAVIOR OF ISOXAZOLIDINYL EPOXIDES: A COMBINED NMR AND DFT STUDY

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Isoxazolidinyl epoxides **2** can be prepared from 2,3-dihydroisoxazoles **1** by modified procedure (Figure 1) using *in situ* generated 3,3-dimethyldioxirane which enables the scale-up of former synthesis.^[1] These unusually stable epoxides represent a new valuable structural class of *N,O*-containing heterocycles that allow highly stereoselective access to 3,4-*trans*-hydroxyisoxazolidines with a variety of substituents at C-5.

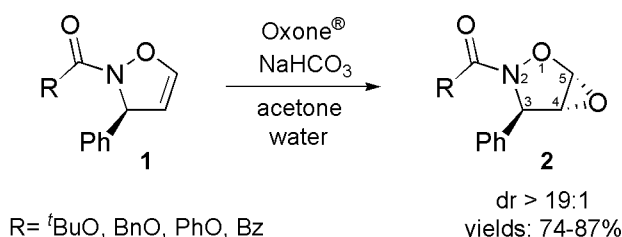


Figure 1: Epoxidation reaction of 2,3-dihydroisoxazoles.

Variable temperature NMR study revealed an existence of the dynamic equilibrium between two rotamers of isoxazolidinyl epoxide **2b** (R = BnO).^[2] The barrier to carbamate C-N bond rotation was found to be 14.0 kcal/mol using computer line-shape simulation method.

Further investigation including the influence of carbamate protection (R-group), C-3 aromatic ring substitution and solvent on conformer population and rotational barrier as well as DFT calculations will be presented.

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Acknowledgement:

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P5 - ISATIN (*para*-NITROPHENYL) HYDRAZONES: INSIGHT INTO VIS-VIS PHOTOSWITCHING MECHANISM (SPECTRAL STUDY)

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In the last three years, hydrazones found important application in various areas of photochemistry [1-3]. This study focuses on the comprehensive investigation of photoswitching behaviour of two isatin (*para*-nitrophenyl) hydrazones as new Vis-Vis (NIR) photochromic switches (Figure 1). Switching mechanism was studied by ¹H NMR and UV-Vis spectroscopy and nanosecond laser flash photolysis.

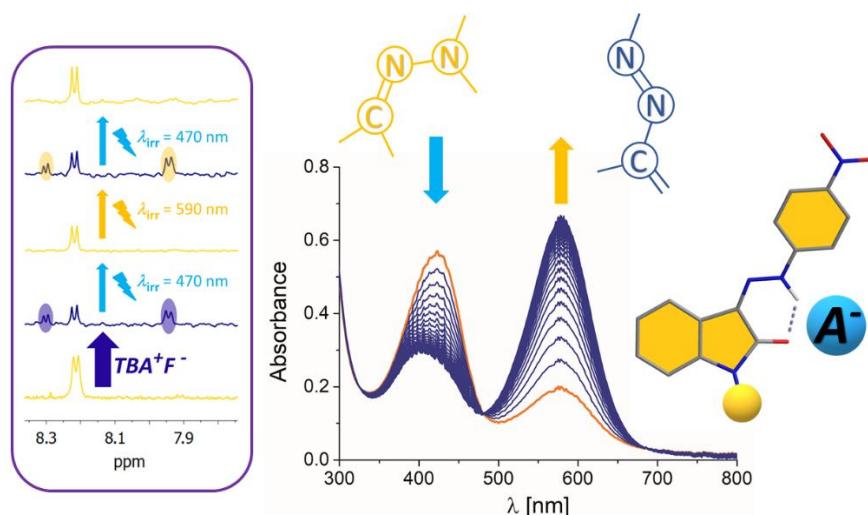


Figure 1: Photoswitching behaviour of isatin (*para*-nitrophenyl) hydrazones

Interestingly, the photochromic behavior of hydrazone/*F* photochromic system results from mutual *hydrazo* (keto) and *azo* (enol) tautomer photoconversion. To the best of our knowledge, this is the first report on reversible light-induced *azo/hydrazo* tautomerism. Facile photochromic behavior through a large anion concentration region can be observed in the presence of less basic *Cl*⁻ anion, although the amplitude of photoswitching decreases compared to the hydrazone/*F* photochromic system. This behavior clearly indicates the crucial role of an anion basicity in stabilization of the blue *azo* form.

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Acknowledgements

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P6 - NMR AEROSOLOMICS AS A TOOL TO DISTINGUISH VARIOUS TYPES OF AEROSOL SAMPLES

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Atmospheric aerosols are a small but very important part of the Earth's atmosphere. The proportion of inorganic and organic compounds in aerosol particles seems to be equal on average.^{1,2} While the inorganic composition of aerosols is well explored, knowledge about the organic part is still very limited. It is well known that the major part of organic aerosol compounds is represented by polar, water-soluble organic compounds (WSOC).¹ NMR spectroscopy was for the purpose of aerosol chemistry "discovered" only recently³ as it is rather insensitive method. Nevertheless, NMR has undergone rapid development and sensitivity gain of late.

Aerosolomics provides complex evaluation of aerosol composition and compound concentration.⁴ It is exploiting metabolomic approach, which is applied to aerosol samples. In NMR aerosolomics the assignment of dominant signals is based on precise chemical shift of the compound which enables identification of organic compounds in given aerosol sample. For this purpose, a comprehensive library of high-res ¹H NMR spectra of organic compounds that are known to be present in aerosol particles is essential. The database of the ChenomX NMR Suite program⁵ contains about 70 compounds that have also been found in aerosol samples according to the literature. Up to now, 50 new compounds attributed to aerosol have been added to the database; the largest gap was found in aromatic carboxylic acids (12), compounds with sulphur (11) and amines (8). Additionally, about 30 new organic compounds (mainly hydroxyl carboxylic acids) were found in aerosol samples. These compounds were present in the original ChenomX library and have not been found in aerosol samples yet.

In the recent study, the summer and winter aerosol samples were analyzed using NMR aerosolomics approach. The samples were collected in Prague-Suchbát during summer 2008 and winter 2009 in two different particle size fractions – PM_{2.5} and PM₁₀. Around 50 compounds were identified in each aerosol spectrum owing to the comprehensive library. The profile of 86 identified compounds, which were identified in the samples altogether, served as an input data for statistical analysis. Multivariate statistical analysis clearly discriminates the two groups studied. Furthermore, it is possible to determine the most significant compounds.

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P7 - NMR SPECTROSCOPY OF THE RAT HEART IN DIABETES

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Diabetes mellitus (DM), defined by elevated glycemic markers, is a major risk factor for cardiovascular disease, which is the most common cause of death among adults with DM. When using animal models, DM is usually induced by administering streptozotocin that targets pancreatic β -cells. In a study that should lead to the characterization of mitochondria in rat hearts under various conditions and after preconditioning we performed a metabolomic detection of intracellular cardiac metabolites by ^1H NMR spectroscopy.

First, we performed untargeted analysis using CRAFT method that extracts signal amplitudes directly from an FID without the need to Fourier transform spectra. The principal component analysis of such data separated the two groups.

Next, we tried to identify and quantify as many individual metabolites as possible using Chenomx NMR Suite. We were able to identify more than 20 metabolites of which the most prominent were taurine, creatine, lactate, glutamine, glutamate, inosine, alanine and glycerol. The differences between metabolites from healthy and DM rats were not significant except for creatine and glutamate that were decreased in diabetes. The presence of 3-hydroxybutyrate and niacinamide was also detected but their role will have to be studied further.

Acknowledgement

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P8 - AN INVESTIGATION OF CEREBRAL METABOLIC PROFILE IN THE RAT MODEL OF AGE-RELATED DEMENTIA

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Aging is a phenomenon that all living organisms inevitably face. Every year 9.9 million people globally suffer from dementia, an indicator of the aging brain [1]. In the quest for biomarkers of the onset and progression of dementia *in vivo* ¹H MRS and NMR-based metabolomic study was performed on the animal model of brain aging induced by D-galactose. D-galactose accelerates brain-aging in animal and causes neurodegeneration, inflammatory response and disordered neurotransmitter metabolism [2].

We performed a comparative study of rat brain metabolites by *in vivo* as well as *in vitro* MRS. The aim was to quantify and interpret changes in the neurochemical profile of important cerebral regions which are responsible for cognitive activity in order to predict the progression in the early stages of neurodegeneration. The selected regions from the left hippocampus and the cortex were first measured at 4.7 T by *in vivo* localised ¹H MRS. We also wanted to see if higher magnetic fields could detect more metabolites, so we used quantitative ¹H NMR spectroscopy at 14.1 T on tissue extracts from the same regions of the brain. We simultaneously tested the effect of a neuroprotective drug Huperzin A (a centrally active acetylcholinesterase inhibitor). The experiment used 3 rat groups: control group treated with saline (N = 9), D-galactose-induced neurodegeneration group (N = 10) and a similar group treated with Huperzin A (N = 10). We were able to quantify 13 cerebral metabolites that were statistically analysed for both brain regions separately.

No significant, only marginal differences in brain metabolites were seen after 8 weeks of D-galactose administration. However, after one month of simultaneous Huperzin A treatment a significant decrease in some important cerebral metabolites (NAA, glutamate, myo-inositol) has been found. The same results were obtained by *in vitro* NMR but in addition we found a significant decrease in GABA levels that indicate the progression of neurodegeneration. In our comparative analysis we found that both significant and marginal changes of metabolites mapped by *in vitro* and *in vivo* ¹H MRS methods correlated very well. The most important finding of our study was that a systemic treatment with Huperzin A in rats is not an effective therapy, when the drug after 4 weeks of D-galactose administration have been given (the brain degeneration already started) for age-related neurodegeneration model.

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Acknowledgement

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P9 - NMR STUDY OF EXTRACTION OF BUTYRIC ACID BY IONIC LIQUID

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Ionic liquids (ILs) have received substantial attention in basic and applied research in the last two decades. In our work we are focused on the design of the IL suitable for the extraction of organic acids (potential platform chemicals) from diluted streams in bio-refineries. In this presentation results of NMR study of the system composed of the Trihexyl-(tetradecyl) phosphonium decanoate (Cyphos IL-103), butyric acid (BA) and water will be shown. ¹H, ¹³C and ³¹P NMR was used for the determination of the equilibrium composition of the systems with different amount of BA as well as for elucidation of the way how individual compounds of the system mutually interact.

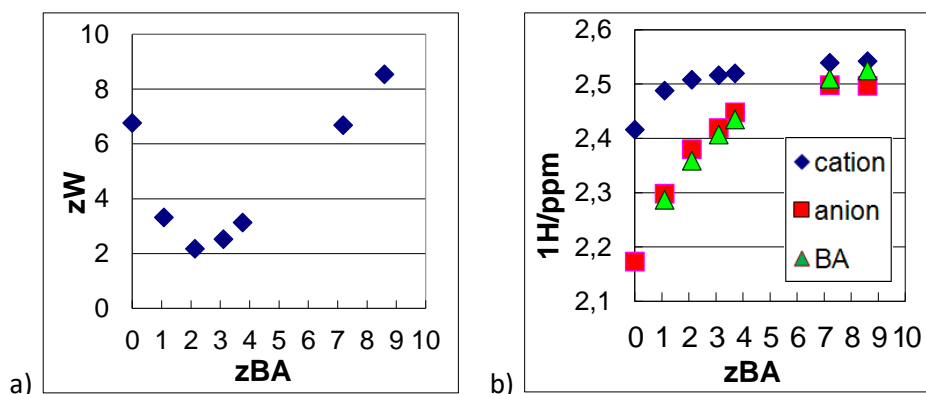


Figure 1: Influence of BA loading of IL on the equilibrium water loading of IL (a); and on NMR chemical shift of protons close to polar groups of IL and BA (b). zW and zBA are number of water and BA molecules per one ionic pair of IL (IL loadings), respectively.

Transport properties of the individual components (anion, cation of IL, BA and water) of the studied systems as expressed by their selfdiffusion coefficients were determined by ¹H (*Doneshot*) and ¹³C (*Dbbpsteinept*) detected PFGSE experiments.

The obtained NMR data are discussed in the terms of our recently proposed model of extraction of carboxylic acid with phosphonium ILs [1-2].

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Acknowledgment

This work was supported by the project APVV-15-0494



P10 - COMPARISON OF SIMULTANEOUS AND POSTPONED TREATMENT OF D-GALACTOSE INDUCED NEURODEGENERATION IN RAT MODEL WITH HUPERZINE A: IN VIVO ¹H MRS STUDY

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Rat model of neurodegeneration is generally accepted in research of this group of disorders. However, it is not natural for rats to acquire significant neurodegeneration process resembling Alzheimer disease, which is main aim of our study. To do so, we decided to use long-term subcutaneous application of D-galactose (D-gal) to our experimental animals as it was proved to cause signs of neurodegeneration by several authors [1]. Our study also included huperzine A (hup) application to see its effect on neurodegeneration process and its reflection in ¹H MRS. Hup is a drug from the category of acetylcholine-esterase inhibitors, which are part of common medical therapy of Alzheimer disease [2].

The main goal of this study is to compare two different schemes of application of hup to experimental animals and its reflexion on the neurochemical profile measured by localized ¹H MRS. It utilizes two of our previous studies where dosage of D-gal and hup was the same (D-gal: 300 mg/kg per day; hup: 0,1 mg/kg per day), but the timing was different. In the first experiment we let the rats 30 days to develop some grade of neurodegeneration by injecting D-gal without the application of the remedy (hup). After this period we continued with the application of D-gal for another 30 day, but this time hup was also administrated. Temporal design of the second study was different – D-gal and hup were injected together for the whole duration of the experiment. The main instrument to detect metabolic changes in rat brain was localized ¹H MRS, with VOI set to hippocampi.

In the study where the treatment with hup was postponed statistically significant decrease in relative concentrations of glycerophosphocholine+phosphocholine was observed when comparing D-gal group with D-gal+hup group. Concentrations of these two metabolites were significantly lower also when comparing D-gal+hup group with controls. In paired test of D-gal+hup group decrease in absolute glutamate and N-acetylaspartate (NAA) concentrations was observed. In the study where hup and D-gal were administrated simultaneously statistically significant increase in the relative concentrations of myo-inositol was observed in D-gal treated group (paired test). In this setting paired test of D-gal+hup treated group showed significant increase in relative concentrations of glutamate (glu) and glutamate+glutamine.

The result above show, that changes in metabolic profile of D-gal model of neurodegeneration are not consistent in some parameters, e.g. myo-inositol concentrations were significantly changed in the second study in D-gal group, but not in the first study, though the design of the treatment was the same for D-gal group in both cases. Although this paper doesn't deal with *in vitro* measurements (which we used to verify our MRS results), we should mention that it showed significant changes in myo-inositol concentrations, that we were not able to detect by *in vivo* MRS. Another inconsistent parameter was glu concentration in D-gal+hup group. While in the first study it decreased, in second study there was significant increase in its concentration suggesting different effect of hup on glu metabolism in relation to different temporal application scheme. The first study also shows significant decrease in NAA concentration (marker of neuronal count and function in MRS) in D-gal+hup group. This was not observed in the second study, thus implying a question if hup is able to accelerate neurodegeneration in specific temporal application schemes.

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P11 - REACTION MONITORING: HOW TO DEAL WITH NON-STATIONARY 2D FID?

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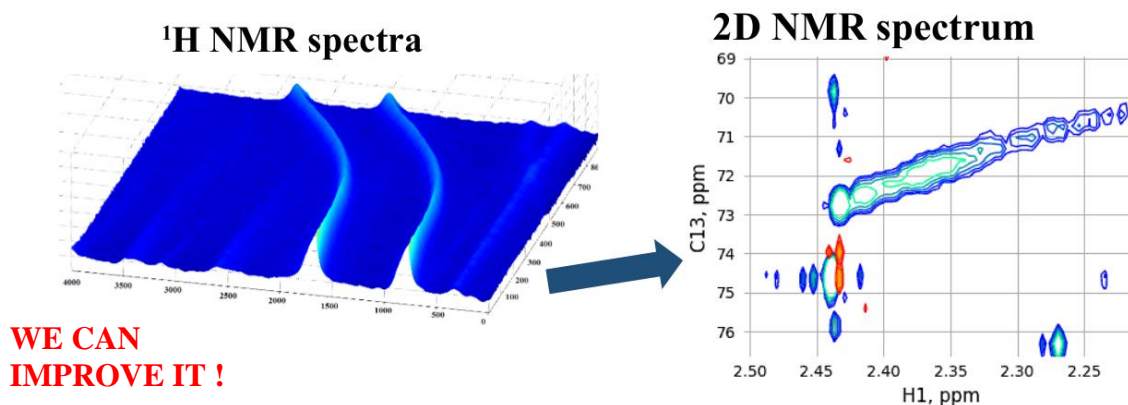
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Monitoring the chemical reaction with NMR is a commonly used method of studying its mechanism. Often intermediate products do not appear in the post-reaction mixture. A particularly interesting and informative is the case when chemical shifts vary in the during the reaction. This change can provide information, e.g. about reaction kinetics or about the influence of conditions, such as change in temperature or pH, on the course and the result of the reaction.

Most often, one-dimensional (1D) spectra are used to monitor the reaction. Unfortunately, despite numerous advantages, such as high sensitivity and the ability to observe even rapidly occurring changes in the reaction mixture, they do not allow to determine the structure of products and intermediate products. 2D spectra are therefore a perfect complement to the analysis of the reaction mixture. However, the sampling of 2D FID can take too long comparing to the rate of studied changes, which can lead to several distortions in a spectrum (see Figure below). Yet, the problem can be alleviated by the application of interleaved acquisition of 1D and 2D spectra and non-uniform sampling.

We show, how a chemical reaction, affecting positions of the peaks in a spectrum, can be followed non-standard measurement methods [1-2]. With the use of TReNDS software [3], we have been able to implement interleaved NUS of 2D and 1D spectra and use the latter to correct the variations affecting the former.

Additionally, we demonstrate how conventionally sampled 2D spectra are affected by changes of peak position and how the effect can be suppressed.



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P12 - INDIRECT DETECTION OF ^{195}Pt NUCLEI IN SOLIDS VIA PROTONS

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Pt element is extensively used for applications, such as catalysis or the treatment of cancer. The NMR spectra of this heavy spin-1/2 isotope by solid-state NMR are broadened by large chemical shift anisotropy (CSA), which can exceed 10^4 ppm. Acquisition of this wide-line spectrum is challenging since (i) the signal intensity is spread over a wide spectral range, decreasing the signal-to-noise ratio, (ii) standard, high power rectangular pulses are insufficient for uniform excitation of these broad pattern and (iii) the width of the spectrum can exceed the probe detection bandwidth. Nevertheless, it has been recently demonstrated that these challenges can be alleviated through the indirect detection of ^{195}Pt nuclei via protons.^[1] As the conventional Cross-Polarization under Magic-Angle Spinning (CPMAS) experiment suffer from too limited bandwidth, dipolar-mediated Heteronuclear Multiple Quantum Coherence (D -HMQC) sequence has been used to indirectly detect ^{195}Pt nuclei via protons. We have analysed the performances and the optimization of these $^1\text{H}\{-^{195}\text{Pt}\}$ D -HMQC experiments using various excitation schemes for ^{195}Pt channel, such as single rectangular pulse and trains of rotor-synchronized rectangular pulses in the form of DANTE schemes. Such analysis has used numerical simulations of the spin dynamics as well as experiments on the chemotherapeutic drug, cis-platin.

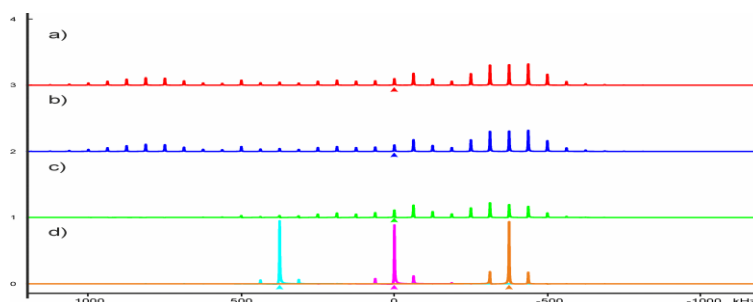


Figure 1. Simulated ^{195}Pt projection of constant-time $^1\text{H}\{-^{195}\text{Pt}\}$ D -HMQC 2D spectra with on-resonance ^{195}Pt irradiation at 0 ppm with $B_0 = 18.8$ T and CSA interaction $\Omega = 1.47$ MHz. (a) Dirac $\pi/2$ -pulses; (b) D_1^4 trains or (c) HPs with $\nu_1 = 200$ kHz and $t = t_{\text{tot}} = 1.25$ μs ; and (d) SLP with $\nu_1 = 42$ kHz and $t = 24$ μs . In (d), the projections observed with SLP excitation at $\pm 6\nu_R$ are also shown.

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P13 - ¹³C NMR STUDY OF MONOSACCHARIDE CONFORMATION

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Determination of monosaccharide conformation in solution based on the analysis of NMR spectra is often complicated by inherent saccharide flexibility and by averaging of NMR observables according to the conformer populations. Conformational analysis of hydroxymethyl rotamers (*gg*, *gt* and *tg*, Fig. 1) is usually performed by inspection of ³*J*(H5,H6R), ³*J*(H5,H6S) vicinal coupling constants followed by their analysis according to their known Karplus-type behavior or their simulation at the quantum-chemistry level. Similarly, heteronuclear vicinal coupling constant ³*J*(H1,CH₃) can be employed in conformational analysis of *g+*, *g-* and *t* (Fig. 1) conformers arising from rotation around glycosidic bond.

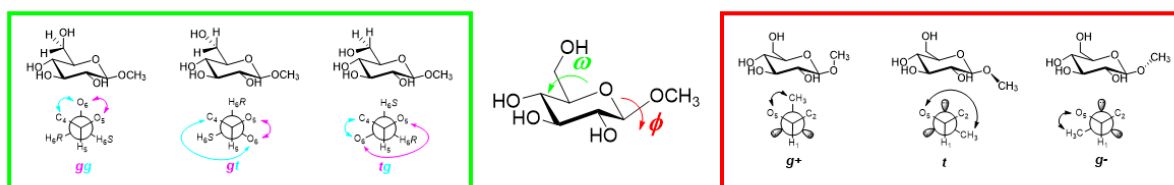


Figure 1: Various conformations of methyl β-D-glucopyranoside originating from rotation of hydroxymethyl group (ω) or rotation around glycosidic bond (φ)

Less attention is devoted to application of ¹³C NMR in conformational analysis of carbohydrates although it was noticed earlier in solid-state ¹³C NMR study¹ and by theoretical calculation² that the chemical shift of carbon C6 is sensitive to changes in torsion angle ω. In this contribution, we will present utilization of ¹³C chemical shifts as an alternative tool in conformational analysis of monosaccharides. In our study, we performed a combination of molecular modeling (molecular dynamics or systematic scanning of torsion angles) for conformer identification followed by DFT calculation of ¹³C chemical shifts of individual conformers. Calculated ¹³C chemical shifts were then correlated with observed values. In the series of simple methyl glycosides of glucose and galactose we will show how sensitive are ¹³C chemical shifts to relatively small changes in molecular geometry and how they can be used in prediction of conformations depicted in Fig.1.

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Acknowledgement

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P14 - INSIGHTS INTO A G-QUADRUPLEX FORMATION IN THE RANKL GENE REGULATORY REGION

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Osteoporosis is a chronic disease that decreases bone density, which is a consequence of unbalanced bone remodelling caused by upregulated expression of the RANKL gene. Precise molecular mechanisms by which the activity of the RANKL gene is regulated are not yet elaborated¹. Recently, a 20 nucleotide long G-rich sequence has been found in the regulatory region of the RANKL gene. G-rich regions can adopt different non-B DNA secondary structures such as G-quadruplexes. Since the formation of G-quadruplexes in gene regulatory sequences can act as transcription regulators, we evaluated the potential of identified G-rich sequence to form G-quadruplex(es).

Wild-type sequence RANwt, d(G₄AG₃AGCG₃AGAG₃), folds into diverse G-quadruplex conformations presumably due to four consecutive guanines in the first G-tract. Simple G1-to-T1 modification (RAN1) results in a single G-quadruplex structure as evidenced by 12 well-resolved signals in the imino region of the 1D ¹H NMR spectrum. Detailed analysis of NMR spectra revealed the formation of a parallel topology with three stacked G-quartets connected with propeller loops (Figure 1). Interestingly, one of the G-quartets involves G16 (bold in the RANwt sequence), which was initially expected to be positioned in a loop since it is not part of G-tract. G16 adopts *syn* glycosidic conformation, which pushes residues G14 and A15 into a bulge that links the middle and one of the outer G-quartets. To the best of our knowledge, a structure with a guanine residue present in a bulge has not yet been reported.

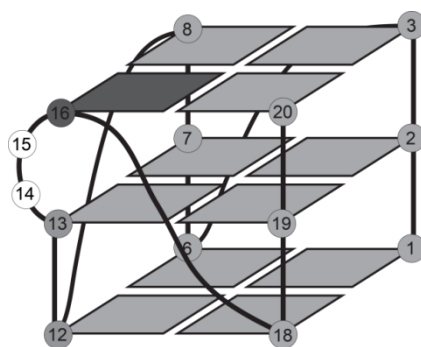


Figure 1: RAN1 topology.

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P15 - STUDY OF SELF-ASSEMBLY OF SOME TRITERPENE DERIVATIVES

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Investigation of supramolecular self-assembly of amphiphilic conjugates of a triterpenoid acid carried out using a combination of spectroscopic (NMR, UV-Vis, IR, ECD and VCD) and microscopic (AFM, TEM and SEM) techniques, some of which applied in our previous investigation as well [1]. Nevertheless, most of the methods deal with the final aggregate, i.e., the substance that aggregates and forms a gel or another form of self-assembled material. Only a few methods (cryo-TEM, AFM, ESEM, ...) can provide direct insights on the sequence of molecular self-assembly, i.e., starting from a system with small particles and leading to an organized superstructure. Recently, we showed that, the stepwise aggregation process can be monitored using NMR spectroscopy methods [2,3]. In this contribution we present the results obtained from variable temperature (VT) pulse field gradient (PFG) diffusion ordered NMR spectroscopy (DOSY) studies on the self-assembly of amphiphilic triterpenoids.

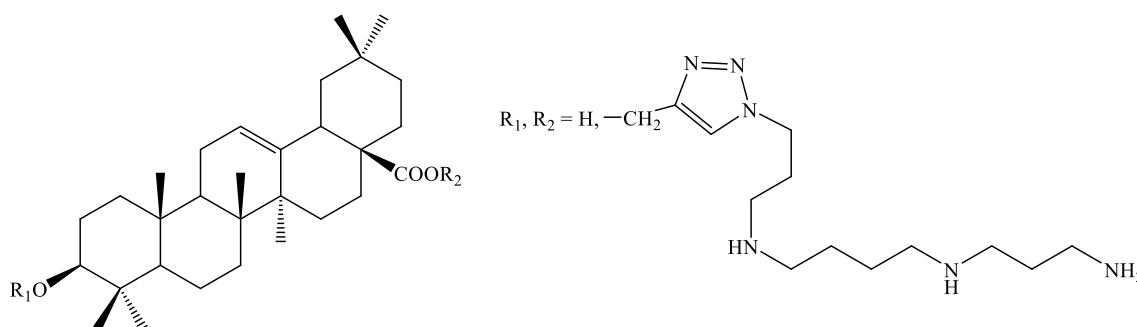


Figure 1: A general formula of oleanolic acid conjugates under study

The results obtained from DOSY NMR experiments were compared with other complementary techniques including cryo-TEM, FT-IR, CD, and VCD combined with molecular modeling.

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P16 - NMR STUDY OF PHASE TRANSITIONS IN ACETIC ACID AND ITS CHLORINATED ANALOGS

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Crystallization is a phenomenon that has not been fully understood yet. It strongly depends on molecular structure, and many intermolecular interactions of different kind and strength are involved in this process. Acetic acid and its chlorinated derivatives are simple molecules, that can serve as model compounds for a broad spectrum of carboxylic acids. We measured temperature dependence of chemical shifts during crystallization and melting. In order to assess solvation effects, NMR spectra of gaseous acetic acid were acquired and we observed signals of gaseous form and the condensate simultaneously.

Liquid structure of acetic acid is still debated up to this day as there are several papers focused both theoretically and experimentally with inconsistent conclusions. There are works [1,2] supporting the hypothesis of cyclic dimers as in gas phase (Fig. 1), on the other hand several papers [3,4] confirmed a presence of linear chains analogous to those found in crystalline phase (Fig. 2). Structure in liquid state was also tackled with a help of DFT calculations. It seems there is no single dominant structure eg. monomer or dimer, but rather a mixture of both structures or a mixture of other oligomeric forms.

A care should be taken when interpreting signals in the spectra. Especially magic angle spinning measurement is prone to various artifacts arising from temperature inhomogeneity within the sample volume. The results can also be easily misinterpreted when even a small amount of water is present in the sample.

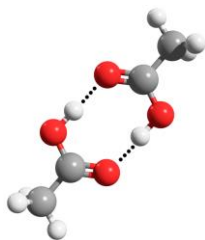


Figure 1: Cyclic dimer of acetic acid present in gas phase.

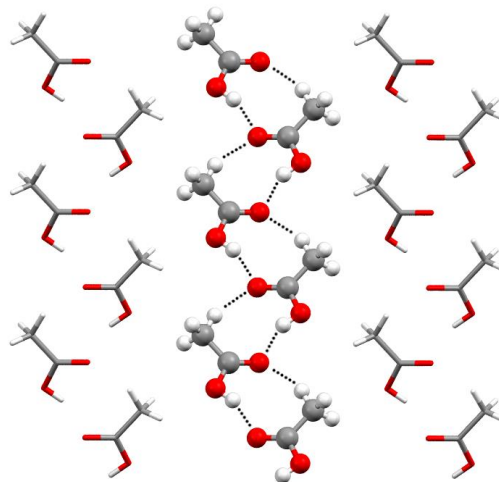


Figure 2: Crystal structure of acetic acid. Linear chain motif is emphasized

Acknowledgement

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P17 - DETERMINATION OF COMPOSITION AND ANOMERIC RATIO IN MIXTURES OF FLUORINATED OLIGOSACCHARIDES

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Deoxofluorinated carbohydrates result from replacement of one or more hydroxyl groups in carbohydrates by fluorine. These molecules play a prominent role among carbohydrate mimics due to change of electronical properties coupled with minimal steric impact caused by deoxofluorination [1].

Deoxofluorinated oligosaccharides can be prepared by reaction of selectively fluorinated glycosyl donors with appropriate glycosyl acceptors. Glycosylation frequently gives a mixture of both anomers of the target oligosaccharide. Knowledge of anomeric diastereoselectivity in glycosylation is crucial for development of stereoselective glycosylation procedures.

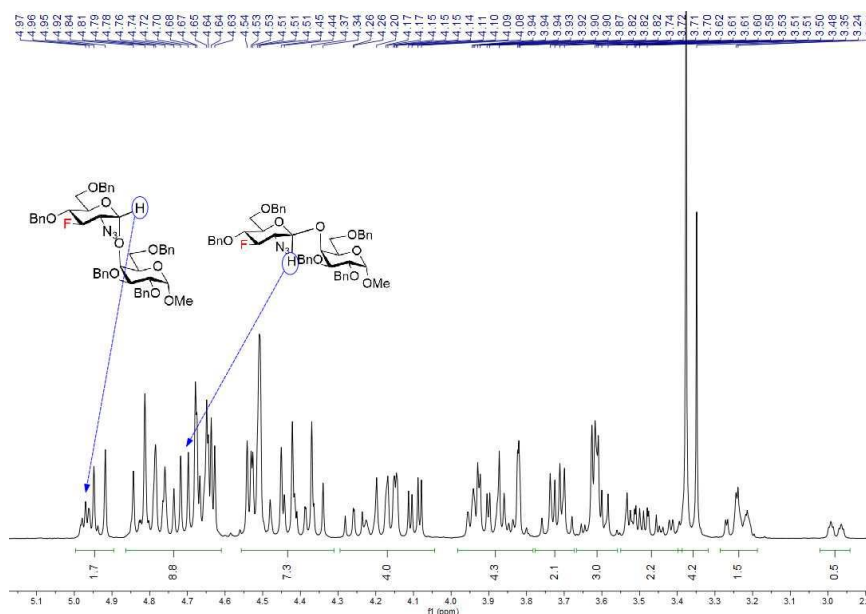


Figure 1: ^1H NMR spectrum (5.1-2.9 ppm) of methyl 4-O-(2-azido-4,6-di-O-benzyl-2,3-dideoxy-3-fluoro- $\alpha\beta$ -D-glucopyranosyl)-2,3,6-tri-O-benzyl- α -D-galactopyranoside

Plenthora of glycosylation reactions was performed and sophisticated NMR approach for characterization of the coressponding products was aplied. 1D ^{19}F and ^1H NMR were utilized for accurate derermination of anomeric ratios and 2D NMR techniques as HSQC, HMBC and COSY were used to assign the signals of individual anomers. In some cases, spectrum simulation was necessary to obtain the desired parameters.

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P18 - INTERMOLECULAR INTERACTIONS STUDIED BY NMR SPECTROSCOPY

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Interactions between biomolecules and drugs are mostly controlled by weak intermolecular forces, particularly hydrogen bonds. The induced fit model postulates that exposure of a biomolecule to a substrate causes the biomolecule or substrate to change its shape or structure in order to allow mutual binding. The structural adaptation involves mostly conformational changes or a change of the tautomeric form. The conformational changes may open the binding pocket or expose the biomolecular binding pattern to the substrate. The tautomeric change, on the other hand, modifies the binding pattern (hydrogen bond acceptor to hydrogen bond donor and vice versa).¹

We studied structural adaptations of modified nucleic acid bases induced by intermolecular interactions. Two rotamers depending on the orientation of methylamino group are present in substituted pyrimidine **1**. Separated signals for each rotamer can be observed in ¹H NMR spectra. The rotamer ratio can be influenced by interactions with compounds able to form hydrogen bonds with one rotamer of **1** only. Compounds with the acceptor-donor-acceptor (ADA) structure can form three hydrogen bonds with rotamer **1B**. I.e., addition of substituted thymine (**2**) changes the ratio of rotamers in favour of rotamer B (dimer **1B-2**). NMR experiments with variable temperature and concentration of interacting partners may be used for the determination of free energy of complex formation. Similarly, intermolecular interactions change the tautomer equilibria of modified nucleobases significantly.

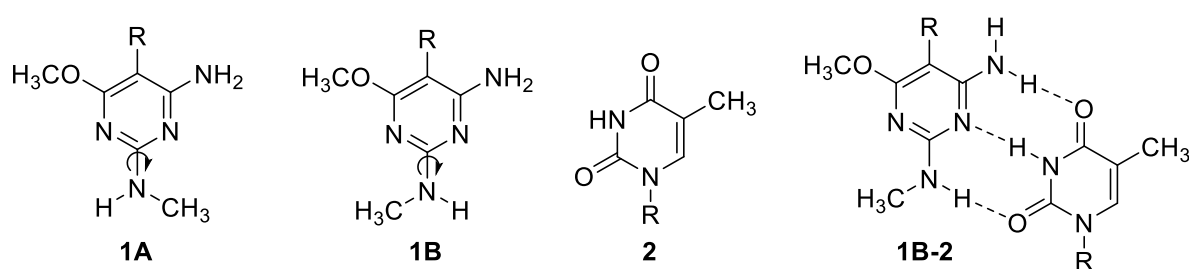


Fig. 1 The two rotamers **1A** and **1B**, the binding partner **2** and formed complex **1B-2**

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P19 - STRUCTURAL INVESTIGATIONS OF SOME COMPLEXES OF PCl_5 AND SbCl_5 WITH NITROGEN-CONTAINING BASES

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Group 15 atoms, often called pnictogens (Pn), appear in a wide range of oxidation states from –III to +V. Like halogen (X) atoms or other *p*-block elements, Pn atoms exhibit an interesting ability to interact simultaneously with electrophiles and nucleophiles. The current explanation is based on the concept of the σ -hole, developed by Politzer and Murray [1], where an interpretation of interactions of the elements in the highest oxidation states with a lack of lone electron pairs is also possible. The σ -hole and halogen bonding phenomena have found applications in crystal engineering, preparation of porous structures or synthesis of species with presumed biochemical relevances.

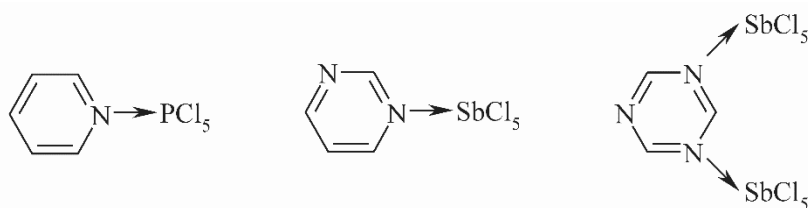


Figure 1: Examples of complexes studied

We have prepared a set of PnCl_5 (Pn = P or Sb) complexes with various nitrogen-containing bases (e.g. substituted pyridines; pyrazine, pyrimidine, *s*-triazine; Figure 1) due to the presumed presence of halogen bonding [2]. Both NMR and sc-XRD characterization of these compounds will be discussed in detail within the poster presentation. Some of these compounds have been already studied from a theoretical point of view by us in order to bring to light the true nature of observed halogen bonds [3].

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P20 - DETERMINATION OF THE RELATIVE CONFIGURATION OF LILY ALKALOIDS

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Thirty years ago Eisenreichová *et al.* isolated and characterized several pyrroline-pyrrolidine and pyrroline dimer alkaloids from Madonna lily (*Lilium candidum* L.) [1–3]. For three of these alkaloids (**1–3**, Fig. 1.) only the constitution is known, their stereochemistry has not been elucidated by anybody. Thus we endeavored to solve the relative configurations of **1–3** by NMR.

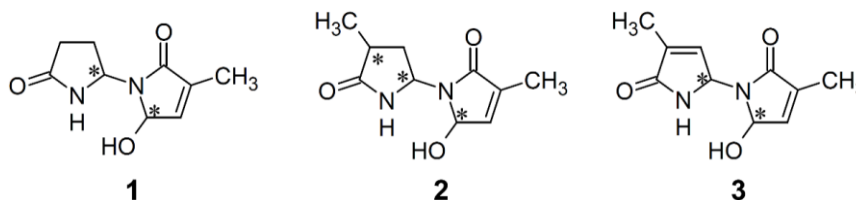


Figure 1: Selected lily alkaloids of which we determined the relative configuration

To determine the relative configuration of the compounds, we first prepared racemic lily alkaloids **1–3** as isomeric mixtures in multi-step syntheses starting from pyrrole. The stereochemical investigation of them was rather challenging because of the rotatable C-N single bond between the stereogenic centers. Consequently, reliable determination of the stereochemistry could not be performed without prior knowledge about the conformational behavior of these molecules.

To tackle the problem, a concerted use of distance measurements by selective 1D ¹H-¹H NOESY and conformational analyses at the B3LYP-D3/6-31+G** level of theory was necessary for all diastereomers of **1–3**. After elucidating all synthetic isomers, we were able to deduce the relative configurations of the natural products by comparing our proton NMR chemical shift data to those in the literature.

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P21 - METAL-FREE AEROBIC OXIDATION OF UNACTIVATED BENZYLIC SUBSTRATES. FLAVIN PHOTOCATALYSIS STUDIED BY ELECTROCHEMISTRY, NMR, EPR, MASS SPECTROMETRY AND DFT

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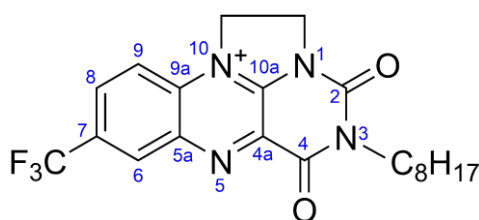
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Direct oxidation reaction of C-H belongs to the recent challenges in organic chemistry. There are still limited number of mild oxidative procedures that do not need stoichiometric or at least a catalytic amount of toxic metals. Oxidations with molecular oxygen, which are not involved in the latter, can be successfully driven by mimicking the action of flavoenzymes with systems based on flavinium salts[1]. A novel approach in order to activate the flavin, on the basis of “quaternization” and visible-light irradiation, will be presented. We used a photo-excited 1,10-ethyldien-3-octyl-7-trifluoromethylalloxazinium (see Scheme 1) to oxidize the benzylic compounds with high oxidation potentials ($> +2.5$ V vs SCE), which would be otherwise a very hard task.



Scheme 1: Flavinium structure used as a photocatalist for oxidation of the unactivated benzylic substrates.

Spectroscopic techniques like magnetic resonancies (NMR, EPR) as well as mass spectrometry, together with the help of density functional theory (DFT) computations, enabled us to identify products as well as intermediates of the complex pathways (involving flavin radicals)[2] and thus to propose the oxidation mechanism, which will be presented.

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P22 - RDCS - POWERFUL TOOL FOR ENANTIODISCRIMINATION AND SPATIAL STRUCTURE ANALYSIS OF INHERENTLY CHIRAL CALIX[4]ARENES DERIVATIVES

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The aim of this work is the application of residual dipolar couplings (RDCs) in the investigation of enantiodiscrimination and in the study of the conformational behavior of new inherently chiral calix[4]arene derivatives. Using liquid crystal phase for the measurement of RDCs to obtain structural information has become more popular recently^{1,2}. Moreover, the homopolypeptide-based alignment media can induce different orientations of enantiomers as the result of their helical structures, and thus to generate a diastereomorphous interaction leading to different orientations and RDCs of individual enantiomers³.

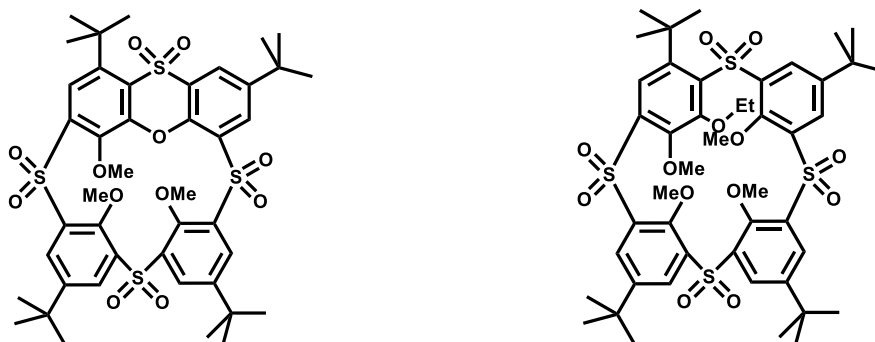


Figure 1: The studied calix[4]arene derivatives

We are going to present the use of polyglutamate alignment media PBLG and PBPMG⁴ to determine unambiguously which of the possible spatial structures (*cone*, *partial cone*, *1,2-* or *1,3-alternate*) the studied compounds adopt (Figure 1). Furthermore, the enantiodiscrimination capability of the two media will be examined on the systems with the inherent chirality for the first time.

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P23 - IN VIVO MRI DIFFUSION TENSOR IMAGING IN METABOLIC MODEL OF NEURODEGENERATION OF THE RAT BRAIN

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Fractional anisotropy and mean diffusivity - parameters computed from diffusion tensor imaging (DTI) allow us to quantify microstructural changes in early stages of neurodegeneration in rat brain. Our goal is to develop fast and reliable examination protocol for *in vivo* DTI measurement of rat brain. This measurement is used to compute tractography, detect changes in brain microstructure and correlate quantitative data with volumetric, spectroscopic and behavioral tests in metabolic model of neurodegeneration. Visualisation and assesment of white matter tracts using tractography shows potential in early detection of neurodegeneration in human [1].

MRI measurements were performed at 4,7 T 400 mT/m horizontal bore magnet with small animal holding system on adult Wistar rats using dual channel surface coil. Multislice coronal fast spin echo brain sequences were measured with 10 or more non-colinear diffusion gradients, b values were optimised for evaluation of white matter and perivascular spaces. Quantification of DTI data was performed using DSIstudio software [2]. The animal model of neurodegeneration consists of control group of normal rats daily injected with saline and experimental groups daily injected with D-galactose or D-galactose applied with neuroprotective drug [3]. Thanks to optimization of scanning protocol and even distribution of diffusion gradients through space [4], current experimental setting showed better signal to noise ratio and lower variance in diffusion parameters compared to previous versions. High sensitivity of measurement is needed to detect subtle changes in early stages of neurodegeneration. Measurements are also used as a standard for future experiments. Main limitation of *in vivo* DTI measurements is scanning time, future development of high-angular resolution diffusion imaging atlas will require more diffusion orientations to be examined, extending measurement time. Echo planar imaging offers one solution in cost of limited spatial resolution and severe scanning artifacts.

Although significant changes in experimental groups have been shown by *in vivo* spectroscopy and volumetry, quantification of *in vivo* DTI limited by measurement time had not shown significant results yet. Relatively small volume of rat brain white matter, high resolution of scan needed and time limitation of *in vivo* measurements are obstacles to overcome.

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P24 - IN VIVO DIFFUSION TENSOR IMAGING OF THE BRAINS OF STRESSED RATS

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AIM: Stress is the most important triggering factor for the development of various psychiatric disorders, but the underlying neurobiological events are not completely understood. Stress exposure can affect neuroplasticity and structural integrity of limbic brain areas. Here, we used diffusion tensor imaging (DTI) to study the temporal dynamics of stress induced structural changes in the brains of laboratory rats.

METHOD: Young adult male Sprague-Dawley rats (control group: 16 animals, stress group: 16 animals) were subjected to restrain stress (6 hours/day) for 21 days. DTI and T2-weighted images were acquired with a 4.7T Bruker PharmaScan pre-clinical MR scanner. Baseline measurements were performed before stress and the protocol was repeated three times: one week (acute stress), three weeks (chronic stress) after stress initiation and two weeks after the end of the stress (recovery). A pre- and post-processing pipeline was built up by using FMRIB Software Library for both DTI and T2-weighted measurements.

RESULTS: Diffusion data were corrected for eddy currents and subject movements by the detection and the replacement of positive and negative outliers and then fractional anisotropy (FA), mean diffusivity (MD), eigenvalues ($L_{1,2,3}$) and eigenvectors ($V_{1,2,3}$) were calculated. After manual corrections, different brain areas were used for diffusivity and volumetric analysis.

CONCLUSION: We have developed an image processing pipeline for volumetric and diffusion analysis in order to identify rat brain areas affected by stress.

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