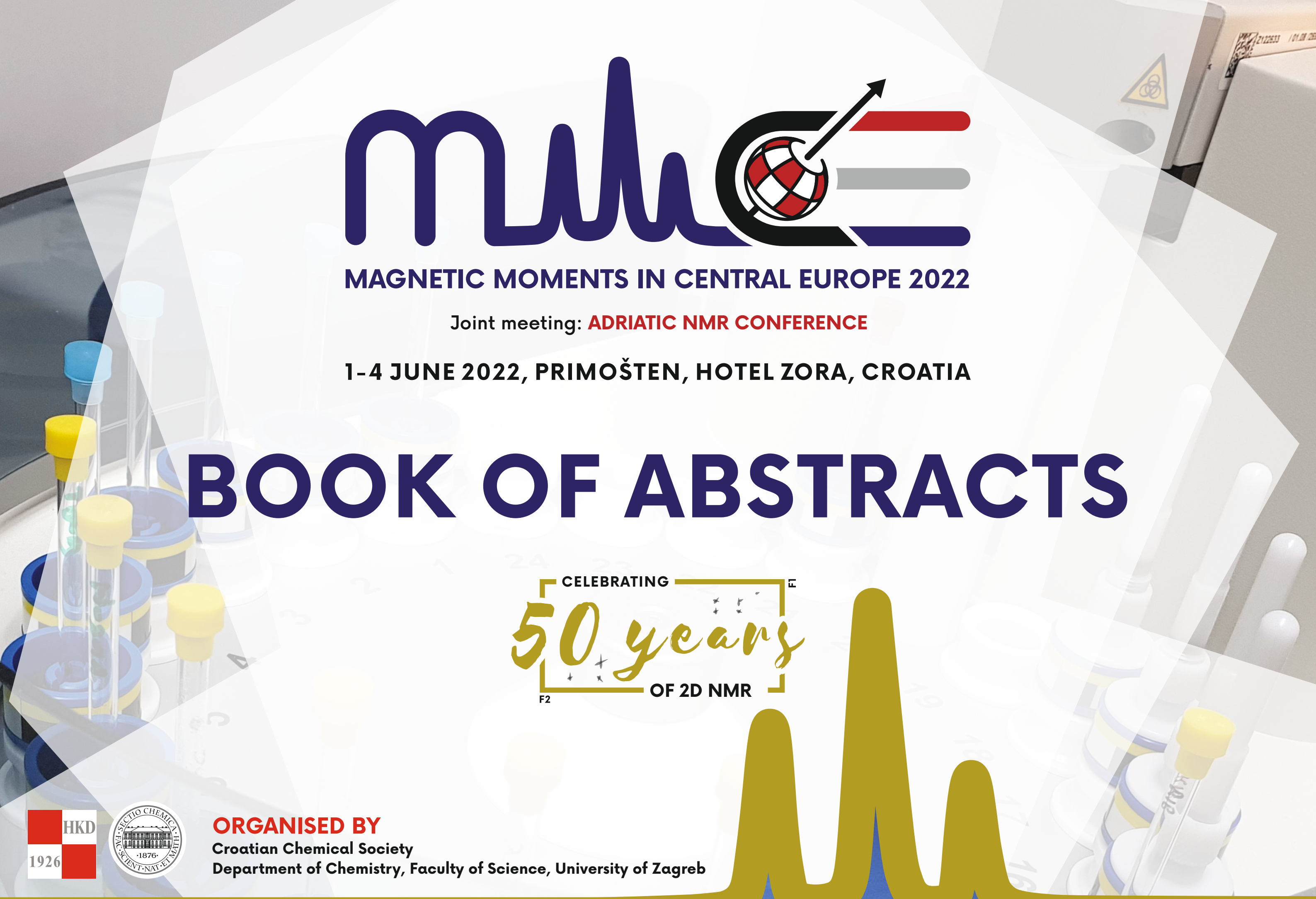

# MAGNETIC MOMENTS IN CENTRAL EUROPE 2022

Joint meeting: ADRIATIC NMR CONFERENCE

1-4 JUNE 2022, PRIMOŠTEN, HOTEL ZORA, CROATIA

# BOOK OF ABSTRACTS

CELEBRATING
50 years
OF 2D NMR

**ORGANISED BY**
Croatian Chemical Society
Department of Chemistry, Faculty of Science, University of Zagreb

Hotel Zora
PRIMOŠTEN

TOGETHER WE ARE CREATING
*Magnetic Moments*
IN PRIMOŠTEN

We are happy to welcome you on **MMCE 2022** to celebrate **50 years of 2D NMR**

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**Department of Chemistry, Faculty of Science, University of Zagreb**

HKD
1926

·1876·

We are happy to welcome you on **MMCE 2022** to celebrate **50 years of 2D NMR**
**mmce2022.hkd.hr @mmce2022 #mmce2022**

Dear Participants,

Magnetic Moments in Central Europe 2022 is taking place from 1 to 4 June 2022. The Conference is organized jointly with the Adriatic NMR Conference to celebrate **50 years of 2D NMR** proposed in Baško Polje, and to share *magnetic moments* in the charming city of Primošten, on the unique Adriatic coast of Croatia. The conference will be held at the Congress centre of the hotel Zora, in Primošten.

MMCE was conceived in 2007 with the vision of providing a unique knowledge-sharing event for NMR scientists and students in Central Europe and beyond. The Conference's aims have been to address all types of theoretical and applied NMR spectroscopy - from the fundamental theory and small-molecule structure determination in solution and solid state, new pulse sequences, software and hardware development, mixture analysis, metabolomics, benchtop NMR etc., to biological systems. The conference also offers Tutorial talks which are expected to provide a distilled wisdom on topics that can be captivating and instructive to both students and senior NMR scientists.

We strongly believe that the MMCE and Adriatic NMR will foster the exchange of knowledge and experience among students and scientists and will serve as a forum for extensive networking opportunity. With confidence that it will be a memorable event, we are delighted to welcome you to Primošten.

*Organizing Committee*

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MAGNETIC MOMENTS IN CENTRAL EUROPE 2022

# PROGRAMME

## TUESDAY, MAY 31

| | |
|---|---|
| 16:00 – 17:00 | **REGISTRATION** |
| 17:00 – 19:00 | **BRUKER WORKSHOP:** *From Benchtop to High Field NMR* |
| 19:00 – 19:30 | **PRE-CONFERENCE LECTURE – Frances Separovic:** *NMR Structural Studies of Antimicrobial Peptides in Model Membranes and Live Bacteria* |

## WEDNESDAY, JUNE 1

| | |
|---|---|
| 8:00 – 9:00 | **REGISTRATION** |
| 9:00 – 9:15 | **OPENING** |
| **CHAIR: Predrag Novak** | |
| 9:15 – 10:00 | **HISTORICAL LECTURE – In memory of Ray Freeman (1932–2022)**<br>**Geoffrey Bodenhausen:** *Early Days of Two-dimensional Fourier Transform NMR* |
| 10:00 – 10:45 | **HISTORICAL LECTURE**<br>**Norbert Müller:** *Some Memories About NMR at ETH Zurich in the mid-1980s* |
| **Section: SMALL MOLECULES (I)** | |
| **CHAIR: Nikola Bregović** | |
| 10:45 – 11:15 | **Christina Thiele:** *Recent Advances in Polypeptidic Thermo-Responsive Alignment Media for Organic Compounds* |
| 11:15 – 11:30 | **COFFEE BREAK** |
| 11:30 – 12:00 | **Jelena Parlov Vuković:** *Challenges of NMR Spectroscopy in Crude Oil and Alternative Fuels Analysis* |
| 12:00 – 12:30 | **Andrea Usenik:** $H_2O$ *vs.* $D_2O$*: Thermodynamics Beyond NMR* |
| 12:30 – 12:45 | **Eliška Procházková:** $^{31}$*P NMR Spectroscopy in Stereochemical Analysis* |
| 12:45 – 13:00 | **Petra Cuřínová:** *Structurally Forced Ion Binding Affinity in a Urea-based Macrocycle* |
| 13:00 – 14:30 | **LUNCH BREAK** |

### Section: BIOMOLECULES (I)

**CHAIR: Wiktor Kozminski**

| | |
|---|---|
| **14:30 – 15:00** | **Paul Schanda:** *Dynamic Protein Machineries Studied by Solution- and Solid-state NMR* zoom |
| **15:00 – 15:30** | **Peter Podbevšek:** *How Oxidative Lesions Affect DNA Secondary Structure* |
| **15:30 – 16:00** | **Gyula Batta:** *Dynamics in ‘Rigid’ Disulfide Proteins* |
| **16:00 – 16:15** | **COFFEE BREAK** |

### Section: SOLID STATE NMR

**CHAIR: Alexej Jerschow**

| | |
|---|---|
| **16:15 – 16:45** | **Guido Pintacuda:** *Fast Biomolecular NMR with Fast MAS (Without and With DNP)* |
| **16:45 – 17:15** | **Martin Dračínský:** *NMR Crystallography of Molecular Solids* |
| **17:15 – 17:45** | **Marek Potrzebowski:** *The World of Peptides Viewed Through the Solid State NMR Spectroscopy Window* |
| **17:45 – 18:15** | **Pinelopi Moutzouri:** *Pure Isotropic Proton Solid State NMR* |
| **18:15 – 18:30** | **Marta Dudek:** *Determining Molecular Conformation in Organic Crystals Using Combined Solid State NMR – Crystal Structure Prediction (CSP) Approach* |
| 19:00 | **WELCOME MIXER** |

## THURSDAY, JUNE 2

### Section: METHODS AND APPLICATIONS

**CHAIR: Norbert Müller**

| | |
|---|---|
| **9:00 – 9:45** | **TUTORIAL – Gareth Morris:** *Pure Shift NMR Methods* zoom |
| **9:45 – 10:15** | **Horst Schmidt-Böcking:** *Otto Stern – the Prime Father of the NMR-Method* zoom |
| **10:15 – 10:45** | **Craig Butts:** *Adventures in (Machine) Learning to Determine 3D Structure with NMR Spectroscopy* |
| **10:45 – 11:15** | **Alexej Jerschow:** *Nuclear Spin Singlet State Relaxation Mechanisms from Experiment and Molecular Dynamics Simulations* |
| **11:15 – 11:30** | **COFFEE BREAK** |

**CHAIR: Jan Sykora**

| | |
|---|---|
| **11:30 – 12:00** | **Dušan Uhrín:** *SHARPER NMR: Our Journey to Remarkable Sensitivity Gains* |
| **12:00 –12.30** | **Krzysztof Kazimierczuk:** *Non-stationary NMR spectroscopy* |
| **12:30 – 12:45** | **Ewa K. Nawrocka:** *Temperature Coefficients as a Tool for Spectral Assignment* |
| **12:45 – 13:00** | **Ján Tarábek:** *Stability of Triarylamine Radical Cations Studied by In-situ Voltammetry with Electron Paramagnetic Resonance (EPR)* |
| **13:00 – 14:30** | **LUNCH BREAK** |

| | |
|---|---|
| **CHAIR: Vilko Smrečki** | |
| **14:30 – 15:15** | **TUTORIAL – Klaus Zangger:** *Selective Excitation* |
| **15:15 – 15:45** | **István Timári:** *NO Relaxation Delay (NORD) Spectroscopy: Rapid Sequential Acquisition of NMR Experiments* |
| **15:45 – 16:00** | **Javier Agustín Romero:** *Supervised Classification Methods in NMR for Resonance Mapping of IDPs* |
| **16:00 – 16:15** | **COFFEE BREAK** |
| **Section: NMR METABOLOMICS** | |
| **CHAIR: Andres Moreno** | |
| **16:15 – 16:45** | **Calin Deleanu:** *Some Tips and Tricks for NMR Metabolomics and Lipidomics* |
| **16:45 – 17:15** | **Svetlana Simova:** *Sugars Containing Food – NMR Applications* |
| **17:15 – 17:30** | **James Eills:** *Metabolic NMR Without the Magnet* |
| **17:30 – 17:45** | **Lenka Michálková:** *NMR-Based Metabolomic Analysis of Blood Plasma as a Pancreatic Cancer Diagnostic Tool* |
| **17:45 – 18:00** | **Štěpán Hornik:** *NMR Aerosolomics: an Overview* |

## FRIDAY, JUNE 3

| | |
|---|---|
| **Section: BIOMOLECULES (II)** | |
| **CHAIR: Gyula Batta** | |
| **9:00 – 9:45** | **Lucia Banci:** *In-cell NMR for the Understanding of Physiological Process at Molecular Level* zoom |
| **9:45 – 10:15** | **Harald Schwalbe:** *NMR Contributions Against SARS-CoV-2: Studying the Genome and Proteome of SARS-CoV-2* |
| **10:15 – 10:45** | **Markus Schade:** *NMR-aided Design of Clinical Stage Protein-Protein-Interaction Inhibitors: The Mcl-1 Success Story* |
| **10:45 – 11:00** | **Witold Andrałojć:** *Lanthanide Binding Aptamer as a Tool for Paramagnetic NMR Spectroscopy of Nucleic Acids* |
| **11:00 – 12:15** | **COME TO SEE MY POSTER! + COFFEE BREAK + *LEDO* SURPRISE** |
| **Section: SMALL MOLECULES (II)** | |
| **CHAIR: Ana Čikoš** | |
| **12:15 – 12:30** | **Thomas Schwarz:** *Novel High-Resolution Structural Models of Membrane Bound α-Synuclein* |
| **12:30 – 12:45** | **Radek Pohl:** *$^{15}$N SPY in Conformational Analysis of Disaccharides* |
| **12:45 – 13:00** | **Zuzana Osifová:** *Diketo-ketoenol Tautomers in Curcuminoids* |
| **13:00 – 14:30** | **LUNCH BREAK** |
| **16:00** | **SOCIAL ACTIVITIES – GUIDED TOUR AND WINE TASTING** |
| **20:00** | **CONFERENCE DINNER** |

# SATURDAY, JUNE 4

| | |
|---|---|
| **Section: PHARMACEUTICALS** | |
| **CHAIR: Goran Landek** | |
| **9:00 – 9:30** | **Silvia Davalli:** *NMR in Drug Development: Role, Challenges and Process Chemistry Sustainability* |
| **9:30 – 10:00** | **Ana Čikoš:** *Macrocyclic Disruptors: The NMR Side of the Story* |
| **10:00 – 10:30** | **Marc-Antoine Sani:** *NMR Studies of an Antimicrobial Peptide: from In Vitro to In Situ* |
| **10:30 – 11:00** | **Julien Orts:** *Advanced NMR Approaches for Drug Discovery* zoom |
| **11:00 – 11:15** | **Áron Szigetvári:** *On the Utility of Nonuniformly Sampled Two-dimensional NMR Spectra in the Pharmaceutical Industry* |
| **11:15 – 11:30** | **COFFEE BREAK** |
| **Section: APPLICATIONS — SMALL MOLECULES (III)** | |
| **CHAIR: Tomislav Jednačak** | |
| **11:30 – 12:00** | **Andrés Moreno:** *Multinuclear Magnetic Resonance in Food Composition and Quantitative Analysis* |
| **12:00 – 12:30** | **Vito Gallo:** *Can NMR spectroscopy Pave the Way for Community-built Analytical Systems?* |
| **12:30 – 12:45** | **Aleš Růžička:** *Power of $^{11}$B NMR Spectroscopy in Studies of Heteroboranes* |
| **12:45 – 13:00** | **Markéta Tichotová:** *$^{31}$P NMR with In Situ Irradiation for Study of Self-immolation* |
| **13:00** | **CLOSING** |

**LEGEND:**

| | | | |
|---|---|---|---|
| **PLENARY LECTURES** | **INVITED LECTURES** | **SECTION LECTURES** | **TUTORIAL** |

# POSTER SESSION

| | |
|---|---|
| **P01** | **Dimitris Argyropoulos, Mikhail Elyashberg**<br>Evaluation of the Benefit and Informing Capability of 2D NMR Experiments for Computer-Assisted Structure Elucidation |
| **P02** | **Tea Babić, Andrea Usenik, Katarina Pičuljan, Nikola Bedeković, Josip Požar**<br>Understanding the Attraction Between Hydrophobic Cavities and Benzene Derivatives |
| **P03** | **Nicholle G. A. Bell, Alan R. Smith, Dušan Uhrín, Richard York**<br>$^{19}$F-Centered NMR Spectroscopy for the Analysis of Complex Mixtures |
| **P04** | **Aurimas Bieliauskas, Viktorija Dargytė, Eglė Arbačiauskienė, Algirdas Šačkus**<br>NMR-Based Structure Elucidation of Novel Polycyclic Heterocyclic Compounds Obtained via MCRs from 1*H*-pyrazol-4-yl Carbaldehydes |
| **P05** | **Mirko Cevec, Janez Plavec**<br>NMR, CD and UV Studies of *NRAS* RNA G-Quadruplexes |
| **P06** | **Ema Chaloupecká, Martin Kurfiřt, Lucie Červenková Šťastná, Jindřich Karban, Martin Dračínský**<br>Long-Range C–F Couplings in Conformational Analysis of Disaccharides |
| **P07** | **Ana Čikoš, Dijana Jureša, Leo Štefan**<br>The Use of $^{1}$H-qNMR for Determination of Benzoyl Peroxide in Gel Suspension – a Pilot Study |
| **P08** | **Amalija Ernečić, Sunčica Roca, Nikola Bregović, Viktoriia Lazarenko, Marina Šekutor, Marija Alešković**<br>Adamantane Ammonium Salts as Guests for the Cyclodextrin Host: Something Old, Something New |
| **P09** | **Luka Fotović, Marin Liović, Nikola Bedeković, Katarina Pičuljan, Vladimir Stilinović**<br>*Anti-Electrostatic* or Not – A SS-NMR Determination of Proton Position in a Halogen and Hydrogen Bonded Tetrameric Supramolecule |
| **P10** | **Vito Gallo, Piero Mastrorilli, Mario Latronico, Biagia Musio, Giuseppe D'Orazio, Rosa Ragone**<br>NMR Studies on the Tacticity of Functional Polymers |
| **P11** | **Dessislava Gerginova, Svetlana Simova**<br>Chemical Profile of Mead - The Oldest Alcoholic Beverage |
| **P12** | **Diego J. González-Serrano, Covadonga Lucas-Torres, Milad Hadidi, José Carlos Orellana, Beatriz Cabañas, Andrés Moreno**<br>NMR Study of Glycosyl $^{15}$N-Anilines |
| **P13** | **Nina Habanová, Radek Pohl**<br>Rapid Quantitative Determination by $^{13}$C-NMR of the Composition of Acetylglicerol Mixtures as Byproduct in Biodiesel Synthesis |
| **P14** | **Milad Hadidi, Covadonga Lucas-Torres, Eva M Sánchez-Palomo, Miguel A. González, Diego J. González-Serrano, José C. Orellana, Andrés Moreno**<br>Nuclear Magnetic Resonance Applications to Compositional Analysis of Spanish Black Garlic |
| **P15** | **Anna Hruzíková, Lucie Mužíková Čechová, Sille Štěpánová, Markéta Tichotová, Aleš Růžička, Václav Kašička, Eliška Procházková**<br>Azopyrimidine Photoswitches and Their Interactions With Beta-Cyclodextrin |
| **P16** | **Tomislav Jednačak, Ivana Mikulandra, Monika Kapustić, Iva Kušec, Kristina Smokrović, Ema Hošnjak, Ivo Piantanida, Marta Košćak, Klaus Zangger, Predrag Novak**<br>Interactions of Macrozones With Their Biological Receptors |
| **P17** | **Nikolina Kalčec, Antonio Ljulj, Lucija Božičević, Barbara Pem, Valerije Vrček, Frances Separovic, Ivana Vinković Vrček**<br>Combined NMR and Computational Study of L-DOPA and Dopamine Oxidation Upon Interaction With Gold Nanosurface |
| **P18** | **Eszter Kalydi, Milo Malanga, Szabolcs Béni**<br>Complete Resolution of Protonation Equilibria of Totalsymmetric Polycarboxylic Cyclodextrin Derivatives Using $^{1}$H NMR-pH Titration and Advanced Evaluation |
| **P19** | **Martina Lenarčič Živkovič, Martin Gajarský, Petr Stadlbauer, Jiři Šponer, Lukáš Trantírek, Janez Plavec**<br>Insight into Formation Propensity of Pseudocircular DNA G-Hairpins |
| **P20** | **Damjan Makuc, Fazilet Gürer, Tamilselvan Mohan, Karin Stana Kleinschek, Janez Plavec**<br>NMR Characterization of Cellulose Nanocomposite Bioscaffolds for Regenerative Medicine |

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|---|---|
| **P21** | **Valentina Petrelli, Giorgio Rizzo, Gianluca Maria Farinola, Teresa Sibillano, Cinzia Giannini, Vito Gallo, Piero Mastrorilli**<br>$^{1}$H-$^{13}$C CP/MAS NMR Analysis of Silk Fibroin Regenerated Using Lanthanide Salts |
| **P22** | **Matija Modrušan, Lucija Otmačić, Lucija Glazer, Nikolina Vidović, Nikola Cindro, Giovanna Speranza, Gordan Horvat, Vladislav Tomišić**<br>Anion Binding by Linear Penta–Homopeptides Comprised of Amino Acids with Nonpolar Side Chains |
| **P23** | **José Carlos Orellana, Covadonga Lucas-Torres, Diego J. González-Serrano, Milad Hadidi, Beatriz Cabañas, Andrés Moreno**<br>Quantitative Determination of 1,2- and 1,3-Diglycerides and Other Minor Constituents by 31P-Nuclear Magnetic Resonance Analysis. A Rapid Comparative Study of Olive Oil Degradation by Conventional and Microwave Heating. |
| **P24** | **Piotr Paluch, Marta K. Dudek**<br>Solid-State NMR Reveals Crystal Structure of Elusive Polymorphs of Meloxicam With the Help of Crystal Structure Prediction Calculations |
| **P25** | **Piotr Paluch, Rafal Augustyniak, Mai-Liis Org, Kalju Vanatalu, Ats Kaldma, Ago Samoson, Jan Stanek**<br>NMR Assignment of Methyl Groups in Immobilized Proteins Using Multiple-Bond $^{13}$C Homonuclear Transfers, Proton Detection, and Very Fast MAS |
| **P26** | **Barbara Panić, Miro Sambolec, Ivana Biljan**<br>NMR Insights into the Structural Features of Triazine-Based Porous Organic Polymers |
| **P27** | **Helena Pelantová, Blanka Šedivá, Thi Tra My Nguyen, Miroslava Kacířová, Lenka Maletínská, Marek Kuzma**<br>Mouse Model of Tau Pathology Thy-Tau22: NMR-Based Metabolomic Characterization |
| **P28** | **Lenka Poštová Slavětínská, Miloš Buděšínský, Özlem Bahadir Acikara, Josef Cvačka**<br>Determination of the Relative Configuration of New Sesqiuterpene Glycosides with Many Chiral Centres |
| **P29** | **Andrea Usenik, Katarina Leko, Vesna Petrović Peroković, Željka Car, Rosana Ribić, Katarina Pičuljan, Marko Hanževački, Josip Požar**<br>The Thermodynamics of Hydrophobicaly Driven Inclusion; β-cyclodextrin vs. Cucurbit[7]uril |
| **P30** | **Alma Ramić, Ines Primožič**<br>Synthesis and Characterization of Cinchonidine and Cinchonine C9-Epimers |
| **P31** | **Markus Rotzinger, Karina Stadler, Klaus Zangger**<br>Interactions of Macrolides and Human Serum Albumin via DOSY |
| **P32** | **Monika Škrjanc, Damjan Makuc**<br>NMR Studies of Slovenian Honey |
| **P33** | **Kristina Smokrović, Ema Hošnjak, Saranyarat Sanemit, Hana Čipčić-Paljetak, Saša Kazazić, Ivana Mikulandra, Predrag Novak**<br>Synthesis and Characterization of Nickel(II) Macrozone Complexes Derived from 4-formylbenzoic Acid and 3-formylsalicylic Acid |
| **P34** | **Ondřej Socha, Martin Dračínský**<br>Dimerization of Acetic Acid in the Gas Phase |
| **P35** | **Nikola Bedeković, Luka Fotović, Katarina Pičuljan, Vladimir Stilinović**<br>Ordered and Disordered Cocrystals of Halogenopyridines and Halogenobenzoic Acids Studied by Combining X-Ray Diffraction and SS-NMR Spectroscopy |
| **P36** | **Jakub Radek Štoček, Martin Dračínský, Ivana Císařová, Tomáš Slanina, Ondřej Socha**<br>Temperature Induced Salt to Cocrystal Transformation |
| **P37** | **Sunčica Roca, Amalija Ernečić, Nikolina Višić, Marija Alešković, Nikola Bregović, Marina Šekutor**<br>Exploring Structural and Thermodynamic Properties of Various Diamantane Amine–Cyclodextrin Complexes |
| **P38** | **Marko Trajkovski, Janez Plavec**<br>NMR Studies of G-Quadruplex-Ligand Binding |
| **P39** | **Bianka Várnai, Zsófia Garádi, Dóra Ujj, Erzsébet Varga, Tamás Sohajda, Szabolcs Béni**<br>Molecular Interactions in Remdesivir-Cyclodextrin Systems Probed by NMR and CE |
| **P40** | **Julia Wieruszewska, Aleksandra Pawłowicz, Zofia Gdaniec, Witold Andrałojć**<br>DNA Construct Optimization for the NMR Structural Studies of the 8-17 DNAzyme |
| **P41** | **Amadeusz Woś, Dorota Gudanis, Witold Andrałojć, Zofia Gdaniec**<br>Biophysical Characterization of a Systematic Set of Model DNA G-Quadruplexes |

# NMR STRUCTURAL STUDIES OF ANTIMICROBIAL PEPTIDES IN MODEL MEMBRANES AND LIVE BACTERIA

Frances Separovic

School of Chemistry, University of Melbourne, Melbourne, Australia
fs@unimelb.edu.au

Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, their primary target. Such studies are important to provide high-resolution details within a model system but correlation with *in vivo* situations remains speculative, especially in view of the complex modulation observed with slight changes in conditions such as pH, temperature, lipid composition or peptide concentration. Studying AMPs in live bacteria is, therefore, important but presents several challenges, such as sensitivity and bacterial lifetime. Studies of AMPs in live *E. coli* or *S. aureus* bacteria using solid-state NMR techniques will be presented. The impact of the AMP maculatin 1.1 (Mac1) on bacteria was monitored by $^{31}$P NMR while structural details on the peptide were obtained using dynamic nuclear polarization (DNP) enhanced $^{13}$C and $^{15}$N solid-state NMR experiments. Finally, a novel strategy to perform in-cell DNP NMR experiments was established by using spin-labelled peptides;[2] and $\{^{15}N\}^{13}C$ REDOR measurements have been performed to measure the distance between several pairs of $^{13}$C=O and $^{15}$NH within the Mac1 amino acid sequence, which indicate a transmembrane helical structure in bacteria.

**Acknowledgements.** This work has been supported by the Australian Research Council.

## REFERENCES

[1] S. A. Overall, S. Zhu, E. Hanssen, F. Separovic, M.-A. Sani, *Int. J. Mol. Sci.* **2019**, 20, 181 (12 pages).

[2] S. Zhu, E. Kachooei, J. R. Harmer, L. J. Brown, F. Separovic, M.-A. Sani, *Biophys. J.* **2021**, 120, 4501-4511.

# EARLY DAYS OF TWO-DIMENSIONAL FOURIER TRANSFORM NMR

Geoffrey Bodenhausen

Ecole Normale Supérieure, Paris
Geoffrey.Bodenhausen@ens.fr

When I decided to leave Zürich in the autumn of 1974, after passing the last exams of my diploma in chemistry at ETH, I did not have a clue what I was heading for. I had visited Oxford to meet Ray Freeman in July of that year. He had taken me to have sandwiches in a wonderful pub on the Thames – I believe it was called The Trout Inn – but I was intimidated by Ray's presence, unfamiliar with colloquial English, more comfortable with "Sir" rather than with "Ray". I was delighted by the Oxford setting, feeling intuitively that it would do me a lot of good, after four years in a rather frigid atmosphere in Zurich, where I had completed a Master's thesis under the supervision of Richard Ernst. Its subject (Overhauser effects with multiple selective irradiations) seemed to be a bit exotic, and I did not feel that Richard cared much about the subject.

When I arrived at the Physical Chemistry Laboratory (PCL) on South Parks Road, I met David Turner, who was at that time a "Part II" student (similar to a Master's). David had unpacked and fired up a brand-new Varian CFT 20 spectrometer, which was designed for carbon-13 NMR at 20 MHz, with a proton channel at 80 MHz designed for noise decoupling. This was our only machine for the next three years. In the following months, Gareth Morris was to join our group, initially as Part II student, before starting on his DPhil, soon to be followed by Reinhard Niedermeyer. Malcolm Levitt joined the team when I had just submitted my thesis.

Ray Freeman did not spend much time in the lab, for he was deeply involved in teaching tutorials, i.e., meetings with a handful of students, mysterious rituals that seemed surreal by ETH standards. But Ray often came to coffee or tea breaks in the Common room of the PCL, around 11 am or 4 pm, less frequently around 1 pm for lunch, consisting of a bowl of soup and a roll with cheese, all served by a charming tea lady named Gladys. It is in the Common room that most interesting things happened. When we were particularly excited, we would discuss non-stop from 11 am to 5 pm, over coffee, soup and tea.

Indeed, these were exciting times. We learned to master the timing of crazy pulse sequences on our CFT 20, which had never been intended for anything sophisticated. We programmed in octal code, since there was nothing like a compiler. The entire memory of the Varian computer was limited to a mere 16 k words. The external Diablo disk had a capacity of 1 Mbyte, and we had to instruct the computer to save and retrieve data by indicating the physical addresses of the tracks on the disk.

Ray had a remarkable intuition for the behaviour of classical magnetization vectors and their trajectories. Some of his talents would be revealed after my stay in his lab, when composite pulses became fashionable. In my days, our greatest achievement was a simple sequence of equidistant pulses that was to become known as 'Delays alternating with nutation for tailored excitation' (DANTE).[1] The pinnacle of my skills was reached when I managed to program in real time two such sequences running with different intervals simultaneously, although this was never used for anything.

Then came the totally unexpected advent of two-dimensional Fourier transformations. Initially suggested by Jean Jeener, the idea came to us through a TAMU newsletter by Richard Ernst.

(Only many years later did I become aware of Jean Jeener's lecture in Baško Polje.) This was to become a true passion for me. I remember a social gathering where I pulled an early 2D spectrum from my pocket to show it to the bewildered attendants, much to their dismay, for they must have thought that I suffered from some weird delusions. We discovered all sorts of things, such as artefacts that Ray affectionately dubbed "ghosts" and "phantoms". The invention of phase-cycles allowed us to get rid of both ghosts and phantoms.[6] We liked to speak of Exorcycle, after a silly movie called 'the Exorcist'. We also invented what later came to be known as 'time proportional phase increments'.[8]

We spent most of our time exploring 'J spectra', where the evolution period contained a refocusing pulse to remove line-broadening effects due to inhomogeneous fields.[2,3] (My inability to shim the magnet provided a powerful motivation!) To our surprise, we noted that spin-echo spectra could feature negative lines.[7] We managed to explain these, and Gareth did a great job at adapting spectral simulations ('Son of Laocoon'.[11]) We found some curious effects of interference with sample spinning[15] that would later become relevant in solids.

We re-invented heteronuclear correlations, by invoking the role of populations where information could be stored temporarily.[9,10] The original work by Maudsley and Ernst did not mention this concept. Indeed, the question in which order pulses should be applied to protons and carbon-13 nuclei is immaterial, because the operators that represent the corresponding rotations commute. But this sort of reasoning was far beyond our understanding.[16] In retrospect, the most remarkable feature of my days in Oxford is that we invented so many novel things without a sound theoretical basis! Little by little, we learned to work with density matrices,[11] and came to realize that unitary rotations could be expressed by manually calculating products of 4×4 matrices…

Ray maintained friendly relationships with his former employer, Varian Associates in Palo Alto, especially though Howard Hill, who came to see us in Oxford once in a while. This contributed to fruitful collaborations.[15] In the course of the years, I have tried to foster collaborations between academia and industry in various areas, such as optical 2D spectroscopy with laser pulses, 2D microwave spectroscopy, 2D-ESR, and 2D ion cyclotron resonance mass spectroscopy (FT-ICR-MS). Not surprisingly, many of these attempts have been less successful than 2D NMR, not only because their advantages were less spectacular, but because the instrument manufacturers were attracted to more profitable projects.

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# SOME MEMORIES ABOUT NMR AT ETH ZURICH IN THE MID-1980S

Norbert Müller

Johannes Kepler University Linz, AT & University of South Bohemia, České Budejovice, CZ
Norbert.Mueller@jku.at

Probably the most formative research experience of my career was the 12 months or so I spent at ETH-Zurich. In 1984 and 1985 I was a post-doc with a position shared between the research groups of Richard R. Ernst and Kurt Wüthrich. With my background in organic chemistry and molecular mechanics acquired at the university of Vienna the learning curve in "hardcore" NMR was a steep but also very rewarding one. In retrospect this was the most exciting and rewarding period of my scientific life. While I did not stay long enough at ETH to give an authoritative report, I am glad to share some of my memories and recollections from that time, which was period of rapid development of NMR concepts and applications of 2D NMR methodology and also a unique opportunity to meet exceptional scientists, who were concentrated at that particular period in that remarkable environment and are famous in the NMR-community today. Some of the "hot" topics at the Laboratory of Physical Chemistry and the Institute of Molecular Biology and Biophysics at that time, which still have a sustained impact on the NMR research community today were Cartesian product operators,[1] coherence transfer pathways,[2] composite pulses,[3] protein structure from 2D NMR (protons only!)[4] and multiple quantum filters.[5] To that last topic I was able to make some moderately useful contributions at that time[6–8], which turned out to be the foundation for some of my later research topics.

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# IN-CELL NMR FOR THE UNDERSTANDING OF PHYSIOLOGICAL PROCESS AT MOLECULAR LEVEL

Lucia Banci

CERM and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy

In-cell NMR, i.e. high resolution NMR spectra of biomolecules in intact, living cells, represents one of the highest impact applications of magnetic resonance.

These experiments allow to obtain information on the conformational and functional properties of biomolecules at atomic resolution in conditions as close as possible to the physiological ones. In-cell NMR allows also to monitor protein-protein interactions and to follow functional processes, as well as protein maturation and post-translational modifications. A further striking application is its use for drug screening in real time at cellular level, in human living cells.

Methodological aspects and innovations will be discussed and a few examples of the striking power of this approach for the characterization of the metal transport processes, for the assessment of the protein redox state in the cellular environment, and for the study of effective drug screening will be presented. Particular focus will be on the cellular uptake and target binding in the cellular milieu of drugs and leads and on the meaningful differences observed between drug-target binding in living cells versus in vitro.

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# RECENT ADVANCES IN POLYPEPTIDIC THERMORESPONSIVE ALIGNMENT MEDIA FOR ORGANIC COMPOUNDS

Christina M. Thiele,[a] Dominic S. Schirra,[a] Max Hirschmann[a,b]

[a] Technische Universität Darmstadt, Darmstadt, Germany
[b] Present address: Kungliga Tekniska Högskolan (KTH), Stockholm, Sweden
✉ cthiele@thielelab.de

Anisotropic NMR parameter become increasingly important in organic structure elucidation for the determination of conformation and relative configuration of natural products, synthesized compounds and catalysts.[1]

To obtain such anisotropic NMR parameter, suitable alignment media are necessary. The use of lyotropic liquid crystals based on helically chiral polymers is especially intriguing as they additionally allow for enantiodiscrimination.[2]

We have recently synthesized several homopolypeptides,[3] which form lyotropic liquid crystals that are suitable for the measurement of anisotropic NMR observables and show excellent enantiodiscrimination. Furthermore, the thermoresponsive properties of these homopolypeptides can be utilized to induce different orientations at different temperatures, which alleviates the need to use more than one alignment medium in cases of ambiguity.

The intriguing properties of these new thermoresponsive alignment media will be described in this presentation. Furthermore, we will shed light on the processes responsible for the thermoresponsivity.[4]

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# CHALLANGES OF NMR SPECTROSCOPY IN CRUDE OIL AND ALTERNATIVE FUELS ANALYSIS

Jelena Parlov Vuković

CTL INA d.d., Zagreb, Croatia
✉ jelena.parlov-vukovic@ina.hr

An understanding of crude oil and alternative fuels composition is of outmost importance in research and development of production processes, in production control and in product quality assessment.[1-4] In this talk challenges and possibility of NMR spectroscopy in analysis of different mineral samples will be demonstrated. It will be shown that NMR spectroscopy coupled with statistical methods is a valuable tool for the investigation of physicochemical properties and aggregation of asphaltenes, most complex and polar oil components which cause different problems in oil industry.[1,2]

Recently, the need to develop alternative fuels has become essential for reducing the impact of global warming and climate changes on the planet as well as the limited resources of crude oil and natural gas. Some distinctive examples of using NMR to study renewable fuels will be discussed.[3,4]

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# $H_2O$ *vs.* $D_2O$: THERMODYNAMICS BEYOND NMR

Andrea Usenik

Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
ausenik@chem.pmf.hr

Hydrogen bonding plays a crucial role in water structuring. Due to disruption of the strong hydrogen bond network, solvation of non-polar species (and surfaces) is thermodynamically unfavorable. This leads to a significant affinity of macrocyclic receptors (e.g., cucurbiturils (CBs) and cyclodextrins (CDs)) towards hydrophobic species in water. Recent studies have shown that dehydration of the guest and the host cavity are the main driving forces for hydrophobically driven inclusion. In these systems, high complex stability is usually due to favorable complexation enthalpy, commonly ascribed to cavity dehydration and the reestablishment of bulk water hydrogen bonds. Since the latter are known to be stronger in $D_2O$ versus $H_2O$, a considerable impact on the complexation thermodynamics is to be expected in these cases. However, the inclusion complex stability constants for these systems are usually assessed by $^1H$ NMR spectroscopy in $D_2O$ which does not provide information on the possible isotope effect on the enthalpic and entropic contribution to the reaction Gibbs energy. Therefore, we have systematically explored the difference in binding affinities of various CBs and CDs with hydrophobic guests in $H_2O$ and $D_2O$ by means of ITC in a wider temperature range (5–65 °C). The obtained results were discussed based on the structure of hosts and guests, as well as the possible influence of the differences in $H_2O$ and $D_2O$ structuring and related hydration and physical properties. The obtained results provide important insights into the isotope effects on the complexation thermodynamics in protic solvents with variable protium and deuterium abundance.

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# DYNAMICS IN LARGE PROTEIN ASSEMBLIES

Paul Schanda

Institute of Science and Technology Austria, Am Campus 1, A-3400 Klosterneuburg, Austria
✉ paul.schanda@ist.ac.at

Studies of protein dynamics in large assemblies, and of the constituent components before assembly, is of great interest for understanding biological mechanisms at the atomic level. In this presentation, I will show recent data from our research, combining solid-state NMR, solution-state NMR, MD simulations and a host of other techniques to shed light onto two biological questions. In the first part I will provide insight into a 500 kDa large enzyme, the TET aminopeptidase. By combining magic-angle-spinning NMR with a host of biophysical methods we show that a highly flexible loop, which is even undetected in crystal structures, is a key functional element. Its dynamics allows substrate passage, and a conserved motif within this loop plays an important role in substrate stabilisation. In the second part, we focus on a very different type of assembly: the encounter of a chaperone with its client protein. We demonstrate that such chaperone-client complexes are characterized by extensive dynamics, and that these dynamics are the key for chaperone function. Subtle changes in the sequence of the chaperone can greatly impact the binding of client proteins, and thereby confer specificity to chaperone

# HOW OXIDATIVE LESIONS AFFECT DNA SECONDARY STRUCTURE

Peter Podbevšek

Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia
peter.podbevsek@ki.si

Guanine rich regions can adopt non-canonical four-stranded DNA structures called G-quadruplexes. Contiguous runs of guanines are especially susceptible to oxidation and contain the highest frequency of 8-oxo-7,8-dihydroguanine ($^{\text{oxo}}$G) - a major product of reactive oxygen species (ROS). We have analyzed the effect of $^{\text{oxo}}$G on human telomeric (hTel) and promoter (bcl2) G-quadruplex structures. While substituting most G positions with $^{\text{oxo}}$G proved detrimental, some positions within G-rich sequences were found to retain the G-quadruplex structure. Accommodation of $^{\text{oxo}}$G at sites in *syn* or *anti* in non-substituted hTel G-quadruplex requires a minor structural rearrangement or a major conformation shift, respectively. Nevertheless, thermal stability of resulting G-quadruplex structures was typically reduced.[1] However, in an isolated case a reduction of structural polymorphism and a surprising boost in thermal stability of a bcl2 G-quadruplex with $^{\text{oxo}}$G was also observed.[2] This was achieved by distinct hydrogen bonding properties of $^{\text{oxo}}$G, which facilitate formation of an antiparallel basket-type G-quadruplex with a three G-quartet core and a G·$^{\text{oxo}}$G·C base triad. $^{\text{oxo}}$G could act as an epigenetic modification, which alters DNA secondary structure and subsequently regulates gene expression by altering the binding of transcription factors to the DNA. This suggests a potential novel regulatory role of oxidative stress in gene transcription.

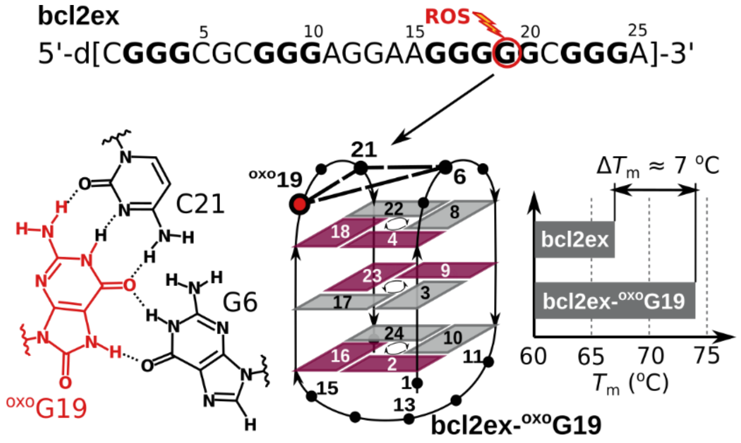

**Figure 1.** $^{\text{oxo}}$G stabilizes the bcl2 G-quadruplex through extensive hydrogen bonding with loop nucleotides

**Acknowledgements.** This work was supported by the Slovenian Research Agency [J1-1704, P1-0242].

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# DYNAMICS IN ‘RIGID’ DISULFIDE PROTEINS

Gyula Batta,[a] András Czajlik,[a] Ádám Fizil,[a] Dorottya Hajdu,[a] Ágnes Batta,[a]
Gai Jiawei,[a] Zoltán Gáspári,[b] László Galgóczy,[c] Florentine Marx[d]

[a] Structural Biology Research Group, Dept. of Chemistry, University of Debrecen, Debrecen, Hungary
[b] Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary
[c] Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[d] Institute of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria
batta@unideb.hu

Small disulfide proteins (50-60 aa) like PAF are efficient antifungals and some have anti-*Candida* (PAFC) or anti-corona virus activity (PAF, PAFB).[1] However, their mode of action is not yet fully understood.[2] Their β-barrel tertiary structures are stabilised by 3-4 disulfide bridges lending apparent rigidity to the structures. Still, intrinsic dynamics persists as shown[3] by NMR $^{15}$N-relaxation, $^{15}$N-CEST and MD calculations that are now supported by stress induced reversible unfolding and natural abundance $^{13}$C relaxation studies. Besides thermal unfolding we show DMSO induced transitions in PAF and variants as detected by NMR and DSC microcalorimetry.

Partially unfolded reversible states can be biologically relevant, e.g. connected to disulfide shuffling or other thiol related transitions, while dynamic intermediates can be preferred for conformational-selection mode of molecular recognition. Practical consequences may have impact on the validation of MD simulations or protein concentration measurements.

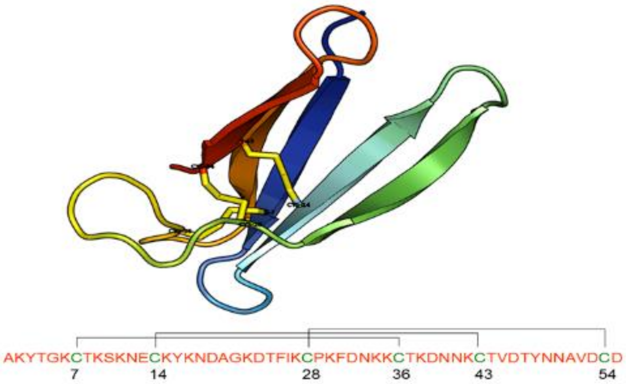

**Figure 1.** Solution structure of the antifungal protein PAF with three disulfide bonds.

**Acknowledgements.** The research was supported by the EU and co-financed by the European Regional Development Fund under the projects GINOP-2.3.2-15-2016-00008 and GINOP-2.3.3-15-2016-00004, and also by the Hungarian National Grant OTKA ANN 110 821, the Austrian Science Fund (I3132-B21) to F.M. L.G. is financed by the FK 134343 project of the Hungarian National Research, Development and Innovation Office - NKFIH.

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# FAST BIOMOLECULAR NMR WITH FAST MAS (WITHOUT AND WITH DNP)

Guido Pintacuda

Lyon High-Field NMR Center (CNRS/ENS Lyon/UCBL), Villeurbanne, France
guido.pintacuda@ens-lyon.fr

For large molecules, ultrafast (100 kHz) spinning narrows spectral resonances better than Brownian motion does for solution NMR, removing a fundamental barrier to the NMR study of large systems with $^1$H detection. Nonetheless, performing the assignment of all resonances remains a rate-limiting step in protein structural studies, and even the latest optimized protocols fail to perform this step when the protein size exceeds ~20 kDa. We introduce two approaches that address this issue, simultaneous parallel detection[1] and projection spectroscopy of hyperdimensional datasets,[2] allowing to accelerate acquisition and data analysis and at the same time to lift the molecular size barrier of the targets amenable to NMR analysis.

We additionally discuss the applicability of $^1$H detection methods under cryogenic conditions to perform structural studies with DNP.

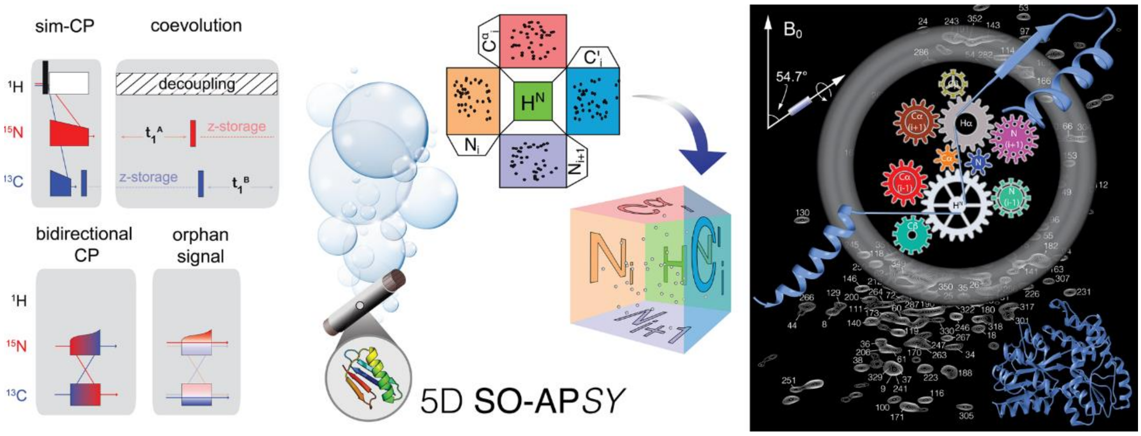

**Figure 1.** Left: scheme of radiofrequency building blocks for multiple pathway coherence transfers, key for the simultaneous acquisition of a single self-consistent data set composed of multiple $^1$H-detected 3D spectra. Middle: correlations of five resonance frequencies of nuclear spins within one NMR experiment are now feasible by using projection spectroscopy at 100 kHz MAS. Right: 100 kHz magic-angle spinning NMR allows automatic fingerprinting of large proteins, as demonstrated here on the 42.5 kDa maltose binding protein, the largest protein assigned to date in the solid state.

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# NMR CRYSTALLOGRAPHY OF MOLECULAR SOLIDS

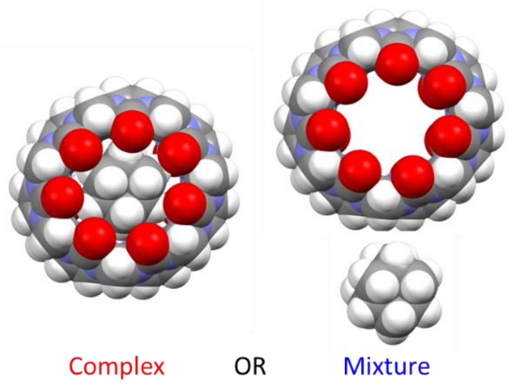
Martin Dračínský,[a] Jakub Radek Štoček,[a] Guillaume Bastien,[a] Carina Santos,[a] Jiří Kaleta[a]

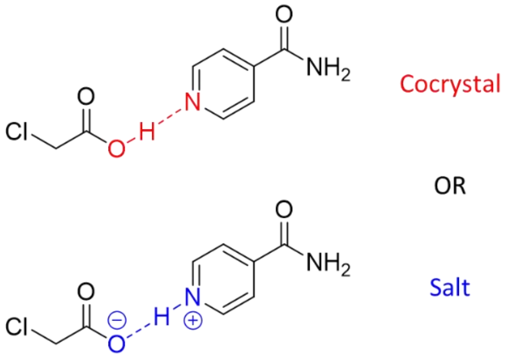
[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
✉ dracinsky@uochb.cas.cz

Modern chemistry, biology and materials science are based on the knowledge of structure on the molecular level. X-ray crystallography is the most widely used technique for determination of structures with atomic resolution. Single crystal X-ray diffraction (XRD) has been called the "gold standard" for solid-state structure determination,[1] which has been central to many achievements in chemistry and biology. However, there are two major limitations of this method. First, hydrogen atoms are very difficult to characterize by this technique. Another well-known limitation of XRD is that it requires a highly ordered crystalline sample and is inherently limited in the structural characterization of disordered or amorphous samples.

An alternative experimental technique for atomic-level characterization of solids is solid-state NMR spectroscopy (SS-NMR). NMR spectroscopy is very sensitive to the local environment of hydrogen atoms. Furthermore, SS-NMR does not require a long-range order in the studied materials and is, therefore, suitable for characterization of disordered and amorphous samples. The progress of SS-NMR methods has led to the development of NMR crystallography, which combines experimental SS-NMR data with theoretical simulations to obtain otherwise inaccessible insights into the structure and dynamics of solid materials.

Here, several recent examples of NMR-crystallography studies overcoming the limitations of XRD will be discussed, such as investigation of mechanochemically prepared 2D and 3D arrays of molecular machines[2,3] or investigation of temperature-induced salt-to-cocrystal transformation of pharmaceutical solids.[4] We will also discuss the importance of nuclear quantum effects for the structure of solids with short hydrogen bonds.





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# THE WORLD OF PEPTIDES VIEWED THROUGH SOLID STATE NMR SPECTROSCOPY WINDOW

Marek J. Potrzebowski

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland
marekpot@cbmm.lodz.pl

Short peptides have recently attracted increasing attention in biology, chemistry of new materials, and medicine due to their specific features. The wealth of possible applications, the complexity of the systems studied and the need to understand the correlation between structure, dynamics and functions have prompted many research groups to seek universal analytical tools that provide maximum information with minimum effort. One of such instrumental technique that comes close to fulfilling these requirements is solid state NMR spectroscopy. The advantage of solid state NMR spectroscopy is that specific structural and dynamic aspects can be accurately described by using different pulse sequences and different viewing windows.

In the first part of the MMCE paper, the usefulness of tripeptides as standards for the developing of new NMR sequences will be briefly discussed. In particular attention will be paid to the problem of local molecular motion analysis, in particular for aromatic residues.

The second part will be devoted to demonstrating the power of solid-state NMR spectroscopy in the analysis of supramolecular structures formed by self-assembling peptides. Currently, there is an increasing interest in the use of self-organizing peptides in the development of new types of soft matter, such as hydrogels and organogels. Gelation and crystallization processes are intertwined, yet it is still not clear in what way. In this work, the attention is focused on understanding the relationship between these two phenomena with an aim to deliver a rational explanation for the fact of distinct behaviour of two cTyrTyr (cYY) diastereoisomers.

In the final part, we will present a unique and very effective approach for peptide cyclization in the condensed matter, a method combining the achievements of mechanochemistry and the topochemical concept. The progress of mechanosynthesis is controlled by advanced solid state NMR spectroscopy. The power of the method is proved with the use of four linear peptides Tyr-(D)Ala-Phe-Gly (signal sequence of neuropeptide Dermorphin), Tyr-Pro-Phe-Phe-OH (analogue of neuropeptide Endomorphin-2), ProPheProPhe·HCl and O-Benzyl derivative of Tyr-(D)Ala-Phe-Gly. Each of the used peptides adopts in the solid state a pseudocyclic conformation that meets the topochemical criteria allowing for the formation of new peptide bonds and the synthesis of cyclic compounds.

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# PURE ISOTROPIC PROTON SOLID STATE NMR

Pinelopi Moutzouri,[a] Bruno Simões De Almeida,[a] Manuel Cordova,[a,b]
Daria Torodii,[a] Lyndon Emsley[a]

[a] Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[b] National Centre for Computational Design and Discovery of Novel Materials MARVEL, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
pinelopi.moutzouri@epfl.ch

Coherent averaging methods are at the heart of modern NMR spectroscopy ranging from simple spin echoes to remove heteronuclear *J*-couplings in solution, to rotor-synchronized symmetry-based pulse sequences to modulate dipolar couplings under MAS in solids. However, coherent averaging schemes are never perfect, and residuals are always present.

In solids, the workhorse of coherent averaging is magic-angle-spinning. Physically spinning the sample around an axis tilted at 54.74° with respect to the main magnetic field direction averages the interactions that have a second rank spatial dependence (such as dipolar couplings), while preserving the isotropic interactions (such as chemical shifts). However, for abundant spins, such as protons, imperfect averaging leads to residual dipolar broadening. Even after careful calibration of the magic angle and at the fastest MAS rates (around 100 - 150 kHz) possible today, the spectra obtained are hundreds of Hertz broader than their isotropic linewidths.

Here, instead of optimizing an averaging scheme, we introduce a new approach which parametrically maps the residual terms deriving from the imperfect averaging of MAS and removes them in a k-space representation, allowing the extraction of the pure isotropic signal. For example, by recording a series of MAS spectra at different spinning rates, we can model the parametric behaviour of the intrinsic pure isotropic shift terms in comparison to the residual broadening terms.

We illustrate the method on a series of eight organic solids using either a parametric fitting approach[1] or a machine-learning based model. In both cases we are able to produce pure isotropic spectra that are significantly narrower (by a factor of 7 on average) than the fastest MAS spectrum we can record (at 100 kHz). We also illustrate how we can expand this approach to produce high resolution two-dimensional NMR spectra.

**Acknowledgements.** Financial support from Swiss National Science Foundation Grant No. 200020_178860 is acknowledged.

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# OTTO STERN-THE PRIME FATHER OF THE NMR-METHOD

Horst Schmidt-Böcking

Universität Frankfurt, Institut für Kernphysik, Max-von-Laue-Str. 1, 60438 Frankfurt, Germany
schmidtb@atom.uni-frankfurt.de

In 1919, the theoretically trained physical chemist Otto Stern developed at the University of Frankfurt the so-called Molecular Beam Method MBM. This method makes it possible to measure transverse momentum changes (deflections caused by e.g. an external magnetic force) with extremely high resolution for individual atoms flying in a vacuum. In 1922, together with Walther Gerlach in Frankfurt, Stern was able to prove for the first time that certain atoms have a magnetic moment and that these moments are quantized in size and direction. This was the first direct experimental proof of angular momentum quantization in atoms. By improving the MBM, he was able to measure the magnetic moment of the proton and the deuteron for the first time in 1933 at the university Hamburg. He thus became the pioneer in the investigation of inner nuclear and elementary particle structures. Since Otto Stern was a Jew, these successful pioneering experiments in nuclear physics were practically ended when he was forced by the NAZI regime to emigrate to the USA. However, his pupil Isidor Rabi and Rabi’s students continued Stern’s experiments at Columbia University in New York and later at MIT in Boston and developed the foundations of the NMR method.

# ADVENTURES IN (MACHINE) LEARNING TO DETERMINE 3D STRUCTURE WITH NMR SPECTROSCOPY

Craig Butts

University of Bristol, School of Chemistry, Cantocks Close, Bristol, BS8 1TS, UK
✉ Craig.Butts@bristol.ac.uk

Interpreting NMR spectra has become somewhat of an art form in chemistry, particularly when applied to molecular structure elucidation. Skilled practitioners will often pore over several, often complex, NMR spectra for hours, days and even weeks to work out the connectivity (2D structure), stereochemistry and conformation (3D structure) of challenging new molecules. Our team develop tools that help with the steps in this process: creating new NMR experiments that provide different or more quantitative information than existing methods; applying quantum chemical calculations (usually DFT) to accurately predict the NMR properties of candidate molecular structures; developing machine learning tools that can accelerate predictions by 10,000-fold and thus potentially allow us to screen hundreds of thousands of molecular structures to find good fits to the experimental NMR spectra. The potential (and limits!) of these approaches will be illustrated by the case of the structure reassignment of natural products that have billions of potential 3D geometries and exploring how machine learning is starting to compete with DFT as a method to be used in these approaches.

# NUCLEAR SPIN SINGLET STATE RELAXATION MECHANISMS FROM EXPERIMENT AND MOLECULAR DYNAMICS SIMULATIONS

Alexej Jerschow

Department of Chemistry, New York University
✉ alexej.jerschow@nyu.edu

Nuclear spins states have been shown to exceed spin-lattice relaxation times several fold, with impressive demonstrations of singlet lifetimes of more than an hour in organic molecules in solution. Over the years, several relaxation mechanisms have been identified, including dipolar coupling, chemical shift anisotropy, paramagnetic relaxation, spin rotation and spin-internal motion, and the scalar relaxation of the second kind. While in principle, many of the mechanisms are well understood, estimating their size can be difficult. Furthermore, multiple experimental examples have been found that decidedly defy expectations.

We present here work on directly estimating singlet relaxation mechanisms from molecular dynamics simulations. Here we show calculations for intermolecular mechanisms and find good agreement with experiment. It is particularly surprising to see that such mechanisms as intermolecular coupling to $^{35}$Cl and $^{37}$Cl nuclear spins (of the chloroform solvent) could be rate limiting for singlet states. In addition, we also show work on $^{31}$P spin singlets, and compare their lifetimes to those from molecular dynamics trajectories and ab initio calculations of chemical shift anisotropy tensors, which show good agreement.

Calculations of this sort may help in the design of particularly long-lived singlet states, or could be used to identify new probes for dynamics.

# SHARPER NMR: OUR JOURNEY TO REMARKABLE SENSITIVITY GAINS

Dušan Uhrín,[a] George Peat,[a] Claire L. Dickson,[a] Patrick J. Boaler,[a]
Guy Lloyd-Jones,[a] Meghan E. Halse,[b] and Ana Silva Terra[b]

[a] EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK
[b] Department of Chemistry, University of York, York, UK
dusan.uhrin@ed.ac.uk

The SHARPER (Sensitive, Homogeneous And Resolved PEaks in Real time) NMR experiment, proposed originally in the context of reaction monitoring, removes all homonuclear and heteronuclear splittings from a selected signal by pulsing only on the observed nucleus. SHARPER compensates for magnetic field inhomogeneity, producing very narrow singlets.[1] A recent extension of SHARPER allowed simultaneous monitoring of a reactant and a product.[2]

Optimised acquisition and processing of spectra further improved sensitivity as demonstrated by the implementation of SHARPER on benchtop NMR spectrometers.[3] Hyperpolarised benchtop $^{19}$F NMR, a combination of SHARPER with the parahydrogen-based SABRE, provided an additional 2 to 8 fold SNR improvement beyond that achieved through hyperpolarisation alone. By incorporating the SHARPER acquisition mode into existing NMR experiments we can dramatically enhance their sensitivity by collapsing parts (whole spectrum) into a singlet (Fig. 1).

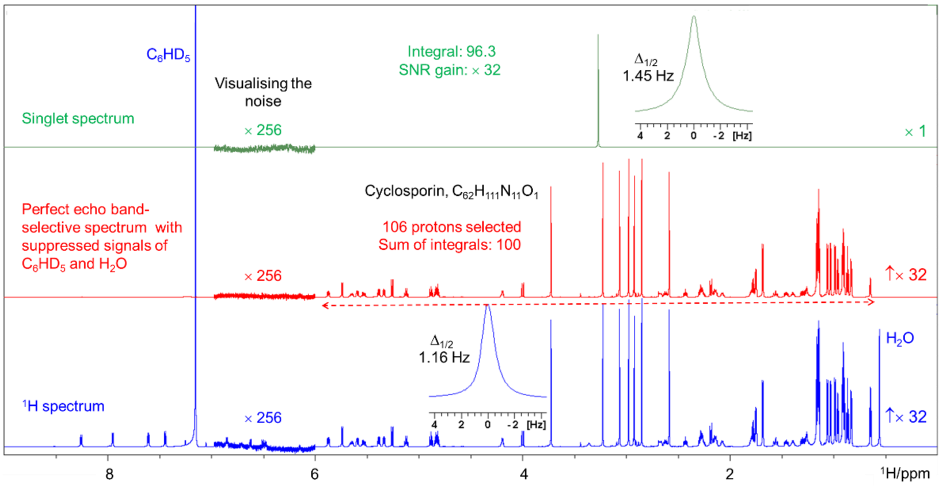

**Figure 1.** Collapsing a large part of an 800 MHz $^1$H NMR spectrum of cyclosporine into a singlet.

**Acknowledgements**. We thank Juraj Bella (UoE) for maintaining NMR spectrometers, Dr Stuart Kennedy (The Falcon Project Ltd and) for access to a Spinsolve 60 MHz NMR spectrometer and Dr Craig Eccles (Magritek Ltd) for technical assistance. EP/S016139/1, EP/R030065/1, EP/M020983/1 and EP/R028745/1.

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# NON-STATIONARY NMR SPECTROSCOPY

Krzysztof Kazimierczuk

Laboratory of NMR Spectroscopy, Centre of New Technologies, University of Warsaw
k.kazimierczuk@cent.uw.edu.pl

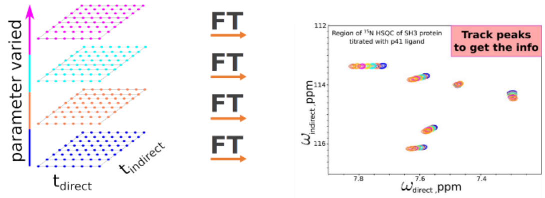
The implicit assumption behind a routine NMR spectroscopy is that the parameters of an FID signal (frequencies, amplitudes, decay rates) are independent of time. NMR experiments, therefore, have to be performed under highly stable conditions. For example, the temperature has to be precisely controlled, and chemical reactions in the sample have to be avoided.

However, a series of NMR experiments with different environmental conditions is often used to monitor changes to molecular structure. For example, a series of spectra acquired at certain temperature values or ligand concentrations, changed from spectrum to spectrum, may be used to study temperature-induced protein unfolding.

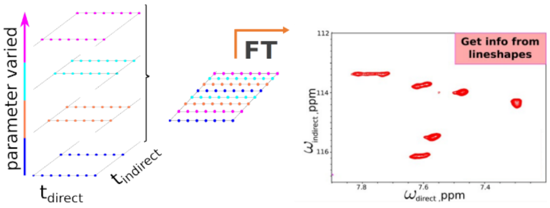
My hypothesis, confirmed in several studies,[1–4] is that NMR experiments do not need to be conducted in a stable environment. The environmental conditions can be changed e.g. between the measurment of consecutive indirect dimension points. Such a *non-stationary signal* recorded for different temperatures, pH values, sample compositions and so on can actually replace a series of conventional spectra and be recorded in a time corresponding to the measurment of a single stationary spectrum.

In my presentation, I will summarize the recent progress and future plans in developing non-stationary NMR methods. I will discuss the possible applications in structural studies of large and small molecules, metabolomic screening and other fields.

**Conventional series of 2D NMR spectra**



**Non-stationary 2D NMR**



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# NO RELAXATION DELAY (NORD) SPECTROSCOPY: RAPID SEQUENTIAL ACQUISITION OF NMR EXPERIMENTS

István Timári,[a] Tamás Milán Nagy,[b] Ole W. Sørensen,[c] Katalin E. Kövér[b,d]

[a] Department of Organic Chemistry, University of Debrecen, Debrecen, Hungary
[b] MTA-DE Molecular Recognition and Interaction Research Group, Debrecen, Hungary
[c] Copenhagen, Denmark
[d] Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary
✉ timari.istvan@science.unideb.hu

Since NMR is an inherently low-sensitivity method, it is a matter of constant attention to find ways for improving sensitivity and/or reducing measurement time. A few years ago, the idea of sequentially exploiting two different pools of magnetization in a concatenated experiment was proposed for saving spectrometer time since the two pools can relax simultaneously in a single delay.[1] More recently, we have demonstrated that it was possible to omit the relaxation delay using the NORD (NO Relaxation Delay) strategy.[2] NORD experiments are laid out not only to operate with two orthogonal pools of magnetization but also to have one of the pools relaxing, while the other is being used and vice versa, or to save magnetization in individual modules for succeeding scans in accordance with the Ernst angle concept. A typical set of experiments in small-molecule NMR ($^{1}$H–$^{13}$C HSQC or H2OBC/2BOB, $^{1}$H–$^{13}$C HMBC and $^{1}$H–$^{1}$H TOCSY) is combined in novel NORD sequences.[3] The result is a significant sensitivity gain to the least sensitive of the three, HMBC, and thus a highly welcome saving in instrument time. It will be presented in the lecture that the NORD strategy in combination with the concatenation of two or three experiments delivers complete hetero- and homonuclear correlation maps within minutes for small and medium-sized molecules (e.g. carbohydrates, steroids) in moderate concentration. Although the proposed NORD experiments can serve most of the needs across a wide range of NMR applications, but if needed, it is also possible to replace any modules by another one relying on the same pool of magnetization.

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# SOME TIPS AND TRICKS FOR NMR METABOLOMICS AND LIPIDOMICS

Calin Deleanu[a,b]

[a] "C.D. Nenitescu" Centre of Organic Chemistry, Romanian Academy, Bucharest, Romania
[b] "Petru Poni" Institute of Macromolecular Chemistry, Romanian Academy, Iasi, Romania
✉ calin.deleanu@yahoo.com

To date there are only two analytical techniques, namely, MS and NMR spectroscopy, which are suitable for large metabolomics and lipidomics screenings of various pathologies and for epidemiological studies. Metabolomics is currently a well-established topic both in education and in a wide range of research areas. Lipidomics has already provided new insights into health and disease monitoring. However, NMR lipidomics is much less explored in comparison with NMR metobolomics.

With the advent in NMR-based human phenotyping, as well as urine and plasma-based medical diagnosis and health status monitoring, several large groups have become well-established in NMR metabolomics. Recent studies have proven impressive interlaboratory reproducibility of NMR spectra when employing trained personnel and the latest industry standard solutions.[1,2]

In addition to dedicated metabolomics groups, NMR metabolomics has penetrated many groups with nonexclusive or even marginal metabolomics interests. Our group is a good example where interests in various NMR topics coexist. We have also evaluated the NMR reproducibility for metabolomics in a "real-life" situation when combining both industry standard NMR solutions and multipurpose NMR equipment and we compared the reliability of NMR metabolomics data when involving both dedicated NMR operators and chemistry users from outside the NMR group.[3]

The paper presents examples of NMR metabolomics and lipidomics applications in lifesciences. Some entry-level experimental tips and tricks are presented in order to ensure reliable NMR metabolomics and lipidomics data.

**Acknowledgements.** This work has been supported by the Ministry of Research and Innovation, CNCS - UEFISCDI, project PN-III-P4-ID-PCCF-2016-0050 (5DnanoP), within the PNCDI III program.

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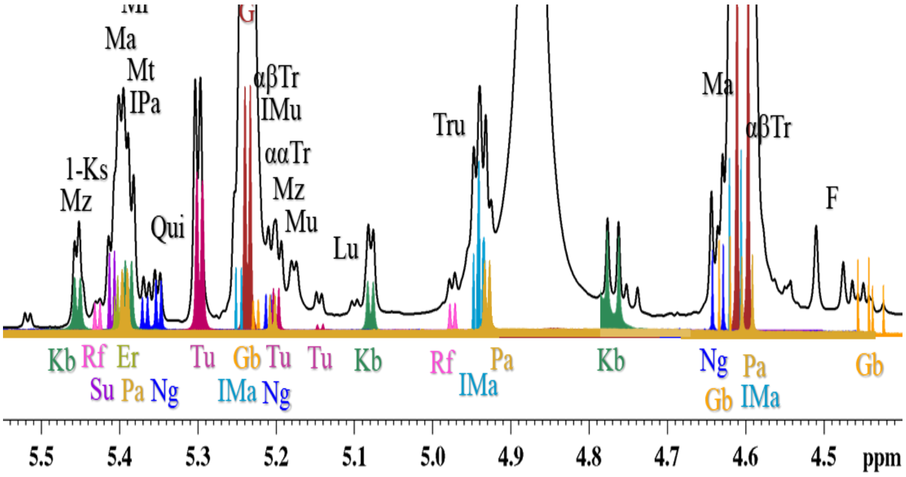
# SUGARS CONTAINING FOOD – NMR APPLICATIONS

Dessislava Gerginova, Svetlana Simova

Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences, Sofia, Bulgaria
✉ Svetlana.Simova@orgchm.bas.bg

Saccharides, or sugars belong to the most abundant classes of biomolecules. Various types of saccharides, including mono-, oligo- and polysaccharides are traditionally studied by NMR spectroscopy, as one of the major tools to foster the advance of food chemistry. The structure, conformation and dynamics of mono- and oligosaccharides are of particular importance because of their biological relevance.



Determination of quality, authenticity and shelf life of food products are gaining increasing interest in recent years due to the importance for human health. Adulteration and improper labelling are still often encountered despite the efforts of the regulatory authorities. Combining NMR spectroscopy and chemometrics provides detailed insight in the characteristic properties, allowing distinction in the origin of various products.

Recent results to determine small differences in the chemical profile of sugar containing food – honey, jam, mead and wine will be presented. Elucidation of composition, determination of similarities and common components and proof of botanical, geographical and entomological origin will be discussed.

**Acknowledgements:** This research was supported by Project No BG05M2OP001-1.002-0012, Center for Competence "Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products", funded by the Operational Program "Science and Education for Smart Growth" 2014-2020, co-financed by the European Union through the European Regional Development Fund. Research equipment of Distributed Research Infrastructure INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation.

# STRUCTURAL BIOLOGY AT THE RIBOSOME

Harald Schwalbe

Center for Biomolecular Magnetic Resonance, Goethe-University Frankfurt/M., 60438 Frankfurt, Germany
Instruct-ERIC
✉ schwalbe@nmr.uni-frankfurt.de

The ribosome is the large translation molecular machine. Integrated structural biology approaches have provided key insight into the molecular mechanism of how genetically coded information is translated into proteins.

In this contribution, we will present our work in looking at the conformation of mRNA adjacent to the entry channel of the ribosome (1,2) and at the conformation of the protein nascent chain within the ribosomal exit tunnel (3,4).

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# NMR-AIDED DESIGN OF CLINICAL STAGE PROTEIN-PROTEIN-INTERACTION INHIBITORS: THE MCL-1 SUCCESS STORY

Markus Schade,[a] David Hargreaves,[b] Rodrigo J. Carbajo,[a] Michael S. Bodnarchuk,[a]
Philip B. Rawlins,[b] Martin Packer,[a] Sébastien L. Degorce,[c] Alex Hird,[c]
Jeffrey Johannes,[c] Elisabetta Chiarparin[a]

[a] Chemistry, Oncology R&D, AstraZeneca, Cambridge CB4 0WG, UK.
[b] Discovery Sciences, AstraZeneca, Cambridge CB4 0WG, UK.
[c] Chemistry, Oncology R&D, AstraZeneca, Waltham, Massachusetts 02451, USA
✉ Markus.Schade1@astrazeneca.com

The structure-based design of small-molecule inhibitors targeting protein-protein interactions (PPIs) remains a huge challenge as the drug must bind typically wide and shallow protein sites. A PPI target of high interest for hematological cancer therapy is Myeloid cell leukaemia 1 (Mcl-1), a pro-survival guardian protein from the Bcl-2 family. Despite previously considered undruggable, six small molecule Mcl-1 inhibitors have recently entered clinical trials.

Here we report the free in solution NMR conformational analysis of the clinical stage Mcl-1 inhibitors AZD5991, AMG-176 and S64315, and compare the free 3D conformers with the protein-bound conformers determined by crystallography.[1] Our data reveal high plasticity of the Mcl-1 protein and a marked ligand-induced pocket deeping. NMR-based free ligand conformer analysis demonstrates that such unprecedented induced-fit is uniquely achieved by designing highly rigid inhibitors, preorganized in their bioactive conformation. By elucidating key chemistry desgin principles, this work provides a roadmap for targeting the largely untapped PPI class more successfully.

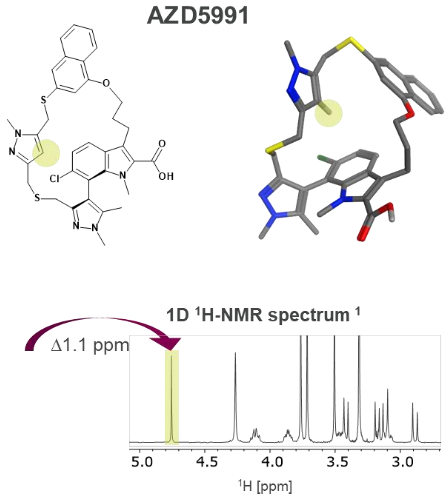

**Figure 1.** The free in solution conformer of Mcl-1 inhibitor AZD5991 shows a characteristic $^1$H-NMR fingerprint signal (green background), indicating aromatic ring packing and pre-structuring in the bioactive, protein-bound conformation. The arrow highlights the upfield shifted $^1$H signal compared with the 2D-HOSE predicted value.

**Acknowledgements.** This work has been supported by Astrazeneca.

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# NMR IN DRUG DEVELOPMENT: ROLE, CHALLENGES AND PROCESS CHEMISTRY SUSTAINABILITY

Silvia Davalli, Andrea Bernardelli, Chiara De Santi, Stefano Provera, Lucilla Turco

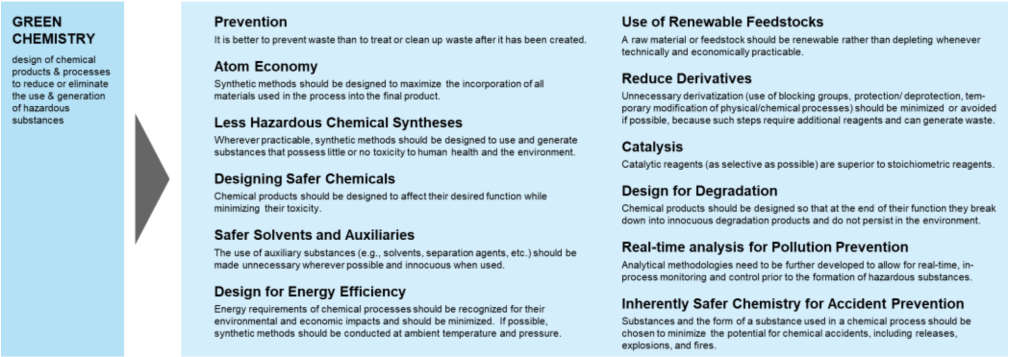
Aptuit (Verona) srl, an Evotec Company, Italy
silvia.davalli@evotec.com

Solution NMR plays a key role in the Pharmaceutical Research area as a powerfull tool for Identity testing and structural elucidation. Active Pharmaceutical Ingredient (API) synthesis requires process optimization to meet ICH requirements and reach sustainability. In this route scouting phase the generation of analytical information and specifically of NMR data is essential. Meanwhile during the GMP manufacturing stage, NMR applications range from materials identity test to full characterization for regulatory documentation.

In the recent years, quantitative NMR (qNMR) has been recognized as a powerful tool, alternative or orthogonal to other techniques. The application of qNMR can provide significant contribution in the process chemistry sustainability.

The Green Chemistry concept [1] can be defined as the "design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances" and has been increasingly used in chemistry and pharmaceutical industries, by application of its twelve principles, which ultimately allow to both decrease the environmental impact and reduce the economic costs. The eleventh principle of the Green Chemistry [2] is about real-time analysis for pollution prevention, and shows how an analytical technique, such as NMR, can contribute to reduce time, costs and pollution in an early-phase Active Pharmaceutical Ingredient's (API) manufacturing process.

For its low environmental impact and robustness NMR spectroscopy is a useful and low time-consuming technique to monitor scale-up reactions for both qualitative and quantitative purposes.



**GREEN CHEMISTRY**
design of chemical products & processes to reduce or eliminate the use & generation of hazardous substances

**Prevention**
It is better to prevent waste than to treat or clean up waste after it has been created.

**Atom Economy**
Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

**Less Hazardous Chemical Syntheses**
Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

**Designing Safer Chemicals**
Chemical products should be designed to affect their desired function while minimizing their toxicity.

**Safer Solvents and Auxiliaries**
The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

**Design for Energy Efficiency**
Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

**Use of Renewable Feedstocks**
A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

**Reduce Derivatives**
Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

**Catalysis**
Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

**Design for Degradation**
Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

**Real-time analysis for Pollution Prevention**
Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

**Inherently Safer Chemistry for Accident Prevention**
Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

**Figure 1.** The twelve principles of Green chemistry.

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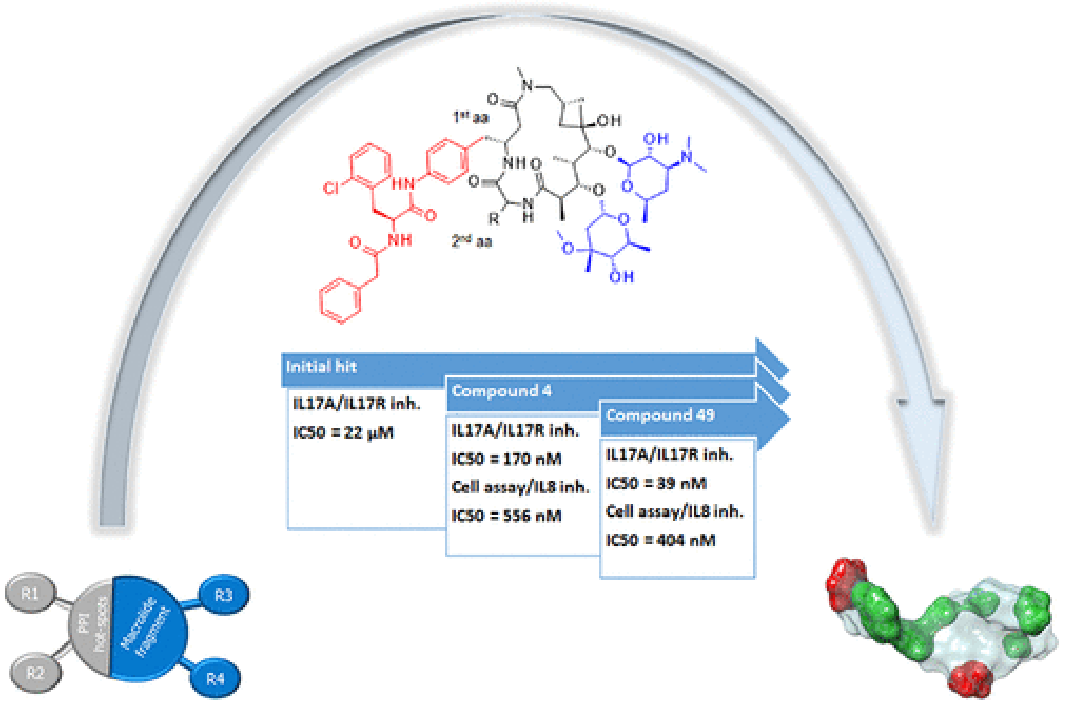
# MACROCYCLIC DISRUPTORS: THE NMR SIDE OF THE STORY

Ana Čikoš

Ruđer Bošković Institute, NMR Centre, Bijenička cesta 54, 10000 Zagreb, Croatia
ana.cikos@irb.hr

Interleukin 17A has a significant role in inflammatory response of the body, but also is involved in pathophysiology of autoimmune diseases, such as psoriasis. Protein-protein interactions (PPI) of IL-17A with its receptor IL-17RA are crucial for its activity. Due to their size, PPI targets are very difficult to inhibit using conventional small heterocyclic molecules. The aim of this project was to discover a macrocyclic disruptor which could modulate the IL-17A/IL-17RA binding. This talk is about the role NMR spectroscopy (coupled with computational methods) played at the very beginning of the project which resulted in an nM range inhibitor, as well as the explanation of its binding mode.[1]



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# NMR STUDIES OF AN ANTIMICROBIAL PEPTIDE: FROM *IN VITRO* TO *IN SITU*

Frances Separovic,[a,*] Vinzenz Hofferek,[b] Anthony Duff,[c]
Malcom J. McConville,[b] and Marc-Antoine Sani[a]

[a] School of Chemistry, Bio21 Institute, University of Melbourne, Melbourne, VIC 3010, Australia
[b] Department of Biochemistry and Molecular Biology, Bio21 Institute of Molecular Science and Biotechnology, University of Melbourne, Melbourne, VIC 3010, Australia
[c] National Deuteration Facility, Australian Nuclear Science and Technology Organisation, Kirrawee DC, NSW 2232, Australia
msani@unimelb.edu.au

Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, their primary target. Such studies are important to provide high-resolution details with a model membrane system but correlation with *in vivo* situations remains speculative, especially in view of the complex modulations observed with slight changes in sample conditions (pH, temperature, lipid composition or peptide concentration). Studying AMPs in live bacteria is, therefore, attractive but presents several challenges, such as sample longevity.

Using dynamic nuclear polarization (DNP) enhanced solid-state nuclear magnetic resonance (ssNMR), we report the impact of an antimicrobial peptide (AMP), on lipid membrane and macromolecular components (i.e., proteins and nucleic acids) of *Escherichia coli*. Global scanning of the cellular components was achieved by monitoring the nitrogen ($^{15}$N) signals by cross polarization (CP) magic angle spinning (MAS) NMR of whole bacteria grown in isotopically enriched media ($^{15}$N, $^{13}$C and $^{2}$H 98% enriched isotopes). The different $^{15}$N chemical shifts of cellular components served as an atomic marker for monitoring the action of the AMP maculatin 1.1 on *E. coli* (Fig. 1). The enhanced $^{15}$N ssNMR signals from nucleic acids, proteins and lipids identified a number of unanticipated physiological responses to peptide stress, revealing that membrane-active AMPs can have a multi-target impact on bacterial cells.

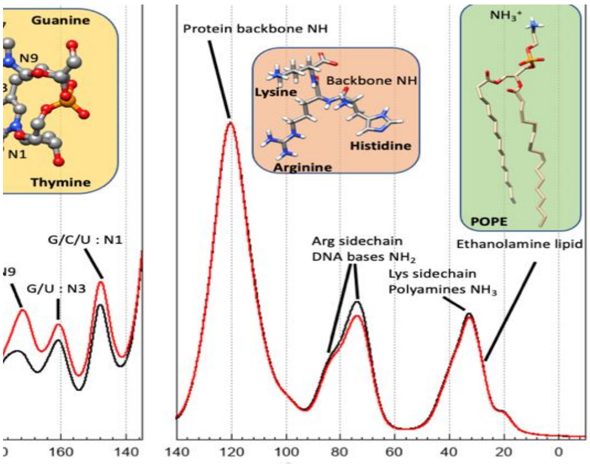

**Figure 1** DNP-enhanced $^{15}$N CPMAS spectra of untreated *E. coli* cells (black line) and in the presence of Mac1 at 15:1 w/w ratio (red line). The left panel is scaled 4-fold compared to the right panel to increase visibility. The DNA bases, amino acids with nitrogen containing sidechains and the phospholipid palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) structures are displayed in the inserts with nitrogen (blue), oxygen (red), phosphorous (orange), carbon (grey) and hydrogen (white) atoms.

# ADVANCED NMR APPROACHES FOR DRUG DISCOVERY

Julien Orts

University of Vienna, Austria
✉ julien.orts@univie.ac.at

X-ray crystallography molecular replacement (MR) is a highly versatile tool for the detailed characterization of lead compound and binding modes in the pharmaceutical industry. The two major limitations of its application to drug research are (i) the availability of a similar protein structure, and (ii) obtaining well-diffracting crystals of the ligand-protein complexes of interest. While nowadays the first point is often not a limitation anymore, obtaining well-diffracting crystals might be difficult. In such situations structure determination of protein-ligand complexes by liquid-state NMR is a good option. Unfortunately, the established standard structure determination protocol is in general time-consuming, and a shortcut using available structural data as in the case of MR in X-ray crystallography is not available.

Here, I present *N*MR$^2$ (**NMR M**olecular **R**eplacement), a MR-like approach in NMR to determine the structures of the binding pockets of ligands at atomic resolution. The calculation of structures of protein-ligand complexes relies on the collection of unassigned semi-quantitative inter-molecular NOE distance restraints and on previously solved structures.[1] The *N*MR$^2$ method uses a high throughput structure calculation protocol, rather than a docking-scoring simulation. It is fast since it requires only a few days of measuring time and bypasses the time-consuming sequential assignment steps for the protein.

I will present multiple *N*MR$^2$ applications covering several ligand topologies ranging from peptidomimetic to small molecules that bind strongly or weakly to protein receptors. We also report how *N*MR$^2$ can make use of partially labelled protein using methyl-specific isotope labelling. Our findings demonstrate that *N*MR$^2$ may open an avenue for the fast and robust determination of the binding pocket structure of ligand-protein complexes at atomic resolution.

In the second part, I will present our last findings regarding our effort towards the development of a PET tracer for amyloids fibrils.

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# MULTINUCLEAR MAGNETIC RESONANCE IN FOOD COMPOSITION AND QUANTITATIVE ANALYSIS.

Andrés Moreno, Covadonga Lucas-Torres, María Moreno, Milad Hadidi,
Diego J. González-Serrano, José C. Orellana

Departamento of Química Inorgánica, Orgánica y Bioquímica, Área de Química Orgánica. Universidad de Castilla-La Mancha. Facultad de Química, Avenida Camilo José Cela 10, 13071. Ciudad Real, Spain
✉ andres.moreno@uclm.es

NMR spectroscopy is emerging as an alternative analytical tool in several applied fields, including Food Science.[1,2] With NMR, it is possible to identify many compounds in a complex mixture simultaneously and non-destructively. Very often, the mixture can be analyzed with minimal or no treatment, which is a very desirable feature in terms of speed of the analysis, recovery of the analyte, and operator time. Quantification of single constituents in mixtures is also possible through integration of the area of the NMR signal. One of the most serious drawbacks of this technique is its scarce sensitivity, although great strides have been accomplished in the recent years.

“Manchego”, a popular Spanish cheese, is produced from pasteurized or raw dairy milk according to a regulation approved by the European Union. The cheese has a protected denomination of origin (PDO) mark, which strictly defines the geographical area of its production.

NMR spectroscopy allows to establish a quantification methodology of each of the components found in different cheeses, using an internal standard and/or a derivatizing agent.

Moreover, in this oral communication an NMR study of other food as, wine and olive oil will be presented.

This research marks the first characterization of some food with DO “La Mancha” using NMR spectroscopic analysis. The global analysis of metabolites in wine could provide useful information on different quality markers of red wine from Castilla-La Mancha region. This study further demonstrates the possibility of NMR-based metabolomic research to characterize wine quality and applied fermentation methods and product origin.

The study demonstrates the efficiency of the 31P-NMR technique to detect and quantify DG and other minor components of olive oil and provides a new way to detect oxidized olive oils. Also, in order to apply the study to the quotidian chemistry, important differences between thermal and microwave oxidation were found.

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# CAN NMR SPECTROSCOPY PAVE THE WAY FOR COMMUNITY-BUILT ANALYTICAL SYSTEMS?

Vito Gallo[a,b]

[a] Politecnico di Bari, DICATECh, via Orabona 4 – CAMPUS, I-70125, Bari, Italy
[b] Innovative Solutions S.r.l., Zona H 150/B, I-70015, Noci (BA), Italy
vito.gallo@poliba.it

NMR spectroscopy is gaining ever-growing importance in analytical chemistry. In the last decade, quantitative NMR (qNMR) and non-targeted approaches allowed for great improvements in both quantification of molecules in complex mixtures and identification of product features in suitable sample pools. Metrological traceability of products can be successfully achieved by qNMR even when no certified reference materials are commercially available. Along with quantification and purity assessment of molecules, NMR is emerging also as a powerful tool in food chemistry, especially when searching for product features such as cultivar, geographical origin, typicality of the production process, etc.

The great application potential of NMR spectroscopy derives from the fact that, based on theory, the ratio between a signal generated by the molecule under investigation and the signal of a reference molecule depends exclusively on the corresponding mole ratio. In other words, when a given sample is analyzed by different spectrometers, the same output is obtained in terms of signal ratios independently of the hardware configuration. This offers the unique opportunity to develop community-built analytical systems capable of identifying sample features and quantifying a number of molecules.

In this presentation, based our previous studies carried out with different NMR spectrometers (300 to 700 MHz),[1,2] the first examples of community-built analytical systems will be shown. In particular, the case study of a data-driven grape juice identification system will be presented along with the advantages and the limitations of using non-targeted NMR analyses performed at different magnetic fields. Moreover, quantification of metabolites in grape juices by using a community-built calibration tool will be also shown.[3] The feasibility of NMR spectroscopy to generate statistically equivalent NMR signal ratios from a number of different spectrometers will be demonstrated also for other complex mixtures such as aqueous extracts of wheat and flour.[4] Finally, potential use of benchtop NMR in both quantification of standard molecules and valorization of food biodiversity will be introduced.

**Acknowledgements.** This work has been supported by Ministero dell'Università e della Ricerca (Program Agreement ACPR20_00189)

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# $^{31}$P NMR SPECTROSCOPY IN STEREOCHEMICAL ANALYSIS

Markéta Tichotová,[a,b] Aneta Ešnerová,[b] Lucie Tučková,[a] Ivana Císařová,[b]
Ondřej Baszczyňski,[b] Eliška Procházková[a]

[a] Institute of Organic Chemistry and Biochemistry, the Czech Academy of Sciences, 16000 Prague, Czech Republic
[b] Faculty of Science, Charles University, 11628 Prague, Czech Republic
prochazkova@uochb.cas.cz

Phosphorus is a fascinating element playing a crucial role in nature. It is a key component in nucleic acids (DNA, RNA), in energy storage (ATP/ADP system), or signal transduction. The P-containing compounds are also important in medicinal chemistry, e.g. nucleoside prodrugs with antiviral effects or drug delivery systems based on phosphate self-immolative linkers. The compounds with chiral center on phosphorus atom, so called P-chirogenic molecules, are crucial for enantioselective catalysis and in coordination chemistry. The correct determination of stereochemistry (configuration, conformation) is substantial for structure-activity relationship studies.

$^{31}$P isotope is a magnetically active nucleus with a high natural abundance, therefore, incorporation of $^{31}$P NMR parameters into the analysis can provide the key structural information when the conventionally used NMR methods based on $^{1}$H, $^{13}$C NMR parameters fail.

In this work, we prepared model P-containing compounds, extracted $^{31}$P NMR parameters, such as chemical shifts and internuclear interactions, and correlated them with quantum-chemical calculations. $^{31}$P chemical shift is rather sensitive to even minor structural changes (e.g. solvation), which may be confusing in some cases. Spin-spin interactions of $^{31}$P with the neighbouring nuclei ($^{1}$H, $^{13}$C) provided the key connectivity and geometry information due to Karplus-like dependence of *J*-couplings on dihedral angles. Residual dipolar couplings were investigated as additional parameters used to obtain the stereochemical information. [1]

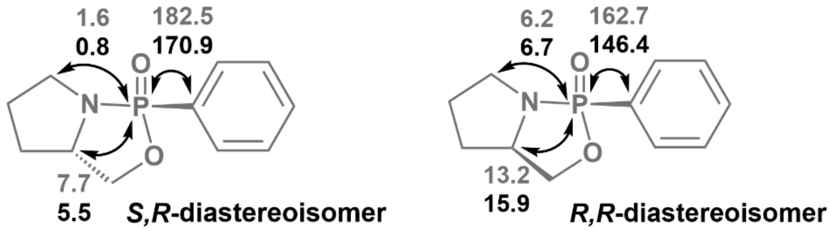

**Figure 1.** Experimental (grey) and DFT-calculated (black) $^{13}$C-$^{31}$P *J*-couplings used for determination of relative configuration. The absolute configuration was confirmed by X-ray diffraction data.

**Acknowledgements.** This work has been supported by the Czech Science Foundation (grant No. 21-23014S).

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# STRUCTURALLY FORCED ION BINDING AFFINITY IN A UREA-BASED MACROCYCLE

Petra Cuřínová,[a] Fabricio Ramírez Cortéz,[b] Václav Eigner,[b] Michal Himl[b]

[a] Institut of Chemical Process Fundamentals of CAS, v.v.i, Rozvojová 135, 165 02 Prague 6, Czech Republic
[b] University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic
curinova@icpf.cas.cz

Urea-based receptors are commonly used as potent supramolecular tools for anion recognition.[1,2] Here we report on a cyclization-driven change of complexation preferences of a urea-based receptor. Using $^1$H NMR complexation studies, an unexpected conformation of the receptor was proposed with an electron-rich cavity formed by urea oxygens and hydrogen bond donating outer part of the macrocycle. This unique structure gives the receptor selectivity for heavier alkali metal cations, rubidium and caesium. For suitable ion pairs the obtained macrocycle functions as a ditopic receptor, causing the formation of infinite hydrogen-bound chains. Similar structures were also confirmed in the gas phase and in the solid state using high resolution mass spectrometry and X-ray crystallography, respectively.

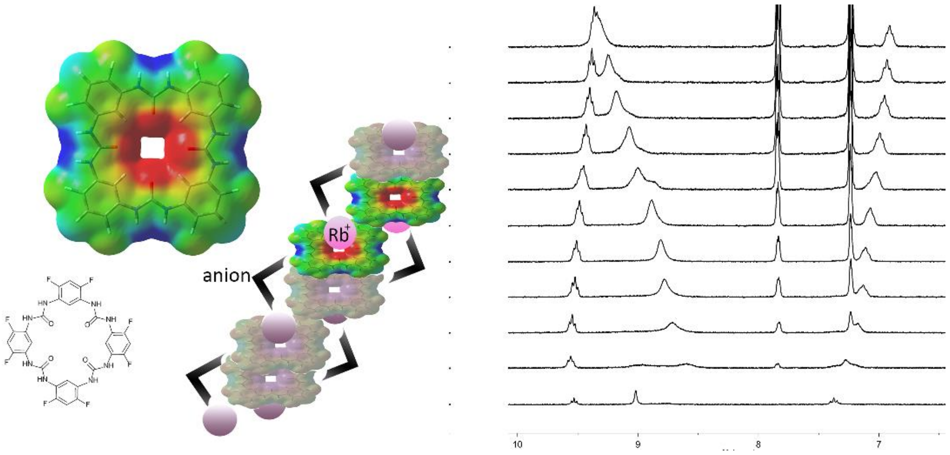

**Figure 1.** A urea-based ditopic receptor, left: electron density distribution in the macrocycle structure (DFT) and schematic structure of the complex with rubidium benzoate, right: $^1$H NMR titration with rubidium benzoate.

**Acknowledgements.** This work has been supported by the Czech Science Foundation grant number 20-07833S.

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# DETERMINING MOLECULAR CONFORMATION IN ORGANIC CRYSTALS USING COMBINED SOLID STATE NMR – CRYSTAL STRUCTURE PREDICTION (CSP) APPROACH

Marta K. Dudek, Piotr Paluch

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112,
90-363 Lodz, Poland
mdudek@cbmm.lodz.pl

Many organic crystals are notoriously challenging to characterize structurally at the atomic level due to their tendency to crystallize as microcrystalline powder. Here, we demonstrate the benefits and limitations of using crystal structure prediction (CSP) calculations in combination with solid-state NMR spectroscopy to fully describe crystalline structures of so far uncharacterized polymorphic forms of pharmaceutical-like systems. Such molecules usually display fairly high level of flexibility, resulting in a large number of possible molecular conformations which have to be accounted for in a CSP search.[1] While solid-state NMR spectroscopy can successfully assist in limiting this search space, a bunch of ambiguities in the interpretation of experimental data often lessens its usefulness. We show how a combined theoretical and experimental approach used in a step-by-step manner helps in circumventing the disadvantages of each of the methods alone. With it, we were able to describe polymorphic forms of furazidin and meloxicam,[2] as well as of two new cocrystals of linezolid (Figure 1a) and of a monohydrate of a cyclic dipeptide (Figure 1b). Using this set of examples, together with cases in which the CSP-NMR approach failed to deliver a decisive answer, lessons can be learned about the existing boundaries and weak points of this method.

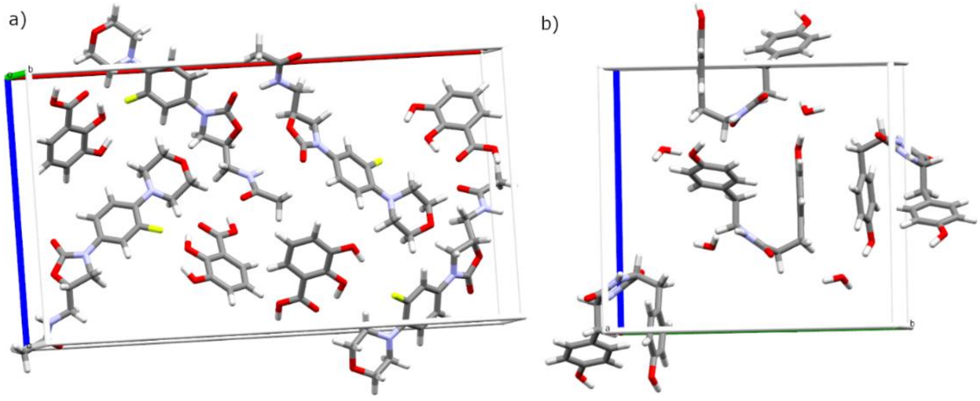

**Figure 1.** Crystal structures of linezolid:2,3-dihydroxybenzoic acid cocrystal (a) and a monohydrate of cyclic-L-Tyr-L-Tyr dipeptide revealed by combined solid-state NMR – crystal structure prediction approach.

**Acknowledgements.** This work was financially supported by Polish National Science Center (UMO-2018/31/D/ST4/01995). PL-GRID is gratefully acknowledged for providing computational resources.

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# TEMPERATURE COEFFICIENTS AS A TOOL FOR SPECTRAL ASSIGNMENT

Ewa K. Nawrocka,[a,b,*] Mateusz Urbańczyk,[b,c] Kamil Koziński,[b] Michał Jadwiszczak,[b] Piotr J. Leszczyński,[b] Krzysztof Kazimierczuk[b]

[a] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-089 Warsaw, Poland
[b] Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland
[c] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
* e.nawrocka@cent.uw.edu.pl

Assignment of NMR spectra is needed to solve various problems e.g. to identify compounds in metabolomic mixtures or to confirm the structures of new compounds after chemical synthesis. 1D and 2D NMR provide information about chemical shifts and correlations. However, some ambiguities in assignment process may be still present. Thus, having more nuclei-specific spectral parameters would be useful.

In protein studies, researchers often measure temperature coefficients (TCs), i.e. the rates of change of chemical shifts with temperature. They indicate the exposure of amide protons to solvent exchange, presence of low-populated excited states and other phenomena. We show, that TCs can be used in the analysis of small molecules, such as metabolites,[1] and to support the spectral assignment of groups of similar compounds.

Therefore, we propose simple $^{1}$H variable-temperature (VT) measurements whose time can be the same or even shorter than the those of the conventional 2D spectra. We present two approaches: 1) TCs for metabolites identification in natural mixture (plasma) based on serum's TCs and 2) TCs from one assigned molecule (Figure 1A) transferred to another, very similar molecule. We show, that TCs are consistent and reproducible between samples. For metabolomics studies (Figure 1B), combining with Radon transform processing[2] provided extra increase in signal-to-noise ratio. We believe that in future, it might be one of the trustworthy stages of analysis.

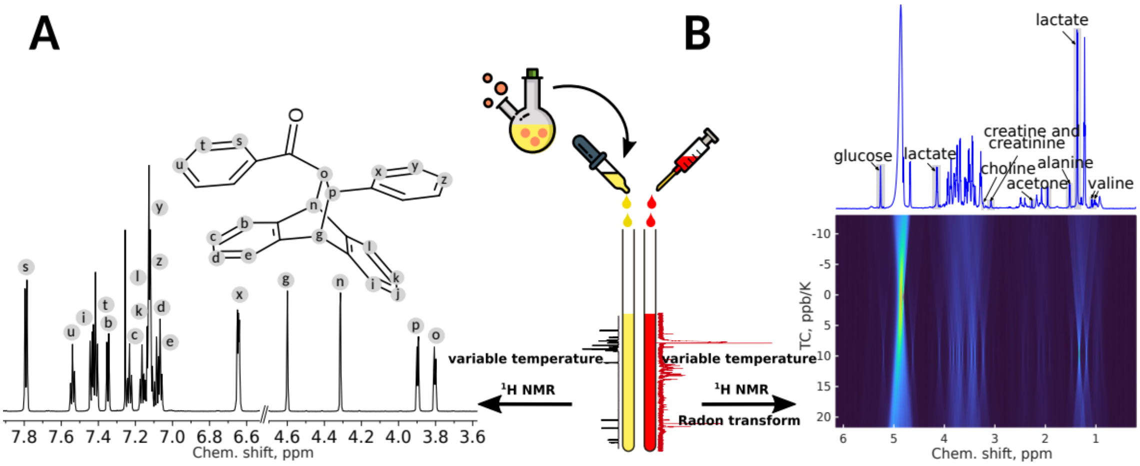

**Figure 1.** Usefulness of variable temperature $^{1}$H NMR in spectral assignment for organic synthesis (A) and metabolomics (B).

**Acknowledgements.** This work has been supported by the National Science Centre (OPUS grant 2019/35/B/ST4/01506 and OPUS 18 grant 2019/35/B/ST5/04528).

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# STABILITY OF TRIARYLAMINE RADICAL CATIONS STUDIED BY IN-SITU VOLTAMMETRY WITH ELECTRON PARAMAGNETIC RESONANCE (EPR)

Ján Tarábek, Denisa Hidasová, Tomáš Slanina

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, Prague, Czech Republic
jan.tarabek@uochb.cas.cz

Triarylamines are one of the most prominent group in organic electronic applications such as light-emitting diodes or solar cells in which they usually act as a hole-conducting/transporting material.[1] They can undergo one-electron oxidation (chemically, electrochemically or even photochemically) forming relatively stable radical cation, that can serve as an oxidant in the additional reactional pathways.[2,3] The crucial role of radical cation is affected by the follow-up reactions like dimerization. Substituents like *tert*-butyl on aromatic rings may dramatically hinder the dimerization via σ-bond character. Moreover, the propeller-conformation of aromatic rings suppress the stacking of radical cationic species (e.g. π-dimerization). Otherwise, with planar structure stacking appears to be very likely present. However, also radical cations with almost planar configuration or slightly helical can exhibit quite high stability, even higher than those for propeller-shaped triarylamines. This will be shown by the results obtained via combined *in-situ* voltametric EPR technique as the dimerization gives rise to quenching of the radical cations (see Figure 1) and intensity decline of the corresponding EPR spectra. Therefore, the stability/reactivity can be characterized by the kinetic rate constants, accordingly.

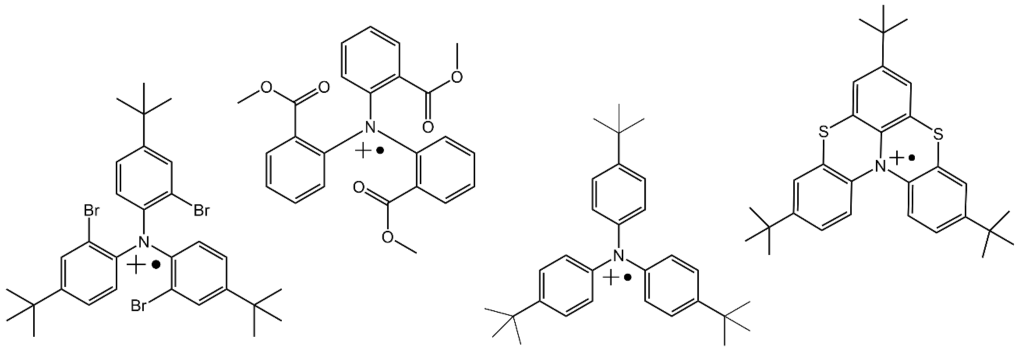

**Figure 1.** Structure of triarylamine radical cations as a subject of the combined in-situ voltametric EPR (Electron Paramagnetic Resonance) stability studies.

**Acknowledgements.** This work has been supported by the Czech Science Foundation Project 19-20467Y and by the Czech Academy of Sciences RVO-61388963

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# SUPERVISED CLASSIFICATION METHODS IN NMR FOR RESONANCE MAPPING OF IDPS

Javier A. Romero, Paulina Putko, Krzysztof Kazimierczuk, Anna Zawadzka-Kazimierczuk

Centre of New Technologies, Stefana Banacha 2c, Warsaw, Poland
j.romero@cent.uw.edu.pl

Machine Learning (ML) methods are being used more and more in every branch of science, and NMR is no exception. Famous examples include deep neural networks for peak picking and spectral deconvolution [1] and AUTOMAP for image reconstruction in MRI [2]. But as the field of ML keeps expanding and gaining worldwide recognition, its methods and techniques are being incorporated into every-day research tasks. I will present the case of resonance assignment in Intrinsically Disordered Proteins (IDPs). IDPs play an essential biological role, but their research is challenging. The sequential assignment procedure can be particularly difficult since the set of heteronuclear spectra used are usually crowded and signals overlap. Therefore, the chains of sequentially-linked residues are short and to map them on the protein's sequence one has to efficiently recognize which amino-acid types the residues correspond to. This is essentially a classification problem: we aim at assigning amino acid types to resonance signals (residues).

I will show how the mapping process can be assisted, to the point of almost fully automation, by basic ML classification methods. In particular, I will present Linear Discriminant Analysis (LDA) and its close relative Quadratic Discriminant Analysis (QDA), along with K-Nearest Neighbors (KNN) and Support Vector Machines (SVM). I will show how each of these methods can be used for protein mapping, how to choose a consistent training set and define criteria to choose which is the best method for classification.

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# METABOLIC NMR WITHOUT THE MAGNET

James Eills,[a,b,c] Román Picazo-Frutos,[b,c] Oksana Bondar,[d] Eleonora Cavallari,[d]
Carla Carrera,[e] Sylwia Barker,[f] Marcel Utz,[f] Silvio Aime,[d] Francesca Reineri,[d]
Dmitry Budker,[b,c,g] John W. Blanchard[h]

[a] Institute for Bioengineering of Catalonia, Barcelona, Spain
[b] Johannes-Gutenberg University, Mainz, Germany
[c] Helmholtz-Institute Mainz, Mainz, Germany
[d] Center of Molecular Imaging, University of Turin, Turin, Italy
[e] Institute of Biostructures and Bioimaging, Turin, Italy
[f] University of Southampton, Southampton, U.K.
[g] University of California at Berkeley, Berkeley, U.S.A.
[h] NVision Imaging Technologies, Ulm, Germany
✉ jeills@ibecbarcelona.eu

Zero- to ultralow-field (ZULF) NMR is a modality of NMR experiment performed in the absence of a strong magnetic field. In this regime, Larmor precession is suppressed, and other interactions such as J-couplings dominate. This grants three important advantages: the low frequency signals readily penetrate metals and conductive materials, magnetic susceptibility-induced line broadening from sample inhomogeneity is suppressed, and no bulky superconducting magnet is needed. In this work we form the biomolecules [1-$^{13}$C]fumarate and [1-$^{13}$C]pyruvate in aqueous solution via parahydrogen-induced polarization. We acquire the low-frequency ZULF signals using optical magnetometers, with Helmholtz coils surrounding the sample to apply magnetic field pulses. This is all contained within a mu-metal shield to attenuate Earth's field. With this apparatus we are able to acquire zero-, ultralow-, and low-field spectra. The conversion of fumarate into malate, and pyruvate into lactate, are metabolic processes observed *in vivo* in hyperpolarization-enhanced magnetic resonance imaging experiments.[1,2] We show that ZULF NMR can be used to study metabolism by observing these two biochemical reactions. This work paves the way to a heretofore unexplored class of biomedical imaging applications.

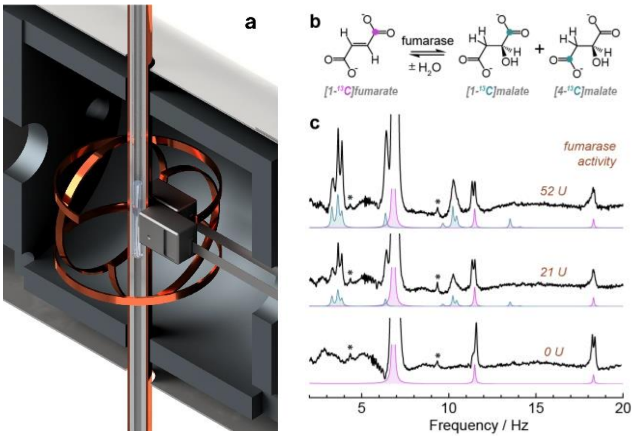

**Figure 1.** (a) The ZULF setup used in this work. (b) The transformation of fumarate into malate. (c) Zero-field spectra of hyperpolarized [1-$^{13}$C]fumarate after addition of the enzyme fumarase (given in enzyme units U), with simulated fumarate and malate spectra beneath.

**Acknowledgements:** This work has been supported by European Union's Horizon 2020 Research and Innovation Programme.

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# NMR-BASED METABOLOMIC ANALYSIS OF BLOOD PLASMA AS A PANCREATIC CANCER DIAGNOSTIC TOOL

Lenka Michálková,[a,b] Štěpán Horník,[a] Vladimír Setnička,[b] Jan Sýkora,[a] Bohuš Bunganič[c]

[a] Institute of Chemical Process Fundamentals of the CAS, Prague, Czech Republic
[b] Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic
[c] Department of Internal Medicine, 1st Faculty of Medicine of Charles University and Military University Hospital, Prague, Czech Republic
michalkova@icpf.cas.cz

Pancreatic cancer (PC) is well known for high mortality, the 5-year survival reaches only 5 %. This is mainly caused by asymptotic course or unspecific symptoms. The poor prognosis can be improved by early diagnosis. In this context, the relationship of PC to diabetes mellitus should be investigated as diabetes or impaired glucose tolerance had been observed in 80 % of PC patients. This specific type of diabetes mellitus is characterized with increased risk of PC development and it is called pancreatogenic diabetes (T3cDM). Since its development is similar to the most prevalent type 2 diabetes mellitus (T2DM), T3cDM is often misdiagnosed. T3cDM patients are associated with an up to 7-fold increased risk of PC development. [1] The differentiation of T3cDM-T2DM among recent-onset diabetes mellitus (RODM) patients may play crucial role for the early PC diagnosis and thus improving the prognosis of this fatal disease. NMR metabolomics may facilitate the solution of this complicated problem of recent clinical diagnosis. [2,3]

In this work, $^1$H NMR metabolomic analysis of blood plasma was used as an alternative approach for early PC diagnosis in RODM patients. Based on concentration profile of 58 metabolites, a discrimination of PC patients from long-term T2DM patients was achieved. A specific biomarker panel of eight metabolites was proposed and successfully tested for PC diagnosis against T2DM patients and healthy controls. Moreover, a prediction model for the identification of risk individuals for PC development in RODM group was developed and the patients likely suffering from pancreatogenic diabetes were identified. Six of 59 RODM patients had similar metabolic characteristics as PC patients and their health conditions were therefore re-examined. The found pathological changes correlated reasonably with our findings. Recent results also indicated subtle metabolic changes among individual PC clinical stages that could be used for their differentiation in future.

**Acknowledgements:** This work has been supported by TK02010035 project provided by Technology Agency of the Czech Republic.

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# NMR AEROSOLOMICS: AN OVERVIEW

Štěpán Horník,[a] Petr Vodička,[a] Petra Pokorná,[a] Jaroslav Schwarz,[a] Vladimír Ždímal,[a] Jan Sýkora[a]

[a] Institute of Chemical Process Fundamentals of the CAS, v. v. i., Prague, Czech Republic
✉ hornik@icpf.cas.cz

NMR Aerosolomics offers a different analysis approach of water-soluble organic compounds (WSOCs) from complex aerosol mixtures, inspired by the profiling of substances in metabolomics. To this end, an extensive library of compounds commonly found in atmospheric aerosols was created. The method was evaluated on a set of 21 samples of aerosol particulate matter ($PM_{2.5}$ and $PM_{10}$) collected in summer 2008 and winter 2009 at a Prague suburban site. Approximately 60 compounds were identified in each sample. Three compounds were identified for the first time in atmospheric aerosols and the presence of four other theoretically predicted species was confirmed. In total, the number of identified compounds reached 79. The concentration values of the identified compounds were then used for statistical analysis. A clear differentiation between summer and winter samples was obtained, while separation by particle size was indicated only in the group of summer samples. Univariate statistical analysis revealed which compounds were responsible for the group separation, and possible sources for these compounds were suggested.[1]

In addition, the method was used in WSOC analysis of size-resolved aerosol particles collected by a 6-stage high-volume cascade impactor collected during summer and winter resulting in four sample series. In each sample, 31-45 compounds were identified, for a total of 73 compounds. Distribution profiles were used to reveal associations between individual compounds and to identify associations with probable sources. The results were supported by multivariate statistical analysis.[2]

Unlike other methods used for the analysis of WSOC in atmospheric aerosols, NMR aerosolomics allows the simultaneous determination of several groups of WSOC. The method offers great potential for source apportionment, which is an important issue in contemporary aerosol science.

**Acknowledgements.** This work has been supported by Technology Agency of the Czech Republic project (TK02010035); by the infrastructure project of the MEYS of the Czech Republic ACTRIS-CZ (grant No. LM2018122); and by the Czech Science Foundation, grant No. 20-08304J.

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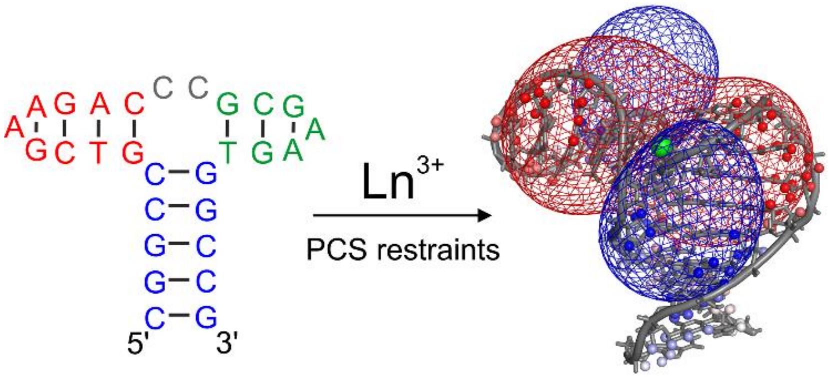
# LANTHANIDE BINDING APTAMER AS A TOOL FOR PARAMAGNETIC NMR SPECTROSCOPY OF NUCLEIC ACIDS

Witold Andrałojć,[a] Julia Wieruszewska,[a] Karol Pasternak[a], Zofia Gdaniec[a]

[a] Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland
wandralojc@ibch.poznan.pl

NMR spectroscopy is among the primary techniques used for high resolution structural studies of nucleic acid systems. Classical approaches to biomolecular NMR structure determination rely of the measurement of inter-proton distances up to 5-6 Å, through the means of the Nuclear Overhauser Effect (NOE). The local nature of these distance restraints can lead to a poor reproduction of some global structural features of the studied molecule (e. g. the degree of helical bending). Long-range NMR structural restraints derived from the presence of a paramagnetic ion in the studied system can radically change this situation and have already found much success in NMR studies of proteins, beyond just structure determination[1]. However, the potential of paramagnetic NMR methods remains largely unrealized for nucleic acids due to lack of general methods to rigidly and site-specifically introduce paramagnetic ions into these systems.

Here we present the high-resolution NMR structure of a lanthanide-binding DNA aptamer and demonstrate that lanthanide ions bound to this system induce large magnitude paramagnetic effects (pseudocontact shifts; PCS), readily interpretable in terms of structural parameters. We propose that the lanthanide binding motif uncovered for this system can be exploited to introduce lanthanide binding sites into a broad range of different DNA and RNA molecules to facilitate their structural and functional NMR studies. The presented structure is also of interest by itself, as it constitutes the first high-resolution structure of a metal-binding aptamer deposited in the PDB, furthering our understanding of high-affinity metal binding by DNA.



**Acknowledgements.** This work has been supported by the National Science Center (Poland) under grant agreements: [UMO-2020/37/B/ST4/03182] and [UMO-2018/31/D/ST4/01467] to W.A.. The calculations were performed at Poznań Supercomputing and Networking Center.

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# NOVEL HIGH-RESOLUTION STRUCTURAL MODELS OF MEMBRANE BOUND α-SYNUCLEIN

Thomas C. Schwarz,[a] Andreas Beier,[a] Karin Ledolter,[a] Thomas Gossenreiter,[b]
Theresa Höfurthner,[a] Markus Hartl,[b] Terry S. Baker,[c] Richard J. Taylor,[c] and Robert Konrat[a]

[a] Dept. of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Austria
[b] Mass Spectrometry Facility, Max Perutz Labs, Vienna BioCenter, Vienna, Austria
[c] UCB Pharma, 208 Bath Road, Slough SL1 3WE, United Kingdom
t.schwarz@univie.ac.at

α-synuclein (αS) is an intrinsically disordered protein (IDP) important in neurodegenerative disorders such as Parkinson's disease and Lewy body dementia.[1] It can adopt a large array of varying structures, some of which can form toxic aggregates.[2] These aggregates interact with cellular membranes and can disrupt them, leading to their incorporation in Lewy Bodies.[3] Despite regulated interaction with the membrane being crucial for αS functionality[4] the interaction has been shown to promote aggregation *in vitro.*[5] Although, high-resolution structural information has been obtained in the SDS-micelle bound state,[6] deriving structures from membrane bound αS has been hindered by the large variation in the structural ensemble and lower resolution techniques have indicated differing states of the protein and the oligomers it forms on membranes.[7,8] To obtain new insight into the structural parameters of membrane bound αS, we combine the use of NMR derived parameters obtained on SDS-micelle and bicelle bound αS with chemical crosslink mass spectrometry (XLMS) on $^{14}N/^{15}N$-labelled α-synuclein mixtures. In contrast to the available micelle bound structure, which focused on the use of nuclear Overhauser effects (NOEs) and residual dipolar couplings (RDCs),[6] our data relies predominantly on paramagnetic relaxation enhancements (PRE) and interference (PRI) measurements for long-range information. These measurements are very sensitive to compact substates of the ensemble, allowing us to detect novel conformations in the membrane bound ensemble of αS. We validate our findings by cross-checking the modeled structures with data obtained from XLMS and discuss their relevance in the context of known mutations and regions relevant for oligomer formation.

**Acknowledgements.** This work has been supported by UCB Biopharma SRL, the Christian Doppler Laboratory for High-Content Structural Biology and mass spectrometry measurements were performed using the Vienna BioCenter Core Facility instrument pool.

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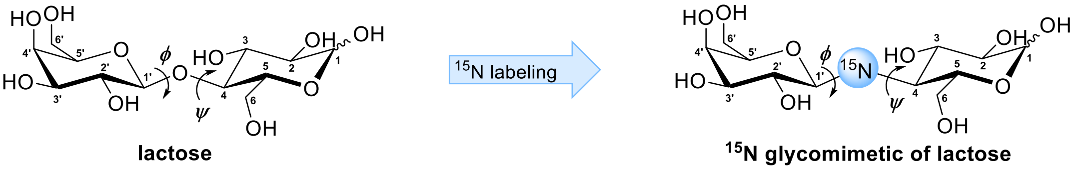
# $^{15}$N SPY IN CONFORMATIONAL ANALYSIS OF DISACCHARIDES

Radek Pohl, Nina Habanová

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic
pohl@uochb.cas.cz

The interaction of carbohydrate-based molecules with proteins is essential for life and controls e.g. fertilization, immune response, or defense against pathogens and cancer. This interaction is driven by the recognition of specific carbohydrate conformation with a protein. Therefore, the development of analytical tools for their conformation prediction both in a free and bound state is of great importance. However, the determination of their conformational properties in solution remains a difficult task due to the structural complexity and inherent flexibility of saccharides.

NMR represents the most used and versatile method in conformational analysis of saccharides and their non-natural derivatives – glycomimetics. *J*-Coupling as a representative of NMR parameters is especially suitable in the conformational analysis since it is sensitive to the local geometric arrangement of interacting nuclei upon structural change. In this contribution, we introduce $^{15}$N labeling directly in the glycosidic linkage, which offers redundant *J*-couplings useful in the conformational analysis (Figure 1). The $^{15}$N labeling is used for the first time, however, $^{13}$C ring labeling has been used for example in conformational analysis of methyl β-lactoside.[1]



couplings sensitive to $\phi$: $^{2}J_{H1',C2'}$, $^{3}J_{H1',C4}$
couplings sensitive to $\psi$: $^{2}J_{H4,C3}$, $^{2}J_{H4,C5}$, $^{3}J_{H4,C1'}$

couplings sensitive to $\phi$: $^{1}J_{C1',N}$, $^{2}J_{C2',N}$, $^{3}J_{C3',N}$, $^{3}J_{C5',N}$, $^{2}J_{H1',N}$, $^{3}J_{H2',N}$
couplings sensitive to $\psi$: $^{1}J_{C4,N}$, $^{2}J_{C3,N}$, $^{2}J_{C5,N}$, $^{3}J_{C2,N}$, $^{2}J_{H4,N}$, $^{3}J_{H3,N}$, $^{3}J_{H5,N}$

**Figure 1.** Redundant *J*-couplings across glycosidic linkage useful for conformational analysis.

The concept will be explained on bis(D-glucosyl)amine and bis(D-mannosyl)amine as examples of (1→1)-disaccharides. We will present in situ preparation of the disaccharides directly in an NMR tube, and their conformational analysis based on a combination of experimental NMR, molecular dynamics simulations, and DFT calculation of NMR parameters.

**Acknowledgements.** This work has been supported by the Czech Science Foundation – project 22-17586S.

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# DIKETO-KETOENOL TAUTOMERS IN CURCUMINOIDS

Zuzana Osifová,[a,b] Aleš Machara,[b] Martin Dračínský[b]

[a] Department of Organic Chemistry, Charles University, Prague, Czech Republic
[b] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
zuzana.osifova@uochb.cas.cz

Curcumin represents a class of natural drugs possessing wide range of pharmacological properties including anti-inflammatory and anti-oxidant activities.[1,2] Curcumin and its derivatives exist in an equilibrium between diketo and ketoenol tautomers. Each of the tautomeric states exhibits dissimilar potency to bind biomacromolecules which affects their pharmacological activities.[3]

In this work, we present comprehensive NMR studies of curcumin analogues in different solvents. We described equilibrium properties of curcumin and its 12 derivatives including pharmaceutical ingredient ASC-JM17 (**1**, Figure 1) in different solvents. Moreover, we separated two tautomers of ASC-JM17 on column chromatography and studied their equilibration in solution. Solid-state NMR and X-ray diffraction studies revealed two new polymorphs of ketoenol tautomer of ASC-JM17 (**1KE**, Figure 1).

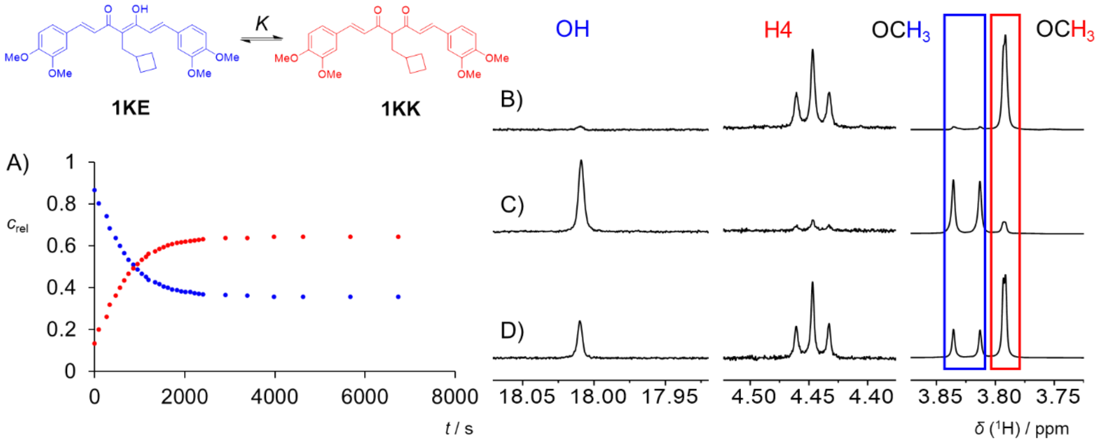

**Figure 1.** A) The keto-enol equilibrium of compound **1** and the time-dependence of relative concentration, $c_{rel}$, of **1KK** and **1KE** obtained from $^1$H NMR spectra after dissolution of the pure **KE** form. The OH, H4 and methoxy-group region of $^1$H NMR spectra of B) lyophilizate containing over 95 % of the **KK**, C) the crystalline **1KE** and D) the equilibrium mixture in $d_6$-DMSO at room temperature. Note that the first NMR experiment, $t$ = 0, was recorded about 3 minutes after dissolution of the compounds.

**Acknowledgements.** This work has been supported by Czech Science Foundation (grant no. 22-153745)

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# ON THE UTILITY OF NONUNIFORMLY SAMPLED TWO-DIMENSIONAL NMR SPECTRA IN THE PHARMACEUTICAL INDUSTRY

Áron Szigetvári,[a] Csaba Szántay Jr,[a]

[a] Spectroscopic Research Department, Gedeon Richter Plc., Budapest, Hungary
✉ szigetvaria@richter.hu

We present some intriguing examples of NMR structure elucidation – with a special pharmaceutical industrial flair. A common feature of these problems is that the use of nonuniformly sampled 2D NMR spectra substantially contributed to their successful solution. We discuss the difficulties that we encountered during the acquisition and interpretation of the 2D NMR spectra of these samples, and we suggest a novel and efficient way to overcome them.

The examples presented involve a) the determination of the constitution of a complex molecule; b) the unambiguous resonance assignment of a molecule consisting of several identical functional groups in different positions; c) the verification of the substitution pattern in aromatic rings when “routine” techniques fail due to severe signal overlap; d) proof of a four-site conformational exchange in a severely mass-limited sample; e) detection of trace impurities in a starting material used in the production of a drug substance. The theoretical background and the experimental conditions of the examples are discussed in detail in our recent publication.[1]

Áron Szigetvári’s PhD thesis (under preparation) is partly based on the presented topic.

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# POWER OF $^{11}$B NMR SPECTROSCOPY IN STUDIES OF HETEROBORANES

Jan Vrána,[a] Josef Holub,[b] and Aleš Růžička[a]

[a] Department of General and Inorganic Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic
[b] Institute of Inorganic Chemistry, Czech Academy of Sciences, 250 68 Řež, Czech Republic
ales.ruzicka@upce.cz

In the hundred-year-history of boron hydrides and their successors (polyhedral boranes, carboranes and other heteroboranes) and derivatives, the number, molecular shape, reactivity and applications of these species have become enormous, thus establishing a self-consistent field of chemistry. Areas of possible applications of these species ranging from energy/hydrogen storage, synthetic organic chemistry, catalysis and medicine to preparation of new materials.

In contrast to organic chemistry, the reaction mechanisms involving boranes and boron clusters can be very complex since there are very small energy differences between many intermediates and transition states, thus reactions of boron hydrides can involve many competing pathways.

In this work, we would like to illustrate the power of 1D and 2D $^{11}$B NMR spectroscopy for understanding numerous boron cluster transformations and structural properties.[1](Figure 1).

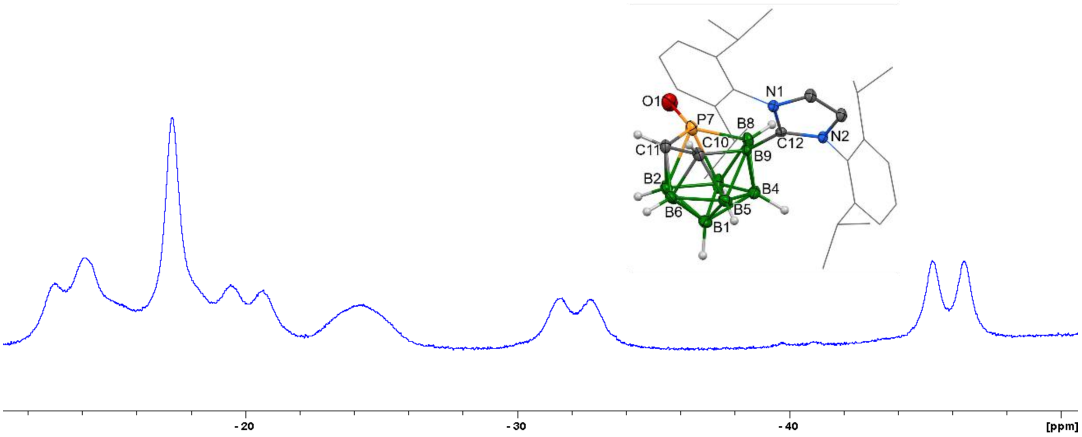

**Figure 1.** An example of $^{11}$B NMR spectrum and structure of shown compound.

**Acknowledgements.** Authors wish to thank the Czech Science Foundation grant nr. 22-03945S.

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# $^{31}$P NMR WITH *IN SITU* IRRADIATION FOR STUDY OF SELF-IMMOLATION

Markéta Tichotová,[a] Petr Šimon,[b] María García Gallardo,[b] Ondřej Baszczyňski,[a,b]
Eliška Procházková[a]

[a] Institute of Organic Chemistry and Biochemistry, The Czech Academy of Sciences, Flemingovo nám. 542/2, 166 10 Prague, Czech Republic
[b] Faculty of Science, Charles University, Hlavova 2030/8, 128 43 Prague, Czech Republic
marketa.tichotova@uochb.cas.cz

Self-immolation (SI) is a fragmentation of a molecule via intermolecular cyclization upon external stimuli. The activated intermediate spontaneously cyclizes while releasing a cargo which is used in a range of applications, such as smart materials or drug delivery systems. The phosphorus-based SI linkers stand above the "classical" carbamate linkers. They allow attachment of additional substituent, and, therefore, can be ideal candidates for a double cargo delivery.

We synthetized series of phosphate-based SI linkers able to release two cargos (Fig. 1). The SI was initiated by UV light, and the reaction course was monitored by $^{31}$P NMR spectroscopy with *in situ* irradiation in real time. The structure of the intermediates was determined directly *in situ* combining $^{31}$P and $^{13}$C NMR spectra. Structural modifications allowed us to drive the sequential release of two cargos.[1] This structure-activity relationship study enabled us to fine-tune the velocity of SI from 1 day up to 5 minutes, thus, greatly widened possible applications of the phosphate-based SI linkers. Moreover, these results were used for a rational design of new SI systems for a delivery of amine-containing drugs.[2]

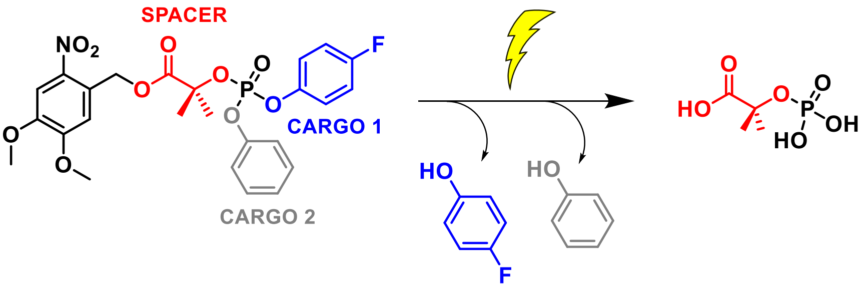

**Figure 1.** An example of a α-hydroxyisobutyrate SI spacer studied in this work.

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# PURE SHIFT NMR METHODS

Gareth A. Morris

University of Manchester, Manchester, UK
g.a.morris@manchester.c.uk

Homonuclear spin-spin couplings are simultaneously a great strength and a great weakness of NMR spectroscopy. A great strength because of the wealth of information they convey about chemical structure and stereochemistry, and a great weakness because the multiplet structure they cause greatly increases the frequency width of the NMR signals for a given chemical site. Multiplet structure is a particular challenge in $^1$H NMR because of the narrow (*ca*. 10 ppm) range of typical $^1$H chemical shifts, which means that all but the simplest species show spectra in which the multiplet signals from different chemical sites overlap. From the early days of $^1$H NMR it was recognized[1] that it would be immensely useful to be able to switch off the effects of homonuclear couplings, as it subsequently became possible to do for heteronuclear couplings with broadband decoupling, to give a $^1$H spectrum with just one peak per chemical site – a "pure shift" spectrum (see Figure 1). Over the last 20 years a series of developments[2-4] have made it possible to approach this ideal very closely, improving the resolution of $^1$H NMR by almost an order of magnitude. This tutorial lecture will describe the development of pure shift NMR methods, survey the current state of the art, and show some applications.

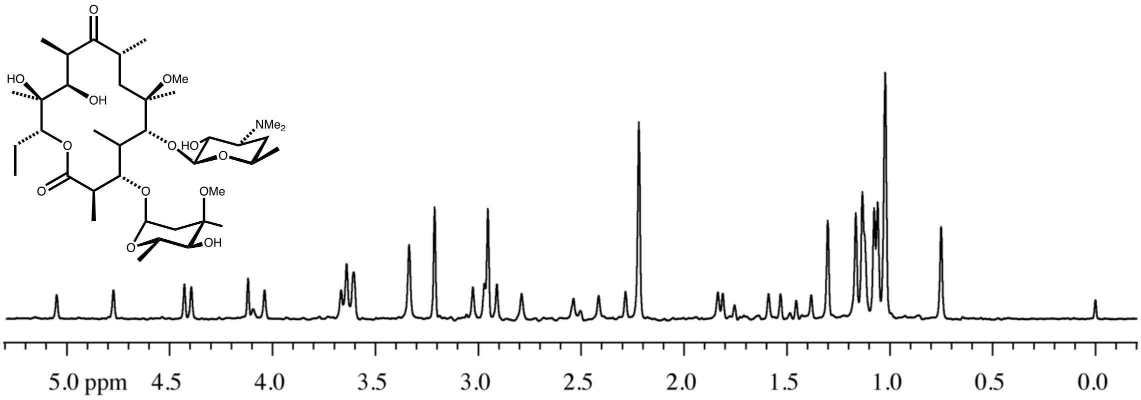

**Figure 1.** 500 MHz $^1$H NMR spectrum of the antibiotic clarithromycin.

**Acknowledgements.** This work has been supported by the Engineering and Physical Sciences Research Council.

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# TUTORIAL ON SELECTIVE EXCITATION

Klaus Zangger

Institute of Chemistry, University of Graz, Graz, Austria
klaus.zangger@uni-graz.at

Most radiofrequency pulses used in Fourier transform NMR experiments rely on a uniform excitation of all signals across the whole spectral window. However, for several reasons the selective excitation of individual signals or spectral regions is necessary. A number of techniques have been established to achieve such a frequency selective excitation, using either trains of high power (hard) pulses or shaped low power (soft) pulses.[1,2] This tutorial talk describes the use of selective pulses for various applications like excitation, refocusing, transforming multidimensional spectra to 1D counterparts or restricting the magnetization transfer pathway. With the use of selective pulses, homonuclear experiments can be carried out in a way that is otherwise only possible in heteronuclear spectroscopy.

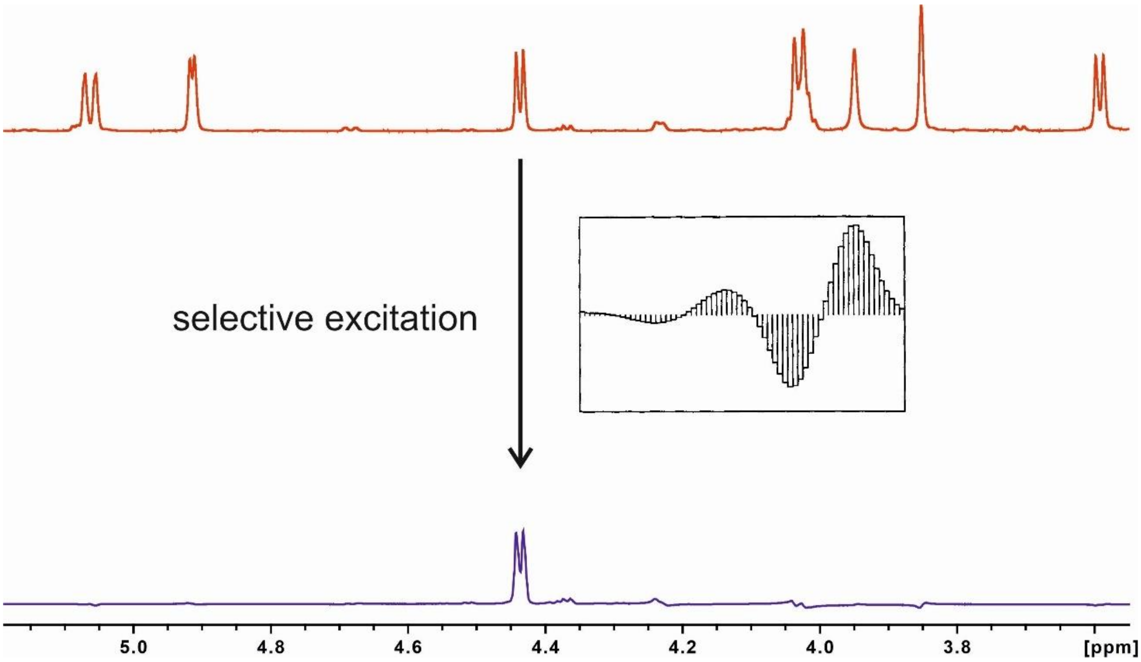

**Figure 1.** Selective excitation

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# EVALUATION OF THE BENEFIT AND INFORMING CAPABILITY OF 2D NMR EXPERIMENTS FOR COMPUTER-ASSISTED STRUCTURE ELUCIDATION

Dimitris Argyropoulos and Mikhail Elyashberg

Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, Canada
✉ dimitris.argyropoulos@acdlabs.com

The most significant improvement in the 50-year history of Computer-Assisted Structure Elucidation (CASE)[1,2] came after the introduction of routine 2D NMR experiments in the 1990s. The increased information content of these experiments allowed substantially more complex problems to be addressed than ever before. With the simultaneous advances in computing, CASE has now become an established tool for resolving the unprecedented structures of natural products.[3] Today, further advancements in CASE involve the evaluation of advanced NMR experiments and the determination of the minimum set of experiments required to solve a structure. Although these answers vary with problem complexity, we begin to address both questions through a few examples in this poster.

In the first example, we use the very simple molecule 2-Ethylindanone to examine the benefits of basic NMR experiments and their effect on calculation time. Then, we examine the benefits and potential consequences of using modern experiments like LR-HSQMBC, in addition to the traditional HSQC and HMBC using the complex natural product Spirodactylone.[4] The influence of manually adjusting atom properties (e.g., hybridization and connection to heteroatoms) is also examined using this molecule. Finally, a xanthone-class natural product[5-6] is used to consider the inter-relation between HMBC and INADEQUATE in CASE.

Details of spectra, calculations, and a comparison of the achieved improvements in performance will be shown.

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# UNDERSTANDING THE ATTRACTION BETWEEN HYDROPHOBIC CAVITIES AND BENZENE DERIVATIVES

Tea Babić, Andrea Usenik, Katarina Pičuljan, Nikola Bedeković, Josip Požar

Department of Chemistry, Faculty of Science, University of Zagreb,
Horvatovac 102a, 10 000 Zagreb, Croatia
tea.babic@chem.pmf.hr

Amongst macrocyclic receptors, cucurbit[7]uril (CB7) and β-cyclodextrin (β-CD) stand out as good receptors for hydrophobic species in aqueous solutions.[1] In contrast to the frequently investigated hosting of aliphatic compounds, the inclusion of aromatic guests within these hosts is far less explored. Taking this into account, we have studied the complexation of various benzene derivatives with CB7 and β-CD in water by means of isothermal titration microcalorimetry and in a wide temperature range (5−65 °C). ROESY and $^1$H NMR experiments were carried out to investigate the inclusion of 4-*tert*-butylphenol into the host cavities. The inclusion of guests within the CB7 cavity in vacuo was also studied by performing DFT calculation using the bp86/tzvp level of theory. The examined host-guest systems exhibited distinct $\Delta_r H°$ and $\Delta_r S°$ temperature dependence, resulting in weak variation of standard Gibbs energy with temperature. The complexation thermodynamics at lower temperatures was in agreement with the classical interpretation of the hydrophobic effect, whereas the binding at higher temperatures was in line with the non-classical rationale. [1,2]

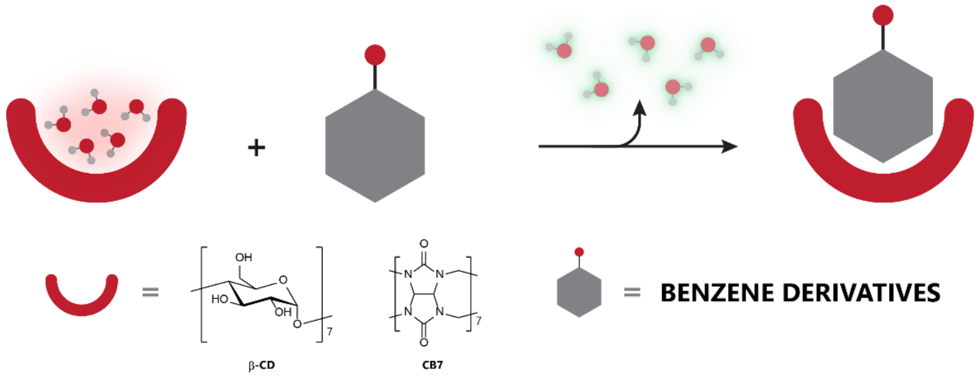

**Figure 1.** Schematic representation of the complexation process.

**Acknowledgements.** This research was fully supported by the Croatian Science Foundation (project MacroSol, IP-2019-04-9560) and European Regional Development Fund (project CluK, KK.01.1.1.02.0016).

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# $^{19}$F-CENTERED NMR SPECTROSCOPY FOR THE ANALYSIS OF COMPLEX MIXTURES

Nicholle G. A. Bell[a] Alan R. Smith,[a] Dušan Uhrín,[a] Richard York[a]

[a] EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, United Kingdom
Nicholle.Bell@ed.ac.uk

We have developed NMR methodology that uses $^{19}$F as a ‘spy’ for the structure determination of mono-fluorinated compounds. The so-called $^{19}$F-centred NMR analysis consists of a complementary set of broadband, phase-sensitive NMR experiments that utilise the substantial sensitivity of $^{19}$F and its far reaching couplings with $^{1}$H and $^{13}$C to obtain a large number of NMR parameters.[1]

The assembled $^{1}$H, $^{13}$C and $^{19}$F chemical shifts, values of $J_{HF}$, $J_{HH}$, and $J_{FC}$ coupling constants and the size of $^{13}$C induced $^{19}$F isotopic shifts constitute a rich source of information that enables structure elucidation of fluorinated moieties and even complete structures of molecules. This novel approach performs particularly well in the structure elucidation of fluorinated compounds embedded in complex mixtures, eliminating the need for compound separation or use of standards to confirm the structures.

We illustrate the $^{19}$F-centred structure determination process and demonstrate its power by elucidating the structures of disinfectant by-products produced by chloramination of a single mono-fluorinated phenolic compound.[2]

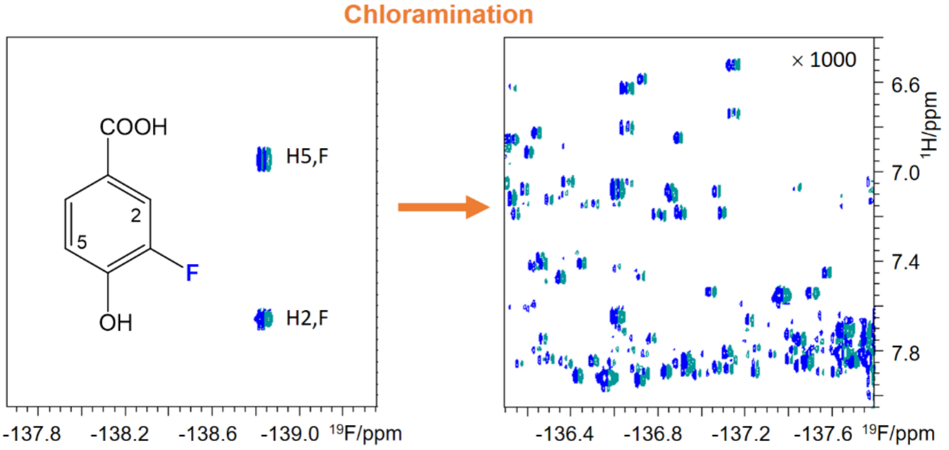

**Figure 1.** 2D $^{1}$H, $^{19}$F HETCOR spectrum before and after chloramination of a single compound.[2]

**Acknowledgements.** This work is supported by the NERC soil security programme, NERC E4 DTP, Scottish Water and EPSRC funding. The authors would like to thank Juraj Bella and Dr Lorna Murray for maintenance of the NMR spectrometers at the University of Edinburgh.

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# NMR-BASED STRUCTURE ELUCIDATION OF NOVEL POLYCYCLIC HETEROCYCLIC COMPOUNDS OBTAINED VIA MCRS FROM 1*H*-PYRAZOL-4-YL CARBALDEHYDES

Aurimas Bieliauskas,[a] Viktorija Dargytė,[a,b] Eglė Arbačiauskienė,[a] Algirdas Šačkusa[b]

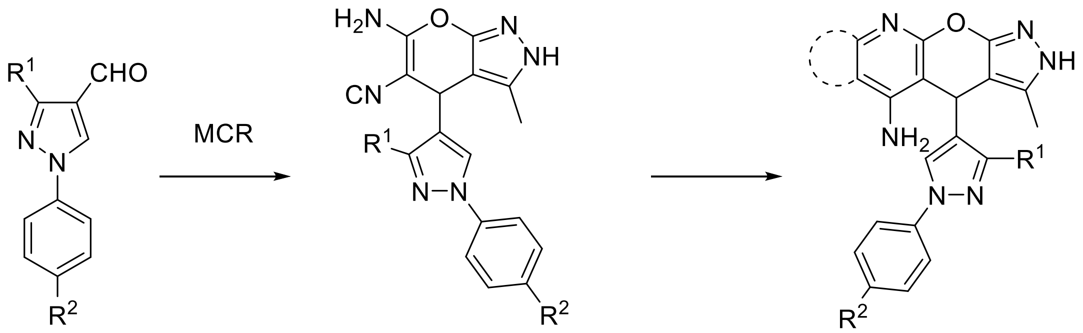
[a] Institute of Synthetic Chemistry, Kaunas University of Technology, K. Baršausko g. 59, Kaunas LT-51423, Lithuania
[b] Department of Organic Chemistry, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas LT-50254, Lithuania
aurimas.bieliauskas@ktu.lt

The potential of Multicomponent reactions (MCRs) where three or more reagents have their atoms incorporated into the final product in a single reaction pot is well-known [1]. MCRs are considered as an alternative to sequential multistep synthesis due to the atom economy and reduced waste generation which is highly valued in Medicinal Chemistry and drug discovery.

In continuation of our interest in the efficient synthesis and investigation of pyrazole-containing polycyclic systems from easily accessible starting materials, herein we report the synthesis of novel pyrazole-containing polycyclic compounds via various multicomponent reactions.

Starting from 3-substituted 1*H*-pyrazole-4-carbaldehydes and employing one-stage four-component reaction, a series of 4-(pyrazol-4-yl)dihydropyrano[2,3-*c*]pyrazoles were obtained, under the Hantzsch MCR conditions. Then, subsequent oxidation were applied for the synthesis of 4-(1*H*-pyrazol-4-yl)pyridines. Moreover, some of the side-products were obtained and their structures were successfully elucidated as well.

The structures of the synthesized compounds were confirmed by various $^{1}H$, $^{13}C$ and $^{15}N$ NMR spectroscopy techniques.



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# NMR, CD AND UV STUDIES OF *NRAS* RNA G-QUADRUPLEXES

Mirko Cevec,[a] Janez Plavec[a,b,c]

[a] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova ulica 19, 1000 Ljubljana, Slovenia
[b] EN-FIST Centre of Excellence, Trg Osvobodilne fronte 13, 1000 Ljubljana, Slovenia.
[c] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia
mirko.cevec@ki.si

Guanine (G)-rich RNA sequences can assemble into a G-quartet structure through hydrogen bond interactions. G-quartets stack with each other and form a G-quadruplex, which is additionally stabilized by monovalent cations such as potassium and sodium ion. RNA G-quadruplexes commonly adopt a parallel conformation in which all four strands are oriented in the same direction. RNA G-quadruplexes are involved in many biological processes, including translation, regulation of alternative splicing and the subcellular transport of mRNAs, and they can be used as therapeutic targets and agents in therapies.

RNA G-quadruplex forming sequence in the 5’ untranslated region (UTR) of *neuroblastoma RAS viral oncogene homologue* (*NRAS*) proto-oncogene has a suppressive role in translation. We will present our results on *NRAS* RNA G-quadruplexes utilizing NMR, CD and UV spectroscopies. Modified RNA G-quadruplexes were further prepared with different G-tract and loop lengths in order to stabilize the two most probable structures.

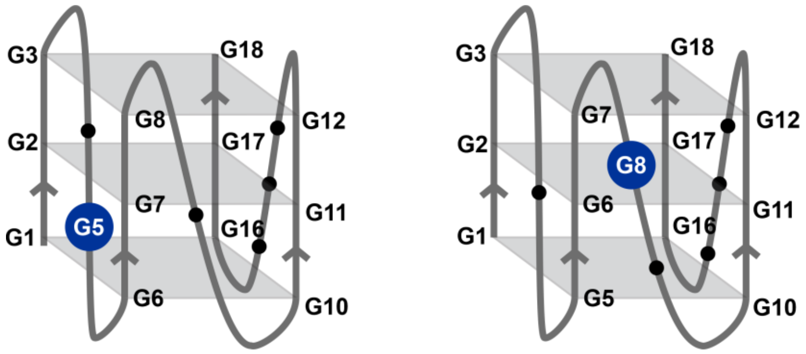


**Figure 1.** The two most probable *NRAS* RNA G-quadruplexes.

**Acknowledgements.** This work has been supported by the Slovenian Research Agency (ARRS).

# LONG-RANGE C–F COUPLINGS IN CONFORMATIONAL ANALYSIS OF DISACCHARIDES

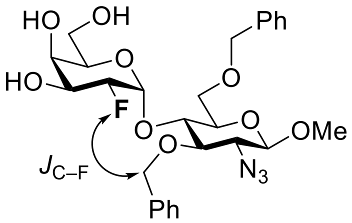
Ema Chaloupecká,[a,b] Martin Kurfiřt,[c] Lucie Červenková Šťastná,[c]
Jindřich Karban,[c] Martin Dračínský[a]

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences,
Flemingovo nám. 2, 166 10, Prague, Czech Republic
[b] Department of Organic Chemistry, Faculty of Science, Charles University,
Hlavova 8, 128 43, Prague, Czech Republic
[c] Institute of Chemical Process Fundamentals, Czech Academy of Sciences,
Rozvojová 135, 165 02, Prague, Czech Republic
ema.chaloupecka@uochb.cas.cz

Carbohydrates are biomolecules responsible for many processes in living organisms and primarily serve as a source of energy and building blocks. In addition, they are also crucial for cellular interactions; they serve, for example, as substrates for lectins – proteins involved in many processes, in which specific recognition is required. The conformation of oligosaccharides is essential for their intermolecular interactions.

Carbohydrates can usually possess multiple conformations and resolving their structure may be challenging. In this work, we studied the conformation of fluorine-containing disaccharides. In their $^{13}$C NMR spectra, we observed a long-range (through-space) fluorine–carbon *J*-coupling, which we used in the conformational analysis.

The experimental data were compared with quantum-chemical calculations. First, we evaluated the accuracy of the C–F coupling calculations on a series of model fluorine substituted bicyclopentanes.[1] Subsequently, we performed a conformation search of selected disaccharides,[2] calculated the C–F couplings and determined the conformation of the disaccharides.



**Acknowledgements.** The research was supported by Czech Science Foundation (grant. no. 22-15374S) and INTERCOST (grant no. LT20052).

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# THE USE OF $^1$H-QNMR FOR DETERMINATION OF BENZOYL PEROXIDE IN GEL SUSPENSION – A PILOT STUDY

Ana Čikoš,[a] Dijana Jureša[b] and Leo Štefan[b]

[a] Ruđer Bošković Institute, NMR Centre, Bijenička cesta 54, 10000 Zagreb, Croatia
[b] JGL d.d Jadran Galenski Laboratorij, Svilno 20, 51000 Rijeka, Croatia
ana.cikos@irb.hr

Clindamycin hydrochloride (CP) and benzoyl peroxide (BPO) are pharmaceutically active substances whose combination in gel suspension (AAG) is used in treatment of mild to moderate acne.[1] According to the pharmaceutical regulation it is necessary to quantify the amount of CP and BPO in the marketed product. While the amount of CP is easily quantifiable, determination of the exact quantity of BPO is a challenge due to its lack of homogeneity.

Drug substance BPO is naturally nonhomogeneous: it is a mixture of water and anhydrous BPO, where hydrophobic BPO is prone to rearrangement to minimize physical contact with water. Using the dry BPO is also not an option since it must contain at least 20.0 % of water to prevent its explosivity.[2]

Since homogeneity of the analytical standard is a critical factor which especially impacts the accuracy of the analysis, the goal of this study was to avoid the external BPO standard and develop a qNMR[3] method for quantification of BPO in gel suspension using CP as internal standard.

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# ADAMANTANE AMMONIUM SALTS AS GUESTS FOR THE CYCLODEXTRIN HOST: SOMETHING OLD, SOMETHING NEW

Amalija Ernečić,[a] Sunčica Roca,[b] Nikola Bregović,[c] Viktoriia Lazarenko,[c]
Marina Šekutor,[a] Marija Alešković[a]

[a] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia
[b] NMR Center, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia
[c] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia
aernecic@fkit.hr

Cyclodextrins (CDs) are important host macrocycles in supramolecular chemistry that possess deep, centrally positioned cavity, a structural feature essential for accommodation of hydrophobic guest molecules *via* their non-polar parts. Because of their properties and bioavailability, CDs have found application in pharmacology, biotechnology, catalysis, nanotechnology, *etc.*[1]

Adamantane derivatives are known guest molecules that fit well into a CD cavity.[1] However, permethylated ammonium salts of adamantane have not been explored so far with this respect. Note that this class of compounds shows excellent binding abilities for cucurbituril hosts (CBs).[2] Inspired by these properties, we decided to broaden the scope of research regarding the binding between 1-substituited adamantane ammonium salts and CDs.[3] As a continuation of our study, herein we evaluated the binding capability of other regioisomers and the effect of other nitrogen-based functionalities on the complex stabilities.

Various NMR spectroscopic techniques and ITC measurements were employed to determine the stability constants and explore host-guest interactions. Computational methods were used to gain more insight into structural features of formed complexes.

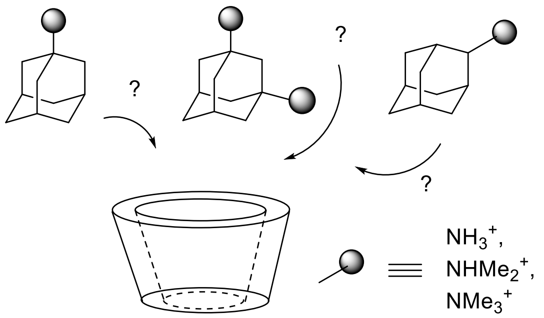

**Figure 1.** Adamantane ammonium salts complexes with CDs.

**Acknowledgements.** This work has been supported by the Croatian Academy of Science and Arts and the Croatian Science Foundation (UIP-2017-05-9653 (DiamMat), IP-2019-04-9560 (MacroSol)).

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# *ANTI-ELECTROSTATIC* OR NOT – A SS-NMR DETERMINATION OF PROTON POSITION IN A HALOGEN AND HYDROGEN BONDED TETRAMERIC SUPRAMOLECULE

Luka Fotović, Marin Liović, Nikola Bedeković, Katarina Pičuljan and Vladimir Stilinović

University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia
lfotovic@chem.pmf.hr

Halogen bond is an attractive interaction in which a halogen atom acts as a Lewis acid. This is explained by the presence of a σ-hole – an area of reduced electron density (and therefore positive electrostatic potential) on a covalently bonded halogen atom. Acceptors of halogen bonds are most commonly molecules containing nitrogen or oxygen atoms and anionic species – i.e. species capable of acting as Lewis bases.

By dissolving 1,4-diiodotetrafluorobenzene (**tfib**) and monohydroiodide salt of 1,4-diazabicyclo[2.2.2]octane (**dabco**HI) in dimethyl sulfoxide (**dmso**), we have prepared a quaternary cocrystal in which the **tfib** molecule acts as a donor of two halogen bonds, with an iodide anion and a **dabco** molecule, and the **dabco** molecule is additionally hydrogen bonded to a **dmso** molecule (Figure 1), with one of the two being protonated. If the proton is located on the **dabco** molecule, then the acceptor of the halogen bond would in fact be a positively charged species (a **dabco**H$^+$ cation), which would make this an unusual type of halogen bond sometimes referred to as *anti-electrostatic* halogen bond. Unfortunately, while X-ray diffraction is generally the most powerful technique for investigating the structure of solids to date, locating hydrogen atoms can sometimes be difficult. So it was in this case, and the position of the proton along the $N_{[dabco]}\cdots H\cdots O_{[dmso]}$ hydrogen bond could not be determined based on the X-ray diffraction data. It has however been successfully determined by applying $^{15}N$ CP-MAS solid state NMR spectroscopy for characterization of **dabco** quaternary cocrystal and comparing the result to the $^{15}N$ CP-MAS spectra of non-protonated (neutral) **dabco** base form and **dabco** diprotonated salt form (**dabco**$H_2$)($ClO_4$)$_2$. Isotropic $^{15}N$ chemical shifts are very sensitive to the local environment of the nitrogen atoms due to hydrogen bond formation and/or protonation. In the cocrystal the $^{15}N$ signal was found to be deshielded by 18 ppm when compared to the one in neutral **dabco** (at 10,95 ppm), which is comparable to the $^{15}N$ chemical shift observed in (**dabco**$H_2$)($ClO_4$)$_2$ (wide signal found at 32 - 33 ppm), corresponding to the protonated **dabco** nitrogen. It can therefore be inferred that the proton is indeed positioned on the **dabco** rather than the **dmso** molecule, and that the halogen bond acceptor in the studied (**dmso**)(**dabco**H)(**tfib**)I supramolecule is indeed a positively charged **dabco**H$^+$ cation.

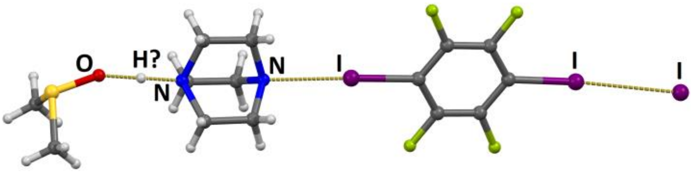


**Figure 1.** The supramolecular tetramer in the quaternary cocrystal (**dmso**)(**dabco**H)(**tfib**)I.

**Acknowledgements.** This work has been supported by the Croatian Science Foundation under the project IP-2019-04-1868.

# NMR STUDIES ON THE TACTICITY OF FUNCTIONAL POLYMERS

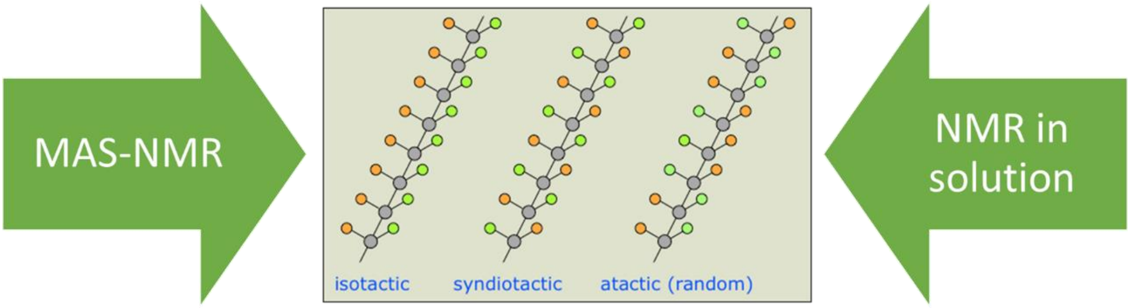
Vito Gallo,[a] Piero Mastrorilli,[a] Mario Latronico,[a] Biagia Musio,[a]
Giuseppe D'Orazio,[a] Rosa Ragone[a]

[a] Politecnico di Bari, DICATECh, via Orabona 4 – CAMPUS, I-70125, Bari, Italy
✉ vito.gallo@poliba.it

Functional materials are defined as materials capable of exerting a special function, which can be intrinsically related to the chemical structure or can be triggered when such materials are exposed to external stimuli, such as electrical, magnetic, or optical signals. This type of material is finding vast technological use in many fields. Indeed, in recent years intense research has been directed toward the development of new materials to be used in the collection and storage of energy, in biomedical applications, and technologies for the mitigation of climate change.[1]

The chemical-physical characterization of this material is fundamental to predicting its behavior in the presence of external stimuli. In this work, high-resolution Nuclear Magnetic Resonance (NMR) is proposed as a valid tool for analyzing the primary structure of the functional material along with the dynamic phenomena it encounters in solution. A special focus is on the investigation of polymer tacticity, which is a function of its stereochemical placement, determining crucial properties as the degree of crystallinity of a polymer, its melting temperature, rheological properties, or its glass transition temperature.

Here are described the results of a spectroscopic study by MAS-NMR and NMR in solution carried out to establish the dual control of molecular weights and tacticity of the functional synthetic polymers obtained in our labs though radical polymerization processes. Free-radical polymerization is one of the synthetic strategies most used in the chemical production of polymeric material on an industrial and laboratory scale, because it allows to convert a wide variety of vinyl monomers into high molecular weight polymeric materials with reduced purification steps and the possibility of use different types of solvents available on the market.



**Acknowledgments.** This work has been supported by Istituto Poligrafico Zecca dello Stato S.p.A.

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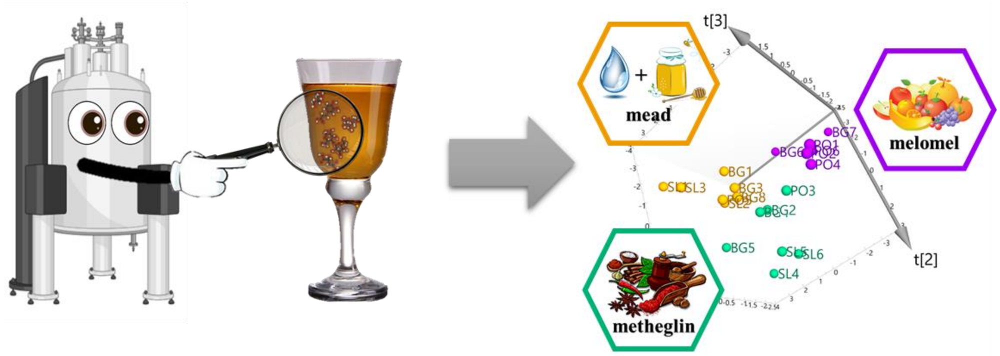
# CHEMICAL PROFILE OF MEAD – THE OLDEST ALCOHOLIC BEVERAGE

Dessislava Gerginova, Svetlana Simova

Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences, Sofia, Bulgaria
✉ Dessislava.Gerginova@orgchm.bas.bg

Mead (honey wine) is the oldest alcoholic beverage known for its antioxidant and therapeutic properties. Traditional mead is made from honey and water via fermentation with yeast. Different types of honey wine can be obtained using fruits, herbs and spices during or after the fermentation. Difficulties in the production process caused by differences in the quality and type of honey has triggered the replacement of mead by other alcoholic beverages. In the last decade the consumption of mead in Europe and America is steadily increasing, however, only scarce information on the chemical composition of the different honey wines is available.

In the present study twenty meads from Bulgaria, Poland and Slovakia produced from different honey types were analyzed by $^{1}$H NMR spectroscopy. A number of compounds – amino and organic acids, alcohols, sugars and phenolic substances were identified and quantified. The chemical profiles of linden, acacia, honeydew and polyfloral honey were compared with the chemical profiles of the corresponding four types of mead using Nightingale's Diagrams. Multivariate chemometric techniques were applied for differentiation of meads according to geographical origin and variety of added ingredients - fruits (melomel) or spices (metheglin).



**Acknowledgements:** This research was supported by the Bulgarian Ministry of Education and Science under the National Research Programs "Young scientists and postdoctoral students", approved by DCM # 577/17.08.2018 using research equipment of Distributed Research Infrastructure INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures.

# RAPID QUANTITATIVE DETERMINATION BY $^{13}$C-NMR OF THE COMPOSITION OF ACETYLGLICEROL MIXTURES AS BYPRODUCT IN BIODIESEL SYNTHESIS

Diego J. González-Serrano, Covadonga Lucas-Torres, Milad Hadidi, José Carlos Orellana, Beatriz Cabañas, Andrés Moreno

Departamento of Química Inorgánica, Orgánica y Bioquímica, Área de Química Orgánica. Universidad de Castilla-La Mancha. Facultad de Química, Avenida Camilo José Cela 10, 13071. Ciudad Real, Spain
✉ andres.moreno@uclm.es

The method widely used to produce biodiesel is transesterification which involves triglycerides from vegetable oils or animal fats and an alcohol in the presence of a catalyst. The reaction yields monoalkyl esters of the fatty acids with excellent diesel-like properties and glycerol as a byproduct. However, a common biodiesel production line utilizes a downstream alcohol recovery and biodiesel purification step, and the final product can be contaminated with partially substituted glycerols, glycerol, free fatty acids, residual alcohol, and catalyst. These byproducts of the biodiesel production are considered contaminants, and they alter the physical-chemical properties of the final product and can create engine problems such as engine deposits, corrosion, and failure. The efficiency of the process steps utilized during the biodiesel production and the quality of the final products can be monitored by measuring the concentration of these contaminants. Currently, primary analytical methods involve chromatography (HPLC, GC), spectroscopy (MS, NIR), and wet chemical techniques (potentiometric, iodometric titration) which are often time-consuming due to sample preparation, extended analysis time, and/or complicated data analysis. Because of these limitations, there is a clear need for a rapid and accurate quantitative analysis technique for biodiesel constituents. Commercially available partly acetylated glycerols (mono and diacetins) are a mixture of glycerol, 1- and 2-acetylglycerol, 1,2- and 1,3-diacetylglycerol, and triacetin. No easy, fast and exact analysis method is available.

In this work, a complete $^{13}$C chemical shift data for all five components allows for the identification of the components in the mixture and thus the determination of the composition is developed. This experimental protocol allows for rapid analysis of biodiesel mixtures of alcohols, glycerol, and mono-, di- and trisubstituted glycerides. Characteristic chemical shift ranges were developed with model compounds and used to fully characterize the conversion of triglyceride samples to biodiesel for two commercial production processes.

**Acknowledgements.** Financial Support from Financial Support from the Consejería de Educación y Ciencia JCCM through project SBPLY/17/180501/000522, JCCM. 2018-2021 and the RTI2018-099503-8-I00. MINCIN, 2019-2022 and from FEDER projects UNCM05-23-039 and UNCM05-23-082 is gratefully acknowledged. Also, thanks to the University of Castilla-La Mancha (Vicerrectorado de Investigación) for the financial research of the research group.

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# NMR STUDY OF GLYCOSYL $^{15}$N-ANILINES

Nina Habanová, Radek Pohl

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic
nina.habanova@uochb.cas.cz

Carbohydrates are structurally diverse biomolecules that secure many important functions in living organisms. They serve not only as source of energy and construction materials, but they also play an important role in specific recognition processes. For instance, a recognition of carbohydrates by lectins (carbohydrate-binding proteins) is responsible for many biological actions including immune response and pathogen virulence. Since the recognition is driven by specific sugar structure, a detailed knowledge of carbohydrate conformation is highly desirable. This work deals with preparation and NMR study of four glycosyl $^{15}$N-anilines as model compounds containing $^{15}$N-label. The labelling offers an extra NMR observables such as $J(^{15}N,H)$ and $J(^{15}N,C)$ coupling constants[1] useful for structural and conformational studies (Fig.1).

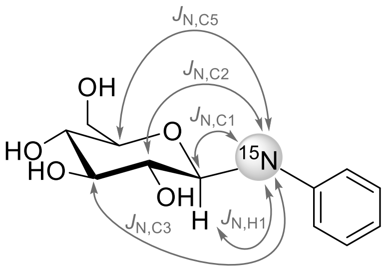

**Figure 1.** Conformationally sensitive *J* mediated via $^{15}$N in β-D-glucosyl $^{15}$N-aniline.

D-Gluco-, D-manno-, D-xylo- and D-lyxopyranosyl $^{15}$N-anilines were synthesized *in-situ* in an NMR tube. Reaction conditions were optimized to achieve almost complete conversion and kinetics of individual reactions was monitored by $^{1}$H NMR. The structure of $^{15}$N-labelled products was studied by $^{1}$H, $^{13}$C and $^{15}$N NMR spectroscopy. Based on the combination of experimental and calculated NMR parameters, it was found that glycosyl $^{15}$N-anilines prefer β-orientation of the aglycone, which can be explained by the reverse-anomeric effect. Moreover, in case of α-D-lyxose derivatives, the change in ring conformation ($^{4}C_{1}$ to $^{1}C_{4}$) was observed. Tools of molecular modeling, molecular dynamics simulations, DFT optimizations, and calculations of NMR parameters were used to describe conformational preferences of glycosyl $^{15}$N-anilines. The dependence of $J(^{15}N,C)$ coupling constants on the orientation of the aglycone will be described in more detail. The knowledge gained for the series of these model compounds will be used next in the study of conformational behavior of $^{15}$N-labelled disaccharides.

**Acknowledgements.** This work has been supported by the Czech Science Foundation – project 22-17586S.

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# NUCLEAR MAGNETIC RESONANCE APPLICATIONS TO COMPOSITIONAL ANALYSIS OF SPANISH BLACK GARLIC.

Milad Hadidi, Covadonga Lucas-Torres, Eva M Sánchez-Palomo, Miguel A. González, Diego J. González-Serrano, José C. Orellana, Andrés Moreno*

Departamento of Química Inorgánica, Orgánica y Bioquímica, Área de Química Orgánica. Universidad de Castilla-La Mancha. Facultad de Química, Avenida Camilo José Cela 10, 13071. Ciudad Real, Spain
andres.moreno@uclm.es

Black garlic is a processed food product obtained by subjecting completely raw garlic to thermal processing that causes interesting chemical reactions, which change the chemical composition of the garlic. This poster will report a nuclear magnetic resonance (NMR)-based comprehensive analysis of raw garlic and black garlic extracts to determine the compositional changes resulting from thermal processing. Thus, 1H-NMR spectra with a detailed signal assignment of most of the components were altered by thermal processing of raw garlic will be showed. For example, the contents of amino acids, etc., during thermal processing. Multivariate data analysis revealed changes in the contents of fructose, glucose, acetic acid, formic acid, pyroglutamic acid, cycloalliin, and 5-(hydroxymethyl)furfural (5-HMF). Therefore, the results will be provided comprehensive information on changes in NMR-detectable components during thermal processing of whole garlic. The aroma of different black garlics has been study by instrumental and sensory descriptive analysis to determine their typist and quality. Significant differences regarding the initial organoleptic characteristics of garlic after a thermal process of 3 months will be showed in the poster.

**Acknowledgements.** Financial Support from the Consejería de Educación y Ciencia JCCM through project SBPLY/17/180501/000522, JCCM. 2018-2021, and projectr RTI2018-099503-8-I00. MINCIN, 2019-2022 and from FEDER projects UNCM05-23-039 and UNCM05-23-082 is gratefully acknowledged. Also, Vicerrectorado de Investigación y Política Científica of the University of Castilla-La Mancha is gratefully acknowledged for providing a project for our research group "Food & Food Waste Chemistry". The Mouse Hill (Sociedad Cooperativa de las Pedroñeras) is gratefully acknowledged for providing the raw and black garlic samples.

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# AZOPYRIMIDINE PHOTOSWITCHES AND THEIR INTERACTIONS WITH BETA-CYCLODEXTRIN

Anna Hruzíková,[a,b] Lucie Mužíková Čechová,[a,c] Sille Štěpánová,[a] Markéta Tichotová,[a,b]
Aleš Růžička,[d] Václav Kašička,[a] Eliška Procházková[a]

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague 160 00, Czech Republic
[b] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague 128 00, Czech Republic
[c] University of Chemistry and Technology, Prague 166 28, Czech Republic
[d] Department of General and Inorganic Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice 532 10, Czech Republic
anna.hruzikova@uochb.cas.cz

Azopyrimidines undergo isomerization from *trans* into the *cis* isomer upon irradiation by UV/vis light.[1] Their physico-chemical properties as irradiation wavelength or *cis* isomer stability can be tuned by relevant structural modification.[2,3] Moreover, based on their structural similarity with azobenzenes, azopyrimidines should create supramolecular complexes suitable for applications, such as development of molecular machines, drug-delivery systems, sol-gel systems or self-assembly materials.

In our work, we prepared a series of azopyrimidine derivatives and investigated their structure and physico-chemical properties by NMR spectroscopy and X-ray diffraction. Complexation properties were screened by electrophoresis. Only beta-cyclodextrin was found to interact with some of the studied compounds (Figure 1). Interactions and arrangement of inclusion complexes were revealed by NOE contacts. The experimental NMR data were supported by quantum-chemical calculations.

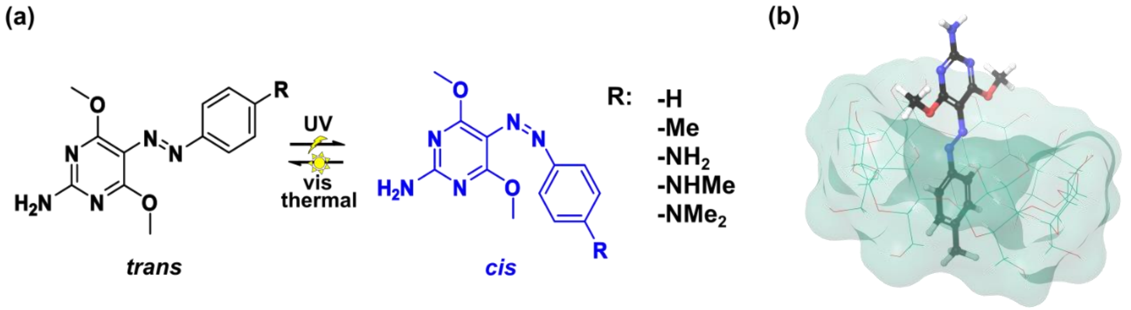

**Figure 1.** Photoswitching of azopyrimidines (a) and inclusion complex formation (b)

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# INTERACTIONS OF MACROZONES WITH THEIR BIOLOGICAL RECEPTORS

Tomislav Jednačak,[a] Ivana Mikulandra,[a] Monika Kapustić,[a] Iva Kušec,[a] Kristina Smokrović,[a] Ema Hošnjak,[a] Ivo Piantanida,[b] Marta Košćak,[b] Klaus Zangger,[c] Predrag Novak[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia
[b] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia
[c] Institute of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstraße 28, A-8010 Graz, Austria
✉ tjednacak@chem.pmf.hr

Macrolides belong to the largest class of antibiotics and are clinically used for the treatment of upper and lower respiratory tract infections.[1,2] They inhibit the synthesis of bacterial proteins by reversible binding to the ribosomal 23S RNA at the peptidyl transferase region, which blocks the exiting tunnel for newly synthesized peptides. Despite the fact that new antibiotics have recently been developed, some bacteria have acquired broad resistance, representing a global medical problem, which can only be resolved by the discovery of novel and more potent drugs. It has been reported that linking known macrolides to bioactive thiosemicarbazones resulted with new conjugates, the macrozones, efficient against multidrug-resistant strains.[3,4] On the other hand, some thiosemicarbazones and their metal complexes showed anti-infective, anti-tumor and anti-inflammatory activity.[5]

In this work, we structurally characterized several azithromycin-derived macrozones and their metal complexes. Furthermore, interactions between the macrozones showing excellent *in vitro* activity and the *E. coli* ribosome were studied by a combination of NMR methods and fluorescence measurements. Transferred nuclear Overhauser effect (trNOE), saturation transfer difference (STD) and WaterLogsy experiments provided a wealth of information about ligand conformations and binding epitopes. Fluorescence measurements revealed binding constants. The obtained results can serve as a good basis for the design and development of macrolide conjugates with enhanced biological properties.

**Acknowledgements.** This research was funded by HRZZ, grant number IP-2018-01-8098 ”Macrozones“

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# COMBINED NMR AND COMPUTATIONAL STUDY OF L-DOPA AND DOPAMINE OXIDATION UPON INTERACTION WITH GOLD NANOSURFACE

Nikolina Kalčec,[a] Antonio Ljulj,[b] Lucija Božičević,[a] Barbara Pem,[a] Valerije Vrček,[b]
Frances Separovic,[c] Ivana Vinković Vrček[a]

[a] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, Zagreb, Croatia
[b] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, Zagreb, Croatia
[c] School of Chemistry, Bio21 Institute, University of Melbourne, Melbourne, VIC, 3010 Australia
nkalcec@imi.hr

The great potential of gold nanoparticles (AuNPs) functionalized with catechols, dopamine and L-DOPA, in biomedical applications prompts the need for evaluation of their interaction at the nano-bio interface.[1] In the presence of gold slats, catechols are known to transform by various pathways including oxidation, cyclization and polymerization.[2] Thus, different transformation patterns should be considered in order to avoid binding the structurally changed molecule to the nanosurface while preventing unpredictable consequences for the health status of an organism. In this study, the fate of functionalization agents upon interaction with gold nanosurface was investigated by combining nuclear magnetic resonance (NMR) spectroscopy and a computational approach. Experimental results revealed that the final form of catechol on the AuNPs nanosurface depends on the molar ratio of reactant used in synthesis. The oxidation of both L-DOPA and dopamine was promoted in the case of molar excess of Au. However, their final products differ as L-DOPA underwent intramolecular cyclization. Computational simulations using density functional theory (DFT) and molecular dynamics (MD) methods revealed the mechanism that stays behind this difference and clarified the most likely candidates, among different oxidation products, for binding to the AuNPs surface. The obtained results represent valuable mechanistic data about the binding events at the surface of AuNPs to encourage their application in biomedicine.

**Acknowledgements.** This work has been supported by the "Research Cooperability" Program of the Croatian Science Foundation funded by the European Union from the European Social Fund under the Operational Programme Efficient Human Resources 2014–2020 (grant HRZZ-PZS-2019-02-4323).

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# COMPLETE RESOLUTION OF PROTONATION EQUILIBRIA OF TOTALSYMMETRIC POLYCARBOXYLIC CYCLODEXTRIN DERIVATIVES USING $^1$H NMR-PH TITRATION AND ADVANCED EVALUATION

Eszter Kalydi,[a] Milo Malanga,[b] Szabolcs Béni[a]

[a] Department of Pharmacognosy, Semmelweis University, Budapest, Hungary
[b] Last affiliation: CycloLab, Cyclodextrin Research and Development Ltd, Budapest, Hungary
kalydi.eszter@pharma.semmelweis-univ.hu

Acid-base properties of polyprotic molecules have great importance in understanding physicochemical behavior and intermolecular interactions. Sugammadex (Suγ) is a widely used cyclodextrin (CD) derivative, applied in the clinical practice as an antidote. Although Suγ has been first reported twenty years ago[1], its acid-base properties have not been characterized yet.

The objective of our work was to determine the whole set of protonation constants (log*K*) of Suγ and its two analogues: sualphadex (Suα) and subetadex (Suβ), differing in cavity size and in the number of thiopropionic acid sidechains.

The acid-base properties of each CD were deduced from a $^1$H NMR pH-titration experiment. The evaluations were performed using a computational method based on the cluster expansion of microscopic protonation constants, developed by Borkovec[2]. In this model, mutual basicity-modifying effects of the carboxylates are handled by pair-interactivity parameters. The derived macroscopic protonation constants of these $C_n$ symmetric molecules were found to cover a wide pH range ensuring negative charge of these selectors even at highly acidic pHs.

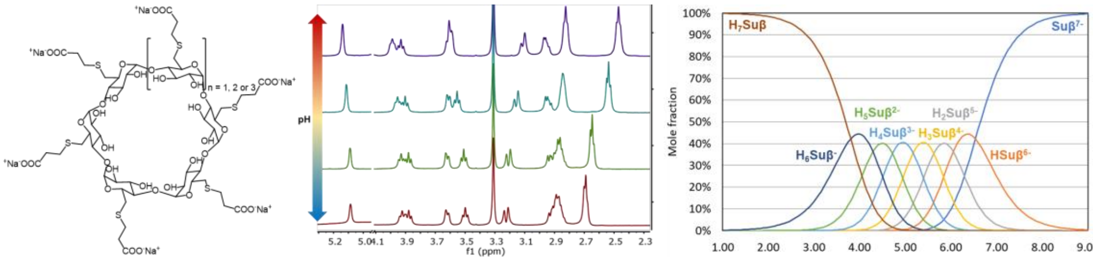

**Figure 1.** The structure of sugammadex-analouge cyclodextrins (left), the pH-dependent $^1$H NMR spectrum of subetadex (middle) and the calculated distribution of its macrospecies (right).

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# INSIGHT INTO FORMATION PROPENSITY OF PSEUDOCIRCULAR DNA G-HAIRPINS

Martina Lenarčič Živkovič,[a,b] Martin Gajarský,[b,c] Petr Stadlbauer,[d] Jiři Šponer,[b,d]
Lukáš Trantírek,[b] Janez Plavec,[a,e,f]

[a] National Institute of Chemistry, Slovenian NMR Centre, Ljubljana, Slovenia
[b] Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[c] National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic
[d] Institute of Biophysics of the Czech Academy of Sciences, Czech Republic
[e] EN-FIST Centre of Excellence, Ljubljana, Slovenia
[f] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia
✉ martina.lenarcic@ki.si

We have shown recently that a short 11-nt long G-rich sequence 5’-d(GTGTGGGTGTG)-3’ that corresponds to the most abundant sequence motif in irregular telomeric DNA from *Saccharomyces cerevisiae*, can fold into an unprecedented pseudocircular G-hairpin (PGH).[1] The first high-resolution structure of PGH revealed a formation of a stable monomeric fold, which is stabilized by dynamic G:G base pairs. The structure is additionally characterized by interesting features, such as chain reversal arrangement of the backbone in the center of the continuous G-tract and 3’-to-5’ stacking of the terminal guanine residues, which is responsible for the pseudocircular nature of PGH.

Herein, we show that despite its ‘circular’ nature, PGHs tolerate single-stranded (ss) protrusions, which is a prerequisite for their function *in vivo* and their applications in biotechnology.[2] The high-resolution NMR structure of prolonged sequence revealed a new member of the PGH family and showed atomistic details on a junction between ssDNA and structured PGH unit. We also identified new sequences capable of folding into one of the two forms of PGH and defined minimal sequence requirements for their formation. Altogether, presented data suggest that PGHs might constitute novel structural class of DNA motifs that can, next to G-triplex and a number of left- and right-handed G-quadruplexes, be formed by G-rich DNA oligonucleotides.

Novel insights into the sequence requirements and structural characteristics of PGHs contribute to our understanding of the structural diversity of G-rich oligonucleotides that could be applied to the prediction of natural and/or the design of artificial recognition DNA elements. Additionally, overrepresentation of sequences able to form PGHs in the evolutionary-conserved regions of the human genome implies their potential functionally important biological role(s).

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# NMR CHARACTERIZATION OF CELLULOSE NANOCOMPOSITE BIOSCAFFOLDS FOR REGENERATIVE MEDICINE

Damjan Makuc,[a] Fazilet Gürer,[b] Tamilselvan Mohan,[b,c]
Karin Stana Kleinschek[c,d], Janez Plavec[a,e,f]

[a] National Institute of Chemistry, Slovenian NMR Centre, Ljubljana, Slovenia
[b] University of Maribor, Faculty of Mechanical Engineering, Laboratory for Characterisation and Processing of Polymers, Maribor, Slovenia
[c] Graz University of Technology, Institute of Chemistry and Technology of Biobased System (IBioSys), Graz, Austria
[d] University of Maribor, Faculty of Electrical Engineering and Computer Science, Institute of Automation, Maribor, Slovenia
[e] EN-FIST Centre of Excellence, Ljubljana, Slovenia
[f] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia
✉ damjan.makuc@ki.si

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

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

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

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

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

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# $^{1}H$-$^{13}C$ CP/MAS NMR ANALYSIS OF SILK FIBROIN REGENERATED USING LANTHANIDE SALTS

Valentina Petrelli,[a] Giorgio Rizzo,[b] Gianluca Maria Farinola,[b] Teresa Sibillano,[c]
Cinzia Giannini,[c] Vito Gallo,[a] Piero Mastrorilli[a]

[a] DICATECh, Polytechnic University of Bari, Italy
[b] Dept. of Chemistry, University of Bari, Italy
[b] CNR Institute of Crystallography, Bari, Italy
✉ p.mastrorilli@poliba.it

Considerable attention has been paid to silk and silk fibroin-based materials for a wide range of applications ranging from textile to biomedicine. The reasons for this interest reside in the excellent mechanical properties characterizing these fibers based on a combination of high tensile strength, large breaking strain and high toughness linked to their favorable interaction with biological systems.

The modifications in secondary and supramolecular structures of silk fibroin-based materials can impact the properties and functions of the resulting fibrous material.

Solid-state $^{1}H$-$^{13}C$ CP-MAS NMR analysis of silk fibroins derived from *Bombyx mori* regenerated using different lanthanide salts as chaotropic agent[1] was carried out to gain insights into possible correlation between conformation behavior and changes in the secondary structure due to treatment with different lanthanides. The analysis, that permitted to quantify the relative contribution of Silk-I and Silk-II in each sample, was performed by exploiting the sensitivity of the to the influence of the secondary structure of the peptide.[2]

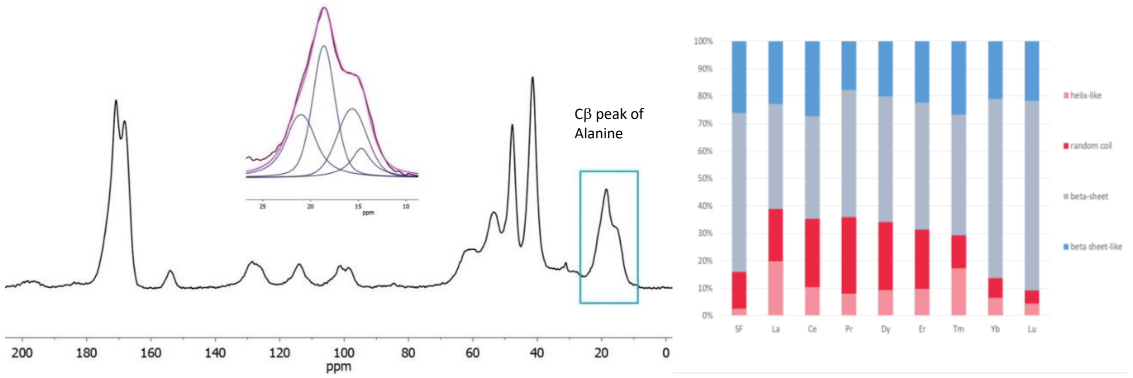

**Figure 1.** *left*: $^{1}H$-$^{13}C$ CP-MAS spectrum (with the profile fitting of the Cβ peak of Alanine) of degummed silk fibroin regenerated using $CeCl_3 \cdot 7H_2O$ as chaotropic agent; *right*: Relative contributions of silk II (blue) and silk I-like (red) in samples regenerated using different Lanthanide salts.

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# ANION BINDING BY LINEAR PENTA–HOMOPEPTIDES COMPRISED OF AMINO ACIDS WITH NONPOLAR SIDE CHAINS

Matija Modrušan,[a] Lucija Otmačić,[a] Lucija Glazer,[a] Nikolina Vidović,[b] Nikola Cindro,[a]
Giovanna Speranza,[c] Gordan Horvat,[a] Vladislav Tomišić[a]

[a] Department of Chemistry, Faculty of Science, Horvatovac 102a, Croatia
[b] Institute of Agriculture and Tourism, Department of Agriculture and Nutrition, Karla Huguesa 8, 52440 Poreč, Croatia
[c] Department of Chemistry, University of Milan, Via Golgi 19, Italy
✉ mmodrusan@chem.pmf.hr

During the last few decades there has been a growing interest for developing linear and cyclic peptides for molecular recognition. Due to the fact that these compounds can mimic biomacromolecules as ion receptors, peptides can be used in medicine and pharmacology for drug discovery.[1] It is well known that cyclic peptides generally exhibit improved metabolic stability and bioavailability, as well as enhanced binding affinity and selectivity toward different substrates compared to their more-flexible linear analogues. The main step in synthesis of such cyclic receptors is cyclization reaction which is often conducted in presence of templating agents. The main factor that affects its success is the conformational preorganization, that is the ability of a linear precursor to bring its reactive termini in the close spatial proximity. We have recently reported that, in addition to metal cations, the chloride anion is also able to act as directing agent for promoting the cyclization of linear peptides.[2,3]

In the scope of this work, we investigated the affinity of two linear homo-peptides **L1** and **L2** (Figure 1), composed of nonpolar amino acids, namely L–leucine and L-isoleucine, towards several anions in acetonitrile by means of $^1$H NMR spectroscopy and microcalorimetry. The obtained results indicated that the anions other that chloride can also serve as very efficient templating agents for the intramolecular head-to-tail linear peptide cyclization.

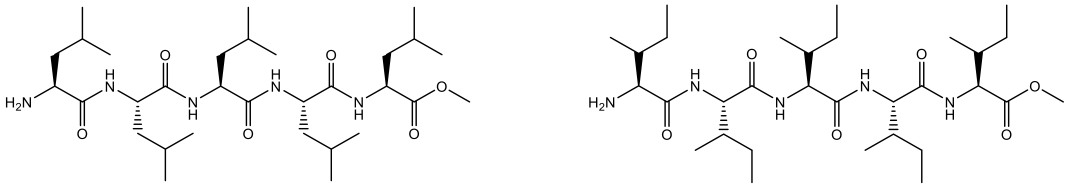

**Figure 1.** Structures of linear peptides **L1** and **L2**.

**Acknowledgements.** This research was fully supported by the Croatian Science Foundation (project MacroSol, IP-2019-04-9560) and European Regional Development Fund (project CIuK, KK.01.1.1.02.0016).

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# QUANTITATIVE DETERMINATION OF 1,2- AND 1,3-DIGLYCERIDES AND OTHER MINOR CONSTITUENTS BY 31P-NUCLEAR MAGNETIC RESONANCE ANALYSIS. A RAPID COMPARATIVE STUDY OF OLIVE OIL DEGRADATION BY CONVENTIONAL AND MICROWAVE HEATING.

José Carlos Orellana, Covadonga Lucas-Torres, Diego J. González-Serrano, Milad Hadidi,
Beatriz Cabañas, Andrés Moreno*

Departamento of Química Inorgánica, Orgánica y Bioquímica, Área de Química Orgánica. Universidad de Castilla-La Mancha. Facultad de Química, Avenida Camilo José Cela 10, 13071. Ciudad Real, Spain
andres.moreno@uclm.es

An efficient methodology for the identification and quantification by $^{31}$P NMR of minor components in fresh and thermal degraded oil have been developed. The olive oil degradation could be different depending on the heat transfer mechanism. For this purpose, conventional and microwave thermal degradation of olive oil has been performed.

As shown in this study, conventional thermal degradation, with a convective heat transfer, favors triacylglycerols hydrolysis, while the dielectric heating mechanism of microwaves enhances diacylglycerols isomerization. It is worth to take into account this important result, from the point of view of the shelf life of the olive oil. Regarding the NMR technique, the derivatization with a phosphorus reagent of the minor olive oil components, helps us making them visible in 31P NMR spectra, enhancing their importance over the major triacylglycerols.

This study proves how a simple and fast method could be useful for identification and quantification of different compounds in a single spectrum.

The $^{31}$P NMR spectra of the degraded olive oils showed that the conventional heating is more harmful that the microwave technique, increasing greatly the diacylglycerol and free fatty acid contents, as well as the amount of degradation compounds such as oxidation derivatives, which damage the olive oil quality. However, the main process that takes place when using microwave heating is the isomerization between diacylglycerols, what gives a potential longer shelf life to the olive oil.

**Acknowledgements.** Financial Support from the Consejería de Educación y Ciencia JCCM through project SBPLY/17/180501/000522, JCCM. 2018-2021, RTI2018-099503-8-I00. MINCIN, 2019-2022 and from FEDER projects UNCM05-23-039 and UNCM05-23-082 is gratefully acknowledged.

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# SOLID-STATE NMR REVEALS CRYSTAL STRUCTURE OF ELUSIVE POLYMORPHS OF MELOXICAM WITH THE HELP OF CRYSTAL STRUCTURE PREDICTION CALCULATIONS

Piotr Paluch, Marta K. Dudek

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112,
90-363 Łódź, Poland
mdudek@cbmm.lodz.pl

Meloxicam (MLX, Figure 1) is a nonsteroidal anti-inflammatory drug commonly used in rheumatic diseases. Patent literature shows there are four neat polymorphs of this drug, yet to date only one of these forms, the commercially available form I, has been characterized structurally, with the other neat forms II, III and V remaining elusive. Structural characterization of these forms is hampered by difficulties in their crystallization, as well as by the fact that when they do appear, it is in their powder forms, which excludes their characterization *via* single crystal X-Ray diffraction.

Having developed new crystallization routes leading to elusive forms of MLX which yielded microcrystalline powders, in this work we demonstrate their structural characterization by solid-state NMR spectroscopy. Figure 1 shows $^{13}$C and $^{15}$N CPMAS NMR spectra of four polymorphs of MLX. The spectra indicate that forms II and III are Z’=2 structures, whereas form V has at least Z’=4. It is also visible that forms II, III and V display distinct hydrogen bonding pattern from that found in the known form I, which was also confirmed by 2D NMR spectra registered at a very fast MAS (60 kHz). Interestingly, a visible change to the $^{13}$C chemical shift of N-$CH_3$ group, which in form I is engaged in a tetrel bond with an oxygen atom from $SO_2$ moiety suggests, that in the elusive forms this bond is no longer observed. These experimental NMR observations were a basis for CSP calculations, which resulted in finding crystal structures of forms II and III of MLX.

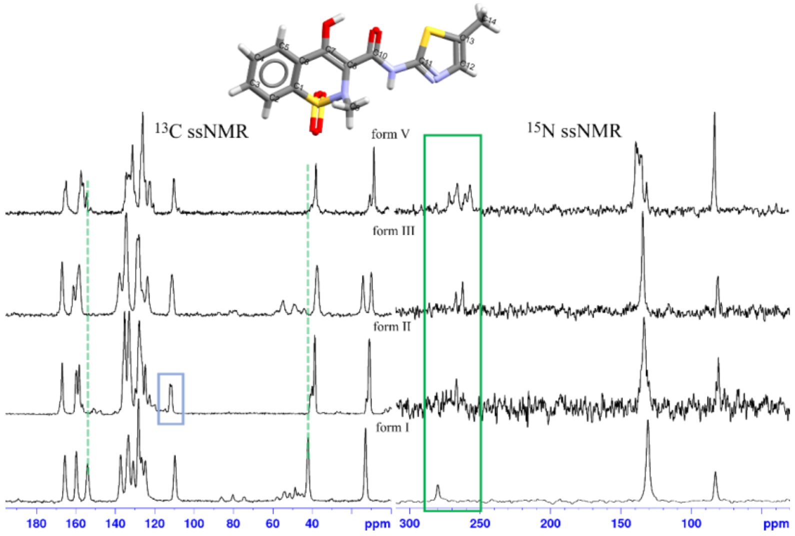

**Figure 1.** Chemical structure of MLX and $^{13}$C and $^{15}$N CPMAS NMR spectra registered for four neat polymorphs of MLX.

**Acknowledgements.** This work was financially supported by Polish National Science Center (UMO-2018/31/D/ST4/01995). PL-GRID is is gratefully acknowledged for providing computational resources.

# NMR ASSIGNMENT OF METHYL GROUPS IN IMMOBILIZED PROTEINS USING MULTIPLE-BOND $^{13}$C HOMONUCLEAR TRANSFERS, PROTON DETECTION, AND VERY FAST MAS

Piotr Paluch,[a,b] Rafal Augustyniak,[a] Mai-Liis Org,[c] Kalju Vanatalu,[c] Ats Kaldma,[c]
Ago Samoson,[c] and Jan Stanek

[a] Faculty of Chemistry, University of Warsaw, Warsaw, Poland
[b] Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland
[c] Tallin University of Technology, Tallinn, Estonia
✉ piotr.paluch@gmail.com

In nuclear magnetic resonance spectroscopy of proteins, methyl protons play a particular role as extremely sensitive reporters on dynamics, allosteric effects, and protein–protein interactions, accessible even in high-molecular-weight systems approaching 1 MDa. The notorious issue of their chemical shift assignment is addressed here by a joint use of solid-state $^{1}$H-detected methods at very fast magic-angle spinning, partial deuteration, and high-magnetic fields. The suitability of a series of RF schemes is evaluated for the efficient coherence transfer across entire $^{13}$C side chains of methyl-containing residues, which is key for establishing connection between methyl and backbone $^{1}$H resonances. The performance of ten methods for recoupling of either isotropic $^{13}$C–$^{13}$C scalar or anisotropic dipolar interactions (five variants of TOBSY, FLOPSY, DIPSI, WALTZ, RFDR, and DREAM) is evaluated experimentally at two state-of-the-art magic-angle spinning (55 and 94.5 kHz) and static magnetic field conditions (18.8 and 23.5 T). Model isotopically labeled compounds (alanine and Met-Leu-Phe tripeptide) and ILV-methyl and amide-selectively protonated, and otherwise deuterated chicken α-spectrin SH3 protein are used as convenient reference systems. Spin dynamics simulations in SIMPSON are performed to determine optimal parameters of these RF schemes, up to recently experimentally attained spinning frequencies (200 kHz) and $B_0$ field strengths (28.2 T). A resolution enhancement provided by 4D spectroscopy with non-uniform (sparse) sampling is demonstrated to remove ambiguities in simultaneous resonance assignment of methyl proton and carbon chemical shifts.

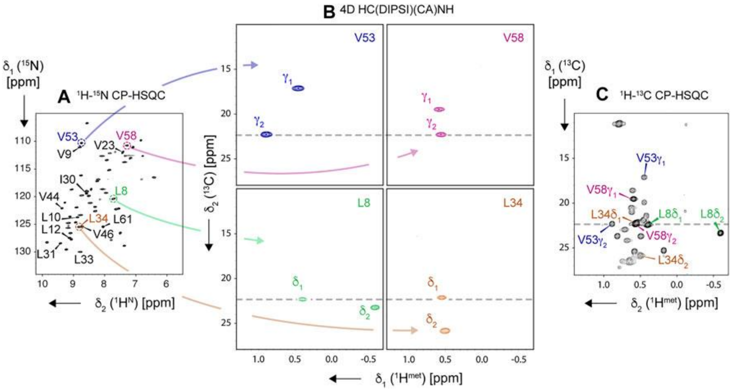

**Figure 1.** Resolving ambiguity of methyl $^{1}$H and $^{13}$C resonance assignment using 4D HC(DIPSI)(CA)NH experiment, demonstrated on the “ILV” sample of SH3 protein.

**Acknowledgements.** This work was financially supported by Polish National Agency for Academic Exchange (Contract No. PPN/PPO/2018/1/00098). PL-GRID is is gratefully acknowledged for providing computational resources.

# NMR INSIGHTS INTO THE STRUCTURAL FEATURES OF TRIAZINE-BASED POROUS ORGANIC POLYMERS

Barbara Panić,[a] Miro Sambolec,[a] Ivana Biljan[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
barbara.panic@chem.pmf.hr

Carbon dioxide ($CO_2$) is the primary greenhouse gas and its emissions into the atmosphere are the major cause of climate changes, global warming and rising sea levels. Porous organic polymers (POPs) are very promising candidates for $CO_2$ storage because of their high thermal and chemical stability, low density, large surface area and permanent porosity. Recent studies revealed that incorporation of nitrogen-rich functionalities (*e.g.* azo and triazine) into the framework of POPs improves interactions between POPs and $CO_2$ molecules and increases the $CO_2/N_2$ selectivity.[1–3]

Here, we synthesized several new triazine-based azo-bridged POPs by condensation reactions of 2,4,6-tris(4-nitrophenyl)-1,3,5-triazine and various aromatic diamino and triamino compounds under basic conditions. The obtained polymers are insoluble in common organic solvents and have very high thermal stability. Powder XRD data indicated amorphous nature of triazine-based azo-bridged polymers. IR spectra showed bands which could be attributed to vibrations of the triazine units, the unreacted terminal nitro and amino groups, and the bands of low intensity which could be assigned to the stretching vibrations of new azo bonds. Finally, the formation of new triazine-based azo-bridged polymers was confirmed by inspection and comparison of $^{13}C$ CP/MAS NMR spectra of polymers and starting compounds.

**Acknowledgements.** This work has been fully supported by Croatian Science Foundation under the project IP-2020-02-4467.

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# MOUSE MODEL OF TAU PATHOLOGY THY-TAU22: NMR-BASED METABOLOMIC CHARACTERIZATION

Helena Pelantová[a], Blanka Šedivá[b], Thi Tra My Nguyen[a], Miroslava Kacířová[c],
Lenka Maletínská[c], Marek Kuzma[a]

[a] Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic
[b] Department of Mathematics, University of West Bohemia, Pilsen, Czech Republic
[c] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic
✉ pelantova@biomed.cas.cz

The prevalence of obesity is increasing worldwide, leading to diverse comorbidities and increasing the cost of their cure. Obesity can cause insulin resistance (IR) not only in the periphery but also in the brain. In addition, obesity supports low-grade inflammation in the adipose tissue and the liver and neuroinflammation in the hypothalamus. Both IR and low-grade neuroinflammation resulting from obesity seem to intensify neurodegenerative changes in brains affected by Alzheimer's disease (AD). Besides beta-amyloid plaques, hyper and abnormally phosphorylated tau protein, which is prone to form neurofibrillary tangles and finally to cause synapse failure, is another main AD hallmark. The mouse transgenic model THY-Tau22, expressing mutated human tau protein, was established to elucidate the role of tau pathology and related pathological changes in the brains of patients with AD.[1] Recently, the study determining the impact of high-fat (HF) diet-induced obesity on peripheral IR, age-related behavioral and memory changes, and neuroinflammation in the brain of THY-Tau22 mice has been published.[2]

In this work we present the first metabolomic characterization of THY-Tau22 model. THY-Tau22 mice and their wild-type C57Bl/6J controls, both males and females, were fed standard and HF diet, establishing 8 experimental groups. The urine and plasma samples, collected at the age of 7 and 11 months, were analyzed by $^1$H NMR spectroscopy. The acquired data were subjected to multivariate and univariate statistical analysis to identify a set of discriminating metabolites. Individual experimental groups were compared in terms of genotype, diet, sex, and age. Subsequently, the levels of detected metabolites were correlated with selected parameters of IR and neuroinflammation, reported in the previous study.[2] The aim was to determine how is neuroinflammantion and peripheral IR, observed in this model, reflected in the plasma and urine metabolic composition.

**Acknowledgements.** This work has been supported by Czech Science Foundation: grant No. 20-00546S

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# DETERMINATION OF THE RELATIVE CONFIGURATION OF NEW SESQIUTERPENE GLYCOSIDES WITH MANY CHIRAL CENTRES

Lenka Poštová Slavětínská,[a] Miloš Buděšínský,[a] Özlem Bahadir Acikara,[b] Josef Cvačka[a]

[a] Institute of Organic Chemistry and Biochemistry, v.v.i, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[b] Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey
slavetinska@uochb.cas.cz

From the aerial parts of *Scorzonera latifolia, a* series of natural compounds was isolated and their structures derived by NMR and MS analysis. Three of these compounds were identified as new glycosides (M-1, M-2, P-1) consisting of a sesquiterpene aglycone containing 6 or 5 chiral centers and a disaccharide part.

Models of 32 or 16 possible enantiomeric pairs of these tricyclic aglycones were build up and their geometries were optimized. Complete set of the observed vicinal *J*(H,H) was then compared with torsion angles in calculated model stereoisomers. Calculated stereoisomers showing large deviations were filtered off and only those fitting the range of torsion angles were used further for configuration estimation. The NOE between H-5 and H-11 and its presence or absence in the studied compounds and the model was a next step, which eliminated improper stereoisomers and confirmed the correct relative configurations.

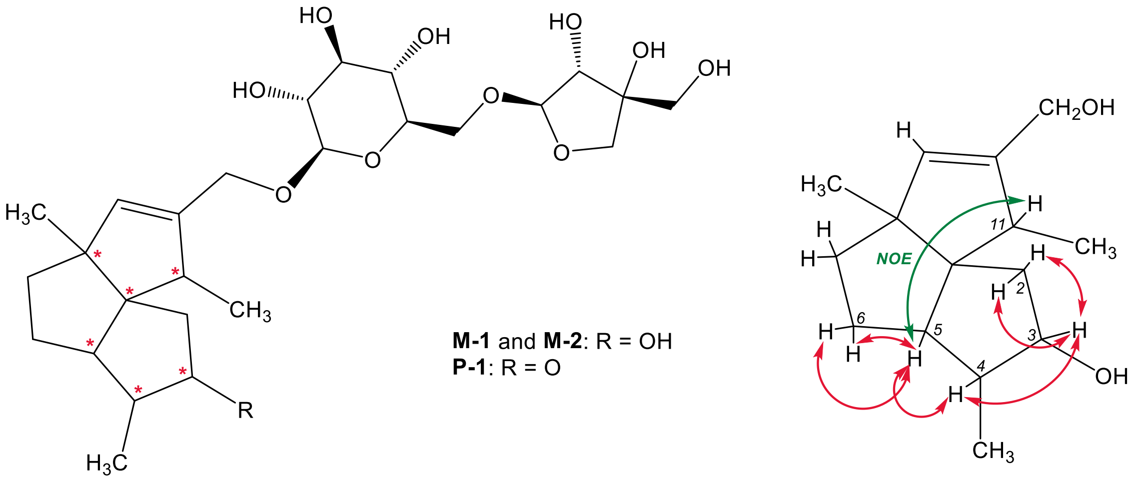

**Figure 1.** Structures of new sesquiterpene glycosides M-1, M-2, P-1 and their aglycone used for molecular modeling.

# THE THERMODYNAMICS OF HYDROPHOBICALY DRIVEN INCLUSION; β-CYCLODEXTRIN VS. CUCURBIT[7]URIL

Andrea Usenik,[a] Katarina Leko,[a] Vesna Petrović Peroković,[a] Željka Car,[a] Rosana Ribić,[b] Katarina Pičuljan,[a] Marko Hanževački,[c] and Josip Požar[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
[b] University center Varaždin, University North, Jurja Križanića 31b, Varaždin, Croatia
[c] The University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom
pozar@chem.pmf.hr

Cyclodextrins and cucurbiturils are very good receptors of hydrophobic species in water. The product stability (log$K$ > 10 for complexes involving neutral guests and cucurbiturils were reported) is a consequence of strongly exothermic association, rationalized by non-classical hydrophobic effect (release of energetically rich cavity water).[1] However, our recent study of the adamantyl-based guest inclusion within β-cyclodextrin (**β-CD**) revealed an almost complete compensation of $\Delta_r H°(T)$ and $-T\Delta_r S°(T)$ contributions to log$K$ temperature dependence.[2] The results could be explained by the Frank-Evans model[3] of adamantane hydration at lower (classical hydrophobic effect) and gradual "iceberg melting" at higher temperatures.

We have therefore decided to study the complexation of both (poly)cyclic and linear aliphatic compounds (Figure 1.) with cucurbit[7]uril (**CB7**) and **β-CD** in a wide temperature range, both experimentally (ITC, ROESY NMR spectroscopy) and computationally (classical, solvent-explicit MD simulations). The investigated guests were chosen with the aim of supressing all thermodynamic contributions to hosting apart those associated with the hydrophobic effect.

The study provided missing pieces to a jigsaw puzzle explaining the thermodynamic contributions to standard complexation Gibbs energies, as well as insights into hydration of guest and host cavities. Apart from that, intriguing differences concerning the enthalpic and entropic effect of **CB7** and **β-CD** cavity dehydration were revealed.

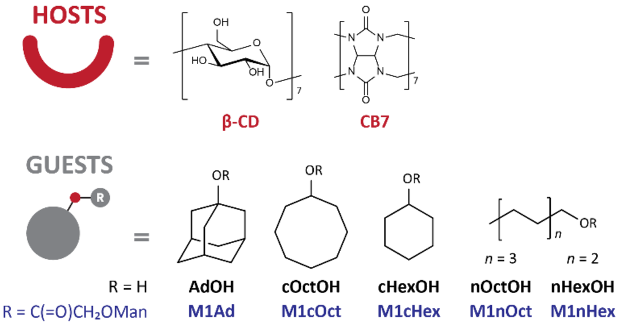

**Figure 1.** Structures of investigated host and guest molecules.

**Acknowledgements.** This research was fully supported by the Croatian Science Foundation (project MacroSol, IP-2019-04-9560) and European Regional Development Fund (project CIuK, KK.01.1.1.02.0016).

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# SYNTHESIS AND CHARACTERIZATION OF CINCHONIDINE AND CINCHONINE C9-EPIMERS

Alma Ramić, Ines Primožič

Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia
alma.ramic@chem.pmf.hr

Cinchonidine and cinchonine are, together with quinine and quinidine, natural alkaloids isolated from the bark of the *Cinchona* tree.[1] *Cinchona* alkaloids have five stereocenters but they occur in *pseudo*-enantiomeric pairs which have different configurations only on C-8 and C-9 atoms. They are called privileged catalysts because stereochemistry of the product in organocatalytic asymmetric reactions usually depends only on the configuration of the C-8 and C-9 stereogenic centers. These alkaloids and their derivatives are used as stereoselective organocatalysts in asymmetric synthesis as well as chiral solvating agents for NMR spectroscopy, or chiral stationary phases for chromatographic separation of various analytes.[2,3] In this study, Mitsunobu reaction was employed for the preparation of C-9 epimers of cinchonidine and cinchonine as well as for preparation of C-9-(amino)-9-(deoxy) cinchonidine and cinchonine derivatives. Course of the reactions was monitored by $^1$H NMR spectroscopy. The structure of products of Mitsunobu reaction were determined by means of $^1$H and $^{13}$C 1D and 2D NMR spectroscopy techniques such as COSY, HSQC and HMBC.

**Acknowledgements.** This work has been supported by the Croatian Science Foundation, Project No. IP-2016-06-3775 ADESIRE.

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# INTERACTIONS OF MACROLIDES AND HUMAN SERUM ALBUMIN VIA DOSY

Markus Rotzinger,[a] Karina Stadler,[a] Klaus Zangger[a]

[a] University of Graz, Institute of Chemistry, Graz, Austria
markus.rotzinger@uni-graz.at

Macrolide antibiotics are a group of antimicrobial agents, which has been commonly administered for 70 years. In an effort to circumvent macrolide antibiotic resistances several derivatives have been developed, among those are the ketolide antibiotics. This group exhibits the desired traits, while also retaining a high therapeutic index and efficacy. However, ketolides also display more severe side effects such as potential hepatotoxicity, ultimately limiting the application of this group of drugs.[1]

In order to potentially explain the diverging pharmacokinetic behavior, binding affinity studies of macrolide and ketolide antibiotics with human serum albumin (HSA) were conducted. HSA is the most abundant protein in the blood plasma and as such responsible for the transport of endo- and exogenic compounds. The binding strength of drugs to HSA is frequently measured to gauge the bioavailability of a compound, in this case via diffusion ordered spectroscopy (DOSY). The method consists of the comparison of the diffusion coefficients of the free drug molecule and the diffusion coefficient of the drug in the state bound to the protein. From these values the mole fraction partition coefficients ($K_p$) were calculated, indicating how strongly the drug binds to HSA.[2] Applying high resolution DOSY the $K_p$ values showed the highest interaction strength for telithromycin followed by erythromycin and lastly azithromycin aglycon.

**Acknowledgements.** This work has been supported by the Austrian science fund FWF grant P30230

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# NMR STUDIES OF SLOVENIAN HONEY

Monika Škrjanc, Damjan Makuc

National Institute of Chemistry, Ljubljana, Slovenia
monika.skrjanc@ki.si

Honey is a natural sweetener that people have been using since ancient times. It is produced by honey bees (*Apis mellifera*) from the nectar or honeydew of various floral sources. These affect its chemical composition, that can be affected also by bees themselves which secrete enzymes into the emerging honey. The wide range of sugars in honey is a result of the activity of these enzymes. Glucose and fructose present the majority of the sugar content and are produced by the hydrolysis of sucrose. In addition to sugars, honey contains other groups of molecules, such as proteins, vitamins, amino acids, minerals, organic acids, phenolic compounds, terpenes, flavonoids and glycosides.[1] NMR can be used to achieve both the structural characterization of an individual molecule, and the chemical characterization of a mixture of compounds. With 2D NMR spectra the structure of molecules is determined at the atomic level. Isomeric forms and configurations which are common for sugars, exhibit different chemical shifts in NMR spectra, allowing them to be easily separated. 1D $^1$H NMR spectra are mainly used for quantitative analysis. The advantages of NMR spectroscopy in food analysis are simple sample preparation and non-targeted analysis, as not only the selected substances in the sample are detected, but all of the present compounds. Substances that are not commonly found in honey are therefore immediately noticed.[2]

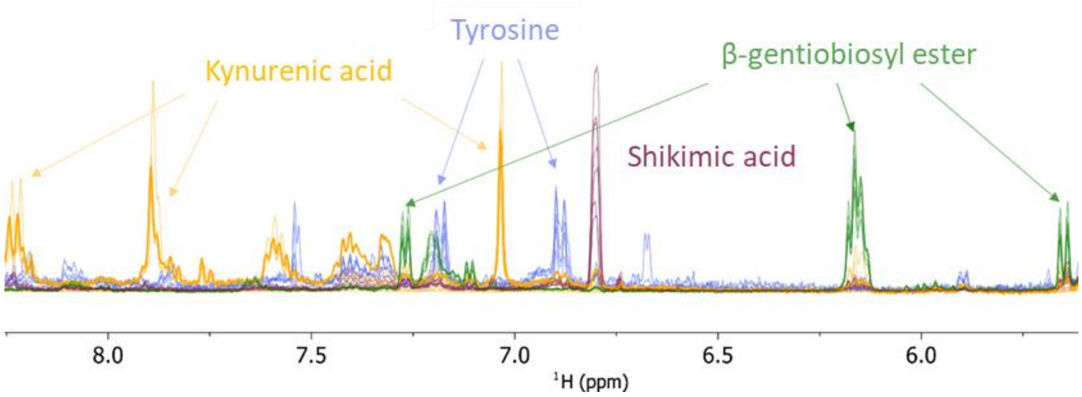

**Figure 1.** Aromatic region of stacked 1D $^1$H spectrum of different types of honey. Green – linden honey, red – honeydew honey, yellow – chestnut honey, blue – buckwheat honey.

**Acknowledgements.** This work has been supported by Slovenian Beekeepers' Association.

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# SYNTHESIS AND CHARACTERIZATION OF NICKEL(II) MACROZONE COMPLEXES DERIVED FROM 4-FORMYLBENZOIC ACID AND 3-FORMYLSALICYLIC ACID

Kristina Smokrović,[a] Ema Hošnjak,[a] Saranyarat Sanemit,[a] Hana Čipčić-Paljetak,[b]
Saša Kazazić,[c] Ivana Mikulandra,[a] Predrag Novak[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia
[b] Center for Translational and Clinical Research, University of Zagreb School of Medicine, 10 000 Zagreb, Croatia
[c] Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, POB 180, 10002, Zagreb, Croatia
ksmokrovic.chem@pmf.hr

Macrozones, i.e. thiosemicarbazone azithromycin derivatives, are a novel hybrid class of potential anti-infective macrolide compounds (Figure 1.a).[1] It has been well established that complexation of transition metals by the thiosemicarbazone moiety increases antibacterial activity of such ligand.[1] Therefore, we investigated the effect of nickel(II) complexation of macrozones on their antibacterial activity. Nickel(II) complexes of several thiosemicarbazones with 4-formylbenzoic and 3-formylsalicylic acid were prepared and structurally characterized via NMR spectroscopy and single-crystal X-ray diffraction (Figure 1.b). Nickel complexes were then coupled to the 4’’-aminopropyl azithromycin derivative, and the nickel(II) macrozone complexes were characterized using NMR spectroscopy and MS spectrometry. Prepared compounds were screened against a panel of selected Gram-positive and Gram-negative bacterial strains.

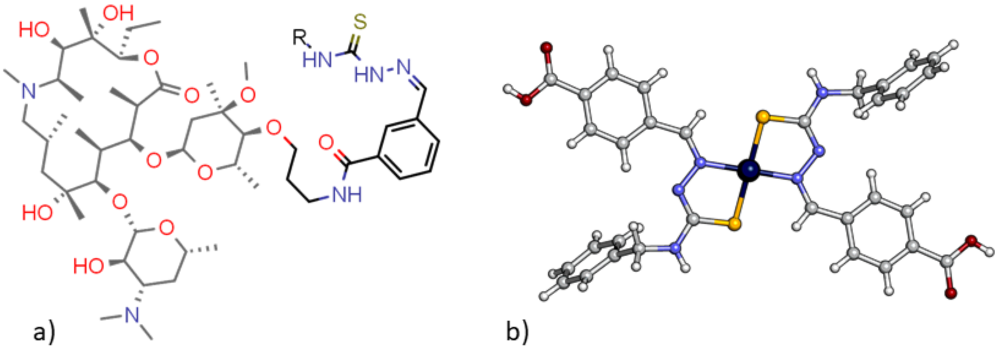

**Figure 1.** a) General structural formula of 4’’- macrozone derivative b) Ball-and-stick model of nickel(II) thiosemicarbazone complex derived from 4-formylbenzoic acid and 4-benzyl-3-thiosemicarbazide.

**Acknowledgements.** This work has been supported by *Croatian Science Foundation* (HRZZ-IP-2018-01-8098)

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# DIMERIZATION OF ACETIC ACID IN THE GAS PHASE

Ondřej Socha, Martin Dračínský

Institute of Organic Chemistry and Biochemistry, AS CR, Prague, Czech Republic.
ondrej.socha@uochb.cas.cz

Due to the nature of the carboxylic group, acetic acid can serve both as a donor and acceptor of hydrogen bond. Therefore, it can be used as a model system for studies of intermolecular interactions.[1] Acetic acid is expected to form cyclic dimers with two strong hydrogen bonds and their presence in the gas phase was confirmed experimentally.[2,3] Trimeric and various oligomeric structures have been also hypothesized in vapors[4] as well as in liquid acetic acid.[5] In this work, a combination of gas phase NMR experiments and advanced computational approaches is employed in order to validate the basic dimerization model of gaseous acetic acid. Experimental thermodynamic parameters are in excellent agreement with the *MP2* and *CCSD(T)* calculations. Further analysis, however, revealed slight disparity between the theoretical model and our experimental data, especially at lower temperatures. This supports the hypotheses about the presence of other, entropically disfavored, supramolecular structures at low temperatures. NMR calculations unveiled yet another discrepancy regarding the chemical shifts of the dimeric form.

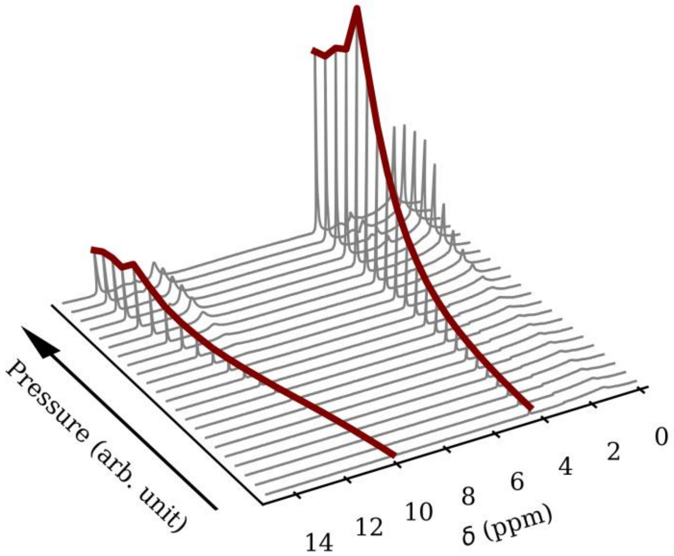

**Figure 1.** Pressure dependence of $^1$H NMR spectra of acetic acid.

**Acknowledgements.** This study was supported by Czech Science Foundation, grant no. 20-01472S and by the Charles University, project GA UK no. 1494119.

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# ORDERED AND DISORDERED COCRYSTALS OF HALOGENOPYRIDINES AND HALOGENOBENZOIC ACIDS STUDIED BY COMBINING X-RAY DIFFRACTION AND SS-NMR SPECTROSCOPY

Nikola Bedeković, Luka Fotović, Katarina Pičuljan and Vladimir Stilinović

University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia
vstilinovic@chem.pmf.hr

Among X-ray crystallographers, disorder in crystal structures is notorious for adding a measure of misery into their professional lives. Disordered crystal structures are difficult to study and properly model, and different types of disorder can be virtually indistinguishable by X-ray diffraction experiments. For this reason, the study of crystal disorder is generally avoided. This is unfortunate, as the presence, nature, and characteristics of disorder in crystal structures provide invaluable insight into the nature of the solid state, in particularly the molecular dynamics in solids, molecular aggregation upon nucleation and growth of the crystals, as well as interplay between the effects of entropy and enthalpy on crystal stability. Using solid-state NMR spectroscopy alongside X-ray crystallography allows one to tackle the problems of studying disordered crystals in more detail.

The system we have selected for this study are the cocrystals of *m*-halogenobenzoic acids with *m*-halogenopyridines (**Xbzac**H–**X'py**, X, X' = Cl, Br, I). All nine cocrystals comprise molecular complexes with the acid carboxyl group hydrogen bonded to the pyridine nitrogen atom. Four of these (with X, X' = Cl, Br) are isostructural and crystallise with the molecules are in positional disorder so that the orientation of the complexes is apparently random, i.e. the complexes are disordered about inversion centres. However, if either one of X or X' is an iodine atom (a stronger halogen bond donor than Cl or Br), the structure becomes ordered. In the case when both X and X' were iodine atoms, however, the structure was again found to be disordered. Solid-state $^{15}$N CP-MAS NMR spectroscopy was successfully used to complement X-ray data in determining the position of the proton involved in the hydrogen bond between the carboxylic group and the pyridine nitrogen (which could not be located based the X-ray data). *m*-Iodopyridine (**mIPy**) and *m*-iodopyridine hidrogeniodide (**mIPy**-HI) were chosen as reference substances for the non-protonated and protonated forms of the **X'py** moiety in **Xbzac**H-**X'py** analogues, respectively. Also, the broadening of the $^{13}$C signals in the disordered structures, as compared to the ordered ones, seem to confirm the random orientation of the **Xbzac**H–**X'py** complexes in the crystal structures.

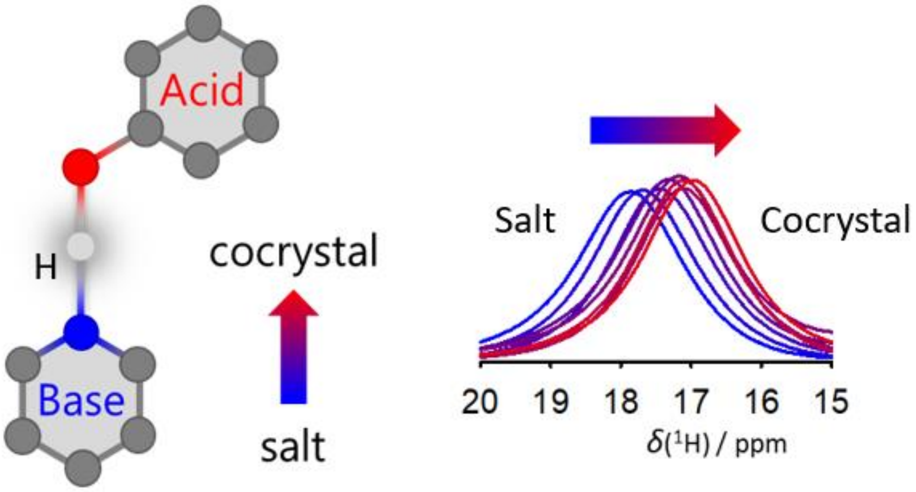
**Acknowledgements.** This work has been supported by the Croatian Science Foundation under the project IP-2019-04-1868.

# TEMPERATURE INDUCED SALT TO COCRYSTAL TRANSFORMATION

Jakub Radek Štoček,[a,b] Martin Dračínský,[a] Ivana Císařová,[c] Tomáš Slanina,[a] Ondřej Socha[a]

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Czech Republic
[b] Department of Organic Chemisty, Faculty of Science, Charles University, Czech Republic
[c] Department of Inorganic Chemisty, Faculty of Science, Charles University, Czech Republic
✉ jakub.stocek@uochb.cas.cz

Many efforts have been recently devoted to the design and investigation of multicomponent pharmaceutical solids, such as salts and cocrystals.[1,2] Experimental distinction between these solid forms is often challenging. Here, a combination of solid-state NMR spectroscopy, X-ray diffraction and diffuse-reflectance measurements with DFT calculations that include nuclear quantum effects (NQEs) provides evidence of temperature induced hydrogen-atom shift in cocrystals with short hydrogen bonds. We have demonstrated that for the predictions of the salt/cocrystal solid forms with short hydrogen bonds, the computations have to include NQEs (particularly hydrogen nuclei delocalization) and temperature effects. We also show that the transformation of a cocrystal into a salt does not occur as a sharp phase transition but rather a smooth shift of the positional probability of the hydrogen atoms.



**Acknowledgement.** This work has been supported by Czech Science Foundation (Grant no. 22-15374S).

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# EXPLORING STRUCTURAL AND THERMODYNAMIC PROPERTIES OF VARIOUS DIAMANTANE AMINE–CYCLODEXTRIN COMPLEXES

Sunčica Roca,[a] Amalija Ernečić,[b] Nikolina Višić,[a] Marija Alešković,[b]
Nikola Bregović,[c] Marina Šekutor[b]

[a] NMR Center, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
[b] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
[c] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia
✉ sroca@irb.hr

Diamondoid ammonium salts are an intriguing class of compounds capable of forming ultra-stable complexes with cucurbituril (CB[n]) hosts in aqueous environment.[1] The host–guest chemistry of diamantane derivatives with different hydrophobic cavities, *i.e.*, cyclodextrins (CDs), has not been explored as much.[2] We have therefore embarked on a study of inclusion complexes consisting of cage ammonium salts and CDs.[3]

Herein we present the synthesis of a new series of diamantane amine derivatives and showcase the results of their binding with β- and γ-CDs. We used a diffusion NMR $^1$H-$^1$H DOSY method to estimate the percentage of complexation based on the calculated diffusion coefficients. The binding strength of complexation was revealed by $^1$H NMR titrations, and the structural features of the complexes were assessed by the $^1$H-$^1$H NOESY NMR experiments. Equilibrium constants and other thermodynamic parameters were additionally confirmed by ITC whereas computational studies revealed key interactions within the inclusion complexes.

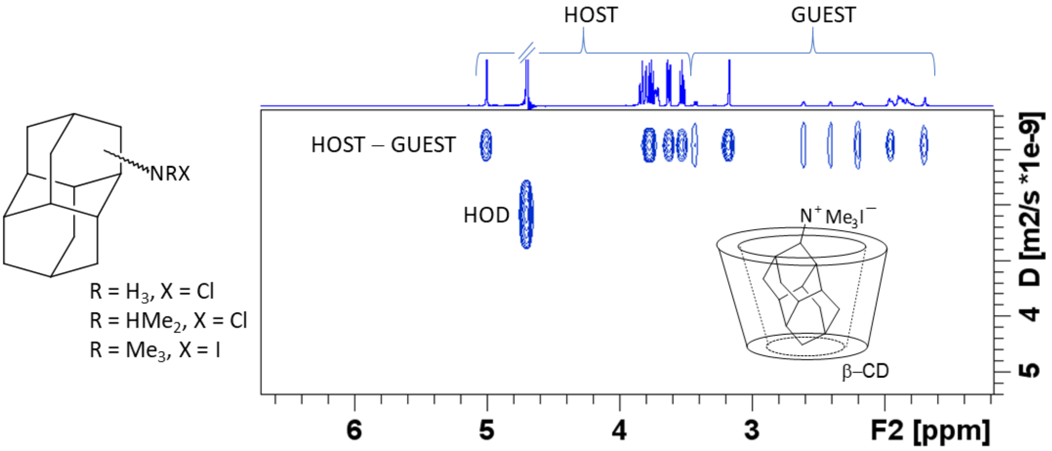

**Figure 1.** Diamantane amine derivatives and DOSY NMR spectra of the shown complex.

**Acknowledgements.** This work has been supported by the Croatian Academy of Science and Arts and the Croatian Science Foundation (UIP-2017-05-9653 (DiamMat), IP-2019-04-9560 (MacroSol)).

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# NMR STUDIES OF G-QUADRUPLEX-LIGAND BINDING

Marko Trajkovski,[a] Janez Plavec,[a,b,c]

[a] Slovenian NMR centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[b] EN→FIST Centre of Excellence, Trg OF 13, SI-1000 Ljubljana, Slovenia
[c] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, p. p. 537, SI-1000 Ljubljana, Slovenia
marko.trajkovski@ki.si

Various biological events rely on DNA remodeling, wherein unfolding of double-stranded helix gives rise to non-canonical structures, including G-quadruplexes that can form within G-rich segments. Discrete features of G-quadruplexes as well as their folding dynamics can alter cell processes, such as promote or hinder enzymes' activities via either stimulating or stalling assembly of the required factors.[1] Considering their fascinating regulatory roles, G-quadruplexes represent attractive targets for treatment of different diseases and especially cancers.[2] Moreover, the novel medical strategies envisage the use of small-molecular drugs that may selectively bind and modulate G-quadruplexes formation within specific G-rich DNA segments, thereby gaining control over aberrant gene transcription, preclude reversal of telomere shortening inherent to immortal cancer cells, etc.

Herein G-quadruplex – ligand interactions will be discussed by considering NMR-based insights from our recent studies on different G-rich DNA oligonucleotides and small-molecules with potential therapeutic values.[3,4] The structural details aim to contribute to better understanding of binding modes in context of G-quadruplex polymorphism as well as ligands' chemical functionalities.

**Acknowledgements.** This work has been supported by Slovenian Research Agency (ARRS) (grant no. P1–0242 and J1-1704)

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# MOLECULAR INTERACTIONS IN REMDESIVIR-CYCLODEXTRIN SYSTEMS PROBED BY NMR AND CE

Bianka Várnai,[a] Zsófia Garádi[a], Dóra Ujj,[b] Erzsébet Varga,[b] Tamás Sohajda,[c] Szabolcs Béni[a]

[a] Department of Pharmacognosy, Semmelweis University, Budapest, Hungary
[b] CycloLab, Cyclodextrin Research and Development Ltd, Budapest, Hungary
[c] CarboHyde Inc, Budapest, Hungary
bnkvarnaivgmail.com

Remdesivir (REM), the first antiviral drug (Veklury™) approved by the FDA for the therapy of COVID-19 possesses low aqueous solubility, thus the final formulation necessitates a solubilizing excipient. For this purpose, the randomly substituted sulfobutylether-β-cyclodextrin (SBEβCD) is used [1]. In this study we aimed at determining the intermolecular interactions between REM and various β- and γ-cyclodextrin (CD) derivatives by NMR spectroscopy to understand their interactions at the atomic level. Moreover, as the synthesis of REM provides a diastereomeric mixture of $R_P$- and $S_P$-REM bearing different pharmacological properties, we also aimed to explore the selectivity of various CDs towards REM diastereomers.

$^1$H NMR titrations were performed to determine the stability constants of various CDs and REM at pH 2.0. Job's method was used to determine the stoichiometries and it resulted in a 1:1 complex in each case. The NMR data revealed that the anionic sulfobutylether-sidechains of the hosts contribute to a significant stability enhancement (γCD < SBEγCD < βCD < SBEβCD < per-6-SBEβCD), and the primary side sulfobutylation of the host plays a critical role in this process [2]. The complex structures were proposed based on the 2D ROESY NMR data. The applicability of CD derivatives to separate $R_P$- and $S_P$-REM was also investigated. Based on capillary electrophoresis (CE) experiments it was found that sugammadex (SGM) provides the highest resolution ($R_s$ = 5.9 at 20 mM SGM) of REM diastereomers and their separation is driven by stability differences. The separation mechanism in CE has also been supported by NMR results investigating the individual REM isomers with SGM.

**Acknowledgements.** Supported by the New National Excellence Program of the Ministry of Innovation and Technology (ÚNKP-21-3-I-SE-52).

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# DNA CONSTRUCT OPTIMIZATION FOR THE NMR STRUCTURAL STUDIES OF THE 8-17 DNAZYME

Julia Wieruszewska,[a] Aleksandra Pawłowicz,[a] Zofia Gdaniec,[a] Witold Andrałojć[a]

[a] Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznań, Poland
julia.joanna.wieruszewska@gmail.com
apawlowicz@ibch.poznan.pl

DNAzymes are synthetic enzymes based on DNA, able to catalyze several chemical reactions – especially when substrates are nucleic acids. Thanks to their resistance to degradation, DNAzymes show promise for future use in biotechnology and medicine, as biosensors or therapeutics, e.g. for gene silencing. Unfortunately, their mechanisms of catalytic action has not yet been fully understood, hindering their practical use. The 8-17 DNAzyme, which is the main focus of our research, is able to catalyze the cleavage of RNA strands at precisely defined points in the sequence. It is a DNA metalloenzyme active in the presence of divalent metal ion cofactors such as $Pb^{2+}$, $Zn^{2+}$ or $Mg^{2+}$. Surprisingly, the research into 8-17 structure using low resolution techniques, such as CD spectroscopy and FRET, revealed that it appears to possess two very distinct active conformations depending on the metal cofactor present[1]. Namely, while $Pb^{2+}$ appears to bind directly to the apo-enzyme, interaction with other activating ions is accompanied by a large scale structural change, towards a more compact fold. When the crystal structures of the apo- and $Pb^{2+}$-bound forms of 8-17 DNAzyme were finally obtained, they turned out to indeed be identical, while displaying an unexpectedly intricate network of tertiary interactions[2]. However, the second catalytically active conformation of the DNAzyme – induced by cofactors such as $Zn^{2+}$ or $Mg^{2+}$– remains elusive to crystallization.

In our research we set out to study the folding of 8-17 DNAzyme in the presence of $Zn^{2+}$ using biomolecular NMR methods in solution. Given the large size of 8-17 DNAzyme constructs usually employed and molecular weight limitations of NMR, a careful optimization of the 8-17 DNAzyme construct to be studied turned out to be a necessary prerequisite for any such investigations. Through a process of gradual truncation and sequence-optimization of the DNAzyme's target-binding arms we were able to arrive at a variant composed only 35 nucleotides (with the classic 8-17 constructs counting >65 nt), without affecting its enzymatic capabilities or metal binding, as assessed by gel electrophoresis and CD spectroscopy. The shortened construct turned out adopt a single NMR spectral form in the presence of $Zn^{2+}$ with sharp lines allowing for resonance assignments and distance restraint derivation without isotope enrichment. Restrained MD simulations are now ongoing of elucidate the elusive $Zn^{2+}$-bound form of the 8-17 DNAzyme.

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# BIOPHYSICAL CHARACTERIZATION OF A SYSTEMATIC SET OF MODEL DNA G-QUADRUPLEXES

Amadeusz Woś,[a] Dorota Gudanis,[a] Witold Andrałojć[a], Zofia Gdaniec[a]

[a] Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Noskowskiego 12/14
61-704 Poznań, Poland
awos@ibch.poznan.pl

The principles underlying the folding of G-quadruplexes remain unexplained thus hindering the analysis of biological processes involving them, as well as, efforts toward their application in nanotechnology. The topological architecture of G4 structures depends on many factors and has not been fully understood.[1] The aim of our research is to understand the relationship between the G4 structures and their nucleotide sequences in order to allow for the design of G-quadruplexes with desired conformations. In order to study the relationship between loop length and conformational preferences we have systematically investigated a set of all 64 two-tetrads G4 containing thymidine–only loops of 1-4 length ($GGT_{1\text{-}4}GGT_{1\text{-}4}GGT_{1\text{-}4}GG$). We applied a combination of biophysical tools (NMR, CD and UV-VIS spectroscopies) to characterize the G4 folds and native gel electrophoresis to determine the number of structures formed in each case. All 64 sequences form G-quadruplexes both in $Na^+$ and $K^+$ buffers, although their stabilities vary significantly. For sequences adopting a single form structural studies are ongoing.

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