ADRIATIC NMR

June 16–18, 2017, Mali Ston, Croatia

BOOK OF ABSTRACTS

The Adriatic NMR Conference is
organised by the Department of Chemistry,
Faculty of Science, University of Zagreb, Croatia

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June 16–18, 2017, Mali Ston, Croatia

ADRIATIC NMR CONFERENCE

Mali Ston, Pelješac, 16–18 June 2017.

BOOK OF ABSTRACTS

IMPRESSUM

ORGANIZER

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CONFERENCE VENUE

"Vila Koruna"
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http://www.vila-koruna.hr/
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Dear Participants,

the Organizing committee welcomes you to the Adriatic NMR conference in Mali Ston at the Pelješac peninsula, 16 to 18 June 2017.

The Conference will provide an interactive forum for presenting various aspects of using NMR spectroscopy, from theory to practical applications encouraging the exchange of ideas and facilitating collaboration among people interested in NMR. It is our goal to gather researchers, application scientists, instrumentation developers and students from universities, research institutes and industry to discuss the topics on the frontiers of NMR spectrosopy.

Bearing in mind the charming ambient of Pelješac peninsula, and the unique environment of Ston and Mali Ston, situated 50 km northweast of Dubrovnik, the Adriatic NMR conference will surely be an inspirational scientific event. Furthermore, Pelješac has an ancient maritime tradition and is well know for the fine vineyards (the excellent sorts of wine, "Dingač" and "Postup", are famous all over the world) which will create a memorable personal experience.

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ORGANIZING COMMITTEE

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KATARINA PIČULJAN
Department of Chemistry, Faculty of Science, University of Zagreb, Croatia

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PROGRAMME

FRIDAY, JUNE 16		
8:00-10:00	**REGISTRATION**	
CHAIR: PREDRAG NOVAK		
10:00-10:40	**PL-1**	**Janez Plavec:** *Solution-state Structures of G-rich DNA Regions*
10:40-11:20	**PL-2**	**Ivana Biljan:** *Structural Features of Human Prion Protein Variants Revealed by NMR*
11:20-11:35	**COFFEE BREAK**	
CHAIR: JANEZ PLAVEC		
11:35-11:55	**IL-1**	**Martina Lenarčič Živković:** *NMR Structure of a G-hairpin in Telomeric DNA of Yeast*
11:55-12:25	**PL-3**	**Kavita Dorai:** *NMR Investigations of the Drosophila Melanogaster Metabolome: Insights Into Circadian Clock, Aging and Immune Response*
12:25-13:05	**TD-1**	**Klaus Zangger:** *Tutorial on Pure Shift NMR*
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16:00-16:20	**IL-3**	**Sunčica Roca:** *Characterization of Silver(I) Complexes with Halo-substituted Derivatives of Pyridine*
16:20-16:35	**COFFEE BREAK**	
CHAIR: PAWEŁ DYDIO		
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SATURDAY, JUNE 17

CHAIR: KLAUS ZANGGER		
9:00-10:40	**PL-6**	**Vladislav Tomišić:** *Coordination Chemistry of Calix[4]arenes: Thermodynamic and Structural Points of View*
9:40-10:20	**PL-7**	**Paweł Dydio:** *Strategies Toward Selective Catalysis: Inspirations from Nature*
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CHAIR: KAVITA DORAI		
10:35-10:55	**IL-5**	**Nikola Bregović:** *NMR Spectroscopic Characterization of Acid-base Equilibria in Aprotic Organic Solvents*
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13:00-15:00	**LUNCH BREAK**	
15:00	**EXCURSION**	

SUNDAY, JUNE 18

CHAIR: DRAŽEN VIKIĆ TOPIĆ		
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10:40-11:20	**PL-8**	**Vlatka Vajs:** *Applied NMR Spectroscopy*
11:20-11:35	**COFFEE BREAK**	
11:35-11:55	**IL-7**	**Tomislav Jednačak:** *DOSY NMR Spectroscopy in the Analysis of Complex Oil Mixtures*
11:55-12:15	**IL-8**	**Thomas Zellhofer:** *Benchtop NMR - New Updates in a Progressing Technology*
12:15-12:45	**TD-4**	**Adolfo Botana:** *Drugs, Procreation, Rocks and Rolls*

LEGEND:

PLENARY LECTURES	**INVITED LECTURES**	**TUTORIALS AND DEMONSTRATIONS**

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PLENARY LECTURES

INVITED LECTURES

TUTORIALS AND DEMONSTRATIONS

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LECTURES

SOLUTION-STATE STRUCTURES OF G-RICH DNA REGIONS

Janez Plavec,[a,b,c]

[a] Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia
[b] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana
[c] EN-FIST Centre of Excellence, Ljubljana, Slovenia
*E-mail: janez.plavec@ki.si

Human telomeres are essential for chromosomal stability and genomic integrity and are composed of thousands of double-stranded TTAGGG repeats featuring a 3'-terminal single-stranded overhang of ca. 200 nucleotides. It is widely accepted that telomere maintenance plays a vital role in tumorigenesis thus offering a possible strategy in anticancer therapy. The G-rich overhang has a high propensity to fold into four-stranded helical secondary structures known as G-quadruplexes. The stabilization of telomeric G-quadruplexes by small molecule ligands can alter response pathway and the release of some of shelterin proteins from telomeres. G-quadruplex ligands as new anticancer agents have captured extensive attention. On the other hand, regulatory regions in chromatin are characterized by nucleosome depletion that allows access of proteins directing gene transcription, replication and epigenetic plasticity. G-quadruplex DNA structures have recently been visualized in human cells. Employing G-quadruplex-promoting conditions, several hundreds of thousands polymerase-stalling sequences have been observed in the human genome in vitro.

Detailed structural information can be obtained by NMR experiments. The imino proton spectral region of G-quadruplexes offers important insights into their structural diversity. Use of 2D homo- and heteronuclear NMR experiments enables assignment of signals corresponding to well-defined folds through proton correlations with aromatic C6/C8 carbon resonances. Structural determinations are furthermore based on detailed analysis of NOESY spectra offering distance restrains.

[1] P. Galer, B. Wang, P. Šket, J. Plavec, *Angew. Chem. Int. Ed.* **2016**, *55*, 1993.
[2] A. Kotar, B. Wang, A. Shivalingam, J. Gonzalez-Garcia, R. Vilar, J. Plavec, *Angew. Chem. Int. Ed.* **2016**, *55*, 12508.
[3] S. Čeru, P. Šket, I. Prislan, J. Lah, J. Plavec, *Angew. Chem. Int. Ed.* **2014**, *53*, 4881.
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STRUCTURAL FEATURES OF HUMAN PRION PROTEIN VARIANTS REVEALED BY NMR

Ivana Biljan,[a,*] Gregor Ilc,[b,c] Gabriele Giachin,[d] Giuseppe Legname,[d] Janez Plavec[b,c,e]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb
[b] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[c] EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia
[d] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Via Bonomea 265, Trieste I-34136, Italy
[e] Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia
*E-mail: ibiljan@chem.pmf.hr

Prion diseases are a group of fatal neurodegenerative disorders that can be of sporadic, genetic or acquired origin. The key molecular event in prion diseases is the conformational conversion of the physiological cellular prion protein, PrP^{C}, into a disease-associated form, prion or PrP^{Sc} (scrapie PrP). Understanding of the earliest stages of the conformational changes leading to spontaneous generation of prions in genetic prion diseases, which are linked with mutations in the human prion protein gene, may benefit from structural characterization of various human (Hu) PrP variants.

We determined NMR structures of the truncated recombinant HuPrPs (residues 90-231) with pathological Q212P [1] and V210I [2] mutations associated with Gerstmann-Sträussler-Scheinker (GSS) syndrome and familial Creutzfeldt-Jakob disease (CJD), respectively, and of HuPrP with naturally occurring E219K polymorphism [3] considered to act protectively against sporadic CJD. Comparison of 3D structures of HuPrP variants with the WT HuPrP revealed that mutations do not affect global architecture of the protein. However, 3D structures of HuPrPs with pathological Q212P and V210I mutations highlighted several common structural perturbations. These include disruption of hydrophobic contacts at the α_2-α_3 inter-helical interface, loosening of tertiary contacts between the β_2-α_2 loop and the C-terminus of helix α_3 and increased exposure of hydrophobic residues to solvent. In addition, we determined NMR structure of HuPrP(V210I) under physiological pH conditions which was found to exhibit higher structural stability when compared to the structure of the same protein at pH 5.5 [4].

[1] G. Ilc, G. Giachin, M. Jaremko, L. Jaremko, F. Benetti, J. Plavec, I. Zhukov, G. Legname, *PloS One* **2010**, *5*, e11715.
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[3] I. Biljan, G. Giachin, G. Ilc, I. Zhukov, J. Plavec, G. Legname, *Biochem. J.* **2012**, *446*, 243.
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NMR INVESTIGATIONS OF THE DROSOPHILA MELANOGASTER METABOLOME: INSIGHTS INTO CIRCADIAN CLOCK, AGING AND IMMUNE RESPONSE

Kavita Dorai

Department of Physical Sciences, Indian Institute of Science Education and Research (IISER) Mohali Punjab 140306 India

NMR metabolomics is an excellent tool to answer interesting questions about the effects of environment and stress conditions on the metabolic response of an organism. We use drosophila melanogaster as a model organism to examine questions about its circadian clock, immune response and aging. We were able to identify several metabolites whose concentrations vary with diurnal rhythms over a 24 h time period in fruitflies. 1D and 2D NMR experiments were performed on whole-body extracts sampled from flies that experienced strong time cues in the form of both light and temperature cycles and were able to identify fourteen metabolites whose concentrations vary throughout this period [1]. We also used NMR-based metabolomics to test the hypothesis that the metabolic networks of fly lines selected for enhanced immunity will exhibit better resistance to bacterial infection than the metabolic networks of control fly lines. We show that a population of fly lines selected for enhanced immunity as compared to a control population, responded differentially to to injury and to infection by a pathogen. We also investigated how the immune response to injury or pathogen infection varies with age.

[1] N. Gogna, V. J. Singh, V. Sheeba, K. Dorai, *Mol. BioSyst*. **2015**, *11*, 3305**.**

THE STRUCTURE BEHIND THE DRUG, THE MAN BEHIND THE STRUCTURE: SCRUTINIZING MOLECULES BY NMR AND MS WITH A HUMAN EYE

Csaba Szántay, Jr.

Spectroscopic Research Division, Gedeon Richter Plc. H-1475 Budapest 10., P.O.B. 27, Hungary
E-mail: cs.szantay@richer.hu

The fundamental innovation behind any drug is the molecular structure of the drug substance itself. Besides that, the need for determining molecular structures (such as related molecules, metabolites or degradants) crops up in numerous respects during the discovery, the development, the clinical studies, the patenting, the quality control, etc., of a drug. But how exactly are these structures determined, and how sure can we be about their correctness?

In this presentation I will take a conceptual look at the roles that the two most powerful and frequently used methods, nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) play in the structure determination of small molecules in an innovative pharmaceutical industrial setting. The discourse will not only focus on the capabilities of modern NMR and MS, but also on the inherent but often overlooked role of the "human element" in these investigations. On that pretext, and in a somewhat philosophical stance, I will outline a unique way of thinking about and practicing science in general, and NMR and MS in particular. This approach, called "Anthropic Awareness", has been cultivated for a while in our research facility and has proved to be highly useful not only in our everyday professional lives, but also in our nonprofessional everyday lives. The aim is to develop a keen mindfulness of how our human nature secretly influences our thoughts in science in general, and on how this influence can lead even the smartest and most knowledgeable scientist into what we call "Mental Traps", resulting in cognitive mistakes ranging from widely held scientific misconceptions to faulty personal or team-level deductions. By understanding and analyzing the nature of these Mental Traps, one can develop the enlightening faculty of detecting and avoiding them both in one's own and others' thoughts. Based on a freshly published book written by our team,[1] in this talk I will briefly discuss the reasons behind the Mental Traps, as well as some of the most important Traps that are relevant to the application of NMR and MS.

[1] Cs. Szántay, Jr, (Ed), Anthropic Awareness: the human aspects of scientific thinking in NMR spectroscopy and mass spectrometry. New York: Elsevier, 2015.

STRUCTURAL STUDIES OF PROTEIN-PROTEIN AND PROTEIN-LIPID INTERACTIONS DETERMINING THE FATE OF FAT

Andras Boeszoermenyi,[a,b] Peter Hofer,[b] Krishna Mohan Padmanabha Das,[b] Roland Viertlmayr,[b] Claudia Radler,[b] Haribabu Arthanari,[a] Achim Lass,[b] Rudolf Zechner,[b] Klaus Zangger,[c] Karina Preiss-Landl,[b] Monika Oberer[b]

[a] BCMP, Harvard Medical School, Boston, USA
[b] Institute of Molecular Biosciences, University of Graz, Austria
[c] Institute of Chemistry, University of Graz, Austria

Triacylglycerols (TGs) are stored in lipid droplets (LDs) from where they can be mobilized on demand during a process termed lipolysis. Lipolysis is a highly regulated and results in the production of free fatty acids that serve as energy substrates for β-oxidation, precursors for membrane lipids, and signaling molecules. Adipose triglyceride lipase (ATGL) catalyzes the first step by hydrolyzing TGs to diacylglycerols and fatty acids (FAs). The protein comparative gene identification-58 (CGI-58) stimulates the enzymatic activity of ATGL, the protein G0S2 inhibits ATGL. It is unknown whether CGI-58-mediated activation of lipolysis occurs due to increased access to the substrate, conformational changes induced in ATGL, or increased product release (e.g. channeling the produced FA away from the reaction site).

In this work, we demonstrate that an N-terminal region (residues 10-31) of CGI-58 is essential for CGI-58 localization to LDs and concomitantly CGI-58-mediated activation of ATGL. The tryptophan-rich N-terminal peptide serves as an independent LD anchor. The NMR structure of a peptide comprising this LD anchor bound to dodecylphosphocholine micelles as LD mimic reveals that the left arm forms a concise hydrophobic core comprising tryptophans and two adjacent leucines. We also identify fatty acid-binding proteins (FABPs) as interaction partners of CGI-58 using co-immunoprecipitation, microscale thermophoresis, and solid phase assays. We mapped the interaction surface on FABPs using chemical shift titration experiments. The interaction of FABP and CGI-58 leads to additional stimulation of ATGL-catalyzed TG hydrolysis in a CGI-58-dependent manner. As implied by their names, FABPs bind FAs with high affinity. Besides their crucial role as energy substrates and membrane lipid precursors, FAs also act as important signaling molecules and can bind to members of the PPAR family of nuclear receptors. The nuclear import of FAs has been shown to involve FABPS to activate PPAR dependent genes. Consequently, these findings support the concept that FAs produced by lipolysis are bound to FABPs and translocated to the nuclease to act on PPAR target gens, thus providing a direct link between lipolysis and lipid signaling.

COORDINATION CHEMISTRY OF CALIX[4]ARENES: THERMODYNAMIC AND STRUCTURAL POINTS OF VIEW

Vladislav Tomišić

Division of Physical Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
E-mail: vtomisic@chem.pmf.hr

Calixarenes are macrocyclic oligomers which consist of four or more phenolic residues linked by methylene group in the ortho position. Many calixarene derivatives are known to be very efficient, and, in some cases, selective binders of ions and neutral molecules.

In this lecture the comprehensive investigation of several calix[4]arene complexation reactions will be described. The thermodynamic data (complex stability constants and derived reaction Gibbs energies, reaction enthalpies and entropies) determined by means of UV and NMR spectroscopies, fluorimetry, potentiometry, conductometry, and microcalorimetry will be correlated with structural results obtained by X-ray crystallography, NMR, and computational methods (DFT and molecular dynamics). The intra- and intermolecular hydrogen-bonding and solvent effects (especially specific solvent-solute interactions) on the equilibria of binding reactions will be particularly addressed.

The results of the above-mentioned studies have clearly indicated how remarkable and complex the influence of the solvent on the ion-hosting abilities of the calixarene derivatives, and macrocycles in general, can be. They have also suggested that integrated and comprehensive experimental and computational investigations can provide a rather detailed insight into the ligand properties and reactivities, i.e. the factors governing the complexation processes. This can serve as a basis for targeted design of efficient and selective supramolecular receptors.

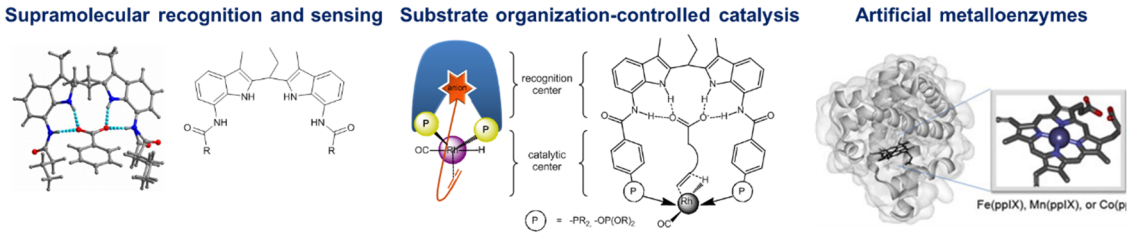
Acknowledgement
This work has been fully supported by Croatian Science Foundation under the project IP-2014-09-7309 (SupraCAR).

STRATEGIES TOWARD SELECTIVE CATALYSIS: INSPIRATIONS FROM NATURE

Paweł Dydio

University of Strasbourg, CNRS, ISIS UMR 7006, F-67000 Strasbourg, France
E-mail: dydio@unistra.fr; www.dydiolab.com

Nature serves as an endless source of inspiration for science. Given the properties of enzymes – natural catalysts – such as high activities and selectivities, these natural catalytic systems serve as attractive models for the design of new catalysts for abiological reactions. Additionally, the cooperative mode of operation of multiple enzymes, if applied to traditional catalysis, opens new possibilities in synthetic chemistry.



I will present my contributions to the catalytic systems that have been designed to mimic the properties of natural catalytic systems and to repurpose natural systems to conduct artificial functions. The role of the NMR tools in the course of the design and the development of these systems will be presented. I will discuss the studies on substrate preorganization-controlled catalysis, cofactor-controlled catalysis and artificial metalloenzymes.[1] I will also present our current interest into the nature-inspired complex system catalysis. I will discuss the construction of complex networks of catalytic reactions with emerging properties of the system.

[1] Review: *Chem. Sci.* **2014**, 2135. Selected contributions: *Angew. Chem. Int. Ed.* **2011**, 396; *J. Am. Chem. Soc.* **2013**, 10817; *Angew. Chem. Int. Ed.* **2013**, 3878; *J. Am. Chem. Soc.* **2014**, 8418; *ACS Catal.* **2013**, 2939; *Nat. Protoc.* **2014**, 1183; *J. Am. Chem. Soc.* **2011**, 17176; *Organometallics*, **2016**, 1956; *Nature*, **2016**, 534, 534; *Science*, **2016,** 354, 102; *J. Am. Chem. Soc.*, **2017**, 139, 1750; *ACS Cent. Sci.*, **2017**, 302.

APPLIED NMR SPECTROSCOPY

Vlatka Vajs

Faculty for Chemistry and ICTM, University of Belgrade

This lecture is concerned with the application of spectroscopy (IR, NMR, MS, LC/ESI MS TOF, GC/MS and microanalysis) with the emphasis on NMR, through examples from the Center for instrumental analysis (Faculty for Chemistry and Center for Chemistry ICTM, University of Belgrade).

A variety of samples have been analysed during a long standing scientific work as well as within collaboration with industry and other institutions such as Ministry for Home Affairs, Drug Agency, OPCW (Organization for Prohibition of Chemical Weapons), pharmaceutical firms from Serbia (Hemofarm, Galenika etc.).

Analysis of medicinal plant crude extracts, narcotics, counterfeit drugs, fake dietary supplements and chemical weapons have been selected as examples to be presented. It is demonstrated how by interpretation of spectra (with accent on NMR) one can reach valuable and widely applicable results.

NMR STRUCTURE OF A G-HAIRPIN IN TELOMERIC DNA OF YEAST

Martina Lenarčič Živković,[a,*] Janez Plavec[a,b,c]

[a] Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia
[b] EN-FIST Centre of Excellence, Ljubljana, Slovenia
[c] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia
*E-mail: martina.lenarcic@ki.si

Besides well-known double helical structural motif, DNA can form a variety of uncanonical structures that are being intensively studied due to their role in cellular regulation [1]. Most attention has been focused on G-rich sequences that form G-quadruplexes, although recent discoveries of G-triplex [2] and left handed G-quadruplex [3] clearly show that G-rich DNA folding landscapes are more complex than previously thought. G-hairpins were for a long time considered only as folding intermediates of G-quadruplexes [4] since the formation of a stable G-hairpin from a natively occurring DNA sequence has never been experimentally observed.

A G-rich sequence motif found in telomeric DNA of *Saccharomyces cerevisiae* (yeast), 5'-d(GTGTGGGTGTG)-3', folded into a novel type of mixed parallel/antiparallel fold-back DNA structure [5]. Seven well-resolved imino proton resonances observed in a 1D ^{1}H NMR spectrum were assigned to a loop-forming thymine and six guanine residues connected by uncanonical hydrogen bonds. Although the sequence is only 11 nucleotides long, it forms a thermodynamically stable structure with a complex topology which includes a chain reversal placing residue G7 between G5 and G6 and 5'-to-3' stacking of terminal residues G1 and G11. Inspection of a rather intricate NOE pattern in NOESY spectra confirmed that the structure is stabilized by three dynamic G:G base pairs that switch between different base-pairing arrangements. Comprehensive evaluation of interesting dynamic properties by NMR experiments was complemented by extensive MD simulations that supported the dynamic nature of G:G base pairs. The structure reveals previously unknown folding principles of G-rich sequences and extends the list of potential biotechnological applications of DNA.

Acknowledgments: This study was supported by Slovenian Research Agency (ARRS, grant nos. P1-424 and J1-6733). The contributions of Martin Gajarský and Lukaš Trantirek are gratefully acknowledged.

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[2] V. Limongelli et al., *Angew. Chem. Int. Ed.* **2013**, *52*, 2269-2273.
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APPLICATION OF MOLECULAR SPECTROSCOPY METHODS IN QUALITY CONTROL OF MEDICINES AND ANALYSIS OF COUNTERFEITS

Lovorka Pitarević

Agency of Medicinal Products and Medical Devices of Croatia, Official Medicines Control Laboratory Division-OMCL, Ksaverska cesta 4, 10000 Zagreb, Croatia
E-mail: lovorka.pitarevic@halmed.hr

Identification of API (active pharmaceutical ingredient) is one of the parameters in quality control in the specification of finished pharmaceutical product. It is accomplished by diverse analytical methods and techniques which mainly consist of one or both chromatographic and molecular spectrometry methods. Infrared and UV-VIS are commonly used techniques but NIR is becoming more “popular” because it can be also used in quantitative determinations and it is more in the spirit of “green chemistry”.

Analysis of counterfeit drugs is not a routine job and for qualitative and quantitative determinations of a counterfeits a combination of different analytical methods and techniques is the best approach. Spectroscopic methods such as FT-IR, NIR and Raman spectroscopy are used for qualitative determinations. Sometimes, results produced by infrared spectroscopy do not yield definite answer regarding identification so NMR spectroscopy is needed and used. A quantitative determination of a counterfeit both for API and for excipients is mostly done by LC-MS.

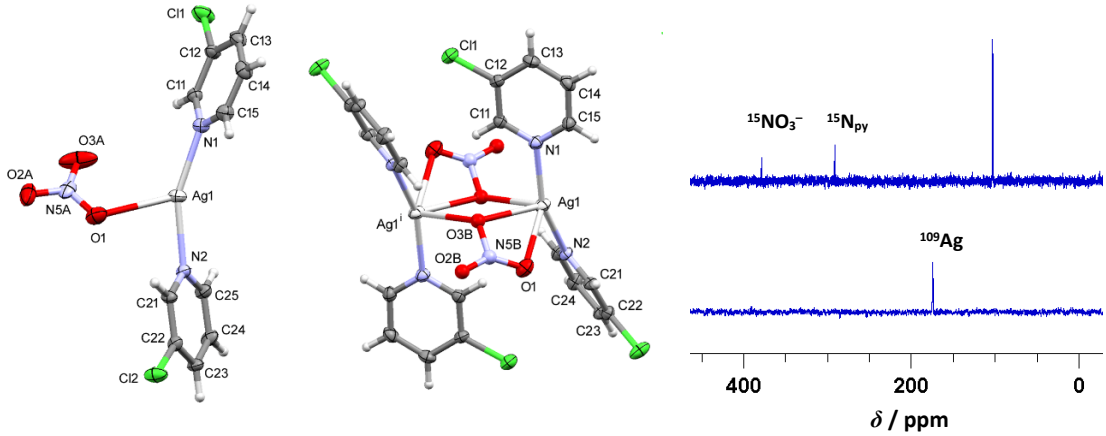
CHARACTERIZATION OF SILVER(I) COMPLEXES WITH HALO-SUBSTITUTED DERIVATIVES OF PYRIDINE

Sunčica Roca

NMR Centre, Ruđer Bošković Institute, Bijenička c. 54, HR-10000 Zagreb, Croatia
E-mail: sroca@irb.hr

The coordination chemistry of silver(I) complexes is very active research area, since these compounds possess antimicrobial [1] and antitumor properties [2]. The well-known flexibility of the coordination sphere of the silver atom as well as different role/assistance of the nitrate ion (bridging, monodentate, bidentate ligand) provide the possibility of forming different structured compounds. The halogen atoms (Cl, Br, I) substituted on the pyridine ring (the basic scaffold for many biologically active substances), moderate compounds properties and behavior through halogen and hydrogen bonding. All this together gives rise to design structure-property relationships.

Presentation shows structural analysis and antibacterial activity results obtained for new $AgNO_3$ complexes with halopyridine ligand of the general formula $[Ag(NO_3)(Xpy)_2]$ and $[Ag(NO_3)(X_2py)_2]$. The isolated products were characterized by means of mass spectrometry, multinuclear NMR spectroscopy (^1H, ^{13}C, ^{15}N, ^{109}Ag) in solution and by X-ray diffraction data on single crystal.



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STRUCTURAL CHARACTERIZATION OF hG0S2: NEW INSIGHTS INTO THE MOLECULAR BASIS OF THE REGULATION OF INTRACELLULAR LIPOLYSIS

Krishna Mohan Padmanabha Das,[a] Andras Boeszoermenyi,[b,c] Lisa M. Wechselberger,[a] Roland Viertlmayr,[a] Melissa Ann Graewert,[d] Alejandro Panjkovich,[d] Klaus Zangger,[e] Gerhard Wagner,[b] Haribabu Arthanari,[c] Monika Oberer[a,*]

[a] Institute of Molecular Biosciences, University of Graz, Austria
[b] BCMP, Harvard Medical School, Boston, USA
[c] Cancer Biology, Dana Farber Cancer Institute, Boston, USA
[d] EMBL, Hamburg, Germany
[e] Institute of Chemistry, University of Graz, Austria
[*] E-mail: m.oberer@uni-graz.at

In response to increased energy requirements of the body, triacylglycerols (TGs) stored in the adipose tissue are hydrolyzed and mobilized by intracellular lipases leading to the release of fatty acids to the circulation which in turn acts as precursors for phospholipid synthesis and as substrates for β-oxidation. Tight regulation of this process is ensured by a series of protein-protein interactions, the molecular basis of which remains elusive. G0/G1 switch gene 2 (G0S2) inhibits adipose triglyceride lipase (ATGL), which catalyzes the first step of lipid catabolism (lipolysis).[1] G0S2 driven inhibition of lipolysis improves glucose tolerance and insulin sensitivity, and protects from cancer-induced cachexia and non-alcoholic fatty liver disease (NAFLD).[2] The structural characterization of the mechanism by which G0S2 inhibits ATGL is therefore of key therapeutic importance. We have previously demonstrated that a 32 amino acid long largely hydrophobic stretch of G0S2 is responsible for the inhibition of ATGL.[3] Here, we explore the structural characteristics of human G0S2 (hG0S2) using multiple biophysical methods such as NMR and SAXS. The hydrophobic character, the tendency to aggregate and structural heterogeneity of hG0S2 make structural analysis of the protein a challenging task, which demands unconventional and novel strategies. We used selective labeling in combination with ILV-methyl NOESY experiments to assign close to 70% of backbone and side-chain resonances and analyzed the dynamic behavior of assigned residues. Our solution studies in the presence of DPC micelles demonstrate that the N-terminal micelle-bound region of hG0S2 is largely alpha helical and it is involved in the interaction with ATGL, whereas the C-terminal region is flexible and unstructured. SEC-MALLS studies indicate the presence of a functional dimer within the micelle, which was further verified by NOESY experiments. In this presentation, we will show our advancements towards the 3D structure of hG0S2 in micelle-bound form with inputs from both NMR spectroscopy and SEC-SAXS experiments.

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NMR SPECTROSCOPIC CHARACTERIZATION OF ACID-BASE EQUILIBRIA IN APROTIC ORGANIC SOLVENTS

Nikola Bregović,[a,*] Dajana Barišić,[b] Vladislav Tomišić[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia
[b] Laboratory for Green Synthesis, Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
* E-mail: nikola.bregovic@chem.pmf.hr

Acid-base reactions are ubiquitous in chemistry, often playing the key role in biological systems, in the development of new pharmaceuticals or materials, etc. Although (de)protonation reactions in water are among the most studied equilibria, analogous investigations in non-aqueous media are rather scarce. This is partly due to experimental difficulties regarding the potentiometric measurement of pH, but also because the complexity of the corresponding equilibrium systems increases in such solutions.[1] Namely, the protonation constants are much higher in organic solvents in comparison to water, and many acids are prone to homoconjugation (interaction with their conjugated bases) and dimerization.[2]

NMR spectroscopy has been extensively used as a tool for quantitative thermodynamic characterization of diverse reactions.[3] However, its utilization for the study of (de)protonation equilibria commonly includes measurement of pH (pD) dependence of ^{1}H NMR spectra, and it is in most cases limited to aqueous systems.[4]

In this work, the characterization of acid-base behavior of rather important and extensively studied organic (acetic acid) and inorganic (phosphoric acid) acids in dimethyl sulfoxide and acetonitrile was carried out by means of several experimental methods (UV and NMR spectroscopies, ITC, and conductometry). In addition, the deprotonation of aromatic urea derivatives acting as anion receptors was studied in DMSO using competitive NMR titrations. The focus of the talk will be on the results obtained by NMR spectroscopy which will be compared to those gathered by other techniques. The concepts, advantages, and limitations of the NMR–spectroscopic investigations of proton transfer, homoconjugation, and dimerization equilibria of the studied systems will be discussed in detail.

Acknowledgement
This work has been fully supported by Croatian Science Foundation under the project IP-2014-09-7309 (SupraCAR).

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LC-SPE/CRYO NMR ANALYSIS OF A RAW MATERIAL IN THE PREPARATION OF DRUG ETODOLAC

Iva Habinovec,[a,*] Željka Car,[a] Ivana Biljan,[a] Katarina Pičuljan,[a] Tomislav Jednačak,[a] Nives Galić,[a] Predrag Novak[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia
*E-mail: ihabinovec@chem.pmf.hr

Etodolac, 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic acid, is a non-steroidal antiinflamatory antirheumatic drug. A key starting material in the synthesis of etodolac is 7-ethyltryptophol which is accessible on the market in various degrees of purity. Depending on the synthetic pathway for 7-ethyltryptophol, commercially available material comprises many different impurities which can cause formation of coproducts in the synthesis of Etodolac thus complicating the purification of the final product.[1] Therefore, to develop an optimal purification procedure of Etodolac, it is important to know the structures of impurities in 7-ethyltryptophol.

Classical methods for separation and isolation of impurities, such as preparative or semi-preparative liquid chromatography, are time and solvent consuming. Nowadays, hyphenated NMR techniques are becoming faster, more efficient and more sensitive tool for determination of impurities and degradation products in pharmaceuticals and natural products.[2,4]

In this lecture application of LC-SPE/cryo NMR methodology in the isolation and structural identification of impurities in 7-ethyltryptophol will be discussed.

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DOSY NMR SPECTROSCOPY IN THE ANALYSIS OF COMPLEX OIL MIXTURES

Tomislav Jednačak,[a,*] Jelena Parlov Vuković,[b] Martina Kveštak,[a] Klaus Zangger,[c] Simon Glanzer,[c] Vilko Smrečki,[d] Davor Kovačević,[a] Predrag Novak[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia
[b] Product Development Department, Refining and Marketing Business Division, INA-Industrija Nafte d.d., Lovinčićeva bb, HR-10002, Zagreb, Croatia
[c] Institute of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstraße 28, A-8010 Graz, Austria
[d] NMR Centre, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia
*tjednacak@chem.pmf.hr

Crude oil, its residues and products are one of the most complex compound mixtures in nature. Over the past couple of years, one of the main goals in petroleum industry has been to transfer heavy crude oil residues into light crude oil distillates. Asphaltenes, which are the heaviest and least reactive molecules in crude oils, cause many problems during transport and production. Owing to complex asphaltene structures, their characterization posts a major challenge in petroleum industry [1,2].

In this research, concentration dependent diffusion measurements have been performed to determine the lowest aggregation concentration of asphaltenes and influence of magnetic field strength on aggregation process. Asphaltene components were separated by DOSY NMR technique according to their different diffusion coefficients. Their hydrodynamic radii and molecular weight were then estimated by using Stokes-Einstein equation. Changes in diffusion coefficients reflected the formation of different asphaltene types. It is expected that the presented results will contribute to better understanding of asphaltene aggregation process.

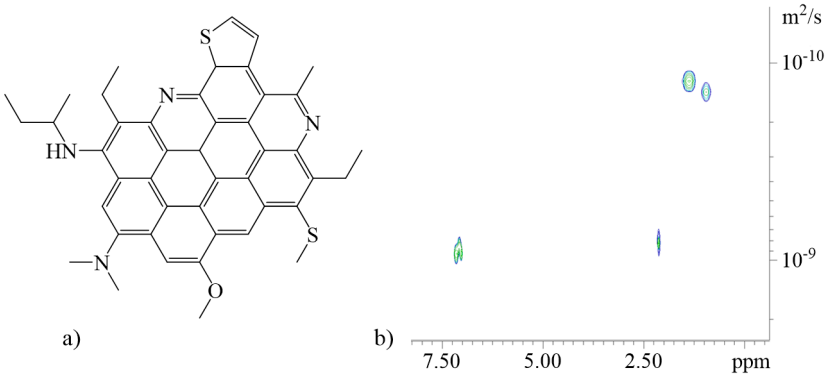

Figure 1. a) A typical asphaltene structure; b) representative DOSY NMR spectrum of vacuum residue sample (γ = 20,28 g L^{-1}) recorded in toluene-d_8 at 25 °C.

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BENCHTOP NMR – NEW UPDATES IN A PROGRESSING TECHNOLOGY

Thomas Zellhofer

Indpendent, D – 65 779 Kelkheim, Germany
E-mail: zellhofer-consulting@t-online.de

The first generation of NMR spectrometers used large electromagnets with a weight of more than one ton. Smaller permanent magnets were developed in the 1960s – 70s at proton resonance frequencies at 60 to 90 MHz using continuous wave method.

Superconducting magnets up to 1 GHz were developed over decades of years to achieve stronger magnet fields for higher resolution and increased sensitivity. These instruments are expensive investments and require building facilities and special operational conditions an in addition high running maintenance costs. As a result, these instruments are dedicated for the use of research groups on a high specialized level.

Since the early 2000s there is a renaissance in permanent-magnet technology and design which allow development of much smaller NMR instruments with useful resolution and sensitivity for education, research and industrial applications.

Samarium-cobalt and neodymium magnets in particular are strong enough for instruments up to 90 MHz. These smaller design, which operate at room temperature allow to be placed on a bench and are safe to operate in a typical lab environment. There is a wide range of NMR experiments which could be done with benchtop NMR systems e.g. 2D-DOSY, HETCOR, HMQC, HMBC and reaction monitoring by using the sensitive nuclei ^{1}H and ^{19}F.

PURE SHIFT NMR TUTORIAL

Klaus Zangger

Institute of Chemistry, University of Graz, Austria

The majority of NMR spectra contain at least one proton frequency axis. Compared to other NMR detectable nuclei, ^{1}H spectra typically suffer from low resolution and severe signal overlap, mainly due to extensive scalar coupling between protons. Homonuclear broadband decoupling, which leads to a collapse of ^{1}H signals into singlets (pure shift spectra) vastly increases the resolution [1], which in some cases corresponds to a theoretical signal dispersion of NMR spectrometers at several GHz [2]. One of the most often used approaches for homonuclear broadband decoupling in the indirect dimension of two- and multidimensional NMR spectra uses frequency-selective pulses during a weak gradient field [3]. Slice-selective decoupling can also be used during acquisition, resulting in FIDs which can be processed like a regular data set. The introduction of homonuclear broadband proton decoupling in the direct and indirect dimensions of two- and three-dimensional NMR spectra significantly enhances their resolution and allows the assignment of signals that are not amenable by the same experiments without proton decoupling. In this tutorial, basic concepts as well as pros and cons of real-time pure NMR are discussed. Although, the signal dispersion of pure shift spectra is far superior to conventional proton NMR spectra, the sensitivity is significantly reduced. Strongly coupled signals typically produce serious artifacts in basically all kinds of pure shift NMR spectra. Finally, interrupted acquisition which is at the heart of real-time NMR methods produces decoupling sidebands in the spectra. Depending on the pure shift method used, all these disadvantages can be overcome, at least to some extent.

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SPE-NMR: REVIVAL OF AN OLD TECHNIQUE FOR THE ANALYSIS OF WINE

Sandra Loss,[a]* Markus Godejohann,[b] Yasmin Jaradat,[b] Manfred Spraul,[b]

[a] Applications, Bruker Biospin AG, Industriestrasse 26, 8117 Fällanden, Switzerland
[b] Applications, Bruker Biospin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany
*E-mail: Sandra.Loss@bruker.com

NMR is a very powerful analytical tool for statistical analysis of complex mixtures. However, in some cases concentrations of interesting compounds are very low and it can be helpful to get rid of the solven to overcome problems connected to dynamic range and solvent suppression. SPE (solid phase extraction) is a valuable technique to achieve this and, in combination with LC, MS and NMR, can also be used to reveal the structure of individual metabolites.

On the example of wine analyzes the presentation will give an overview of the use of theses techniques.

SENSITIVITY AND (NON) LINEARITY IN NMR SPECTROSCOPY

Norbert Müller,[a,b] Matthias Bechmann,[a] Stephan Ginthör,[a] Maria Theresia Pöschko,[a] Victor V. Rodin,[a] Judith Schlagnitweit,[a,c] Hervé Desvaux[d]

[a] Institute of Organic Chemistry, Johannes Kepler Univ., Linz, Austria
[b] Faculty of Science, Univ. of South Bohemia, České Budějovice, Czech Republic
[c] Present address: Dept. of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
[d] NIMBE, CEA, CNRS, Univ. Paris-Saclay, CEA/ Saclay, Gif-sur-Yvette, France

Since the invention of NMR sensitivity has been the main issue in limiting its wide spread application. Numerous technological advances have advanced sensitivity forward in smaller or larger steps. Today a number of approaches based on hyperpolarisation are on the horizon for the main stream NMR labs. However, with sensitivity enhancement techniques one often has to give up a huge advantage of NMR over other techniques: linearity with respect to the number of spins and being a "*primary ratio method*, i.e. the fact that we can determine relative concentrations without substance specific calibration. As a recent example of nonlinearity at the low end of sensitivity it is reported that the spin noise response of small signals be amplified by a nonlinear effect, which occurs when a large radiation damped signal overlaps with the small ones. For example this occurs as enhanced detection of secondary isotope (two-bond coupling) C-13-satellites on top of a large main isotopomer C-12-peaks. The enhancement effect is owed to radiation damping and to the temperature difference between the sample and the detection coil of cryogenically cooled probes causing non-linearities in spin noise response.[1] In the spin noise power spectra they are clearly discernible as bumps on the broad dip of the main isotope peak, which is opposite to the relative signs observed in small flip angle spectra.[2] These experimental observations are completely explained by a new theory of spin noise in NMR probes, which comprises multiple spin systems, the tightly coupled receptor circuit, and, most importantly, the pre-amplifier.[3] Secondary ^{13}C-isotope effects on chemical shifts and coupling constants can thus be determined at natural abundance by spin noise detection.

Acknowledgment

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DRUGS, PROCREATION, ROCKS AND ROLLS

Adolfo Botana

JEOL (U.K.) Ltd., Welwyn Garden City, United Kingdom
E-mail: adolfo.botana@jeoluk.com

According to international regulations [1] all impurities above 0.1 % of the concentration of the active ingredient in a drug must be identified and quantified. Moutzouri et al. [2] demonstrated that it is possible to quantify impurities at these low levels using a ^{19}F detected experiment with ^{1}H and ^{13}C decoupling in a time-shared acquisition. Here we present a probe that can perform these experiments with a high frequency coil that can switch automatically between double tuning to both fluorine and proton, and single tuning to either proton or fluorine, thus maximizing the sensitivity depending on the experiment.[3]

Current spectrometers provide capabilities that are generally underutilized. Here we present an experiment that generates four different spectra in a single acquisition in order to minimize the acquisition time of multiple spectra.

There are a number of reasons why solid state NMR is not used as frequently as liquid state NMR. Here we present some solutions to maximize the potential of solid state NMR, including an automated sample loading using standard sample changers used for liquid state samples.

Spectral interpretation suffers several issues such as baseline rolls and phase rolls. However, obtaining the desired information directly from the FID avoids these problems. JEOL software is incorporating this approach, known as CRAFT,[4] to optimize automated spectral interpretation.

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POSTERS

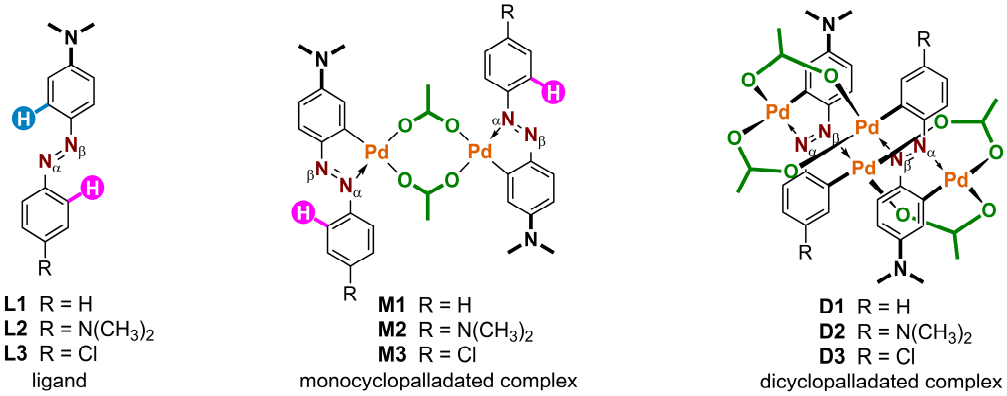
CYCLOPALLADATION OF 4,4'-DISUBSTITUTED AZOBENZENES BY PALLADIUM(II) ACETATE

Dajana Barišić,[a] Ana Budimir,[b] Marina Juribašić Kulcsár,[a] Janez Plavec,[c] Manda Ćurić[a,*]

[a] Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia
[b] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia
[c] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
* E-mail: curic@irb.hr

Transition-metal mediated activation of a carbon–hydrogen bond is a common method for synthesis of cyclometallated compounds.[1] Cyclopalladated compounds have been vastly investigated due to their unique chemical and physical properties that, when fully explored, enable them for application ranging from catalysts and active units in sensors to cancer treatment agents.[1b] Our group focuses on cyclopalladated azobenzenes that show promising photophysical properties in solution and in solid state.[2]

Here we report an NMR spectroscopic study of cyclopalladation of 4,4'-disubstituted azobenzenes **L1**-**L3** (Scheme 1) by palladium(II) acetate in DMF-d_7. ^{1}H monitoring of the reaction enabled an insight into the reaction progress and allowed identification of mono- and dicyclopalladated acetate products (major isomers are shown in Scheme 1).



Scheme 1. Ligands and major isomers of mono- and dicyclopalladated azobenzenes.

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SYMMETRICAL AND ASYMMETRICAL CARBOHYDRAZIDES: A SOLID-STATE AND SOLUTION STUDY

Mirta Rubčić,[a,*] Jana Pisk,[a] Marina Cindrić,[a] Višnja Vrdoljak[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102 a, HR-10000 Zagreb, Croatia.
*E-mail: mirta@chem.pmf.hr

Carbohydrazide, *i.e.* 1,3-diaminourea, represents a versatile building block for the design of symmetrical and asymmetrical urea-based componds.[1] Introduction of specific groups into the structure allows one to fine-tune the desired properties of such systems. For example, pyridine functionalized carbohydrazides proved to be an effective anion receptors, with the ability to stabilize small anion-water clusters.[2] On the other hand, monosubstituted pyridine based derivatives showed to be exquisite ligands for the design of dynamic metal-organic frameworks with intriguing anion-switchable properties.[3]

Within this investigation we explored the synthetic opportunities towards selected mono- and bis-substituted carbonohydrazides, both symmetrical and asymmetrical ones, derived from 2,3- and 2,4-dihydroxybenzaldehyde, pyridinecarbaldehyde and carbohydrazide.[4] Dihydroxybenzaldehydes in combination with carbohydrazide yielded preferably bissubstituted symmetrical products, whereas in the case of pyridine based aldehydes monosubstituted derivatives were also isolated. The latter were then utilized as starting materials for the synthesis of bissubstituted asymmetrical compounds. All isolated products were thoroughly investigated in the solid-state by means of infrared (IR) spectroscopy, thermal analysis, powder X-ray diffraction (PXRD) and when suitable *via* single-crystal X-ray diffraction (SCXRD). The results point out to interesting differences in conformation between the symmetrical and asymmetrical derivatives in the solid state. Finally, the obtained compounds were explored in solution by appropriate one- and two-dimensional NMR techniques.

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G-QUADRUPLEX FORMATION IN RANKL GENE

Jan Rozman,[a,*] Martina Lenarčič Živković,[a] Janez Plavec[a,b,c]

[a] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia
[b] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, Ljubljana, Slovenia
[c] EN-FIST Centre of Excellence, Trg Osvobodilne fronte 13, Ljubljana, Slovenia
*E-mail: jan.rozman@gmail.com

Pathogenic conditions that affect the skeleton, such as osteoporosis, disrupt balanced bone remodelling by accelerating differentiation and maturation of osteoclasts. The pivotal regulator of osteoclast activity is receptor activator of NF-κB (RANK) expressed by osteoclasts that interacts with RANK ligand (RANKL) formed by osteoblasts. The interaction initiates a cascade of intracellular signalling events and promotes bone resorption that in the case of excessive activity leads to osteoporosis. Little is known about precise molecular mechanisms of regulation of RANKL expression. [1–3]

One of the potential ways of gene regulation is facilitation or inhibition of transcription by different *non-B* secondary structures formation in the promoter regions of DNA. [4,5] These regions show a potential to adopt G-quadruplex structures, which are typically comprised of two or three stacked planar G-quartets. Guanines are connected by Hoogsteen hydrogen bonds and stabilized by cations located in the center of G-quartets or between them. H-bonds, base stacking and ionic interactions effectively stabilize these structures.[6]

A G-rich region in the RANKL gene promoter sequence with potential to adopt G-quadruplex structures was found using bioinformatics. Identified 20-nt long sequence, 5'-GGGGAGGGAGCGGGAGAGGG-'3 (RanWT), folded into diverse structures presumably due to four consecutive guanines in the first G-tract. G1 to T1 mutation resulted in a single-fold conformation characterized by 12 well-resolved signals in the imino region of the 1D ^{1}H NMR spectrum. Determined topology revealed the formation of G-quadruplex with three stacked G-quartets connected with propeller loops that link two outer quartets. In contrast to expectations, one of the G-quartets involves G16, initially presumed to be loop-forming residue since it was not part of G-tract. This special feature is pushing residues G14 and A15 into the bulge linking the middle and outer 3' G-quartets. To the best of our knowledge, this is the first G-quadruplex structure where guanine residue is present in the bulge.

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DISENTANGLING SCALAR COUPLING PATTERNS BY REAL-TIME SERF NMR

Nina Gubensäk, Walter Fabian, Klaus Zangger*

Bio-NMR, Institute of Chemistry, Heinrichstrasse 28 Graz Austria

NMR spectroscopy is one of the most frequently used techniques for the structural characterization of small to medium sized organic molecules and biomolecules. Because of their widespread occurrence, high natural abundance and high sensitivity, ^{1}H nuclei are often used in this process. Structural information is obtained from the resonance frequencies and scalar coupling patterns. However, due to the limited chemical shift range of protons, the signals are often overlapped, rendering the extraction of structural information difficult or sometimes impossible.

Here we present a real-time (single scan) experiment, which allows the recording of one-dimensional spectra showing all scalar couplings to one selected signal only.[1] Signals not directly coupled to the selected spin are reduced to singlets (see Figure 1). This not only greatly simplifies the spectra, but in many cases would be the only way to extract individual coupling constants from 1D spectra.

This real-time selectively refocused NMR experiment is achieved by spatially selective homonuclear broadband decoupling [2,3] combined with selective refocusing [4]during acquisition. It allows the unperturbed extraction of scalar coupling constants from the highly resolved acquisition dimension of NMR spectra.

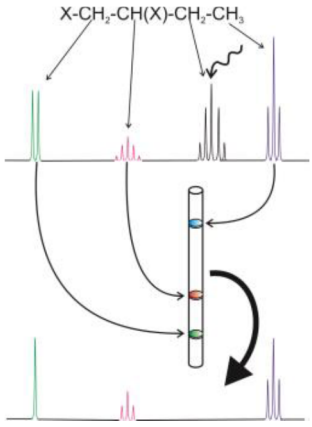

Figure 1 Schematic representation of the real-time SERF NMR experiment

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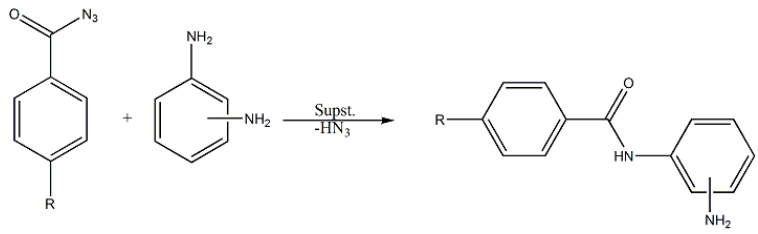
MECHANOCHEMICAL SYNTHESIS OF AMIDE-BASED SUPRAMOLECULAR ANION RECEPTORS

Martina Tireli,[a] Filip Lešić,[b] Nikola Bregović,[b] Krunoslav Užarević,[a,*]

[a] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
[b] Department of Chemistry, Faculty of Science, Horvatovac 102a, 10000 Zagreb, Croatia
* E-mail: Krunoslav.Uzarevic@irb.hr

Traditional synthetic procedures in organic chemistry are generally very successful but global problems of pollution and growing energy demand have compelled the chemical industry to search for new, more efficient and "greener" synthetic methods. Mechanochemical reactions are induced by the direct absorption of mechanical energy and they are mainly promoted by hand grinding or mechanochemical milling. Mechanochemical organic reactions are carried out without the need for solvents or conventional catalysts, and most often feature higher product yield in shorter reaction time.[1] Contrary to the wide knowledge about organic reactions in solution, mechanisms of mechanochemical reactions are still mostly unknown. Only the recently developed *in situ* monitoring techniques[3] started to unveil the mechanisms of organic mechanochemical reactions, leading to wider application of this methodology in modern material and pharmaceutical industry.[4]

Here we present mechanochemical reactions between acyl azide and primary amine as a model nucleophilic substitution reaction on the carbonyl group. We have examined the influence of different additives on reaction time and yields using *in situ* Raman spectroscopy monitoring and the products were confirmed by 1H and 13C NMR spectroscopy. As amide derivatives are widely investigated as receptors for anions,[5] we studied affinity of the prepared compounds towards biologically important acetate and dihydrogen phosphate anions in solution using 1H NMR spectroscopy. Tables and



Scheme 1.

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STRUCTURAL ANALYSIS OF α-HYDRAZINO PEPTIDES

Luka Kavčič,[a] Gregor Ilc,[a,d] Kristina Vlahoviček-Kahlina,[b] Ivanka Jerić,[b] Janez Plavec[a,c,d,*]

[a] Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia
[b] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia
[c] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia
[d] EN-FIST Centre of excellence, Ljubljana, Slovenia
* E-mail: janez.plavec@ki.si

Highly selective protein-protein interactions are essential for many physiological processes and their modulation is gaining increasing interest in the field of drug development. Peptidomimetics that mimic protein-like secondary structures can adapt to large surfaces, which makes them attracting inhibitors of these interactions. Substitution of natural amino acids with α-hydrazino analogs, which are derivatives of β-amino acids with nitrogen replacing the C_β atom, can result in rearrangement of intramolecular hydrogen bonds and formation of novel conformations and secondary structures.[1]

Interaction of the central tumour suppressor protein p53 with its repressor MDM2 is one of the most studied systems. A series of hydrazino peptides as peptidomimetics were synthesized based on the minimal octapeptide p53-derived sequence with μM affinity for MDM2. By varying the number and the position of the α-hydrazino acids, we anticipate to finely modulate this interaction, as was shown in the previous study of hydrazino peptides with DNA and RNA.[2]

Our goal was to determine the structural properties of hydrazino peptides with NMR spectroscopy in DMSO-d_6. Comparison of chemical shifts for H_N, H_α and C_α resonances of the α-hydrazino peptide with its parent equivalent indicates that the hydrazino group affects the local chemical environment. The presence of resolved sets of signals for H_N and H_α protons of flanking residues as well as separate sequential assignments using 2D NOESY spectrum revealed the presence of multiple conformational forms in solution. Based on the previous work on α-hydrazino peptides,[3] we propose E/Z isomerism of the hydrazide link to account for observed multiple forms.

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NMR AND COMPUTATIONAL STUDY OF MONOMER-DIMER EQUILIBRIUM OF AROMATIC DINTROSO COMPOUNDS

Ivana Biljan,[a,*] Igor Rončević,[a] Petar Bibulić[a], Hrvoj Vančik[a]

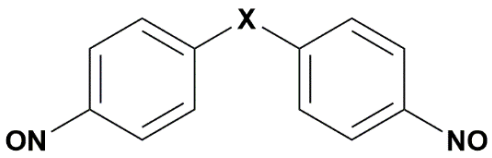
[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb
*E-mail: ibiljan@chem.pmf.hr

Aromatic C-nitroso compounds can exist as monomers or *Z*- and *E*-azodioxides (dimers). The preferred form in solution at ambient temperature is monomeric while lowering of temperature or crystallization favors dimerization. Azodioxides undergo solid-state photodissociation to corresponding monomers under cryogenic conditions, which again dimerize by warming.

Aromatic C-nitroso compounds with two or more nitroso groups could be used as building blocks for supramolecular structures that could be disassembled or reassembled by external stimuli such as UV radiation or heat.

In the present work, we studied solution-state monomer-dimer equilibrium of several new aromatic dinitroso compounds that differ in spacer between two aromatic rings (Scheme 1). In order to observe formation of dimers, we recorded ^{1}H and COSY NMR spectra in chloroform-d_5 at ambient and low temperatures. Inspection of NMR spectra revealed that by lowering of temperature new signals appear in the spectra that could be assigned to dimers. Signals of dimers disappear after warming the solution to room temperature.

The NMR spectra of monomers and dimers were also modelled using density functional theory. Solution-phase (SMD) optimizations, followed by GIAO calculations, were done at the ωB97-XD/6-311G(d,p) level of theory. In case of dimers, multiple conformers had to be considered in order to obtain a good agreement (MUE < 0.1 ppm) between calculated and experimental spectra.



X = CH_2, CH_2CH_2, O, C(O), Ø

Scheme 1.

Acknowledgements
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^{1}H NMR ADULTERATION STUDY OF HEMPSEED OIL OF DIFFERENT GEOGRAPHICAL ORIGIN USING INTERVAL AND MERGED-INTERVAL REGRESSION PROCEDURES

Ozren Jović,* Katarina Pičuljan

Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
*E-mail: ozren.jovic@chem.pmf.hr

Adulteration of high-priced, health beneficial hempseed oil of different geographical origin was studied by combination of ^{1}H NMR spectroscopy and multivariate statistical analysis. The fatty acid composition of hempseed oil corresponds to the ideal ratio of essential fatty acids (EFAs) required by human body, which is roughly 3:1 of omega-6 to omega-3. 15 hempseed oil samples were adulterated with 6 samples of sunflower oil, 4 samples of rapeseed oil and 4 samples of sesame oil. Each particular edible oil sample varies in producer and country of origin. Including binary mixtures there were altogether 105 oil samples. The aim of this study was to determine the content of each studied hempseed oil sample using established methodology regarding iodine value [1] and fatty acid composition [2], and to select optimal variables and establish optimal regression model for prediction of adulteration with already mentioned adulterant oils.

Obtained results show that although hempseed oil samples vary considerably in iodine value (154.0 – 165.5) and omega-3 fatty acids (15.7 – 20.0 %), interval ridge regression [3] and first-break forward interval partial-least square regression (FB-FiPLS) [4] obtain significantly lower root-mean error of prediction than PLS applied to the whole considered NMR spectral region (6 – 0 ppm) in most studied cases. To the best of our knowledge merged-interval regression procedures are for the first time applied to NMR data. Obtained prediction accuracy for volume fraction of each adulterant oil in binary mixtures (1.4 – 3.0 %, $0.991 < R^2 < 0.998$) are promising enough to conclude that ^{1}H NMR combined with the inspected chemometric procedures can be used to effectively quantify adulteration in hempseed oils, even when many samples of different geographical origin are considered for both hempseed oil and adulterant oils.

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TRIMETHYLSILYL TAG FOR THE CHARACTERISATION OF PROTEIN-LIGAND INTERACTIONS BY NMR

Walter Becker,[a,b] Christoph Nitsche,[a] Luke Adams,[c] Bim Graham,[c]
Klaus Zangger,[b] Gottfried Ottinga*

[a] The Australian National University, Research School of Chemistry, Canberra, ACT 2601, Australia
[b] University of Graz, Institute of Chemistry, 8010 Graz, Austria
[c] Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia
*E-mail: gottfried.otting@anu.edu.au

Fragment-based drug discovery (FBDD) is a widely used approach in pharmaceutical and biotechnological companies. Nuclear magnetic resonance (NMR) spectroscopy is an important experimental method in FBDD, as it enables the screening of large compound libraries to identify weak binders in a high throughput manner. Limitations arise from requirements in protein size, amount and availability of isotopic labelling, especially in the absence of structural information.

Here we present a new tool for measurement of ligand binding affinity and characterisation of ligand binding site. The approach is based on a small molecule tag, trimethylsilyl methyl 2-iodoacetate (TMS), that reacts with the thiol group of a solvent-exposed cysteine residue. The TMS tag generates a narrow and intense singlet resonance in the 1D ^{1}H-NMR spectrum that can easily be detected without any isotope labelling. The chemical shift of the signal is near 0 ppm, where there are very few protein resonances. To demonstrate the potential of the TMS tag we used the Zika virus protease NS2B-NS3[1] and the human prolyl isomerase FK506 binding protein (FKBP).[2] Both proteins are established drug targets. In the case of NS2B-NS3 we mutated the natural cysteine residues (C80, C143) to serine and substituted V36 near the active site by a cysteine. In FKBP we mutated the natural cysteine residue (C22) to serine and mutated R18 to cysteine. Following ligation of the TMS probe to the cysteine residues we acquired 1D ^{1}H-NMR spectra with and without inhibitors. Changes in lineshape and chemical shift of the TMS signal upon titration of the Zika virus protease with an inhibitor (4-nitrophenyl 4-guanidinobenzoate hydrochloride) revealed a dissociation constant K_d of about 20 µM. The same K_d value was measured for TMS tags attached at different sites. The ligand-induced chemical shift changes in the TMS signal decreased in size with increasing distance of the TMS group from the active site. The FKBP inhibitor 4-hydroxy-*N*-(4-hydroxyphenyl)benzamide showed a K_d value of about 200 µM. In both protein targets, the TMS signal provided very clear evidence for fast or slow ligand exchange. In the case of slow exchange, integration of the TMS signals in the free and ligand-bound protein was greatly facilitated by its location in a spectral region with few protein resonances.

In conclusion, the TMS tag provides an inexpensive and sensitive tool to assess ligand binding in target proteins without any isotope labelling using straightforward 1D ^{1}H-NMR spectra. As far as the chemical shift perturbation of the TMS signal is related to the distance from the ligand binding site, the tag also provides information about the approximate location of the ligand on the protein.

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AUTHORS AND PARTICIPANTS

AUTHORS

PARTICIPANTS

Barišić, Dajana
Becker, Walter
Biljan, Ivana
Botana, Adolfo
Bregović, Nikola
Cindro, Nikola
Dorai, Kavita
Dydio, Paweł
Gubensäk, Nina
Habinovec, Iva
Jednačak, Tomislav
Jović, Ozren
Kavčič, Luka
Lenarčič Živković, Martina
Loss, Sandra
Mihoci, Marijana
Munier, Jean Pierre
Müller, Norbert
Nastasić, Vladimir
Novak, Predrag
Oberer, Monika
Padmanabha Das, Krishna Mohan
Parlov Vuković, Jelena
Pičuljan, Katarina
Pitarević, Lovorka
Plavec, Janez
Požar, Josip
Pupić-Bakrač, Mirko
Ripamonti, Angelo
Roca, Sunčica
Rozman, Jan
Rubčić, Mirta
Stipaničić, Siniša
Szántay, Jr., Csaba
Tireli, Martina
Tomišić, Vladislav
Vajs, Vlatka
Vančik, Hrvoj
Vikić-Topić, Dražen
Zangger, Klaus
Zellhofer, Thomas
Žižek, Slavko

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