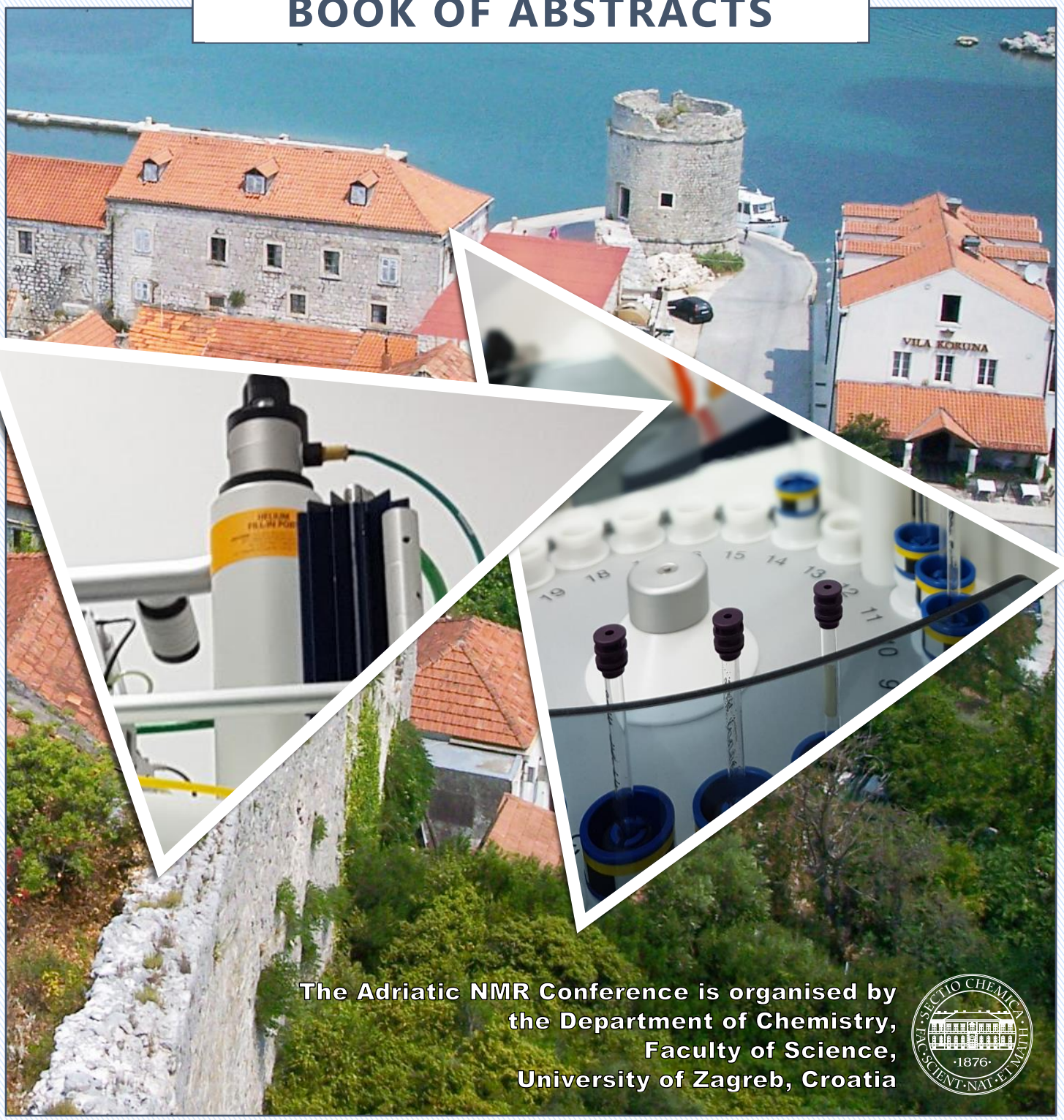
ADRIATIC NMR

June 15–17, 2018, 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 15–17, 2018, Mali Ston, Croatia

ADRIATIC NMR CONFERENCE

Mali Ston, Pelješac, 15–17 June 2018.

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IMPRESSUM

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

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Dear Participants,

the Organizing committee welcomes you to the Adriatic NMR conference in Mali Ston at the Pelješac peninsula, 15 to 17 June 2018.

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 northwest 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.

Organizing Committee

ORGANIZING COMMITTEE

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In memory of Professor Zlatko Meić

This conference is dedicated to late professor Zlatko Meić who was one of the pioneers of NMR in Croatia. He was a distinguished scientist with remarkable achievements in the field of spectroscopy. He published more than 100 papers and book chapters in esteemed international journals and gave many inspiring lectures at scientific conferences and academic institutions worldwide. He was a great teacher, unselfishly trying to help his students but also encouraging their autonomy and self-direction in solving problems.

We will remember him by his outstanding erudition, subtle humour and open mindedness and we will always keep a good memory of him in our minds.

Predrag Novak

ADRIATIC NMR

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PROGRAMME

FRIDAY, JUNE 15		
8:00 – 9:30	**REGISTRATION**	
CHAIR: KLAUS ZANGGER		
9:30 – 10:10	**PL-1**	**Robert Konrat:** *NMR Reveals Order in Disordered Proteins*
10:10 – 10:50	**PL-2**	**Roberta Pierattelli:** *Un-structural Biology by NMR Spectroscopy*
10:50 – 11:10	**IL-1**	**Anita Kotar:** *Small-molecule Optical Probe DAOTA-M2 Interacting With a Parallel G-quadruplex*
11:10 – 11:30	**COFFEE BREAK**	
CHAIR: MARIO SCHUBERT		
11:30 – 12:10	**PL-3**	**Janez Plavec:** *Exploration of Structures of G-rich DNA Regions by NMR*
12:10 – 12:30	**TD-1**	**Francesca Benevelli (Bruker):** *AVANCE NEO – Breakthrough in Multi-Receive NMR Technology*
12:30 – 13:10	**PL-4**	**Gregor Ilc:** *NMR Pathway to Structural Comparability Assessment of Biosimilar Compounds*
CHAIR: LEO FRKANEC		
13:10 – 13:50	**POSTER SESSION 1**	
13:50 – 15:50	**LUNCH**	
CHAIR: ROBERTA PIERATTELLI		
15:50 – 16:30	**PL-5**	**Klaus Zangger:** *Enhancing Time and Frequency Resolution by Restricted Acquisition*
16:30 – 16:50	**IL-2**	**Sebastian Tassoti:** *High Resolution for Chemical Shifts and Scalar Coupling Constants: The 2D Real-time J-upscaled PSYCHE-DIAG*
16:50 – 17:10	**COFFEE BREAK**	
CHAIR: JANEZ PLAVEC		
17:10 – 17:30	**IL-3**	**Jelena Parlov Vuković:** *Applications of NMR Spectroscopy in Petroleum Science*
17:30 – 17:50	**IL-4**	**Lovorka Pitarević:** *Use of NMR Spectroscopy in Quality Control of Pharmaceutical Products*

SATURDAY, JUNE 16

CHAIR: VLADISLAV TOMIŠIĆ		
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9:40 – 10:20	**PL-7**	**Leo Frkanec:** *Application of NMR Methods in Supramolecular Chemistry and Molecular Self-assemblies*
10:20 – 10:40	**IL-5**	**Josip Požar:** *NMR Spectroscopy and Thermodynamics of Supramolecular Host-Guest Reactions; Calixarenes and Cyclodextrins*
10:40 – 11:00	**IL-6**	**Nikola Cindro:** *Synthesis and Complexation Properties of Novel Glycoconjugated Calix[4]arenes*
11:00 – 11:20	**COFFEE BREAK**	
CHAIR: NIKOLA BREGOVIĆ		
11:20 – 11:50	**IL-7**	**Thomas Zellhofer:** *The Stern-Gerlach Experiment – Nearby 100 Years Ago*
11:50 – 12:10	**IL-8**	**Ivana Biljan:** *Dimerization of Aromatic C-nitroso Compounds: Insights From NMR Spectroscopy*
12:10 – 12:30	**IL-9**	**Valerije Vrček:** *Mechanistic Insights Into Chemical Reactions Via "In Situ" NMR*
CHAIR: NIKO RADULOVIĆ		
12:30 – 13:10	**POSTER SESSION 2**	
13:10 – 15:00	**LUNCH**	
15:00	**EXCURSION**	

SUNDAY, JUNE 17

CHAIRS: PREDRAG NOVAK, DRAŽEN VIKIĆ-TOPIĆ **This section is dedicated to Professor Zlatko Meić (1938 – 2017)**		
9:30 – 9:45		**Predrag Novak and Dražen Vikić-Topić:** *Introduction*
9:45 – 10:25	**PL-8**	**Vilko Smrečki:** *Deuterium Isotope Effects in ^{13}C NMR Spectra of Mono- and Binuclear Aromatic Compounds*
10:25 – 11:05	**PL-9**	**Tomislav Biljan:** *NMR Spectroscopy and Generic Pharma R&D: Past, Present and the Future*
11:05 – 11:25	**COFFEE BREAK**	
CHAIR: ROBERT KONRAT		
11:25 – 11:45	**TD-2**	**Yusuke Nishiyama (JEOL):** *Ultrafast Magic Angle Spinning Solid-state NMR: Methods and Applications*
11:45 – 12:25	**PL-10**	**Mario Schubert:** *Posttranslational Modifications in Proteins Studied by NMR Spectroscopy*
12:25 – 13:05	**PL-11**	**Niko Radulović:** *Two NMR-oriented Approaches to the Identification of Natural Products Directly From Their Mixtures*
13:05 – 13:25	**IL-10**	**Jelena Tošović:** *Determination of Chlorogenic Acid Structure Using Combined Experimental and Theoretical NMR Study*
13:25 – 13:45	**IL-11**	**Mario Vazdar:** *Arginine "Magic": Guanidinium Like-Charge Ion Pairing from Aqueous Salts to Cell Penetrating Peptides*
13:45	**CLOSING**	
13:50	**LUNCH**	

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

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P2	**Katarina Vazdar, Ivanka Jerić:** Amino-β-Lactams in Ugi Reaction: Synthesis and NMR Analysis of Functionalized Peptidomimetics
P3	**Niko S. Radulović, Milica M. Todorovska, Dragan B. Zlatković, Nikola M. Stojanović:** NMR Determination of Enantiomeric Purity of 5-Phenyl-1,3-oxazolidine-2-thione From Reseda Luteola
P4	**Barbara Pem, Valerije Vrček, Marija Ćurlin, Darija Domazet Jurašin, Ivana Vinković Vrček:** NMR Study of Nano-bio Interface: a Case of Interaction Between Gold Nanoparticles and Biothiols
P5	**Branimir Bertoša, Marija Cvetnić, Josip Požar, Nikola Cindro, Vladislav Tomišić:** Monte Carlo Conformational Search of Glycoconjugated Amide-based Calix[4]arenes
P6	**Vojč Kocman, and Janez Plavec:** Multiple Possible Folds in the Regulatory Region of the *PLEKHG3* Gene
P7	**Katarina Leko, Andrea Usenik, Nikola Cindro, Vladislav Tomišić:** Synthesis and Complexation Properties of Fluorescent Phenanthridine-based Calix[4]arene Derivatives
P8	**Urška Slapšak, Giulia Salzano, Gregor Ilc, Gabriele Giachin, Giuseppe Legname, Janez Plavec:** Structure of Mule Deer Prion Protein Provides Insight Into Chronic Wasting Disease
P9	**Nina Gubensäk, Tea Pavkov-Keller, Thomas Eichmann, Stefan Schild, Klaus Zangger:** New Structural Insights Into the Gram-negative Lipid Transporter YrbC

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P11	**Eduard Stadler, Georg Gescheidt:** New Ways for Investigating Photochemical Reactions With NMR
P12	**Ivana Mikulandra, Tomislav Jednačak, Jelena Parlov Vuković, Mateja Djetelić Ibrahimpašić, Klaus Zangger, Vilko Smrečki, Predrag Novak:** Concentration Dependence of Asphaltene Aggregation Monitored by DOSY NMR
P13	**Iva Habinovec, Predrag Novak, Katarina Pičuljan, Tomislav Jednačak, Ivana Rubić, Ivana Mikulandra, Ivan Grgičević, Mirjana Bukvić Krajačić, Nina Gubensäk, Klaus Zangger:** Impurity Profiling of Azithromycin Conjugates by LC-SPE/cryo NMR Methodology
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P17	**Tomislav Gregorić and Leo Frkanec:** Structure Characterization of Self-Assembled Novel Amino Acid Fumaramides by NMR
P18	**Martin Walenta, Kathrin Buchberger, Alexander Pöcheim, Sebastian Tassoti, Olaf Kunert, and Klaus Zangger:** Differentiating Alcohols From Ethers by Deuterium Isotope Effects
P19	**Tamara Rinkovec, Nikolina Vidović, Nikola Cindro, Giovanna Speranza, Gordan Horvat, Vladislav Tomišić:** Thermodynamic and Structural Studies of the Complexation of Homocyclopeptides With Halide and Structural Anions in Acetonitrile

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LECTURES

NMR REVEALS ORDER IN DISORDERED PROTEINS

Robert Konrat

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Despite the accepted belief that protein function is encoded in the three dimensional (3D) structure of a protein *Intrinsically Disordered* (IDPs) have attracted a lot of attention over the last decade due to both their fascinating structural properties and their involvement in important physiological and pathological processes. The inherent structural flexibility of IDPs requires the application of appropriate experimental methods since X-Ray crystallography cannot access the distribution of conformational states of these proteins. Over the last decade an NMR based methodological framework has emerged to characterize the structural dynamics of IDPs. In particular, paramagnetic relaxation enhancement (PRE) has matured into a well-established technique to probe residual structures and local compaction of the polypeptide chain as well as long-range transient contacts, although the existence of concerted motions and cooperatively folded segments cannot be detected. To circumvent this problem we recently proposed a novel technique coined paramagnetic relaxation interference (PRI) based on cross-correlation effects between pairs of spin labels in doubly-labelled protein systems. Additionally, cross-correlated NMR relaxation (CCR) has been established as a useful tool to study structure and dynamics of proteins in solution. These effects have been shown to be a valuable source of information about structure and dynamics of proteins, since their concerted effect is related to their relative geometry. Although the situation is more complex in IDPs it will be demonstrated that this approach is also feasible in conformationally flexible proteins. Given the sensitivity of CCR experiments to subtle structural changes, it can be expected that CCR will be able to make a substantial contribution to the study of the dynamic nature of IDPs in solution.

UN-STRUCTURAL BIOLOGY BY NMR SPECTROSCOPY

Roberta Pierattelli

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The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated as intrinsic disorder, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved.

In recent years many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases.

The general properties of IDPs cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in their investigation, being the only method that allows a high resolution description of their structural and dynamic features in solution. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information.

We will illustrate how NMR can help in describing the importance of intrinsic disorder to encode in a relatively short polypeptide many functional modules.

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EXPLORATION OF STRUCTURES OF G-RICH DNA REGIONS BY NMR

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DNA can adopt various secondary structures including duplexes, triplexes, quadruplexes and i-motifs as well as other branched architectures. The canonical Watson-Crick paired duplexes play major roles in genetic inheritance and gene expression. Other structures have been associated with many different biological functions of DNA. The most well studied alternative DNA structures are G-quadruplexes. They are formed by G-rich sequences and consist of four-stranded columnar structures. G-quadruplexes are stabilized by the stacking of multiple Hoogsteen-hydrogen-bonded G-quartets. Notably, cations residing in the center of G-quartets contribute additional stabilizing factor through electrostatic interactions. The G-rich sequences have been identified in numerous regions of human genome including chromosomal telomeres and many gene promoters, which play important roles in DNA recombination, replication, transcription, translation, and many other critical biological processes. Discoveries of these sequences have led to significant interest in finding ways to control or modulate formation of G-quadruplexes. Our laboratory has been using NMR extensively to uncover structural details of G-quadruplexes in relation to sequence details [1-4], presence of cosolutes [5] interaction with ligands [6] and even expand the structure and sequence complexity of DNA four-stranded architectures by discovering AGCGA-quadruplexes [7].

[1] M. Marušič, J. Plavec, *Angew. Chem. Int. Ed.* **2015**, *54*, 11716–11719.
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[5] M. Trajkovski, T. Endoh, H. Tateishi-Karimata, T. Ohyama, S. Tanaka, J. Plavec, N. Sugimoto, *Nucleic Acids Res.* **2018**, 46, 4301.
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NMR PATHWAY TO STRUCTURAL COMPARABILITY ASSESSMENT OF BIOSIMILAR COMPOUNDS

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The patents for the first generation of approved biological drugs have either already expired or are about to expire in the near future, providing an open market for biosimilars that are expected to reduce costs of treatments and thus allow greater accessibility for biologic therapies. Biosimilar compounds are defined as complex proteins and nucleic acids that are highly similar to already approved reference product by Food and Drug Administration. They must contain no clinically meaningful differences from the reference products and only minor differences in inactive components are allowed. In order to evaluate and compare reference products and their biosimilars, an array of analytical and biophysical methods are required with high accuracy, precision and robustness.

We are extensively trying to advance the application of NMR methods to the structural characterization of protein biologics. Our results have demonstrated that advanced 2D-NMR can yield a precise and unique “fingerprint” of the higher-order structure among biosimilars. 2D-NMR spectroscopy is one of the few approaches that can yield complete assignment of 3D structure across the entire molecule at atomic-level resolution. The final goal is to translate complex NMR spectral data into similarity scores that are used to estimate the degree of similarity between the biosimilar and reference product.

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ENHANCING TIME AND FREQUENCY RESOLUTION BY *RESTRICTED ACQUISITION*

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

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[3] S. Glanzer, K. Zangger, *J. Am. Chem. Soc.* **2015**, *137*(*15*), 5163–5169.
[4] G. Wagner, P. Sakhaii, W. Bermel, K. Zangger, *Chem. Commun.* **2013**, *49*(*30*), 3155–3157.

STIMULI-RESPONSIVE SUPRAMOLECULAR SWITCHES BASED ON HIGH AFFINITY HOST-GUEST BINDING PAIRS

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Water soluble macrocyclic receptors showing high affinity and selectivity for target guest molecules hold great potential to be employed in a wide range of applications such as sensing, biochemical assays, separation, self-assembled functional materials, functional surfaces, molecular machines and many others. While it is widely recognized that stimuli-responsiveness expands the functionality of some target applications based on high-affinity host-guest pairs; controlling their association/dissociation with external inputs such as pH, light, temperature or electrons becomes more challenging as stability increases. In the last years we have been focused on macrocycles such as cucurbiturils, cyclodextrins and calixarenes to develop high-affinity host-guest complexes that respond to external stimuli. In this lecture I will highlight and discuss some examples from our group to illustrate the journey from moderate to ultrastable stimuli-responsive host-guest complexes and their applications in pseudo-rotaxanes, self-sorting systems and in the complexation and stimulated release of functional molecules such as drugs and fragrances.[1–7]

[1] A. Acuña, N. Basilio, M. Parajo, J. C. C. Mejuto, J. Perez-Juste, P. Taladriz-Blanco, L. Garcia Rio, *Org. Biomol. Chem.* **2018**, *14*, 9105.
[2] A. Zubillaga, P. Ferreira, A. J. Parola, S. Gago, N. Basílio, *Chem. Commun.* **2018**, *54*, 2743.
[3] M. A. Romero, N. Basílio, A. J. Moro, M. Domingues, J. A. González-Delgado, J. F. Arteaga, U. Pischel, *Chem. - A Eur. J.* **2017**, *23*, 13105.
[4] N. Basílio, J. Mendoza, S. Gago, A. J. Parola, *Chem. Commun.* **2017**, *53*, 6472.
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APPLICATION OF NMR METHODS IN SUPRAMOLECULAR CHEMISTRY AND MOLECULAR SELF-ASSEMBLIES

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Self-assembly of small organic molecules is one of the most important methods of synthesis of organic nano-structured materials. Based on the rules of supramolecular chemistry, the bottom-up approach to design functional objects at nanoscale is currently producing highly sophisticated materials oriented towards a growing number of applications. These structuring features are nowadays well understood and can be finely controlled in order to introduce and tune functional properties of self-assembled nanomaterials used for a very wide range of applications. Further, such systems exhibit a complex array of morphologies and dynamics and have a huge potential for utilization in various areas of nanotechnology, in development of new materials, diagnostic or "smart" systems for drug delivery, in medicine or in tissue engineering.[1,2]

In recent years, various chiral bis (amino acid) oxamides and other structural types that include amino acid subunits have been investigated as low molecular weight gelators with a particular focus on stereochemical effects and solvent influence on gel systems. [3–5] The molecular self-assembling and supramolecular interactions in the new supramolecular systems were studied by various spectroscopic methods e.g. NMR, FTIR, CD, that proved the self-aggregation of molecules. The contribution made by using of various NMR methods in the characterization of such systems will be shown through several examples.

[1] E. Busseron, Y. Ruff, E. Moulin, N. Giuseppone, *Nanoscale* **2013**, *5*, 7098.
[2] T. Pospišil, L. Ferhatović Hamzić, L. Brkić Ahmed, M. Lovrić, S. Gajović, L. Frkanec, *Biomater. Sci.* **2016,** *4*, 1412.
[3] L. Frkanec, M. Žinić, *Chem. Commun*. **2010**, *46*, 522.
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DEUTERIUM ISOTOPE EFFECTS IN ^{13}C NMR SPECTRA OF MONO- AND BINUCLEAR AROMATIC COMPOUNDS

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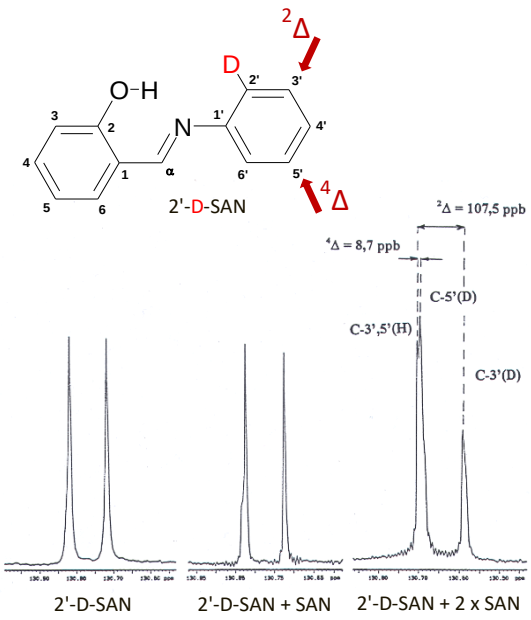
†dedicated to the Memory of the late Professor Zlatko Meić

Isotopic substitution produces changes in the reactivity of a molecule and causes a redistribution of molecular internal vibrational and rotational energy. In NMR, it causes changes in chemical shifts (nuclear shielding), coupling constants and relaxation times. The most studied by far are deuterium effects on nuclear shielding owing to the large fractional change in mass on isotopic substitution and relative ease of their determination from high resolution NMR spectra.

$$^{n}\Delta\,[^{13}C(^{2}H)] = \delta^{13}C\,(^{1}H) - \delta^{13}C\,(^{2}H)$$



The focus of this talk will be put to unsaturated systems containing one or two bridged phenyl groups where isotopic perturbation can be transmitted many bonds away from the isotope substitution site. The sign alternation and specific magnitudes of such long-range isotope effects will be discussed in terms of subtle charge shifts throughout the molecule as a consequence of bond shortening upon isotopic substitution. Moreover, the linear correlation between long-range isotope effects and molecular torsional angle can serve as a conformational probe [1] for studied type of molecules.

[1] P. Novak, D. Vikić-Topić, V. Smrečki, and Z. Meić, Isotope Effects in NMR Spectra as a Structural Tool for Organic Molecules, in *New Advances in Analytical Chemistry* (Ed.: Atta-ur-Rahman), Harwood Academic Publishers, Amsterdam, **2000**, 135–168.

NMR SPECTROSCOPY AND GENERIC PHARMA R&D: PAST, PRESENT AND THE FUTURE

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Historical overview, current status and future directions for NMR spectroscopy use in generic pharma R&D and industry in general will be presented including regulatory requirements. Some examples of NMR spectroscopy use in real-life solving of problems during development of generic API (active pharmaceutical ingredient) and final dosage forms will be given. Examples will include organic synthesis particularly fluorine and boron compounds; metal catalysts and also polymorphs and solid dispersions.

POSTTRANSLATIONAL MODIFICATIONS IN PROTEINS STUDIED BY NMR SPECTROSCOPY

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The presence of post-translational modifications (PTMs) and degradation products in proteins can be a serious problem, especially in therapeutic proteins. Detecting and quantifying them is very important but also challenging.

A spontaneous, nonenzymatic PTM is succinimide (Snn), which can form spontaneously in prone primary protein sequences, resulting typically in an equilibrium between Snn and its hydrolysis products isoaspartate (isoAsp) and aspartate. We used 2D NMR spectroscopy to assign all ^{1}H and ^{13}C chemical shifts of Snn and isoAsp in model peptides and found characteristic chemical shift correlations. Chemical shift reference data suitable for comparison with data of denatured proteins were obtained in 7 M urea (pH 2.3). The characteristic 2D NMR fingerprint of Snn was used to detect and quantify this PTM in the model protein lysozyme, a biotherapeutic, and the recombinant, non-glycosylated Fc part of immunoglobulin G1 [1].

Glycosylation is the most abundant but also most complex PTM, because of the vast variability of branches, linkages and extensions. However, functionally relevant are only parts of the glycans: glycoepitopes. Identifying the type glycoepitopes in a sample is a challenge. A new search algorithm for chemical shifts of an observed spin system will be presented that finds matching chemical shifts in a chemical shift database. Since carbohydrate chemical shifts are very sensitive to the sugar type, and type of extensions, the type of monosaccharide and its environment can be predicted.

Studying PTMs by NMR spectroscopy is a promising method to analyze proteins and peptides from natural sources, recombinant expression, or chemical synthesis.

[1] L. Grassi, C. Regl, S. Wildner, G. Gadermaier, C. G. Huber, C. Cabrele, M. Schubert. *Anal. Chem.* **2017**, *89,* 11962–11970.

TWO NMR-ORIENTED APPROACHES TO THE IDENTIFICATION OF NATURAL PRODUCTS DIRECTLY FROM THEIR MIXTURES

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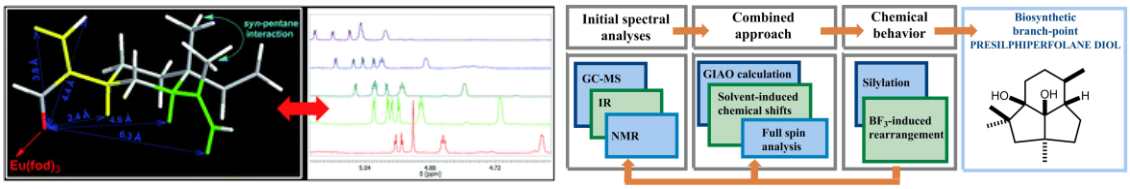
Nature offers an inexhaustible pool of biologically relevant molecules crafted by evolution and working in unison. Extensive analyses of mixtures of naturally occurring molecules of plant and microbial origin enable us to locate the possible organisms for bioprospecting. However, the classical approaches leave much "unidentified" and "unassigned" compounds. Metabolites displaying highly overlapped and higher order NMR signals are unattractive targets and require the application and development of innovative analytical methodologies. We investigated the potential usefulness of lanthanide-induced shift reagents for the resolution and assignment of overlapped ^{1}H NMR signals originating from different components of a complex natural mixture (i.e. for qualitative analysis). The incremental addition of Eu(fod)$_3$ leads to a simplification of NMR spectra in terms of signal overlap and removal of chemical shift degeneracy, allowing the mining of crucial data from the shifted NMR spectra. 2D-NMR spectra (^{1}H–^{1}H-COSY, NOESY, HSQC and HMBC) of the sample mixed with Eu(fod)$_3$ prove to be particularly valuable in this respect. This approach was applied in the case of a rare bioactive sesquiterpene elema-1,3,11(13)-trien-12-al. Additionally, we have demonstrated that combining solvent-induced removal of chemical shift degeneracy and theoretical (DFT-GIAO) prediction of NMR spectra with the analysis of ^{1}H NMR splitting patterns can facilitate structural elucidation of organic molecules with difficult-to-interpret NMR data. The approach was developed and its usefulness illustrated in the challenging case of a new natural triquinane sesquiterpene, presilphiperfolane-7α,8α-diol. Thus, herein we report on a new chromatography-free methodology that could be of value in structure elucidation of unknown compounds even if they are not available in pure state and present an approach that decreases the probability of an erroneous identification, and allows an unambiguous stereochemical elucidation and full NMR assignment.



SMALL-MOLECULE OPTICAL PROBE DAOTA-M2 INTERACTING WITH A PARALLEL G-QUADRUPLEX

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G-quadruplexes (G4s) are important nucleic acid secondary structures, formed by guanine-rich DNA oligonucleotides.[1] G4s have been associated with regulation of diverse biological processes like genomic instability and regulation of gene expression. Because of their important regulatory functions, G4s could have a potential as targets for antitumor therapies.

We studied the interactions between a G-quadruplex from promoter region of *c-myc* gene and a small-molecule optical probe DAOTA-M2 (Figure 1). It has a significantly longer fluorescence lifetime upon binding to G4s in comparison to double- and single-stranded nucleic acids [2]. Thus, DAOTA-M2 can be used to monitor G4s *in vitro* and in live cells. NMR structural study revealed that DAOTA-M2 forms a well-defined complex with the G-quadruplex at 1:2 binding stoichiometry (PDB ID: 5LIG) [3]. The insights into binding characteristics of DAOTA-M2 provide a structural rationale for its unique fluorescence behavior upon binding to G4s and stimulate development of novel probes with improved selectivity and fluorescence response.

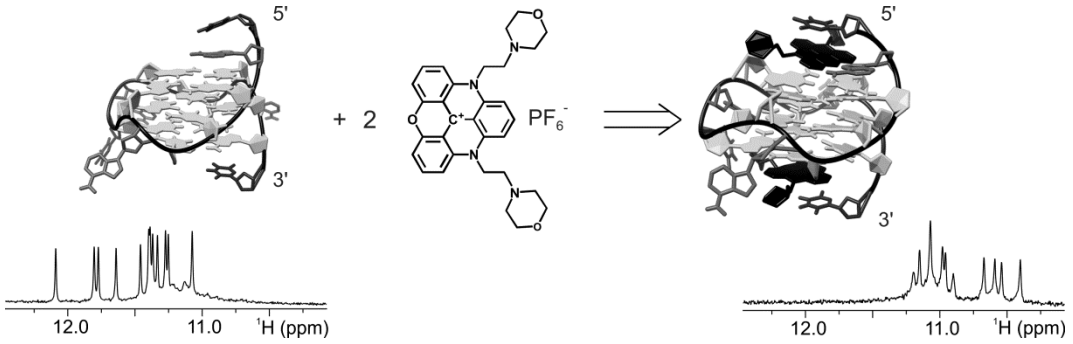

Figure 1. The imino region of ^{1}H NMR spectra of G-quadruplex alone and in complex with DAOTA-M2.

[1] T. Tian *et al.*, *Chem*. **2018**, doi: 10.1016/j.chempr.2018.02.014.
[2] A. Shivalingam *et al.*, *Nat. Commun.* **2015**, *6*, 8178.
[3] A. Kotar *et al.*, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 12508–12511.

Acknowledgements: The study was done in collaboration with the group of Professor R. Vilar, Imperial College London.

HIGH RESOLUTION FOR CHEMICAL SHIFTS AND SCALAR COUPLING CONSTANTS: THE 2D REAL-TIME J-UPSCALED PSYCHE-DIAG

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NMR spectra yield two main sources for structural information: resonance frequencies and scalar coupling patterns. Two factors often prevent facile determination of the coupling patterns and *J*-values: the limited chemical shift range of proton signals leads to overlaps, and small coupling constants are hidden within the linewidth of common 1D experiments.

High resolution of chemical shift can be achieved by so-called pure shift experiments, but these approaches collapse all coupling patterns to singlets, losing all information about couplings. Recently, the DIAG experiment [1] was used to only decouple the indirect dimension of a 2D spectrum, generating signals with retained coupling patterns along the diagonal. Drastic reduction of the spectral window in the indirect dimension allows high resolution of the chemical shift, however the resolution of the traces in the direct dimension is limited.

On the other hand, determination of small coupling constants has been facilitated by the proposal of real-time *J*-upscaling. [2] This method allows the acquisition and interpretation of small couplings by allowing for a period of scalar coupling, but not chemical shift evolution. Applied to a regular 1D proton spectrum, *J*-upscaling often is limited by signal overlap due to the upscaling of multiplets even in small molecules. It can however be applied to the direct dimension of a 2D experiment.

Combination of the DIAG experiment and *J*-upscaling yields 2D NMR spectra in which the chemical shift is resolved in the indirect dimension with ultrahigh resolution, whereas the scalar coupling information can be determined from the direct dimension of the spectra in high resolution.

[1] A. Cotte, D. Jeannerat, *Angew. Chem. Int. Ed.* **2015**, *127*, 6114–6116.
[2] S. Glanzer, K. Zangger, *J. Am. Chem. Soc.* **2015**, *137*, 5163–5169.

APPLICATIONS OF NMR SPECTROSCOPY IN PETROLEUM SCIENCE

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Complex chemical composition and physical properties of oil and oil products make their complete characterization very difficult. Components present in oil samples differ in structure, size, polarity and functionality. NMR spectroscopy plays a significant role in analysis and identification of complex hydrocarbons mixtures of oil samples.

In this presentation the application of NMR spectroscopy for analyzing gasoline and diesel fuels, middle and high fractions, residues and asphaltenes will be described.[1] By using NMR spectroscopy it is possible to determine gasoline composition and presence of benzene and oxygenates as well as some important physical characteristics of gasoline such as the research octane number, for example. An application of different NMR techniques made it possible to characterize diesel fuels and middle and higher oil distillates from various refineries.[2] Data so obtained can be used in combination with statistical methods to predict fuel properties and to monitor production processes in oil industry. NMR spectroscopy has proven useful in analysis of biomasses and biofuels. Furthermore, techniques such as DOSY for characterization of asphaltenes will be discussed as well.[3]

[1] J. Parlov Vuković, V. Srića, P. Novak, *Kem. Ind.* **2012**, 61 (11–12) 513–522.
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[3] J. Parlov Vuković, T. Hrenar, P. Novak, J. Plavec, M. Friedrich, *Energy & Fuels* **2017**, *31*(*8*), 8095–8101.

USE OF NMR SPECTROSCOPY IN QUALITY CONTROL OF PHARMACEUTICAL PRODUCTS

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Quality control of finished pharmaceutical product in the shelf-life consists of diverse analytical methods that are validated by manufacturer and approved by national authority. Using system suitability tests the given analytical methods are transferred to the laboratories. Laboratories in charge of monitoring the quality of pharmaceutical products in shelf life are usually a part of national governments in EU, which is also the case in Croatia.

Nuclear magnetic resonance spectroscopy is not at all common as a method of choice during quality control of finished pharmaceutical products for identification of active substances or impurities. It is due to different reasons but mainly NMR is not a part of analytical procedures for finished product that are part of the drug master file, DMF.

On the other hand, NMR as a method for identification of active substances used in production of the finished product is a main part of DMF. So, in the process of approval of complete drug documentation qualified persons evaluate very complex NMR spectra to be sure that suitable substances are used in the production process.

Further on, analysis of counterfeit drugs is a very particular and challenging task, NMR among other analytical methods and techniques found its place in it, especially in the qualitative analytical part.

NMR SPECTROSCOPY AND THERMODYNAMICS OF SUPRAMOLECULAR HOST-GUEST REACTIONS; CALIXARENES AND CYCLODEXTRINS

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The lecture will be dedicated to the role of NMR spectroscopy in the physico-chemical investigations of host-guest complexation reactions, namely the complexation of alkali-metal cations with calix[4]arenes, and the inclusion of lipophilic moieties within hydrophobic cavity of cyclodextrins.

In the first part of the lecture, comprehensive investigations of calix[4]arene complexation reactions in several solvents will be described. The thermodynamic data (complex stability constants and derived standard reaction Gibbs energies, reaction enthalpies and entropies) determined by various experimental methods will be correlated with the results of structural investigations. The solvation effects on the equilibria of binding reactions will be discussed in detail. For that purpose, thermodynamic cycles involving the reactant and the complex transfers will be prepared and explained. The role of NMR spectroscopy in revealing specific solvation effects, which can influence the complexation equilibria remarkably, will be addressed.

In the second part of the lecture, the thermodynamics of various guest inclusion within the nonpolar cavity of cyclodextrins in several strongly hydrogen-bonding solvents will be reviewed. The influence of the guest structure, size, and conformational freedom on the complex stability will be discussed. The effect of temperature and solvent on the complexation reactions will be analysed in detail. The importance of NMR investigations for the rationalization of the corresponding thermodynamic complexation parameters will be revealed. Finally, the thermodynamics of solvophobically driven complexation involving non-polar solutes and cyclodextrins in organic solvents and water will be addressed.

Acknowledgements: This work has been supported by Croatian Science Foundation under the project IP-2014- 09-7309 (SupraCAR), and by European Regional Development Fund and Croatian Ministry of Science and Education under the project Met4Pharm (RC.2.2.08-0024).

SYNTHESIS AND COMPLEXATION PROPERTIES OF NOVEL GLYCOCONJUGATED CALIX[4]ARENES

Nikola Cindro,[a,*] Josip Požar, [a] Dajana Barišić,[a] Nikola Bregović,[a] Katarina Pičuljan,[a] Renato Tomaš,[b] Leo Frkanec,[c] Vladislav Tomišić[a]

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Calixarenes are a class of supramolecular cavitands that can be easily functionalized to give receptors for ionic and neutral species. Structural evolution of these compounds has led to more selective receptors with improved physico-chemical properties. Most of the synthesized calixarene ionic receptors are not sufficiently soluble in water which makes their use quite limited [1]. Water-soluble calixarenes usually own this property to the introduced easily-ionized sulfonic groups. In the scope of this work novel neutral and water-soluble ligands **1**–**3** (Figure 1) were designed and prepared, and their complexation with alkali-metal cations was investigated. Glucose was embedded in the structure as hydrophilic domain whereas secondary and tertiary amides served as cation-binding sites. The latter motifs were used since the calixarene derivatives comprising such groups were previously proven to form highly stable complexes [2]. Complexation reactions with alkali-metal cations were studied in water, methanol, and formamide thus giving insight into the solvation as well as intra- and intermolecular hydrogen bonds effects. Several techniques were used, such as UV spectrophotometry, isothermal titration calorimetry and NMR spectroscopy.

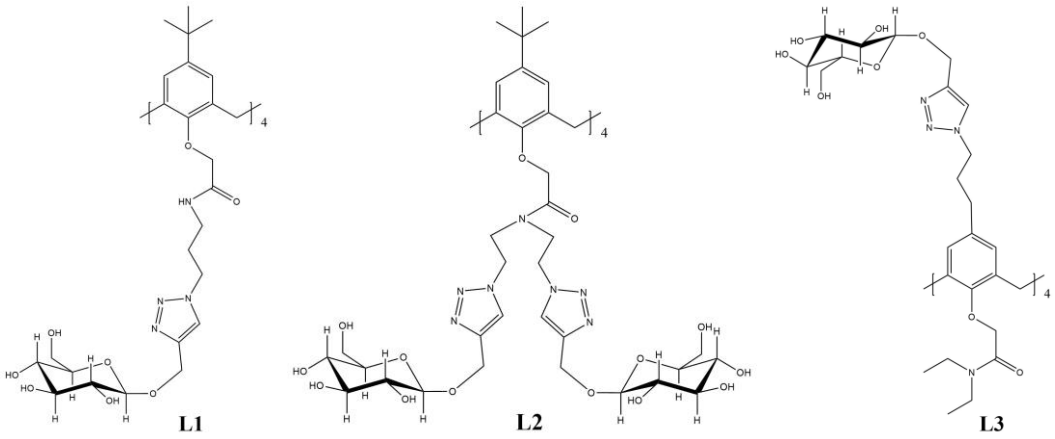

Figure 1. Structure of L1-3

[1] N. Cindro, J. Požar, D. Barišić, N. Bregović, K. Pičuljan, R. Tomaš, L. Frkanec, V.Tomišić, *Org. Biomol. Chem.* **2018**, 16, 904.
[2] G. Horvat, V. Stilinović, B. Kaitner, L. Frkanec, V. Tomišić, *Inorg. Chem.* **2013**, 21, 12702.

THE STERN-GERLACH EXPERIMENT – NEARBY 100 YEARS AGO

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The basic development for NMR have be done at the Institute of Physics at the Johann Wolfgang Goethe University in Frankfurt (Main). Max von Laue was the head of this institute from 1914 – 1919 and he brought pioneers to his group with Max Born and Alfred Landé, as they created the basics in quantum physics. The outstanding contributions came from Otto Stern (1914 – 1921 the Inst. of Physics) and his co-worker Walter Gerlach; they followed the hypothesis of Niels Bohr, that the rotating impulse of electrons follows quantum rules. The proof was performed with a simple microscope and a high vacuum pump in 1919. This was the start-up for NMR, which has today so many applications in chemistry, pharmacy and material science with more than 10 000 scientists working with this method worldwide.

[1] Horst Schmidt-Böcking, Karin Reich, *Otto Stern – Physiker, Querdenker, Nobelpreisträger*, Societäts-Verlag, Frankfurt (Main) **2011**.

DIMERIZATION OF AROMATIC C-NITROSO COMPOUNDS: INSIGHTS FROM NMR SPECTROSCOPY

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Aromatic C-nitroso compounds can dimerize by forming *Z*- or *E*-azodioxides. In the solid state, aromatic C-nitroso compounds are usually present as dimers, and in solution monomer-dimer equilibrium is established. At room temperature the equilibrium is usually shifted towards the nitroso monomers, while *Z*- and/or *E*-azodioxides can be observed only at low temperatures.

Our studies revealed that aromatic C-nitroso compounds are also able to form asymmetrical dimers or heterodimers. The selectivity in formation of heterodimers was examined by investigating cross-dimerization of several *p*- and *m*-substituted nitrosobenzene derivatives with the parent nitrosobenzene in solution by one- and two-dimensional variable temperature ^{1}H NMR, and in solid state by ^{13}C CP-MAS NMR and IR spectroscopy. It was found that in solution and in solid state the selectivity is different. The selectivity is in both media affected by the electron-donating ability of the substituent, and the differences can be explained by the influence of molecular arrangements in the crystal lattice.

Recently, we investigated solution-state monomer-azodioxide equilibria and conformational freedom of several new aromatic dinitroso derivatives by using one- and two-dimensional variable temperature ^{1}H NMR spectroscopy and quantum chemical calculations. Compounds with multiple aromatic nitroso groups are interesting because they can form oligomeric and polymeric structures through azodioxide bonds, which makes them promising candidates for design of new supramolecular systems. Inspection of NMR spectra revealed that the studied compounds dimerize by lowering the solution temperature yielding the mixture of monomers and *Z*- and/or *E*-azodioxides. Experimental NMR results were validated by DFT calculations enabling assignments of proton chemical shifts of azodioxy species. The DFT was used to predict the geometric (*Z*/*E*) preference of the azodioxy dimers revealing higher stability of the *Z*-form.

Acknowledgement: This work was supported by the Croatian Science Foundation, grant no. 7444, ORGMOL.

MECHANISTIC INSIGHTS INTO CHEMICAL REACTIONS VIA "IN SITU" NMR

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^{19}F and ^{1}H NMR experiments were performed to study the mechanism of selected chemical reactions. In the case of the acylation (ferrocenoylation) of purine and pyrimidine nucleobases, the attention has been focused on the regioselectivity/ regiospecificity of the respective reaction. It has been shown that the reaction centre and the product isomers ratio cannot be determined from the isolated weights alone, but the analysis of "in situ" NMR spectra is required.

In the case of chlorination of the 5-fluorouracil, "in situ" NMR data has been generated under actual reaction condition [2]. The existence of the presumed chlorinated intermediates has been confirmed spectrophotometrically, which is necessary to scheme out the full reaction profile.

The two cases demonstrate that the information obtained by "in situ" NMR approach may provide the answers to challenging mechanistic or kinetic studies, or may contribute to the optimization of synthetic routes.

Acknowledgement: This work is sponsored by the Croatian Science Foundation (IP-2016-06-1137).

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DETERMINATION OF CHLOROGENIC ACID STRUCTURE USING COMBINED EXPERIMENTAL AND THEORETICAL NMR STUDY

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Chlorogenic acid (5-*O*-caffeoylquinic acid, **5CQA**) is an ester of the caffeic and quinic acids which can be isolated from natural plants such as coffee, pears, plums, potatoes, tomatoes, carrots and oilseeds [1]. This compound proved to exhibit anticarcinogenic, antimutagenic and glucose lowering effects [2], antioxidative activity for human low-density lipoprotein [3], as well as anti-obesity properties and ability to improve lipid metabolism [4]. To determine the most stable conformer of chlorogenic acid in DMSO solution a detailed conformational analysis was performed. The lowest-energy conformers were further used for simulation of ^{1}H-NMR and ^{13}C-NMR at B3LYP-D2/, B3LYP-D3/, and M06-2X/6-311+G(d,p) levels of theory in combination with the CPCM solvation model. Very good agreement between experimental and simulated NMR spectra was achieved in the case of all three applied methods [5,6]. This finding indicates correct arrangement of the atoms in the **5CQA**. It was found that the structure is characterized with five intramolecular hydrogen bonds, where four of them are located on the quinic moiety and one is located on the benzene ring (Figure 1). A very interesting finding is that carboxylic hydrogen is not directed towards carboxylic oxygen, but towards the oxygen of the proximate hydroxyl group. Our results are in excellent agreement with the experimentally obtained NMR studies from Forino et al. [7] (Figure 1), indicating that all three applied theoretical models are capable of quantifying subtle differences among conformers.

Acknowledgement: This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia (project no. 172016).

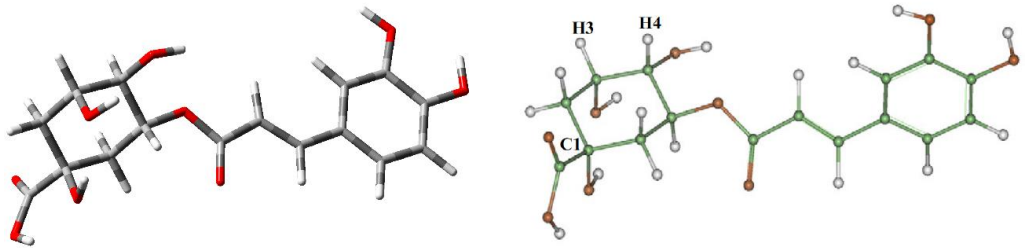

Figure 1. The most stable conformer of 5CQA in DMSO obtained at the B3LYP-D3/6-311+G(d,p) level of theory (left) and structure obtained experimentally by Forino et al. [7] (right).

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ARGININE "MAGIC": GUANIDINIUM LIKE-CHARGE ION PAIRING FROM AQUEOUS SALTS TO CELL PENETRATING PEPTIDES

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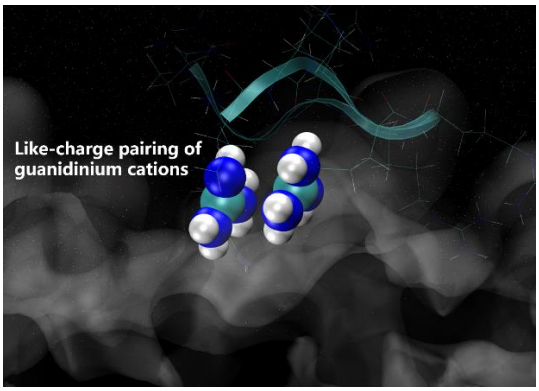

It is a textbook knowledge that charges of the same polarity repel each other. For two monovalent ions in the gas phase at a close contact this repulsive interaction amounts to hundreds of kilojoules per mole. In aqueous solutions, however, this Coulomb repulsion is strongly attenuated by a factor equal to the dielectric constant of the medium. The residual repulsion, which now amounts only to units of kJ/mol, may be in principle offset by attractive interactions. Probably the smallest like-charge pair, where a combination of dispersion and cavitation forces overwhelms the Coulomb repulsion, consists of two guanidinium cations in water. Indeed, by a combination of molecular dynamics and electronic structure calculations and electrophoretic as well as spectroscopic experiments we have demonstrated that aqueous guanidinium cations form thermodynamically stable like-charge ion pairs.

The importance of pairing of guanidinium cations in aqueous solutions goes beyond a mere physical curiosity and has significant biochemical implications. For example, arginine-arginine pairing has been frequently found in structural protein databases. In particular, when strengthened by a presence of negatively charged glutamate, aspartate, or C terminal carboxylic groups, this binding motif helps to stabilize peptide or protein dimers and is also found in or near active sites of several enzymes, as suggested by molecular dynamics simulations, SAXS and NMR experiments.

The like-charge pairing of the guanidinium side chain groups may also hold the key to the understanding of the arginine "magic", i.e., the extraordinary ability of arginine-rich polypeptides to passively penetrate across cellular membranes. Unlike polylysines, which are also highly cationic but lack the ease in crossing membranes, polyarginines do not exhibit mutual repulsion. Instead, they accumulate at the membrane, weaken it, and may eventually cross in a concerted, "train-like" manner. This behavior of arginine-rich cell penetrating peptides can be exploited when devising smart strategies how to deliver in a targeted way molecular cargos into the cell.

AVANCE NEO – BREAKTHROUGH IN MULTI-RECEIVE NMR TECHNOLOGY

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NMR experiments involving multiple receivers provide a unique way of increasing the sensitivity and information content of data recorded in a given period of time [1–4].

AVANCE NEO represents the latest generation in the AVANCE series product line. The electronics is based on a 'transceive' principle, meaning each NMR channel has both transmitter and receiver capabilities. Thus, each channel is its own independent spectrometer with the full RF generation, transmission and receive infrastructure.

This architecture provides high flexibility and allows the implementation of multi-receive experiments in a straight-forward manner. Examples of multiple receiver operation are shown in routine analysis of small molecules and structure elucidation of biomolecular systems. The design of the experiments and the optimal applications for multiple receivers are discussed.

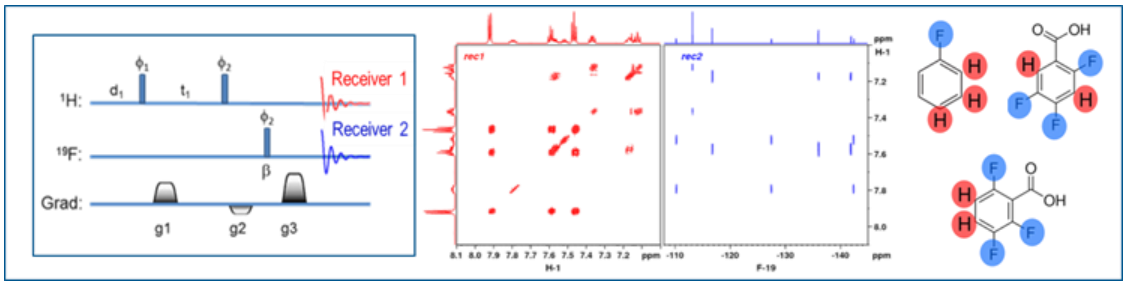

Figure 1. 2D H-H COSY and F-H COSY spectra of a mixture of three ^{19}F-labelled aromatic compounds recorded in parallel using the pulse sequence shown.

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NMR

Innovation with Integrity

ULTRAFAST MAGIC ANGLE SPINNING SOLID-STATE NMR: METHODS AND APPLICATIONS

Yusuke Nishiyama[a,b]

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Solid-state nuclear magnetic resonance (NMR) is a versatile technique to get atomic level information of nonsoluble and noncrystalline samples of any shape and size. Nevertheless, solid-state NMR spectra, in general, are broad due to the presence of orientation-dependent anisotropic interactions. Such spatial anisotropy can be removed by spinning the sample at the magic angle. However, the extent to resolution and sensitivity of the NMR spectrum relies on the sample spinning rate. In the recent past, with the invent of ultrafast sample spinning (~120 kHz) technology the solid-state NMR community has witnessed a rapid growth in the structural and dynamics studies of chemical, biological, materials, pharmaceutical samples, etc.[1–3] In the presentation, I will discuss the technological development of ultrafast spinning technology and its applications towards the structural characterization of various pharmaceutical samples at natural abundance.

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DOI: 10.1016/j.ssnmr.2016.06.002

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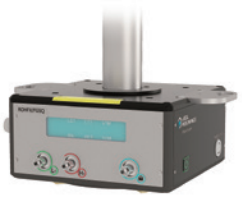
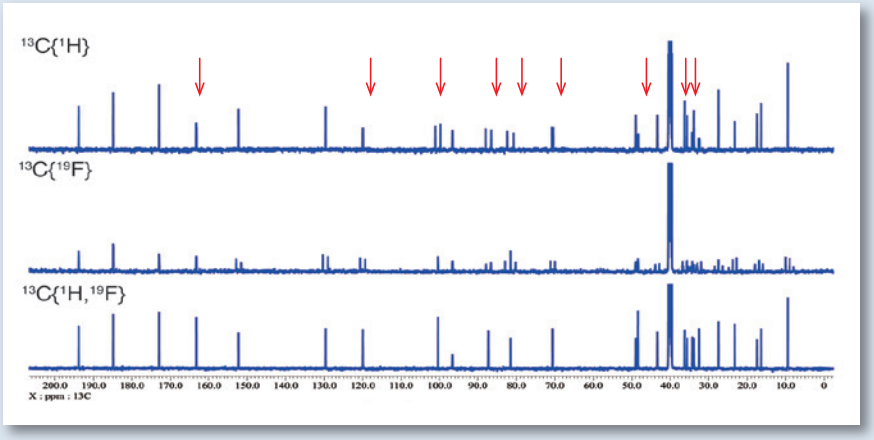


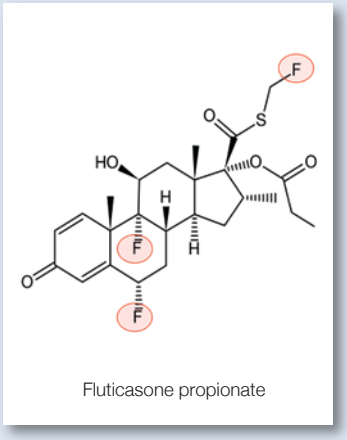
■ Specifications

Magnetic field	400 MHz~600 MHz
Observation range	^{1}H,^{19}F,^{31}P~^{15}N*
Spectrometer	JNM-ECZ400S JNM-ECZR series
Software	Delta.V.5.2 or later

∗ include ^{39}K and ^{109}Ag at 400 MHz and 500 MHz



Fluticasone propionate /DMSO-d6 128scans



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Measured by 500 MHz ROYALPROBE HFX and JNM-ECZR

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POSTERS

ROLE OF AN INTRINSICALLY DISORDERED REGION (IDR) OF TRAD – A COUPLING PROTEIN INVOLVED IN THE R1 / F PLASMID TRANSFER MECHANISM

Krishna Chaitanya Bhattiprolu, Evelyne Schrank, Ellen Zechner, Klaus Zangger,[a,*]
Jun Lu, Barbara Pedrycz, Mark J. N. Glover[b,*]

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[b] Department of Biochemistry, University of Alberta, Edmonton, Canada
*corresponding authors

Bacterial conjugation is one of the primary mechanisms by which the genetic information is transferred among bacteria. In the current project, we are working with gram negative bacteria which use Type IV Secretion System (T4SS) for the gene transfer leading to a rapid dissemination of antibiotic resistance and virulence factors among the bacterial community. The DNA transfer is assisted by complex macromolecular machineries known as Relaxosome, Transferosome and Coupling protein.

Relaxosomal complex consists of all the enzymes which are directly or indirectly involved in the nicking, unwinding and processing of the conjugative plasmid in the cytoplasm. Whereas, Transferosomal complex consists of membrane bound and periplasmic proteins which are involved in conjugative pore formation, T4SS pilus formation and retraction. For a successful recruitment of the conjugative plasmid to the randomly formed conjugative pore, there must be a direct interaction between the Relaxosome, which resides in the bacterial cell's center or quarter center and the membrane bound components. This recruitment mechanism is the least well-understood aspect.

The third complex known as coupling protein, are membrane bound and assist the 'coupling' of the Relaxosome to the membrane bound components. Our results show a novel mechanism by which the hexameric ATPase coupling protein, TraD interacts with the relaxosomal nucleoprotein complex, TraM - dsDNA. These are the only results which structurally confirm an interaction between the relaxosomal nucleoprotein complex and the membrane bound coupling protein in F-Like plasmid systems. One of our main objectives is to structurally characterize the multimeric nucleoprotein complex, TraD-(TraM-dsDNA).[1–4] I will present some of our findings which reveal mechanistic details in the substrate translocation during bacterial conjugation.

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AMINO-β-LACTAMS IN UGI REACTION: SYNTHESIS AND NMR ANALYSIS OF FUNCTIONALIZED PEPTIDOMIMETICS

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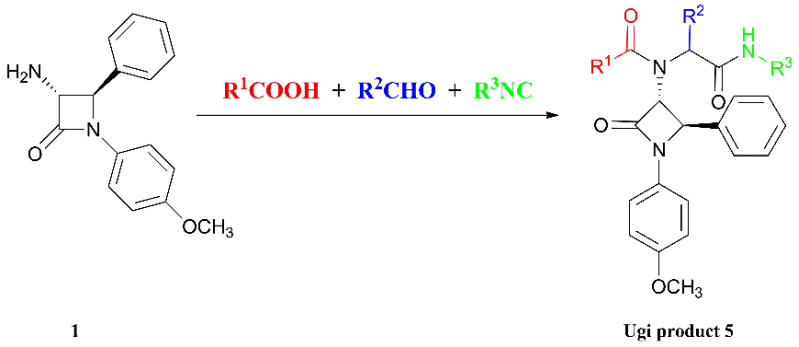
Multicomponent reaction (MCR) chemistry is among the most prominent methodologies for creating diverse compounds in an economical, efficient and environmentally friendly way.[1] The efficiency of MCR chemistry has already been proven in terms of natural product synthesis[2] and drug discovery processes. Among various MCR reactions, the Ugi reaction is especially attractive since it gives rise to complex peptide-like structures in one easy synthetic step.[3] Despite having been known for its medicinal and biological importance,[4] the β-lactam moiety harbours other excellent qualities. In particular, the β-lactam ring can provide amino acid building blocks or can be expanded into macrocyclic skeletons.[5] This excellent feature makes the β-lactam moiety compatible with the MCR chemistry, thus allowing the increase in final product complexity.

Here we present a multicomponent Ugi reaction using amino-β-lactam synthon **1** as basis for diastereoselective synthesis of functionalized peptidomimetics and give an insight in their structural characteristics using NMR analysis, Scheme 1.



Scheme 1. Ugi reaction on amino-β-lactam **1**

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NMR DETERMINATION OF ENANTIOMERIC PURITY OF 5-PHENYL-1,3-OXAZOLIDINE-2-THIONE FROM *RESEDA LUTEOLA*

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Nikola M. Stojanović[b,*]

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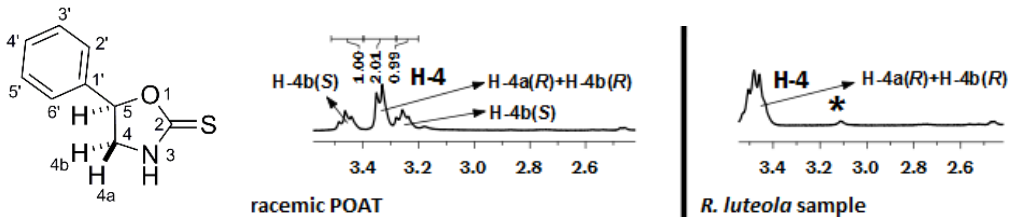
5-Phenyl-1,3-oxazolidine-2-thione (5-POAT), a known goitrogen, was isolated from the autolyzate of the flowers of *Reseda lutea* L. (Resedaceae). This compound naturally predominantly occurs as the *R*-enantiomer; however, recently *S*-enantiomer was found to be dominant in some samples of watercress [1]. This motivated us to investigate the enantiomeric nature of the sample at hand. We were drawn by the easiness of an NMR titration with chiral lanthanide shift reagents (CSR). There are no previous literature reports on chiral 1,3-oxazolidine-2-one resolution by such reagents. We supposed that a CSR (such as $Eu(hfc)_3$) would bind reversibly to 5-POAT and form two diastereomeric complexes with proton signals appearing at different shifts. Incremental addition of $Eu(hfc)_3$ to racemic 5-POAT ((±)5-POAT) resulted in a complete separation of H-4 proton signals of the two enantiomers. Enantiomeric excess could be calculated from the ratio of signal integral of H-4a(*S*) or H-4b(*S*) (from (+)5-POAT) and one half of the integral of H-4a+b(*R*) (from (-)5-POAT) from ^{1}H shifted spectra. These results clearly proved the validity of the method, i.e. that CSR can be used to successfully separate signals of (-) and (+)5-POAT. Enantiomeric purity of compound isolated from the autolyzate of *R. luteola* was studied by utilizing the same CSR, where upon close inspection only one set of signals could be observed. We concluded that 5-POAT isolated from *R. luteola* indeed consisted of only one enantiomer, (-)5-POAT. In this way, for the first time, we demonstrated that $Eu(hfc)_3$ forms distinct diastereomeric complexes with enantiomers of 5-POAT that are suitable for NMR-based determination of enantiomeric excess of this compound and probably of related ones.



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NMR STUDY OF NANO-BIO INTERFACE: A CASE OF INTERACTION BETWEEN GOLD NANOPARTICLES AND BIOTHIOLS

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The growing interest in utilization of gold nanoparticles (AuNPs) for diagnostics and treatment of various diseases [1] generates the need to examine their interactions with biological systems. Upon contact with biological media, NPs are immediately coated with a layer of adsorbed biomolecules called “corona” [2]. However, detailed knowledge still lacks about the nature and mechanisms behind the nano-bio interactions. One of the possible binding mechanisms is through the thiol groups, considering the high affinity of sulphur for metals. Endogenous biothiols such as cysteine and glutathione are therefore useful as models for studying the mechanism of nano-bio interactions [3].

In this study, 1H NMR was employed to study the interactions of AuNPs with cysteine and glutathione. Nanoparticles were synthesized by reduction of tetrachloroauric acid with sodium borohydride in the presence of cysteine or glutathione as stabilizing agents. 1H NMR spectra were recorded immediately after mixing the reagents, and subsequently every 20 minutes until AuNPs formation finished. Process of interaction between CYS and GSH with the AuNPs surface was observed by recording chemical shifts of signals corresponding to protons close to the thiol group, along with the loss of resolution and peak broadening. Furthermore, the intermediate spectra were captured, with distinct peaks corresponding to both bound and unbound biothiols. The results suggest the thiol group of CYS and GSH is the most responsible for biothiols-nanoparticle binding. In addition, AuNPs stabilized with CYS and GSH were characterized by means of size distribution, surface charge and visualized by transmission electron microscopy.

Acknowledgment: This study was financially supported by the HRZZ-IP-2016_06_2436 grant.

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MONTE CARLO CONFORMATIONAL SEARCH OF GLYCOCONJUGATED AMIDE-BASED CALIX[4]ARENES

Branimir Bertoša,[a,*] Marija Cvetnić,[a] Josip Požar,[a] Nikola Cindro,[a] Vladislav Tomišić,[a]

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Calixarenes are well known as efficient cation receptors. Affinity of calixarene derivatives towards cations in different solvents has been extensively studied. However, the major drawback in their wide application as cation receptors is their insolubility or low solubility in water. In order to overcome this obstacle, calix[4]arenes with carbohydrate units attached to the lower rim, that are soluble in water, were recently synthetized [1]. In order to gain better insight in molecular basis of their affinity and selectivity towards cation complexation, conformational analyses of several such ligands that are soluble in water were performed. Their conformational space was studied in water and in methanol. Conformational searches were conducted using Monte Carlo Multiple Minimum (MCMM) algorithm available within MacroModel software [2]. Solvent effects were modelled using continuum solvent models. In some cases, explicit solvent molecules, water or methanol, were present during the simulation in order to model the competition between sodium cation and solvent molecule for complexation. Cluster analyses of obtained results, as well as detailed analyses of intramolecular hydrogen bonds were performed. Computational results were compared and validated with available experimental data which consisted of different titration methods and NMR data.

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MULTIPLE POSSIBLE FOLDS IN THE REGULATORY REGION OF THE *PLEKHG3* GENE

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The human *PLEKHG3* gene is expressed in many areas of the brain and represents a potential candidate contributing to the risk of autism. In the regulatory region of this gene there are multiple GGG-tracts separated by AGCGA-repeats. These types of oligonucleotides are predicted to form G-quadruplexes. Contrary to expectations, we show that no G-quadruplexes are formed by such AGCGA-rich oligonucleotides. Instead, they adopt tetrahelical folds very different from G-quadruplexes [1]. The folds adopted by AGCGA-rich oligonucleotides can be further subdivided into two groups [2]. One structural group is characterized by a core comprised out of four GC base pairs and contains GA base pairs in N1-N7, carbonyl-amino geometry on each side of the GCGC-core. The other structural group is characterized by an opposite arrangement where the core contains two GAGA-quartets and two GC base pairs on each side of the GAGA-core. Both structural groups contain GG base pairs in N1-carbonyl symmetric geometry.

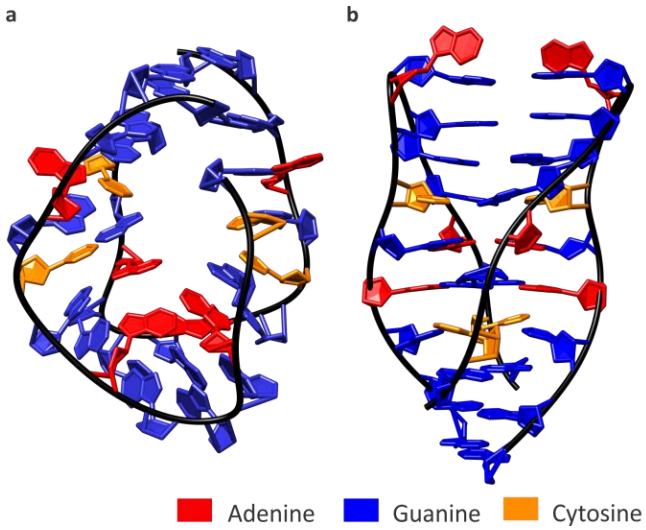

Figure 1: Two structural groups of AGCGA-rich folds. Two high-resolution structures containing a (**a**) GCGC-core and (**b**) GAGA-core.

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SYNTHESIS AND COMPLEXATION PROPERTIES OF FLUORESCENT PHENANTHRIDINE-BASED CALIX[4]ARENE DERIVATIVES

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Calixarenes are macrocyclic oligomers consisted of four or more phenolic residues linked by methylene bridges in the *ortho* position and can be easily functionalized to give receptors for various ionic or neutral species. Calixarenes bearing fluorescent moieties can be considered as potentially very sensitive fluorimetric ion sensors due to the high sensitivity of fluorescence spectroscopy and high affinity of these macrocyclic ligands towards cations.[1–3] In the scope of this work, novel fluorescent phenathridine-based calix[4]arene derivatives **L1** and **L2** were designed and prepared (Figure 1) and their complexation properties towards metal cations in several solvents were investigated. Phenanthridine moities were introduced at a lower calixarene rim and served both as fluorescent probes and parts of cation-binding site. Stability constants of the corresponding complexes were determined by means of fluorimetry, UV spectrophotometry, NMR spectroscopy and isothermal titration calorimetry.

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

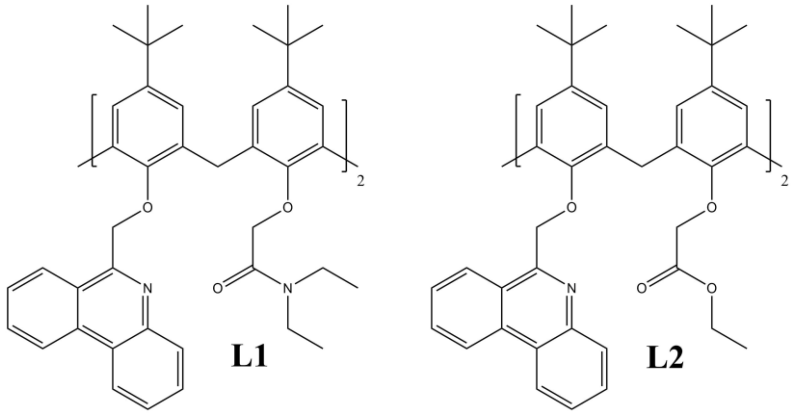

Figure 2. Structures of calix[4]arene derivatives L1 and L2.

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STRUCTURE OF MULE DEER PRION PROTEIN PROVIDES INSIGHT INTO CHRONIC WASTING DISEASE

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Chronic wasting disease (CWD) is the most efficiently transmitted prion disease of free-ranging wildlife, including elk, mule deer, white-tailed deer, red deer, reindeer and moose[1]. The ability of prion proteins to selectively infect some mammalian species rather than others is known as species barriers, which are impacted by the existence of different prion strains. For instance, in deer and elk the presence of conformational PrP^{Sc} strains was confirmed based on different disease progression in species[2].

In this study, we highlight the importance of PrP structure in prion susceptibility, and how a polymorphism in position 226, in which elk PrP contains glutamate and deer PrP contains glutamine, might influence prion susceptibility and pathogenesis. We have determined the high-resolution structure of the mule deer prion protein (mdPrP) (residues 94-233). We then compare the structure to previously published cervids PrPs structures (i.e. elk PrP (ePrP) and white-tailed deer PrP (wtdPrP)). Although the overall structures of the mammalian prion proteins are similar, we observe several local structural variations in the examined structures. The most remarkable differences are located in the α2-α3 loop with a different rearrangement of residues together with hydrogen-bond formation that create a tighter packing of the α1 helix and α2-α3 loop in mdPrP structure in comparison to ePrP and wtdPrP. Additionally, in mdPrP hydrophobic interactions of amino acid residues at the end of α3 helix and β2-α2 loop lead to a lower solvent accessibility of Ser^{225} and Gln^{226} which are related to the CWD transmissibility. We found that a single amino acid variation can alter the PrP structure and this can have important consequences on the different pathogenesis of CWD prions.

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NEW STRUCTURAL INSIGHTS INTO THE GRAM-NEGATIVE LIPID TRANSPORTER YRBC

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The lipid composition and asymmetry of the outer membrane of Gram-negative bacteria plays a crucial role in pathogenesis and intercellular interactions. The establishment and maintenance of this complex system is yet poorly understood.

Recent studies presented a VacJ/Yrb ABC (ATP binding cassette) transport system which is conserved among Gram-negative bacteria.[1] The periplasmic protein YrbC is essential in this mechanism, since it transports accumulated phospholipids from the outer to the inner membrane which is highly important for the maintenance of the membranes barrier function.

The structure of YrbC (2.2Å) from *Hämophilus influenza*, including a bound phospholipid, could be solved using crystallography and molecular replacement. Analysis of the ligand via nuclear magnetic resonance NMR and mass spectrometry MS revealed a clear preference of YrbC for phosphoglycerol over phospahtidylethanolamin, although these lipids are present in equally amounts in the expression system *Escherichia coli.* Analysis of lipid ligands from two YrbC orthologues from *Vibrio cholerae* and *E. coli* showed the same outcome.

The structure of YrbC *H. influenzae* reveals new insights into its binding preferences and furthermore the binding cavity and its chemical environment. These findings can be useful to understand how the VacJ/Yrb ABC transport system works, which by itself may have a pathophysical role *in vivo* since proper function of the outer membrane is crucial for bacterial adaption to harsh environments, like colonization of a new host, as well as antiobiotic resistance.

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DINUCLEAR Mo(VI) COMPLEXES WITH 4-AMINOBENZOYLHYDRAZONE DERIVATIVES: A SOLID-STATE AND SOLUTION STUDY

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An important part of supramolecular chemistry refers to the coordination-driven self-assembly. Recently, the design and synthesis of supramolecular coordination complexes have attracted significant attention in various fields due to the interesting molecular architectures and/or a wide variety of properties, e.g. redox, magnetic, catalytic or optical. Although polydentate ligands have played a substantial role in coordination chemistry of molybdenum(VI), their use as structural elements in metallosupramolecular chemistry has received considerably less attention. Therefore, three polydentate aroylhydrazone ligands ($H_2L^{1–3}$) were selected to investigate if they could serve as organic linkers for the *cis*-octahedral complex containing the $\{MoO_2\}^{2+}$ core.[1] Hydrazones were prepared by the reaction of 4-aminobenzhydrazide with salicylaldehyde (H_2L^1), 3-methoxysalicylaldehyde (H_2L^2) or 4-methoxysalicylaldehyde (H_2L^3). Dinuclear dioxidomolybdenum(VI) complexes of selected hydrazones, $[MoO_2(L^{1–3})]_2$, were synthesized by the reaction of $[MoO_2(acac)_2]$ and the corresponding ligand. All isolated complexes were characterized in the solid state by means of elemental and thermogravimetric analysis, infrared spectroscopy and X-ray diffraction methods (PXRD and SCXRD when suitable). Furthermore, the complexes were explored by one- and two-dimensional NMR spectroscopic techniques in dmso-d_6. In all complexes, the doubly deprotonated form of aroylhydrazone is coordinated to $\{MoO_2\}^{2+}$ core through the *O*,*N*,*O* donor atoms. The sixth coordination site of molybdenum is occupied by the amino nitrogen of the neighboring hydrazone.

[1] This work has been fully supported by Croatian Science Foundation under the project IP-2016-06-4221.

NEW WAYS FOR INVESTIGATING PHOTOCHEMICAL REACTIONS WITH NMR

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A typical setup for studying photochemical processes with NMR spectroscopy usually comprises optical fibers to guide the light from its source to the NMR-active volume, as is shown in Figure 1, left [1]. This approach has two main disadvantages: First, the light intensity reaching the sample is rather low and non-uniform, causing long conversion times (see Figure 2, right [2]). Second, this setup needs to be assembled and carefully inserted into the spectrometer for each measurement. We have placed high power LEDs, usually applied in industry, directly inside the NMR probe head using custom-made devices.

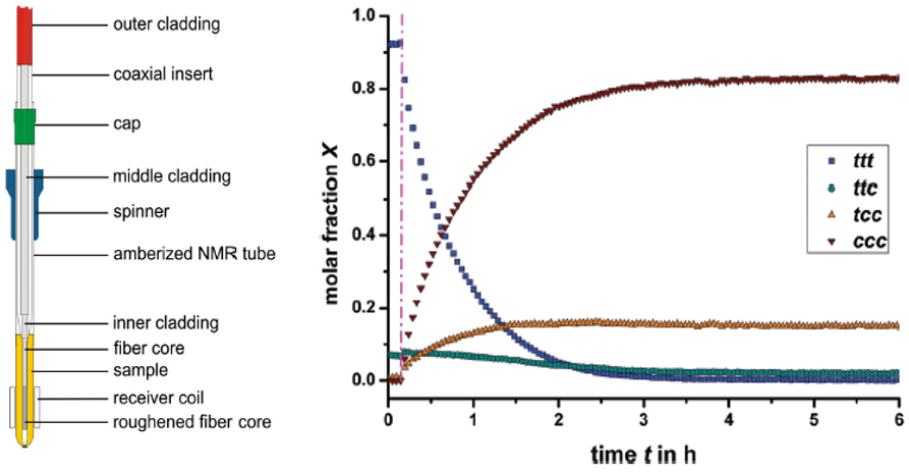

Figure 1. Typical fiber-setup (left). Kinetic profiles of the isomerization of azobenzene benzene-tricarboxamides (right). Note the necessary illumination time in hours. Graphs taken from References 1 and 2.

We discuss several aspects of our new setup: Its development and implementation, sample mixing and illumination homogeneity, determination of light intensity with model systems, kinetic investigations of light switchable compounds and suitable pulse sequences for measuring chemically induced dynamic polarization (CIDNP).

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CONCENTRATION DEPENDENCE OF ASPHALTENE AGGREGATION MONITORED BY DOSY NMR

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Asphaltenes are the heaviest, most polar and least reactive molecules in crude oil, primarily consisting of carbons and hydrogens, some heteroatoms, such as sulfur, nitrogen, oxygen and traces of transition metals [1,2]. They may aggregate during the downstream and upstream processes and cause many problems in production, refining and transportation. Asphaltene aggregation highly depends on the chemical nature of the crude oil, temperature and pressure.

In this research, concentration dependent diffusion measurements were carried out to determine the lowest aggregation concentration of Middle Eastern asphaltenes and to study how the addition of V(IV), Fe(III) and Ni(II) affects the aggregation process. Asphaltene components were separated by DOSY NMR technique according to their diffusion properties. The influence of T1 relaxation on average diffusion coefficients was investigated by inversion recovery experiments. Changes in diffusion coefficients reflected the formation of different asphaltene types. It is expected that the presented results could throw more light into the asphaltene aggregation mechanism.

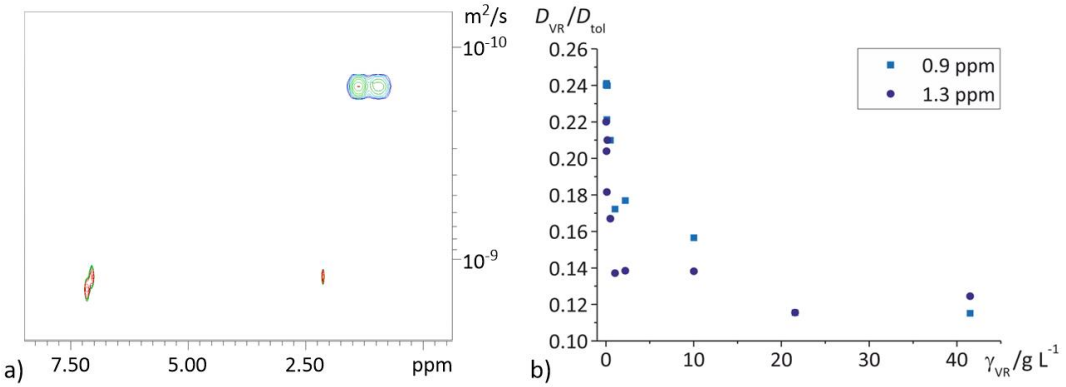

Figure 1. a) DOSY NMR spectrum of a vacuum residue sample (21,56 g/L) recorded in toluene-d_8; b) relative diffusivities (D_{VR}/D_{tol}) as a function of sample concentration.

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IMPURITY PROFILING OF AZITHROMYCIN CONJUGATES BY LC-SPE/CRYO NMR METHODOLOGY

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Azithromycin belongs to an azalide subclass of 15-membered macrolide antibiotics. It was synthesized in the early 1980s as a semi-synthetic derivative of erythromycin.[1] With a much improved pharmacokinetic properties over erythromycin, azithromycin became the most widely used broad-spectrum antibiotic.[2] Thiosemicarbazones belong to a large group of thiourea derivatives and are well known as antibacterial, antiviral, anti-inflammatory, antifungal and anticancer therapeutics.[3] Furthermore, a new multi-functional thiosemicarbazones were designed to treat Alzheimer and malaria.[4,5] Conjugation of the azithromycin and thiosemicarbazone derivatives resulted in novel compounds which showed good activity against some Gram positive and Gram negative bacterial strains.

Modern approach to the drug impurity profiling is based on the use of hyphenated systems, such as LC-NMR and/or LC-MS.[6] One of the most efficient and powerful tools for on-line isolation and identification of compounds in complex mixtures in the pharmaceutical industry is the LC-SPE-NMR system. In this study, LC-SPE/CRYO NMR technique was used for impurity profiling of novel azithromycin conjugates.

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BIOTRANSFORMATION OF SILVER NANOPARTICLES IN VIVO

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Excellent microbicidal properties of silver nanoparticles (AgNPs) instigated their use in medicine, cosmetics, textile and food industries. However, detailed information on their fate and effects on the human body is so far unknown. [1] When exposed to biological media, AgNPs are prone to intimate interactions with different biomolecules and biological structures. [2] This study demonstrates possible biotransformation patterns of AgNPs after *in vivo* exposure.

Fate of AgNPs was investigated in ultrapure water, cell culture medium, phosphate buffer, artificial lysosomal fluid, artificial gastric fluid, and liver homogenates by means of agglomeration and dissolution behaviour. For this purpose size distribution, surface charge, Ag^+ release and interaction with albumin and glutathione were evaluated using dynamic and electrophoretic light scattering methods, transmission electron microscopy, atomic absorption spectroscopy and proton nuclear magnetic resonance spectroscopy (1H NMR). For the first time, this study evidenced *in vivo* synthesis of AgNPs in the liver resulting from the interaction of Ag^+ with glutathione. First, the binding of glutathione to the surface of AgNPs was investigated on the NPs synthesized by sodium borohydride reduction of silver nitrate. The process of NP formation and glutathione adsorption was tracked by 1H NMR in 20-minute time intervals. Initial and final spectra differ significantly in chemical shifts of C7, indicating the binding through the thiol group. In the second part, AgNPs were synthesized using glutathione as both the reducing and capping agent. This eliminated the interference from sodium borohydride, and demonstrated the potential mechanism of *in vivo* AgNP formation from ionic silver.

Acknowledgment: This study was financially supported by the HRZZ-IP-2016_06_2436 grant.

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THE SOLVATION INFLUENCE ON THE COMPLEXATION OF ALKALI-METAL CATIONS WITH CALIX[4]ARENE DERIVATIVES

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The *p-tert*-butyl calix[4]arenes with carbonyl-containing substituents at the lower rim are known to be effective receptors for alkali-metal cations. Apart from the chemical nature of the attached functionalities, and the cation size, the complexation thermodynamics is strongly influenced by the reactant and the product solvation.[1,2]

In the first part of the work the thermodynamic parameters of alkali-metal cation complexation with a simple calix[4]arene ketone derivative (**L**) in methanol, ethanol, *N*-methylformamide, *N,N*-dimethylformamide, dimethyl sulfoxide, and acetonitrile were determined.[1] The compound **L** was found to be rather efficient receptor for alkali-metal cations. The cation binding was enthalpically controlled with the peak affinity for Na^+. To examine the possible inclusion of the investigated solvent molecules into the hydrophobic basket of the receptor and its sodium complex detailed spectrophotometric, calorimetric and ^{1}H NMR titrations in chloroform were carried out. The solvent binding was much more favorable in the case of Na**L**$^+$ when compared to free ligand. The sodium complex exhibited the highest affinity for the acetonitrile, which can, at least in part, explain the highest stability of the complexes in this solvent.

In the second part of the work, the inclusion of acetonitrile molecule in the hydrophobic cavity of a more rigid tertiary amide calix[4]arene derivative and the corresponding sodium complex was investigated. The obtained data provided an insight into the influence of the calixarene flexibility on the thermodynamics of adduct formation.

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

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CHARACTERISATION OF EXCHANGE OF SOLVENT LIGANDS ON DICYCLOPALLADATED AZOBENZENES IN SOLUTION

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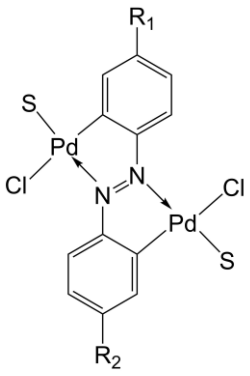
Organopalladium compounds, usually containing one palladium center per ligand, are one of the most popular transition-metal complexes investigated in organometallic chemistry.[1] Their rich chemistry enables easy preparation and wide scope of possible transformations in order to achieve desired physical properties that qualify them for potential application in organic synthesis and catalysis, supramolecular chemistry and as new materials.[1] As introducing the second palladium to investigated compounds broadens the scope of their applications and transformations, dicyclopalladated azobenzenes, which exhibit strong light absorption and emission in the visible region, recently became one of the main research focuses in our group.[2]

In this work we have investigated exchange of solvent ligands (S = DMSO, DMF or pyridine) coordinated to palladium centers in seven dicyclopalladated azobenzenes (**C1**–**7**, Scheme 1) in DMF and DMSO by means of spectrophotometric and ^{1}H NMR titrations. Special consideration was paid to varying substituents in 4,4'-positions of azobenzene moiety as their electronic properties significantly influence spectral properties of the studied dicyclopalladated complexes. In the case of pyridine as a coordinated solvent, corresponding equilibrium constants have been determined. Results will be discussed in terms of solvent donor abilities as well as the electron-donating and electron-withdrawing properties of substituents in the 4,4'-positions of the azobenzene.



C1 $R_1 = N(CH_3)_2$, $R_2 = N(CH_3)_2$
C2 $R_1 = N(CH_3)_2$, $R_2 = H$
C3 $R_1 = NHC_6H_5$, $R_2 = H$
C4 $R_1 = H$, $R_2 = H$
C5 $R_1 = I$, $R_2 = H$
C6 $R_1 = N(CH_3)_2$, $R_2 = Cl$
C7 $R_1 = N(CH_3)_2$, $R_2 = NO_2$

S = DMF, DMSO or pyridine

Scheme 1. Structures of dicyclopalladated complexes C1–7.

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STRUCTURE CHARACTERIZATION OF SELF-ASSEMBLED NOVEL AMINO ACID FUMARAMIDES BY NMR

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Synthesis of new materials is one of the very important areas of science that special chemistry deals with. High technology has requested for new materials that will be used for medical purposes, biomaterials, sensors, liquid crystalline materials and electronic materials[1]. Supramolecular chemistry has found interest in the synthesis of new materials because it provides a wide range of possibilities for generating new materials as self-organized nanomaterials because of non-covalent interactions such as hydrogen bonds, π-π stacking or Van der Waals forces. We investigated the possibility of polymerization of supramolecular gels[2] from the class of low molecular weight gelators, mono(vinyl-amino acid) fumarate with UV light and benzophenone as a photoinitiator. These compounds are gelator of various organic solvent but the but polymerization occurs only in acetonitrile. Accordingly with this, we investigated this system by characterizing it with ^{1}H, ^{13}C, temperature dependent NMR spectroscopy and FTIR spectroscopy.

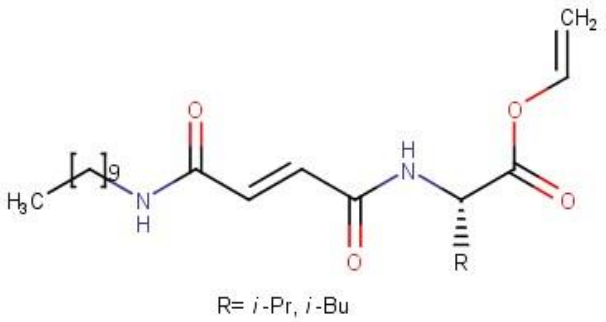

Figure 1. New fumaroyl based low molecular weight gelators

[1] E. Busseron, Y. Ruff, E. Moulin, N. Giuseppone, *Nanoscale* **2013**, *5*, 7098.
[2] L. Frkanec, M. Žinić, *Chem. Commun*. **2010**, *46*, 522.

DIFFERENTIATING ALCOHOLS FROM ETHERS BY DEUTERIUM ISOTOPE EFFECTS

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The measurement of deuterium isotope effects on ^{13}C-atoms in alcohols, ethers and amines is proven to help with an easier assignment without using complex or time consuming multidimensional correlations. Here we show that the protonation state of oxygen and nitrogen can be determined in a variety of organic solvents when accurate isotope effects are used. Two almost identical samples need to be prepared with the only difference being the presence of 10 % v/v D_2O or H_2O. By comparing the two independently measured ^{13}C-chemical shifts it is possible to differ between protonated heteroatoms (alcohols, primary and secondary amines) and non-protonated ones (ethers and tertiary amines) through the differences in isotope shifts (DIS values). DIS was selected to be the difference of the chemical shifts in parts per million between the ^{13}C shift observed in H_2O and the upfield shift in D_2O ($\delta^{13}C_{H2O} - \delta^{13}C_{D2O}$). As an example, erythromycin is shown to prove the concept of this approach (Figure 1).

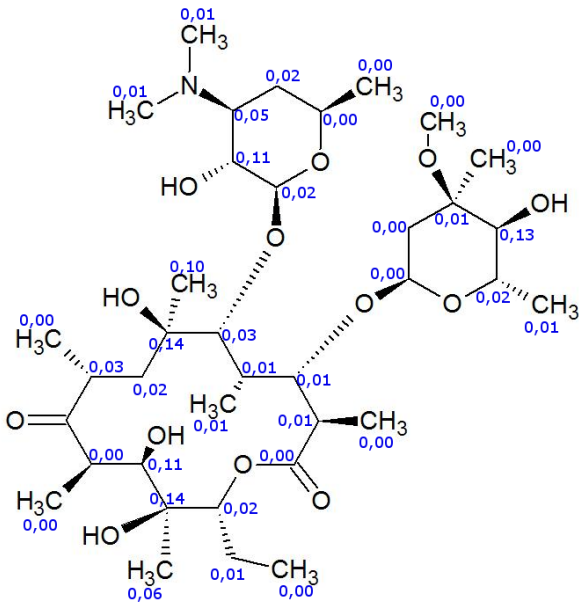

Figure 1. Chemical structure of erythromycin. (DIS values are blue in ppm upfield shift)

THERMODYNAMIC AND STRUCTURAL STUDIES OF THE COMPLEXATION OF HOMOCYCLOPEPTIDES WITH HALIDE AND STRUCTURAL ANIONS IN ACETONITRILE

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The development of artificial anion receptors that mimic natural systems in their ability to efficiently and selectively bind a target anion has recently become an area of intense focus within supramolecular chemistry.[1] Cyclic peptides present promising synthetic scaffolds for selective anion binding due to the amide group hydrogen bond properties, and also because of the macrocyclic ring flexibility and the variability of the subunits.[2–4] By the combination of the thermodynamic and structural characterization of the cyclopeptide-anion complexes formed in the solution it is possible to obtain a detailed insight into the energetics of complexation and in the relationship of the receptor structure and its affinity towards particular anion.

In this work, two cyclopeptide receptors were studied, **L1** that contains five lysine subunits with amino groups of the side chains protected by a BOC group (Lys-BOC) and **L2** that is comprised of six Lys-BOC subunits. These receptors bind anions directly through interactions with the peptide backbone or with the carbamate protons of BOC groups. Complexation affinities of **L1** and **L2** ligands toward halogen (Cl^-, Br^-, I^-) and structural anions (ClO_4^-, HSO_4^-, $H_2PO_4^-$, NO_3^-, NO_2^-, SCN^-) in acetonitrile were investigated by means of mass spectrometry, ^1H NMR and isothermal microcalorimetric titrations. More information about the structural characteristics of the cyclopeptide binding sites and microscopic image of the anion binding processes was gained by molecular dynamics simulations with explicit solvent molecules.

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