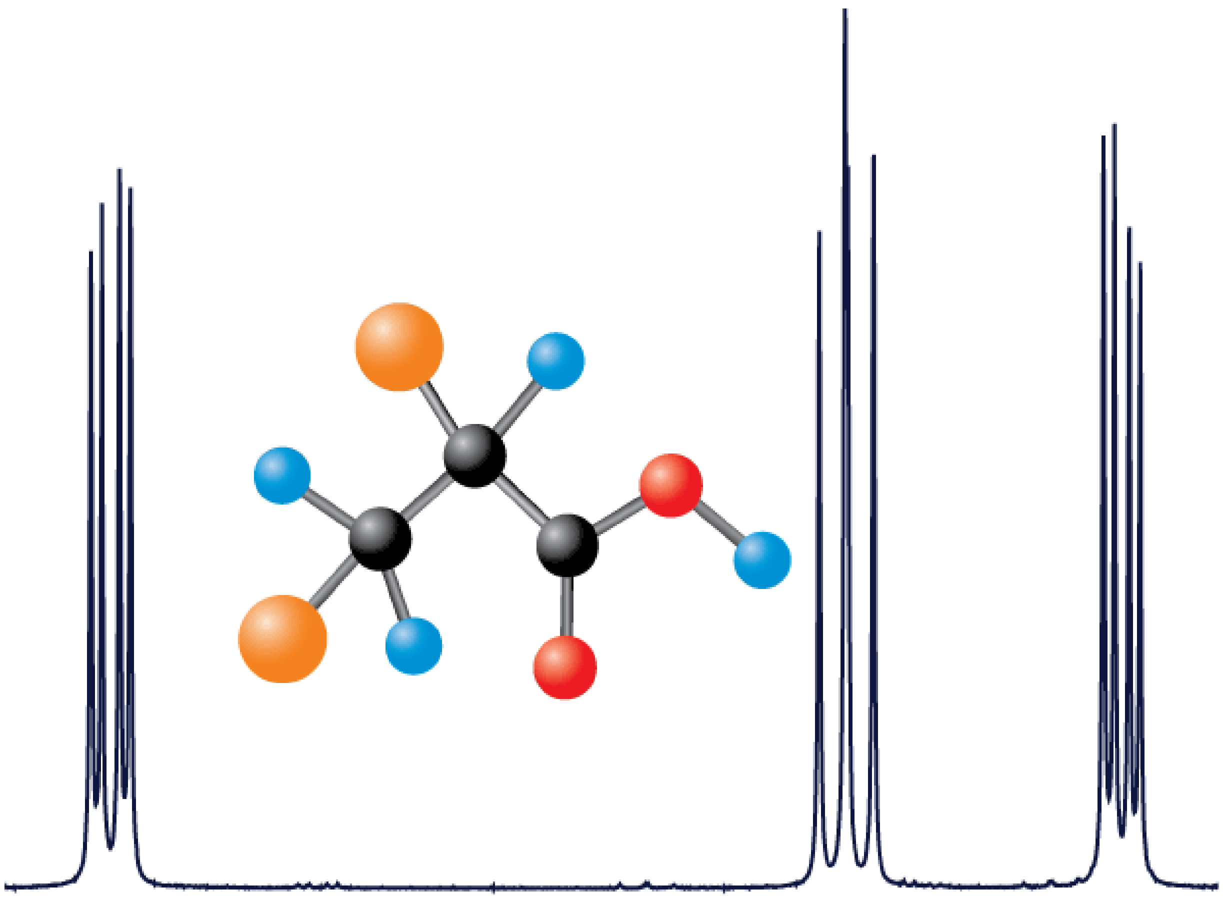


SMASH 2015

Conference Program

September 20th-23rd, 2015
Baveno, Italy

SMASH 2015 NMR Conference

Dear SMASH 2015 Attendees,

We welcome you to the 2015 Small Molecules Are Still Hot conference in the gorgeous location of Baveno, Italy. The town sits on the shore of Lago Maggiore about 90 kilometres NE of Milan and its Roman culture can be traced back to 1st Century BC, with its basis in the wine trade for many centuries. Famous for being a health and thermal resort, Mount Camoscio rising behind the town holding the famous red granite cave from which comes the Fonti di Baveno mineral springs.

SMASH is a scientific meeting highlighting NMR spectroscopy and applications, in addition to other analytical technologies, used in small molecules research. We are very proud that SMASH brings together cutting-edge science from both academia and industry in one international meeting. The compact and friendly nature of this meeting provides an excellent networking opportunity for students and professionals, attracting the leading spectroscopists in this field.

The formal program for SMASH begins on Sunday evening with registration, mixer and dinner. On Monday evening we are delighted to be presenting the 2nd James N. Shoolery Award to Professor Gareth Morris (University of Manchester) for his career-long contributions of key developments in small molecule NMR spectroscopy and following the presentation he will give an award lecture summarising his historical and ongoing contributions to our field. This year's program has oral sessions cover the breadth of our NMR science, ranging from methods for molecular structure and dynamics determinations through computation and low field NMR to metabolomics and drug discovery, finishing off with the enticingly titled "Quo Vadis NMR" session. Intermingled with these are workshops on quantitative 2D NMR, the FDA & pharmacopeia and an all-encompassing "Future of NMR" vendor panel. Posters will be up throughout the meeting, with Monday and Tuesday sessions to meet and speak with the presenters. Building on recent tradition, following SMASH there will be a "Reaction Characterisation & Monitoring" workshop on Thursday, starting with a dinner on Wednesday night.

In addition to wandering around the town and partaking of the beautiful surroundings near the hotel, on Tuesday afternoon there is an optional boat trip and tours of the remote Isla near to Baveno for those who aren't immersed in science, networking and the chance to catch up with friends during the free afternoon (which is followed by the second poster session in the evening – so make sure you get back for that!).

On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2015.

Sincerely,

Craig Butts and Silvia Mari
Co-Chairs, SMASH 2015 NMR Conference

SMASH 2015 NMR Conference Program

Sunday, September 20th

05:00 PM - 07:00 PM Registration
08:00 PM - 09:00 PM Mixer
09:15 PM - 11:00 PM Dinner

Monday, September 21st

09:00 AM - 09:15 AM Opening Remarks
09:15 AM - 11:00 AM **Exploring the Unknown - Molecular Structure Determination**
Chair: Tim Claridge, *University of Oxford, (UK)*

Isotope Filtered nD NMR Spectroscopy for Structure Determination of Molecules in Complex Mixtures
Dusan Uhrin, *University of Edinburgh (UK)*
Elimination of Scalar Interactions in the 13C or 1H Dimensions of 2D Spectra, with Application to 13C-Enriched Products
Damien Jeannerat, *University of Geneva (CH)*
NMR Characterization of Complex Natural Products: Assigning Novel, Proton-Deficient Alkaloid Scaffolds
Kirk Gustafson, *National Cancer Institute (US)*
Development and Evaluation of Experiments for Oligosaccharide Structure Elucidation using Phosphitylated Derivatives (Upgraded Poster)
Clark Ridge, *U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (US)*

11:00 AM - 11:30 AM Break
11:30 AM - 01:15 PM **Spinning in Silico - NMR and Computation**
Chair: Michela Ghitti, *S. Raffaele Scientific Institute, (IT)*

Challenges in Bio-Molecular Research: On Comparing Simulated with Experimental Data
Wilfred F. van Gunsteren , *Swiss Federal Institute of Technology, ETH (CH)*
Information-Driven, Fully Flexible HADDOCKing of Protein-Ligand Interactions
Alexandre M.J.J. Bonvin, *Bijvoet Center for Biomolecular Research (NL)*
Dynamics in Solids Studied by NMR Crystallography and MD Simulations
Martin Dračínský, *Institute of Organic Chemistry Biochemistry (CZ)*
Determination of Peptide Solution Conformations Using RDC (Upgraded Poster)
Dennis Anderson, *Pfizer, Inc. (US)*

01:15 PM - 02:45 PM Lunch & Vendor Discussions
02:45 PM - 04:30 PM **Poster Session 1 (even numbers)**
Co-chairs: Krish Krishnamurthy, *Chempacker LLC, (US)*
Elisabetta Chiarparin, *AstraZeneca, (UK)*
04:30 PM - 05:00 PM Break
05:00 PM - 06:30 PM Workshop
Future of NMR
Coordinated by: Craig Butts and Silvia Mari
07:30 PM - 08:30 PM **Presentation of the James N. Shoolery Award and Lecture**
SMASH 2015 Recipient: Professor Gareth Morris
08:30 PM - 11:00 PM Dinner/ Social Hour

Tuesday, September 22nd

09:00 AM - 10:45 AM **Molecules on the Move - NMR of Reacting Systems**
Chair: Christina Thiele, *Technische Universität Darmstadt, (DE)*

Recent Applications of NMR Reaction Monitoring in a Pharmaceutical Development Environment
Mark Zell, *Pfizer, (US)*

***In situ* NMR Interrogation: Probing the Mechanism of Action of Reagents in Synthesis**
Guy Lloyd-Jones, *University of Edinburgh (UK)*

Dynamic Non-Uniform Sampling
Kris Kazimierczuk, *Centre for New Technologies (PL)*

Slice-Selective NMR Spectroscopy as Versatile Tool for Chemists (Upgraded Poster)
Michael John, *Institut für Anorganische Chemie, Georg-August-Universität (DE)*

10:45 AM - 11:15 AM Break

11:15 AM - 01:00 PM **Finding a Noodle in an NMR Soup Bowl - Food and Health**
Chair: David Chang *Metabolomic Technologies, (CA)*

Statistical Correlation Spectroscopy and Spectral Filtering Applied to NMR Spectra
Tim Rudd, *Diamond Light Source Ltd (UK)*

Linear Models to Survive Between 64K Points and my Food Science Collegues
Luca Laghi, *University of Warsaw, (PL)*

Rapid Quantification of Polysaccharides in Foods: A Small Molecule Based Chemometric qNMR Approach
Niels de Roo, *Unilever (DK)*

Using off- and on-line SPE-NMR in Bioactive Metabolomics (Upgraded Poster)
Sofia Moco, *Nestle Institute of Health Sciences, Natural Bioactives and Screening (CH)*

01:00 PM - 02:00 PM Lunch

02:00 PM - 07:00 PM Free Time & Vendor Discussions

07:00 PM - 08:30 PM **Poster Session 2(odd numbers)**
Co-chairs: Krish Krishnamurthy, *Chempacker LLC, (US)*
Elisabetta Chiarparin, *AstraZeneca, (UK)*

Wednesday, September 23rd

08:45 AM - 10:35 AM **Medicines in Magnets - Innovative Approaches to Drug Discovery**
Chair: Carla Marchioro, *Research4Rent, (IT)*

Biophysical Characterisation of GPCR-Ligand Interactions for Drug Discovery
Rob Cooke, *Heptares (UK)*

Applications of High-Field Solid-State NMR to Small Organic Molecules
Dinu Iuga, *University of Warwick (UK)*

Anti-inflammatory Drugs by NMR in Ordered Media: Insights into Conformation and Stereochemistry (Upgraded Poster)
Maria Enrica Di Pietro, *Universita' della Calabria (IT)*

A Comparison of Fluorine Ligand-Based NMR Screening with other Biophysical Techniques: Advantages and Limitations
Claudio Dalvit, *Université de Neuchâtel (CH)*

10:30 AM - 11:00 AM Break

11:00 AM - 12:30 PM Workshop/Tutorial (concurrent)

Modernizing the Pharmacopeias - NMR tests for drug quality
Coordinated by: Marco Guerrini and David Keire, *Ronzoni Institute (IT)*
, US Food and Drug Administration (US)

2D qNMR
Coordinated by: Patrick Giraudeau, *Université de Nantes (FR)*

12:30 PM - 02:00 PM Lunch, Free Time & Vendor Discussions

02:00 PM - 03:45 PM **Small but Beautifully Formed - Low Field NMR**
Chair: Andrew Coy, *Magritek, (NZ)*

A Single Chip Multi-dimensional NMR Spectrometer
Jeffrey Paulsen, *Schlumberger-Doll Research (US)*

Automated Data Evaluation and Modeling of Simultaneous ^{19}F-^{1}H Benchtop NMR Spectra for Online Reaction Monitoring
Michael Maiwald, *BAM Federal Institute for Materials Research and Testing (DE)*

Analysis of Counterfeit Drugs and Dietary Supplements with Low-Field Benchtop NMR Spectroscopy
Anna Gerdova, *Oxford Instruments (UK)*
Quantification of Composite β-Lactam/β-Lactamase Inhibitor Antibiotics with 60 MHz Benchtop NMR (Upgraded Poster)
Susie Riegel, *Nanalysis Corp. (CA)*

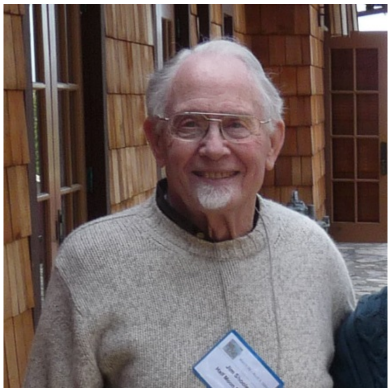
03:45 PM - 04:15 PM Break

04:15 PM - 06:00 PM **Quo Vadis NMR - Emerging Methods & Exotica**
Chair: Stanislav Sykora, *Extra Byte, (IT)*
To Tune or Not to Tune, That's the Question: A Revolution in NMR Sensors!
Aldrik Velders, *Wageningen University (NL)*
What's Left to Spin? Spinning Samples, Coils and Magnets for High-Resolution NMR of Heterogeneous Samples
Dimitrios Sakellariou, *CEA-CNR, Paris (FR)*
Microcoils for Inline/Insitu Monitoring
Maria Victoria Gomez, *University of Castilla-La Mancha (ES)*
The Power of Variable Field Relaxometry Applied to Small Molecules
Rebecca Steele, *Stelar (IT)*

06:00 PM - 06:15 PM Closing Remarks

The James Shoolery Award

In 2014, SMASH established the James Shoolery Award as a grant, in honor of James N. Shoolery, to recognize the important contributions by an individual to the field of small molecule NMR spectroscopy.



In 1952, Jim Shoolery joined Varian Associates to set up an applications laboratory for NMR spectroscopy. His main initial goals were to develop applications of NMR in chemistry and to educate the wider chemistry community in the potential value of NMR spectroscopy in their research. In pursuit of these goals during the 1950's, he published a series of highly popular ads entitled "NMR at Work," initially in *Analytical Chemistry* and later on the back page of the *Journal of the American Chemical Society*. These illustrated a wide range of applications of NMR in chemistry and were based on work that he carried out in the applications lab. He also wrote a number of "Technical Information Bulletins" to help spectrometer owners in the operation of their instruments. Finally, he gave numerous lectures at conferences and research laboratories and at the annual NMR and EPR workshops that Varian Associates held in Palo Alto starting in 1958. In a 1993 article on the early history of NMR, he estimated that about 20,000 scientists had attended these different lectures by the end of the 1950's.

At the same time, Jim interacted with the R & D division of Varian on NMR instrument improvement, including the progression of ^{1}H operating frequency on Varian spectrometers from 30 to 40 to 60 and finally to 100 MHz by 1959. He was also involved in important technical improvements, including sample spinning, shim coils, spin decoupling, a flux stabilizer, and an electronic integrator. However, even with these improvements, the HR series of spectrometers were still extremely tricky to operate, requiring a significant amount of training, operating experience and patience. Jim realized that NMR spectroscopy would not reach its full potential as an analytical technique in chemistry until a spectrometer was developed that would be much easier to use, similar to the routine IR spectrometers that were already available from other manufacturers. Therefore, in 1957, Jim teamed with Emery Rogers of the marketing division of Varian to propose to the R & D division the development of a lower cost NMR spectrometer, which could use calibrated chart paper, which was rugged and reliable, and which could be run by graduate students and laboratory technicians with no training other than that provided by the spectrometer manual. He was heavily involved in this project, which resulted in 1961 in the introduction of the Varian A-60. This was a truly revolutionary development whose ease of operation triggered a dramatic increase in the use of NMR spectroscopy by chemists, in general, and by organic chemists, in particular. To illustrate its impact, the 1960 volume of the *Journal of Organic Chemistry* contained only one paper reporting the use of NMR while the 1967 volume included 220 papers, which used NMR data. In 2011, the seminal role of the A-60 in the development of NMR as a valuable analytical technique was recognized by the American Chemical Society as a National Historical Chemical Landmark in a ceremony at the Agilent facility in Santa Clara.

After the initial demonstration of FT NMR at Varian, Jim was involved in the development of the CFT-20 and FT-80 Varian spectrometers. These followed in the footsteps of the A-60 in being low cost and easy-to-use instruments for chemistry labs. In 1972, his book, “A Basic Guide to NMR,” was published by Varian Associates and helped to educate many young chemists in the use of NMR. Later, with the development of multi-pulse sequences and 2D NMR, Jim was among the first to recognize the great value of these techniques for identifying unknown organic chemical structures, particularly in the natural products field. Jim, along with Steve Patt, developed the APT sequence for spectral editing ^{13}C spectra of organic compounds and, through the 1980’s, he collaborated with a number of natural products groups in establishing structures and assigning spectra of the compounds which they had isolated. He also, in 1984, published an important review article in the *Journal of Natural Products*, which clearly demonstrated the value of modern NMR techniques in the natural products field.

SMASH 2015 Scholarship Recipients

The following students received a scholarship to attend SMASH 2015

- **Jessica Bame**, University of Bristol, United Kingdom
- **Nicholle Bell**, University of Edinburgh, United Kingdom
- **Rupashree Dass**, University of Warsaw, Poland
- **Maria Enrica Di Pietro**, Università della Calabria, Italy
- **Claire Dickson**, University of Bristol, United Kingdom
- **Ruth Dooley**, University of Edinburgh, United Kingdom
- **Niels Geudens**, Ghent University, Belgium
- **Simon Glanzer**, University of Graz, Austria
- **Boris Gouilleux**, Université de Nantes, France
- **Julian Greindl**, Universität Regensburg, Germany
- **Andrew Hall**, University of Bath, United Kingdom
- **Florian Hastreiter**, University of Regensburg, Germany
- **Aaron Hernandez-Cid**, University of Manchester, United Kingdom
- **Jonas Kind**, Technische Universität Darmstadt, Germany
- **Sandeep Kumar Mishra**, Indian Institute of Science, India
- **Thomas Leman**, University of Bristol, United Kingdom
- **Martin Leyendecker**, Technische Universität Darmstadt, Germany
- **Núria Marcó García**, Universitat Autònoma de Barcelona, Spain
- **Lucio Mauri**, Ronzoni Institute, Italy
- **Francesca Nardelli**, Università Vita-Salute San Raffaele, Italy
- **Nilamoni Nath**, Max Plank Institute for Biophysical Chemistry, Germany
- **Ikenna Ndukwe**, University Of Bristol, United Kingdom
- **Jan Novotny**, Masaryk University CEITEC MU, Czech Republic
- **Jane Power**, The University of Manchester, United Kingdom
- **Eliška Procházková**, Institute of Organic Chemistry and Biochemistry, Czech Republic
- **Polyssena Renzi**, Universität Regensburg, Germany
- **Eduardo Gomes Rodrigues de Sousa**, FARMANGUINHOS/FIOCRUZ/UFRJ, Brazil
- **Laetitia Rouger**, CEISAM, France
- **Andreas Seegerer**, University of Regensburg, Germany
- **Kawarpal Singh**, Institut Fur Technische Und Makromolekulare Chemie, Germany
- **Susann Weißheit**, Technische Universität Darmstadt, Germany

Thanks to our sponsors for their generous support.

SMASH 2015 NMR Conference Acknowledgements

The SMASH 2015 Conference gratefully acknowledges the support provided by the following companies:

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Martin Will
Sanofi-Aventis

Monday, September 21st
09:15 AM - 11:00 AM

Exploring the Unknown - Molecular Structure Determination

Chair: Tim Claridge

Speakers:

Dusan Uhrin
University of Edinburgh (UK)

Damien Jeannerat
University of Geneva (CH)

Kirk Gustafson
National Cancer Institute (US)

Clark Ridge (Upgraded Poster)
U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (US)

Isotope Filtered nD NMR Spectroscopy for Structure Determination of Molecules in Complex Mixtures

Nicholle G. A. Bell[1], Adam A.L. Michalchuk[1], John W. T. Blackburn[1], Margaret C. Graham[2] and **Dušan Uhrín**[1]

1. EastChem School of Chemistry, University of Edinburgh, Edinburgh, UK
2. School of Geosciences, University of Edinburgh, Grant Institute, Edinburgh, UK

Even the most powerful multidimensional (nD) NMR methodologies alone cannot solve structures of compounds contained in mixtures of thousands of small molecules. Some form of "spectroscopic separation" is therefore required. To achieve this, we have developed isotope filtered nD NMR spectroscopy [1] that allows structural investigation of isotopically tagged molecules in complex, inseparable mixtures.

Incorporating isotopically labelled moieties within targeted functional groups of small organic molecules opens a unique possibility for structure characterisation of molecules in complex mixtures. Although isotopic tagging has been used in the analysis of complex mixture in the past, we propose a new paradigm, whereby the tags are used to collect direct NMR information about the tagged molecules. Rather than focusing on the chemical shifts of the tags only, we use the tags to collect the chemical shifts and coupling constants of the parent molecules. We illustrate this approach by ^{13}C-methylation of hydroxyl and carboxyl groups in combination with purpose designed nD NMR experiments.

Couplings between the nuclei of the tag and those of the parent molecule are used to transfer the polarisation in nD NMR experiments providing multiple correlated chemical shifts and coupling constants of methylated compounds. The obtained information enables to solve the structures of derivatised molecules. In these experiments, the signals from unlabelled molecules are eliminated, providing much needed simplification of NMR spectra of complex mixtures.

Our methodology is aimed at the analysis of the aromatic moieties of humic substances (HS) - the main organic component of soil and amongst the most complex mixtures on Earth. We have recently applied it to characterise major substitution patterns of phenolic moieties of fulvic acid isolated from a peat soil [2].

Our approach is not limited to studies of HS, neither is the methylation as the way of introducing labels to act as spies on the parent molecules. We are currently exploring several different approaches based on the principles outlined here.

1. Bell, N.G.A., Murray, L., Graham, M., Uhrín, D., NMR methodology for complex mixture 'separation'. *Chem. Commun.* **50**, 1694-1697 (2014).
2. Bell, N.G.A., Michalchuk, A.A.L., Blackburn, J.W. T., Graham, M. C, Uhrín, D, Isotope-Filtered 4D NMR spectroscopy for structure determination of humic substances, *submitted*

Elimination of Scalar Interactions in the ^{13}C or ^{1}H Dimensions of 2D Spectra, with Application to ^{13}C-Enriched Products

Damien Jeannerat

Chemistry Department, University of Geneva, Switzerland

The Zangger-Sterk decoupling scheme found many applications to decouple direct and indirect detected dimensions of 2D experiments.[1] In order to adresses the low senstivity of these experiements, we applied a multiple-modulation of the selective pulse.[2] The problem is that modulation can causes artifacts when a multiple of the distance between modulation sites corresponds to the difference in chemical shifts of pairs of coupled nuclei. These artifacts can be drastically reduced using a non-equidistant modulation (nemo) of the selective pulse.[3]

We applied nemo-ZS decoupling to the indirect proton dimension of a special experiment called “DIAG” because it only records the diagonal of a TOCSY experiment. Such spectrum can be used to produce “decoupled” 1D ^{1}H spectra and provide scalar coupling constants in first-order systems.

The modulated ZS can also be used to simplify carbon-carbon coupling patterns in HSQC spectra of ^{13}C enriched compounds. Application to ^{13}C enriched cholesterol will be presented.[4]

1. Zangger, K., Prog. NMR spectrosc., 86, 1, 2015.
2. Cotte, A., Angewandte Chemie, int. ed., in press, 2015.
3. Foroozandeh, M., Giraudeau, P., Jeannerat, D., Magn. Reson. Chem. 51, 808, 2013
4. Shivapurkar, R., Souza, C. M., Jeannerat, D., Riezman, H., J. Lip. Res., 52, 1062, 2011.

NMR Characterization of Complex Natural Products: Assigning Novel, Proton-Deficient Alkaloid Scaffolds

Kirk R. Gustafson, Susanna T. S. Chan, and Dennis Milanowski

Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD USA

NMR provides powerful structural elucidation tools that are particularly well suited for natural products studies. Comprehensive spectroscopic characterization of a native metabolite is often sufficient to fully assign a new structure. However assignment of novel molecular architectures that incorporate numerous heteroatoms and proton-deficient fused ring systems can be challenging. Two new families of marine alkaloids were recently discovered that each had quite novel structural features. One series of alkaloids had two pyrimidine rings and an imidazole ring fused to generate an unprecedented tetracyclic core with embedded guanidine and amidine functionalities. The other class of metabolites incorporated multiple halogen substituents and six fused heterocyclic rings to generate a new structural motif. Structure characterization of these proton-deficient alkaloid scaffolds was quite challenging using only HMBC heteronuclear correlation data, which generally provides only two- and three-bond correlations with good sensitivity. A highly sensitive complimentary experiment, the recently reported LR-HSQMBC pulse sequence, was used to obtain very long-range ($\geq {}^4J_{CH}$) heteronculear correlations. The LR-HSQMBC data revealed numerous ${}^4J_{CH}$ and several ${}^5J_{CH}$ heteronuclear coupling pathways that were pivotal in linking together remote segments of the alkaloid structures. Comprehensive NMR analyses ultimately allowed the complete structural elucidation of the new marine alkaloids. Continued development, refinement, and application of enhanced NMR capabilities is crucial for the successful assignment of novel structural scaffolds and functional group arrays that are often found in natural products.

Development and Evaluation of Experiments for Oligosaccharide Structure Elucidation Using Phosphitylated Derivatives

Clark D. Ridge and Eugene Mazzola

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College, MD, USA

Carbohydrates, oligosaccharides, and other polysaccharides can be difficult to characterize by NMR due to the crowded nature of the proton spectrum. Spectral overlap and strong coupling can make it difficult to separate out the ring-proton multiplets, which are important for determining the proton-proton coupling constants used to identify the sugar subunits of oligosaccharides. Structural elucidation of such compounds can in some cases be aided by applying derivatization in combination with specialized NMR sequences. One such scheme uses carbon-13 labeled samples and doubly selective NMR experiments. [1,2] This method works well but can take up to 24 hours to prepare a sample and requires access to specialized equipment. This reaction can be replaced by a phosphorous labeling scheme originally used in the quantitative analysis of edible oils. The main advantage of using the phosphorous derivatization is the speed of the reaction. The phosphitylation reaction can be done in a few minutes in an NMR tube with no separate equipment. This reaction tends to go to completion and, with some adjustments can possibly be used to quantitate sugars in a similar manner to its use in edible oils.

The carbon-13 experiments used 100% labeled acetic anhydride with carbon-13 at both carbonyl positions to completely acetylate a sample. This "iso-tagging" allowed for several specialized pulse sequences used to selectively excite the ring protons through the coupling to the carbon-13 on the acetyl group. These experiments offer very good resolution and decent sensitivity but, as mentioned above, the reaction to produce these derivatives involves several steps before preparing an NMR sample and can take a significant amount of time. Another group of experiments has been developed for quantitative analysis of edible oils and their constituent molecules using a phosphitylation reaction. [3,4] This reaction is similar to the acetylation but uses a phosphorus reagent that leaves a phosphorous-31 nucleus where the carbon-13 would be in the acetylation experiments. This makes the molecules accessible by similar selective pulse experiments used in the iso-tagged case. The resolution improvement is not as good as in the acetylation case but the speed of the reaction makes it more practical in some situation. Some adjustments need to be made for use on carbohydrates compared to the method as used on edible oils. Experimental details and results will be presented on several model compounds such as maltitol. Advantages and drawbacks compared to the iso-tagging methods will be discussed as well as reaction optimization and pulse sequence considerations. Also, efforts at quantification of sugars will be discussed.

1. Meng, X.; Nguyen, W.; Nowick, J.,; Shaka, A., J. Mang. Reson., 2010, 203 (1), pp 73-80
2. Sun, Y.; Ridge, C.; del Rio, F.; Shaka, A.; Xin, J., Signal Processing, 2011, 91 (8), pp 1838-1851
3. Christophoridou, S.; Dais, P., J. Agric. Food Chem. 2006, 54 (3), pp 656-664
4. Dais, P.; Spyros, A., Magn. Reson. Chem., 2007, 45, pp 367-377

Monday, September 21st
11:30 AM - 01:15 PM

Spinning in Silico - NMR and Computation

Chair: Michela Ghitti

Speakers:

Wilfred F. van Gunsteren
Swiss Federal Institute of Technology, ETH (CH)

Alexandre M.J.J. Bonvin
Bijvoet Center for Biomolecular Research (NL)

Martin Dračínský
Institute of Organic Chemistry Biochemistry (CZ)

Dennis Anderson
Pfizer, Inc. (US)

Challenges in Bio-Molecular Research: On Comparing Simulated with Experimental Data

Wilfred F. van Gunsteren

Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH, 8093 Zuerich, Switzerland

Computer simulation of the dynamics of bio-molecular systems by the molecular dynamics technique yields the possibility of describing structure-energy-function relationships of molecular processes in terms of interactions at the atomic level. This is one of the reasons why computation based on molecular models is playing an increasingly important role in biology, biological chemistry, and biophysics. Since only a very limited number of properties of bio-molecular systems is actually accessible to measurement by experimental means, computer simulation can complement experiment by providing not only averages, but also distributions and time series of any definable – observable or non-observable – quantity, for example conformational distributions or interactions between parts of molecular systems. Present day bio-molecular modelling is limited in its application by four main problems: 1) the force-field problem, 2) the search (sampling) problem, 3) the ensemble (sampling) problem, and 4) the experimental problem. These problems, or rather challenges, will be discussed and in particular the pitfalls of comparing simulated with measured data will be illustrated using different examples. Perspectives may be outlined for pushing forward the limitations of computational modelling of bio-molecular systems.

1. Angew. Chem. Int. Ed. 45, 4064–4092, 2006.
2. Biochem. Soc. Trans. 36, 11-15, 2008.
3. Curr. Opin. Struct. Biology 18, 149-153, 2008.
4. Mol. Sim. 38, 1271-1281, 2012.
5. Angew. Chem. Int. Ed. 52, 2820-2834, 2013.

www.igc.ethz.ch

Information-Driven, Fully Flexible HADDOCKing of Protein-Ligand Interactions

Jeff Grinstead[2], Annin F. Ramsing[2], T. Wormwood[2], J.P.G.L.M. Rodrigues[1],
and **Alexandre M.J.J. Bonvin**[1]

1. Utrecht University, Faculty of Science - Chemistry, CH Utrecht, the Netherlands
2. University of Puget Sound, Tacoma WA, USA

Computational docking of small-molecule ligands into proteins is an important tool for modern drug discovery. Despite that some conformational differences are observed between free and ligand-bound states, protein conformational flexibility is often ignored in protein-small molecule docking programs. Our integrative, information-driven docking approach HADDOCK [1,2], which does account for flexibility in both receptor and ligand, has been mainly applied to protein-protein, protein-DNA/RNA, and protein-peptide docking problems. Here we present the results of its application to a benchmark of 76 protein-ligand complexes [3], concentrating on unbound cases (56 of those native complexes) [4].

HADDOCK sampling performance (ligand-RMSD < 2Å) on the benchmark set of Native cases (redocking) was 75-84% depending on the protocol, and generated a top-scoring solution in 67-72% of all 76 Native cases (90% if we consider only cases for which at least one acceptable solution was generated (51/57 cases)).

For the Unbound cases (each Native case represented by an apo-structure and/or a structure crystallized with a different ligand), the sampling success of HADDOCK was 60.7% (35/56 cases) and a top-scoring solution was generated for 42.9% of the 56 Native cases (24/56 cases) (68.6% if we consider only cases for which at least one acceptable solution was generated (24/35 cases)). HADDOCK Unbound docking performance is relatively insensitive to conformational differences in the input protein up to ~1.5Å interface RMSD.

This demonstrates that HADDOCK can handle a large variety of complexes, from small molecules to large macromolecular assemblies consisting of multiple (>2) components, allowing for the integration of experimental or predicted information to guide the flexible docking process.

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Dynamics in Solids Studied by NMR Crystallography and MD Simulations

Martin Dračínský[1], Michal Šála[1] and Paul Hodgkinson[2]

1. Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
2. Chemistry Department, Durham University, Durham, UK

A combination of molecular dynamics (MD) simulations and calculations of NMR parameters with solid-state NMR experiments brings important information about dynamics in solid materials. Dynamic processes may range from fast vibrational and librational motions to larger scale conformational changes in disordered materials. Various MD simulation techniques have to be used to simulate motions with different timescales (classical MD, DFT-MD, metadynamics). Several examples of recent applications of MD simulations coupled with NMR calculations and experiment will be discussed.

Molecular motion has a significant effect on all NMR parameters (chemical shifts, CSAs, couplings). Re-orientation of NMR tensors by molecular motion reduces the magnitudes of NMR anisotropies, and inclusion of molecular dynamics can significantly improve the agreement between calculated and experimental quadrupolar couplings. Furthermore, crystal structures derived by diffraction methods are affected by molecular motion, and neglecting this effect may lead to significant errors of calculated NMR parameters of solids [1].

The influence of nuclear quantum effects (zero-point vibration, delocalisation of the positions of light nuclei) on chemical shifts has also been explored by a combination of path integral molecular dynamics (PIMD) and NMR calculations. The results confirm a significant contribution of the nuclear delocalisation to the isotropic shielding values, and the implications for "NMR crystallography" will be discussed. For example, the correction of carbon isotropic shielding obtained by PIMD depends strongly on the number of attached hydrogen atoms, which has an important influence on the overall correlation between the calculated and experimental data [2].

Water molecules in some solid hydrates can be very mobile. NMR experiments together with molecular dynamics simulations and NMR calculations have been used to investigate mobility of water molecules and sodium ions in solid hydrates of two nucleotides. The structure of guanosine monophosphate system (GMP) is relatively rigid, with a well-ordered solvation shell of the nucleotide, while the water molecules in uridine monophosphate system (UMP) were shown to be remarkably mobile even at –80 °C [3]. The disorder of water molecules was confirmed by ^{13}C, ^{31}P, ^{2}H, and ^{23}Na solid-state NMR experiments. The experiments were complemented with NMR calculations on an ensemble of structures obtained from DFT-MD simulations. The MD simulations confirmed higher water mobility in the UMP system and the calculated chemical shifts and quadrupolar couplings were consistent with the experimental data. The disordered solvation shell in UMP is likely to be a good model for solvated nucleotides in general, with fast reorientation of water molecules and fluctuations in the hydrogen-bond network.

1. Dračínský, Martin, Hodgkinson, Paul, CrystEngComm, 15, 8705-8712, 2013.
2. Dračínský, Martin, Hodgkinson, Paul, Chem. Eur. J., 20, 2201-2207, 2014.

3. Dračínský, Martin, Šála, Michal, Hodgkinson, Paul, CrystEngComm, 16, 6756-6764, 2014.

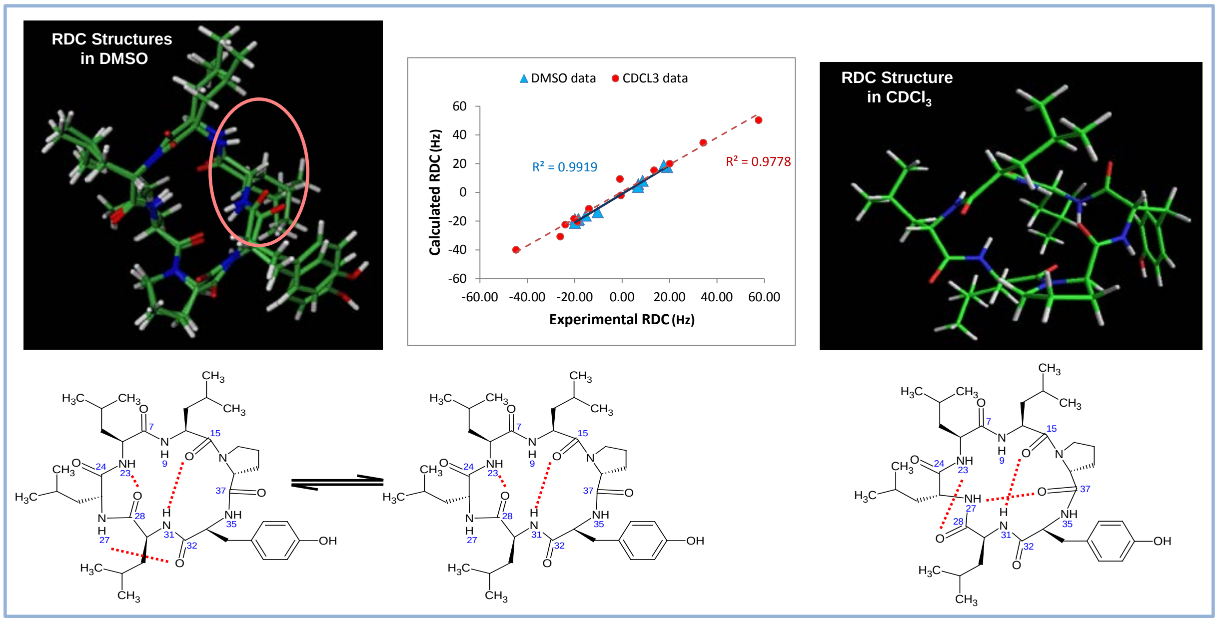
Acknowledgement:
This study was supported by the Czech Science Foundation (grant no. 15-11223S and 13-24880S).

Determination of Peptide Solution Conformations Using RDC

Dennis Anderson

Analytical Research and Technology, WWMC, Pfizer Inc., Groton, CT 06340, USA

Recently, the intense interest in peptides is demonstrated as promising therapeutic candidates due to their wide involvement in physiological processes. When peptide design has emerged as an independent discipline in drug discovery, it becomes more and more critical to understand the molecular properties of these beyond-5-rule molecules. The characterization of peptide solution conformations is proven to be the best way to insight into their activity, permeability, metabolic stability, clearance and bioavailability to guide design. Here we report the technology development and application of residual dipolar coupling (RDC) in combination with nuclear Overhauser effect (NOE) to determine the solution structures of peptides in different organic solvents. The technology development enabled our new capability to aid ligand based drug design using solution conformations of ligands, especially when it is challenging to obtain co-crystal structures to do structure based drug design (SBDD).



The comparison of the solution structures of peptide PF-06413600 in DMSO-d6 and CDCl3. The red dotted lines indicate the IMHB observed through temperature coefficients of chemical shifts.

Monday, September 21st
5:00 PM - 6:30 PM

Workshop

Future of NMR

Coordinated by:
Craig Butts and Silvia Mari, SMASH 2015 Co-Chairs

Future of NMR

Coordinated by:

Craig Butts and Silvia Mari, SMASH 2015 Co-Chairs

Discussion on Sustainability and Service Needs for NMR

We will update colleagues on the state of play following the closure of the Agilent NMR business, including availability of parts, service support (Agilent and 3rd party), software updates, hardware compatibility and upgrade pathways.

Vendor Panel for Future Prospects

A discussion, including a panel of vendors (all are welcome to participate) will examine over-arching directions for NMR for the next decade, for example: low-field NMR; smaller chip-based spectrometer architectures; open source operating systems; beyond cryogens; in-vivo spectroscopy etc.

Monday, September 21st
7:30 PM - 8:30 PM

Presentation of the James N. Shoolery Award

SMASH 2015 Recipient:

Professor Gareth Morris
University of Manchester, UK

Professor Gareth Morris

Gareth Morris was successively an undergraduate in Chemistry, postgraduate student, and research fellow at Magdalen College Oxford from 1972 to 1981, first as a Demy and subsequently as a graduate student (supervised by Ray Freeman) and as a Fellow by Examination. He worked on a remarkable number of, now crucial, underpinning pulse sequence elements during this time, including DANTE and INEPT (with the first INEPT experiments being performed in the few weeks between the submission of his thesis and leaving for a year's postdoc with Laurie Hall at the University of British Columbia as an Izaak Walton Killam postdoctoral fellow). In 1979 he returned to Oxford, working with Ray and colleagues on a variety of 1D and 2D methods and applications.

In 1982 he moved to Manchester, where he was successively Lecturer, Reader and Professor of Physical Chemistry. In these 30+ years he has made seminal contributions in gradient shimming, diffusion-ordered NMR spectroscopy including the use of multicomponent diffusion matrices to enhance diffusional separation; the analysis and correction, both by methodological and hardware improvements and by post-processing software, of limitations to performance in high resolution NMR equipment; and the improvement of spectral resolution by new one- and two-dimensional pure shift NMR techniques, and their extension to structural biology.

He was awarded the RSC Corday-Morgan prize and medal in 1988, a Leverhulme Fellowship in 1996, and the RSC Industrially-Sponsored Award in Magnetic Resonance Spectroscopy in 2001, and was an invited professor at the Université Pierre et Marie Curie, Paris, in 2010. In 2011 he was awarded the Russell Varian Prize for NMR, and in 2014 was elected a Fellow of the Royal Society.

Tuesday, September 22nd
09:00 AM - 10:45 AM

Molecules on the Move - NMR of Reacting Systems

Chair: Christina Thiele

Speakers:

Mark Zell
Pfizer, Inc. (US)

Guy Lloyd-Jones
University of Edinburgh (UK)

Kris Kazimierczuk
Centre for New Technologies (PL)

Michael John (Upgraded Poster)
Institut für Anorganische Chemie,
Georg-August-Universität (DE)

Recent Developments in the Use of Online NMR Reaction Monitoring in the Pharmaceutical Industry

Mark T. Zell and David A. Foley

Pfizer Worldwide Research & Development, Pfizer Inc., Analytical Research and Development, Groton, CT, USA

The use of NMR spectroscopy to monitor chemical reactions has become increasingly popular, as it provides a quantitative picture of all of the NMR active species in a reaction mixture. NMR is well suited for interrogating transient intermediates, providing kinetic information via multiple NMR active nuclei, and providing quantitative information for monitoring mass balance.

We make use of a wide range of NMR systems in our reaction monitoring work, ranging from 60 to 600 MHz depending on the application to be investigated. We have recently begun utilizing smaller, more flexible NMR systems for reaction monitoring to take advantage of their increased portability. These lower field NMR systems are “cryogen free”, utilizing either permanent magnets (60 MHz) or new generation HTS superconducting magnets (200 MHz), eliminate the need for costly cryogens, and allow the NMR to be performed in the chemistry lab where a superconducting NMR spectrometer may not be available.

An overview of our work utilizing NMR for reaction monitoring will be outlined, and the application of 60 MHz permanent magnet, 200 MHz HTS magnet, and 400, 500, and 600 MHz superconducting magnet-based NMR systems to reaction monitoring will be discussed.

In situ NMR Interrogation: Probing the Mechanism of Action of Reagents in Synthesis

Guy Lloyd-Jones[1], Paul Cox[1], Jorge Gonzalez[1], Tom Corrie[1], Andrew Leach[2], and Martin Burke[3]

1. School of Chemistry, University of Edinburgh, UK
2. John Moores University, Liverpool, UK
3. Chemistry Department, University of Illinois, Urbana Champaign, USA

The presentation will focus on the in situ analysis of reactions of interest to the synthetic chemist, such as cross-coupling, asymmetric rearrangements, etc. The work aims to gain a better grasp of the fundamental physical and chemical processes that facilitate and govern the reaction of interest, either directly or via modulation of undesired side reactions.

The approach is predominantly mechansitic elucidation via analysis of reaction kinetics and other physical organic parameters, obtained by NMR in concert with strategic istopic labelling, and augmented by computatational analysis and other spetcroscopic / spectrometric methods.

Some recent examples include the study of Suzuki-Miyaura couplings,[1] the investigation of gold-catalysed aryl silane coupling processes,[2] and borane displacement from amines and phosphines.[3] The presentation will predominantly include as yet unpublished work.

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2. Ball, Liam, T., Lloyd-Jones, Guy C., Russell, Christopher A., *J. Am. Chem. Soc*., 136, 254-264, 2014
3. Lloyd-Jones, Guy C., Taylor, Nicholas, P., , *Chem. Eur. J*., 21, 5423-5428, 2015

Dynamic Non-Uniform Sampling

Rupashree Dass[1], Wiktor Koźmiński[1], and **Krzysztof Kazimierczuk**[2]

1. Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland
2. Centre of New Technologies, University of Warsaw, Warsaw, Poland

Non-uniform sampling (NUS) has become a popular solution to accelerate multidimensional NMR experiments. The majority of standard signal acquisition software allows to run experiments in the NUS mode. Also numerous NUS processing methods are available. Among them, sparsity-based reconstructions, known also as compressed sensing (CS), have recently become popular [1].

Importantly, NUS can be exploited in a way different from the usual undersampling of the full Nyquist grid, i.e. to implement time-resolved multidimensional experiments with extraordinary temporal resolution. The original idea of Mayzel et al. [2] assumed, that NUS of the indirect dimensions is performed in parallel to some chemical reaction or physical process occurring in the sample. Then, overlapping subsets of the acquired dataset are used for the reconstruction of the series of multidimensional spectra corresponding to various moments of the process.

Our group developed CS methods dedicated for the acquisition and processing of the time-resolved data. The examples of both artificially induced processes [3] and uncontrolled reactions [4] have been given. Currently, the idea is being extended to the experiments where the observed change in the spectrum is induced not by the changes in the molecular structure, but by varying the magnitude of the coherence transfer. The examples of applications, as well as the main principles of the method, referred to as dynamic NUS, will be mentioned during the talk.

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2. Mayzel, Maxim, Rosenlöw, Joakim, Isaksson, Linnéa, Orekhov, Vladislav Y. J. Biomol. NMR, 58(2), 129–39,2014
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Slice-Selective NMR Spectroscopy as Versatile Tool for Chemists

Michael John, Ann-Christin Pöppler, Thomas Niklas, and Dietmar Stalke

Institut für Anorganische Chemie, Georg-August-Universität, Göttingen, Germany

Slice-selecive excitation (SSE) is a fundamental part of most current MRI methods [1,2] as well as several more recent NMR techniques such as pure-shift NMR [3] or single-scan 2D NMR [4]. SSE is accomplished by band-selective rf pulses in the presence of a magnetic field gradient. Due to the widespread availability of NMR probes with single-axis gradient coils, we believe that SSE also has an enormous potential in chemistry when simply employed to obtain localized "classical" NMR spectra. For highly sensitive nuclei such as ^{1}H, ^{7}Li, ^{19}F or ^{31}P, a series of 20 spectra of individual horizontal 1-mm-slices is obtained in less than 2 min.

Recently we demonstrated the method on cross-linked polystyrene sticks that are unidirectionally swollen by a solvent (toluene-d8) and at the same time penetrated by a lithium amide dissolved in that solvent [5]. Based on the ^{2}H and ^{7}Li signal integrals and quadrupolar splittings observed in the individual slices, concentration and strain profiles could be constructed, respectively, which were followed over time. The strain profiles turned out to be an excellent way to assess the homogeneity of the resulting gel which is of major importance if the gel is to be used as alignment medium for RDC measurements [6].

Meanwhile we have extended our study to cross-linked ethyl and butyl acrylate polymers, which present highly promising alignment media for more polar solvents and solutes [7]. As a key result of this work, it was found that polymerization in the presence of a RAFT (reversible addition-fragmentation chain transfer) agent leads to significantly better homogeneity of the resulting gel.

Additionally we demonstrated two more "chemical" applications of slice-selective NMR: a "single-shot" NMR titration, where the concentration of the titrated component follows a gradient over the sample rather than being incremented and fully mixed step by step, and a "single-shot" reaction monitoring experiment, where two reaction components diffuse towards each other within the active rf coil region [8]. The latter experiment could show the build-up and decay of a reaction intermediate in the slices located at the reaction front.

Currently, we use slice-selective NMR spectroscopy in gel matrices to separate the components of a mixture of compounds due to their different molecular size or polarity. This way we hope to establish slice-selective NMR as a method to obtain diffusion coefficients as an alternative to the DOSY method.

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2. Lauterbur, Paul C.; Kramer, David M.; House, Waylon V. Jr.; Chen, Ching-Nien, J. Am. Chem. Soc., 97(23), 6866-6868, 1975.
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8. Niklas, Thomas; Stalke, Dietmar; John, Michael, Chem. Commun., 51, 1275-1277, 2015.

Tuesday, September 22nd
11:15 AM - 01:00 PM

Finding a Noodle in an NMR Soup Bowl - Food and Health

Chair: David Chang

Speakers:

Tim Rudd
Diamond Light Source Ltd (UK)

Luca Laghi
Università di Bologna (IT)

Niels de Roo
Unilever (DK)

Sofia Moco (Upgraded Poster)
Nestle Institute of Health Sciences, Natural Bioactives and Screening (CH)

Statistical Correlation Spectroscopy and Spectral Filtering Applied to NMR Spectra

Timothy R. Rudd

National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire, UK

The analysis of heterogeneous and structurally varying polymers presents a challenge that cannot be met by direct comparisons with a single standard sample [1]. This makes the validation of heterogeneous material and the subsequent identification of possible contaminants problematic.

A series of related approaches have been developed to meet this challenge, based on considering the polymer as a statistical population, applying methods to ascertain the differences between a library of bona fide spectra and a test spectrum, these methods are based on two-dimensional correlation spectroscopy (2D-COS).

The 2D-COS analyses are a development of the technique pioneered by Isao Noda [2], in this circumstance a difference covariance matrix is formed using a library of NMR spectra and the NMR spectrum of a test sample, in its simplest form the 2D-COS spectrum of a set of spectra is the covariance matrix of those spectra.

The subtraction of the covariance matrix formed by the library of bona fide spectra from a covariance matrix formed by the aforementioned library of bona fide spectra with the addition of a test spectrum reveals features in the test sample that are not present in the library of bona fide spectra. This approach is coined Two-Dimensional Correlation Spectroscopy-filtering (2D-COS-f) [3]. This method has been expanded, this involves utilising a difference covariance matrix in combination with iterative random sampling, and is capable of revealing contamination in pharmaceutical heparin to a high level of sensitivity irrespective of the nature of those features, while also providing a measure of the natural variability of the material being investigated, in this case heparin, to compare against the features found in the test sample [4].

These techniques are not limited to 1D-NMR spectra, they can be applied to 2D-NMR spectra, for example 2D-COS applied to HSQC spectra (HSQC correlation spectroscopy – HSQCcos) has allowed information regarding the sequence of heparin to be uncovered, without having to use traditional enzyme depolymerisation techniques [5]. Furthermore, applying spectral filtering to HSQC spectra goes some way to negating the signal dispersion problem that occurs in the 1D-NMR equivalent [6].

These techniques are suitable to any situation in which a comparison of a single entity to a family of heterogeneous entities, particularly natural products and biosimilars, needs to be made, and will find application in pharmaceutical monitoring, manufacturing quality control, materials science, biotechnology, and metabolomic investigations.

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Linear Models to Survive Between 64K Points and my Food Science Collegues

Luca Laghi

Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521 Cesena, Forlì-Cesena, Italy

In metabolomics studies, the quest for transforming potentially informative nuclear magnetic resonance (NMR) spectra in actual information on a sample requires several steps, one of which being, sooner or later, the assignment of the signals. In my last year of activity, the shoulder to shoulder work with many colleagues involved in food science, microbiology and human health led me through many of these steps, and forced me to make choices, some of which proved unsuccessful. In the present communication I'd like to describe metabolomics works I was involved in, focusing on pros and cons of the decisions that can be made at each key step.

Contrary to most of the other spectroscopic techniques, high resolution NMR is particularly suited, when employed in metabolomics studies, for targeted investigations, where a priori selected metabolites are observed. It is obvious that such approach intentionally neglects significant portions of the spectrum. The rationale under this exclusion is that a feature of a spectrum has good chances of giving robust information when specific chemical, biological or physical meanings can be obtained from it, otherwise it represents noise.

The use of the entire spectrum, thus of the entire source of information available, requires an untargeted approach. In a limited number of cases this can be done by considering the spectrum like a fingerprint, for example by looking for overall similarities among samples. This choice was recently made by Bordoni et al. [1], to follow the fate of a cured meat based product upon digestion.

Nevertheless, in most circumstances the discovery of a connection between spectra features and sample characteristics passes through signals assignment. From a logistic point of view this step is most conveniently performed right after spectra acquisition and preprocessing, by using the same software created for targeted analysis. An example is given by the work by De Filippis et al. [2]. Indeed, the data matrices so generated are easily handled, are ready for discussion with the other stakeholders of the study and are suitable for the enrichment of versatile databases. Moreover many software tools designed for the purpose can be user guided, thus taking advantage from the innate ability of humans for trends recognition.

Unfortunately signals assignment right after spectra acquisition and preprocessing corresponds again to arbitrary data reduction, where the choice of the signals worth attention is basically eye driven, so that peaks which rise above the surrounding (because big or resonating in poorly crowded spectra portions) are easily over considered.

More unbiased importance is attributed to signals when their identification is performed before signals assignment. This is typically done through multivariate analysis, preferably with variable selection as in the case of (iECVA) [3] and sparse PLS [4].

A balance between the versatility of visual inspection for signals assignment and the need for unbiased information may be based on the identification of NMR signals by means of univariate statistical analysis. Examples will be presented as a base for a discussion about pros and cons of this (low tech) approach.

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Rapid Quantification of Polysaccharides in Foods: A Small Molecule Based Chemometric qNMR Approach

Ewoud van Velzen[1], Donny Merkx[1], Kavish Thakoer[1], **Niels de Roo**[1], Christian Grun[1], Yvonne Westphal[1], and John van Duynhoven[1,2]

1. Unilever Research and Development, Vlaardingen, The Netherlands
2. Laboratory of Biophysics, Wageningen University, Wageningen, The Netherlands

Food polysaccharides show a wide structural diversity and already provide benefits when used at low levels. Taken together with strong matrix interactions these factors complicate the quantitative assessment of polysaccharides in complex food products. Whereas NMR presents an ideal tool for quantification of low-molecular weight species, its deployment for absolute quantitative assessment of polysaccharides in complex mixtures is less straightforward [1]. This is primarily due to broadened and overlapping lineshapes, which compromises NMR signal integration. This can be circumvented by hydrolysis of polysaccharides mixtures, which enhances spectral resolution and allows for straightforward, rapid and absolute quantification [2]. Using PULCON, Quantum Mechanical Total Lineshape Fitting (QMTLS), and corrections for both non-immediate polymer hydrolysis and recovery of monosaccharides accurate and precise monosaccharide compositions could be obtained for a wide range of food polysaccharides. Hydrolysis however discards secondary structure, which compromises polysaccharide identification in mixtures. Hence we combined quantification of monosaccharide compositions (C) with known stoichiometries (S_t^T) from polysaccharides identified from ^{1}H NMR lineshape analysis of the intact polysaccharides:

$$C_p = C\, S_t\, (S_t^T\, S_t)\text{-}1 + \varepsilon$$

The vector of polysaccharide compositions (C_p) could be obtained via an Alternate Least Squares optimisation with non-negativity constraints. Using this approach we achieve identification and absolute quantification of polysaccharides in complex food matrices by NMR, without the use of analytical standard mixtures.

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Using off- and on-line SPE-NMR in Bioactive Metabolomics

Killian Barry, Martine Cabo, Denis Barron, and **Sofia Moco**

Nestle Institute of Health Sciences, Natural Bioactives and Screening, Lausanne, Switzerland

Plants are rich sources of novel bioactives. The biochemical elucidation of these is imperative to understand their bioactivity and mechanism-of-action. State-of-the-art spectrometric and spectroscopic technologies are used to assist in the separation, enrichment and structure elucidation of bioactives in complex mixtures. NMR has therefore a pivotal role in all biochemical analysis of extracts and biological samples. Knowing the endogenous abundance of bioactive molecules, namely secondary metabolites from plants, are in the sub-micromolar range, metabolite enrichment is unavoidable to conduct identification studies.

Here, we present how solid phase extraction (SPE) can be efficiently combined with NMR, as a sample preparation strategy to enrich certain classes of compounds in mixtures. Using an automated robotic station, sample preparation routines were implemented to reproducibly enrich semi-polar compounds in plant extracts, before NMR analysis. On these enriched samples, metabolomics studies were then conducted, unravelling lower-abundant compounds, previously masked by highly abundant central metabolism intermediates, such as sugars. In addition, the SPE-treated extracts could be used for further structure elucidation of bioactive components using online LC-SPE-flowNMR.

We believe the usage of SPE on NMR applications can be efficient for the analysis of bioactives in diverse biological samples (e.g. plant extracts, cellular extracts, biofluids) by enriching lower-abundant or specific classes of compounds, in an automated way. With the variety of solid materials available, many classes of compounds can be targeted, as well as solvent systems. These enriched samples can be directly analysed by NMR or further processed for comprehensive structure elucidation using NMR-hyphenated techniques, speeding up bioactive discovery studies.

Wednesday, September 23rd
08:45 AM - 10:35 AM

Medicines in Magnets - Innovative Approaches to Drug Discovery

Chair: Carla Marchioro

Speakers:

Rob Cooke
Heptares (UK)

Dinu Iuga
University of Warwick (UK)

Maria Enrica Di Pietro (Upgraded Poster)
Universita’ della Calabria (IT)

Claudio Dalvit
Université de Neuchâtel (CH)

Biophysical and Structural Studies of Engineered GPCRs for Drug Discovery

Robert Cooke

Heptares Therapeutics, United Kingdom

Recent years have witnessed dramatic advances in the approaches available for GPCR drug discovery. High resolution X-ray structures are now available for approximately 30 GPCRs, allowing the mechanisms of activation to be deduced, and enabling structure-based drug discovery approaches to be used for the first time.

Many GPCR structural studies have utilised the StaR® technology developed by Heptares, where the introduction of a small number of mutations locks the GPCR in an active or inactive state and stabilises the receptor sufficiently to allow purification and crystallization. In addition to structural studies, the StaR approach enables other biophysical approaches to investigate ligand binding. Locking the receptor in a specific pharmacological form allows the generation of a reagent ideally suited for screening for molecules matching that pharmacology. The increased stability resulting from the StaR approach also permits screening in harsher conditions than may otherwise be feasible.

Structure-based drug discovery is an established technique for soluble proteins, but, until recently, its utility for GPCRs has been limited by their instability. Heptares has now used structure-based approaches for several GPCRs, combining screening of StaR proteins with structural and modelling studies to drive forward medicinal chemistry efforts. The presentation will include examples of these, and a perspective on the outlook for GPCR structure-based drug discovery.

Applications of High-Field Solid-State NMR to Small Organic Molecules

Dinu Iuga

Department of Physics, University of Warwick, Coventry, UK

The UK 850 MHz Solid-State NMR Facility has been operational since 2010 and has enabled applications across chemistry, materials science, life sciences and environmental sciences. Being equipped with a large number of probes, including very fast magic-angle spinning (MAS) and special probes (like DOuble Rotation) the Facility provides unique possibilities to investigate, develop and apply advanced solid-state NMR methods for the structural characterization of organic molecules at natural abundance.

The increased sensitivity provided by high field combined with the enhanced resolution of ^{1}H spectra obtained when the solid samples are spun very fast at the magic angle enhances experiments like two-dimensional double quantum high-resolution ^{1}H [1] and opens the door for indirect detection of low gamma nuclei (like ^{14}N), methods long envied by the solid-state NMR community on the their liquid-state peers. In this presentation, the benefits and limits of very fast MAS (up to 100 kHz) on the resolution of ^{1}H spectra will be exemplified on a dipeptide [2]. Further it will be shown how indirect detection of ^{14}N resonances reveal one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed. Finally application of these methods to the structural characterization of the hemihydrate of 2′, 3′-*O*-isopropylidineguanosine [3], identification of intermolecular hydrogen bonding in the solid-state structure of an indomethacin-nicotinamide cocrystal [4] and characterization of nitrogen functional groups in pharmaceutical compounds like cimetidine [5], nicotinamide palmitic acid cocrystal [6] and acetaminophen-polyvinylpyrrolidone solid-dispersion [6] will be shown.

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Anti-inflammatory Drugs by NMR in Ordered Media: Insights into Conformation and Stereochemistry

Maria Enrica Di Pietro[1], Giuseppina De Luca[1], Christie Aroulanda[2], Giorgio Celebre[1], and Denis Merlet[2]

1. Università della Calabria, Dip. Chimica e Tecnologie Chimiche, Lab. LXNMR_S.C.An., Rende (CS), Italy
2. Université Paris-Sud, ICMMO, Equipe LRMN, Orsay, France

Naproxen (2-(6-methoxy-2-naphthalen)propanoic acid), flurbiprofen (2-(2-fluoro-4-biphenyl)propanoic acid) and ibuprofen (2-(4-(2-methylpropyl)phenyl)propanoic acid) are three popular non-steroidal anti-inflammatory drugs belonging to the family of 2-arylpropionic acids, commonly known as profens. Profens are among the most important drugs in common use and are widely employed as therapeutic agents for the treatment of pain and inflammation. Their anti-inflammatory and antipyretic action as well as the gastrointestinal side-effects are based on the blockage of cyclooxygenase enzyme (COX) with following inhibition of downstream prostanoid species.[1] However, it has been reported that such drugs may bind some other proteins with different mechanisms and orientations and new targets are likely to be individuated in the future.

When a given flexible ligand binds to a receptor, and initiates then a biological effect, it has to adopt a “bioactive conformation” which is in some way complementary to its target protein.[2] The determination of the bioactive conformation of profens is hence an important challenge since it may help in gaining knowledge about the drug-protein complex, and thus be used as a template when designing novel anti-inflammatory agents with desired properties. However, this is often far from being a trivial task since in lots of cases the bioactive conformation does not correspond to the global energy minimum in the free state and lies instead at or very close to a local minimum on the potential energy surface.[2-3] In addition, the bioactive conformation depends on the receptor or receptor subtype the drug is bound to and on the specific medium.[4] Overall, this means that structures experimentally determined by single crystal X-ray crystallography or global minima found for an isolated molecule by in vacuo calculations do not always well represent the protein-bound conformation in shape and are then less useful in the context of drug design than generally assumed.[2,5]

A valid drug design approach for flexible ligands lies in the exploration of a wide conformational space highlighting all the possible minima that the molecule can assume. Indeed, when searching the bioactive conformation, it is recommended to consider a more extended and diverse set of conformations rather than a single conformer.[6] In this optic, NMR spectroscopy in partially ordered media shows many strong points. Contrary to techniques currently of choice for structure-based drug design, namely solid-state NMR spectroscopy and X-ray crystallography, this methodology allows (i) to probe molecular structure in solution, which is closer to the physiological environment of bioactive molecules, and (ii) to investigate the molecular internal motions and then the flexibility, leading to an equilibrium between global and local minima. In this contribution we show how the measurement of residual dipolar couplings (RDCs), extracted from NMR spectra in partially ordered media and interpreted via proper theoretical models, can give valuable insights into the structural and conformational features of profens.[7] Fig.1 reports the probability distribution together with the structure of the energetically-admissible conformers experimentally found for S-(+)-naproxen and R-(-)-flurbiprofen, dissolved in a weakly ordering liquid crystal phase composed of the synthetic homopolypeptide poly-γ-benzyl-L-glutamate (PBLG) and an organic co-solvent.

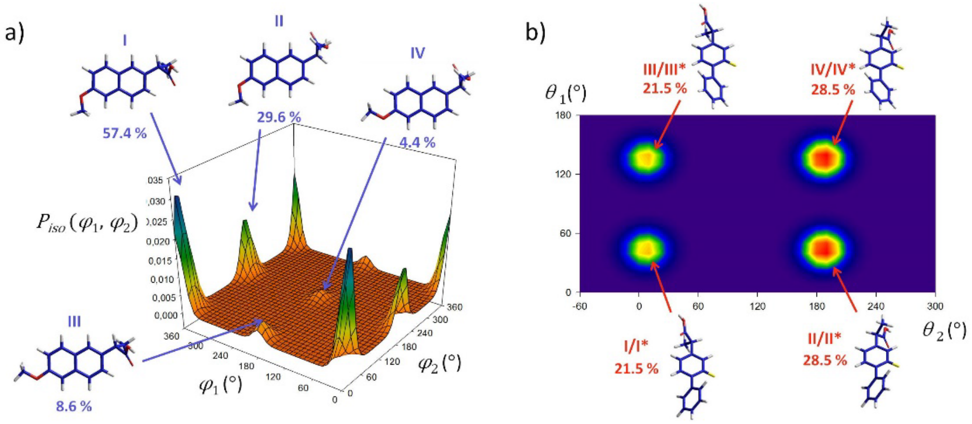

Figure 1. Experimental probability distribution Piso(φ1,φ2) and Piso(θ1,θ2) obtained for (a) S-naproxen and (b) R-flurbiprofen, both dissolved in PBLG/THF-d8, including structures of minimum energy conformers and their relative abundance.

The potential of PBLG ordered media have been largely demonstrated for various purposes, but probably the most exploited application is in the discrimination of S- and R-isomers and measurement of enantiomeric excesses.[8] These properties gains a major role in the study of pharmaceutically active molecules like profens, where enantiomeric discrimination and quantification are crucial goals. Indeed, profens are chiral molecules and individual enantiomers differ in their pharmacological activity. Despite the anti-inflammatory activity due to COX inhibition is largely stereospecific for the S-enantiomers, more recent works proved R-isomers can have different appealing activities, thanks to alternative binding modes to COX enzymes or inhibition of non-conventional targets.[9-11] Combining orientational order and chirality, PBLG phases give the opportunity to both discriminate enantiomers and investigate their order and conformational surface. In this contribution we report preliminary results obtained for racemic ibuprofen in PBLG/$CDCl_3$. A series of 1D and 2D NMR experiments allowed us to discriminate between the enantiomers using order-sensitive NMR observables, namely ^{13}C chemical shift and a considerable number of short- and long-range ^1H-^1H and ^1H-^{13}C RDCs (fig.2).

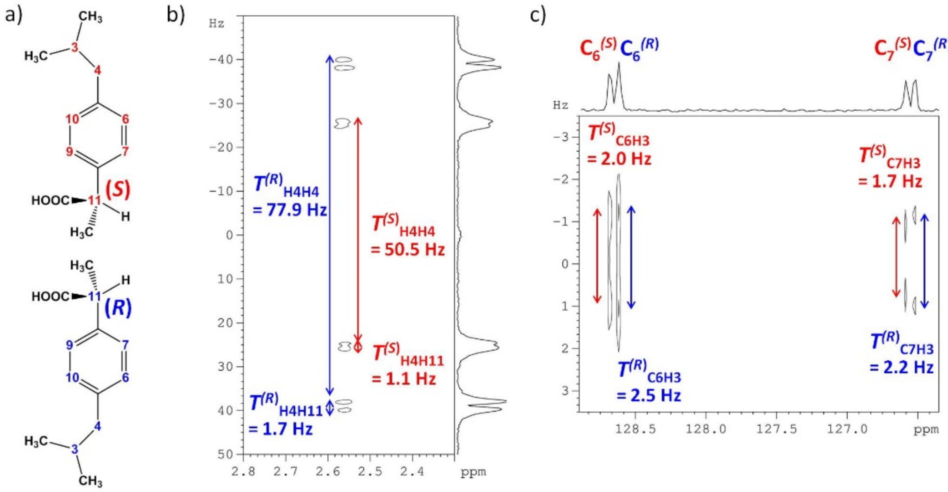

Figure 2. (a) Structure and labeling of R- and S-enantiomers of ibuprofen. Examples of 2D (b) ^1H-^1H SERF and (c) ^{13}C-^1H HETSERF spectra recorded on racemic ibuprofen in PBLG/$CDCl_3$.

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Ackowledgements

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A Comparison of Fluorine Ligand-Based NMR Screening with Other Biophysical Techniques: Advantages and Limitations

Claudio Dalvit[1] Marina Veronesi[2] and Anna Vulpetti[3]

1. University of Neuchatel, Neuchatel, Switzerland
2. Fondazione Istituto Italiano di Tecnologia, Genova, Italy
3. Novartis Institutes for Biomedical Research, Basel, Switzerland

Fluorine ligand-based NMR screening is becoming increasingly popular in drug discovery projects carried out in academia and pharmaceutical industries. Experiments such as the direct and competition binding assay FAXS (Fluorine chemical shift Anisotropy and eXchange for Screening) and the functional assay n-FABS (n-Fluorine Atoms for Biochemical Screening) are robust and efficient methods for the identification and the K_I or IC_{50} determination of chemical fragments binding to the receptor of interest. In addition, their versatility allows for novel applications to complex chemical and biological systems. The principles, along with theoretical and experimental analysis of these methodologies are presented and a detailed comparison is made with other biophysical techniques frequently used in fragment based drug discovery. Advantages and limitations of the two ^{19}F NMR-based methodologies are discussed and described with examples taken from drug discovery projects.

Wednesday, September 23rd
11:00 AM – 12:30 PM

Workshop / Tutorial (concurrent)

Modernizing the Pharmacopeias - NMR Tests for Drug Quality

Marco Guerrini*, Ronzoni Institute (IT)
David Keire, US Food and Drug Administration (US)

2D qNMR

Patrick Giraudeau*, Université de Nantes (FR)

* Coordinator

Modernizing the Pharmacopeias - NMR tests for drug quality

Coordinated by:
Marco Guerrini and David Keire, Ronzoni Institute (IT)
US Food and Drug Administration (US)

For certain complex drugs the high resolution and information content of NMR spectroscopy is a key tool for assuring their quality. However, the application of NMR as a compendia test is rare. In this session we will examine the present use of NMR in the US and EU pharmacopeias and possible future application of NMR methods for drug quality testing. Examples of 1D and 2D approaches will be presented which could be possible additions to the present pharmacopeias.

2D qNMR

Coordinated by:
Patrick Giraudeau*, Université de Nantes (FR)

This workshop will cover the recent approaches yielding quantitative results from 2D NMR spectra. This will include both acquisition and processing strategies, described in a tutorial fashion with practical examples. A significant part of the workshop will be dedicated to Q&A from the audience.

Wednesday, September 23rd
02:00 PM – 03:45 PM

Small but Beautifully Formed - Low Field NMR

Chair: Andrew Coy

Speakers:

Jeffrey Paulsen
Schlumberger-Doll Research (US)

Michael Maiwald
BAM Federal Institute for Materials Research and Testing (DE)

Anna Gerdova
Oxford Instruments (UK)

Susie Riegel (Upgraded Poster)
Nanalysis Corp. (CA)

A Single Chip, Multi-dimensional NMR Spectrometer

Dongwan Ha[1] **Jeffrey Paulsen**[2] Yi-Qiao Song[2] and Donhee Ham[1]

1. School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA
2. Schlumberger-Doll Research Center, Cambridge MA, USA

Multi-dimensional NMR spectroscopy has long been an essential technique in the chemist's tool kit for chemical and structural analysis. To meet the requirements of sensitivity, spectral resolution and complex RF control these applications demand, NMR instrumentation has traditionally required large superconducting magnets and spectrometer electronics; thus largely limiting their use to within dedicated laboratory spaces. Over the past decade, magnet technology has improved to the point where miniaturized permanent magnets have the sufficient strength and homogeneity for the NMR spectroscopy of small molecules,[1] thus overcoming a critical limitation to its routine in-field, on-demand and on-line application. In contrast, spectrometer electronics with the flexible pulse sequence capabilies have remained compartatively bulky in size, largely limiting 'miniaturized' NMR spectroscopy to benchtop applications.

To fully complement advances in magnet technology, we have developed a 4-mm^2 NMR spectormeter on a chip.[2] This extreme miniaturization has largely been enabled by adapting the same kinds of mixed analogue and digital chip technologies that were originally developed for today's smart phones. Our chip has the same sensitivity as today's larger spectrometers, and achieves 6μs 90^o pulses when powering NMR micro-coils. It furthermore supports advanced pulse programing features including composite pulses, 32-pulse phases, and complex looping structures, while multiple chips can be included and synchronized within the same chip package for multiple-nuclear or -coil measurements. As we will show, the chip is easily capable of supporting common multi-dimensional NMR experiments. Ultimately, along with advances in magnet technology, the minaturization this chip technology achieves should not only enable truly handheld NMR spectroscopy for small molecules, but also serve to improve existing NMR applications such as massively parallel NMR and MRI experiments, process monitoring, NMR chemometrics, and microfluidics.

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Automated Data Evaluation and Modeling of Simultaneous ^{19}F-^{1}H Benchtop NMR Spectra for Online Reaction Monitoring

Michael Maiwald[1], Nicolai Zientek[1,4], Clément Laurain[1,2], Klas Meyer[1], Andrea Paul[1], Dirk Engel[5], Matthias Kraume[4], Gisela Guthausen[3]

1. BAM Federal Institute for Materials Research and Testing, Berlin, Germany
2. École Nationale Supérieure de Chimie de Lille, Villeneuve D'ascq Cedex, France
3. Pro2NMR, Institute of Mechanical Process Engineering and Mechanics and Institute of Biological Interfaces, KIT, Karlsruhe, Germany
4. Department of Chemical Engineering, Technische Universität Berlin, Berlin, Germany
5. S-PACT GmbH, Aachen, Germany

Benchtop nuclear magnetic resonance spectroscopy currently develops to an important analytical tool for both quality control and process monitoring. [1] In contrast to high resolution online NMR (HR-NMR), benchtop NMR can be operated under rough environmental conditions. A continuous re-circulating stream of reaction mixture from the reaction vessel to the NMR spectrometer enables a non-invasive, volume integrating online analyses of reactants and products. Here we investigated the esterification of 2,2,2-trifluoroethanol with acetic acid to 2,2,2-trifluoroethyl acetate both by ^{1}H HR-NMR (500 MHz) and ^{1}H and ^{19}F MR NMR (43 MHz and 40 MHz) as a model system. The parallel online measurement was realized by splitting the flow, which allowed the adjustment of quantitative and independent flow rates, both in the benchtop NMR probe as well as in the HR-NMR probe, in ad-dition to a fast bypass line back to the reactor. [2,3]

One of the fundamental acceptance criteria for online benchtop MNR spectroscopy is a robust data treatment and evaluation strategy with the potential for automation. The NMR spectra were treated by an automated baseline and phase correction using the minimum entropy method. The evaluation strategies comprised (i) direct integration, (ii) automated line fitting, (iii) indirect hard modeling, and (iv) partial least squares regression (see Fig. 1). To assess the potential of these evaluation strategies for benchtop NMR, prediction results were compared with the line fitting data derived from the quantitative HR-NMR spectroscopy. Although superior results were obtained from both IHM and PLS-R for ^{1}H benchtop NMR, especially the latter demands for elaborate data pretreatment, whereas IHM models needed no previous alignment. [2,3]

Diverse data processing strategies are known in NMR. Four out of this variety of mathematical approaches were applied on data obtained during a sample esterification reaction by HR and benchtop NMR spectroscopy. Similar results in terms of concentrations were obtained which reflect the reaction progress consistently. All data analysis methods in general and especially chemometric models require a constant signal quality over the duration of the experiment, i.e., line shape, symmetry, and ideally Gaussian-Lorentzian shape.

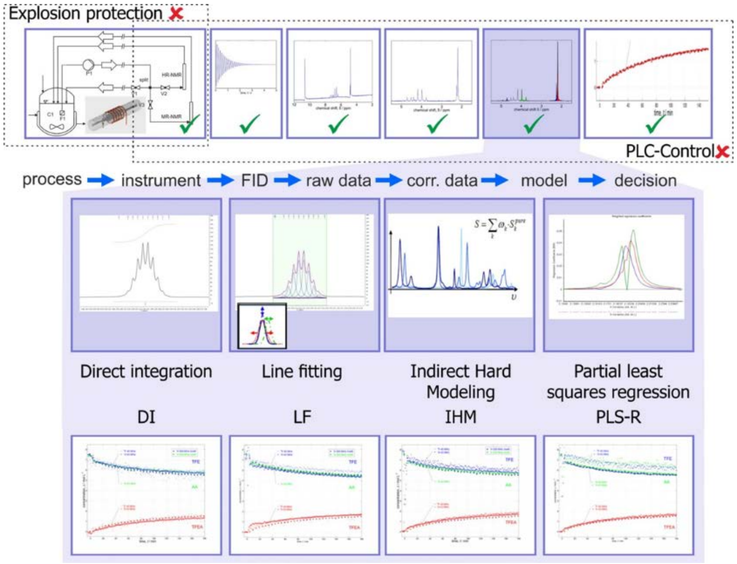

Figure 3: Work flow for data acquisition and data processing in MR-NMR spectroscopy. After receiving 19F and 1H raw data (as free induction decay, FID) the data is Fourier transformed and has to be corrected with regard to its base line and phase. Different mod

Finally it turns out that the instrumental stability and robustness against environmental influences are important prerequisites for a successful data analysis and even more important than the highest potential dispersion, especially in the case of PLS-R. Indirect Hard Modeling came out with reliable results for the observed reaction, both with low scattering results - especially for ^{19}F data - as well as accurate numbers. This is due to the redundant information which the model receives from the combination of all lines of an individual reactant, which is the major difference between IHM and direct integration or line fitting. This effect is even more obvious, when ^{1}H and ^{19}F data are combined into a single IHM models, which results in increased robustness. In comparison to the separate IHM models, data are distributed more homogeneously and at the same time, accuracy is improved in a combined ^{19}F-^{1}H model. All in all, IHM is superior to simple linefits of individual compounds. In the case of IHM, potential developments include the implementation of prior knowledge of the multiplet structure, flexible line shape func-tions and an improvement of the optimization algorithm. Similar arguments hold for the line fitting approach. Line shifts pose problems and are addressed by suitable line alignments algorithms. Overlap of lines poses problems especially to DI and to a smaller extent on line fitting, in which cases IHM and PLS-R can successfully be applied. A unified data structure, not only for the raw data but also for switching between modular data preparation and data analysis tools is preferable for future developments since it smoothes the way for automated applications.

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Analysis of Counterfeit Drugs and Dietary Supplements with Low-Field Benchtop NMR Spectroscopy

Guilhem Pages[1], **Anna Gerdova**[2], David Williamson[2], Veronique Gilard[1], Robert Martino[1] and Myriam Malet-Martino[1]

1. Groupe de RMN Biomedicale, Université Paul Sabatier, Toulouse, France
2. Oxford Instruments Industrial Analysis, Tubney Woods, Abingdon, Oxon, UK

Counterfeit drugs may contain ingredients that are not stated on the label or have inappropriate quantities, or none, of active ingredients. Dietary supplements are products between medicines and foods and have to be free of pharmaceautical components. Both of these groups impose risk to the consumer's health as well as pharmaceutical industries. A low-cost, rapid and effictive way to distinguish counterfeit drugs and dietary supplements from the genuine ones is required.

In this work we evaluated potential of a bench-top cryogen-free 60MHz NMR spectrometer to study counterfeit antimalarial and erectile drugs as well as sexual enhancement and weight loss dietary supplements. We demonstrate that the adulterants can readily be detected and in most of the cases identified in both drugs and dietary supplements. Some of the analyzed antimalarial drugs showed no presence of active ingredient.

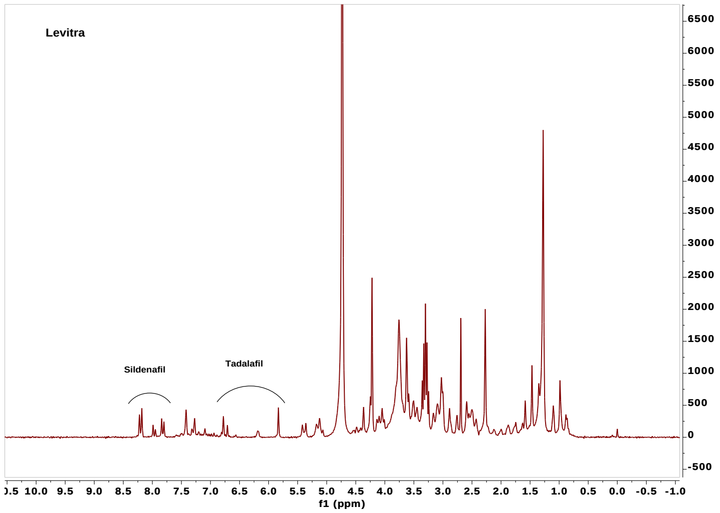

Figure 4: 60MHz NMR spectrum of erectile drug Levitra containing adulterants sildenafil and tadalafil.

We also demonstrate that the quantification by the internal standard method can be done on the low-field NMR spectrometer and leads to results similar to those obtained with high field 500MHz NMR [1].

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Quantification of Composite β-Lactam/β-Lactamase Inhibitor Antibiotics with 60 MHz Benchtop NMR

Susanne D. Riegel

Nanalysis Corp., Calgary, AB, Canada

Over time, the efficacy of traditional β-lactam antibiotics (e.g., the penam and cephem families), has decreased.[1] Bacterial resistance is rooted in the production of β-lactamase, an enzyme that effectively destroys the active four-membered β-lactam ring. Pairing these antibiotics with a β-lactamase inhibitor (e.g., tazobactam, clavulanic acid) has been shown to increase antimicrobial activity as much as 32-fold.[2]

The components of these so-called fusion drugs are prepared, isolated and certified individually. Once the purity of the individual component drugs has been authenticated, the composite drug can be formulated at a specific ratio. NMR spectroscopy is a non-destructive, innately quantitative analytical technique having straightforward sample preparation and rapid thoroughput, and it is an ideal tool to authenticate this molar ratio. The recent development of permanent-magnet based benchtop NMR spectrometers provides an attractive tool to simplify this authentication process. Although low-field spectrometers have lower resolution and sensitivity than their high-field counterparts, 60 MHz benchtop NMR provides an excellent screening tool to increase regulation workflow without occupying precious time on a high-field spectrometer that might otherwise be used for more demanding tasks such as complex structural elucidation, dynamics and interaction studies, and higher precision impurity analyses.

Herein, we describe a piperacillin/tazobactam case study to exhibit the utility of 60 MHz benchtop NMR spectroscopy for structural confirmation and targeted quantitation of composite antibiotics. We compare 60 and 400 MHz data and discuss the relative merits of various forms of manual and automated data analysis.

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Wednesday, September 23rd
04:15 PM - 06:00 PM

Quo Vadis NMR - Emerging Methods & Exotica

Chair: Stanislav Sykora

Speakers:

Aldrik Velders
Wageningen University (NL)

Dimitrios Sakellariou
CEA-CNR, Paris (FR)

Maria Victoria Gomez
University of Castilla-La Mancha (ES)

Rebecca Steele
Stelar (IT)

To Tune or Not to Tune, That's the Question: A Revolution in NMR Sensors!

Aldrik Velders[1,2], Vittorio Saggiomo[2], Victoria Gomez[1], Raluca Fratila[3], and Stan Sykora[4]

1. University of Castilla-La Mancha, Spain
2. University of Wageningen, The Netherlands
3. University of Zaragoza, Spain, 4. Ebyte, Castano Primo, Italy

Commercially-available NMR probes are, in general, limited to the requests and needs of the main stream user and cost roughly in the order of 20 to 200 kE. Probe heads typically house a sample holder, air and temperature control accessories, RF circuitry and a few transceiver coils. Classically, the transceiver coils are tuned to the specific Larmor frequency of a nuclide of interest at a particular magnetic field strength; consequently, to measure a nuclide at different magnetic field strengths different NMR probes are required. An NMR department typically possesses a dozen of probes.

The costly aspects of NMR probes as well as the need to special (mass and/or volume-limited) options, has pushed the search for alternative approaches for NMR detection. Inspired by the pioneering work of Olson et al. on solenoidal microcoils [1] and Massin et al. on planar microcoils, we have investigated the concept of microcoils for their fascinatingly high mass sensitivity, required among other applications for lab-on-a-chip and other microfluidic applications.[3]

In this talk, examples will be given of the different home-built NMR probes that we have explored in the past years, with different NMR coil geometries and detection volumes in the nL-to-µL range. We typically use a microcoil of a few millimeters diameter, located on top of a microfluidic channel, thus defining the so-called “NMR-chip” that can detect down to picomole quantities of material. One fascinating aspect of the planar microcoils is their broad-band character that allows acquisition of homo- and heteronuclear 1D & 2D NMR data with a single non-tuned microcoil [4]. Such broad-band coils can form the heart of a universal NMR probe that can be used at any magnetic field strength. In a new microfluidic manufacturing protocol we have now turned to solenoidal coils as well, which offers yet another horizon for low-cost NMR probe designs. [5]

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What's Left to Spin? Spinning Samples, Coils and Magnets for High-Resolution NMR of Heterogeneous Samples

Dimitrios Sakellariou

CEA-CNR, Paris, France

Magic Angle Sample spinning is one of the cornerstones of solid-state NMR. Most of anisotropic interactions can be eliminated upon rapid sample rotation, leading to narrow high-resolution NMR spectra. There are however many situations where the sample cannot, or should not be spun rapidly, for example in living tissues, in porous media, in heterogeneous reactors and in energy materials. In these cases the equivalent option of Magic Angle Field Spinning seems very appealing. Many efforts have been made to produce a sufficiently strong and homogeneous magnetic field, rotating at the magic angle, without for the moment reaching the required homogeneity and stability for resolving ^{1}H resonances.

We have recently shown that pure permanent magnets can provide solenoidal fields having sub-ppm high spectral resolution. Furthermore, we have demonstrated high-resolution Magic Angle sample spinning at 0.9T, resolving the resonances from small mobile molecules in various heterogeneous samples such as seeds and rock cores. Some years ago we have introduced the idea of magic angle Coil spinning for improving sensitivity in mass limited samples. This methodology will be reviewed in the light of inductive coupling which can be used in yet another innovative approach based on spinning the magnetic field around a static sample.

Here we present the first wide-bore, preclinical pure permanent magnet for magic angle field spinning imaging and spectroscopy experiments on static heterogeneous samples. The structure of the magnet is inspired by a hybrid, interleaved design. The field strength is 1.0T and the vertical spinning frequency is limited for safety reasons to 6Hz. Additional instrumentation (shims, pulsed field gradients and radio frequency coils) are spinning together with the magnet.

Microcoils for Inline / Insitu Monitoring

M. Victoria Gomez[1], Antonio M. Rodriguez[1], Alberto Juan[1], Antonio de la Hoz[1], Raluca Fratila[2], Rosa Sánchez[1], Jose M. Mateo[1] and Aldrik Velders[1,3]

1. University of Castilla-La Mancha, Ciudad Real, Spain
2. University of Zaragoza, Zaragoza, Spain
3. University of Wageningen, Wageningen, Spain

The use of miniature NMR detectors, termed “microcoils”, is a way to overcome a “weakness” of this powerful and non-invasive analytical tool, i.e., its intrisically low sensitivity. This is due to the fact that the sensitivity of a given coil geometry is inversely proportional to coil diameter [1]. Therefore, the amplitude of the NMR signal is optimal when the sizes of the coil and sample match, resulting in detection volumes in the low µL- nL range [2].

We use a microcoil of a few milimeters diameter, located on top of a microfluidic reactor (microreactor), thus defining the so-called “NMR-chip” with a detection volume of 25 nL. These NMR-chips have enabled monitoring of supramolecular interactions at picomole level by 19F-NMR [3], monitoring of an in-line microwave-assisted chemical reaction (optimizing reaction conditions in a rapid manner) [4], as well as acquisition of multinuclear 1D and 2D NMR data with a single non-resonant, and non-tuned. microcoil [5].

We will illustrate the hyphenation of these NMR detectors to other energy sources for activation of chemical reaction, i.e. a continuous-flow microreactor platform or UV-VIS devices, for in-line and/or in-situ monitoring of all reactions, and report a new procedure to rapidly extract information from a chemical reaction. In comparison with conventional approaches, this method reduces by a factor of 12 the time necessary for the determination of kinetic parameters, such as reaction order, activation energy, reaction rate constant, and pre-exponential factor. The novel procedure is based on the capability of the NMR chip to analyse very small portions of the reaction volume before the reaction, at its onset, and during its steady state phase. The data can be then fitted to a reaction conversion model. We also present a new setup for NMR in-situ monitoring of chemical reactions, suitable in situations requiring the elimination of dead-volumes.

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The Power of Variable Field Relaxometry Applied to Small Molecules

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Fast Field Cycling (FFC) relaxometry is a particularly powerful variable field relaxometry technique and one of the most efficient means for investigating molecular dynamics of small molecules as well as more complex molecular systems. Indeed the time scales of intra- and inter-molecular motions affect nearly all aspects of molecular function and the properties of an entire system.

FFC NMR relaxometry measures the spin-lattice relaxation dispersion profile efficiently over nearly five decades in Larmor frequency from 10 kHz to 42 MHz and higher in a fully automated mode. The relaxation dispersion profile, $R1(\omega) = 1/T(\omega)$ versus the Larmor frequency ω, reports the power spectrum of the fluctuations that modulate couplings between magnetic moments by rotational and translational reorientation [1,2].

Indeed, relaxometry has been applied to study the dynamics of small molecules and their interactions with larger molecules or more complex systems as a function of both temperature and magnetic field strength, with the latter being done most efficiently using FFC instruments.

Here we will discuss the application and utility of FFC relaxometry to paramagnetic molecules in solution (including MRI contrast agents and selective relaxometric agents), studies of the role and dynamic behaviour of small molecules in pharmaceutical/cosmetic/chemical systems (hydration, polymorphism, gels, emulsions, micelles, catalysts etc.), studies of liquid crystal phases, studies of the dynamics of water in a variety of more complex systems (protein solutions, biological tissues, cell membranes, porous materials, soils, etc.), as well studies of other small molecules such as hydrocarbons in complex systems (rocks).

While relaxometry of pure compounds with extremely low molecular weight may appear somewhat trivial (no dispersion), the behaviour of the same molecules in complex systems is a quite different story.

Moreover, many larger molecules which are still considered small by today's standards (hydrocarbons, oils, fats and other fatty acid derivatives, soaps, tensioactives and most surfactants, oligomers, oligosaccharides, etc.) can be directly studied, both alone and in composite systems, by variable-field relaxometry methods.

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Poster Sessions

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2:45 PM - 4:30 PM

Tuesday, September 22nd
7:00 PM - 8:30 PM

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Exploring Nutritional Contribution to a Metabolic Disease in Horses by NMR-Based Metabonomics

Lucia Pappalardo[1], István Pelczer[2], Pablo A. Hoijemberg[2], and Sarah L. Ralston[3]

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A major aim of our long-term project is to characterize the metabonome of a metabolic disease, osteochondritis dissecans (OCD), in horses and to find correlations with nutritional intakes using NMR-based analysis of their blood. Over several years we have identified a number of metabolites and other components (specific amino acids, lipo-proteins, etc.), which differed consistently between OCD and control cohorts in various conditions. Based on these findings we have developed recommendations to alter the feed to compensate for these changes in hope that it could reduce the occurrence of OCD (Patent pending, 2015). A version of this feed formula was used for the first time in 2013 on a farm that had had a 25 to 30% incidence of OCD in its foal crop annually for over 6 years. The hypothesis was that the new feed would reduce the incidence of OCD and significantly alter not only the metabonome of all horses (the controls included), but that the specific metabonome of the OCD horses would also reflect the changes in the feed formula.

There was one cohort in the earlier set on the original feed (2011) that was directly comparable with the experimental cohort of 2014 on altered feed in terms of the conditions under which the samples were collected, age and environment. In September 2011 twenty matched pairs of yearling Standardbred horses were sampled at a training facility. Their blood (serum) samples were collected using standard procedures [1]. Each pair of horses had the same sire and similarly bred dams. They had been raised on the same farm and fed the same pelleted ration formulated for growing horses since weaning. The only difference between members of each pair was that one had had surgical correction of hock OCD lesions 2 to 7 months before the samples were collected, while the other had no radiographic evidence of lesions. In September 2014 serum samples were again obtained from yearlings born in January-April, 2013 on the same farm. They had been fed a new formulation of pelleted feed all of their lives. Ten OCD and 14 controls were sampled, all in the same facility as in 2011, under similar conditions. The same type of hay (Alfalfa) was fed in both years. Blood samples were handled as before.

In this poster we present the results of NMR-based metabonomic analysis comparing the spectra of 2011 and 2014 horses, both for the controls and those with OCD, and analyze the variations between controls and OCD horses. The analysis used carefully processed 1H-NMR data and multivariate statistics, as well as statistical total correlation spectroscopy (STOCSY). The results are preliminary, yet promising. The incidence of OCD in the overall population of 200+ foals born on the farm and fed the new ration was 15% down from a consistent 25 to 30% in the foal crops of previous years. However further cycles of applying the new feed formulation with refinements to the formula will be necessary for long-term, solid statistical conclusions with respect to its efficacy in reducing the incidence of this metabolic disorder.

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2

Discussing Structural Proposals by Theoretical and Experimental NMR

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The structure determination of natural products by NMR remains one of the biggest challenges in chemistry. Although NMR correlation data is relatively accessible, the interpretation can still be very hard. But, the use of NMR in this process is not only limited by the experimental part. Frequently, molecules are found that are very similar in their constitution, and actually could not have been distinguished by NMR. In these cases complimentary methods are needed, that might be chosen based on the structural proposals.

The identification of such cases is a challenge on its own, and can only be achieved using computer software to interpret the experimental (or better theoretical) data. Over the past years we have found several of these molecules in the literature that deserve more attention. Some of the most challenging will be shown, together with suggested complimentary methods, as far as possible.

Caffeine (1,3,7-trimethylxanthine, C8H10N4O2) is a well-known alkaloid, which was charac-terised originally by total synthesis. In total, only 8 of it's 14 heavy atoms are carbons, which dramatically reduces the number of possibly observable NMR correlations. In this special case actually, no COSY or 1,1-ADEQUATE correlations are observed. This turns this molecule into an special challenge for NMR.

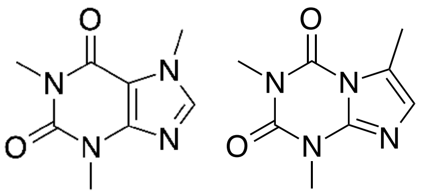

Figure 1: Caffeine and var-Caffeine, note the exchanged heavy atoms marked above.

The analysis of theoretical caffeine NMR correlation data reveals 2 constitutions that are compatible with the complete correlation dataset. Both differ only in the positions of 2 heavy atoms (that are switched). The inverse analysis with the theoretical NMR correlation data set from the alternative molecule also includes caffeine in the solution set, which is favoured by the molecular modelling filter.

Overall the theoretical analysis of caffeine and the proposed structural alternative alt-caffeine shows only small differences in the predicted proton and carbon NMR spectra. Additionally, the predicted 15N chemical shifts for both molecules also do not allow for a distinction. Finally, the theoretical correlation data analysis of the alt-caffeine also comes up with caffeine, rated better by the molecular modelling component of our structure generator. Hence a clear distinction between the two by NMR alone seems very unlikely.

To reach a final verdict, alt-caffeine was synthesised and experimental data acquired. As expected, the patterns of correlation peaks are very similar between both molecules, the only difference being the 1,1-ADEQUATE peak observed for alt-caffeine. As for the chemical shifts, the only major change that is expected according to DFT calculations is for the methyl group in the five membered ring.

When we inspect the experimental chemical shifts themselves, we see a 1.3 ppm difference for the 1H chemical shift for the methyl group. We also see two changes in the carbon spectra. One change is for the carbon that changed position into the five membered ring, a change that was predicted to be small and turned out to be >25 ppm. The other change is for the methyl group that in alt-caffeine is connected to a Carbon, and therefore has a chemical shift that is almost 25 ppm lower then in caffeine.

3

Accurate Determination of Binding Parameters by 1D ^{1}H Relaxation Dispersion Experiments

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Protein functions are closely linked with their motions. Particularly, recent developed relaxation dispersion experiments sensitively probed motions within the supra-τc window, which are critical for binding processes.[1] Although the study of ligand binding is critical for the discovery and development of new active drugs, the determination of binding kinetic constants is still cumbersome.

Here we present a 1D 1H R1 experiment on the ligand side, from which off-rates can be easily extracted.[2] This method has advantages over previously proposed 13C dispersion techniques.[3] First, 1H spectra does not require expensive, and unpractical, isotope-labeled samples; second, the acquisition time is significantly shorter; and third, the 1H nucleus is differently sensitive from 13C to the conformational state, e.g. rotameric states, reducing the weight of bound ligand slow dynamics (if any) that might obscure the binding event.

We show the applicability of the method with two different proteins: the soluble protein kinase A and Ca-stabilised microtubules. The ligands we chose in both cases show no dispersion when free in solution at 277 K. In both cases, upon the addition of protein, significant dispersion profiles were detected and importantly, distant protons within the ligands could be fit to the same kinetic parameters, which are consistent with the reported dissociation constants (assuming the association as diffusion controlled; kon ≅ 108 M-1 s-1).[4]

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4

NMR, Chemometric and GCxGC Studies of Crude Oils, Vacuum Residues and Vacuum Gas Oils

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NMR spectroscopy in combination with statistical methods was used to study vacuum residues and vacuum gas oils from 32 crude oils of different origin. Two chemometric metodes were applied. Firstly, principal component analysis on complete spectra was used to perform classification of samples and clear distinction between vacuum residues and vacuum light and heavy gas oils were obtained. To quantitatively predict the composition of asphaltenes, principal component regression models using areas of resonance signals spaned by 11 frequency bins of the ^{1}H NMR spectra were build.

A comprehensive two dimensional gas chromatography (GCxGC) was applied to identify different classes of hydrocarbons present in a selected crude oil. Pseudo two-dimensional DOSY NMR experiments were then used to assess the composition and structural properties of asphaltenes in a selected crude oil and its vacuum residue on the basis of their different hydrodynamic behavior and translational diffusion coefficients. DOSY spectra showed the presence of several asphaltene aggregates differing in size and interactions they formed. It has been shown that NMR spectroscopy coupled with statistical methods and GCxGC is a valuable tool for the investigation of crude oil and asphaltene samples.

5

NMR Spectroscopic Investigations of Intermediates in Dienamine Catalysis

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Organocatalysis is one of the three major areas of catalysis in chemistry. The huge number of transformations that can be achieved by the catalysis of small organic molecules is continuously increasing, but mechanistic studies on the underlying mechanism of most of the organocatalytic reactions are still very rare. During the last decade the catalytic functionalization of carbonyl compounds, like saturated and unsaturated aldehydes through enamine and dienamine catalysis was investigated synthetically by a large number of working groups around the world. [1,2] Based on these results it is possible to alpha- or gamma-functionalize various carbonyl compounds by using different amino catalysts, like the Jørgensen-Hayashi type organocatalysts and electrophiles without knowing the exact reaction mechanism.

Therefore the proposed catalytic cycle of the dienamine catalysis of alpha,beta unsaturated aldehydes with a Jørgensen-Hayashi type organocatalyst was investigated by nuclear magnetic resonance spectroscopy.[3,4] The investigation yielded a detailed description of the structure of the already known dienamine intermediates by selective 1H-1D-TOCSY, 1H-1D-NOESY, coupling constant analysis and other 2D-NMR experiments, which include an exact conformational characterization of the catalyst substructure and the diene system. These experimental findings were in a very good agreement with theoretical calculations at different levels of theory. In addition a new previously unknown intermediate could be detected and described. These carbinolamines were detected for the very first time. The detection of carbinolamines enables a new insight into the formation pathway of the dienamines and these species represent an additional intermediate in the proposed catalytic cycle.

Furthermore the formation of different 3E, and 3Z-dienamines were monitored for up to 20 hours with 1H-NMR-kinetic-experiments. The impact of solvent, substitution pattern of the alpha,beta-unsaturated aldehyde and the catalyst structure were screened and serveral structural features were investigated. The focus of the investigation was mainly on the 3E/3Z-ratio of the second double bond within the dienamine system. This 3E/3Z-ratio is suspected to be the key of the enantioselectivity of the electrophilic attack in the dienamin catalysis, in which ee-values up to 97% were achieved.[3,4,5]

In the context of all these experiments, surprisingly a strong influence of the used acidic co-catalysts was found that shows a large impact on the formation of the 3Z-dienamine but not on the 3E-dienamine of 2 pentenal and a Jørgensen-Hayashi type organocatalyst.

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6

NMR Spectroscopic Investigations in Brønsted Acid Catalysis

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In recent years, the use of chiral Brønsted acids as metal free catalysts has been reported for several enantioselective transformations like Mannich-type reactions [1], Strecker reactions [2] or transfer hydrogenation of imines [3,4]. Despite the importance of hydrogen-bonding interactions and π-interactions for the stabilization and structure of catalyst-substrate complexes, the understanding of these interactions is still limited. In order to improve the enantioselectivity of Brønsted acid catalyzed transformations, several theoretical studies have been reported concerning the mechanism of the transfer hydrogenation of imines [5,6]. But due to the lack of experimental accessibility, the investigation of these kind of reactions is still a challenging topic.

To investigate the Brønsted acid catalyzed transfer hydrogenation of imines, low temperature NMR spectroscopic studies have been performed. Therefore, in addition to traditional NMR solvents used in low temperature NMR spectroscopic investigations, such as deuterated dichloromethane and toluene, special deuterated mixtures of freon gases have been synthesized. By using these mixtures, which stay in liquid form even at extremely low temperatures, measurements at a temperature range of 110K – 170K are possible.

In order to improve the rather limited understanding of the nature of catalyst-substrate complexes, several chiral model systems were used to elucidate the structural differences of the complexes present in solution. Therefore, a variety of different Brønsted acid catalysts used in synthesis and several 15N-labeled imines were selected to characterize the hydrogen-bond in the existing complexes. The 15N and 1H chemical shifts and coupling constants were investigated in addition to the detection of 2D and 3D correlations caused by intermolecular 2hJH,P and 3hJN,P couplings.

Additionally, the structural differences of the complexes present in solution were characterized with the help of 2D 1H19F-HOESY in addition to 1D selective and standard 2D 1H,1H-NOESY spectra. The characterizations of these structures are supported by theoretical calculations and show a surprising flexibility for some catalyst-substrate complexes, even at low temperatures.

With the help of direct experimental data provided by low temperature NMR-spectroscopic investigations in combination with theoretical calculations, the understanding of Brønsted acid catalyzed transfer hydrogenations of imines can greatly improve.

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7

NMR Spectroscopic Investigation of Dispersive Interactions in Palladium Compexes

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Phosphoramidite ligands represent an important class of privileged ligands. Until recently chelating ligands were considered to increase enantioselectivity due to the necessity of the resulting rigidity [1]. However in the last years a different trend towards the use of monodentate ligands could be observed. Feringa achieved in the copper-catalyzed 1,4-addition of diethylzinc to α,β-unsaturated carbonyl compounds ee values up to 98 % using a copper complex with monodentate ligands [2]. These ligands most commonly contain a biaryl backbone based on a BINOL or biphenol scaffold and a very flexible chiral amine sidechain. They are considered to improve enantioselectivity since they can form supramolecular interactions due to their flexibility.

In our working group we developed a practical method to quantify these interactions, the supramolecular balance [3]. Palladium complexes in a stoichiometry of PdL2Cl2 are used. The free energy ΔG0 of the equilibria for two combinations (AB and A*B) of three ligands – two enantiomeric ligands A and A* and one enantiopure ligand B – can be compared. Therefore the homocomplexes PdA2Cl2 and PdA*2Cl2 are enantiomers to each other and have the same value of ΔG0 whereas ΔΔG0 equals zero. PdB2Cl2 appears in both equilibria and therefore ΔΔG0 also equals zero. Consequently only the heterocomplexes contribute to the value of ΔΔG0. In the case that the heterocomplexes possess identical stereoelectronic properties (e(A/B) = e(A*/B)) only the non-covalent supramolecular interactions (s(A/B) ≠ s(A*/B)) contribute to ΔΔG0. The equilibrium constants can be easily obtained by integration of the corresponding signals in the 31P spectra and thereof the value of ΔΔG0. The supramolecular balance has been applied only for one set of unsubstituted chiral phosphoramidite ligands. In our investigations we are using a combination of 1D and 2D spectra and especially NOESY spectra to assign the rather complex 1H spectra.

In recent studies the applicability of the balance was proven for more bulky ligands although there is a change in the structure of the homocomplexes to the heterocomplexes [4]. Investigation of the bulkier ligands revealed a higher unbalancement to the side of the heterocomplexes, but similar values for the dispersive interaction. With this method at hand investigating further substituted ligands – either at the biaryl scaffold or at the amine sidechain, especially asymmetric amines – may give insight on improvement of catalyst design for various synthesis.

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8

Using Off- and On-line SPE-NMR in Bioactive Metabolomics

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Plants are rich sources of novel bioactives. The biochemical elucidation of these is imperative to understand their bioactivity and mechanism-of-action. State-of-the-art spectrometric and spectroscopic technologies are used to assist in the separation, enrichment and structure elucidation of bioactives in complex mixtures. NMR has therefore a pivotal role in all biochemical analysis of extracts and biological samples. Knowing the endogenous abundance of bioactive molecules, namely secondary metabolites from plants, are in the sub-micromolar range, metabolite enrichment is unavoidable to conduct identification studies.

Here, we present how solid phase extraction (SPE) can be efficiently combined with NMR, as a sample preparation strategy to enrich certain classes of compounds in mixtures. Using an automated robotic station, sample preparation routines were implemented to reproducibly enrich semi-polar compounds in plant extracts, before NMR analysis. On these enriched samples, metabolomics studies were then conducted, unravelling lower-abundant compounds, previously masked by highly abundant central metabolism intermediates, such as sugars. In addition, the SPE-treated extracts could be used for further structure elucidation of bioactive components using online LC-SPE-flowNMR.

We believe the usage of SPE on NMR applications can be efficient for the analysis of bioactives in diverse biological samples (e.g. plant extracts, cellular extracts, biofluids) by enriching lower-abundant or specific classes of compounds, in an automated way. With the variety of solid materials available, many classes of compounds can be targeted, as well as solvent systems. These enriched samples can be directly analysed by NMR or further processed for comprehensive structure elucidation using NMR-hyphenated techniques, speeding up bioactive discovery studies.

9

Organic Fluorine Involved Intramolecular HBs in the Derivatives of Imides: An NMR and DFT based Theoretical Calculations

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The involvement of organic fluorine in the intramolecular Hydrogen bond of the type N−H•••F−C, is a rare occurrence of its kind. In the present study such type of hydrogen bonding interaction is detected in the derivatives of imides, in a low polarity solvent, by extensive utility of one and two dimensional multinuclear NMR techniques. The observation of 1hJFH, 2hJFN, and 2hJFF through-space couplings, where the spin polarization is transmitted through hydrogen bond, provides unambiguous evidence for the existence of intra-molecular HB. This is further established by the solvent induced perturbations and the variable temperature NMR experiments. Any possibility of self or cross dimerization is discarded by 1H DOSY NMR experiment. The Density Function Theory (DFT) based Quantum Theory of Atom in Molecule (QTAIM) and Non Covalent Interaction (NCI), calculations further substantiated the NMR experimental results.

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10

Still Shimming or Already Measuring? – Monitoring Fast Reactions by NMR

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In order to enable monitoring of rapidly occurring reactions Zangger et al. recently presented a simple scheme for 1D NMR experiments with continuous data acquisition, without interscan delays, using a spatially-selective and frequency-shifted excitation approach.[1] This scheme allows acquisition of proton spectra with temporal resolutions on the millisecond timescale. Such high temporal resolutions are desired in the case of reaction monitoring using stopped flow setups. In regular 1H NMR-spectra without spatial selection the line width increase for a given shim setting with changes in sample volume, susceptibility, convection and temperature or concentration gradients as magnetic field homogeneity is disturbed. Concerning reaction monitoring this is unfortunate as after injection of a reactant into an NMR sample, shimming prior to acquisition is necessary to obtain narrow signals. As even automatic shim routines may last up to minutes, fast reactions can hardly be monitored online without large hardware dead times in a single stopped flow experiment.

Besides high temporal resolution, a major benefit of the spatially-selective and frequency-shifted continuous NMR experiment arising from spatially-selective excitation is a reduced effect of magnetic field inhomogeneties on the obtained line shapes as pointed out by Freeman and demonstrated by Zangger.[1-2]

Here we present the utilization of this technique for observation of reactions in small molecule systems where chemical conversion and longitudinal relaxation occur on the same timescale. By means of the alkaline ethyl acetate hydrolysis, a stochiometric reaction, we show advantages of spatially-selective excitation on both temporal resolution and line shapes in stopped flow experiments. Results were compared with data obtained by non-selective small angle excitation experiments.

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11

Visualizing Unresolved Scalar Coupling by J-Upscaled NMR

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NMR spectroscopy is one of the most frequently used techniques for the structural characterization of small to medium sized organic and biomolecules. Because of its high natural abundance, widespread occurrence and high sensitivity, 1H nuclei are often used in this process. Resonance frequencies and scalar coupling patterns can provide important structural information. Often the quality of complex multiplet structures are significantly reduces by field inhomogenities. Automatizes shimming programmes and extensive manual shimming are in most cases not sufficient to reduces the signal line width to a desirable level. The aim of this work is to provide a method which allows the extraction of small homonuclear scalar coupling constants with high resolution.

We present an approach[2], which allows the real-time (single scan) up-scaling of homonuclear coupled multiplets by a user-defined factor, whereas the chemical shift values are left untouched. The up-scaling technique is based on a recently developed instant homonuclear decoupling method [1]. The basic concept of real-time J-upscaling utilizes the cutting of regular data acquisition into various “data chunks”. In between these chunks we manipulate chemical shift evolution whereas J-evolution stays untouched. In this J-up-scaling block, chemical shift evolution is refocused as well as field inhomogentities. Therefore the coupling to linewidth ratio is significantly increased and scalar coupling patterns, which are hidden within the linewidth of a regular spectrum can be revealed.

For example by increasing the coupling constant up by a factor of 7 in n-propanol it was possible to observe and measure a splitting difference of 0.7 Hz. This was not possible in the regular NMR spectrum where the line width is on the order of 1.8 Hz. An example of the resolution enhancement for scalar coupling determination is shown in Figure 1 in nicotinic acid.

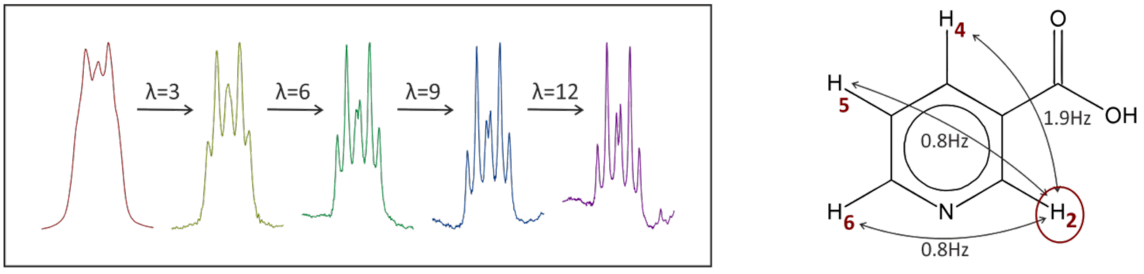

Figure 5: Starting from a distorted peak at 9.1 ppm (H-2 proton), J-upscaling reveals a triple doublet structure according to 4J and 5J homonuclear coupling constants. Note that the two central peaks are separated by only 0.3 Hz

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Determination The Equilibrium Constant& Rate Constant of Keto-Enol Tatumarizm of 5-methyl-3(2H)-Furanone by 45.5, & 400 MHz NMR Spectrometers.

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Inotilone is an effective medicinal molecule that is strongly defends the Arthritis and inflammation of the Human boon's joints (1). Obviously, it had been synthesized by myriad techniques. One of the most short, effective, and economical synthetic mood has an intermediate molecule is called 5-methyl-3(2H)-Furanone that chemically is unstable (2). The reason for that because, it behaves a keto-enol tatumarizme. By future industrial and medicinal importance of the Inotilone to be intended for diagnostic and therapeutic substance, the physical& chemical studies are highly necessary to guide researchers and manufacturers to synthesis this pharmaceutical molecule efficiently. This poster will represent the equilibrium constant (K) and kinetic rate constant (k) of 5-methyl-3(2H)-Furanone with its tautomer 5-methyl-Furane-3-ol by using Quantitative method of H1NMR of 400 MHz spectrometer NMR and 45.2 MHz benchtop NMR instrument at 15,25 ,35,45, and 50 Co. Clearly, the internal standard analytical method has been used in this study. In addition, the poster will exhibit the comparison of the method of two mentioned spectrometer. At the end, the poster will show the optimal instrumental techniques that are recommended to study the physical behaviors of these molecules.

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Membrane Interactions of Cyclic Lipodepsipeptides

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Cyclic lipodepsipeptides (CLPs) are a diverse group of secondary metabolites produced by various bacteria with important biological functions, but with yet unresolved molecular mechanisms. Our previous efforts have gone towards characterizing with NMR the conformation and self-assembling properties of a collection of CLPs known as the viscosin group. [1-4] CLPs increasingly attract attention because of their antifungal and antibiotic properties through membrane permeabilization. A full understanding of their membrane interactions is essential to elucidate the exact working mechanism of CLPs.

Comprehensive structural information in a membrane environment can be obtained by liquid-state NMR of model membrane systems, such as micelles and isotropic lipid bicelles. Like micelles, isotropic bicelles display favourable NMR relaxation properties, while possessing structural characteristics of lipid bilayers. [5] Specifically, the orientation and insertion depth of CLPs in a membrane environment is investigated using diffusion NMR and paramagnetic relaxation enhancement. The latter is achieved by introducing paramagnetic probes at various locations. By introducing a water-soluble paramagnetic complex to a bicelle sample, NMR signals from nuclei closer to the aqueous phase can be identified. Covalently linking paramagnetic radicals to the lipid molecules at different positions reveal the orientation and insertion depth of the peptides in the bilayer.

The NMR results are further supplemented with other experimental techniques, including fluorescence to study membrane permeability and fusion, circular dichroism and infrared spectroscopy. We have also performed all-atom molecular dynamics (MD) simulations of CLPs within lipid membranes, which can be confronted with the experimental NMR results

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NMR and Chemometric Analysis of a Library of Crude Heparin Samples

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2. U.S. Food & Drug Adiministration, Center for Drug Evaluation and Research (CDER), Division of Pharmaceutical Analysis (DPA), St Louis, MO, USA.

High resolution NMR is a well established technique for the analysis of complex mixtures [1]. It can be used as a tool for the identification and the quantification of the compounds within the mixture [2], but the spectra can also be directly used for the comparison of test samples against a reference [3]. In most cases the reference cannot be represented by a single sample, as a library of bona fide samples must be considered to account for the normal/natural variation of the mixture. Statistical tools are then necessary to compare the test samples to the library.

Crude heparin is a complex mixture extracted for pig intestinal mucosa, providing a key step in the production of API heparin. It is interesting because in many circumstances (with some notable exceptions) the first steps in the extraction of heparin from the animal are carried out in a myriad of small faciliteies and the crude is sold to the pharmaceutical industry by the weight on a activity basis, but without the warranty of a GMP process. Since at present the control of the supply chain of the crude heparin doesn't appear to be feasible and considering the risk associated with the contamination of the crude, in particular with non-porcine material, it is important to develop test on the crudes able to define appropriate specifications and capable to reveal possible contaminations [4].

Crude heparin is composed mostly of glycosaminoglycans (GAGs), such as heparin, heparan sulfate (HS), dermatan sulfate, chondroitin sulfate and hyaluronc acid, but it also contains variable quantities of other compounds, such as DNA/RNA fragments and proteins. The most abundant GAG contained in the crude, heparin/HS, is a copolymer of an uronic acid and an aminosugar whose structure already has a great degree of complexity, due for example to a multiplicity of possible substiution patterns along the chains [5].

In this study an analytical protocol for the analysis of crude heparins was developed coupling NMR spectroscopy and chemometric methods. 1H and 1H-13C heteronuclear correlation spectra (HSQC) of a library of 60 samples of crude heparins were registered, including samples that reached the US marked since 2008. HSQC spectra were used quantitatively to evaluate the GAG composition and the corresponding substitution pattern of crude heparins [6,7]. Test samples have been compared using both quantitative analysis and standard and multivariate statistical methods, similarly to what previoulsy described for pharmaceutical grade heparins [8,9]. The results obtained define a model of the crude heparin.

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15

Rapid Identification of Known Compounds in Mixtures

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Natural extracts from plants and microorganisms still constitute invaluable sources of biologically active metabolites for the development of drugs or cosmetics. The major challenge in the search for such metabolites arises from the extreme complexity of plant extracts or culture media which contain a wide diversity of molecules with distinct physical and chemical properties. At present, even if modern analytical and purification techniques are routinely available in most laboratories, a considerable work is still necessary to isolate and elucidate individual metabolite structures from crude natural extracts.

The aim of the present work is to develop a dereplication strategy for the identification of natural metabolites directly within mixtures [1]. Exploiting the polarity range of metabolites, the principle is to rapidly fractionate a multigram quantity of a crude extract by centrifugal partition extraction (CPE). The obtained fractions of simplified chemical composition are subsequently analyzed by 13C NMR. After alignment of 13C signals across spectra, hierarchical clustering analysis (HCA) is performed for the pattern recognition of elution profiles. As a result, strong correlations between 13C signals of a single structure across the fraction series are visualized in order to build chemical shift clusters. Each cluster is assigned to a molecular structure with the help of a locally built 13C NMR chemical shift database. The proof of principle of this strategy was achieved on a simple model mixture of commercially available plant secondary metabolites and then applied to a bark extract of the African tree Anogeissus leiocarpus Guill. & Perr. (Combretaceae).

Recent applications of this workflow include the study of extracts from the lichen Pseudevernia furfuracea and from the flowering plant Tephrosia purpurea. The fractionnation of the acidic depsides from Pseudevernia furfuracea was successfully carried out by the alternative pH-zone refining centrifugal partition chromatograpy (CPC) method [2]. The few unknown compounds from Tephrosia purpurea were identified by means of the structure elucidation software LSD [3, 4].

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16

NMR-Spectroscopic Investigation on Ternary Complexes in Brønsted Acid Catalysis

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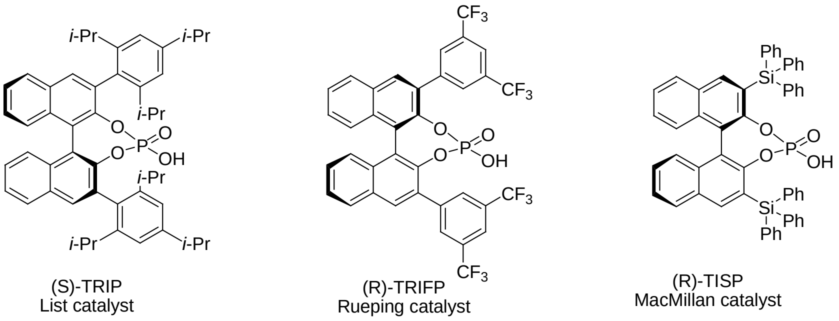
The development of small organic molecule hydrogen-bond donors has received tremendous attention over the past years with the constant challenge to reach the hight levels of control that Nature can achieve. Between these compounds, Brønsted acids have proven to be highly efficent and versatile catalysts for an ever expanding list of synthetic transformations [1]. A privileged class of asymmetric Brønsted acid catalysts is constituted by BINOL (1,1'-binaphtol) derived phosphoric acids (Figure 1), which usage started ten years ago thanks to the pionering works of Akijama [2]. Albeit, the increasing number of publications in this filed, elucidating the exact mechanism involved in chiral phosphoric acid catalyzed systems is not straightforward due to the large number of possible interactions that can occur between the catalyst and the substrates. Early works in this area were reported by Gschwind and Rueping employing NMR experiments to distinguist between ion-pairing and hydrogen bonding [3]. But the picture is still complex because of the role that the substituents on the subtrate, Brønsted acidity of the catalyst and the type of solvent play to determine the nature of the interactions.



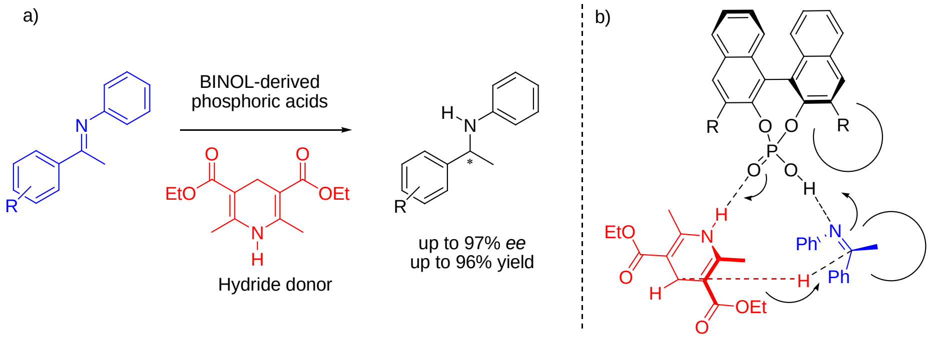
(S)-TRIP
List catalyst

(R)-TRIFP
Rueping catalyst

(R)-TISP
MacMillan catalyst

Figure 1: Examples of BINOL-derived phosphoric acids.

In this frame, our research group focused its attention to the NMR-spectroscopic elucidation of C=N bond asymmetric transfer hydrogenation reaction mechanism. The hydrogenation of ketoimines in the presence of Hantzsch esters as hydride source and BINOL-derived phosphoric acids as catalysts is, in fact, a powerful reaction developped by the parallel work of List and Rueping groups in 2005 (Scheme 1a) [4,5]. Analogous to Nature's NADH, Hantzsch esters have been shown to serve the role of small NADH analogues. Despite the importance of this reaction, which allows to synthesize chiral amine in a straightforward way, in high yield and high stereoselectivity, a compleate understanding of its mechanism is still lacking. Only proposed or calculated transition states are known in literature.



Scheme 1: a) Biomimetic hydrogenation of ketoimines; b) Postulated ternary complex by Goodman.

In 2008, Goodman and Himo proposed by DFT calculations a three points interactions model, in which a highly organised transition state is requested to reach high level of stereocontrol [6,7,8]. According to their model, there must exist at least three interactions between the catalyst and the substrates and one of them should be stabilising. From here, they postulated the existance of a ternary complex between the BINOL-derived catalyst, the imine and the Hantzsch ester (Scheme 1b).

This proposed dual activation mode raised the question, in our reserch group, of the existance in the ground state of this ternary complex, which structure we targeted to clarify by employing different NMR tecniques. 15N-labelled Hantzsch-1,4-dihydropyridine esters and ketoimines, and low temperatures were employed to elucidate the possible hydrogen bonding of both species to the catalyst, which can act both as hydrogen bond donor and acceptor at the same time. The partecipation of the Hantzsch ester to the ternary complex is clearly shown by the progressive low field shift of its NH proton chemical-shift, which strongly suggest the involvment of this group in a hydrogen-bond with the phospate moiety of the catalyst. On the other hand, proton transfer from the catalyst to the imine and strong hydrogen bonds are indicated by the low field signals detectables in the 1H-spectrum. Our effort to the experimental proof of the ternary complex will be presented in this poster.

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17

Improved Methodologies for NOE Distance Determination for Small Molecules

Bame, J.R.; Burns, M.; Essafi, S.; Bull, S.P.; Bain, E.; Dower, L.; Harvey, J.N.; Aggarwal, V.K.; and Butts, C.P.

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The quantitative use of the nuclear Overhauser effect for interproton distance determination has various applications for small molecules. Relative NOE intensities have been used to derive accurate NOE distances with errors as low as 3% by the Butts group.(1) These NOE-derived distances were used in the analysis of conformationally biased alkane products of a newly developed iterative homologation of boronic esters.(2) NOE distance analysis and 1H-1H and 1H-13C scalar coupling measurements profiled the conformation of the n-10 carbon chain and the all-syn alcohol 1 and the syn-anti MoM ether 2 were determined to take on helical and linear configurations respectively, as predicted by computation.

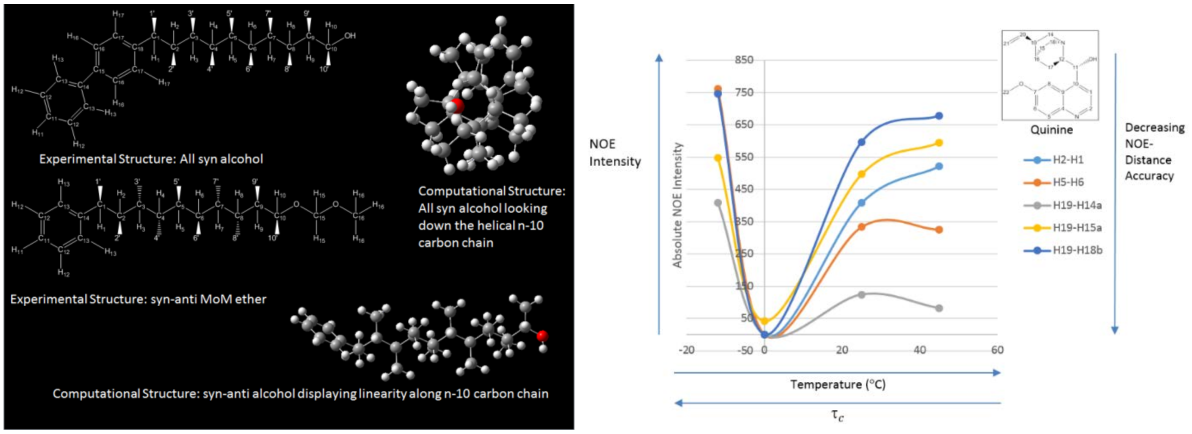

Figure 1: (Left) All syn alcohol and syn-anti MoM ether experimental and computational structures. (Right) Effects of Temperature on Rigid NOE Correlations in Quinine.

Recent work aims to improve accuracy of NOE-derived distances via perturbation of τ_c values through temperature. Decreasing temperature, increases τ_c which in turn affects overall NOE intensity(3) and also the accuracy of subsequently derived-NOE distances. This temperature-dependence has been tested on a selection of small molecules (quinine, camphor, erythromycin and clindamycin) of varying size and rigidity. This study aims to determine whether temperature-dependence of τ_c provides a tool to optimise the accuracy NOE-derived distances.

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18

Extended Acquisition Time (EXACT) NMR

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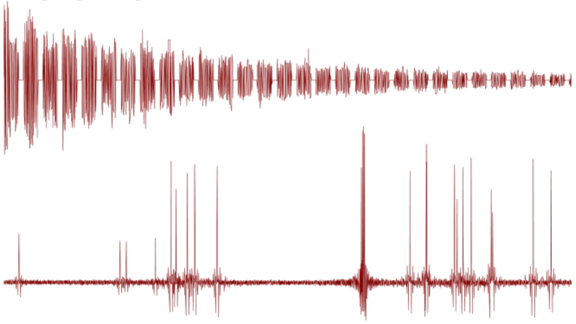
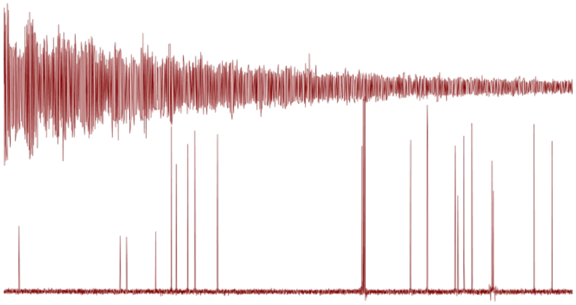
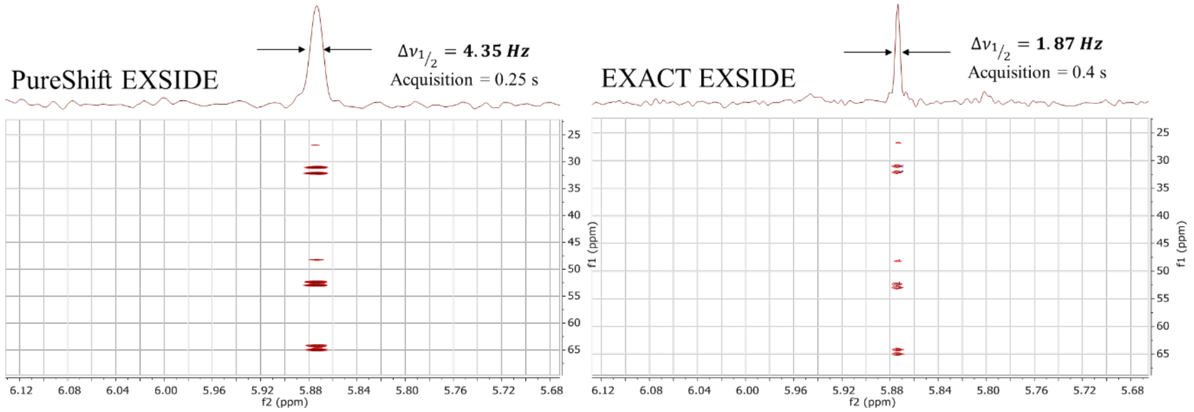
The overall duration of NMR acquisition time (AQ) is a function of the dwell time, DW (defined by the Nyquist condition) and the total number of specified data points, TD (AQ = DW*TD), which in turn defines the frequency resolution of the spectrum after Fourier transformation. The duration of acquisition time is, however, limited by spin relaxation and extensive scalar coupling, further reducing spectral resolution and sensitivity of 1H NMR spectra. To improve NMR spectral resolution and sensitivity, multiplets are collapsed into singlets by homonuclear decoupling either by concatenation of short ‘data chunks’, duce a new method EXACT (EXtended ACquisition Time) which not only retains the full length of the acquisition time but offers the flexibility of extending this period as well. The key here is to keep the active spins unperturbed during the J-refocusing period but achieving decoupling by pulsing on the passive spins. Data points (with zero intensity) are acquired during the J-refocusing period and these points are subsequently reconstructed with the Mestrenova Iterative Soft Thresholding (MIST) algorithm to give an FID which reflects the ‘true’ T2* relaxation, maximising resolution and sensitivity. Substantial improvements in linewidths can be made – with less than 2 Hz linewidths achievable in both 1D and 2D EXACT experiments.

^{13}C NMR FID of strychnine with missing point at 75% sampling and spectra after Fourier Transform



Reconstructed ^{13}C NMR FID of strychnine and spectra after Fourier Transform





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19

Beyond Karplus: Using a Quantitative Method to Determine Stereochemistry from Geminal Coupling Constants

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University of Bristol

An equation designed to predict geminal carbon-proton coupling constants (2JCH) in organic molecules is being empirically derived using a large selection of 2JCH calculations (at the hybrid DFT MPW1PW91/6-311G(d,p) level). The equation takes the form of a Karplus-like curve and is designed to take into account any substituents that affect the 1H-C-13C coupling pathway, most importantly substituents on the α carbon (13C), as these have been shown to produce a strong effect on 2JCH, with the effect dependant on the dihedral angle (φ) between the substituent and the coupled proton. Using a set of ethane models, with various electronegative substituents (C,N,O,F,P,S,Cl,Br) in various substitution patterns and with φ being varied in steps of 30° between 0° and 360° a database of 2JCH values was generated and used to create a quantitative prediction tool. The equation will be tested against experimental results from a preliminary library of organic molecules to confirm its validity, with correction factors introduced to deal with any issues that are found. This tool will enable chemists to exploit 2JCH values in much the same way as 3JHH and Karplus are used currently, however it should be noted that there are many more 2JCH values in most organic molecules than 3JHH So we believe 2JCH could become an important tool in aiding the structural elucidation of molecules via NMR.

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Increasing the Quantitative Bandwidth of NMR

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19F NMR is widely used by chemists in the analysis of drugs and their impurities. Typical drugs have only one or two fluorine atoms, but many protons. Thus in comparison to 1H NMR, 19F NMR offers spectra of significantly reduced complexity, simplifying interpretation. Quantitative analysis using 19F, and other nuclei such as 13C that have wide chemical shift ranges, requires constant-phase broadband excitation over the full spectral width. This can be problematic, since due to the limited radiofrequency power available for pulsed excitation, resonance offset effects distort both signal intensities and signal phases. Because only a small spectral width can be excited uniformly using conventional excitation (e.g. a hard 90° pulse), current practice is to make separate measurements for different regions of the fluorine spectrum, using a quantitation standard with an appropriate chemical shift in each case. This is cumbersome and it can be difficult to find appropriate standards. It would be greatly preferable to find a way to achieve quantitative excitation across the full chemical shift range.

To compensate for resonance offset effects, composite and/or shaped pulses are commonly used. However, even the best of these methods fall far short of the bandwidths required[1], so swept-frequency “chirp” pulses are needed. In principle, the combination of a 90° and a 180° chirp pulse of appropriate relative amplitude can be used to excite very wide bandwidths[2,3]. Unfortunately, only part of the range excited is usable, because the refocused signal phase still varies in a nonlinear fashion with frequency. A further, hidden, problem is that the signal phase is extremely sensitive to B1 amplitude, so that B1 inhomogeneity causes large (> 30%) losses in signal, even with modern probes.

A new broadband sequence, CHORUS (CHirped, ORdered pulses for Ultra-broadband Spectroscopy), has been developed to compensate for this B1¬ sensitivity and to correct the phase errors. CHORUS adds a second 180° chirp pulse, to deliver very uniform, constant-phase excitation over bandwidths of hundreds of kHz, with no undue B1 sensitivity and hence no loss in sensitivity. The accuracies of signal amplitudes and absolute integrals, and their repeatability and robustness, are very satisfactory for quantification purposes; in experimental tests over bandwidths of 100 kHz, drug quantitative analyses showed accuracy and reproducibility better than 1%.

Potentially, CHORUS can be a real asset to a range of users, offering much more efficient quantification than simple 90° excitation and allowing a spectrum to be analysed as a whole, with a single standard. For a given RF amplitude, the frequency range over which CHORUS can achieve 98% excitation is about 16 times greater than that for a hard 90° pulse.

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21

Investigation of a Threefold Photochromic System with In-situ Irradiation and Online NMR

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Photochromic compounds like azobenzenes are widely used for the synthesis of stimuli responsive materials which are capable of changing their physical properties reversibly upon irradiation with light. Using light sensitive materials offers high spatial and temporal control of the stimulus required for the induced change in the properties. Typically UV/Vis measurements are used to examine absorption wavelengths of photoisomers in photochromic systems. NMR spectroscopy as a complementary method can give information on the structure and the distribution of different species of the photochromic system.

Here we show the capabilities of online NMR with in situ irradiation for the investigation of the photochromic properties of a compound containing three azobenzene moieties. Irradiation of the sample was applied in situ by using high power LEDs and a waveguide.[1] Four different photoisomeres (ttt (all-trans), ttc, tcc and ccc (all-cis)) where identified. The evolution of the distribution upon irradiation of the stable ttt-isomer as well as the distribution of all photoisomers in a photo stationary state where examined by using 1H-NMR spectroscopy. In addition thermal and light-induced relaxation were observed and could directly be monitored. The composition can be tuned by irradiation with light of the corresponding wavelength. By using fast ASAP-HSQC[2] and real time BIRD decoupled HSQC[3] experiments 13C chemical shifts for all different photoisomers can be obtained.

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Pure Shift NMR, from BIRD to Chirps

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The emergence and development of practical pure shift methods in the last two decades has set a new standard for resolution in NMR, especially 1H NMR. Pure shift methods produce highly resolved NMR spectra by suppressing the multiplet structure caused by scalar coupling, leading to a chemical shift-only spectrum. This is achieved by use of a J-refocusing pulse sequence element, which can isolate a subset of active spins from their coupling partners. Two common approaches are the early BIlinear Rotation Decoupling (BIRD) method [1], which relies on dilute heteronuclei for selecting the active spins, and the elegant method of Zangger and Sterk [2], which combines spatial and frequency selection of spins. The latest pure shift pulse sequence element, Pure Shift Yielded by Chirp Excitation (PSYCHE), relies on statistical separation of spin populations by the use of low flip angle swept-frequency chirp pulses. It offers a significant improvement in performance over existing methods, as demonstrated for both 1D [3] and 2D [4] experiments. Methods using PSYCHE are rapidly being adopted as routine tools, in part due to their sensitivity, generality and convenience of setup. In this presentation, the theoretical and practical aspects of pure shift methods in general and PSYCHE in particular will be discussed, together with some recent developments.

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23

Unravelling the Structures in Complex Mixtures: Isotope-filtered nD NMR Spectroscopy

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One of the major challenges confronting NMR spectroscopy today is the elucidation of structures contained within mixtures. While a mixture of a tens of molecules can be resolved by standard nD NMR methods, these methods fail when the number of individual molecules reaches the hundreds. Furthermore, if these molecules are similar in terms of chromatographic properties, hyphenated NMR techniques cannot afford the solution. For inseparable mixtures it becomes mandatory to implement some form of 'spectroscopic separation'. This can be achieved by a combination of chemical tagging and specifically designed NMR experiments [1,2].

Isotopic tagging of mixtures has been employed in the past, though only for inspection of the signal of the tags. If instead, we use the tags to report on the chemical shifts and coupling constants of the parent molecules, a new paradigm is born. Towards this end we present here isotope-filtered nD NMR spectroscopy as a methodology to unravel the structures of small molecules contained within complex mixtures.

We illustrate this approach by 13C-methylation of hydroxyl and carboxyl groups in combination with a suite of purposely-designed nD NMR experiments. The incorporated tags act as molecular spies to probe the surrounding chemical environment, utilising the polarisation transfer pathways shown in Fig. 1.

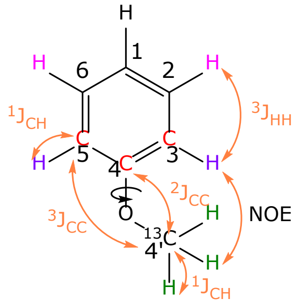

Fig.1. Couplings that can transfer the polarisation between the tag and the parent molecule in a 13C-methylated phenol. These correlations utilized in nD NMR experiments provide multiple correlated chemical shifts and coupling constants. The obtained data are used to piece together the structures of aromatic moieties. In these experiments, the signals from unlabelled molecules are eliminated, bestowing much needed simplification on NMR spectra of complex mixtures.

These methods were designed for the most complex mixture on this Earth - Humic Substances (HS). Defined as the material created by the microbial degradation of plant and animal matter, they represent the main organic component of soil (and natural waters). The importance of these substances in the natural world is immeasurable. Not only are vital to the structural integrity of soil but have major roles in biogeochemical cycles, such as the carbon cycle, and govern the fate and mobility of nutrients and contaminants. Full comprehension of their roles, however, is hindered by a lack of the knowledge of their molecular make-up.

Studied as the operational fractions fulvic and humic acid, based on solubility in different pH environments, these HS samples have thus far been studied by standard high resolution techniques, such as Fourier transform ion cyclotron resonance mass spectrometry as well as an array of NMR techniques. However, neither technique has afforded unambiguous structure identification of individual molecules. Since carboxyl and hydroxyl groups are the prevailing functionalities of HS compounds, they are ideally suited to be studied by our approach. With this in mind, we performed a suite of X-filtered nD NMR experiments, including a 4D HCCH3 experiment (Fig 2), to study the aromatic component of a 13C methylated fulvic acid isolated from a Scottish peaty soil.

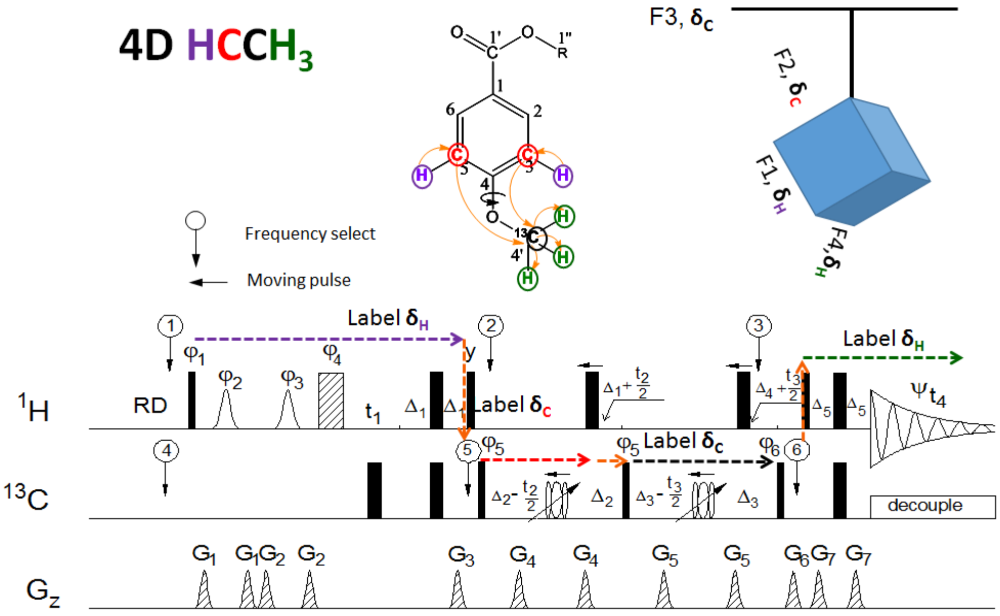

Fig. 2. Pulse sequence of the 4D HCCH3 experiment. The polarization transfer pathway is labelled by colour coded dashed lines corresponding to the labelling of nuclei on the structure shown in the inset [2].

A partial 4D HCCH3 spectrum of 13C methylated FA shown in Fig. 3 illustrates how isotope filtration combined with the dispersion of signals in a 4D space leads to comprehensive structural information. Additional 3D 13CH3O-filtered experiments were designed to complement the 4D experiment:3D INEPT-INADEQUATE-HSQC [1]/3D CqCH3 and 3D CH3-NOESY/3D CH3-NOESY-TOCSY. These NMR experiments utilize the various couplings shown on Fig. 1. The NOESY and TOCSY based experiments also provide 3JHH or 4JHH couplings, hence yielding additional valuable structural information.

Using our methodology we were able, for the first time, to characterise the major phenolic moieties in a HS sample. From the 30 different structure/structural moieties identified, a new understanding of their source and degradation mechanisms within this peaty landscape emerged.

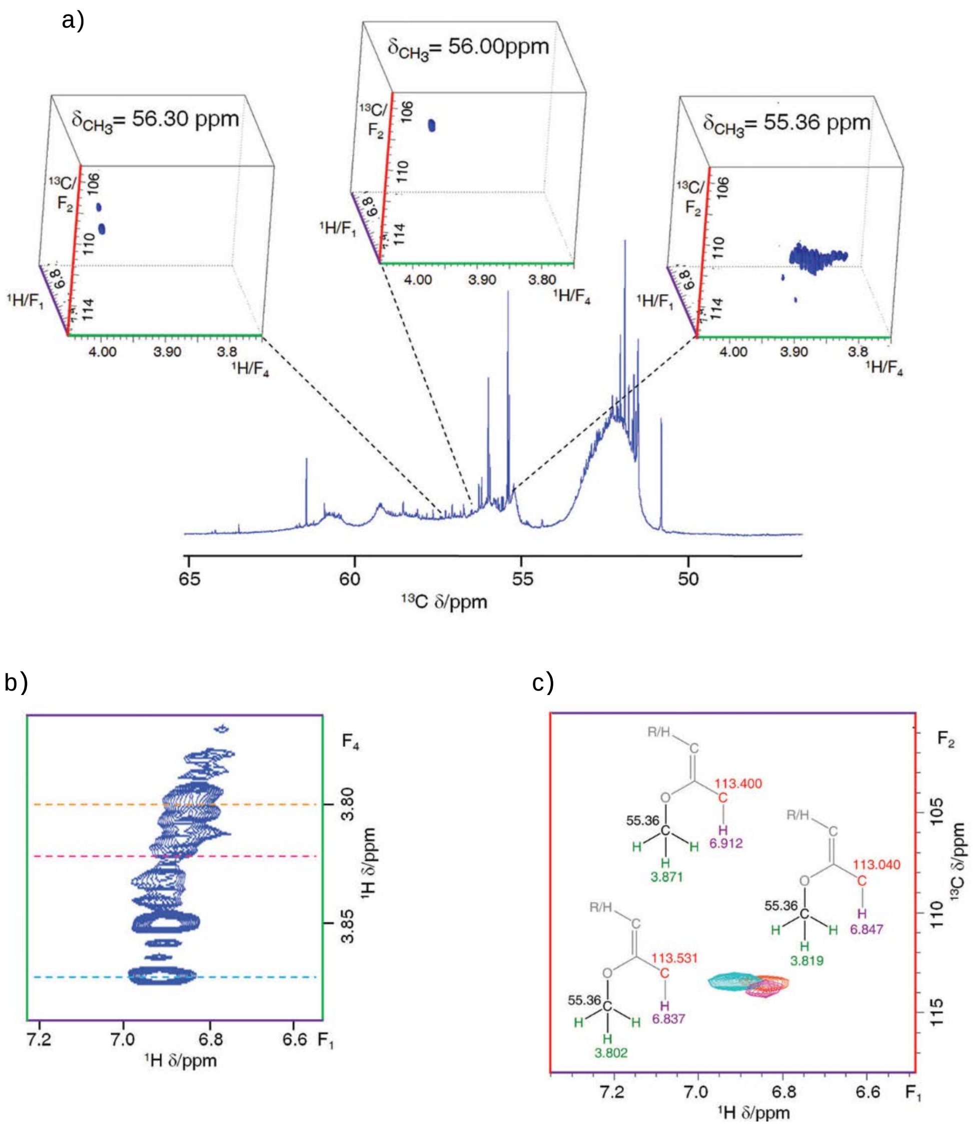

Fig. 3. a) Exemplar 3D cuboids extracted from a 800 MHz 4D HCCH3 spectrum of 13C-methylated FA at 13C(H3) chemical shifts indicated by the dashed lines; b) An F1F4 (Har(C)H3) projection of a cuboid extracted at 55.36 ppm; c) An overlay of three 2D F1F2 (HarCar) planes extracted from this cuboid at proton methoxy chemical shifts indicated by the coloured dashed lines in (b). Insets show the identified structural fragments together with their chemical shifts [2].

Our approach is not restricted to the investigation of environmental matrices. It can be applied to an array of mixtures produced by petrochemical, pharmaceutical or food/beverage industries. Tagging strategies other than methylation can introduce an arsenal of spies on the chosen parent molecules. We are currently exploring several different approaches based on the principles outlined here.

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Desktop NMR Spectroscopy for Quality Control and Reaction Monitoring

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Chemical reaction kinetics can be followed in real time by NMR spectroscopy when passing the reaction mixture through the magnet and acquiring spectra in stopped flow or continuous flow mode. Due to shorter feed lines, desktop NMR spectrometers [1] provide better time resolution than high-field NMR spectrometers. Moreover, hazardous reactions can be monitored by placing the instrument under the fume hood [2]. We report kinetic studies of variety of chemical reactions with time-resolved single-shot 1H NMR spectroscopy including first studies on the kinetic isotope effect, which aims at understanding the chemically selective signature of deuterium depletion in difference crude oils [3-4] as this effect reports on the evolution of the earth over a time range of up to 150 million years. The variation in reaction rate with changing isotope ratio at the reaction site provides information about the primary isotope effect of the reaction, which also depends on temperature and pressure. Compact 1H and 19F NMR spectroscopy is implemented to study the temperature dependence of the reaction rate constant for the hydrolysis of acetic anhydride and ethyl trifluoroacetate in H2O, D2O and D2O/H2O mixtures. The first order rate constants have been determined in the readily available temperature range from 278 K to 326 K in an effort to understand the effect of temperature on the kinetic isotope effect and the activation energy. The observed ratio kD2O/kH2O he increase in chemical shift also depends upon the oil-biodiesel ratio, which provides an environment, which changes during the course of the reaction.

Finally, the capability of 1H and 13C low-field NMR spectroscopy for quality control of raw rubber was tested [1]. Depending on the manufacturing process and the manufacturing batch, variations between samples can be identified in the spectra of rubber solutions, which can translate into variations of material properties of final products. Styrene-butadiene rubber (SBR) samples produced by solution and emulsion polymerization were compared as well as samples from different polymer lots produced by the same polymerization technique. Differences found in 1H NMR spectra of rubber produced by different techniques could be detailed in 13C spectra and assigned to different copolymer sequence statistics, which result from different reaction conditions that give rise to different copolymerization parameters.

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Ultra High-Resolution HSQC: Application to the Efficient and Accurate Measurement of Heteronuclear Coupling Constants

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Universitat Autònoma de Barcelona

Digital resolution and signal resolution are two important concepts in multidimensional NMR spectroscopy. A key parameter defining the total acquisition time of a 2D NMR experiment is the number of variable t1 evolution times required to achieve a satisfactory digital resolution along its indirect F1 dimension. In this study, the success in implementing spectral aliasing along the indirect F1 dimension of HSQC experiments is demonstrated by the easy and fast measurement of heteronuclear coupling constants from the indirect dimension of 2D HSQC spectra, without any significant increase of the experimental time. It is also shown that these gains can be further improved with the large spectral resolution achieved by the collapse of the J(HH) multiplet structures to singlets by broadband 1H homodecoupling along the F2 dimension.

The resulting 2D cross-peaks exhibit ultra simplified multiplet patterns from which the efficient measurement of J coupling values is performed in a straightforward manner. Experimental data are provided for the simultaneous determination of the magnitude and the sign of J(CX) and J(HX) coupling constants from a single cross-peak (X = 19F, 31P or 2H).

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Extending Long-Range Heteronuclear NMR Connectivities by HSQMBC-COSY and HSQMBC-TOCSY Experiments

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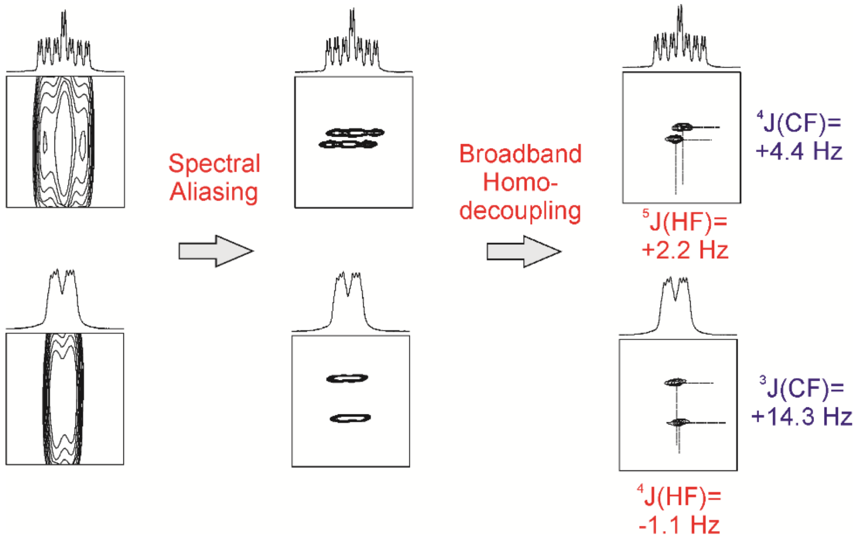
The detection of long-range heteronuclear correlations presenting J(CH) coupling values smaller than 1-2 Hz is a challenge in the structural analysis of small molecules and natural products. HSQMBC-COSY and HSQMBC-TOCSY pulse schemes are evaluated as complementary NMR methods to standard HMBC/HSQMBC experiments. Incorporation of an additional J(HH) transfer step in the basic HSQMBC pulse scheme can favor the sensitive observation of traditionally missing or very weak correlations and, in addition, facilitates the detection of a significant number of still longer-range connectivities to both protonated and non-protonated carbons under optimum sensitivity conditions. A comparative 1H-13C study is performed using strychnine as a model compound and several examples are also provided including 1H-15N applications.

A rapid acquisition strategy in terms of enhanced resolution per time unit for the simple and efficient determination of multiple coupling constants is proposed. The use of 13C spectral aliasing combined with pure shift NMR techniques allows accurate measurements of the magnitude and the sign from ultra-high resolved 2D cross-peaks.



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Ultrahigh Resolution ^1H-^1H Coupling Measurements in Crowded Spectra

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Homonuclear couplings are a double-edged sword. They deliver a wealth of structural information, but equally they are detrimental to spectral resolution, impeding their accurate measurement. One way to disentangle individual couplings from complex spectra is the SERF experiment, which delivers a 2D J-resolved spectrum containing only selected couplings [1]. A variant of this experiment, G-SERF, uses the Zangger-Sterk pulse sequence element to deliver simultaneously all the individual couplings to one selected resonance [2]. Other recent variants incorporate band-selective and Zangger-Sterk real-time pure shift acquisition [3,4]. However, all these methods can break down in crowded spectra, either because of signal overlap or because chemical shift differences between coupled spins are too small.

Here, we present the PSYCHEDELIC (Pure Shift Yielded by CHirp Excitation to DELiver Individual Couplings) experiment, derived from the PSYCHE pure shift method [5]. It delivers simultaneously all individual couplings to a selected proton, with minimal constraints on spectral overlap and chemical shift differences. It provides the usual pure shift resolution, high sensitivity, spectral purity and ease of experimental set-up of PSYCHE, making it of great value to measure homonuclear scalar couplings or RDCs in crowded spectra.

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Anti-Inflammatory Drugs by NMR in Ordered Media: Insights into Conformation and Stereochemistry

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Naproxen (2-(6-methoxy-2-naphthalen)propanoic acid), flurbiprofen (2-(2-fluoro-4-biphenyl)propanoic acid) and ibuprofen (2-(4-(2-methylpropyl)phenyl)propanoic acid) are three popular non-steroidal anti-inflammatory drugs belonging to the family of 2-arylpropionic acids, commonly known as profens. Profens are among the most important drugs in common use and are widely employed as therapeutic agents for the treatment of pain and inflammation. Their anti-inflammatory and antipyretic action as well as the gastrointestinal side-effects are based on the blockage of cyclooxygenase enzyme (COX) with following inhibition of downstream prostanoid species.[1] However, it has been reported that such drugs may bind some other proteins with different mechanisms and orientations and new targets are likely to be individuated in the future.

When a given flexible ligand binds to a receptor, and initiates then a biological effect, it has to adopt a “bioactive conformation” which is in some way complementary to its target protein.[2] The determination of the bioactive conformation of profens is hence an important challenge since it may help in gaining knowledge about the drug-protein complex, and thus be used as a template when designing novel anti-inflammatory agents with desired properties. However, this is often far from being a trivial task since in lots of cases the bioactive conformation does not correspond to the global energy minimum in the free state and lies instead at or very close to a local minimum on the potential energy surface.[2-3] In addition, the bioactive conformation depends on the receptor or receptor subtype the drug is bound to and on the specific medium.[4] Overall, this means that structures experimentally determined by single crystal X-ray crystallography or global minima found for an isolated molecule by in vacuo calculations do not always well represent the protein-bound conformation in shape and are then less useful in the context of drug design than generally assumed.[2,5]

A valid drug design approach for flexible ligands lies in the exploration of a wide conformational space highlighting all the possible minima that the molecule can assume. Indeed, when searching the bioactive conformation, it is recommended to consider a more extended and diverse set of conformations rather than a single conformer.[6] In this optic, NMR spectroscopy in partially ordered media shows many strong points. Contrary to techniques currently of choice for structure-based drug design, namely solid-state NMR spectroscopy and X-ray crystallography, this methodology allows (i) to probe molecular structure in solution, which is closer to the physiological environment of bioactive molecules, and (ii) to investigate the molecular internal motions and then the flexibility, leading to an equilibrium between global and local minima. In this contribution we show how the measurement of residual dipolar couplings (RDCs), extracted from NMR spectra in partially ordered media and interpreted via proper theoretical models, can give valuable insights into the structural and conformational features of profens.[7] Fig.1 reports the probability distribution together with the structure of the energetically-admissible conformers experimentally found for S-(+)-naproxen and R-(-)-flurbiprofen, dissolved in a weakly ordering liquid crystal phase composed of the synthetic homopolypeptide poly-γ-benzyl-L-glutamate (PBLG) and an organic co-solvent.

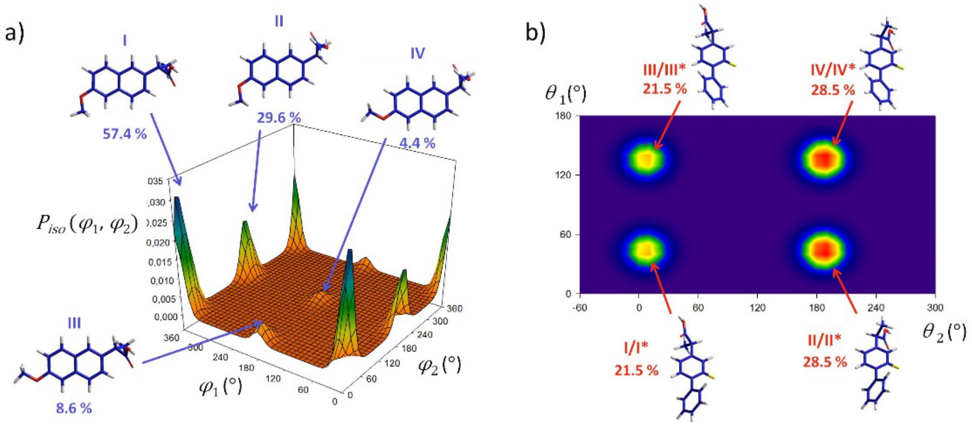

Figure 1. Experimental probability distribution Piso(φ1,φ2) and Piso(θ1,θ2) obtained for (a) S-naproxen and (b) R-flurbiprofen, both dissolved in PBLG/THF-d8, including structures of minimum energy conformers and their relative abundance.

The potential of PBLG ordered media have been largely demonstrated for various purposes, but probably the most exploited application is in the discrimination of S- and R-isomers and measurement of enantiomeric excesses.[8] These properties gains a major role in the study of pharmaceutically active molecules like profens, where enantiomeric discrimination and quantification are crucial goals. Indeed, profens are chiral molecules and individual enantiomers differ in their pharmacological activity. Despite the anti-inflammatory activity due to COX inhibition is largely stereospecific for the S-enantiomers, more recent works proved R-isomers can have different appealing activities, thanks to alternative binding modes to COX enzymes or inhibition of non-conventional targets.[9-11] Combining orientational order and chirality, PBLG phases give the opportunity to both discriminate enantiomers and investigate their order and conformational surface. In this contribution we report preliminary results obtained for racemic ibuprofen in PBLG/CDCl3. A series of 1D and 2D NMR experiments allowed us to discriminate between the enantiomers using order-sensitive NMR observables, namely 13C chemical shift and a considerable number of short- and long-range 1H-1H and 1H-13C RDCs (fig.2).

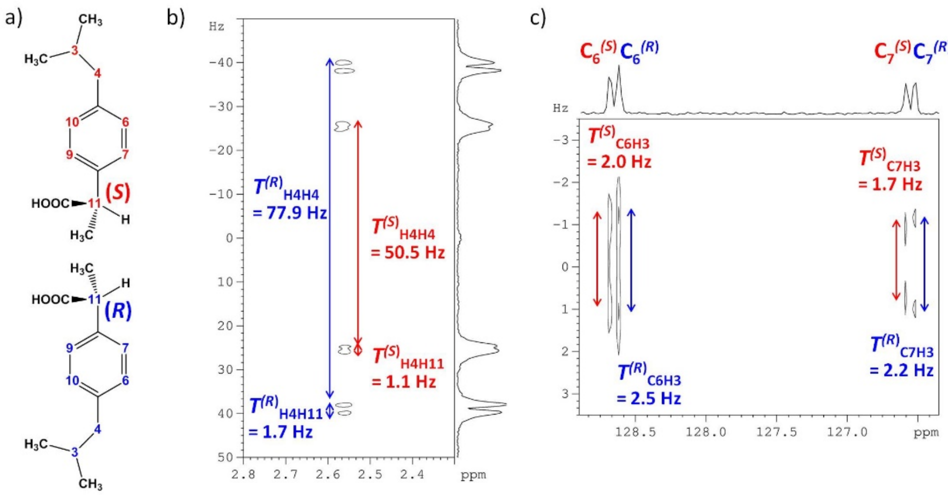

Figure 2. (a) Structure and labeling of R- and S-enantiomers of ibuprofen. Examples of 2D (b) 1H-1H SERF and (c) 13C-1H HETSERF spectra recorded on racemic ibuprofen in PBLG/CDCl3.

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Improving NMR Methods to Solve 3D Molecular Structure in Solution

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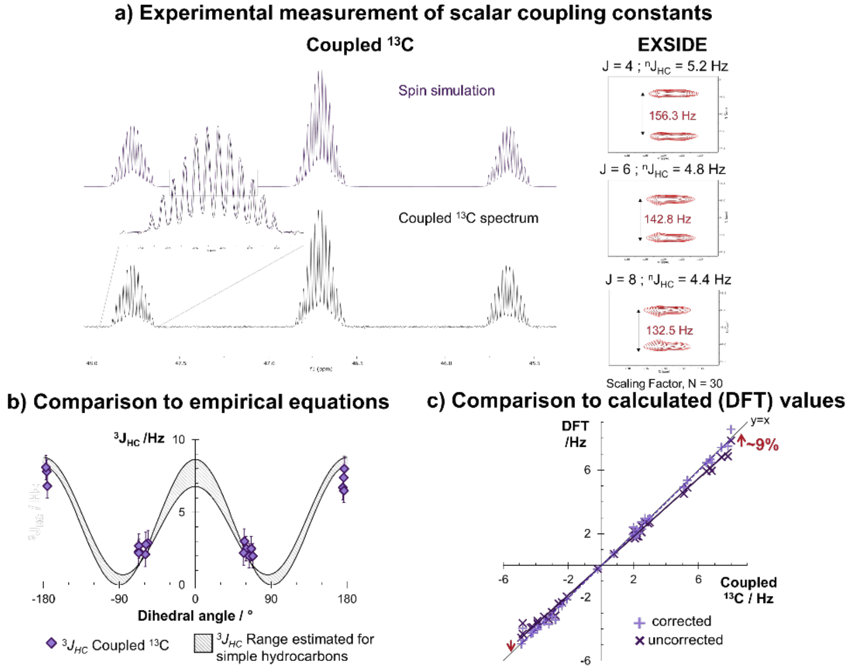
NMR spectroscopy has a wide application in the determination of three dimensional molecular structure and the use of 3-bond scalar couplings is common in solution-state NMR. Scalar coupling between nuclei separated by three bonds can be described empirically by homonuclear (1H-1H) [1] and heteronuclear (1H-13C) [2] Karplus equations relating the magnitude of the scalar coupling constant to the dihedral angle between the nuclei. Computational methods can also predict NMR properties such as chemical shift and scalar coupling constants for a given molecular structure [3].


a) Experimental measurement of scalar coupling constants

b) Comparison to empirical equations

c) Comparison to calculated (DFT) values

Accurate experimental nJHC (n≥1) values were measured from coupled 13C spectra. These values were compared to those measured using the EXSIDE technique in order to assess the accuracy. Data were measured for strychnine, streptomycin and methyl piperidines. The analysis presented here demonstrates that there is room for improvement in both experimental determination and computational prediction of nJHC values. Proposals to address both experimental and computational shortcomings are presented.

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Improving the Quantification of Low Molecular Weight Metabolites Interacting with Human Serum Albumin by ^{1}H-NMR Spectroscopy

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Some amino acids (AAs) in blood have been found to play an important role in the early stages of metabolic-related diseases such as type 2 diabetes and cardiovascular events [1,2]. High-throughput 1H-NMR of serum has been established as the main method for the profiling of these AAs and other low molecular weigth metabolites (LMWMs) in large population studies, as it requires only minimal sample handling and the quantitative property of some NMR methods (qNMR). However, some small molecules bind to macromolecules, especially to the most abundant protein human serum albumin (HSA), and their motional properties are severely affected. Consequently, their signals in 1H-NMR spectra are broadened and sometimes impossible to discern from background signals, making them "invisible" and therefore not measurable [3,4]. Moreover, the commontly used background reduction technique based on "T2-filtered" CPMG sequence could introduce further error in the quantification, as it suppresses part of the signal regardless of the sample-dependent relaxation properties.

Here we offer strategies to avoid such error and improve the profiling of LMWMs interacting with HSA. The first strategy is based on competitive binding. By adding a calibrated quantity of the exogenous molecule TSP (known to bind to HSA), most of the bound LMWMs are released from HSA improving their visibility in qNMR analysis. The second strategy is based on the use of a set of CPMG filters that allows correcting signals of LMWMs by their sample-dependent relaxation properties. Multivariate curve resolution (MCR) techniques are also employed in order to extract signals of LMWMs overlapping the ubiquitous background signals due to macromolecules.

Analysis in serum mimic (20 LMWMs + HSA) demonstrated that TSP allowed the recovery of most of the LMWMs bound to HSA. Further evaluation of the use of TSP in real serum samples showed the complete recovery of most of the compounds and a high recovery of some "NMR invisible" LMWMs (e.g. phenylalanine quantification was increased from 35% to 75% of the total in sample when TSP was added). The lower release compared with the serum mimic suggests further interactions with other macromolecules than HSA and the use of complementary binding competitors.

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NMR-Based metabolomics: Investigating Onset of Diabetes in Humans and Daily Rhythm in Drosophila Melanogaster

Navdeep Gogna[1], Murahari Krishna[2], Anup Mammen Oommen[2], Viveka Jagdish Singh[3], Vasu Sheeba[3], and **Kavita Dorai**[1]

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Diabetes has become an epidemic in the modern world and is an increasing cause of concern in developing countries. It is well known that obesity i.e. high body mass index (BMI) plays a key role in the evolution of insulin resistance and type-2 diabetes mellitus (T2DM). However, the exact mechanism underlying its contribution is still not fully understood. Our work focuses on an NMR-based metabolomic investigation of the serum profiles of diabetic, obese South Indian Asian subjects [1]. 1H 1D and 2D NMR experiments were performed to profile the altered metabolic patterns of obese diabetic subjects and multivariate statistical methods were used to identify metabolites that contributed significantly to the differences in the samples of four different subject groups: diabetic and non-diabetic with low and high BMIs. We were able to linke altered metabolites in the serum of T2DM obese subjects to key cellular processes which are dysregulated in diabetes. We also hypothesize a correlation between these significant metabolites and the associated metabolic pathways underlying the diabetic condition.

In a different direction, we performed an NMR-based investigation of the Drosophila melanogaster metabolome under the influence of daily cycles of light and temperature [2]. 1H 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. Multivariate statistical analysis was used to identify those metabolites whose concentrations oscillate diurnally. We compared metabolite levels at two time points twelve hours apart, one close to the end of the day and the other close to the end of the night, and identified metabolites that differed significantly in their relative concentrations. We were able to identify 13 such metabolites whose concentrations differed significantly between the two time points. Thus, we establish the validity of an NMR-based metabolomics approach in performing quantitative measurements of cycling metabolites in the fruitfly, which is rapidly becoming a very useful model system in the area of metabolomics.

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FlowNMR for In-Operando Investigation of Homogeneous Catalysts

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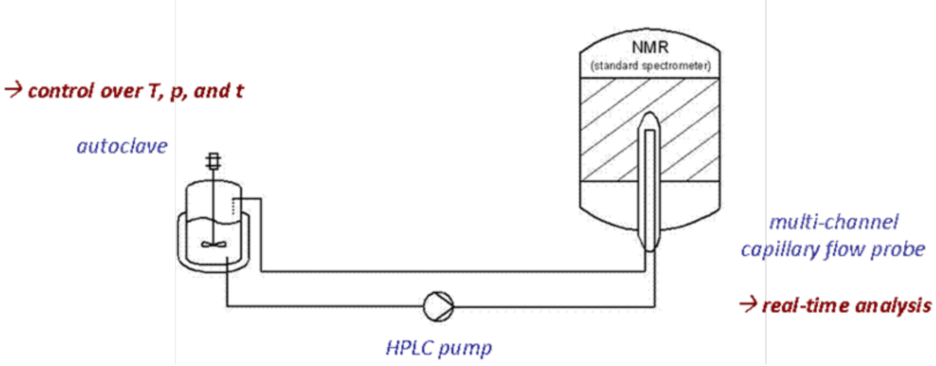
Molecular solution-phase catalysis is a key technology for addressing sustainability issues in the chemical industry. The development and optimization of homogeneous catalysts is, however, often hampered by limited insight into the kinetics of the reaction and the transformation of the catalyst, enforcing empirical optimization. Rational catalyst and reaction development is only possible through thorough understanding of catalyst activation and de-activation mechanisms, potential resting or dormant states, and the kinetics of the productive cycle (i.e. rate-limiting steps). While rather laborious techniques are available to investigate the above-mentioned aspects separately, there is no readily applicable technique that may be used universally in early stages of catalyst development.

This work aims to develop a technique for reaction kinetic analysis based on NMR spectroscopy as one of the most informative solution-phase analysis method available. The basic idea is to couple an autoclave to a capillary NMR probe with HPLC tubing, circulate the reaction mixture through the spectrometer, and continuously acquire spectra as the reaction progresses:



An important feature is that the setup can be run under inert conditions, under pressure of reaction gases, and at various temperatures relevant to transition-metal catalysis. When a multi-channel capillary probe is used with a high-resolution NMR spectrometer, the entire battery of multi-dimensional NMR techniques can be applied to kinetically relevant catalyst species that may only be present under turnover conditions.

Another important aspect is the accessibility of the autoclave during analysis, allowing full control over reaction conditions to be maintained. The external reaction vessel allows the sample to be well mixed and for reagents to be added without interrupting data collection, ensuring that short-lived intermediates can be analysed without delay – an important advantage over traditional analysis in sealed sample tubes where short-lived intermediates may go undetected. Due to the possibility of internal calibration and the structural information content of NMR, this method will prove superior to traditional reaction monitoring techniques.

The above setup has been assembled, characterised and successfully tested. We have demonstrated the principal feasibility and gathered promising results on an enantioselective ketone transfer-hydrogenation reaction catalyzed by chiral ruthenium complexes.

Building on this initial work, the experimental setup has been further developed to include the integration of UV-Vis spectroscopical measurements of the sample to assist with the detection and assignment of highly coloured catalytic intermediates detected during the reaction. The utilisiation of complementary techniques assists in the assignment of intermediates that cannot be assigned by any one technique alone.

In addition to standard NMR techniques, selective excitation of certain regions of the spectra has been applied to enable monitoring of catalytic intermediates present at very low concentrations, allowing the resting state of the catalyst to be identified and concentrations quantified over the course of the reaction.

Kinetic modelling of the data obtained will be performed in collaboration with Dr Jordi Bures of Imperial College, London to better understand the catalytic processes occurring during this reaction. Through collaboration with Bruker we also hope to apply our techniques using a new flow probe currently under development, [1] which would allow the use of a standard NMR probe and the monitoring of a much larger range of nuclei.

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Supramolecular Lyotropic Liquid Cristalline Phases as Alignment Media

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Benzene-1,3,5-tricarboxyamides (BTAs) are known to self-assemble into rod like and helical supramolecules.[1] These stiff aggregates act as mesogenes to form liquid crystalline phases. Thermotropic and lyotropic (in water) liquid crystalline behavior of BTAs have been reported.[2,3] Aggregates of achiral substituted BTAs form racemic mixtures of (M)- and (P)-helices. Introduction of enantiopure side chains leads to helices with only one handedness.[4]

Determining the conformation or relative configuration of small organic molecules is not always possible by classic NMR-spectroscopy via ^3J couplings or NOE data. If this is the case NMR-spectroscopy in weak orienting media gives access to residual dipolar couplings (RDCs) which offer complementary information. RDCs provide angular and distance information with respect to the external magnetic field. To achieve anisotropic conditions lyotropic liquid crystalline phases (LLCs) and anisotropic swollen gels are used as so called alignment media. For anisotropic NMR-spectroscopy of small organic molecules the alignment medium should be compatible with organic solvents and introduce only a low degree of orientation.[5] By using chiral media, the chiral information can be transferred to the analyte. Enantiomers interact diastereomorphously with chiral alignment media and can therefore be differentiated.[6]

Until now mostly polymer based lyotropic liquid crystals and anisotropic swollen gels are used as aligment media in organic solvents. Polymer based systems are affected by several complications when applied as alignment media. In lyotropic liquid crystals the degree of order is limited by the critical concentration of the LLC-phase. A low degree of order is required to ensure weak alignment conditions. Homopolypeptides do need very high molecular weights to achieve low critical concentrations. These high molecular weights can only be gained with highly pure monomers which require a challenging synthesis under strictly inert conditions.[7] Anisotropically swollen gels offer a free scalability for the degree of order but are not always straight forward to prepare. It can be time consuming in case of PBLG which needs about 30 days of preparation time.[8] In addition, fabrication of anisotropic samples by swelling the cross-linked polymer sticks can take up to two months of time.[8]

BTAs are a promising new approach for organic solvent compatible alignment media. They offer several advantages over the commonly used polymer-based alignment media. The synthesis is straight forward and does not require inert conditions.[9] Due to the self-assembling characteristics of BTAs polymerization is not necessary. The properties of the supramolecular BTA system can be widely tuned by varying the side chains[1] or via asymmetric substitution of BTAs[3].

In this work several BTAs were synthesized and tested for their LLC behavior. We could show that BTAs with achiral, aliphatic side chains and asymmetrically substituted BTAs with achiral and chiral, aliphatic side chains form LLCs in organic solvents. To investigate the capability of BTA-LLCs as alignment media, analytes were added to the phases. By fitting the obtained RDCs to structure proposals, the structures of the analytes could be confirmed. This demonstrates BTA-LLCs can be used as alignment media for organic compounds.

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34 Regulation of Quadrupolar Splittings in NMR Experiments on Anisotropic Samples

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It is known that the quadrupolar splitting of solvent resonances is directly linked to the orientational properties of an analyte in NMR alignment media like lyotropic liquid crystals or gels, [1-4] and is fairly sensitive to temperature changes. For well studied systems like PBLG, the magnitude of the splitting can even be used as a measure of sample composition, homogeneity and stability. For reliable measurement of anisotropic NMR parameters, the control of the quadrupolar splitting is essential.

Therefore we propose to use the recently developed "NMR Thermometer" [5] as a means to a) lock on a split solvent signal and b) regulate the quadrupolar splitting of the solvent signal by varying the temperature. Regulating the solvent splitting in such a way enables the comparison of different samples, different magnetic field strengths or time points all scaled to the same strength of the orientational interaction of an analyte in an alignment medium.

Herein we show that the PBLG as established alignment medium for small molecules in organic solvents is suitable to be used with the NMR-Thermometer and the increase of reproducibility in anisotropic sample conditions. Temperature profiles were measured with different concentrations of PBLG and the response to temperature steps was examined. Furthermore it is shown that it is possible to set the quadrupolar splitting to predefined values in various experimental conditions like magnetic field strengths and by vary sample conditions.

The regulation of quadrupolar splitting was then applied to measure RDCs. First experiments were obtained with a rigid organic small molecule (IPC). The obtained results show reproducibility of enantiodiscrimination of (-)- and (+)-IPC in PBLG, we thus propose the quadrupolar splitting is useful to regulate anisotropic sample conditions in combination with the NMR-Thermometer.

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On the Mechanism of Isomerisation of Palladium Pi-allyl Complexes

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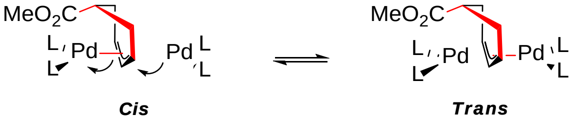
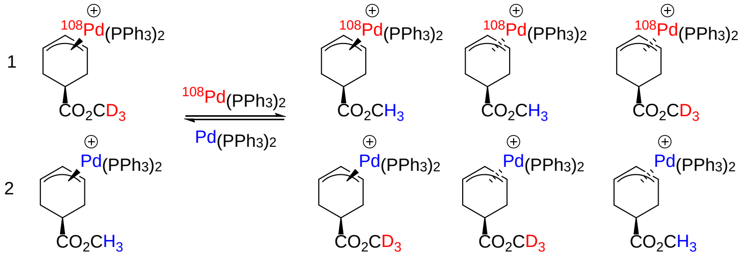
Palladium-catalysed asymmetric allylic alkylation is a key reaction for the formation of both C-C and C-heteroatom bonds. The mechanism involves generation of a palladium π-allyl species, and subsequent attack of a nucleophile. Asymmetry can be induced through the use of chiral substrates, ligands or nucleophiles; and the interconversion between the two enantiotopic faces of the allyl ligand is key in determining the level of enantioselectivity achieved.

Several mechanisms for the intramolecular isomerisation of palladium π-allyl species are well understood, however the intermolecular isomerisation with palladium(0) is less well studied. The previously proposed mechanism involves a nucleophilic displacement of a bis-ligated palladium species by palladium(0) proceeding with inversion of stereochemistry[1], see Scheme 1. This mechanism does not explain the observation that bidentate ligands retard the isomerisation.



Scheme 1: Proposed Mechanism

The mechanism can by probed by simultaneous NMR and mass spectrometry (MS) analysis. Using a diasteretopic palladium π-allyl, (1 and 2, Scheme 2) the rate of isomerisation from cis to trans was monitored by 31P{1H} NMR. Labelling the palladium and allyl allows the rate of displacement to be monitored by MS. Comparison of the rates of cis-trans isomerisation and palladium displacement provides insight into the mechanism. For the proposed mechanism, the rate of cis-trans isomerisation would be twice the rate of palladium exchange; the percentage equilibrium observed by NMR would be twice that by MS.



Scheme 2: Equilibrium Mixture

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Development and Evaluation of Experiments for Oligosaccharide Structure Elucidation Using Phosphitylated Derivatives

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Carbohydrates, oligosaccharides, and other polysaccharides can be difficult to characterize by NMR due to the crowded nature of the proton spectrum. Spectral overlap and strong coupling can make it difficult to separate out the ring-proton multiplets, which are important for determining the proton-proton coupling constants used to identify the sugar subunits of oligosaccharides. Structural elucidation of such compounds can in some cases be aided by applying derivatization in combination with specialized NMR sequences. One such scheme uses carbon-13 labeled samples and doubly selective NMR experiments. [1,2] This method works well but can take up to 24 hours to prepare a sample and requires access to specialized equipment. This reaction can be replaced by a phosphorous labeling scheme originally used in the quantitative analysis of edible oils. The main advantage of using the phosphorous derivatization is the speed of the reaction. The phosphitylation reaction can be done in a few minutes in an NMR tube with no separate equipment. This reaction tends to go to completion and, with some adjustments can possibly be used to quantitate sugars in a similar manner to its use in edible oils.

The carbon-13 experiments used 100% labeled acetic anhydride with carbon-13 at both carbonyl positions to completely acetylate a sample. This "iso-tagging" allowed for several specialized pulse sequences used to selectively excite the ring protons through the coupling to the carbon-13 on the acetyl group. These experiments offer very good resolution and decent sensitivity but, as mentioned above, the reaction to produce these derivatives involves several steps before preparing an NMR sample and can take a significant amount of time. Another group of experiments has been developed for quantitative analysis of edible oils and their constituent molecules using a phosphitylation reaction. [3,4] This reaction is similar to the acetylation but uses a phosphorus reagent that leaves a phosphorous-31 nucleus where the carbon-13 would be in the acetylation experiments. This makes the molecules accessible by similar selective pulse experiments used in the iso-tagged case. The resolution improvement is not as good as in the acetylation case but the speed of the reaction makes it more practical in some situation. Some adjustments need to be made for use on carbohydrates compared to the method as used on edible oils. Experimental details and results will be presented on several model compounds such as maltitol. Advantages and drawbacks compared to the iso-tagging methods will be discussed as well as reaction optimization and pulse sequence considerations. Also, efforts at quantification of sugars will be discussed.

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Automated Structure Verification: Continued Work to Determine Optimum Experiments and Processing

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Advanced methods of chemical structure characterization employ a variety of 2D NMR techniques. However, past practice for the computer automation of this technique, Automated Structure Verification (ASV), primarily employs either 1D 1H NMR only, or a combination of 1D 1H NMR and 2D 1H-13C HSQC [1]. The combination of advancing hardware technology and recent software development make possible the inclusion of a wide array of experimental data in fully automated structure verification work. Inclusion of expanded data types supports more accurate structure verification, decreasing the likelihood that false structures should pass through a verification process.

Recent experimental work has provided a rich array of experimental data on a large variety of structures for chemical samples that are derived from several sources. Included are 1D 1H, 1D 13C, 1D 13C DEPT, 1H-13C DEPT-edited HSQC, unedited 1H-13C HSQC, COSY, TOCSY, HMBC, and H2BC data. Coupling the analysis of such data with the ability to create spectroscopically relevant challenge structures [2] enhances the certainty of the chemist that they have synthesized the correct structure, and the confidence with which any organization can assume that the structure of any component in its library is completely correct.

Analysis of this variety of data sets helps to establish the most effective and efficient experimental processes to ensure that correct structures are rapidly recognized, while incorrect chemically relevant structures are flagged for failure or for further analysis.

Here we present an analysis of several different correlation techniques in order to better understand the value of various NMR experiments in ASV work.

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NMR Characterization of Integrin αVβ6/RGD Cyclopeptides Interactions

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Integrins are heterodimeric cell surface receptors that mediate adhesion to the extracellular matrix and are involved in many severe pathological processes. Among all, αVβ6 integrin plays a key role in the initiation of metastasis and is up-regulated in many types of cancer, but not in normal healthy organs.[1] Therefore αVβ6 is an exciting target for both imaging and treatment, across many common cancer types. Up to date only few αVβ6 selective antagonists have been developed,[2] including long peptides which contain the RGDLXXL/I sequence, such as a TGFβ3 peptide (HGRGDLGRLKK).[3]

As a matter of fact, the rationalization of the effect induced by the RGD flanking amino acids in the interaction with αVβ6 is still an open question. In this context, we wanted to explore the effect of the RGD flanking residues in the binding to αVβ6.

In a recent study we have developed a multistage protocol based on metadynamics and docking to study the modulation of αVβ3-binding selectivity. We carried out a systematic enhanced MD exploration on 400 RGD cyclic head-to-tail pentapeptides (X1-RGD-X2) in order to study the effect the flanking residues X1 and X2 on the cyclopeptides conformational equilibrium. The 400 cyclopeptides were subsequently screened, based on their conformation criteria and computational affinity scoring values for αVβ3, and we ended up with a small library of 16 cyclopeptides that were tested for binding to different integrins (αVβ3, β5, β6, β8, α5β1 and αIIbβ3) in ELISA assays.

Unexpectedly, within this library we identified three cyclopeptides (MRGDW, RRGDF, NRGDW) that showed nanomolar IC50 towards αVβ6.

In order to get structural insights into peptide/integrin interaction, we have performed preliminary Saturation Transfer Difference (STD) and transfer-NOE (tr-NOE) experiments between the soluble extracellular domain of αVβ6 and c(MRGDW) cyclopeptide. The experiments were performed on a sample containing αVβ6 and c(MRGDW) cyclopeptide in a ratio 1:100, and, for comparison purposes, on a sample containing the cyclopeptide only. The STD experiment evidenced the presence of an interaction between c(MRGDW) and αVβ6, and allowed us to perform an epitope mapping of the cyclopeptide protons, which highlighted the proximity of the ligand indole ring to the integrin surface. As concerns the tr-NOE, only for the sample containing c(MRGDW)/αVβ6 mixture we detected negative intramolecular NOEs of the cyclopeptide, which confirms the presence of the peptide/integrin interaction.

Using a combination of computational methods and biological assays we were able to identify three out of 400 pentacyclopeptides that selectively bind to αVβ6.

To the best of our knowledge, these are the first RGD cyclopeptides which showed nanomolar Ki for αVβ6.

The NMR spectroscopy confirmed the presence of the interaction between c(MRGDW) and αVβ6, and allowed us to identify in the indole ring the region of the ligand that is closer to the integrin surface. In the future we will extend this study to the other two cyclopeptides. We plan also to perform NMR competition experiments with TGFβ3 peptide and to perform STD and tr-NOE using directly human cancer cells expressing αVβ6 in order to structurally characterize the interaction in a physiological context.[4]

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Metabolic Profile of Intact mouse Sciatic Nerve Studied by HRMAS NMR

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Context

The tumour suppressor LKB1 codes for a serine/threonine kinase that regulates both metabolism and cell polarity. Germline mutations of this gene are responsible for the Peutz-Jeghers syndrome, a dominantly inherited cancer disorder. The neural crest cells (NCC) constitute a population of highly motile embryonic cells that give rise to a broad array of derivatives including the peripheral nervous system and pigmented cells. Using the Cre-LoxP system (Tyrosinase-Cre), we deleted in a subpopulation of murine NCC the gene coding for LKB1. Mutant mice exhibited hypopigmentation, a progressive hindlimb paralysis and died from intestinal pseudo-obstruction before the sixth postnatal week. Histological analysis of the sciatic nerves revealed a deficit in axonal sorting, hypomyelination and neuronal loss. In this study we aimed to evaluate the consequences of Lkb1 inactivation on sciatic nerve metabolism by High-Resolution Magic Angle Spinning (HRMAS) NMR spectroscopy of intact nerves.

Material and methods

Sciatic nerves from 21 days (P21) wild (WT) and cKO animals were rapidly dissected, cleaned of surrounding fat and connective tissue and immediately frozen in liquid nitrogen. Left and right nerve of each animal were inserted in a disposable insert for HRMAS NMR spectroscopy, and 7 µL of D2O added before sealing the insert.

1H-NMR spectra were acquired with a Bruker Avance III 500 spectrometer (BrukerBiospin, Wissembourg, France) of IRMaGe facility, implanted in the CEA-Grenoble. 1D spectra were acquired at 4 °C with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence synchronized with the 4 KHz spinning rate (inter-pulse delay 250 µs, total spin echo time 30 ms). Resonance assignment was performed as previously described [1,2].

All spectra were pre-processed and then divided in 10-3 ppm buckets from 4.678 to 0.60 ppm using the AMIX software (Bruker). Each bucket was normalized to total spectrum amplitude. These buckets were then loaded to the SIMCA-P software V14 (UmetricsAB, Umea, Sweden) for Principal Component Analysis (PCA) and Orthogonal Partial Least Square–Discriminant Analysis (OPLS-DA).

Results/discussion

By using proton HRMAS NMR spectroscopy of intact sciatic nerves, we were able to highlight variations in both metabolite and lipid levels in cKO mice. We could detect and assign 17 metabolites, however the main spectral contributions were due to total creatine, phospholipid intermediates, taurine and myo-inositol. The multivariate statistical model (OPLS-DA) showed a very good separation between the two mouse groups mainly due to down regulation of all acyl chain groups. However, other compounds related to lipid synthesis or degradation, i.e. phosphoethanolamine, choline and phosphocholine were upregulated while glycerophosphocholine was downregulated. Similarly, beta-D-glucose was decreased while amino-acids alanine and glutamate, myo-inositol and taurine, as well as total creatine were increased. No clear feature could be drawn for N-acetylaspartate, N-acetylaspartateglutamate, glutamine, scyllo-inositol and glycine. Interestingly, the lactate/beta-D-glucose ratio was increased in sciatic nerves of cKO animals thus suggesting that in Lkb1-deficient nerves, glycolysis could be more prominent as compared to oxidative phosphorylation. This is in line with the increase of alanine observed.

The most striking change in 1H HRMAS NMR of sciatic nerve was observed for total creatine When we performed biochemical analysis in protein-free extracts of rat cell line model of Schwann cells (S16) with Lkb1 knock down, we also observed a trend towards increased total creatine, together with a significantly increased PCr/ATP ratio. Adenylates can be hardly resolved by ex vivo 1H-NMR, but biochemical analysis showed that ATP/ADP ratios, energy charge and adenylate concentrations remained unchanged in S16 Lkb1 knock down cells. Thus, these cells did not suffer from acute energy stress, but may have adapted to energetic limitations by increasing the creatine pool. This would also increase, via the creatine kinase reaction, the cellular phosphocreatine that is available as an energy reserve in situations of metabolic stress.

Altogether, these findings highlight the crucial role of LKB1 signaling in the differentiation of Schwann cells, thereby suggesting that perturbations of this pathway may contribute to the aetiology of peripheral neuropathies

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Towards the Development of Diagnostic and Therapeutic Approaches against B. pseudomallei: Structure Based B Cell Epitope Design of BPSL1050 Antigen

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Bulkholderia pseudomallei is the etiological agent of melioidosis, a severe endemic disease in South-East Asia, causing septicemia and organ failure with high mortality rates. Current treatments and diagnostic approaches are largely ineffective. The development of new diagnostic tools and vaccines towards effective therapeutic opportunities against B. pseudomallei is therefore an urgent priority. In the framework of a multidisciplinary project tackling melioidosis through reverse and structural vaccinology, BPSL1050 was identified as a candidate for immunodiagnostic and vaccine development based on its reactivity against the sera of melioidosis patients.

We determined its NMR solution structure and dynamics, and by novel computational methods we predicted immunogenic epitopes that once synthesized were able to elicit the production of antibodies inducing the agglutination of the bacterium and recognizing both BPSL1050 and B. pseudomallei crude extracts.

Overall, these results hold promise for novel chemical biology approaches in the discovery of new diagnostic and prophylactic tools against melioidosis.

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How to Measure Convection and Minimize its Effect for DOSY Experiments

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Convection has always been a significant problem for NMR diffusion measurements, but only recently has the underlying physics been clarified [1]. The classic assumption is that convection is driven by negative vertical temperature gradients, i.e. the bottom of the sample is warmer than the top and hence the warmer, less dense, liquid will rise to the top, causing the convective flow. This is known as Rayleigh-Bénard convection, and is a critical phenomenon determined, amongst other things, by the magnitude of the temperature gradient and the sample geometry [1,2,3]. However, if convection were driven purely by this phenomenon, we would not see convention under positive temperature gradients (i.e. when the bottom of the sample is cooler), nor for very small temperature gradients. In practice, we can observe convection in almost all NMR experiments. It seems to be always there, only the sign and magnitude change! One of the reasons for this is that convection can also be driven by transverse temperature gradients, so-called Hadley convection [2]. As a practical consequence, we need to be able to measure the size of the problem, and develop strategies to minimize its effects.

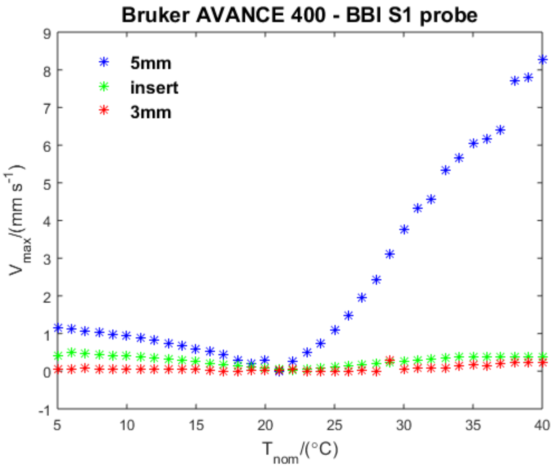

Figure 1: Convection velocity versus temperature for a 400 MHz NMR spectrometer, using three different NMR tubes. The convection is very significant in the standard 5 mm tube, smaller in the insert tube, and practically zero in the 3 mm NMR tube.

DOSY experiments are particularly sensitive to convection, as any movement of, or within, the sample will compromise the measurements. In this work we have investigated the magnitude of convection in a series of spectrometers (Bruker Avance III 400-600 MHz) and a variety of probes (Fig. 1). As 19F measurements are of particular interest to us, we have measured convection using both 1H and 19F experiments, in which the 19F pulse sequence used 1H decoupling during acquisition, an additional source of heating and hence potentially contributing to convection.

Preliminary results suggest that there are large differences in convection between different probes and spectrometers, but that it is almost always present at detrimental levels for a standard 5 mm NMR tube with deuterated chloroform as solvent. The 19F{1H} experiments showed, as expected, a greater tendency to convect. There are several possible ways to deal with convection, but a very efficient and simple way to supress convection here was to restrict the geometry by the use of a 3 mm NMR tube.

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Qualitative Analysis of the Chemical Profile and Quantification of Sugars in Nectarine and Iintegral Grape Juices by NMR

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The sugars comprise an important source of energy and it is a regular part of the human diet. Among the various sources of sugars, one of them is the fruit of grapevine (grape) and their derivatives, mainly in the form of juices, which is even recommend by the medical community due to its important properties. However, the monitoring and control methods applied for juices to certify the quality standards established by law, in Brazil, do not discriminate the individual levels of glucose and fructose. The excess consumption of these compounds can represent a risk for health, for example, a high intake of fructose could result an insulin resistance syndrome and high levels of uric acid triggering a series of damage to the well-being of those who consume. [1] Moreover, it is common to find reports about disrespect to the quality standards of juices, which also represents a potential risk for health, especially in cases associated with a high level of sugars. [2]

In this context, the present study aims to perform a qualitative analysis of the chemical profile and quantification of sugars in nectarine and integral grape juices through NMR experiments using two quantification methods: external and electronic (ERETIC) references. [3]

The 1H and HSQC NMR experimental data of the nectarine and integral grape juices were obtained on a Bruker Avance III 500.13 MHz spectrometer. Each sample was prepared with a total volume of 550.0 µL of juice and 50 µL of DMF (1.0%) and TSP (0.1%). All the unidimensional 1H quantitative NMR experiments were performed using the noesygppr1d pulse sequence (Bruker's library) for suppression of the water.

From the qualitative point of view, it was possible to identify many compounds in both nectarine and integral grape juices, some of them we could highlight as α-glucose, β-glucose, fructose, acetate, tyrosine, malic acid, fumaric acid, valine and methanol, among others. All of these compounds were characterized by corroboration between experimental NMR data and freely Human Metabolome Database (HMDB) data. [4]

The external and electronic (ERETIC) references methods were effective for the quantification, because it permits with a single experiment to quantify individually glucose and fructose, and identify a high concentration of fructose and relevant ethanolic content in the integral juice. From the 22 commercial brands od integral grape juices analyzed, just two have warning messages about the high content of sugars. In addition, an alarming fact is that from the 24 commercial brands of nectarine grape juices analyzed, only three fulfilled the limits established by Brazilian current legislation and two of them had a sucrose content added more than 10 times above the permitted. It is important to highlight that such nonconformity it is not detectable by the official analysis methods currently used by Brazilian regulatory agencies.

The NMR showed that could contribute greatly as an investigative tool for the regulatory agencies, whose official methods evaluate only total sugars content. Most of the nectarine grape juices samples had a sucrose content above of the legislation limit. The NMR data also permitted to characterize many chemical constituents presents in nectarine and integral grape juices.

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43

First Steps Towards Field Integration of Benchtop NMR Spectrometers for Online Monitoring and Process Control

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Online monitoring and process control requires fast and noninvasive analytical meth-ods, which are able to monitor the concentration of reactants in multicomponent mixtures with parts-per-million resolution. Online NMR spectroscopy can meet these demands when flow probes are directly coupled to reactors, since this method fea-tures a high linearity between absolute signal area and sample concentration, which makes it an absolute analytical comparison method being independent on the matrix. Due to improved magnet design and field shimming strategies portable and robust instruments have been introduced to the market by several manufacturers during the last few years. First studies with this technology showed promising results to monitor chemical reaction in the laboratory [1, 2].

Within the project CONSENS, the continuous production of high-value products in small production scale is advanced by introducing MR-NMR spectroscopy. CONSENS is a research and innovation project on integrated control and sensing for sustainable operation of flexible intensified processes.

This poster will present the first steps of the process integration of a benchtop NMR instrument for a lithiation process and outlines further fields of activity and potential challenges. Hereby, the following issues are going to be addressed: explosion-proof housing for the spectrometer, automation of signal processing (data pretreatment, evaluation and communication to the control system), flow cells and measuring con-ditions. Additionally, the first results of the lithiation reaction in lab scale regarding the pure components and reaction mixtures are going to be discussed.

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44

New Method for Lipophilicity Determination by NMR

Raquel Enrech, Magda Bordas, and Adriana Port

ESTEVE, Barcelona, Spain

Lipophilicity, commonly expressed as octanol/water distribution coefficient (LogP) is a crucial parameter in drug development, since it impacts both ADME properties and target affinity of drug candidates. [1]

LogP values are traditionally determined using the shake flask technique, which involves shaking and separating two immiscible solvents in a glass flask in order to analyse the concentration ratio of a solute distribuited in the two phases.

Here we present the use of NMR as a suitable detector for lipophilicity experimental determination. 1H-NMR and WET experiments were used to determinate the LogP of six pharmaceutical compounds, which include acidic, basic and neutral drugs. The partitioning was performed in non-deuterated solvents and a small amount of deuterated DMSO was used as internal standard in both phases. [2,3]

This modified qNMR methodology represents an easy and fast technique for experimental LogP determination, that is comparable to the conventional solute detector HPLC-MS. The advantages of using NMR techniques versus HPLC-UV or potentiometric methodologies, will be also discussed. [4]

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45 Matrix-Assisted Diffusion-Ordered NMR Spectroscopy with an Invisible Matrix

Aaron Hernandez-Cid, Ralph W. Adams, Mathias Nilsson, and Gareth A. Morris

University of Manchester, School of Chemistry, Manchester, UK

Diffusion-ordered spectroscopy (DOSY) is a family of NMR experiments for mixture analysis that disperses the resonances of different species into a second dimension according to their rate of diffusion [1]. However this is of little help for mixtures of species with similar rates of diffusion. A very effective way to improve the dispersion of DOSY in such systems, known as matrix-assisted DOSY (MAD), is to add a co-solute to which the different mixture components bind to different extents, thus perturbing their diffusion differentially. Like chromatography, the method exploits specific chemical interactions to drive diffusion separation. A common problem in MAD is that co-solutes introduce resonances that can overlap with signals of the species of interest. This can be avoided by using perdeuterated surfactants [2] that are almost invisible by 1H NMR or species with a very simple spectrum [3]. Here we report the use of perfluorinated micelles that do not contribute any interfering signals, to separate the 1H signals of mixtures of amino acids.

Figure 1 below shows that the addition of 100 mM sodium perfluruooctanoate to an aqueous mixture of glutamate, histidine and lysine at pH 8 lifts the degeneracy in diffusion coefficient, allowing the signals of the three amino acids to be easily distinguished. Measurements of diffusion coefficient as a function of pH for these and other amino acid mixtures can be modelled successfully in terms of equilibria between free and micelle-bound species in different protonation states, with binding significantly perturbing the ionization equilibria. Results show that binding is primarily driven by charge-charge interactions, but that amphiphilicity also plays a part.

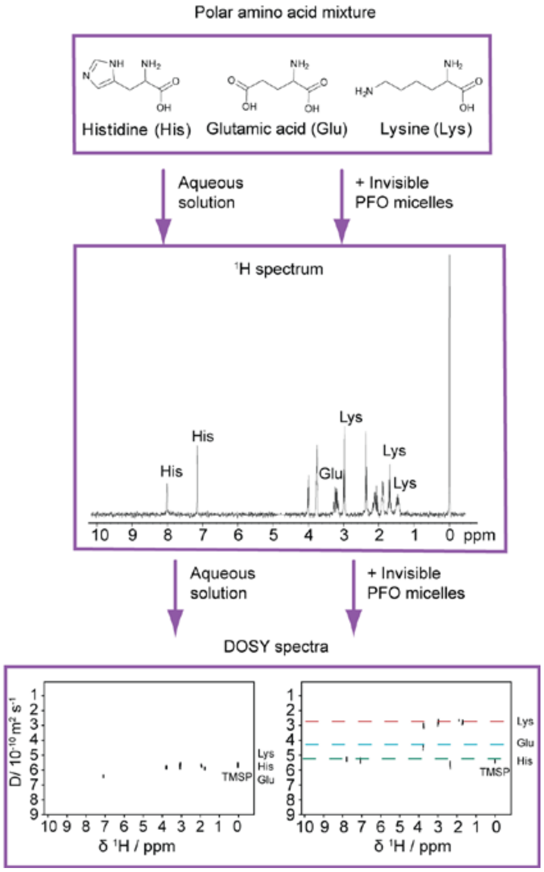

Figure 1. DOSY spectra of a mixture of histidne, glutamic acid and lysine in the presence and absence of PFO micelles.

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46

Anomalous Nuclear Overhauser Effects from Scalar Cross-relaxation of the First Kind

Ilya Kuprov[2], Johannes Kloesges[1], Christopher I. Pearson[1], Tharindi D. Panduwawala[1], Laia Josa-Culleré[1], Barbara Odell[1], Mark G. Moloney[1], David M. Hodgson[1], and **Timothy D. W. Claridge**[1]

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Anomalous NOESY cross-peaks that cannot be explained by dipolar cross-relaxation or chemical exchange are described for a range of small molecules. The origin of these is identified as scalar cross-relaxation of the first kind (SCRFK), as demonstrated by a complete theoretical description of this relaxation process and by computational simulation of the NOESY spectra.

Conventional analysis would have assigned the cross-peaks as being indicative of a chemical exchange process occurring between correlated spins, were it not for the fact that the pairs of nuclei displaying them cannot undergo such exchange.

It is shown that this process relies on the stochastic modulation of J-coupling between correlated protons caused by conformational changes or by the exchange of acidic protons. We demonstrate that in the case of aziridines the effect arises from inversion at the nitrogen center, which is known to be a slow process and occurs at rates appropriate for SCRFK to be operative. For the substituted pyrrolidine analogues studied, the effect is ascribed to the exchange of the amino proton, a process that is highly sensitive to solution conditions which may also be suitable for SCRFK to be detected. This observation of scalar cross-relaxation between protons in NOESY spectra has recently been reported by us [1] and further work exemplifying this effect will soon be submitted [2].

Similar effects may be observed in the popular 1D NOESY experiments, where again the sign of the "NOE peaks" would suggest chemical exchange is occurring, despite scalar cross-relaxation being the true physical process in operation.

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47

The Adventurous Structure Elucidation of an Elusive Degradation Product of Ziprasidone

Zoltán Szakács and János Kóti

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The structure elucidation of impurities and degradation products of small-molecule drugs is often accompanied by unintended detours and false assumptions until the correct solution is found.

Here we describe such a case, involving several challenging complications. The structural problem arose in connection with the alkaline hydrolysis of ziprazidone, an antipsychotic drug substance, which yielded a novel degradation product, the structure of which was initially hypothesized as a cyclic sulfonamide derivative on the basis of LC-MS. Our spectroscopic characterization quickly revealed that we had an isomer in hand, but there were several conceivable structural alternatives that had to be ruled out systematically by combining the results of tedious NMR and MS experiments. The resulting structure was consistent with in-depth MS fragmentation data, nevertheless, it could not be fully characterized by 1H and 13C NMR, partly due to limited solubility, broadened resonance signals, and also because of the high level of acetamide contamination in the sample. In addition, this particular degradant proved to be chemically unstable under acidic circumstances and its acidic decomposition led to unambiguously identifiable products, serving as a posteriori confirmation of the proposed structure.

Further similarly interesting and instructive case studies have been published in our recent book [1].

1. Anthropic Awareness: The human aspects of scientific thinking in NMR spectroscopy and mass spectrometry. Ed. Csaba Szántay Jr., Elsevier, 2015, ISBN: 978-0-12-419963-7.

48

Stability Studies of Acylguanidine BACE1 Inhibitors by HMBC

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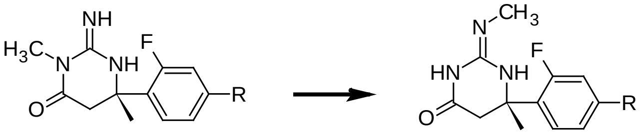
Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia. BACE1 is the rate-limiting enzyme in the processing of APP and the production / deposition of toxic Abeta peptides, and thus the inhibition of b-secretase (BACE1) is believed to represent a potential disease modifying treatment for AD. One of the groups of BACE1 inhibitors is the acylguanidine family, whose inhibitory activity mostly resides on the adequate arrangement of their N-methyl group. Some of these compounds usually undergo a rearrangement in solution by which the N-methyl group ends up in another position, resulting in a loss of activity. In this poster we present a straightforward method to evaluate the right position of that N-methyl group by evaluating the 3J HMBC correlations with its vicinal quaternary carbons.



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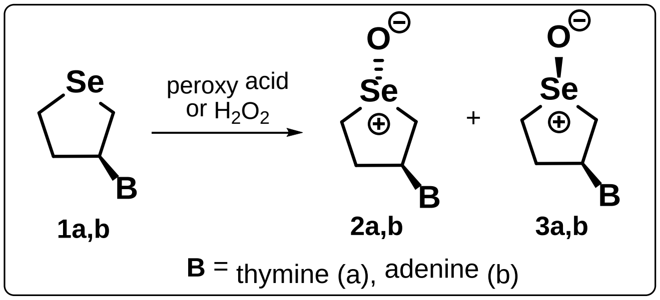
Configuration Determination of SeO in Conformationally Flexible Selenonucleosides

Lenka Poštová Slavětínská, Miloš Buděšínský, Dominik Rejman, and Radek Pohl

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

The knowledge of stereochemistry is essential information e.g. in total synthesis, in development of stereoselective reaction or in searching for new biologically active chiral compound. NMR spectroscopy does a great job in determination of relative configuration without neccessity of isolation of the compound of interest or growing a crystal suitable for X-ray crystallography. NOEs or vicinal coupling constants are the most used NMR parameters performing this task. However, these NMR parameters are not available for some types of asymmetric functional groups such as selenoxides. In this case, a correlation of calculated NMR parameters with observed ones is a method of choice. Recently we have shown [1] that this approach is useful in the configuration determination of N-oxides, phosphinoxides, sulfoxides and selenoxides using conformationally locked six-membered saturated heterocycles as models.

In this poster, we present the assessment of the stereoselectivity of in situ oxidation of conformationally flexible nucleoside analogues 1a,b bearing Se atom in a pseudosugar part of molecule and the determination of the relative configuration of selenoxide products 2a,b and 3a,b.



In situ oxidation of selenanes 1a,b in NMR tube was accomplished by m chloroperoxybenzoic acid (MCPBA) in CDCl3 or by H2O2 in DMF-d7. MCPBA oxidized Se to SeO faster than H2O2 and the stereoselectivity for MCPBA oxidation was 3:1 for 2a:3a, and 6:1 for 2b:3b. We have found that selenoxides 2 and 3 interconvert to each other in DMF-d7 solution reaching equilibrium mixture of diastereoisomers (1:1 for 2a:3a, 2:1 for 2b:3b). This isomerization is most likely mediated by water via hydrated form of selenoxide [2].

The molecular structure of 2 and 3 is rather flexible - five-membered ring can adopt various conformations and nucleobase can be oriented in syn and anti fashion. Therefore, the determination of the relative configuration of 2 and 3 started with conformational analysis and selection of the most stable conformers. Thus, we selected four boundary conformations of tetrahydroselenane five-membered ring with phase angles 0, 90, 180 and 270 degrees and with syn and anti conformation of nucleobase and fully optimized the geometries by DFT B3LYP/6-311++G** method. NMR parameters (1H, 13C and 77Se chemical shifts) were then computed for the ensemble of the most stable conformers, weighted according Boltzmann distribution and correlated with observed NMR data. The poster summarizes results obtained at various levels of calculations reflecting anisotropy of SeO group in determination of selenoxide configuration.

Acknowledgements: The work is supported by the Czech Science Foundation (Grant No. 13-24880S).

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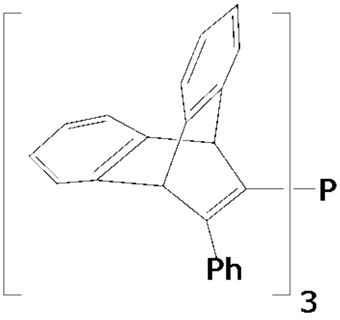
50

2D ROESY and Exchange Studies of a Tertiary Phosphine and Its Derivatives

Corinne Y. Wills, William McFarlane, Simon Doherty, Julian G. Knight, Daniel O. Perry, Nicholas A.B. Ward, Dror M. Bittner, and Michael R. Probert

School of Chemistry, University of Newcastle, Newcastle upon Tyne, UK

The tertiary phosphine TRISKITPHOS and its metal complexes are of interest in the homogeneous catalysis of carbon-heteroatom coupling and cyclisation reactions [1,2].



TRISKITPHOS

We present proton 2D ROESY and chemical exchange experiments over a wide range of temperatures which show that in solution TRISKITPHOS, its oxide and its AuCl complex show hindered rotation about their P-C bonds leading to inequivalence of the two C6H4 rings within each phosphorus substituent, although the substituents themselves remain equivalent. That is, the effective symmetry at lower temperatures corresponds to C3 rather than C3v.

More detailed analysis of the off-diagonal intensities in the ROESY spectra suggests that at low-temperatures in solution the preferred conformations are similar to those found in the solid state by X-ray diffraction.

In addition, in the case of the gold complex the rotation of the phenyl groups is restricted so that each ortho and each meta proton gives a separate signal at temperatures below 200K. For the phosphine itself and its oxide, it appears that this behaviour also occurs but only at even lower temperatures.

Energies of activation derived from the Eyring equation will be reported for the two distinct processes observed in these species.

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51 Solid State NMR Studies of σ-Alkane Complexes: Towards Heterogeneous Catalytic Intermediates

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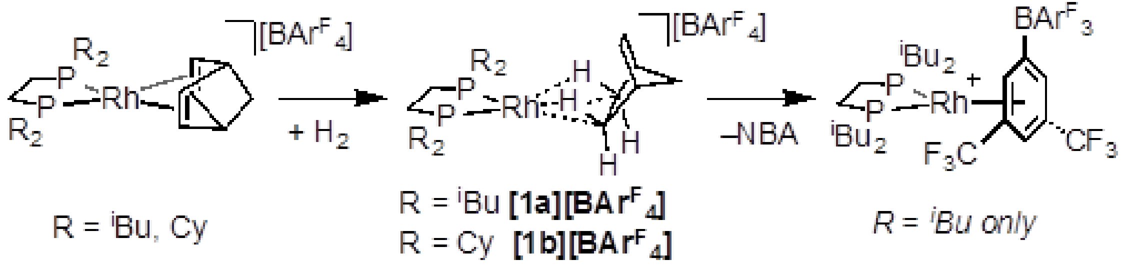
Department of Chemistry, University of Oxford, Oxford, UK

The synthesis of σ-alkane complexes in which an alkane interacts with a metal centre through 3 centre-2 electron M···H-C bonds, are attractive intermediates for the development of new synthetic methodologies for C-H activation processes especially for hydrocarbons [1]. A variety of rhodium bis-phosphine σ-alkane complexes have been synthesised by a solid/gas reaction route often involving single cystal to single crystal transformations [2]. In this work we highlight the application of solid state NMR in conjunction with single crystal X-ray diffraction and DFT calculations to the formation, structure and stability of these species.



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52

The Norcaradiene-Cycloheptatriene Equilibrium in a Series of Azulenones by NMR Spectroscopy; the Impact of Substitution on the Position of Equilibrium

Lorraine Bateman[1], Orla A. McNamara[1], N. Rachael Buckley[1], Patrick O'Leary[1†], Francis Harrington[1], Norma Kelly[1], Sarah O'Keeffe[1], Angela Stack[1], Shane O'Neill[1], Daniel G. McCarthy[1], and Anita R. Maguire [1,2]

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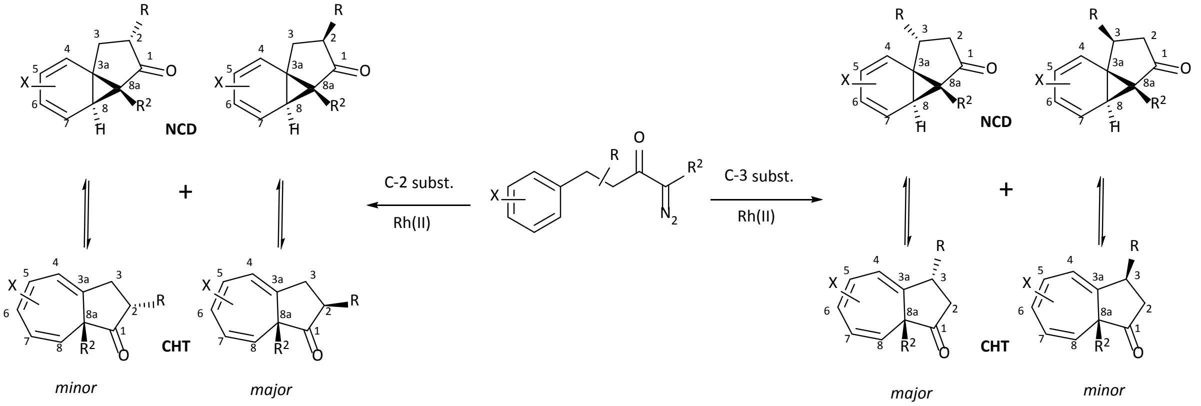
The intramolecular Buchner reaction involving aromatic addition reactions of aryl α-diazoketones, effected by rhodium(II) catalysis, leads to a tricyclic norcaradiene (NCD), which exists in dynamic equilibrium with the more stable bicyclic cycloheptatriene (CHT) valence tautomer through an electrocyclic ring-opening/ring-closing process (Scheme 1). This approach is a versatile route to azulenone systems which are a very interesting series of compounds incorporating NCD-CHT valence isomers. [1,2,3,4] Through careful catalyst and substrate selection, high levels of chemoselectivity and regiocontrol can be exercised. The effect of catalyst and substituent choice on the efficiency, regio- and diastereoselectivity of the reactions of a range of α– and β–substituted α-diazoketones has been reported by the Maguire group.[5,6]



Scheme 1 Cyclisation of precursor α-diazoketones leads to an equilibrating norcaradiene-cycloheptatriene product; major and minor diastereomers shown.

The use of NMR spectroscopy as a tool for studying the position of the NCD-CHT equilibrium in azulenone systems has already been established.[3,4,7] The tautomeric equilibrium in the azulenones is, in general, rapid on a NMR timescale, and hence, time- averaged 1H and 13C NMR signals are observed for the system at room temperature. The position of the tautomeric equilibrium can be readily estimated from the C(8)H

1H NMR and C-8 13C NMR chemical shifts since the C-hybridisation exists between a sp2 environment in the CHT and a sp3 environment in the NCD.[7]

In this study, we have conducted a systematic investigation of the influence of substitution at positions C-2 and C-3 on the azulenone skeleton, as well as the bridgehead position C-8a, based on NMR characterisation (Scheme 1). We have focused in particular, on the impact of the steric and electronic characteristics of substituents on the position of the norcaradiene-cycloheptatriene (NCD-CHT) equilibrium.

In general substituents at C-3 have a stronger impact on the NCD-CHT equilibrium than those at C-2, and, furthermore, when the C-3 substituent is trans- to the bridgehead methyl group the effect is substantially more evident than for the cis- analogues. With the parent unsubstituted azulenone (R, R2 & X=H), the equilibrium lies very much towards the CHT form; introduction of substituents at C(8a) and C-3 (trans) result in a substantial shift towards the NCD tautomer. The minor diastereomers of the azulenones, either cis- or trans- depending on whether C-3 or C-2 substituted, lie almost entirely on the side of the CHT with little sensitivity to substituent size.

Variable temperature (VT) NMR studies, were undertaken on a selection of azulenone systems to enable the resolution of signals for the equilibrating valence tautomers and revealed interesting shifts in the equilibrium. The NCD-CHT equilibrium in the trans C-3 alkyl substituted azulenones, and to a lesser extent the cis C-2 substituted derivatives, is sensitive to temperature, showing a shift towards the NCD tautomer at lower temperatures. In contrast, with the t-butyl substituent, the equilibrium is unaffected by temperature highlighting the conformational impact of the sterically demanding substituent. Signals for the tautomers of the 6-methoxy substituted azulenone were successfully resolved at 183 K allowing full characterisation of each structure by 1H and 13C NMR analysis (Fig. 1). Thus, for the first time in this series, the two interconverting tautomers were successfully resolved by 1H NMR spectroscopy.[8]

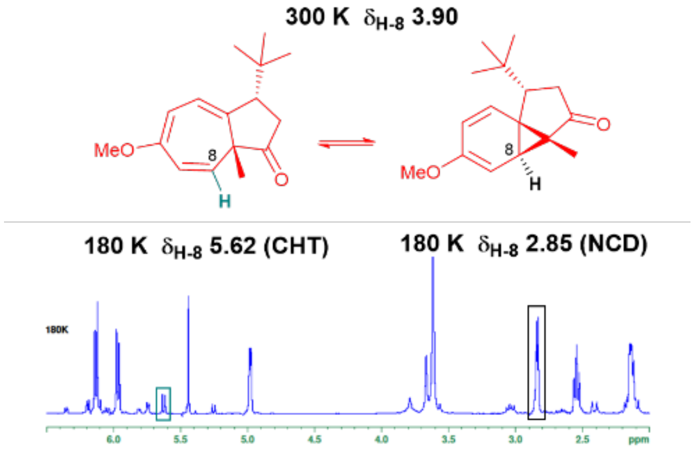


Fig. 1 1H NMR spectrum of 6-OMe-3-t-Bu azulenone at 183 K (CD2Cl2; 500 MHz)

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53

Development and Validation of a Selective NMR Method for Cleaning Validation in a GMP Pharmaceutical Production Plant

Ian Jones

AstraZeneca

New legislation introduced by the FDA in 2014 for pharmaceutical plant cleaning validation meant that visual inspections were no longer acceptable and data from validated sources was needed. For AstraZeneca's Zoladex plant at Macclesfield there was a requirement to develop and validate analytical methods capable of detecting very low levels (~10ppm) of both drug substance (goserelin) and excipient (lactide-glycolide co-polymer) on a variety of plant components and surfaces that have been through a wash cycle.

Selective 1H-NMR experiments have previously been used in trace analysis such as measuring low levels of Potential Genotoxic Impurities (PGI's) in drug products [1].

The poster describes how a selective 1H-NMR experiment has been tailored to provide a GMP validated method and how the method is being used to validate the plant cleaning process.

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54

Application of NMR in Metabolic Phenotyping in the National and Clinical Phenome Centres.

Michael Kyriakides[1,2], Matthew Lewis[1,2], Jake Pearce[1,2], Anthony C. Dona[3], Beatriz Jimenez[1], Elaine Holmes[1,2], and Jeremy Nicholson[1,2]

1. MRC NIHR National Phenome Centre, Imperial College London, London, UK
2. Clinical Phenotyping Centre, Imperial College London, London, UK
3. Sydney Medical School, University of Sydney, Sydney, Australia

Metabolic phenotyping involves the characterization of metabolic profiles with the aim of elucidating diagnostic and mechanistic markers associated to genotype, disease and environmental interaction (food, xenobiotics, pollution etc.). Nuclear Magnetic Resonance (NMR) spectroscopy, together with mass spectrometry, are traditionally the most commonly used analytical platforms for such analyses. The application of NMR spectroscopy in particular has had a significant impact in the field by offering unique advantages due to its non-destructive and reproducible nature. The division of Computational and Systems Medicine at Imperial College London is one of the pioneers of this application of NMR spectroscopy and has recently introduced high-throughput profiling methods for the metabolic characterization of clinical samples. The use of standardised experiments with in-situ automated spectral processing offer increased reproducibility, leading to a guaranteed level of quality control that is necessary for large scale analyses and inter-instrument comparisons. These protocols were originally developed in collaboration with Bruker Biospin (Billerica, MA, USA) for the analysis of urine and blood products (plasma or serum) but they are currently being developed for a variety of sample matrices including synovial fluid, cerebrospinal fluid, faecal water and saliva. In addition to the profiling of biological fluids for the elucidation of mechanistic and diagnostic markers, another novel application of NMR spectroscopy is its use as a screening tool for mass spectrometry, by detecting non-endogenous substances or sample outliers. The presented work illustrates the diversity of implementation of NMR spectroscopy in the field of metabolic phenotyping and the potential of translating this application to improved clinical care.

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NMR Metabolomic Study of Arabidopsis thaliana Wild Type and pp2a-b’γ Mutant

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2. Department of Biochemistry, University of Turku, Finland

Arabidopsis thaliana has become an extremely popular model system for studying many aspects of plant biology ranging from respiration to reproduction [1]. On the other hand, NMR spectroscopy is an invaluable and well-established experimental method to analyze molecular structures and stereochemistry of extracted and isolated natural compounds.

In this study, we will utilize the pp2a-b’γ mutant and NMR spectroscopy for detailed view to defense-active metabolites in Arabidopsis thaliana. The main aim of the study is to reveal effects of age development on defense-associated metabolites with potential impact in plants. To this end, the metabolomes of wild type, constitutively defense-active pp2a-b’γ mutant and a pp2a-b’γ line complemented by 35S-driven expression of the PP2A-B’γ gene will be compared by NMR. Identification of bioactive compounds from plants is drawing increasing attention worldwide. This study will utilize Arabidopsis thaliana to identify novel defense active metabolites. Once the most interesting candidates have been identified, Arabidopsis offers a powerful system for follow-up studies where the underlying biosynthetic enzymes and their metabolic interactions can be identified. On the chemical side of the work, the candidate metabolites may be isolated and thereafter assessed for their chemical properties and potential biotechnological importance.

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56

Unambiguous Determination of Protolimonoids Side Chain Configuration Assisted by Residual Dipolar Couplings and Quantitative NOE

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Limonoids, a type of tetranortriterpenoids, are the compounds responsible for the bitterness in citrus. They are highly oxygenated modified terpenoids and are derived from a 4,4,8-trimethyl-17-furanylsteroid.[1,2] Several biological activities have been attributed to limonoids. These activities range from insecticidal, insect antifeedant, antibacterial, antifungal, anticancer and other pharmacological activities on humans. Limonoids are common in the Meliaceae family.

Nuclear magnetic resonance (NMR) is arguably the most important, analytical tool for the structural elucidation of natural products. The constitution of natural products can be straightforwardly determined using a combination of multinuclear and multidimensional experiments, while their relative configuration is normally determined using 3J(HH or CH) coupling analysis, distances obtained from nuclear Overhauser effect (NOE) and residual dipolar couplings (RDCs).[3]

In the present work, seven new protolimonoids from the heartwood of Xylocarpus rumphii were extracted, purified, acetylated and characterized. The sample of heartwood was air dried and extracted with dichloromethane. A combination of thin layer chromatography and column chromatography over silica gel with hexane/ethyl acetate step gradient elution was used to purify the compounds.

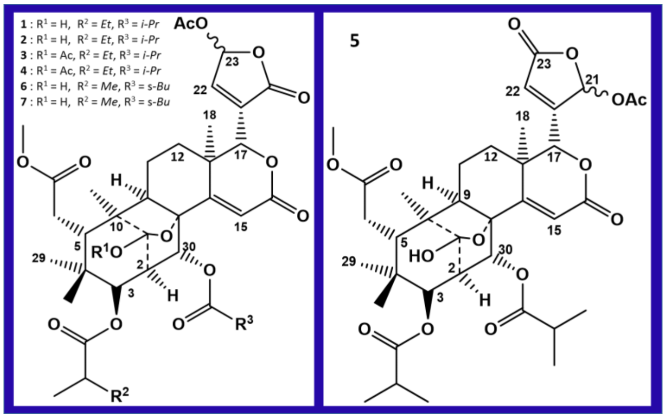

Figure 1. Structure of protolimonoids 1-7.

The seven new protolimonoids that where isolated and characterized are presented in figure 1. The configuration at C23 for compounds 1-4, 6 and 7 could not be solved by conventional NMR methods (3JHH and NOE). Each compound, except for 5, has their corresponding epimer at C23: 2 is the epimer of 1, 4 is the epimer of 3, and 7 is the epimer of 6. Compounds 1, 3 and 6 show the signal corresponding to H23 at 7.04 ppm while for 2, 4 and 7 it resonates at 6.93 ppm.

Configuration at C21 of protolimonoid 5 was determined using a combination of selective and quantitative NOE interactions and molecular modeling. A set of selective 1D NOESY experiments were used to obtain the desired NOE interactions.[4] The two epimeric structures at C21 (5R and 5S) were generated in the Macromodel Suite and a conformational search was performed for both epimers. Each configuration yielded only one preferred conformation, which should show unique NOE interactions for each configuration. Based on this, the quantitative results of the NOE interaction between H22 and H12α,β were enough to determine the configuration at C21 as S.

The configuration at C23 for the other six protolimonoids could not be determined by NOE due to the r-6 dependence of this effect and the distance of the chiral center at C23 with the C18 methyl group. RDCs were used to solve the problem due to their non-local character.[5]

One-bond proton-carbon coupling constants (1JCH) and total proton-carbon splitting in anisotropic conditions (1TCH) were extracted from F1 1H-coupled J-Scaled HSQC spectra.[6] Anisotropic conditions were obtained using cross-linked poly(methyl methacrylate) (PMMA) gels swollen in CDCl3 using reversible compression/relaxation method previously described.[7, 8]

The structures of the two epimers at C23 (6 and 7) were generated and minimized with the hybrid functional B3LYP and 6-31G as the basis set. Singular value decomposition (SVD) fitting of the RDC data of 6 and 7 to the above computer generated structures was performed using MSpin.[9] As a result, the configuration at C23 was determined as R for 6 and S for 7. In consequence, it is now possible to state that all of the other compounds with H23 resonating at 7.04 ppm have R configuration (compounds 1 and 3), while the compounds with H23 resonating at 6.93 ppm have S configuration (compounds 2 and 4).

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The Interaction of O-acetylserine Sulfhydrylase with Peptides: Structural Insight by STD-NMR and Docking

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The last step of cysteine biosynthesis in bacteria and plants is catalyzed by O-acetylserine sulfhydrylase (OASS). In bacteria, two isozymes with similar binding sites, O-acetylserine sulfhydrylase-A (CysK) and O-acetylserine sulfhydrylase-B (CysM), have been identified, whose respective specific functions are still debated. OASS plays a key role in the adaptation of bacteria to the host environment, in the defense mechanisms against oxidative stress and in antibiotic resistance. Since mammals synthesize cysteine from methionine and lack OASS, the enzyme is a potential target for antimicrobials1,2.

In this work STD-NMR was applied to study the interaction of the inhibitory pentapeptide MNYDI with CysK and CysM from Salmonella typhimurium (StCysK and StCysM) and with CysK from Haemophilus influenzae (HiCysK). The structure of HiCysK in complex with MNYDI has already been solved3 and served as an internal control for method optimization. On the contrary, no three dimensional structure of either StCysK or StCysM in complex with reversible ligands has been solved to date.

In all the complexes, STD experiments indicate that protons of Ile5 side chain contribute for a large fraction to the binding energy. This result is in agreement with mutagenesis studies, where removal and/or substitution of the C-terminal Ile of CysE/peptides consistently prevents complex formation4 and with computational analysis.5

In the case of StCysK, the enzyme form showing the highest affinity for MNYDI peptide, a significant saturation of arylic protons, in particular protons 2,6 of Tyr3, was observed in the STD-NMR spectrum. These data are in good agreement with docking studies and structure-activity relationships, that suggested a pivotal role played by an aromatic residue at position P3 for high affinity binding.

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Ritonavir Solution Structure and Relationship to Crystal Polymorphs

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Ritonavir is a potent inhibitor of HIV protease [1] that was introduced in 1996. During development only polymorph form I was found, but in 1998 the more stable form II polymorph spontaneously appeared [2,3]. This less soluble crystal form compromised oral bioavailability and caused the temporary removal of the drug from the market. Polymorphs I and II differ in both hydrogen-bond arrangements and molecular conformation [2]. Here, the dynamic 3D-structure of ritonavir in solution is reported and its relationship to the crystal polymorph conformations is discussed.

The recently reported method for determining dynamic solution structures [4] was used to measure the ritonavir structure. Data was recorded in d8-ethanol to mimic crystallisation conditions. With 19 rotatable bonds, ritonavir oscillates about a great variety of conformations in solution, ranging from largely extended to considerably curled shapes. Many bonds display multi-modal conformational behaviour. NMR analysis is further complicated by slow E/Z conformational exchange at the carbamate group and fast E/Z exchange at the urea group. The structure was solved for the predominant E-configuration of the carbamate (85%), which is present in the form I polymorph.

Although form I and form II have different crystal conformations compared to each other, when considerd bond by bond all but one bond are adopt conformations that are well represented in solution, i.e., ritonavir crystallises in basically preferred conformations for both polymorphs. Interestingly however, the specific set of conformations for all bonds together is not present in solution for either form I or form II, indicating that a global level of conformational strain is induced in the molecule upon crystallisation even though locally there is little strain. This global tension is likely to be a factor in the similar energies between the forms and may relate to why it took so long for the form II polymorph to originally manifest.

In light of these data, comparison between solution conformations and known crystal polymorphs is proposed to be of general usefulness in assessing whether the set of known polymorphs for a drug is likely to contain the most stable polymorph and also whether there are likely to be other metastable forms that are as yet unknown.

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Dynamic Solution Structure of Suvorexant

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Suvorexant is an orexin receptor antagonist approved last year by the FDA for the treatment of insomnia. Although it binds to both orexin-1 (OX1) and orexin-2 (OX2) receptors, most of its physiological benefits in promoting sleep are mediated via its interaction with OX2 [1]. The crystal structure of suvorexant bound to OX2 has recently been published and provides valuable information for understanding the perceived binding mode of this drug [2]. Prior to this, conformational analysis based on the small molecule crystal structure, NMR data, computational chemistry [3] and targeted synthetic approaches had been unable fully to explain how the preferred conformation of suvorexant in solution is related to the activity of conformationally restricted analogues [4] or indeed to the binding of suvorexant to the OX1 and OX2 receptors.

Suvorexant is a challenging molecule to study by NMR because it has a flexible aliphatic 7-membered ring at its core as well as sites of restricted conformational exchange that result in multiple slowly exchanging forms in solution. To complicate the picture yet further, there is a dearth of hydrogens at critical points adjacent to the exchange sites, little chemical shift resolution and each form has its own internal conformational dynamics. Here we present in detail how we have untangled these forms using both new and old NMR methods and precisely measured all the individual dynamic shapes.

The dynamic solution structure of suvorexant gives fresh colour to the static picture presented by X-ray crystallography and resolves many of the questions of the relationship between the solution conformation and binding affinity of suvorexant and its analogues. These data will significantly help the creation of the next generation of orexin antagonists and, furthermore, enable design of selective OX1 or OX2 antagonists.

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Shuttling Motion in Rotaxane Systems Studied by EXSY NMR

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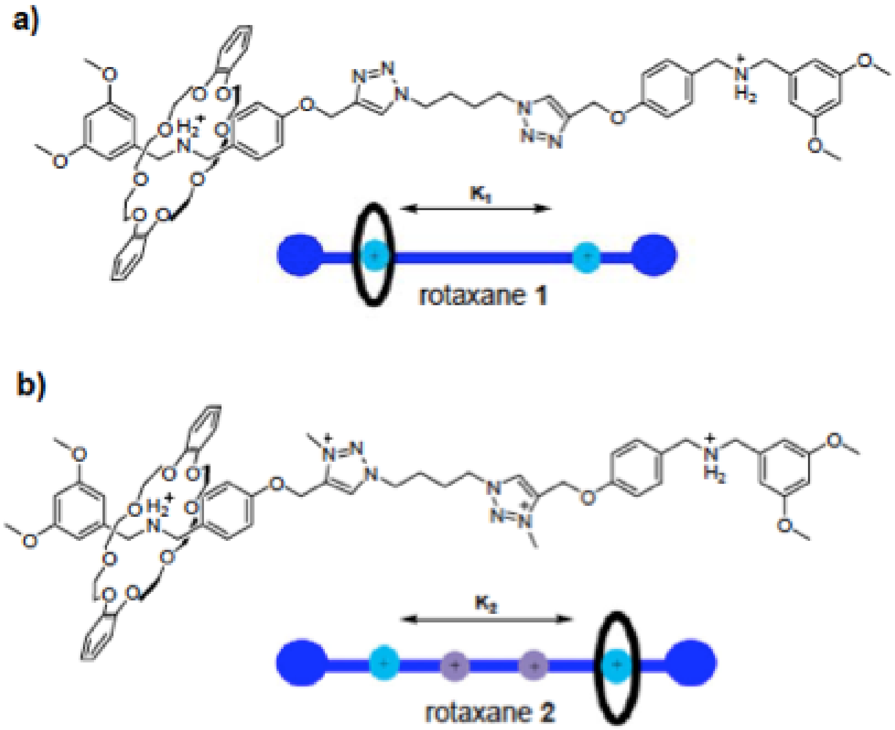
The mechanically-interlocked hydrogen-bonding rotaxane systems [1] used in the present study are composed of a dibenzo-24-crown-8 (DB24C8) macrocycle which can undergo a slow shuttling motion between two dibenzylammonium (DBA) stations (rotaxane 1). The methylation of the triazole moieties of rotaxane 1 gives rotaxane 2 comprising two methyltriazolium stations (MTA). The rate of macrocycle motion between the two DBA stations was monitored by EXSY NMR in order to estimate the exchange constants K1 and K2.



The assignment of resonances for both systems was achieved by combining 2D NMR experiments (TOCSY, HSQC, HMBC, NOESY). For each rotaxane, two sets of signals are observed suggesting the formation of supramolecular complexes between the chains and the macrocycles in which the motion is slow along the molecular axle on the NMR timescale. NOE correlations permit the unambiguous localization of the macrocycle between the two benzyl rings for 1 and 2, as depicted in the scheme above.

Increasing the temperature of the samples 1 or 2 to 70°C (343 K) did not induce the coalescence of each pair of signals.. However, EXSY NMR spectra exhibit cross peak signals due to exchange suggesting that the ring is in motion along the chain. The analysis of the evolution of each cross peak signals observed between a pair of signals depending upon the chosen mixing time affords an estimation of the rate constants using the initial rate method [2].

Molecules	k (333 K) s^{-1}	k (343 K) s^{-1}
1	0.07±0.01	0.13±0.02
2	0.23±0.03	0.38±0.03

The data obtained suggest that the introduction of two charged methyltriazolium stations increases the shuttling motion of DB24C8 between the two DBA stations suggesting that the two charged MTA stations accelerate the movement.

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61 Sparse Principle Component Analysis (PCA) Adapted to NMR Metabolomics

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In NMR metabolomics, sets of spectra of a mixture of metabolites, e.g. blood plasma or urine, from various individuals are analyzed. Peak intensities corresponding to various compounds vary from spectrum to spectrum, which is associated with changes in the composition of a mixture. These variations are usually too complicated to be recognized and explained by sight. Thus, various techniques have been suggested to identify varying peaks of spectra, group them according to the compounds they refer to, find correlations between the changes of concentrations and divide the samples into particular groups. These groups will represent deviations of metabolites possibly connected to illnesses or other factors [1].

One of the techniques used for this aim is Principal Component Analysis (PCA). Mathematically, a data matrix X of dimensions (n x p) of n spectra of metabolites, each consisting of p spectral points, has to be analyzed (usually p>>n). PCA searches for a directions of highest variance in the data and constitues a new basis for such a representation of X that: 1) requires less dimensionality, 2) easily allows to divide the set of samples into meaningful categories. Instead of p dimensions used for initial data representation, only few axes in a suitable basis are sufficient to explain the majority of the variance of spectral points from sample to sample. Such axes are called principal components. They correspond to the eigenvectors of the covariance matrix of data X.

If only a small part of p variables contribute to the meaningful variance of data, which is the case with NMR spectra, it is convenient to use sparse PCA [2]. It looks for principal component vectors with mostly zero entries, which can approximate the eigenvector decomposition of X well enough. Various algorithms were proposed for sparse PCA realization outside the NMR context [3].

Here, we discuss another modification of sparse PCA designed especially for NMR spectra. It takes the assumption of Lorentzian shape of spectral peaks. The idea is based on one proposed in [4], where the authors introduce iterative thresholding on components into a certain variation of PCA (QR algorithm). We suggest substituting subtraction performed by thresholding at each iteration with placing Lorentzian peaks of adjustable widths above a threshold. Thus the components will better fit for the shape of spectra, providing a more accurate approximation and an easily interpretable result.

We discuss the features of the proposed method on the example of both simulated spectra and a real dataset from a medical study.

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DFT Calculations of NMR Chemical Shifts and J(F,C) Coupling Constants of Ciprofloxacin

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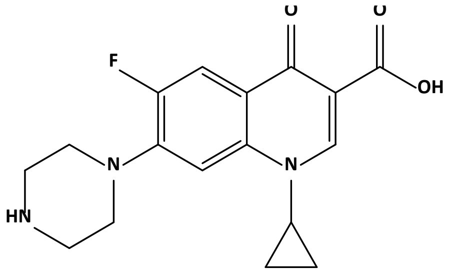
Ciprofloxacin is a fluoroquinolone antibiotic. Recently, a series of novel benzamidomethyl derivatives of ciprofloxacin as potential prodrugs have been synthesized with the aim of higher lipophilicity than the leading compound [1].



To the best of our knowledge, few experimental and theoretical data about C-F coupling constants were reported up to now. That is why, we decided to test the possibilities of several calculation methods and in particular, to analyse computational contributions to the values of the 3J(F,C) coupling constants. A Karplus type relationship was supposed for the F,C coupling constants. The rigid planar structure of the fluoroquinolone moiety excludes variation of the dihedral angles. Yet the two 3J(F,C) showed differences of about 4.5 Hz.

Theoretical computations using different basis sets show the influence of piperazine ring twist on the 3J(F,C) coupling constants. The application of the solvent reaction field using the PCM [2] (Polarizable Continuum Model) method include the effect of the solvent DMSO. The experimental values and the results of the different level of theory were discussed.

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Application of NUS to monitor magnetization transfer

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Nuclear Magnetic Resonance (NMR) spectroscopy is a highly informative tool for chemical analysis. Its extension to multiple dimensions (ND) increases its versatility. Unfortunately, multidimensional NMR is limited by the long acquisition time required. Hence, it cannot be easily employed in quick,serial measurements and thus time resolved spectroscopy was till date limited to 1D NMR. Time-resolved non-uniform sampling (TR-NUS) has been previously shown as a method to monitor chemical reactions on the fly [1,2,3]. The idea behind (TR-NUS) is to apply Compressed Sensing to overlapping subsets of the non uniformly sampled dataset collected using dynamically changing sample. Compressed Sensing algorithm exploits the fact that NMR spectrum is sparse ("mostly empty"). In the resulting set of 2D spectra, time acts as the third pseudo-dimension. This enables one to monitor the changes occurring in a sample with time.

In this project we have further extended the idea by implementing it in a NOESY pulse sequence to monitor the change of magnetization transfer due to NOE with varying mixing time. Incrementation of mixing time in parallel to t1 was initially introduced by Macura et al. [4] as a method to distinguish between J peaks and NOE peaks and was later applied to study NOE build up[5]. In this project Mixing time in the pulse sequence is incremented linearly and in parallel to the random sampling of the FID in indirect t1 dimension. Subsets of these NUS measurements are formed and several spectra corresponding to these subsets are reconstructed using CS. This stack of spectra enables one to plot the NOE build-up curve in a continuous manner and effectively calculate the interproton distances. By artificially extracting points from the FID we have also shown the effect of rate of change of mixing time and consequently on the t1 noise of the spectra. With theoretical considerations and simulations we have shown the effect of sweep rate of mixing time on the signal to noise ratio.

The advantage of the method is the reduced time required to acquire TR-NUS experiment in comparison to several short conventional NOESY experiments. In this work, we discuss the pros and cons of TR-NUS applied to NMR techniques providing with varying peak intensity. Application of TR-NUS in a spectrum with high dynamic range of intensities required special optimizations of a pulse sequence in order to minimize t1 noise and account for the longitudinal relaxation with change in mixing time in every increment. Both method and its optimizations are discussed on the example of the rigid organic molecule strychnine. The resulting interproton distance values were found to be remarkably close to literature [6].

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NMR Characterization of Bcl-2 Family / Meiogynin A Complex for Drug Design

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Apoptosis is an essential physiological process for the selective elimination of cells, which is involved in a variety of biological events. The Bcl-2 family is the best characterized protein family involved in the regulation of apoptotic cell death, consisting of anti-apoptotic, such as Bcl-2, Bcl-xL, and Mcl-1, and pro-apoptotic members, as Bax, Bad, Bid and Bak [1]. A deregulation of apoptosis pathway can promote cancers and autoimmune diseases, which are great public health concern.

Since decade, the regulation of the apoptosis pathway via the Bcl-2 family is a very important area of research in oncology and various therapeutic strategies targeting this family were studied. The most convincing results were obtained by the elaboration of "small molecule inhibitors" called also SMIs. These compounds interact with anti-apoptotic protein and allow the salting out of pro-apoptotic protein, so restoring the apoptosis. At present, three compounds are in clinical trials and about ten in preclinical essays. Theses inhibitors can be classified in two groups: (i) the selective inhibitors of an anti-apoptotic protein as ABT-263 [2], and the compound WEHI-589 [3] for Bcl-xL, or ABT-199 [4] for Bcl-2; (ii) The pan-inhibitors which target several anti-apoptotic proteins (Bcl-2, Bcl-xL, Mcl-1) such as gossypol [5], the acetonapto [6] and ABT-263 derivatives [7]. They are active at the level micro-molar on target protein. The aim of the project is the development of a Bcl-xL and Mcl-1 pan-inhibitors more active than those described in the literature, from a natural product: the meiogynin A [8].

In order to meet our objectives, we used different techniques as: (i) organic chemistry to set up a selective synthesis of the desired compounds, (ii) microbiology and biology to produce the anti-apoptotic proteins and to evaluate the activities of the derivatives on these proteins, (iii) nuclear magnetic resonance (NMR) to determine precisely the binding site of meiogynin A and of the synthetic compounds within the target proteins, and (iv) molecular modelling to make drug design, docking meiogynin A in Bcl-xL and Mcl-1 to provide chemical modifications to make them more active.

In this poster, we focus on structural characterization of the Bcl-xL/meiogynin A complex by NMR and the different steps allow us determine the orientation of ligand on Bcl-xL. For this,we performed titration experiments on the 13C-15N Bcl-xL in presence or in absence of meiogynin A/or derivatives. The HSQC analysis confirmed a specific binding of meiogynin A or derivatives on Bcl-xL. We were able to identify the residues of the protein involved in the interaction with the meiogynin A. This interaction is compatible to an intermediate NMR exchange regime. Moreover the 13C-15N filtered 1D experiment suggests that meiogynin A is an intermediate NMR exchange regime also.

From the titration data, molecular modelling of Bcl-xL/meiogynin A complex data, and NMR paramagnetic enhancement relaxation (PRE) experiments recorded on Bcl-xL in presence of meiogynin A derivative modify by a paramagnetic probe: TEMPO, we were able to orient the meiogynin A and derivatives in the hydrophobic pocket of Bcl-xL.

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Practical Applications of Fast Field Cycling NMR Relaxometry

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The fast field cycling (FFC) NMR relaxometry method allows determination of the spin-lattice relaxation time (T1) continuously over five decades of Larmor frequency. The method can be exploited to observe the T1 frequency dependence of protons, as well as any other NMR-sensitive nuclei, such as 2H, 19F, 7Li, 23Na, etc. in a wide range of substances and materials. The information obtained is directly correlated with the physical/chemical properties of the compound and can be represented as a 'nuclear magnetic resonance dispersion' curve.

Molecular dynamics are critical to understanding intra- and inter-molecular events that affect molecular interactions in condensed phases. The time scales of intra- and inter-molecular events affect nearly all aspects of molecular function and the properties of materials. One of the most efficient means for characterizing molecular dynamics at long time scales is FFC NMR relaxometry. The magnetic field dependence of the nuclear spin-lattice relaxation rate constant, 1/T1, provides a direct measurement of the spectral density functions that characterize molecular motions [1,2].

In this poster we will present some academic and industrial applications showing the relevance of exploiting FFC NMR relaxometry in various materials to study the molecular dynamics or, simply, for fingerprinting or quality control purposes.

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NOMAD – NMR Online Management and Datastore

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In last few decades NMR spectroscopy became a routine tool to investigate the properties of organic molecules for many chemists and biochemists. In order to cope with high demand for NMR experiments, research institutions can possess several NMR spectrometers that are often placed in centralised NMR facility that operates fully or partially in open access environment. Furthermore, modern NMR instruments are usually equipped with sophisticated automation that enables 24/7 operation and hence facilitates high throughput and utilisation. The NMR laboratories set up in this way can run thousands of experiments every month and provide service to hundreds of researches. The software for automation control provided by instrument vendor usually offers rather elaborate functionality that works well only for one isolated instrument with rather small user base and does not address data management and traffic control issues that can arise in modern high throughput NMR labs.

We present here a working prototype of web-based system that provides a resilient searchable data-store and centralised control of automation for 6 Bruker instruments of various vintage in 24/7 open access environment that serves currently about 150 users. The system is controlled using portals that are easily accessible over WWW from anywhere using secure login per individual user via Active Directory Service. A single user friendly portal can be used to check the queue status and submit experiments on any instrument in the lab. The admin portal provides a centralised dashboard for traffic control& queue management, centralised database of users, research groups and experiments, usage activity reports for accounting etc. NMR data are resiliently stored using ZFS file system. Corresponding metadata are organised in MySQL database. That enables an instant one click search and viewing of generated results. Furthermore there are portals for batch submission_posters of NMR samples from teaching labs and upload of manually acquired data. The whole system is still work in progress and further useful features are under development.

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Matrix-Assisted DOSY as a Tool for Chemical Characterization of C. mexicana

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Chemical analysis and standardization of extracts from medicinal plants is one of the most complex tasks in natural products research, due mainly the wide diversity of components, whereas identification and quantitation of the main constituents that could be used as specific markers is an relevant issue [1].

Matrix-Assisted DOSY (MAD) NMR has been established as an useful tool in mixtures analysis [2,3]. The present work deals with the use of MAD experiments. Previously we have shown that introducing a co-solute which differentially interacts with the solutes in a mixture, allowed us to resolve aromatic components in the diffusion dimension [4], using two well established solutes for flavonoid-like compounds: SDS and TFA to assist in the diffusion coefficient separation [5,6].

Our target was the aromatic region focusing on flavonoid components in an aqueous or methanolic extract of Chrysactinia Mexicana A. Gray (Asteraceae). Results showed that using MAD methodology provides reliable results for complex mixtures analysis in the characterization/standardization of medicinal plants.

Acknowledgement: The work was supported by CONACyT (Grant No. 179139).

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Molecular Dynamics in Selected Polyaromatic Compounds Monitored by Measurement of Selected Structural Parameters

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The polycyclic aromatic compounds attract scientific attention namely due to their conjugated π-π aromatic systems which promise many interesting and unusual optical and electronic properties. Some of these compounds were shown to be effective in the development of materials useful e.g. in molecular-based electronics.[1]

The electronic structure of these compounds is studied mainly by theoretical approaches.[2] The theoretical results can be compared and also correlated to some experimentally accessible parameters (e.g. aromaticity vs spin-spin coupling constants and/or bond lengths). The experimental data reflecting current electron distribution between given atoms can be obtained either by detail X-ray structure analysis and/or by NMR spectroscopy. The results can be mutually compared and reveal the key differences in the electronic structures adopted by these molecules in the liquid and solid state. The DFT calculations can explain intra and intermolecular effects leading to different structures in solid and liquid state.

Acknowledgement

For the financial support, the Czech Science Foundation (Project No. 15-12719S) is gratefully acknowledged.

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NMR Aerosolomics: Novel NMR Method for Organic Aerosol Analysis

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Aerosolomics provides complex evaluation of aerosol composition and compound concentration. It is exploiting metabolomic approach, which is applied to aerosols [1]. In our laboratory a novel NMR method for organic aerosol analysis was developed and tested. The method is focused on water soluble organic compounds (WSOC), which is the least examined group of organic aerosols. The potential of NMR spectroscopy for aerosol composition has not been fully exploited and the use of NMR spectroscopy is limited to so called Functional group analysis [2]. Recently 2D NMR techniques were employed, although the analysis is very challenging [3].

NMR metabolomic approach applied in the newly developed method is based on fitting of the 1H NMR spectra of individual compounds into the complex 1H NMR spectrum of the WSOC. The assignment is based on dominant signals and precise chemical shift of the compound. Subsequently the compound spectrum is subtracted from the original aerosol sample spectrum. Application of a metabolomics software, in our case ChenomX 8.0, for this purpose is necessary. The database of ChenomX contains ca. 70 compounds which are also found in WSOC. The rest of compounds found in WSOC is being added to the database continuously.

Both real atmospheric and model samples has been analysed by the method. Around 30 – 35 compounds are usually found in the samples due to the use of lately constructed high performance impactor for sample collection. Most abundant substances are mono- and dicarboxylic acids (formic, acetic, succinic acid) and their derivatives (lactic acid), followed by carbohydrates, anhydro saccharides and sugar alcohols (levoglucosan, fructose, D-threitol) and amines (methylamine, dimethylamine). One of the samples was analyzed on four various NMR spectrometers (500, 600, 700, 800 MHz) and in the 800 MHz spectrum 50 compounds were identified.

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Conformational Analysis of a Tripeptide Catalyst using ROEs, Scalar Couplings and RDCs

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In the study of catalytic reactions, understanding the conformations of the catalyst and intermediates along the reaction pathway is advantageous when expanding substrate scope and optimizing reaction conditions. The tripeptide H-D-Pro-Pro-Glu-NH2 has proven to be a powerful catalyst in 1,4-addition reactions of aldehydes to nitroolefins [1]. Mechanistic studies showed that the reaction pathway proceeds via the expected enamine intermediate [2,3]. However, detailed information about the conformation of the catalyst and the intermediates is still lacking.

Here, we present our investigations of the ground state of the H-D-Pro-Pro-Glu-NH2 catalyst using a combination of NMR spectroscopy and computational methods. In addition to ROE data, we measured vicinal JHH and JCH coupling constants from 1H-NMR, HSQC-HECADE and refocused HMBC. We were also able to determine 1DCH and 2DHH RDCs in a stretched polyvinyl acetate gel in chloroform/methanol.

Three different computational approaches were chosen to find a conformational ensemble that matches the solution NMR data:

First, ensembles were constructed by an extended conformational search (Monte Carlo and Systematic), followed by minimization of the resulting structures in implicit solvent models. Second, the conformational behavior of the tripeptide was studied by molecular dynamics simulations. Third, the experimental data (ROE, J-coupling and RDCs) were implemented as restraints in a simulated annealing procedure with XPLOR-NIH.

The three approaches and the compatibility of the resulting ensembles with the experimental data are discussed.

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NMR Complexation Study of Cycohexylhemicucurbit[8]uril

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Recently synthesized cyclohexylhemicucurbit[8]uril (cycHC[8]) [1] belongs to a widely applied cucurbituril family [2]. CycHC[8] has a barrel shape structure with cavity diameter 8.5 Å and volume 123 Å3. Dimensions and shape of this macrocycle are promising for supramolecular applications.

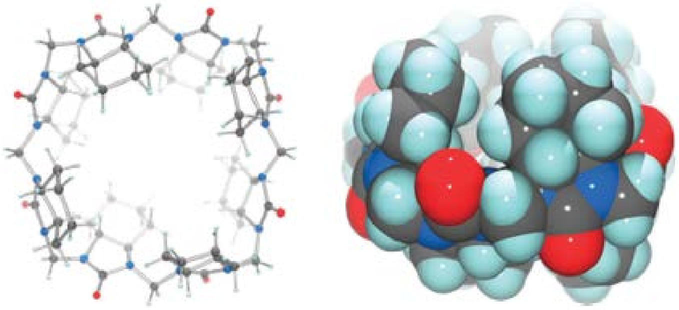

Fig.1. Crystal structure of cycHC[8]

The ability of cycHC[8] to form complexes with neutral molecules and ions (carboxylic acids, PF6-, BF4- etc.) in different solvents was investigated by means of NMR spectroscopy. For complexation study we applied 1D 1H and 19F NMR, DOSY, NMR titration and longitudinal relaxation time measurements.

Solvent screening results showed that complexation in chloroform and methanol with cycHC[8] proceeds differently.

Clear identification of host-guest complexes was observed and stoichiometry of formed complexes as well as association constants will be presented.

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Anti-Obesity Effects of Novel Analogs of Prolactin-Releasing Peptide: NMR-Based Metabolomic Study

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Obesity is currently the most serious and widespread metabolic disease in the Western world. It is associated with many health risks, particularly type 2 diabetes mellitus, increased incidence of cardiovascular diseases, and some types of tumors. However, an effective therapy is still scarce and the need for a new anti-obesity treatment has become more and more acute. It is beneficial to apply a metabolomic approach to investigate pathogenesis and therapy of obesity and associated diseases because the obesity development is manifested also at the metabolic level by changes in the biochemical pathways [1].

Anorexigenic neuropeptides produced and acting in the brain have the potential to decrease food intake and ameliorate obesity [2]. We studied anti-obesity effect of three novel lipidized analogs of prolactin-releasing peptide (PrRP) in a mouse model of diet-induced obesity. Mice of C57BL/6J strain were fed with high-fat diet for three months to develop obesity and then treated subcutaneously by three different palmitoylated PrRP analogs for two weeks. NMR spectra of urine samples, collected before and after treatment, were acquired by CPMG pulse sequence and subjected to multivariate statistical analysis.

Metabolomic fingerprinting based on PLS-DA models indicates the discrimination between groups of treated and control obese animals, caused by changes in metabolites of Krebs cycle, taurine, lactate, and allantoin. Results obtained from parametric t-test and nonparametric Wilcoxon test revealed also significant changes in levels of 1-methylnicotinamide, 3-indoxylsulphate, and branched-chain amino acids. Metabolomic data of urine were also correlated with changes in body weight, glucose, leptin and insulin levels in plasma, and with mRNA expressions of enzymes participating in metabolism of fats.

Acknowledgements

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Quick and Easy NMR Titration Using Slice-selective Experiments to Study Concentration Gradients in Agar Gels

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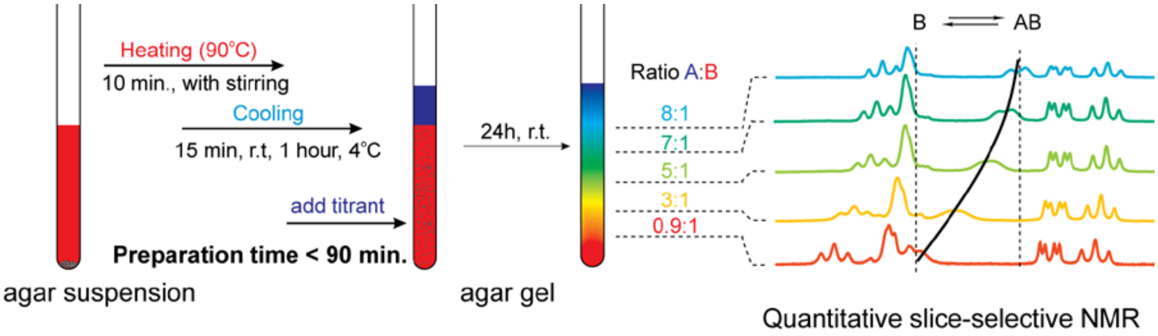
NMR titration is a long recognized method for determination of equilibrium constants or thermodynamic parameters of reactions, but suffers from the drawback of being time consuming. Recently, spatial-selective NMR spectroscopy for reaction monitoring was proposed [1]. The method relies on slow diffusion of one of the reaction components into a polystyrene gel, containing the other, thus resulting in a spatially dependent sample composition along the NMR tube. Following a similar approach we present the use of agar gels as the medium for single-experiment NMR titrations in water. It was used to study the inclusion of paracetamol in cyclodextrine macrocycle as a model reaction. The agar gels benefits from a very simple and reliable sample preparation. Moreover, we observed no interaction between the matrix and the compounds under study and obtained high-resolution spectra, identical to those obtained from solution samples.



One of the main advantages of the proposed method is its speed achieved by performing the slice-selective experiments in an interleaved manner, thus affording the acquisition of quantitative spectra in 1-10 minutes. In addition to the room temperatures measurements, the variable temperature NMR titration has been also investigated, as the agar gels possess the attractive property to lower the freezing point of water making it possible to study water solutions up to -8 C.

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Characterization of Complex Mixtures Containing PAHs

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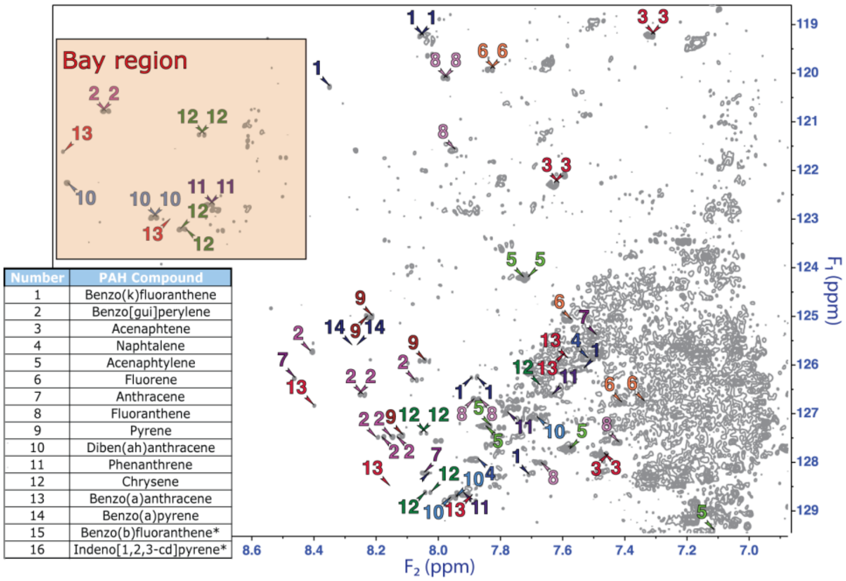
2. Service of toxicology and constructed environment, Geneva, Switzerland

Complex mixtures of polycyclic aromatic hydrocarbon (PAH) are present in many combustion residues and represent a serious health hazards because of the carcinogenicity of some of their components. Their analysis is complex because of the difficulty to separate and uniquely identify their components, in part because some candidates have identical masses.

The 1H-13C HSQC spectra of the crude extracts are very informative and are quite simple to prepare. The strong tendency of carcinogenic compounds to present signals in a relatively sparse region of the HSQC spectrum (see "bay region" in Figure 1) is particularly interesting to identify of the most relevant PAHs[1].

In the perspective of the automatic identification and quantification of large number of samples, we generated a 1H-13C NMR database of the most common PAHs. It was used to identify a number of them in a tire extract and demonstrate that it contained more PAHs than allowed by the EU regulation.[2]



Number	PAH Compound
1	Benzo(k)fluoranthene
2	Benzo[gui]perylene
3	Acenaphtene
4	Naphtalene
5	Acenaphtylene
6	Fluorene
7	Anthracene
8	Fluoranthene
9	Pyrene
10	Diben(ah)anthracene
11	Phenanthrene
12	Chrysene
13	Benzo(a)anthracene
14	Benzo(a)pyrene
15	Benzo(b)fluoranthene*
16	Indeno[1,2,3-cd]pyrene*

Figure 1: Identification of PAH in crude extract of a tire sample.

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Through-space ^{19}F-^{15}N Couplings for the Assignment of Stereochemistry in Flubenzimine

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Flubenzimine (1) is a mite growth regulating acaricide patented by Bayer in 1972 and produced in 1979 under the trademark of Cropotex. The stereochemistry of flubenzimine has never been determined; therefore the structure is commonly represented as in figure 1.

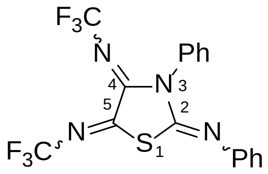

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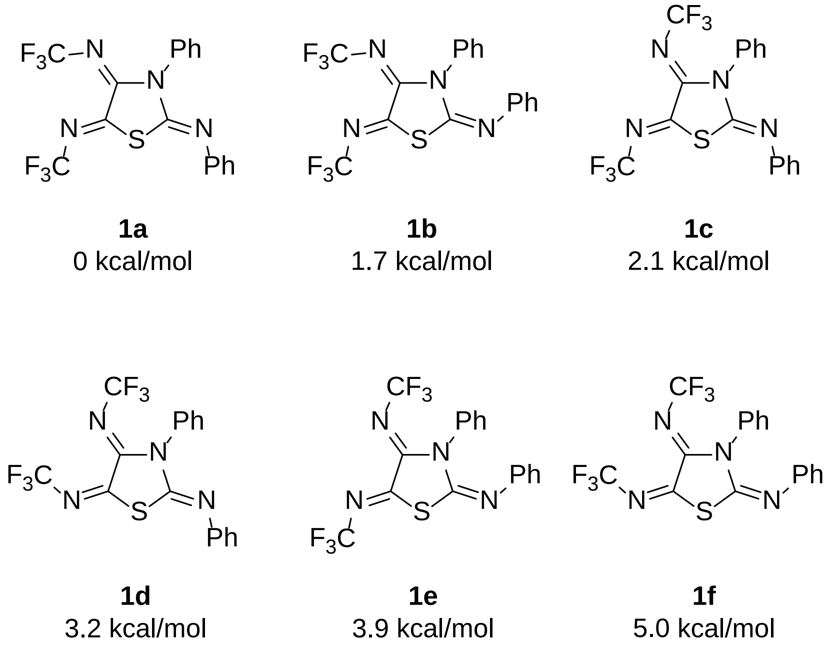
Figure 1. Flubenzimine

An MM2 calculation provides the energies of 6 of the more stable configurations, given in Figure 2. The other possible two, having the two trifluoromethyl groups facing each other, are too high in energy.



1a
0 kcal/mol

1b
1.7 kcal/mol

1c
2.1 kcal/mol

1d
3.2 kcal/mol

1e
3.9 kcal/mol

1f
5.0 kcal/mol

Figure 2. Possible configurations for flubenzimine.

An attempt to measure the chemical shifts of N4 and N5 in a 19F-15N gHMBC experiment revealed that one of the nitrogens couples with the fluorines in both of the trifluoromethyl groups, implying a coupling over two bonds and a coupling over five bonds. The latter is a through-space 19F-15N coupling, possible only in the structures where a trifluoromethyl group on a nitrogen is facing the lone pair of the other, like 1a, 1b, 1d and 1f. Further NMR investigations, described next, limited the possible structures to 1a and 1b.

A 1H-15N gHMBC experiment revealed that the phenyl with the ortho protons at 7.02 ppm is on the nitrogen at 278.3 ppm, and that the phenyl with the ortho protons at 7.69 ppm is on the nitrogen at 159.5 ppm. These chemical shifts allowed the assignment of the nitrogens as N2 and N3, correspondingly. The protons at 7.69 ppm display in a gHMBC experiment optimized for a coupling constant of 3 Hz a coupling with the carbon at 154.7. This carbon displays a cross-peak with the fluorines at -49.2 ppm in a 19F-13C gHMBC spectrum, therefore it is C4 and not C2. It is this trifluoromethyl on N4 that also couples with N5, therefore the structure is one of 1a and 1b. The complete chemical shifts assignments are given in figure 3.

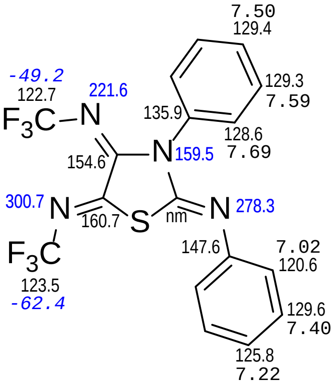


Figure 3. 1H, 19F, 13C and 15N chemical shifts assignment in Flubenzimine.

Measurement of the 19F-15N coupling constants

A 1D band-selective 19F-15N gHSQC experiment revealed the signal of F4 as a superposition of two doublets resulting from coupling with N4 and N5. The linewidths were comparable with the coupling constant, and the doublets were out of phase (figure 4), therefore a spin state selective (S3) approach was used instead.

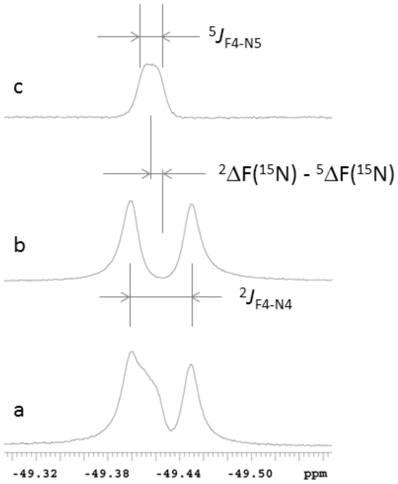


Figure 4. The signal of F4 in 1D gHSQC experiments run without decoupling, non-selective (a), with band-selection for N4 (b) and with band-selection for N5 (c).

The pulse sequence is a modification of the α/β-HSQC [1], in which the band selection is done with excitation sculpturing, and is presented in figure5.

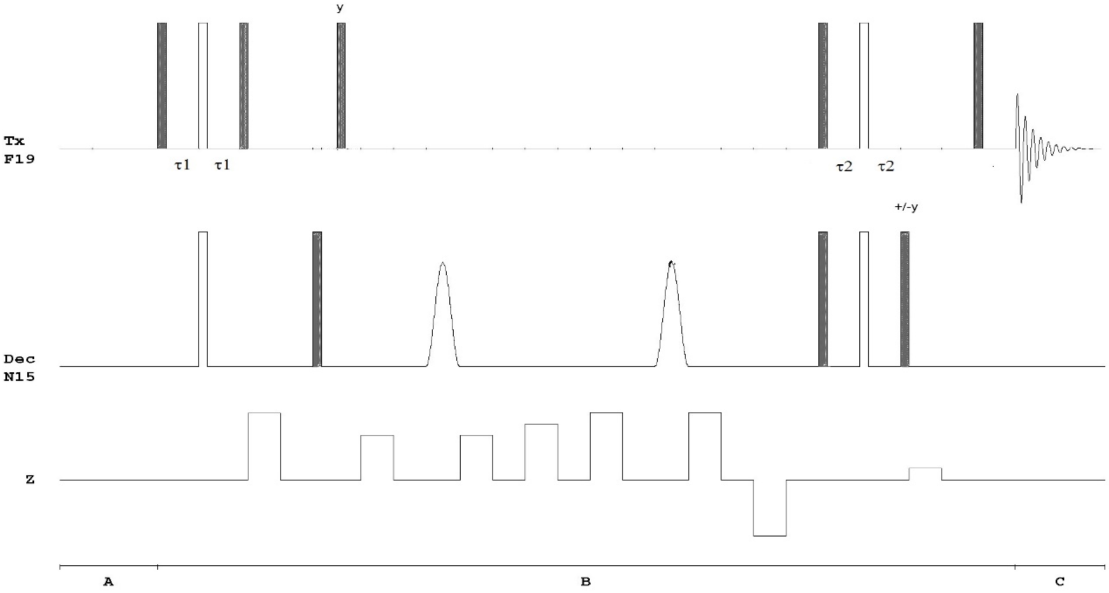


Figure 5. Pulse sequence for the spin state selective bsgHSQC (S3bsgHSQC). The filled pulses are 90°, the white ones (including the shaped pulses) are 180°. The phase of the pulses is x, unless specified otherwise. A two-step phase cycle was used, in which the first 90° decoupler pulse and the receiver are inverted on alternate scans.

The defocusing time τ1 was set to 1/(4JFN). For F3N groups, the best cancellation of the antiphase signals occurs for τ2 around 1/(10JFN) (figure 6).

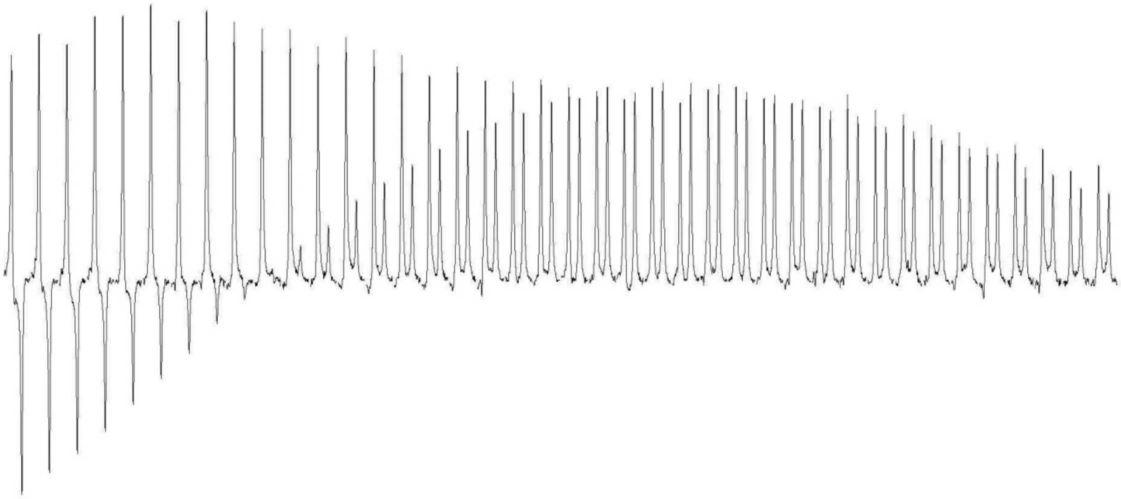


Figure 6. S3bsgHSQC spectra of F5 with selection of N5. $\tau 1$ was optimized for JFN = 20 Hz. $\tau 2$ is 0.0005 s in the first spectrum, left, and incremented with 0.0005s in each spectrum from left to right.

Two spectra were acquired for the +y and –y phases of the last decoupler pulse. The coupling constants, measured as the frequency difference between the two spectra (figure 7) are: 2JF4N4 = 23.2 Hz, 2JF5N5 = 20.0 Hz and 5JF4N5 = 7.8 Hz.

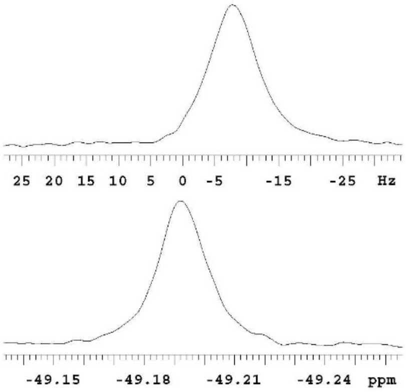


Figure 7. The signal of F4 in theS3bsgHSQC experiment with the phase of the last decoupler pulse +y (top) and –y (bottom). The frequency difference between the two spectra is 5JF4N5 = 7.8 Hz.

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n-FABS Screening from Purified Enzymes to Living Mammalian Cells

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Over the last 10 years Fluorine NMR spectroscopy has gained relevance as a reliable and efficient screening technique against pharmaceutical targets both in academia and industry. The increasing interest for 19F NMR in drug discovery is confirmed by the development of specific fluorinated chemical libraries[1] and optimized 19F NMR screening methods[2]. One of these NMR approaches is the n-Fluorine Atoms for Biochemical Screening (n-FABS)[3,4] which represents a powerful and versatile NMR functional assay. This approach requires the labeling of the substrate (or cofactor) of the enzyme with a fluorine-containing group and allows the direct measurement of the conversion of the substrate (S) into the product (P). n-FABS has been successfully applied to the identification of inhibitors and measurement of their inhibitory strength against a large number of different purified enzymes like caspases, kinases, proteases etc.[5-8]. Aim of this work is to highlight the importance of n-FABS for identifying inhibitors against those targets that are difficult to purify such as membrane proteins. We report our screening results against the membrane-bound enzyme fatty acid amide hydrolase (FAAH), using the purified enzyme, an enriched membrane preparation, and living mammalian cells.

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NMR Imaging Studies Explain the Presence of Isotropic Solvent at Full Degree of Compression in PMMA Gels

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The use of cross-linked polymethylmethacrylate (PMMA) gels[1] in a reversible compression/relaxation protocol[2] proved to be very successful for the measurement of Residual Dipolar Couplings (RDCs) in an fast, reusable, and tunable way.[3-4] When using this methodology, a certain proportion of isotropic solution is always observed even at full compression. This may sound to be a shortcoming, but in many cases, the ability of seeing both the isotropic and anisotropic peaks of the solute leads to the extraction of RDCs from the same spectra. In some particular cases, this isotropic component can superimpose with the anisotropic signal, making the extraction of RDC values difficult. Hence we decided to investigate the physical location of the isotropic gel in the tube in order to improve the physical behavior of the gels.

PMMA gels where synthesized using different amounts of cross-linker. 1D 1H and 2H NMR spectra, as well as 2H 2D Images[5] in the z-axis were taken to monitor the homogeneity of the quadrupolar coupling at different degrees of compression. In addition, the gels were also analyzed by microimaging NMR techniques by selecting 5 mm slices at different heights using a triple-gradient imaging probe.

The 1D images and micro-imaging slices show that the diameter of the fully swollen gel is smaller than the inner diameter of the 5 mm NMR tube and after certain degree of compression they wave and the isotropic solvent is trapped in pockets generated by folding of the gel sticks. The amount of remaining isotropic solvent depends on the cross-link density of the gels as well as on the length of the polymer stick. The amount of isotropic solvent is minimized but not totally removed even at full compression

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Short-term and Long-term Effect of Antidiabetic Interventions on the Urinal Metabolic Profile in Obese Mice

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Obesity has been nowadays regarded as common phenomena but it can be accompanied by several life-threatening defects (hyperinsulinemia, dyslipidemia or hypertension) [1] caused by metabolic interactions throughout the body. Thus, multiple therapeutic interventions hitting particular metabolic pathways might be applied in the treatment of obesity and its concomitant impairments, summarily marked as metabolic syndrome.

Glucose parameters has to be controlled in obese individuals, because obesity is very frequently coupled with type 2 diabetes mellitus (T2DM). Therefore, antidiabetic interventions have to be considered in the comprehensive therapeutic approach. Metformin and vildagliptin or their mutual combination belong to the most prescribed agents applied in the therapy of T2DM. They do not promote weight gain what makes especially the combined therapy very favorable in obese patients [2].

We used NMR metabolomics approach to track the quantitative and qualitative metabolic changes in urine caused by antidiabetic therapy (by metformin, vildagliptin and their combination) in mouse model of diet-induced obesity. Two independent experiments were performed with mice kept on high-fat diet. These experiments differed only in the duration of interventions and their onset.

Urine samples were analyzed by 600 MHz spectrometer using 1D-NOESY, CPMG and J-resolved pulse sequences. Mice were also examined by standard biochemical tests (oral glucose tolerance test - oGTT, measurement of insulin and leptin levels). Whilst two weeks interventions did not show any significant changes in biochemical parameters which would prove the therapy effectiveness, NMR spectroscopy was able to capture differences between obese control and treated group. Prolongation of the therapy up to seven weeks substantially improved discrimination of control mice from treated mice. Particularly, the mice treated with combination therapy achieved in oGTT the same glucose levels as non-obese mice.

PLS-DA combined with parametric t-test and non-parametric Wilcoxon test revealed metabolites responsible for differences between control obese mice and treated mice. The most affected metabolites were lactate, acetate, branched-chain amino acids (e.g. N-isovaleroylglycine), N-phenylacetylglycine, and

N-carbamoylalanine. The treatment hit also the methylamine metabolic pathway including methylamine, dimethylamine and trimethylamine.

Acknowledgements

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Chemical Exchange in Small Molecules studied by STD NMR and SSTD NMR: From Intermolecular Protein-Ligand Interactions to Intramolecular Processes in Organic Molecules

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Chemical exchange underlies very relevant bio- and physicochemical processes in which small molecules participate (e.g. drug-receptor interactions, conformational rearrangements, etc.). In the simplest case, a small molecule is in equilibrium between two different states (two-sites chemical exchange). In NMR we can monitor the motion of the nuclei from one environment to another, accessing to the chemical kinetics of the system. Fast chemical exchange (at least in the NMR relaxation time scale) is particularly interesting as it allows the observation of properties “transferred” from one molecular state to another [1]. In the particular case of saturation transfer experiments, we detect the transfer of polarization from one, selectively, perturbed state to the other, unperturbed, one.

Saturation transfer difference spectroscopy (STD NMR) [2,3] is a powerful technique for the structural and kinetics studies of intermolecular protein-small molecule interactions, and we have used to study relevant protein-ligand systems. What is more, we have developed a novel approach (SSTD NMR) [4] to obtain the kinetics parameters of uni-/intra-molecular chemical exchange processes in challenging small organic and organometallic molecules.

In particular, here we show first our results from STD NMR to characterize the complexes of novel inhibitors of Cholera toxin B (CTB), and provide evidences on how STD NMR is a straightforward tool to verify the proposed “three finger pharmacophore” requirement for the inhibition of the cancer related protein-protein interaction between p53 and MDM2. On the other hand, we demonstrate for the first time how a novel STD NMR based approach (spin saturation transfer difference NMR, SSTD NMR), is a powerful methodology to obtain kinetics parameters in intramolecular processes, exemplified on some organic and organometallic small molecules.

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Application of Homodecoupled 2D Experiments to the Analysis of a Mixture of Carbohydrates

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The signal overlap in proton spectra can significantly hamper their analysis. Homonuclear decoupling [1] leads to a simplification of multiplets into singlets, improving the spectra resolution by almost an order of magnitude.

We applied the DIAG sequence [2] yielding a clean 2D homonuclear spectrum with only diagonal signals. The F1 dimension was homodecoupled either using the modulated Zangger-Sterk (nemo-ZS) [2,3] or the PSYCHE [4] thereby extremely simplifying the spectral analysis. Moreover, the spectra can be acquired in a few minutes when using spectral aliasing.

The 1H-1H correlations can be restored by adding an in-phase magnetization transfer in the mixing period. The TOCSY mixing is the obvious choice [3] but this often results to complex spectra. We therefore introduced F1-homodecoupling in the CLIP-COSY [5] sequence to obtain spectra showing only the correlations of directly-coupled protons. We illustrated the power of these experiments to analyze a mixture of carbohydrates.

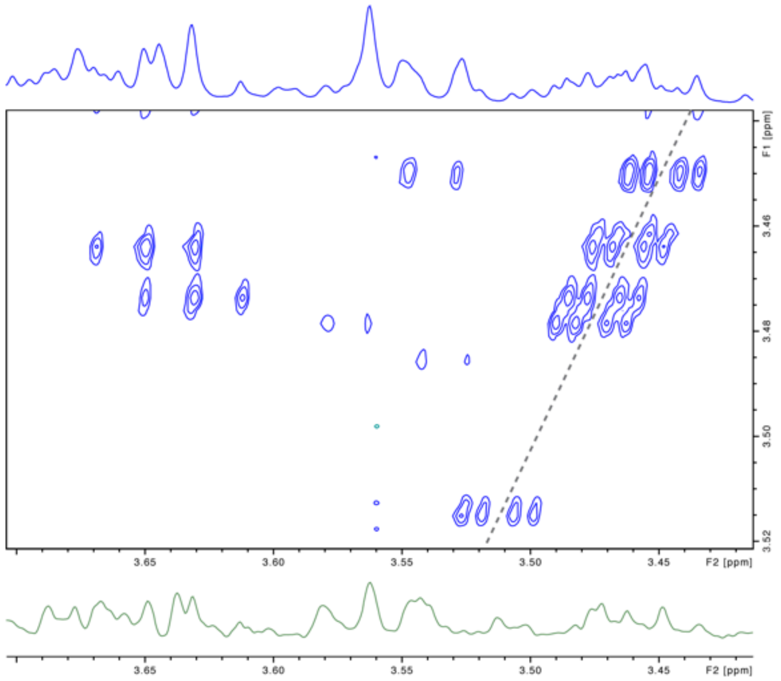

Figure 1: Selected region of a PSYCHE-CLIP-COSY spectrum of a mixture of carbohydrates.

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Organic Purity of Sulfonephthalein Dyes Used for Inorganic Carbon System Analysis

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Investigations were made on a series of seven sulfonephthalein indicator dyes which are currently used for seawater pH analysis (meta-cresol purple, thymol blue, cresol red, phenol red, bromocresol purple, bromocresol green and bromothymol blue) to determine their feasibility for use as a seawater pH reference material. Dye purity was assessed with nuclear magnetic resonance spectroscopy (NMR) and high performance liquid chromatography-mass spectrometry (LC-MS). LC-MS results indicate that meta-cresol purple, phenol red, and thymol blue contain less than 2% of impurities which interfere with seawater pH measurements. LC-MS results revealed that individual batches of the brominated dyes contain between 10 – 40% of other components (impurities) which absorb at the indicator maximum wavelengths. These results suggest that brominated indicators are more susceptible to batch-to-batch uncertainties in pH measurements. Mass spectroscopy and NMR spectra of isolated components were compared to computationally derived NMR spectra of synthetic byproducts to determine the identity of the major impurities.

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Solid State and Liquid NMR Characterization of Zn and Mg Metal Complexes of Diacetylcurcumin

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The study of metal complexes of curcumin, its derivatives and analogs, have reached a substantial level of relevance due to their implication in several fields such as biochemistry, pharmacology, materials science and theoretical chemistry among others. [1].

Our search for homoleptic metal complexes of curcumin and derivatives was directed to the synthesis of metal complexes of the physiological metals Magnesium, Zinc and Calcium.

A first approach was carried out by treatment of variable amounts of diacetylcurcumin (1) and Zn(OAc)2. After equilibration in methanolic solution, the precipitated residues were submitted to CP-MAS (TOSS) measurements. The changes observed in the solid-state NMR spectra were related to the nature of the organic-metal interaction which appear to go from supramolecular to a coordination compound. Changes in both signals shape and chemical shifts were observed going from small ratios metal:organic substrate (0.1:1) up to a well defined spectrum after reaching the 1:2 ratio.

The synthesis of the first pure complex of diacetyl curcumin with magnesium was achieved by crystallization and its structure determined by X ray crystallography. (fig). The liquid NMR spectra of the Mg complex is discussed in relation to the Zn complex in solution.

Furthermore, it was found that stable complexes of diacetylcurcumin with Mg and Zn can be easily observed with good resolution in liquid NMR in DMSO at room temperature. The chemical shifts changes are quite distinctive for each complex in solution as well as those observed for solid state CP-MAS spectra.

The combined use of CP-MAS, liquid state NMR and x-Ray crystallography provides a suitable analytical approach for the study of metal complexation of curcumin derivatives as well as their supramolecular interactions with metals.

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Paramagnetic NMR of Ru(III) Metallodrugs

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To overcome limitations of platinum-based anticancer drugs, new ruthenium compounds have been developed [1]. However, NMR spectra of RuIII-based drugs are complicated by the presence of paramagnetic metal center. The NMR spectra of these systems are usually dominated by Fermi-contact shift contribution to "standard" NMR chemical shift (termed also orbital shift) and short-living excited states related to highly efficient paramagnetic relaxation [2]. In supramolecular or macromolecular systems, the pseudocontact term can play an important role in characterizing 3D structure.

In this contribution, the NMR characterization of systems derived from ruthenium drugs NAMI-A and KP1019, including temperature-dependent NMR spectra, will be presented. In addition, DFT calculations [3] of 1H, 13C, and 15N NMR chemical shifts for these systems will be discussed in relation to the experimental NMR observations. The effects of various substituents of organic ligand, counterions, solvent on propagation of spin density are being investigated.

Acknowledgment: This work was supported by the Czech Science Foundation (15-09381S).

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Unexpected Intermediate with Unique Geminal H2 Transfer Identified by PHIP and Classical NMR

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The detection of intermediates is essential to investigating the mechanistic details of catalytic reactions, but is complicated by their typically low concentration and short lifetimes. Nevertheless, the characterization of elusive intermediates have been reported, including striking recent examples by NMR[1,2]. Recently, Fürstner and coworkers reported the unusual trans-selective hydrogenation of alkynes catalyzed by [Cp*Ru] complexes, which holds promise of being highly enabling [3]. Inspired by previous para-hydrogen induced polarization (PHIP) studies of a similar reaction by Bargon and his coworkers[4], the same approach was used to detect key intermediates in the recently reported catalytic system. During these investigations, previously undescribed hyperpolarized NMR signals were described for the first time. Further NMR examination revealed the structure of these compounds to be carbene intermediates, which was later confirmed by X-ray. Most interestingly, the PHIP experiments demonstrates that hydrogen molecule in these intermediates is transferred to one single carbon atom. The mechanism of this “gem-hydrogenation” mode and the yet unprecedented carbene intermediate was then further investigated by EXSY-NMR and DFT calculations.[5]

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A Gel Stretching Apparatus for the Accurate and Convenient RDC/RCSA Measurement in Small Molecules

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One of the challenges in RCSA measurement arises from the need to control measurement conditions in such a way that isotropic chemical shift changes are minimized upon molecular alignment. Previously, variable angle sample spinning (VASS) has been applied to obtain RCSA data for strychnine (1). Alternatively, a rubber-based gel stretching apparatus (2) was invented and successfully demonstrated for the relative stereochemical assignment of estrone (3). However, these rubber-based systems impose certain limitations. For example, using chloroform as a solvent is precluded due to its incompatibility with the rubber device. Here we introduce an improved method for RCSA measurement, but with the capacity for RDC measurement as well. The method is based on a previously published report of a two-stage NMR tube designed for the RDC and RCSA measurement of biomolecules aligned in polymeric gels (4). Mechanically stretching the gel only causes gel elongation but keeps the chemical composition and concentration constant, thereby providing an ideal means to control the degree of anisotropic alignment. Here, applications are demonstrated for small molecules aligned in a CDCl3-compatible gel, specifically, polymethyl-methacrylate (PMMA), although gels compatible with other solvents can be stretched with the same device as well. Our results have shown that our stretching apparatus affords high data quality, with SVD Q factors of RCSAs and RDCs well below 0.1 for strychnine and estrone.

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Cryptospirolepine: Revising the Structure Using Modern NMR Techniques, DFT Calculations, and CASE Program Capabilities

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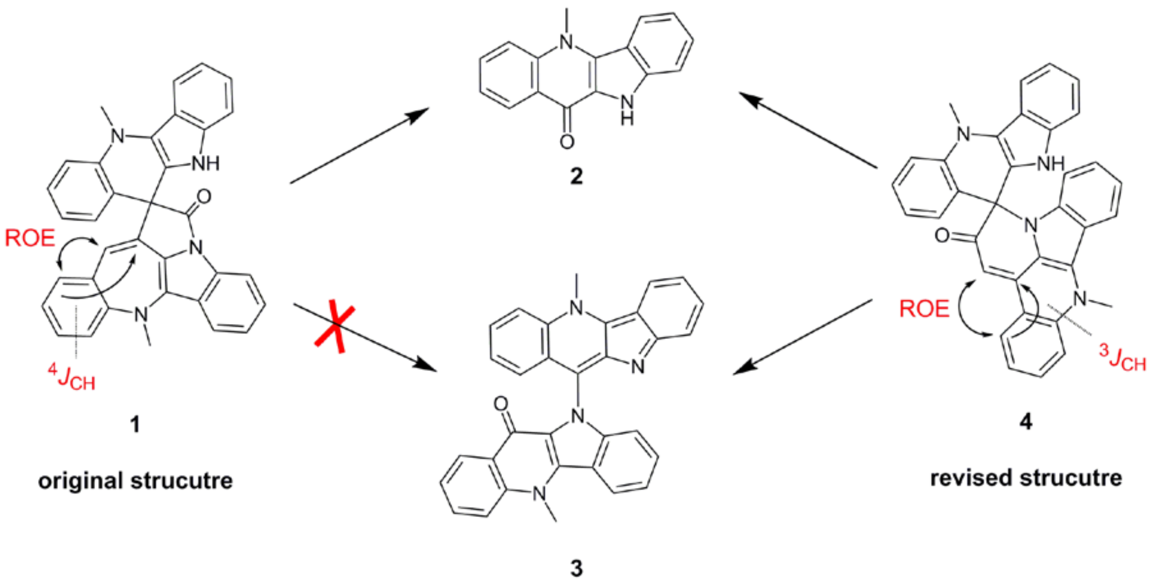
Cryptospirolepine (1) is a complex indoloquinoline alkaloid that was first isolated in late 1991 from extracts of the Ghanian chewing stick, Cryptolepis sanguinolenta. The structure elucidation was reported in 1993 (1) employing what were then state-of-the-art NMR methods and 3 mm micro NMR probe technology. Structure elucidation work done on the molecule preceded the development of 1H-15N HMBC and ADEQUATE NMR methods by several years. Using the NMR methods then available, no correlations were observed to the carbonyl in the HMBC data that could be acquired. The structure elucidation hinged on an ROE observed between a vinyl proton and one of the terminal resonances of a four-spin aromatic system, a putative 4JCH correlation to a quaternary carbon from the same aromatic proton, and positioning the carbonyl to be 4JCH from the nearest proton. Computer-Assisted Structure Elucidation or CASE programs were then in their infancy and incapable of dealing with a molecule of the complexity of cryptospirolepine. A 2002 study of the degraded 1993 sample led to the identification of two compounds, one of which could be rationalized from the assigned structure (2) and a second that couldn't (3) (2).

We have interrogated a 700 µg voucher sample of cryptospirolepine using 1.7 mm MicroCryoProbe™ technology, 2 Hz optimized 1H-13C and 1H-15N LR-HSQMBC, and newly developed pure shift 1,1- and 1,n-ADEQUATE methods. Using this powerful ensemble of NMR methods coupled with state-of-the-art DFT calculations and CASE methods, supplemented by knowledge of the structure of a key degradant of cryptospirolepine whose structure was elucidated more than a decade ago using cryoprobe technology, we now wish to report the revision of the structure of cryptospirolepine (4) (3).



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Benchtop NMR in Teaching Organic Chemistry at University Level

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Chemistry laboratories provide the students an optimal environment for project based learning (PBL) [1], whose basis lies in the real-life application of the research. A project may consist of preparing a particular molecule, whose structure must be confirmed. Thus, an increasing number of analytical characterization techniques have been incorporated into the undergraduate laboratories. Even though NMR is the most commonly used characterization technique in inorganic and organic chemistry, it usually is the least available to undergraduate students. Probably the most significant obstacle for incorporating NMR spectroscopy as part of the laboratory courses is the limited accessibility of high-field NMR. The compact, portable, and easy-to-use benchtop NMR spectrometers provide a convenient and affordable solution [2,3,4].

Figure 1. The benchtop NMR spectrometer used in this work. This Thermo ScientificTM picoSpinTM 45 spectrometer has the size of 20.3 cm x 12.7 cm x 27.9 cm and weighs 4.5 kg.

Within this work the applicability of Thermo ScientificTM picoSpinTM 45 spectrometer (Fig. 1) on the laboratory course in organic chemistry at the level of subject studies (2nd year students) at Department of Chemistry, University of Jyväskylä, Finland, was evaluated. Within the course the students are to perform six synthesis products, whose structures need to be confirmed by spectroscopy. So far the students have just left samples of their products for the laboratory engineer, who has measured the NMR spectra by using the high-field spectrometer in the research laboratory. Being able to acquire their own NMR spectra in addition to carrying out the analysis of the compounds they have made, gives the undergraduate students a more comprehensive picture of the process as a whole as well as increases their interest and enthusiasm towards the laboratory work.

Thermo ScientificTM picoSpinTM 45 spectrometer exploits a fluid capillary for sample injection eliminating the need for expensive deuterated solvents or fragile NMR tubes. It operates at the proton Larmor frequency of 45 MHz. Measurement of 32 scans, which resulted in reasonable quality of the spectra, took less than five minutes. As an example, 1H NMR spectra of 2-chlorobutane measured by Thermo ScientificTM picoSpinTM 45 spectrometer with 64 scans (black) and with 32 scans (blue) as well as by Bruker Avance 300 NMR spectrometer (red), is presented in Fig. 2. As can be seen, the chemical shift values well correspond to the expected ones, and splitting patterns can be resolved by reasonable accuracy. For confirming the structures of the synthesized compounds within the laboratory course the method seems to work well. Moreover, the students found the spectrometer to be easy-to-use, and experienced the ability of performing the measurements of the NMR spectra by themselves particularly educational.

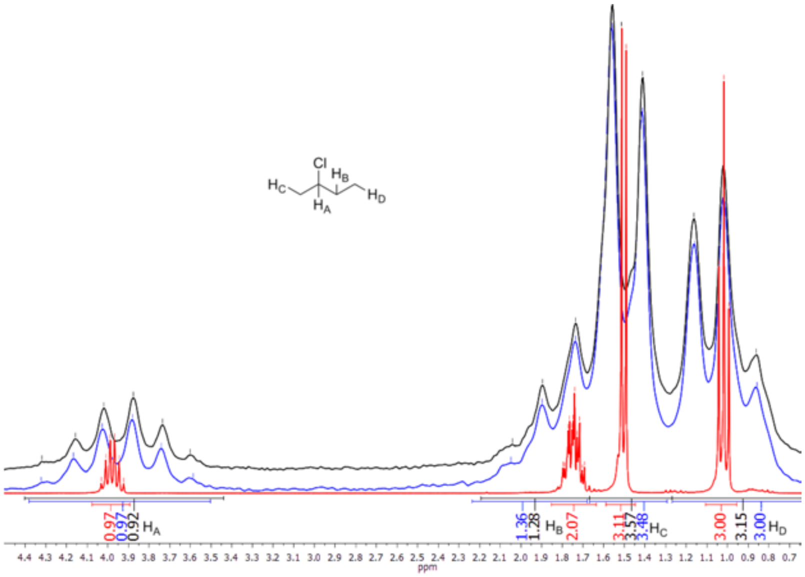

Figure 2. 1H NMR spectra of 2-chlorobutane measured by Thermo ScientificTM picoSpinTM 45 spectrometer with 64 scans (black) and with 32 scans (blue), as well as by Bruker Avance 300 NMR spectrometer (red).

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Small Molecule NMR to Cheaply Sensitively and Non-Invasively Phenotype Humans or Animals

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Research efforts have in recent years concentrated on developing Nuclear Magnetic Resonance (NMR) instrumentation and technology to allow the acquisition of data in a high-throughput manner that is also effective, accurate, precise and reproducible enabling the study of complex biofluids from population samples for biomarkers of disease risk [1]. Proton NMR based metabolic phenotyping studies of urine and blood plasma/serum samples provide important patient biochemical information and permits monitoring of disease progression in an objective manner. Developing highly reproducible methods and standardized protocols that minimize technical or experimental bias, allowing realistic inter-laboratory comparisons of subtle biomarker information is an ongoing challenge for metabolic profiling analytical scientists [2]. The current presentation introduces recently developed protocols that carefully consider major experimental conditions, including sample preparation, spectrometer parameters, NMR pulse sequences, throughput, reproducibility, quality control, and resolution. These results provide an analytical platform that facilitates NMR spectroscopy usage across large cohorts of various types of biofluid samples, enabling intra- and inter-laboratory comparisons of global metabolic profiling that is a prerequisite for personalized healthcare.

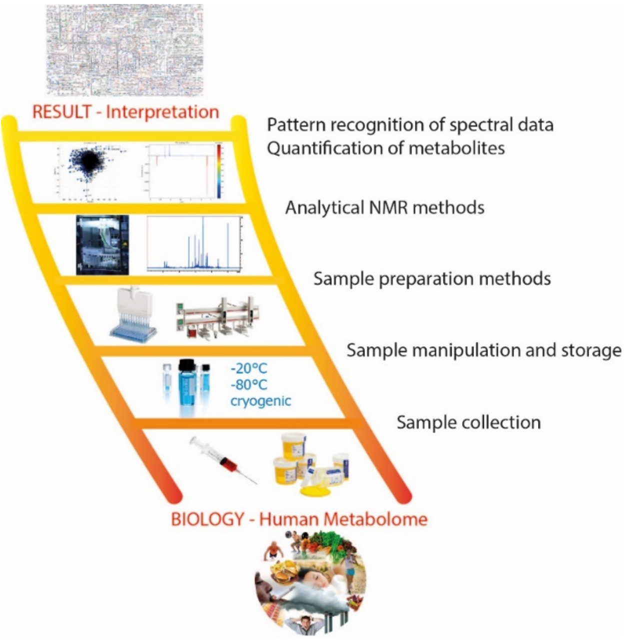

Figure 1. A typical sample workflow highlighting the key stages during which the biological "snapshot" must be preserved in order for a biofluid NMR spectrum to reflect the biology

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Expanding the NMR Palette: Insights on Artificial Charge Separators

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Spurred by worries over climate change, there is increasing interest in mimicking natural photosynthesis for the conversion of solar energy into fuel. The molecular structure and packing of self-assembled Zinc Salphen/NDI dyad and Perylene-based molecules, which are potential, charge separators were studied in detail in the solid state.

While dynamic scattering, availability of diffraction grade crystal, destruction of crystal due to electron beam were the drawback of TEM, difficulty to index the bragg peaks due to overlap is the limitation of the powder XRD. The combination of MAS NMR, TEM, Powder XRD and molecular modeling provide a powerful methodology that can be of use to investigate molecular geometry (and properties) of larger unlabeled - aggregated supramolecular systems. DFT calculations were performed using the CASTEP module in the material studio with GIPAW wave function. Quantum mechanical calculations allow experimental 1H and 13C solid-state NMR spectra to be assigned in a quantitative manner to a specific molecular packing arrangement, starting from the chemical structure of a moderately sized molecule. The incompleteness of SSNMR data is supplemented by data from TEM and powder XRD.

Here we simulated the distance constraints obtained from the LGCP build up curve using Simpson/Spinevolution for the selected carbon atoms. An electron density map of the proposed structure is generated and its projected down in the right orientation followed by fourier transform using EMAN2 software is employed to simulate the electron diffraction pattern. To confirm the model we simulated the powder XRD pattern using Reflex module in the material studio. We described a methodology in which the computational integration of MicroED, Powder XRD and SSNMR to propose a model for a molecule with high molecular mass, with less ambiguity. One of the biggest challenges with smarter crystallography is that it is limited to small molecules but here we proposed structures for molecules with higher atomic weight, which is around 1000gm/mol. This methodology could be extended to understand the mechanism of battery in the near future.

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Who Did It? An Investigation into the Exotic Rearrangement of a Drug Molecule

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We tell the story of a structural and mechanistic investigation into a development drug molecule (AZD9819) that was found to be unstable in ex vivo blood plasma samples.

The drug molecule, a neutrophil elastase inhibitor and pyrazinone derivative, was found to form a mono oxidised species in plasma that seemed quite unrelated to earlier identified metabolites. But where had this oxidation occurred? Structure elucidation proved challenging but strangely we were able to successfully make a synthetic standard before even knowing what the structure was! Subsequent elucidation using NMR and MS fragmentation patterns and assisted by mechanistic chemistry led to a surprise. What we had initially assumed to be a simple oxidised derivative was in fact a dramatically rearranged product involving the transfer of atoms from one end of the molecule to the other.

We proposed a reasonable multistep mechanism for the rearrangement which began with an initial epoxidation. But we had no cause for the formation of this epoxide. Why could we mimic the reaction chemically, but not under the mild conditions present in plasma? If it was the result of metabolism why did this occur only ex vivo? A careful investigation followed and after elimination of several suspects from our enquiries, it led to the surprising guilty party and the role of plasma lipid peroxides.

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Determination of Peptide Solution Conformations Using RDC

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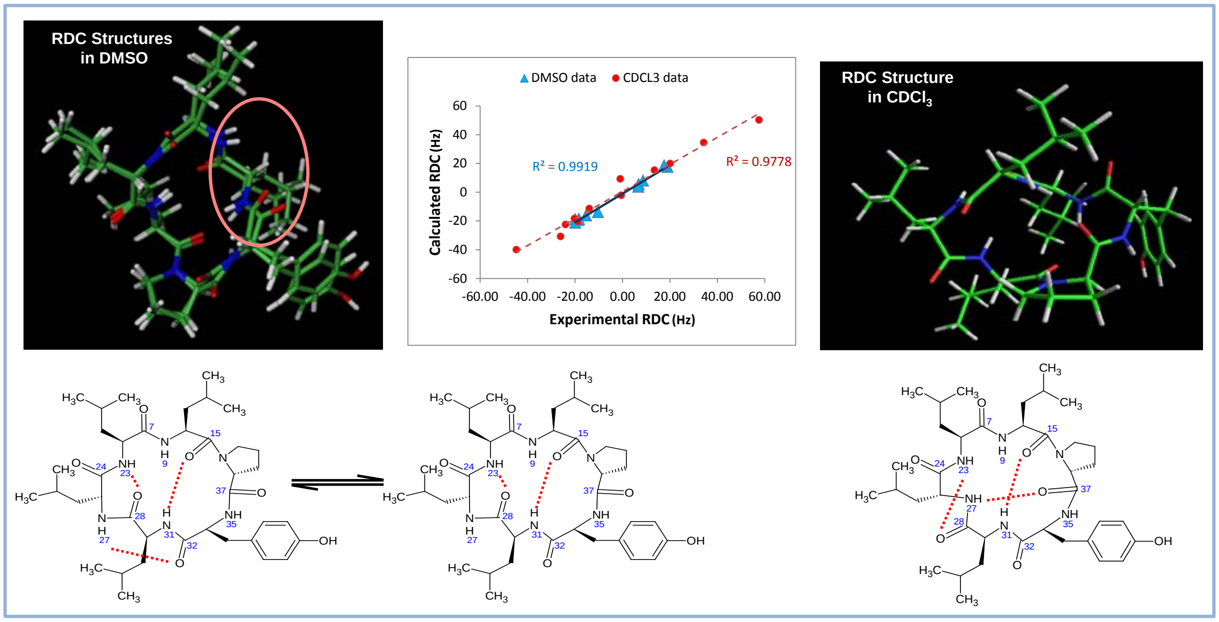
Recently, the intense interest in peptides is demonstrated as promising therapeutic candidates due to their wide involvement in physiological processes. When peptide design has emerged as an independent discipline in drug discovery, it becomes more and more critical to understand the molecular properties of these beyond-5-rule molecules. The characterization of peptide solution conformations is proven to be the best way to insight into their activity, permeability, metabolic stability, clearance and bioavailability to guide design. Here we report the technology development and application of residual dipolar coupling (RDC) in combination with nuclear Overhauser effect (NOE) to determine the solution structures of peptides in different organic solvents. The technology development enabled our new capability to aid ligand based drug design using solution conformations of ligands, especially when it is challenging to obtain co-crystal structures to do structure based drug design (SBDD).



The comparison of the solution structures of peptide PF-06413600 in DMSO-d6 and CDCl3. The red dotted lines indicate the IMHB observed through temperature coefficients of chemical shifts.

92 Recent Developments in the Certification of Organic Reference Materials by Quantitative NMR at the Highest Metrological Level

Markus Obkircher, Michael Weber, **Christine Hellriegel**, Alexander Rueck and Juerg Wuethrich

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In recent years quantitative NMR (qNMR) has become one of the most important tools for the content determination of organic substances or the quantitative evaluation of impurities [1-3]. Since this relative primary method does not require a reference standard of the analytic sample in hands, thousands of different organic compounds can be certified with achieving direct traceability to internationally accepted references such as NIST SRM.

Recent publications have demonstrated the validity, robustness and precision of the qNMR technique [4]. Within Sigma-Aldrich R&D, High-Performance qNMR (HP-qNMR®) was optimized to its maximum level of accuracy using metrological weighing equipment and a specially designed experimental setup for the certification of organic compounds with combined, expanded uncertainties down to 0.1% [4-5].

This new generation of organic certified reference materials (CRM) prepared under ISO 17025 and ISO Guide 34 accreditation, comprise a small number for the application as qNMR internal references and a large series to be used in chromatography [5]. Sigma-Aldrich very recently accomplished an important step towards the implementation of a multi nucleus CRM for qNMR by a successful concept study for 31P qNMR measurements with direct traceability to NIST SRM [6].

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Application of AQARI(Accurate Quantitative NMR with Internal Reference Substance) to the reagents in the crude drug section of the Japanese Pharmacopoeia

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2. National Institute of Health Science
3. Wako Pure Chemical Industries Ltd
4. Tsumura & CO.
5. Tokiwa Phytochemical Co., Lt

In the Japanese Pharmacopoeia (JP), quantitative “assay” is prescribed to ensure safety and efficacy for crude drugs and extracts of Kampo formulations. In most cases, “assay” which is the quantification of marker compounds is carried out using HPLC with the reference substance (RS) possessing purity values. However, it is pointed out that establishing RS derived from natural sources for “assay” is more difficult than the case of synthetic chemicals. Namely, isolation from natural sources requires not only time-consuming steps but also high economical cost and then the determination and control of their purities are difficult unless RS themselves are consumed. Therefore, in many cases commercial reagents are used as RS for HPLC “assay”, despite their absolute purity are not determined.

In this decade, quantitative NMR (qNMR) has been drawing considerable attention in many fields, since it provides accurate quantitative value without RS which is same as the analyte. In addition, qNMR with SI traceability is made possible by using the appropriate protocol. Therefore, qNMR qualifies as a method to evaluate RS.

Since 2008, we have conducted research on AQARI (accurate quantitative NMR with internal reference substance) to determine the purity of reagents used as RS for “assay” in crude drugs and Kampo formulations in JP. As the results of validation studies, when the target reagents having the molecular weight of around 300 and weighed amount of about 10mg are dissolved in 1mL deuterated solvent, AQARI is able to determine the purity with the accuracy of approximately 2 significant digits [1]. Accordingly, AQARI has been adapted as a method for purity determination of reagents used for RS for HPLC quantitative assay on JP16 supplement 2 [2]. Several reagents with purity values determined by AQARI have been commercially provided. By using such reagents and incorporating the purity value into calculation of the quantitative value of crude drug or Kampo extract samples, the obtained quantitative values will be achieved its SI traceability.

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Practical Aspects of Real-time Pure Shift Acquisition

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Developments in NMR are often driven by the insatiable desire for improved resolution and sensitivity. In the 1970s, broadband proton decoupling methods provided a major improvement in carbon-13 detected experiments, by removing the splitting of the observed resonances due to heteronuclear J-coupling interactions with protons. More recently, simplifying proton NMR spectra by collapsing homonuclear multiplets into a sharp single resonance for each chemically non-equivalent environment is the goal of modern pure shift NMR methods. In these, sensitivity is usually sacrificed by observing only a subset of the available magnetization, and/or constructing a pure shift FID using a series of experiments.

We have recently published [1,2] improved acquisition schemes for HSQC that simultaneously improve both resolution and sensitivity. The development of such real-time pure shift methods poses significant practical challenges, particularly when JT2<1. Problems that will be addressed – and their solutions – include (i) phase coherence requirements between acquisition blocks, (ii) implementation of pulsed-field gradients to enforce coherence transfer pathway selection, iii) the need for extended phase cycling, iv) instrument stability and v) water suppression.

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Evaluation of Multivariate Curve Resolution for Macromolecular Baseline Removal in ^{1}H-NMR Spectra

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Introduction

Obtaining a reliable metabolite profile of the low-molecular-weight metabolites (LMWMs) in a 1H-NMR spectrum of serum and plasma samples is severely compromised by the resonances of macromolecules such as albumin, lipids and lipoproteins. The most popular method for avoiding the macromolecular baseline is to apply the CPMG filter, which is able to remove the macromolecule resonances exploiting their shorter transverse relaxation rate [1]. The main problem of working with CPMG spectra is that the resonances of the LMWMs have been reduced by its own spin-spin relaxation time (T2), and obtaining the real concentration of a metabolite is not trivial. While in 1H-NMR spectra the quantification of a metabolite can be obtained directly from the relation between the area of one of its resonances and the area of a reference compound [2], in CPMG spectra each resonance has to be corrected by its own T2 first. Some studies show T2 factors of few resonances of a small set of metabolites and describe how to obtain it, but several quantifications of each resonance are needed in order to accurately calibrate the resonance decay [3]. In this study we present a new methodology for removing the macromolecular baseline and we compare a metabolite profile in the resulting spectra against a metabolite profile in its CPMG spectra.

Methods

We used a set of 10 total serum samples of healthy people in order to evaluate our method. For all the samples we acquired 15 series of CPMG filters (from 0 to 500 ms) and then we selected 8 regions of interest (ROIs) where we applied multivariate curve resolution (MCR) in order to separate the spectra in two components by their relaxation time. After obtaining the two components, we subtracted the part of the spectra related to the macromolecular baseline component (faster decay) from the total spectra in each of the filters, leaving us new spectra free of macromolecule resonances. We applied the metabolite profiling in the MCR-filtered spectra at 0 ms, while we also quantified the signals in each of the filters in order to calculate the T2 of each resonance. The ROIs were chosen to evaluate the performance of MCR in different cases (isolated signals, overlapped regions, low signal-noise ratio) and contain 11 metabolites which are isoleucine, leucine, valine, alanine, glutamine, glycine, tyrosine, glucose, lactate, citrate and formate. We quantified our target signals using the constrained total-line-shape (CTLS) fitting algorithms included in the Dolphin's package [4]. Finally, we compared the correlation between the metabolic profile of these 11 metabolites in the set of MCR-filtered spectra (at 0 ms) against the same profile obtained in the CPMG spectra, and we evaluated the error between the quantification in the MCR-filtered spectra against the calculated by the recovery function obtained from the T2 of each resonance.

Results

As we expected, the correlation pattern of each metabolite through the samples dataset is high (with a correlation coefficient over 0.85 in all cases), but the ratio of intensities between metabolites presented some differences. This is produced by the differences between the T2 of each resonance, where at a determined point (CPMG in that case) some resonances have lost more signal intensity than others. So, MCR-filtered spectra are maintaining the relations between the real amounts of the metabolites present in the samples, which is distorted in the CPMG spectra but can be corrected by the T2 factor of each resonance. The main advantage of the MCR-filtered spectra is that signal intensities do not need recovery factors to be corrected to absolute concentrations, being the area under the curve the real amount of a resonance, which is easily extrapolable to absolute concentration units using a reference compound and applying the Serkova equation [2]. The differences between the absolute concentrations calculated by the fitting in the MCR-filtered spectra against the estimated by the recovery function were under 15% in all the cases, being formate the metabolite with more error due its low signal-noise ratio.

Conclusions

In this study we show a new strategy for removing the resonances of the macromolecules that is at least promising. Even needing a batch of relaxation filters is always faster than quantifying the same resonance in each filter for extracting its T2 and estimating absolute concentrations. In order to optimize the performance of the MCR-filter for high throughput analysis some other studies are needed. Finding the minimum number of filters to give similar results or studying the variability range between samples in order to adjust a global macromolecular pattern could be interesting.

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UF-DQS Optimization Through Theoretical Description and Numerical Simulations

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Ultrafast (UF) NMR, proposed in 2002 by Frydman and co-workers, is a generic approach yielding homo- or heteronuclear multi-dimensional spectra in a single scan [1]. The last decade has seen its analytical performances greatly improved, therefore UF NMR is nowadays an efficient analytical tool which is used in a large array of applications such as metabolomics, real-time reaction monitoring or coupling with HPLC and dissolution DNP [2]. It does suffer, however, from a limited resolution, and innovative strategies are needed to increase information content of UF NMR spectra.

Recently, we showed that double-quantum NMR spectra (DQS) can be recorded in an ultrafast fashion and that they provide significant improvements over classic sequences such as UFCOSY [3]. The lack of auto-correlation peaks in DQ spectra leads to a better peak separation than in UFCOSY, especially for peaks close to diagonal. Nevertheless, both in its UF and conventional version, DQS is less sensitive than its COSY equivalent. This key feature is particularly critical in UF NMR whose intrisic sensitivity is relatively low. Thus, the in-depth understanding of this pulse sequence is essential to optimize the associated experimental parameters, in order to maximize the resulting sensitivity.

We have set up a platform for numerical simulations of ultrafast DQS spectra by adapting a widely used spin dynamic library: Spinach [4]. Numerical simulations, in conjunction with product operator formalism caclulations, make it possible to describe and optimize the key factors governing the sensitivity of the sequence. Two major parameters were studied through theoretical description and numerical simulations: the angle achieved by the reading pulse, and the mixing period added prior to the spatial encoding. For both parameters, the results from theoretical description and simulations perfectly match with experimental data, which is clear evidence that these tools are performant to predict the effect of such experimental parameters in complex spin systems. Predicting the effect of experimental parameters is a convenient way for increasing the intensities of peaks of interest by fine tuning these parameters.

Application of UFDQS on the quantification of taurine in energy drinks will be shown, relying on recently described analytical procedures yielding quantitative results from 2D spectra [5]. The lack of auto-correlation peaks in UFDQS spectra eases the quantification, as signals of interest are masked by the diagonal signals of sugars in the COSY spectrum. Moreover, UFDQS also decreases the amplitude of the ghost peaks arising from “interleaving”, the standard acquisition strategy for UFNMR at high field [6]. The optimization of experimental parameters through numerical simulations lead to a significant enhancement of the sensitivity.

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Quantitive NMR in Hit Evaluation and Lead Optimization

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While structure elucidation and confirmation by NMR are well established activities in chemical and pharmaceutical industrie, the use of NMR for sample quantification remains limited. This is due to (a) existing workflows which do not allow for transfer of NMR results of QC samples to biological test samples and (b) until recently, a lack of efficient software. Many assays in drug discovery – e.g. in the area of eADME, physicochemical and biological profiling - are starting from DMSO stock solutions prepared by different internal or external providers. It is well known that the concentration of these stock solutions is variable due to several reasons. Therefore, in order to improve structure activity relationships (SAR), there is a need to take the real test concentrations into account. This presentation provides an overview on typical qualities of samples in drug discovery, the workflow established at Sanofi Frankfurt, results of concentration determinations by NMR, and the impact of real concentration on structure activity relationships for real life research programs.

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Sensitivity Enhancement in Ultrafast NMR by Optimizing J-Modulation Effects

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Ultrafast (UF) NMR is a generic approach yielding homo-or heteronuclear 2D spectra in a single scan [1]. Since its performances have been significantly improved during the last decade, UF NMR is nowadays an efficient analytical tool which is used in a large array of applications such as metabolomics, real-time reaction monitoring or coupling with HPLC and dissolution DNP [2]. Nevertheless this approach still suffers from an intrinsic low sensitivity, and from the need to compromise between sensitivity, spectral width and resolution. Moreover since most of the UF experiments rely on constant time space-encoding scheme, J-modulation is another source of sensitivity losses. Indeed this effect can lead to large sensitivity losses and even to missing spectral peaks depending on the nature of the spin system. While the effect of J-modulation has already been reported for conventional constant time experiments it has never been described nor optimized in the context of UF NMR experiments [3].

In order to understand and predict the effects of J-modulation in UF experiments, we suggest a theoretical description under product operators formalism which leads to analytical expressions of J-modulation for weakly coupled spin systems. For more complex cases like strongly coupled spin systems, we have set up a platform for numerical simulations of ultrafast spectra by adapting two widely used libraries: Spinach and SpinDynamica [4,5]. The results from simulations and theoretical description perfectly match with experimental data, which is clear evidence that this approach is an efficient predicting tool of the effect of J-modulation in complex spin systems. Predicting J-modulation is also a convenient way for increasing the intensities of peaks of interest by fine tuning a mixing period added prior to the spatial encoding. It is also possible to find a compromise in the case of complex mixture of small molecules, which improves the limit of detection for the sample. Moreover we developed a new excitation block relying on selective pulses which provides sensitivity enhancement by J-modulation even for multiple spin systems within a single scan.

The control of J-modulation on UF constant time experiments provides a significant sensitivity enhancement in UF experiments, thus increasing their general applicability. Spectra recorded through this approach on various samples like small organic molecules and complex metabolites mixtures will be presented.

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Automated Structure Elucidation of Small Molecules

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The 2D NMR-guided computer program COCON is extremely valuable for the constitutional analysis of unknown compounds. In particular, structural proposals made on the basis of the molecular formula and of 2D NMR experiments can be analyzed for the existence of alternative constitutions being in agreement with the same data set. Already back in the 1990s, John Faulkner stated: "Rather than defining a structure that can be shown to fit the data, it is best to examine many possible structures (we would say every) and to treat each proposed structure as a hypothesis that cannot be proved but can only be disproved by incompatible data." [1]. Proton-poor compounds are particularly challenging for an NMR-based structure elucidation. The constitutional analysis of natural products by NMR spectroscopy is usually based on 2D NMR experiments like COSY, HSQC, and HMBC. This connectivity information is used as input for the NMR-based structure generator COCON which both improves and accelerates the process of constitutional assignment.

This contribution will focus on the following topics: 1) consideration of 4J and 5J HMBC correlations within the structure elucidation process of COCON, 2) incorporation of 1,1- and 1,n-ADEQUATE correlations in the automated structure elucidation of small organic molecules, 3) evaluation of the COCON output by C-13 chemical shift calculation, 4) ranking of COCON structural proposals by energy (smi23d), 5) statistical filtering of the COCON structural proposals (smi23d), and 6) consideration of symmetry. Furthermore, COCON was used to find structural alternatives for several known natural products.

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Conformational Features of Small Neurotransmitters in Solution: A Reliable Study *via* NMR in Ordered Media

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Neurotransmitters are small flexible molecules with prominent biological importance since they are chemical messengers that carry, boost and modulate signals between neurons and other cells in our body. Quantitative alterations or imbalances in their biological availability are responsible of various neurological disorders as well as diseases of the nervous system. The molecules perform their biological function by binding to specific receptors on target cell that determine the type of response. Not all the conformations assumed by the neurotransmitter are active towards the receptors. Therefore, the knowledge of the bioactive conformation that it adopts when binds to the receptor and activates it, becomes of utmost importance to clarify its mechanism(s) of action as well as to design suitable analogues with enhanced activity.1 Here, we describe a NMR methodology in solution, combined with the use of weakly ordering media2 to experimentally probe the conformational behavior of the small biogenic amine neurotransmitter 2-aminoethyl imidazole, better known as histamine. The information we look for are contained in the residual dipolar couplings (RDCs) that strongly affect the spectra of the solute dissolved in the partially oriented media. These experimental parameters, that are sensitive to the long-range intramolecular interactions, are averages over torsional internal motions that interconvert the possible conformers. By treating these averaged RDCs via proper theoretical models, it is thus possible to get a rather precise description of the torsional probability distribution. Following this procedure and using the AP-DPD theoretical approach,3 we investigated the conformational behavior of histamine in two different orienting solvents: a) the nematic PBLG/CDCl3 phase4 and b) a stretched gel phase obtained from gelatin swollen in D2O.5 In this contribution, preliminary results on both phases will be outlined.

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Residual Chemical Shift Anisotropy: A Method to Determine the Correct Configuration of Small Molecules

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Among the various NMR anisotropic observables, Residual Dipolar Couplings (RDCs) has become a powerful tool to determine the constitution, configuration and conformation of small molecules [1,2]. While Residual Chemical Shift Anisotropy (RCSA) has been mainly applied to the study of proteins and nucleic acids, its potential for the structural analysis of small molecules has been still scarcely studied [3,4]. Similar to RDCs, RCSAs provide information about the relative orientation of moieties in the molecules, with the additional advantage of also providing this information for non-protonated carbons. We propose a methodology for 13C RCSA measurement based on 13C shifts difference observed between two alignment states which were obtained by reversible compression/relaxation of a PMMA/CDCl3 gel [3,5]. Double delta RCSAs and delta RDCs that are obtained as the differences between two alignment states are fit separately to different configurations of strychnine and estrone; in both cases the correct configuration provided the lower Q factor. For strychnine, both RDC and RCSA alignment tensors are similar with an inter-tensor angle of 14°, whereas for estrone intertensor angle between them is 30°. Noteworthy, for the correct configuration, the combined fit of RDCs and RCSAs to a unique tensor results in an even lowest Q factor.

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Application of LC-NMR in Drug Analysis

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High performance liquid chromatography (HPLC) coupled to nuclear magnetic resonance spectroscopy (NMR) has become one of the most efficient analytical technique for complex mixture analysis. To identify and structurally characterize compounds in a mixture without their physical separation has long been a dream of analytical chemists. LC-NMR hyphenation gives a wealth of structural information on small quantities of substances without the necessity of their isolation and purification.

To analyse complex mixtures is still a chalenging task in drug discovery and development. Our recent results results have demonstrated a successful use of LC-NMR methodologies in solving problems connected to final drug substances1-3. Here, we will shown and discussed examples of using LC-NMR approach for solving purity issues of drugs and their intermediates.

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Unusually High Rotational Barriers in 5-Nitrosopyrimidines with Strong Intramolecular Hydrogen Bonds Studied by NMR Spectroscopy

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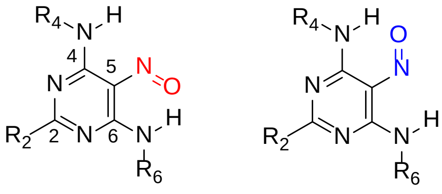
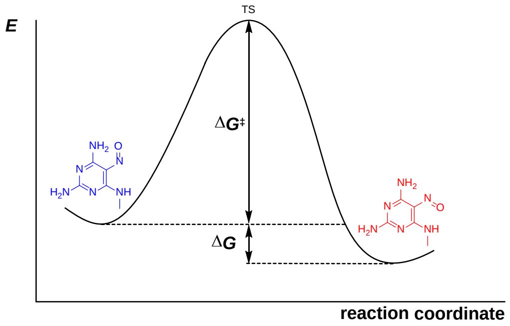
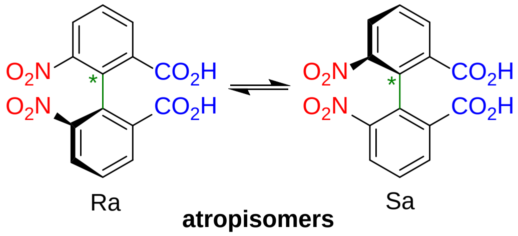
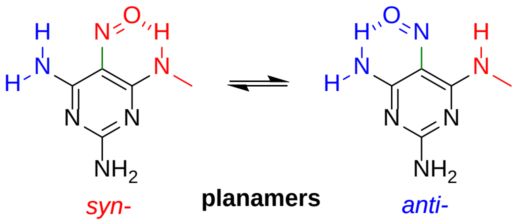
5-Nitrosopyrimidine derivatives display a wide range of biological properties such as cytostatic, antifungal or antimicrobial. Having two H-bond donors in neighbouring positions (4, 6), the nitroso group can form two strong intramolecular hydrogen bonds. Two stable conformers differing in NO group orientation exist. These compounds might work as purine mimics and theoretically target two different metabolic pathways. In our previous work[1,2], we have prepared 5-nitrosopyrimidines with various substituents R_4 and R_6 and we have studied the structure, reactivity and physico-chemical properties of these compounds by NMR and UV/Vis spectroscopy. Two rotamers were observed as two sets of NMR signals (^{1}H, ^{13}C) and solvatochromic effect was found. We observed strong dependence of rotamer ratio in equilibrium on nature of substituents R_4 and R_6, which was supported by DFT calculations. In some cases, it was possible to separate the rotamers from each other and the chemical structure of both separated rotamers in solid state was confirmed by solid-state NMR spectroscopy and by X-ray analysis. Due to their separation, kinetic data could be measured and rotational barriers $\Delta G^{\ddagger}$ higher than 22 kcal/mol were determined.



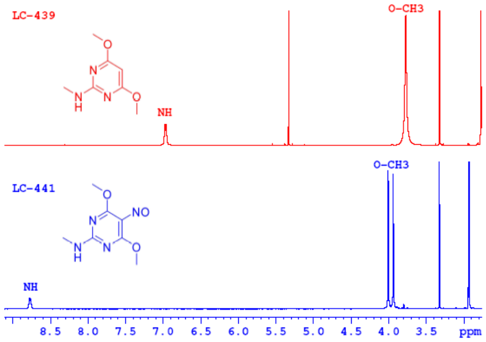


The properties of these compounds have been compared with well-known atropisomers. However, contrary to sterically hindered rotation in atropisomers, our rotamers are planar, achiral and stabilized by strong intramolecular hydrogen bonds. These significant differences led us to suggest a new term "planamerism".

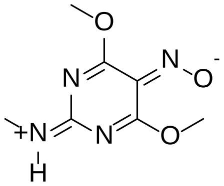




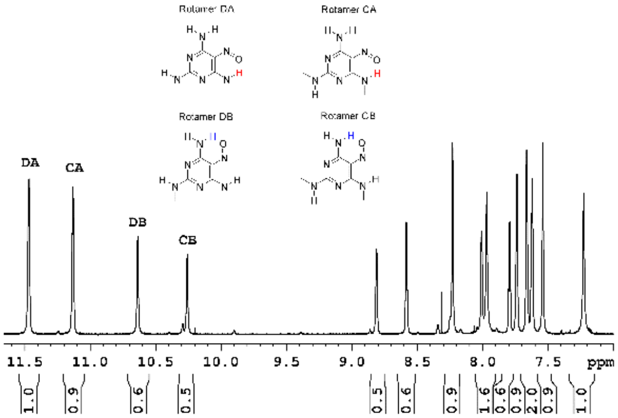
In this work, we prepared a new series of 5-nitrosopyrimidine derivatives with strong intramolecular hydrogen bonds differing in substitution at position 2 on pyrimidine skeleton. We found out that the more electron-donating substituent is (e.g. NH_2 or $NH-CH_3$), the higher the rotational barrier around C5-NO single bond is. For instance, in the case of 4-amino-5-nitroso-6-methylamino derivative with $NH-CH_3$ group in position 2, C5-NO rotational barrier is higher (> 20 kcal/mol) than in the compound with C2-H substituent (16.4 kcal/mol), which was determined by temperature-dependent ^{1}H NMR spectra. We also studied rotational barrier around C2-R_2 bond, which was higher in the 5-nitroso derivative (21.3 kcal/mol) than in the compound without 5-nitroso group (14.5 kcal/mol). The presence of nitroso group has significant influence on the chemical shifts in the whole molecule. For instance, in 5-nitroso derivative, the chemical shift of proton in 2-N**H**-CH_3 group is higher (8.79 ppm) than in the case of derivative without the nitroso group (6.98 ppm). Furthermore, we found out that the C2-NH bond has partially double-bond character, which indicates a “push-pull” system with a partial zwitterionic character of the compound.



Partial zwitterionic character



Due to the unusually high rotational barriers around C5-NO and C2-R^2 bonds in substituted 2,4,6-triamino-5-nitrosopyrimidines, it was possible to observe four rotamers as four sets of signals in NMR spectra (^{1}H, ^{13}C). As an example, a part of ^{1}H NMR spectrum is used to show four signals corresponding to four protons involved in hydrogen bonds in four conformers DA, CA, DB, CB. From the temperature dependent measurement, it is clear that the rotational barrier around C5-NO bond is higher than the C2-NH (19.6 kcal/mol). It is in agreement with DFT calculations (28.6 and 19.4 kcal/mol, respectively).



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Validation of ^{1}H NMR Quantitative Method for Efavirenz Purity

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Currently, much has been said about validation, certification, qualification processes and analytical methodologies in control of safe and reliable quality products and raw materials from the fine chemical and pharmaceutical industries. This fact makes that the analytical methods used for quality control of active pharmaceutical ingredients (API) and components be extremely important for manufacturers and users of these products. For that, standardized methods are used by international organizations like: The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), by government agencies and the Pharmacopoeia. The most recommended techniques for these guides are the chromatography and ultraviolet, visible and infrared spectroscopy, while Nuclear Magnetic Resonance (NMR) spectroscopy which is an important qualitative analysis tool, has only been recommended for quantitative analysis (qNMR) by the US Pharmacopoeia in 2003 [1,2]. The advantage of NMR, compared to other analytical techniques, consists in the fact that it is a primary method of standardization, where the construction of an analytical curve and the use of internal standards identical to the analytes is unnecessary [3]. In addition, another advantage is that the substance can be identified and quantified directly from the spectrum obtained without a reference standard [4]. This is a highly suitable technique for evaluating an API or an excipient and can be used to control a production process and characterization of an entire formulation.

The aim of this study is to develop an advantageous and competitive selective qNMR method for the determination of the assay efavirenz API samples that complies well with the validation requirements based on ICH Q2 (R1).

The method as per ICH Q2 (R1), for following parameters – system suitability, specificity and selectivity, linearity, range, accuracy, precision and intermediate precision, accuracy and robustness [5].

1H NMR spectra were measured on a Bruker Avance 500 spectrometer (500 MHz for 1H) probe with a broadband (BB) 5 mm to 25.0 ± 0.1 ° C using chloroform-d (Cambridge Isotope Laboratories, Inc.) as solvent. The values of the chemical shifts (δ) were reported in ppm using tetramethylsilane (TMS) as reference standard. The technique used to determine the relaxation time (T1) of hydrogen was inversion-recovery sequence ($180 - \tau - 90°$). Measurements were obtained using τ between 0.03 and 12 seconds. The slower T1 value obtained was 4.6 s for the signal of interest. Considering the estimated time in this experiment and an acquisition time (aq) equal to 4.0 s, the relaxation delay was set at 13.8 s (d1=3xT1). For qNMR spectra acquisition, the experiment used was GARP (used to decoupling carbon satellites) and was obtained with the standard sequence of TopSpin program from Bruker. The number of scans (ns) used was 8, with 65,536 data points during the acquisition in a spectral window (sw) of 16 KHz and a receiver gain equal to 322 for validation testing. The spectra were processed with 65536 points (fn), using an exponential multiplication with a lb = 0.3 Hz, automatic baseline correction and manual correction phase. Integrations were also performed manually, using always the same spectral widht.

The linearity of the analytical curve for analyte in the desired concentration range was good (R2 >0.9980). The accuracy obtained was 99.80% according to the range 98-102%. The precision and intermediate precision for the method presented coefficient of variation respectively 1.1% and 1.2%. The robustness was performed by varying the parameters such as number of scans (ns), relaxation delay (d1) and mass of the sample. Results showed no significant deviations indicating that the method is robust for the intended purpose. The method is nondestructive and can be applied for quantification of efavirenz in commercial formulation products. In addition, this method offers an excellent choice over previously described procedures and can be used for routine quality control of EFZ in solid form. Assay results obtained by qNMR were confirmed by comparing with an in-house HPLC method.

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