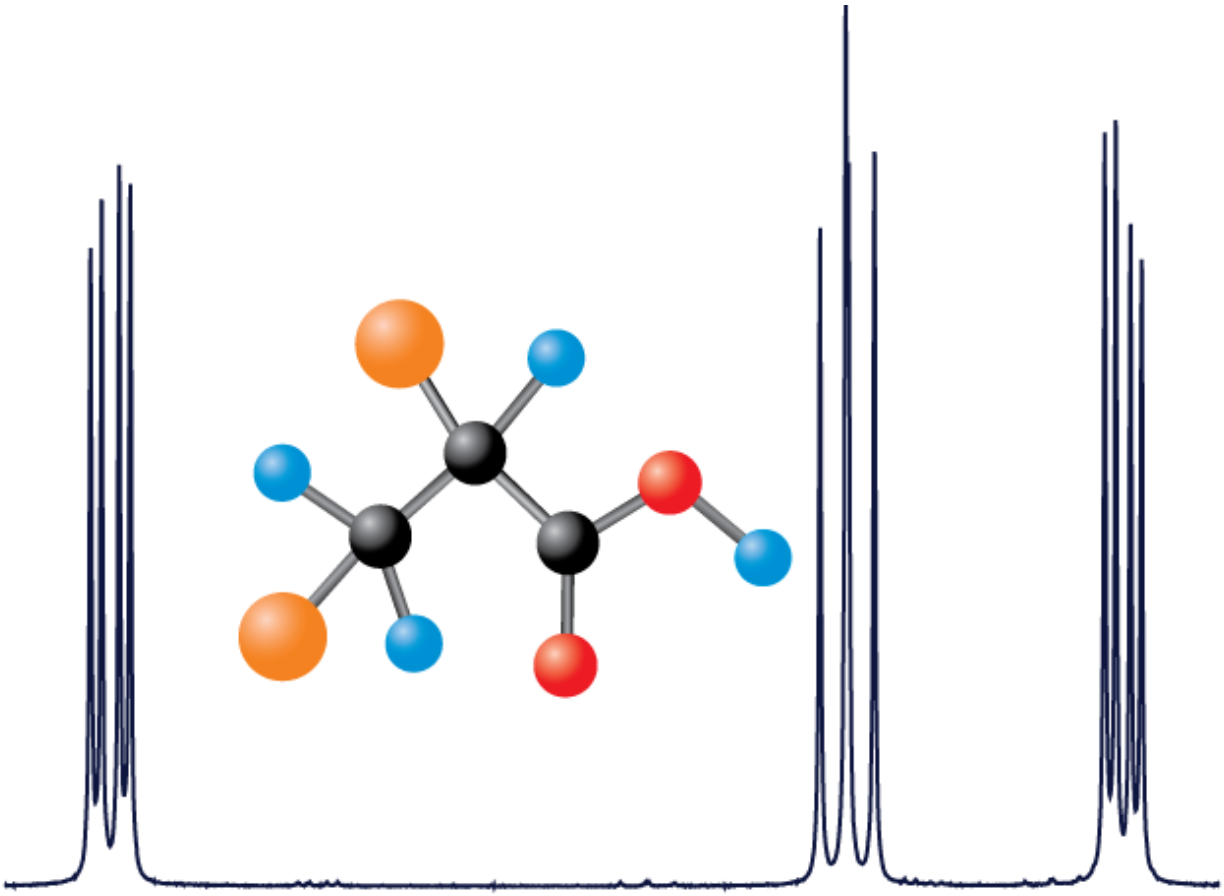

SMASH 2013

Conference Program

September 22nd-25th, 2013

Santiago de Compostela, Spain

SMASH 2013 NMR Conference

Dear SMASH 2013 Attendees,

We want to welcome you to the 2013 **S**mall **M**olecules **A**re **S**till **H**ot NMR conference in Santiago de Compostela, Spain. The conference will be held this year in the wonderful city of Santiago de Compostela. The city was founded in 9th century, being the final destination of the Way of St. James, a leading religious and cultural pilgrimage. Santiago is a UNESCO World Heritage Site and offers many possibilities to all of us from the social, cultural, and scientific viewpoints. The University was created in 1526 and remains one of the most prestigious Universities in Spain. Santiago is perfectly divided in the old and new town. Please, enjoy walking through the narrow and dark medieval streets close to the spectacular Cathedral. You feel the spirit of past ages. Galician gastronomy is outstanding, especially seafood, with special mention to octopus. Numerous restaurants and historical attractions are within walking distance of the conference site, as complement to the science.

We are excited from the program which includes a high quality number of oral sessions and workshops, and time for the posters sessions (and we are amazed by the large number of attendees, students, and posters submissions).

On Monday evening we are delighted to have as after dinner speaker Professor Santiago Alvarez from the Barcelona University which will cover science and cultural aspects.

This year's program has oral sessions titled, *"Less-common NMR-active Nuclei in Small Molecules", "Molecular Interactions by NMR. The Ligand-based Perspective", "Integration of NMR Metabolomic with Other Platforms and Omics", "New Developments in NMR Methodology", "Methods of Analysis for Food Ingredients and Products", "Determination of Stereochemistry by NMR", "Advances in Conformational Analysis and Dynamics by NMR"*. This year's workshops include *"Structural Elucidation: Different Approaches and Insights", "Reaction Monitoring and Quantitation", and "Solid State NMR in Pharma"*. We will have also the occasion to visit the Santiago Parador "Hostal dos Reis Católicos", founded in 1492 by the Catholic Monarchs, Isabella of Castille and Ferdinand of Aragon, where on Wednesday we will have a cocktail to close the SMASH 2013.

During the entire SMASH conference we have also a special focus on students and their progress in the NMR field.

While posters will be up the entire conference, those with even numbers will present on Monday and odd numbers on Tuesday. The free time on Tuesday afternoon will allow additional time to properly visit the Santiago de Compostela town.

To follow the SMASH Conference there will be the "Ultrafast 2D NMR" symposium on Thursday and the "Advances in NMR Methodology and Processing" symposium on Friday.

SMASH is a scientific meeting highlighting Small Molecule work with main focus on the NMR applications, in addition to other analytical technologies, used the small molecules research. The small size of this meeting provides an excellent networking opportunity for students and professionals while attracting leading spectroscopists in this field. This Small Molecule NMR meeting brings together cutting-edge science from Academia and Industry in one international meeting.

On behalf of the Organizing Committee, we want to extend a warm welcome and thank you for attending SMASH 2013.

Sincerely,

Carla Marchioro & Jesús Jiménez-Barbero
Co-Chairs, SMASH 2013 NMR Conference

SMASH 2013 NMR Conference Program

Sunday, September 22nd

8:00 AM - 11:30 AM ACD/Labs NMR Software Symposium
9:00 AM - 4:00 PM Bruker User's Meeting
10:00 AM - 4:05 PM Agilent Technologies User's Meeting
11:00 AM - 5:00 PM Mestrelab Research Software Semina
5:00 PM - 7:00 PM **Registration - Araguaney Grand Hotel**
8:00 PM - 9:00 PM **Mixer - Araguaney Grand Hotel**
9:15 PM - 11:00 PM **Dinner - Hotel San Francisco**

Monday, September 23rd

9:00 AM - 9:15 AM **Opening Remarks**
9:15 AM - 10:45 AM **Less-common NMR-active Nuclei in Small Molecules**
Chair: Fernando Lopez Ortiz, *Almeria University, Spain*

NMR at High Temperature in Molten Inorganic Materials : From the Local Structure to the Dynamics
Catherine Bessada, CNRS-Orleans, France
A Cutaway Drawing of Some NMR Parameters
Bernd Wrackmeyer, University of Bayreuth, Germany
Structure and Polymorphism in Small Molecules and Drugs: Insights from Solid-State NMR of Quadrupolar Nuclei
David L. Bryce, University of Ottawa, Canada
Polymer Networks for the Measurement of Anisotropic NMR Parameters
Ann-Christin Pöppler, Georg-August-Universität Göttingen, Germany

10:45 AM - 11:15 AM **Break**
11:15 AM - 12:45 PM **Molecular Interactions by NMR. The Ligand-based Perspective**
Chair: Gary Sharman, *Eli Lilly and Company Ltd, UK*

Information on Binding from the Ligand Spectrum: An Overview
Gordon C K Roberts, University of Leicester
Reporter-based Ligand Screening by NMR: Applications to 2OG-Dependent Oxygenases
Tim Claridge, University of Oxford, Oxford, UK
A Comprehensive NMR Analysis of the Interaction between DKP-RGD Ligands and Membrane Proteins on Intact Cells (Upgraded Poster)
Ileana Guzzetti, Università degli Studi di Milano, Milano, Italy
A Spin Diffusion Based Method to Determine Protein-Ligand Complex Structures Demonstrated on Enzymes of Pharmaceutical Interest (Upgraded Poster)
Jens Pilger, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

12:45 PM - 2:45 PM **Lunch**
2:45 PM - 4:30 PM **Poster Session**
Co-chairs: Michael Hammer, *University of Regensburg, Germany*
Krish Krishnamurthy, *Agilent Technologies, CA, USA*
4:30 PM - 5:00 PM **Break**
5:00 PM - 6:45 PM **Workshop**
Structural Elucidation: Different Approaches and Insights
Co-chairs: Tim Claridge, *University of Oxford, UK*
Brian Marquez, *Pfizer, USA*
8:30 PM - 11:00 PM Dinner/After Dinner Speaker/Social Hour
Professor Santiago Alvarez, Universitat de Barcelona, Spain

Tuesday, September 24th

9:00 AM - 10:30 AM **Integration of NMR Metabolomic with Other Platforms and Omics**
Chair: Silvia Mari, *R4R, Italy*

Merging Data Coming from Different Metabolomics Platforms
Maria Vinaixa, Universitat Rovira i Virgili, Spain

High Throughput NMR vs MS for Serum Metabolomics: A Comparison of Platforms Through Case Studies
Naomi Rankin, University of Glasgow, Scotland, UK

Can Urine Metabolic Profiling Help to Diagnose or Predict Disorders of the Mother, Fetus and Newborn? (Upgraded Poster)
Sílvia O. Diaz, University of Aveiro, Aveiro, Portugal

^{1}H S-Filter: Quantitative Edition of Singlets in 1D 1H NMR Spectra for the Study of Complex Mixtures (Upgraded Poster)
Manuel Martín-Pastor, University of Santiago de Compostela, Santiago de Compostela, Spain

10:30 AM - 11:00 AM **Break**

11:00 AM - 12:45 PM **Workshop/Tutorial (concurrent)**

Reaction Monitoring & Quantitation
Chair: Silvia Davalli, *Aptuit Verona, Italy*

Solid State NMR in Pharma
Co-chairs: Steven Brown, *University of Warwick, UK*
David L. Bryce, *University of Ottawa, Canada*

12:45 PM - 1:30 PM **Lunch, Free Time & Vendor Discussions**

1:30 PM - 2:30 PM **Student Mentoring Workshop (auditorium)**

3:00 PM - 5:00 PM **Santiago de Compostela Walking Tour (optional)**
(sign-up at registration table)

7:00 PM - 8:30 PM **Poster Session**
Co-chairs: Michael Hammer, *University of Regensburg, Germany*
Krish Krishnamurthy, *Agilent Technologies, CA, USA*

Wednesday, September 25th

9:00 AM - 10:30 AM **New Developments in NMR Methodology**
Chair: Lucio Frydman, *Weizmann Institute, Israel*

Advances in Pure Shift NMR
Peter Kiraly, University of Manchester, UK / Hungarian Academy of Sciences, Hungary

Superconductive NMR Probe Technology for Small Samples
William Brey, National High Magnetic Field Laboratory, USA

Multinuclear Nanoliter 1D and 2D NMR Spectroscopy with a Single Planar Microcoil (Upgraded Poster)
Raluca M. Fratila, University of Twente, Enschede, The Netherlands

Two-Dimensional NMR Spectroscopy on a Desktop Spectrometer (Upgraded Poster)
Andrew Coy, Magritek Ltd, Wellington, New Zealand

10:30 AM - 11:00 AM **Break**

11:00 AM - 12:30 PM **Methods of Analysis for Food Ingredients and Products**
Chair: John van Duynhoven, *Unilever, Netherlands*

Small Molecule Analysis in Food: an Overview and Some Recent Applications
Apostolos Spyros, University of Crete, Greece

Application of Recent Advances in quantitative NMR to Authentication of Beverages
Serge Akoka, University of Nantes, France

CRAFT (Complete Reduction to Amplitude Frequency Table) – Principles and Application to Food Sciences (Upgraded Poster)
Krish Krishnamurthy, Agilent Technologies, Santa Clara, CA, USA
qNMR Targeted Profiling of Food Compounds (Upgraded Poster)
Ewoud J.J. van Velzen, Unilever R&D, Vlaardingen, The Netherlands

12:30 PM - 2:30 PM **Lunch, Free Time & Vendor Discussions**

2:30 PM - 4:00 PM **Determination of Stereochemistry by NMR**
Chair: Ricardo Riguera, *Santiago de Compostela University, Spain*

Recent Achievements of NMR in Chiral Anisotropic Media: NMR Methodology and Applications
Philippe Lesot, Université Paris-Sud 11, France
NMR in Aligned Media, Computation and Chiroptics: from Constitution to Absolute Configuration
Armando Navarro-Vazquez, University of Vigo, Spain
Differentiation of Enantiomers in Complex Systems by NMR Spectroscopy and Chiral Solvating Agents: Applications and Methodology (Upgraded Poster)
Míriam Pérez-Trujillo, Universitat Autònoma de Barcelona, Bellaterra, Spain
Chiral Solvation Revisited: Developing a CSA Method with a Single Enantiomer (Upgraded Poster)
Richard J. Lewis, AstraZeneca R&D Mölndal, Mölndal, Sweden

4:00 PM - 4:30 PM **Break**

4:30 PM - 6:00 PM **Advances in Conformational Analysis and Dynamics by NMR**
Chair: Teo Parella, *Barcelona University, Spain*

Structure and Conformation of Oligosaccharides by NMR Spectroscopy
Göran Widmalm, Stockholm University, Sweden
Anisotropic NMR Parameters for the Configurational Analysis of Small Molecules
Burkhard Luy, Karlsruher Institut für Technologie (KIT), Germany
Configurational Analysis with a Small Number of NOEs – a Test Case (Upgraded Poster)
Matthias Köck, Alfred-Wegener-Institut, Bremerhaven, Germany
Quantification of Free Ligand Conformational Preferences by NMR and Their Relationship to the Bioactive Conformation (Upgraded Poster)
Charles D. Blundell, Conformetrix Ltd., Manchester, UK

6:00 PM - 6:15 PM **Closing Remarks**

7:00 PM - 10:00 PM **Cocktail - Hostal dos Reis Catolicos**

The following are independent activities and not part of the SMASH Program

Thursday, September 26th

8:00 AM - 6:00 PM **Ultrafast 2D NMR Symposium**
Co-chairs: Lucio Frydman, Patrick Giraudeau, Manuel Martin-Pastor and Antonio Herrera

Conference Room, Faculty of Chemistry, University of Santiago de Compostela

Friday, September 27th

8:00 AM - 6:00 PM **Advances in NMR Methodology and Processing**
Chair: Oscar Millet
Conference Room, Faculty of Chemistry, University of Santiago de Compostela

SMASH 2013 Scholarship Recipients

The following students/post docs received a scholarship to attend SMASH 2013

- **Sheraz Ahmad,** Federal University of São Carlos, Brazil
- **M. Álvaro Berbís,** CIB-CSIC, Spain
- **Laura Castañar Acedo,** Universitat Autònoma Barcelona, Spain
- **Abril Castro Aguilera,** Universitat de Girona, Spain
- **Sachin Rama Chaudhari,** Indian Institute of Science, Bangalore, India
- **Sílvia Diaz,** University of Aveiro, Portugal
- **Lauren Emery,** Grinnell College, United States
- **Daniel Finkelstein-Shapiro,** Arizona State University, United States
- **Albert Gargallo Garriga,** Universitat Autònoma Barcelona, Spain
- **Niels Geudens,** Ghent University, Belgium
- **Eduardo Gomes Rodrigues de Sousa,** UFRJ/ Farmanguinhos/FIOCRUZ, Brazil
- **Ileana Guzzetti,** Università degli Studi di Milano, Italy
- **Francisco Juárez-González,** National University of Mexico, Mexico
- **Ligia Llovera,** USB, Venezuela
- **Valeria Mannella,** Dulbecco Telethon Institute c/o S.Raffaele Scientific Inst., Italy
- **Fátima Morales Marín,** Universidad de Granada, Spain
- **Zulay Diney Pardo Botero,** Universidad Complutense de Madrid, Spain
- **Kowsalya Devi Pavuluri,** Indian Institute of Science, India
- **Javier Sastre Martinez,** CIB CSIC, Spain
- **Josep Saurí Jiménez,** Universitat Autònoma Barcelona, Spain
- **Davy Sinnaeve,** Ghent University, Belgium
- **Eduardo Troche Pesqueira,** Universidade de Vigo, Spain
- **Nazish Urooj,** Federal University of São Carlos, Brazil
- **Jan Vícha,** Masaryk University, Czech Republic

Scholarships provided by: Gesellschaft Deutscher Chemiker (GDCh)

- **Hanna Bartling,** University of Regensburg, Germany
- **Michael Hammer,** University of Regensburg, Germany
- **Andreas Kolmer,** TU Darmstadt, Germany
- **Jens Pilger,** Max-Planck-Institute for Biophysical Chemistry, Germany
- **Ann-Christin Pöppler,** Georg-August-Universität Göttingen, Germany

Scholarships provided by: UK NMR Discussion Group (NMR DG) and SMASH

- **Adam Ashley Colbourne,** University of Manchester, United Kingdom
- **Martin Dracinsky,** Durham University, United Kingdom
- **Jonathan Katz,** University of Sussex, United Kingdom
- **Ikenna Ndukwe,** University of Bristol, United Kingdom
- **Godiraone Tatolo,** University of Bristol, United Kingdom

Thanks to our scholarship sponsors for their generous support.

SMASH 2013 NMR Conference Acknowledgements

The SMASH 2013 Conference gratefully acknowledges the support provided by the following companies:

ACD/Labs

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geRMN grupo especializado de resonancia magnética nuclear

GPE

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RSC Advancing the Chemical Sciences NMR DG UK Nuclear Magnetic Resonance Discussion Group

Wilmad LabGlass

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Technische Universitat Darmstadt

Martin Will
Sanofi-Aventis

Monday, September 23rd
9:15 AM - 10:45 AM

Less-common NMR-active Nuclei in Small Molecules

Chair: Fernando Lopez Ortiz

Speakers:

Catherine Bessada
CNRS-Orleans, France

Bernd Wrackmeyer
University of Bayreuth, Germany

David L. Bryce
University of Ottawa, Canada

Ann-Christin Pöppler*
Georg-August-Universität Göttingen, Germany

* Upgraded Poster

NMR at High Temperature in Molten Inorganic Materials : From the Local Structure to the Dynamics

Catherine Bessada[1], Mallory Gobet[1], Georges Moussaed[1], Vincent Sarou-Kanian[1], Anne-Laure Rollet[2], Christian Simon[2], and Mathieu Salanne[2]

1. CEMHTI CNRS, Université d'Orléans, France
2. PECSA, CNRS, Université Pierre et Marie Curie, Paris

Molten salts are liquid mixtures of ionic species at high temperatures. Several kinds of molten salts can be distinguished, depending on the nature of the anions, O^{2-}, Cl^-, F^- and the chemical properties of the liquid. They are well known for their wide range of applications : in metal production, glasses, pyrochemical treatment of nuclear waste, or as primary or secondary coolants in several concepts of generation IV fission and fusion nuclear reactors, and electricity storage devices. The optimization of such processes implies a good knowledge of the physicochemical properties of the molten baths and of their structure in terms of ionic species distribution or speciation. Inorganic molten salts can be strongly organized at unusually long distances due to the predominance of coulombic interactions and described by the formation of an intermediate range ordering.

Because of particular experimental hindrances, the knowledge on molten fluoride salts chemistry has long remained fragmented and limited to a few experimental techniques mainly electrochemical. These systems cumulate high temperatures (from 500 K to 1800 K), corrosive properties towards most of the materials and sensitivity to moisture and oxygen and make the NMR experiment a real challenge. Specific technical developments are required for the sample container, for the heating system adapted to the superconducting magnets and for the protection of the spectrometer and the probe. Thanks to a CO_2 laser heating and a container adapted to corrosive and reactive liquids, airtight and inert chemically towards molten fluorides, NMR measurement can be performed in molten fluorides up to 1500°C [1-3].

Among the different experimental parameters that can be extracted from the NMR spectrum, chemical shifts provide a significant source of structural information including the nature of first neighbors, the coordination numbers, bond lengths and angles. In liquids, the dynamics and the rapid exchange between the different configurations results in a fully averaged local environment giving a single narrow lorentzian line defined by its position. The signal position, or the isotropic chemical shift, is the weighted averaged chemical shift of the different components in the melt. Knowing the chemical shift of the individual species, it becomes possible to extract their distributions depending on the composition [1-3]. This approach of the liquid can be also combined with a more dynamical description with self-diffusion coefficients measurements in situ in the melts. Thanks to the development of an NMR set up based on pulse field gradients

associated with a CO_2 laser heating system, we can measure self-diffusion coefficients up to 1500K [4,5].

Because of the primary importance of transport properties in materials science in a number of domains such as geology, glass industry, electrolytic processes in metallurgy or energy storage, these developments were extended to larger magnetic field gradients (up to 1200G/cm), in order to measure self-diffusion coefficients of different nuclei in a wide range of molten, glassy or even crystalline materials with high ionic conductivity [6,7].

1. Lacassagne V., Bessada C., Florian P., Bouvet S., Ollivier B., Coutures J-P., Massiot D., *J. Phys. Chem. B,* **106,** 1862 (2002)
2. Pauvert O., Salanne M., Zanghi D., Simon C., Reguer S., Thiaudiere D., Okamoto Y., Matsuura H., Bessada C., *J. Phys. Chem. B*, **115**, 9160-9167 (2011)
3. Bessada C., Rakhmatullin A., Rollet A.-L., Zanghi D., *J. Fluor. Chem*., **130**, 45 (2009).
4. Rollet A.-L., Sarou-Kanian V., Bessada C., *Inorg. Chem*., **48(23)**, 10972 (2009)
5. Rollet A.-L., Sarou-Kanian V., Bessada C., C. R. Chimie, **13**, 399 (2010)
6. Ohkubo T., Gobet M., Sarou-Kanian V., Bessada C., Nozawa M. , Iwadate Y., *Chem. Phys. Lett*., **530**, 61 (2012)
7. Wang Z., Gobet M., Sarou-Kanian V., Massiot D., Bessada C., Deschamps M., *J. Chem. Chem. Phys.* **14** 13535 (2012)

A Cutaway Drawing of Some NMR Parameters

Bernd Wrackmeyer

Anorganische Chemie II, Universität Bayreuth, Germany

Among prominent NMR parameters, chemical shifts δX, indirect nuclear spin-spin coupling constants ^{n}J(A,X), and relaxation times T_1, T_2 or T^Q can be listed. Indeed, a wealth of information is revealed by such data. This contribution aims at somewhat neglected issues connected with NMR parameters.

In context with chemical shifts δX or nuclear magnetic shielding σX, the so called isotope-induced chemical shift $^{n}\Delta^{y/x}$A(X) deserves attention. Nowadays, it can readily be measured using NMR spectrometers with moderate to high filed strengths B_0, not only for the effect excerted by $^{1/2}$H on chemicals hifts δX but also for various more heavy isotopes, such as $^{10/11}$B, $^{12/13}$C or $^{14/15}$N, for which examples will be given. Although this is well known in principle, it is not often used, and magnitude and sign of these effects are not well understood. This situation is in contrast with nuclear magnetic shielding itself, which is well understodd and can be related to the respective elecctronic structure.

Indirect nuclear spin-spin coupling constants ^{n}J(A,X) are known to possess a sign which is often neglected. This may be justified for numerous cases, where the sign has been determined and is not likely to change within a series of related compounds. However, there are bonding situations, for which a change in the coupling sign may be conceivabale, and the absolute magnutude of the soupling constant may be misleading. An example is given for a platinum(0) complex conating numerous magnetically active nuclei (^{1}H, ^{13}C, ^{29}Si, ^{31}P, ^{195}Pt). It is shown that sign determinations are straightforward using conventional NMR instrumentation. Clearly, the discussion of coupling signs is greatly aided by the fairly reliable quantum chemical calculations of these parameters.

For routine NMR spectroscopic work on molecules of small to moderate size, NMR measurements of quadrupolar nuclei are often not particularly popular, with few exceptions, if NMR spectra of other spin-1/2 nuclei can be measured conveniently. The obvious reasons are expectedly broad NMR signals and sometimes other unfavourable NMR properties in addition to the nuclear quadrupole moment. Some examples are given for ^{14}N NMR which show that it is wothwhile to spend a little bit of extra time. Other examples are provided by ^{55}Mn NMR.

Structure and Polymorphism in Small Molecules and Drugs: Insights from Solid-State NMR of Quadrupolar Nuclei

Frédéric A. Perras, Kevin M. N. Burgess, Nuiok Dicaire, and **David L. Bryce**

Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada

Many pharmaceutical formulations result in tablet or capsule forms, where the active pharmaceutical ingredient (API) exists as a powdered solid. In addition to X-ray diffraction experiments and ^{13}C CP/MAS NMR spectroscopy, solid-state NMR of various quadrupolar nuclei ($I > ½$) found in drugs may offer valuable insights into structure and polymorphism. Typical quadrupolar isotopes found in APIs include e.g., ^{35}Cl, ^{23}Na, ^{17}O, ^{14}N, ^{33}S. Quadrupolar broadening of the NMR line shapes associated with these nuclei, as well as spectral interpretation, may deter the routine application of quadrupolar solid-state NMR to study small molecules. In this presentation, we address two topics which demonstrate the utility of applying solid-state NMR of quadrupolar nuclei to study small molecules and drugs.

About 85% of pharmaceuticals contain or are manufactured using chlorine. The API may contain chloride as a counterion or chlorine covalently bound to carbon. We have reported previously on $^{35/37}Cl$ SSNMR of chloride ions,[1] and present here our recent work on examining the information available from $^{35/37}Cl$ SSNMR spectra of chlorine which is covalently bonded to carbon atoms in a series of small molecules including the drug chlorothiazide.[2] This is the first comprehensive NMR study of covalently-bound chlorine in organic compounds, and necessitated ultrahigh magnetic fields (21.1 T) and the development of an exact line shape simulation program.[3] Chlorines bound to sp^2 vs sp^3 hybridized carbon atoms are easily distinguished. Furthermore, different molecules in the asymmetric unit can be simply identified, despite the breadth of the NMR spectra.

In the second part of the talk, we demonstrate the utility of high-resolution ^{23}Na double-rotation (DOR) solid-state NMR in identifying and characterizing polymorphs and solvates of the drugs sodium naproxen and sodium valproate.[4] The ^{23}Na quadrupolar coupling constant was found to change markedly depending on the hydration state, and subtle changes in oxygen coordination environment about the sodium cations were also identified. Efforts to crystallize various solvates of sodium naproxen led to the first crystal structure for the heminonahydrated form. The composition of commercial sodium naproxen is also investigated and it is shown that Aleve® is composed of approximately 80% monohydrate solvate. DFT calculations, using the gauge-including projector-augmented-wave formalism, allow for the assignment of ^{23}Na NMR peaks to specific sodium sites in the X-ray crystal structure.

1. R. P. Chapman, C. M. Widdifield, and D. L. Bryce, *Prog. Nucl. Magn. Reson. Spectrosc.* **2009**, *55*, 215.
2. F. A. Perras and D. L. Bryce, *Angew. Chem. Int. Ed.* **2012**, *51*, 4227.
3. F. A. Perras, C. M. Widdifield, and D. L. Bryce, *Solid State Nucl. Magn. Reson.* **2012**, *45-46*, 36.
4. K. M. N. Burgess, F. A. Perras, A. Lebrun, E. Messner-Henning, I. Korobkov, and D. L. Bryce, *J. Pharm. Sci.* **2012**, *101*, 2930.

Polymer Networks for the Measurement of Anisotropic NMR Parameters

Ann-Christin Pöppler, Sebastian Frischkorn, Dietmar Stalke, and Michael John

Georg-August-Universität Göttingen, Department of Inorganic Chemistry, Göttingen, Germany

Anisotropic NMR parameters have gained more and more importance in many fields of analytical organic chemistry and a variety of different alignment media is nowadays available for that purpose.[1] For very reactive compounds like organolithiums, strain induced alignment in a gel (SAG) is the most promising approach. Polystyrene networks represent a rather chemically inert environment and are straightforwardly applicable after only few changes to the original procedure [2] (no initiator, thermal polymerization). Thereby, we were able to prove residual quadrupolar couplings (RQCs) measured for ^{7}Li (I = 3/2) to be a very powerful tool for the discrimination between aggregates with high and low symmetry. The latter show a quadrupolar triplet inside the polymer gel, while ^{7}Li NMR spectra of the former still display a singlet.[3]

While this method works very smoothly for organolithiums and is also applicable to other quadrupolar nuclei, the swelling times required for polystyrene gels are still considerable long (roughly seven days for toluene). Furthermore, organometallic compounds often are part of equilibria[4] or display structural flexibility. Therefore, the temperature range in which gels can be successfully applied comes into focus. We addressed these aspects in different ways:

The temperatures at which quadrupolar splittings can be observed were investigated for polystyrene (PS) and polybutylacrylate (PBA) networks. Furthermore, slice-selective excitation (SSE) was employed to monitor the swelling behaviour of different gels. This is a very efficient technique and enables resolving of the swelling process with spatial resolution. Consequently, the swelling properties of four different polymers sdticks (PS, PS-RAFT, PBA, PBA-RAFT) were studied. The presence of a RAFT (reversible addition fragmentation chain transfer) agent sould result in a more homogeneous and uniform network.[5] First measurements showed interesting and promising results.

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Monday, September 23rd
11:15 AM - 12:45 PM

Molecular Interactions by NMR. The Ligand-based Perspective

Chair: Gary Sharman

Speakers:

Gordon C K Roberts
University of Leicester

Tim Claridge
University of Oxford, Oxford, UK

Ileana Guzzetti*
Università degli Studi di Milano, Milano, Italy

Jens Pilger*
Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

* Upgraded Poster

Information on Binding from the Ligand Spectrum: An Overview

Gordon C.K. Roberts

Henry Wellcome Laboratories for Structural Biology, Department of Biochemistry, University of Leicester, Leicester, UK

As is well known, NMR can provide very detailed information on the structure of macromolecules and their complexes in solution. The emphasis in this field has been predominantly on the determination of the full structure of the complex, which requires quite substantial quantities of the macromolecule in isotopically-labelled form. However, it is possible to obtain valuable information about the binding of small molecules to macromolecules simply from the observation of the small molecule spectrum.

The key to these experiments is that the chemical exchange of the small molecule between the bound and free states should be fast on the NMR timescale – that is, fast relative to the difference in NMR parameters between the two states. This generally limits this kind of experiment to complexes with dissociation constants $K_D \geq 10^{-7}$ M. The information which can be obtained ranges from answering the simple question 'does it bind?' to obtaining detailed information on the conformation of the small molecule in the bound state. There are several useful recent reviews of this field [1-4]; this talk will focus on two broad areas – the use of NMR to detect binding from changes in diffusion coefficients and the use of magnetisation transfer experiments.

When a small molecule binds to a macromolecule, one thing is certain – its rotational and translational diffusion coefficients will change. This provides a convenient way of detecting binding. By editing the spectrum on the basis of diffusion coefficients (see *e.g.* [5]) one can pick out which compounds in a mixture are binding. The use of magnetisation transfer experiments to detect small molecule binding involves (negative) NOEs between protons of the macromolecule and those of the bound small molecule which are detected on the averaged spectrum of the small molecule [1-3]. These 'NMR screening' approaches have been used successfully in the pharmaceutical industry.

The STD experiment has been extended to the determination of the part of the small molecule which is in closest contact with the macromolecule, though care is needed in this more quantitative application [6,7]. NOEs between protons of the bound small molecule can be used, again with care, to determine its conformation in the bound state; this can provide extremely valuable information [4,8]. It must be remembered that interpretation of any experiment designed to obtain structural information on the complex under fast exchange conditions depends on having and appropriate model for the exchange process – are there one or more states of the complex? [4].

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Reporter-based Ligand Screening by NMR: Applications to 2OG-Dependent Oxygenases

Tim D. W. Claridge, Ivanhoe K. H. Leung, Amjad Khan, and Christopher J. Schofield

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Ligand screening methods employing by NMR spectroscopy have become well established techniques for the study of small molecules binding to macromolecular targets and have a role to play in the development of new pharmaceuticals and novel ligands designed as probes to investigate biochemical pathways. The methodologies available generally fall into two classes: “protein observe” where the resonances of the (isotopically labeled) macromolecule are detected or “ligand observe” where the behavior of the small molecule itself is monitored. In the absence of isotopically labeled protein, ligand observed techniques are favored and methods such as saturation transfer difference (STD) and WaterLOGSY are widely employed.

In some cases, however, techniques employing direct observation of the binding ligand can be susceptible to false negatives (high-affinity ligands may give no detectable response on binding) and false positives (non-specific binding to the macromolecule). In such cases the use of “reporter” or “spy” molecules in competition based assays may overcome these limitations whereby the response of the reporter is monitored as it becomes displaced by the ligand of interest under investigation. Herein we describe the application of reporter-based NMR screening methods to members of the so-called 2-oxoglutarate (2OG) dependent oxygenases. This family of proteins comprises Fe(II) dependent enzymes that target either small molecules or complete proteins as substrates for hydroxylation. These enzymes have many essential roles in mammalian biology including, for example, responses to oxygen levels, fatty acid metabolism and epigenetic regulation. Examples of reporter ligand methods will be described, including the use of the co-factor 2OG as a generic reporter for the enzyme family [1] and the use of solvent water itself as a reporter [2].

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A Comprehensive NMR Analysis of the Interaction between DKP-RGD Ligands and Membrane Proteins on Intact Cells

Ileana Guzzetti[1], Donatella Potenza[1], Francesca Vasile[1], Laura Belvisi[1], Monica Civera[1], Ilaria Silvestri[2], Umberto Piarulli[3], and Cesare Gennari[1]

1. Università degli Studi di Milano, Dipartimento di Chimica, Milano, Italy
2. Università degli Studi di Milano, Dipartimento di Biotecnologie mediche e Medicina Traslazionale, Segrate (MI), Italy
3. Università degli Studi dell'Insubria, Dipartimento di Scienza e Alta Tecnologia, Como, Italy

NMR experiments, transferred NOE and Saturation Transfer Difference, were used to shed light on the binding epitope of a small library of DKP - RGD peptidomimetics with integrins alphavbeta3, expressed on the membrane of ECV304 bladder cancer cells, and alpha5beta1, overexpressed on the membrane of MDA-MB-231 breast adenocarcinoma cells.

Integrins are a large family of transmembrane heterodimeric glycoprotein receptors, composed of two non-covalently associated alpha and beta subunits. Once bound to extracellular matrix proteins, they regulate a variety of cellular processes.[1] As a consequence of their role in important physiological phenomena, integrin defects have been implicated in many common diseases. In particular, the observation that alphavbeta3 and alpha5beta1 integrin subtypes are essential for tumor angiogenesis and can be successfully inhibited by small-molecule ligands containing the Arg-Gly-Asp (RGD) motif have turned them into attractive targets for cancer research.[2]

The DKP-RGD compounds studied are characterized by a diketopiperazine (DKP) scaffold introduced into cyclic peptidomimetics containing the Arg-Gly-Asp (RGD) sequence and differ for the configuration of the two stereocenters of the DKP and for the substitution at the diketopiperazinic nitrogens.[3]

The understanding of the interaction of a protein with its ligands requires precise knowledge about the underlying recognition events at a molecular level. A variety of biophysical methods have been developed for this purpose and several NMR spectroscopic techniques have emerged as powerful tools to identify and characterize the binding epitope of ligands to receptor proteins.

In particular, transferred NOE (tr-NOE) has gained momentum, as it provides the basis for a variety of experimental protocols that are designed to detect and characterize binding activity. The observation of tr-NOEs relies on the different behaviour of a small ligand molecule free in solution rather than bound to a receptor protein. In fact, a ligand bound to a large-molecular weight protein behaves as a part of the large molecule and adopts the corresponding NOE behaviour, showing strong negative NOEs, so called tr-NOEs. These tr-NOEs reflect the bound conformation of the ligand. Binding of a ligand to a receptor protein can thus easily be distinguished by looking at the sign and size of the observed NOEs. A NMR spectroscopic technique, complementary to tr-NOE, is Saturation Transfer Difference (STD). This sequence helps identifying the group epitope, revealing which moieties of the ligand molecule are closest

to the receptor in the bound state. The method is based on the transfer of saturation from the protein to the bound ligand which, by exchange, is released into solution where it is detected. The degree of saturation of individual ligand protons (expressed as absolute-STD percent) reflects their proximity to the protein surface and can be used to describe the ligand-target interactions.

Tr-NOE and STD experiments conducted with living cells have showed that the primary interaction between the ligand and the receptor is performed by the guanidine residue of the arginine side chain, the extended conformation of the ligands ensures the formation of the electrostatic clamp (STD effects on protons of the Arg and Asp residues), the opposite configuration at stereogenic centres of the DKP scaffold induces a different binding epitope for the non-RGD moiety of the ligands (diketopiperazine and benzyl substituent).

The NMR results were supported by docking calculations of these ligands in the active sites of alphavbeta3 and alpha5beta1 integrins receptor and were compared to the results of competitive alphavbeta3 receptor binding assays and competitive ECV304 and MDA-MB-231 cell adhesion experiments. The different stereochemistry and the different substitution at the nitrogen atoms of the scaffold strongly influences the conformations adopted in the free-state and the binding mode of these ligands in the active site of the integrin receptor.

Previous conformational studies of these cyclic RGD-peptidomimetics revealed that the highest affinity ligands display well-defined preferred conformations featuring intramolecular hydrogen-bonded turn motifs and an extended arrangement of the RGD sequence [Cbeta(Arg)-Cbeta(Asp) average distance $\geq$ 8.8 Å].

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A Spin Diffusion Based Method to Determine Protein-Ligand Complex Structures Demonstrated on Enzymes of Pharmaceutical Interest

Jens Pilger[1], Adam Mazur[1], Tiziana Masini[3], Peter Monecke[2], Herman Schreuder[2], Bettina Elshorst[2], Gerhard Hessler[2], Anna K. H. Hirsch[3], and Christian Griesinger[1]

1. Max-Planck-Institute for biophysical chemistry, Göttingen, Germany
2. Sanofi Deutschland GmbH, Frankfurt a. M., Germany
3. Rijksuniversiteit Groningen, Bio-organic chemistry, Groningen, Netherlands

Structure-based drug design mainly relies on high-resolution crystal structures of the receptor-ligand complex to obtain the required information for optimizing target binding of small molecules. However, obtaining crystals and structures of sufficient quality cannot be achieved for approximately 40% of pharmaceutically relevant protein targets. For those target proteins that cannot be crystallized, NMR spectroscopy is an alternative. We developed a fast and reliable methodology, termed STI, to use experimental, spin diffusion based NMR data as a scoring function for molecular docking structures: 1) The INPHARMA method [1] derives the relative binding mode of two ligands, given that they target the same binding site 2) Saturation Transfer Difference (STD) [3] yields information on the protein-buried and water-exposed part of the ligand and 3) Transfer-NOE reveals the bound ligand conformation. For every docking model, the NMR peak volumes of all three data sets are back-calculated with our software SpINPHARMA and then correlated with the experimental data. We show on the example of Protein Kinase A (PKA) ligand complexes, that cross validation of docking results against NMR restraints leads to the same binding modes as X-ray crystallography and performs clearly better than docking scoring functions. In the following, the methodology is demonstrated on two enzymes of on-going pharmaceutical research, sEH and DXS.

Soluble epoxide hydrolase (sEH) is an enzyme that hydrolyses the epoxide moiety formed on unsaturated fatty acids like arachidonic acid during metabolism. Inhibition of this reaction has been shown to have favourable effects on cardiovascular diseases [3], making sEH an interesting new drug target. Designing drugs for sEH inhibition is on-going and an early characterized ligand class consists of a central urea moiety positioned in the middle of the binding site, framed by two substituents on the left and right side. For drug optimization of these ligands it is crucial to know their orientation in the binding site, which is shaped like a tunnel with entries to both sides. Four ligands were chosen and binding to sEH was confirmed with STD spectra and INPHARMA peaks were observed between all of the ligand pairs in the presence of sEH. Yet, the application of our STI approach did not yield a clear proposal of the binding modes, as in the case of PKA. Instead, the data give the impression that the ligand substituents can flip around the urea moiety and every ligand is present as two distinct binding modes. To validate these observations, three ligands were successfully co-crystallized with sEH. The crystal structures underline strikingly the observation from NMR: the substituents of the ligands are always present in both binding modes. This interesting observation confirms that our STI approach can also deal with very complex ligand binding behaviours. A new approach of population weighting was also applied to further quantify

the occupancies of each binding modes, as it can be done with X-ray crystallography. The project now continues with stronger sEH binders from the pharmaceutical company Sanofi.

1-deoxy-D-xylulose-5-phosphate synthase (DXS) was chosen as the target of a structure-based drug design project. DXS catalyzes the first and rate-limiting step of the non-mevalonate pathway and employs thiamine pyrophosphate (TPP) as cofactor. Important pathogens such as *Mycobacterium tuberculosis* and *Plasmodium falciparum* use the non-mevalonate pathway for the biosynthesis of the universal precursors of the essential isoprenoids. Given that humans exclusively utilise the alternative mevalonate pathway, the enzymes of the non-mevalonate pathway have emerged as attractive targets for the development of new drugs against infectious diseases like tuberculosis and malaria [4].

To evaluate the applicability of our innovative methodology, we started studying the binding mode of deazathiamine, a derivative of the natural binder TPP. While the binding mode of TPP is known from X-ray crystallography (PDB: 201x), there is no co-crystal structure of deazathiamine with DXS available in the RCSB protein databank. Nevertheless, deazathiamine can be expected to have a very similar binding mode to DXS as TPP, due the high similarity. Yet the measured IC_{50} value of 430 µM for deazathiamine is much lower than that of TPP (14 nM). The molecule **1** was designed *de novo* as a TPP-competitive inhibitor but it is no more a derivative of TPP. **1** was found to possess a promising IC_{50} value (1.81 ± 0.48 mM) and it was the objective to further improve this molecule by better filling the space of the DXS binding pocket in a structure-based approach. In order to optimize or grow **1**, information about its binding mode is necessary, therefore we applied our NMR methodology to this specific case. In the first place, INPHARMA peaks were observed between deazathiamine and ligand **1.** By application of our STI method, the binding mode of both molecules was determined. Hereby the binding mode of deazathiamine is very close to that of TPP, so we were very confident with this binding mode. Based on this complex structure, ligand **1** was optimized. The methyoxy group of **1** was replaced by a trifluoromethoxy group and the right-hand side of 1 was further grown into a benzimidazole group. This new ligand **2** was found to have an IC_{50} of 0.595 mM. The inhibition potency of the original fragment **1** has been therefore improved by four times. A NOESY spectrum of ligand **2** and deazathiamine was recorded and INPHARMA peaks were clearly observed. Applying the STI method, the binding mode of **2** was determined and is similar to the binding mode of ligand **1**, as would be expected.

In conclusion, we have established a robust method to determine complex structures of small molecule ligands based on scoring of docking modes with the spin diffusion based NMR parameters INPHARMA, trNOE and STD. The method was amenable to be applied for pharmaceutical research and structure-based drug design, as demonstrated on the interesting drug targets sEH and DXS.

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Monday, September 23rd
2:45 PM - 4:30 PM

Workshop

Structural Elucidation: Different Approaches and Insights

Tim Claridge, University of Oxford, UK
Brian Marquez, Pfizer, USA

Structure Elucidation - Different Approaches and Insights

Tim Claridge[1] and Brian Marquez[2]

1. University of Oxford, UK
2. Pfizer Inc., USA

The intent of this workshop is to have an open discussion around many aspects of structure elucidation that we all face as practicing NMR spectroscopists. The discussion points that we will address will be the following:

1. Part A- Protocols (how we use data to get to structures)
    - What defines structure? – constitutional, connectivity (planar), stereochemistry.
    - Structure confirmation vs. elucidation vs. characterization
    - Confirmation (ASV)
    - Elucidation programs (CASE)
    - Differences between academia and industry
2. Part B- NMR methodology (methods for generating NMR data)
    - Favored technique variants for elucidation (benefits and down-sides)

We are hoping that attendees will bring questions, ideas, examples, etc. to help facilitate a robust discussion. As we all know many of the above elements are “user preferred” so there is no intent of prescribing methodology nor instruction. However, a discussion/debate around the uses and merits is desired.

Tuesday, September 24th
9:00 AM - 10:30 AM

Integration of NMR Metabolomic with Other Platforms and Omics

Chair: Silvia Mari

Speakers:

Maria Vinaixa
Universitat Rovira i Virgili, Spain

Naomi Rankin
University of Glasgow, Scotland, UK

Sílvia O. Diaz*
University of Aveiro, Aveiro, Portugal

Manuel Martín-Pastor*
University of Santiago de Compostela, Santiago de Compostela, Spain

* Upgraded Poster

Merging Data Coming from Different Metabolomics Platforms

Maria Vinaixa

1. Yanes Laboratory, Universitat Rovira i Virgili, Reus, Spain
2. Metabolomics Platform, Universitat Rovira i Virgili-CIBERDEM, Reus, Spain

Untargeted metabolomics offers a powerful approach to interrogate mechanistic biochemistry in biological systems from a small-molecule standpoint. Untargeted metabolomics allows the study biological systems by measuring, ideally, the largest set of metabolites at the raw signal level and in an unbiased manner. [1]

Due to the wide range of physico-chemical diversity in the metabolites, it is generally necessary to employ multiple analytical platforms so as to widen metabolome coverage. Two main analytical platforms are primarily used to this end: nuclear magnetic resonance spectroscopy and/or mass spectrometry coupled to different chromotographic techniques. Because of their different detection principle, they do not only requiere different experimental conditions (usually adjusted to exploit their complementarity in metabolomics studies) but also they requiere different data analysis approaches. We are going to discuss different work-flows and short-cuts to data conversion, pre-processing, alignment, and normalization for each type of data.

On the other hand, regardless of analytical platform they are derived, untargeted metabolomics datasets are highly-dimensional, with a rather limited number of study subjects as compared to the number of usually multicorrelated variables. This makes statistical analysis of such datasets a challenging task.[2,3] Data analysis strategies to effectively integrate and combine metabolomics data derived from different analytical platforms are going to be illustrated in the context of several untargeted metabolomics case studies. We are going to focus on how to render biologically and chemically meagniful this vast amount of data using tools for its functional interpretation.[4]

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High Throughput Nuclear Magnetic Resonance versus Mass Spectrometry for Serum Metabolomics: A Comparison of Platforms through Case Studies

Naomi Rankin, Emma Boulton, Fraser Morton, Karl Burgess, David Preiss, and Naveed Sattar

University of Glasgow, Scotland, UK

As the clinical field rapidly moves towards treatment based on stratified medicine principles, new methods to profile the health and disease state of populations must be developed. Metabolomic approaches applied to clinical samples are a rapidly growing area of research with significant implications for healthcare and clinical analysis.

Nuclear magnetic resonance (NMR) and Mass Spectrometry (MS) platforms are beginning to provide the high throughput, stability and reproducibility required for studies of large sample cohorts. Sample types commonly consist of serum, plasma, urine, and more rarely CSF, saliva, tissue and cell extracts. Sample collection, storage, preparation must all be carefully controlled. At the University of Glasgow we have access to unique cross-generational cohorts, clinical trial samples, exercise treatment trials and other prospective trials. We are particularly interested in cardiovascular disease, diabetes and obesity.

For clinical NMR analysis we use the high throughput method developed by the Ala-Korpela group. A total of 216 metabolite measures are reported [1]. 117 direct metabolite measures (e.g. lipoprotein subclasses and small molecules) and 99 derived measures (such as Fischer's ratio). The sample is analysed using three different "windows": the LIPO window for lipoproteins in native serum, the low molecular weight metabolite window (LMWM) also on native serum and the LIPID window on the lipid extract [2,3]. The LIPO window is obtained using a 1D-^{1}H-NOESY pulse program, with pre-saturation (noesypr1D). The LMWM window is obtained using a Carr-Purcell-Meiboom-Gill (CPMG) pulse program to allow detection of small molecule components. The LIPID window is performed to detect extracted lipids. The results of all the different windows are brought together and the 99 derived measures are calculated. Self-organising maps are then used to group patients.

For clinical MS analysis, we apply a high throughput ultra-high mass accuracy FTMS platform coupled to hydrophilic interaction liquid chromatography. Specifically, samples were analysed using ZIC-pHILIC chromatography on a RSLCnano (Thermo, UK) coupled to a Q-Exactive (Thermo, UK) high resolution orbitrap MS. Blank samples were inserted at the beginning of the run, followed by six system conditioning samples, three retention time standard samples and batches of samples randomized by patient and time point. Data was analysed using a modified XCMS/MzMatch pipeline (Feihn group (San Diego) Breitling group (University of Manchester) and Glasgow Polyomics (University of Glasgow)). Briefly, samples were grouped, peaks were picked, retention times were aligned, related peaks were annotated and, in cases of known adducts and fragments, were removed from the analysis. The resulting data was either annotated in accordance with accurate mass, or intensified by mass and by reference to a panel of unambiguous retention time standards. Instrument stability is monitored and maintained by reference to recurrent QC analyses.

We discuss the requirements for analysis of large clinical cohorts and compare the benefits and limitations of MS and NMR platforms for high throughput clinical metabolomics.

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Can Urine Metabolic Profiling Help to Diagnose or Predict Disorders of the Mother, Fetus and Newborn?

Sílvia O Diaz[1], António S Barros[2], Elisabete Morais[1], Elisabete Aguiar[1], Iola F Duarte[1], Brian J Goodfellow[1], Isabel M Carreira[3], Eulália Galhano[4], M Céu Almeida[4], Cristina Pita[4], Fátima Negrão[4], and Ana M Gil[1]

1. CICECO Department of Chemistry, University of Aveiro, Aveiro, Portugal
2. QOPNA Department of Chemistry, University of Aveiro, Aveiro, Portugal
3. Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Portugal and CENCIFOR - Forensic Science Centre, Portugal
4. Bissaya Barreto Maternity, Hospital Center of Coimbra, Portugal

Several prenatal disorders have a significant impact on the health of the mother and fetus during pregnancy, as well as of the newborn. In spite of the remarkable advances in recognizing new markers of prenatal disorders [1], fetal malformations (FM) and chromosomal disorders (CD) diagnosis remains lengthy and, ultimately, very invasive, whilst for gestational diabetes mellitus (GDM), preterm delivery (PTD), preeclampsia (PE) and intrauterine growth restriction (IUGR) there are no adequate predictive methods. In this context, urine emerges as a valuable matrix to search for novel diagnostic and predictive signatures of maternal, fetal and newborn disorders due to its non-invasiveness and ease of collection.

In the first part of this presentation, ^{1}H NMR metabolomics of 2nd trimester maternal urine is explored as a possible diagnostic tool of fetal trisomy 21 (T21), FM and specifically malformations of the central nervous system (CNS). PLS-DA models of the ^{1}H NMR spectra were found to be characterised by classification rates (CR) ranging from 94% for T21, 84% for FM and 87% for CNS. These numbers are significantly higher than other non-invasive diagnostic methods used in clinical practice and typically based on biomarkers found in blood (55-75% for T21 [2] and 17-74% for FM [3]) [4].

Secondly, the attempted prediction of poor pregnancy outcomes (PTD, GDM, IUGR and PE) through metabolic profiling of 2nd trimester maternal urine is described, based on a previous report [4]. For this study, urine was collected several gestational weeks (g.w.) before the clinical onset of the disorders. A similar methodology produced PLS-DA NMR models with considerable predictive power, as shown CR ranging from 84% at 11-20 g.w. prior to PTD, 83% at 2-21 g.w. prior to GDM diagnosis, 94% at 2-22 g.w. prior to IUGR diagnosis and, finally, 94% at 18-24 g.w. prior to PE diagnosis). For further compreehensive understanding of GDM, the metabolic trajectory of the disease was traced considering three different stages: pre-diagnosis state, at the moment of diagnosis and during treatment, taking into account a previously established NMR-measured metabolic trajectory for healthy pregnancies [5]. Furthermore, prediction of treatment response (insulin requiring *vs*. non-insulin requiring) through urine metabolic profiling of GDM subjects was attempted, giving promising exploratory results, highlighting the possibility of *a priori* prediction of personalised treatment response.

Finally the impact of prenatal disorders in newborns´ health was explored by profiling urine of babies born from PTD and GDM-affected pregnancies, babies with disturbed fetal growth and neonatal asphixia.

The results hereby presented highlight the potential of urine metabolomics for prenatal diagnosis, still requiring larger cohorts and external validation so that clinical application may be achieved.

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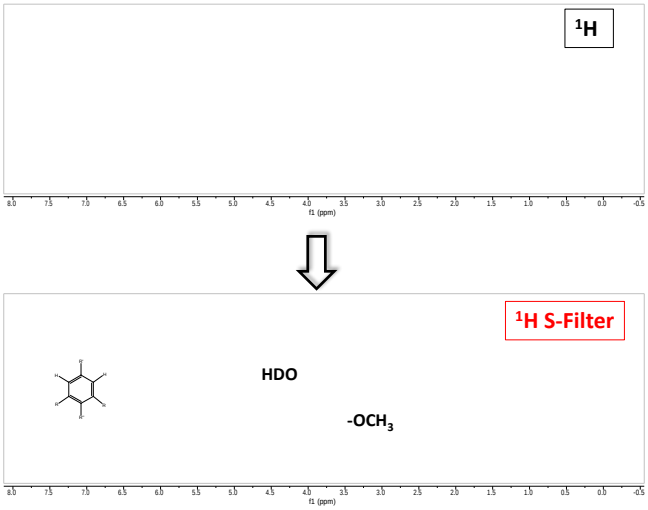
^{1}H S-Filter: Quantitative Edition of Singlets in 1D ^{1}H NMR Spectra for the Study of Complex Mixtures

Manuel Martín-Pastor

Unidade de Resonancia Magnética, RIADT, Campus Vida, University of Santiago de Compostela, Santiago de Compostela, A Coruña, Spain.

NMR is a useful tool for the characterization of complex mixtures of compounds or metabolites. The applications cover a range that includes the analysis of food, biological fluids, tissues and cell extracts [1-2]. The multi-variable statistical analysis of the signals in the spectra with chemometrics methods [3-4] permits to detect patterns in the NMR signals that can be related with a property of interest, e.g. disease [1].

One dimensional NMR experiments relying on ^{1}H are the fastest and more sensitive NMR spectra. Nevertheless, in complex mixtures severe signal overlap often hampers the identification of key metabolites and/or quantitation of their concentration. Several types of NMR experiments have been developed for the edition/filtering of signals in the 1D proton spectrum based in properties such as signal relaxation or translational diffusion [5-7]. However, these methods do not apply too well for complex mixtures of metabolites that typically have similar relaxation and diffusion properties.



In this work, a new experiment called ^{1}H S-Filter is presented for selective edition of singlet peaks in 1D proton spectra. The ^{1}H S-Filter has been tested with a variety of food samples having a complex ^{1}H spectrum, and the results show a clean and robust edition of singlet peaks. Moreover, the spectra retain most of its sensitivity and quantitativity. Future developments of the method could find application in the area of in-vivo localized MR spectroscopy.

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Tuesday, September 24th
11:00 AM - 12:45 PM

Workshops

Reaction Monitoring & Quantitation

Silvia Davalli, Aptuit Verona, Italy

Solid State NMR in Pharma

Steven Brown, University of Warwick, UK
David L. Bryce, University of Ottawa, Canada

Reaction Monitoring & Quantitation

Silvia Davalli

Aptuit Verona, Italy

Quantitative NMR (qNMR) was introduced already more than 50 years ago and nowadays applications cover drugs, natural products, foods and beverages, metabolic profiling/fingerprinting of plant extracts and body fluids, and on-flow monitoring of reaction processes. Interest is still increasing as demonstrated by the increase in the volume of literature as well as by the widely use in industrial settings.

A variety of methodology and pulse sequences have been developed and already validated in terms of specificity, analytical precision and accuracy. Moreover, several experimental protocols (acquisition and processing parameters and sample preparation) have been proposed and discussed, as is unanimously recognized that they are critical for accurate quantification.

Consistent and accurate data processing are even more challenging, due to the high amount of data generated; scripting or other automation features are needed and appropriate software packages are now the limiting step. Currently, many processing packages are available or under development from spectrometer manufacturers and third party developers, thus confirming the interest in the field. This workshop is the occasion to discuss what outcome we expect for these software, what issues we are still facing while processing and interpreting data and what we would like to see as next development.

Solid State NMR in Pharma

Steven Brown [1] and David L. Bryce [2]

1. University of Warwick, UK
2. University of Ottawa, Canada

The importance of solid-state NMR is being increasingly recognised by pharmaceutical industry. This workshop will introduce participants to the power of advanced solid-state NMR experiments for pharmaceutical characterisation (e.g., 2D homonuclear and heteronuclear correlation experiments with high resolution for ^{1}H, as well as quadrupolar nuclei NMR techniques, applicable to compounds at natural isotopic abundance). Within an interactive atmosphere, after introducing the basics of solid-state NMR (and discussing differences and similarities as compared to solution-state NMR) and describing the principles of advanced 2D experiments, we envisage a discussion of recent examples of the application of solid-state NMR to pharmaceutical systems. It is our aim that questions of most current relevance to the pharmaceutical industry that can be addressed by solid-state NMR are at the centre of this workshop.

Wednesday, September 25th
9:00 AM - 10:30 AM

New Developments in NMR Methodology

Chair: Lucio Frydman

Speakers:

Peter Kiraly
University of Manchester, UK
Hungarian Academy of Sciences, Hungary

William Brey
National High Magnetic Field Laboratory, USA

Raluca M. Fratila*
University of Twente, Enschede, The Netherlands

Andrew Coy*
Magritek Ltd, Wellington, New Zealand

* Upgraded Poster

Advances in pure shift NMR

Mathias Nilsson[1,2] and **Peter Kiraly**[2]

1. University of Copenhagen, Faculty of Science, Dept. of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg, Denmark
2. School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK

Scalar coupling is a key source of information on chemical structure, but is also an unwelcome source of complications. In ^{1}H NMR, multiplets caused by homonuclear scalar couplings are often many times the width of a single line, making it very difficult to distinguish individual chemical shifts in crowded spectra. An efficient method to collapse the multiplet structure has long been sought, but only recently have experimental methods for such homonuclear broadband decoupling become practical[1-3]. These "pure shift" or "chemical-shift resolved" or "δ-resolved" methods give resolution improvements approaching an order of magnitude, far in excess of any gains realistically to be expected from increases in static magnetic field.

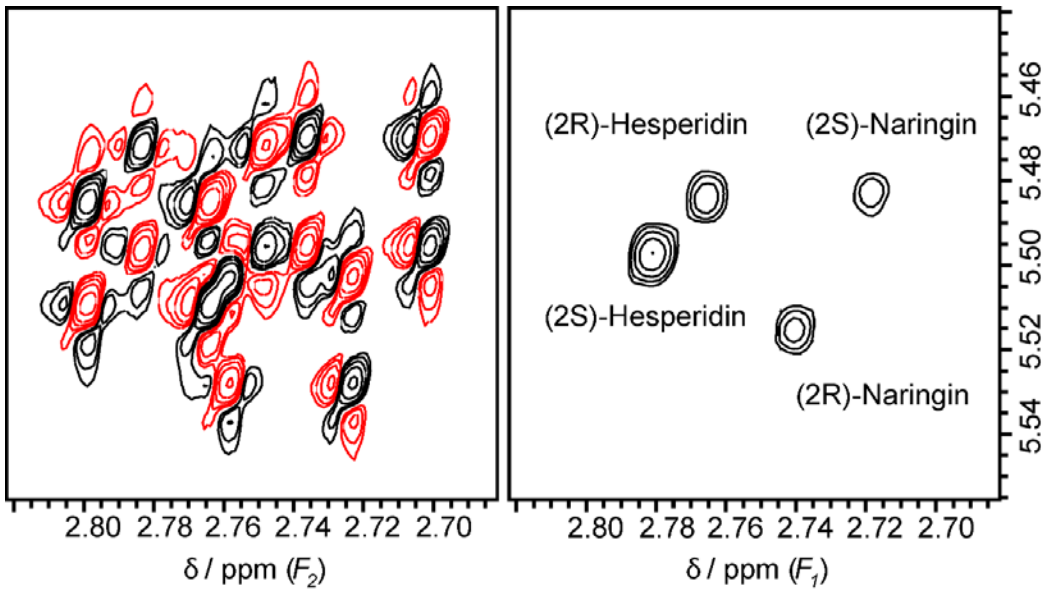

Figure 1. Expansions of cross-peaks between proton 2 and 3 in (a) 3QF-COSY and (b) pure shift (CT) 3QF-COSY covariance spectra, for racemic mixtures of (2RS)-hesperidin, (2RS)-naringin. The improvement in interpretability is striking.

However, the first generation methods[1, 2, 4-13] suffer to a greater or lesser extent from reduced sensitivity compared to conventional measurements. The next generation methods include real-time acquisition[14, 15], which carries a less severe penalty in sensitivity. For certain key experiments there is even a simultaneous improvement in both sensitivity and resolution, as in the pure shift HSQC spectra of **Fig 2**.

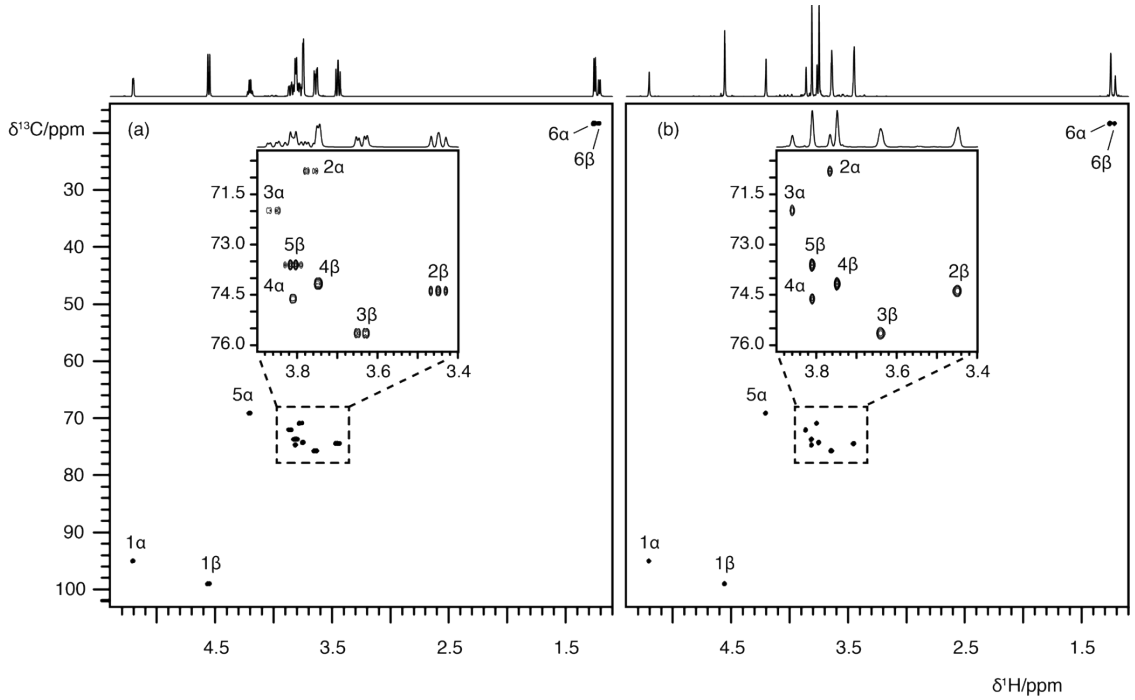

Figure 2. ^{1}H-^{13}C HSQC spectra of D(+)-fucose in D2O: (a) conventional gHSQC, and (b) real-time pure shift gHSQC. 1D traces are integral projections onto the F2 (^{1}H) axis. Data were acquired, processed and plotted with equivalent parameters, to allow quantitative comparison.

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Superconductive NMR Probe Technology for Small Samples

William W. Brey[2], Vijaykumar Ramaswamy[1], Jerris W. Hooker[2], Richard S. Withers, Robert E. Nast[3], and Arthur S. Edison[4]

1. University of Florida, J. Crayton Pruitt Family Department of Biomedical Engineering, Gainesville, FL USA
2. Florida State University, National High Magnetic Field Laboratory, Tallahassee, FL USA
3. Out of the Fog Research, Mountain View, CA, USA
4. University of Florida, Department of Biochemistry & Molecular Biology and NHMFL, Gainesville, FL USA

NMR spectroscopy is a powerful technique for molecular structure elucidation, but suffers from inherently low sensitivity. NMR probes with High Temperature Superconductor (HTS) coils offer extremely high sensitivities [1]. We previously designed a ^{1}H optimized 1-mm triple resonance probe based on HTS coils [2]. More recently we developed a 1.5-mm probe which is optimized for ^{13}C detection sensitivity. The probe has a total sample volume of about 35 microliters with an active volume of 20 microliters and the highest mass sensitivity for ^{13}C detection at any field strength. The probe also has excellent ^{1}H sensitivity and employs a ^{2}H channel lock; ^{15}N irradiation capability can be added in the future. The coils are cooled to about 20 K using a standard Agilent cryogenic refrigeration system, and the sample temperature is regulated near room temperature. Coil design considerations will be discussed in detail. This probe is ideal for directly detected ^{13}C NMR experiments for natural products chemistry and metabolomics applications, for which 35 microliters is an optimal sample volume. The outstanding ^{13}C sensitivity of this probe allowed us to directly determine the ^{13}C connectivity on 1.1 mg of natural abundance histidine using an INADEQUATE experiment. We demonstrated the utility of this probe for ^{13}C-based metabolomics using a synthetic mixture of common natural abundance metabolites whose concentrations ranged from 1 to 5 mM (40 to 200 nmol).

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Multinuclear Nanoliter 1D and 2D NMR Spectroscopy with a Single Planar Microcoil

Raluca M. Fratila[1], M. Victoria Gómez[2], Stan Sýkora[3], and Aldrik H. Velders[1,4]

1. MIRA, University of Twente, Enschede, The Netherlands
2. IRICA, Universidad de Castilla-La Mancha, Spain
3. Extra Byte, Italy
4. BioNanoTechnology Group, Wageningen University, The Netherlands

An intense effort has been devoted over the past decade to the development of microcoil NMR probes for the analysis of mass-limited and volume-limited samples [1]. We previously reported the study of supramolecular interactions using ^{1}H and ^{19}F NMR spectroscopy in a microfluidic chip equipped with a planar transceiver microcoil [2,3]. Recently, the on-line monitoring of a microwave-assisted cycloaddition was also described as a proof-of-concept for the hyphenation of small-volume NMR spectroscopy to other techniques [4].

Herein, we present the design of a second generation of microfluidic chips for nanoliter NMR spectroscopy. [5]. A planar transceiver microcoil integrated in a microfluidic chip (active volume 25 nL) enables the detection of different nuclides in a broadband range of Larmor frequencies (e.g. at 9.4 T from 61 to 400 MHz). All routine 1D and 2D, homo- and heteronuclear experiments can be carried out using our set-up. The possibility of detecting different nuclei combined with the advantages of working on-flow opens the path for many lab-on-a chip applications, including real time monitoring of chemical reactions using sub-microliter volumes of reagents.

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Two-Dimensional NMR Spectroscopy on a Desktop Spectrometer

Bertram Manz[1], Juan Perlo[2], **Andrew Coy**[1], and Federico Casanova[2]

1. Magritek Ltd, 32 Salamanca Road, Wellington, New Zealand
2. Magritek GmbH, Pauwelsstr. 19 - D-52074, Aachen, Germany

Two-dimensional (2D) NMR spectroscopy is a general concept that enables chemical information to be encoded into a second dimension making use of spin-spin interactions like the J-coupling. It is particularly useful to simplify 1D spectra with overlapping signals and to identify coupled chemical groups. While 2D techniques are routinely used at high field they are not exploited on benchtop NMR spectrometer. One of the main reasons for this is the high stability required to sample the data along the indirect dimension. Frequency or phase instabilities between scans result in stripes along the t1 direction also known as t1 noise. Furthermore, the acquisition of meaningful 2D spectra is only possible if the spectrometer provides enough resolution and high signal-to-noise, otherwise the acquisition time for a 2D spectrum can become excessively long.

In this work we demonstrate the performance of 2D NMR on a 1 Tesla permanent magnet benchtop spectrometer. The magnetic field homogeneity of the magnet can be finely shimmed to achieve sub Herz resolution by means of shim coils up to order three, and high field stability is achieved by means of an external lock system. From the large variety of available 2D pulse sequences we tested the performance of J-resolved spectroscopy, correlation spectroscopy (COSY), and double quantum filtered (DQ-COSY). The results presented here demonstrate that 2D NMR is of great assistance for benchtop NMR spectroscopy where the spectrum of small molecules may appear crowded due to the strong coupling limit.

Wednesday, September 25th
11:00 AM - 12:30 PM

Methods of Analysis for Food Ingredients and Products

Chair: John van Duynhoven

Speakers:

Apostolos Spyros
University of Crete, Greece

Serge Akoka
University of Nantes, France

Krish Krishnamurthy*
Agilent Technologies, Santa Clara, CA, USA

Ewoud J.J. van Velzen*
Unilever R&D, Vlaardingen, The Netherlands

* Upgraded Poster

Small Molecule Analysis in Food: an Overview and Some Recent Applications

Apostolos Spyros, Photis Dais, Maria Amargianitaki, Evangelia Ralli and Maria Misiak

NMR Laboratory, Chemistry Department, University of Crete, Heraklion Crete, Greece

The excellent analytical capabilities of NMR spectroscopy have made it an indespensable tool in food analysis during the last few decades. [1] Apart from the qualitative and quantitative chemical characterization of food ingredients, which is important from a nutritional and quality control point of view, NMR spectroscopy has found increased use by providing spectroscopic input data for multivariate statistical analysis methods that can be employed to study a variety of food analysis problems, including authenticity, protected denomination of origin, adulteration, etc. [2]

In this contribution we will present a brief overview of the potential of small molecule NMR in food analysis, focusing on both standard high resolution liquid state NMR, and solid state high resolution-magic angle spinning (HR-MAS) applications.

We will conclude with some examples of NMR applications currently in progress in our laboratory, dealing with the metabolomic analysis and/or authentication of Cretan foodstuff important for the local island economy (wine, graviera cheese, tsikoudia, honey, olive leaves, etc.).

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Application of Recent Advances in Quantitative NMR to Authentication of Beverages

Serge Akoka, P Giraudeau, M. Grand, E. Martineau and G. Remaud

CEISAM, University of Nantes, CNRS, France

Nuclear magnetic resonance spectrometry has been extensively used for analysing complex mixtures such as beverages. Two main approaches are used: the determination of the isotopic profile of major components and the determination of the metabolic profile. In the two cases, high-throughput techniques are welcome.

Concerning the isotopic profiling, Nuclear Magnetic Resonance spectrometry is the only analytical technique which allows the quantification of each isotopomer of a given molecule. Isotopic ^{2}H NMR spectrometry is recognized as a powerful technique for verifying the origin of commercial products [1]. However, certain limitations of the method have been highlighted: (i) a long measurement time; even for a relatively simple molecular structure, obtaining a sufficiently resolved ^{2}H-NMR spectrum required several hours of spectrometer time and (ii) large molecules could not be studied by ^{2}H-NMR because of the complexity of the NMR spectrum. We have recently extended this approach to ^{13}C-NMR [2] and its sensitivity has been improved thanks to the application of multi-pulse sequences [3]. The results obtained show that this strategy can be used effectively to determine the ^{13}C distribution within a given molecule in a very short analytical time and to accurately compare differences in the isotopic distribution between different samples of the given molecule, even if absolute values of specific isotopic deviations cannot always be provided. Results obtained for isotopic profiling of Tequila and wines will be presented.

On the other hand, Nuclear Magnetic Resonance offers a great potential for the quantitative analysis of metabolic samples, but accurate and precise quantitative analysis is often made difficult by a high degree of spectral overlap. Multi-dimensional NMR offers an interesting alternative, as is provides a much better discrimination of resonances. However, its use for quantitative analysis is limited by their long acquisition duration and their multi-pulse character. The last part of the presentation will describe how this drawback could be bypassed using ultrafast 2D NMR [4, 5], which makes it possible to record multi-dimensional spectra in a single scan. Preliminary results obtained on orange juices from different origins will be presented.

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CRAFT (Complete Reduction to Amplitude Frequency Table) – Principles and Application to Food Sciences

Krish Krishnamurthy, David J Russell, and Scott Baggett

Agilent Technologies, Santa Clara, CA, USA

The regulatory guidelines for dietary supplements stipulate that manufacturers provide verification that all products meet established identity, purity, strength, and composition requirements. The evaluation of botanical extracts and dietary supplements using NMR spectroscopy provides researchers with a flexible and powerful tool to comply with regulatory requirements. The intrinsic quantitative nature of NMR is increasingly exploited in areas ranging from complex mixture analysis as in food extracts. Complex NMR spectra are more common than not, and therefore, extraction of quantitative information generally involves significant prior knowledge and/or operator interaction to deconvolute peaks of interest.

We present an algorithm that achieves a Complete Reduction to Amplitude Frequency Table (CRAFT) in an automated and highly time-efficient fashion – thus converting the FID to a frequency-amplitude table. CRAFT tables can be used further for data mining of quantitative information using fingerprint chemical shifts of compounds of interest and/or statistical analysis of modulation of chemical quantity in a biological study (metabolomics). CRAFT approach is quantitative to very low concentrations even in complex mixtures.

Three commercially available dietary supplements containing soybean extract were investigated using ^{1}H, ^{13}C and Pure-shift 1D NMR spectroscopy. The spectra were analyzed using CRAFT. The basic principle behind the CRAFT approach, its application to the soy supplements and its potential for high throughput targeted and untargeted metabolomics will be presented.

qNMR Targeted Profiling of Food Compounds

Ewoud J.J. van Velzen, John P.M. van Duynhoven, Niels de Roo, and Peter Hoos

Unilever R&D, Microbiology and Analytical, Vlaardingen, The Netherlands

NMR has been widely used for the quantitative analysis of common food components. However, accurate quantifications in complex NMR with numerous overlapping signals is still challenging. Even though various computational procedures have been proposed they generally show limitations in analytical accuracy and/or abilities for integrating in (semi-)automated routines[1-3]. Among the different procedures available, the use of interactive targeted profiling using Chenomx[1,4] is often advantageous. Despite the analysis requires critical inspection and close supervision by the NMR researcher, the procedure generally leads to good analytical precision and satisfactory sample throughput. Since the spectral database in Chenomx is specifically optimized for biofluids, the targeted profiling methodology can however not directly be transferred to food systems[5]. Additional calibration is needed to translate the excising response factors into accurate quantitative food models. We demonstrated that the calibrated quantitative food models agreed with established and certified analytical techniques. Agreement (precision) between the different methods was tested by comparing the estimated concentration levels of common food compounds (sugars, organic acids, amino acids, nucleotides etc.) in different food products and food ingredients. In this test the concentrations were unequally distributed in the range 0-3.5%w/w with 95% confidence interval 1.43%w/w to 1.85%w/w at midpoint of the concentration interval (1.64%w/w). We also demonstrated that accuracy levels reached the 5% acceptance limits of two certified reference materials TraceCERT® Maleic Acid (Fluka) and Standard Reference Material SRM3287 (Blueberry Fruit, NIST). Based on this validation study it was concluded that NMR targeted profiling and the traditional analytical methods can be used interchangeably. To make this targeted profiling approach operational in the analytical laboratory the targeted profiling approach was therefore further adjusted. The method now provides a fully automatic calculation module (including the calibration factors), a Chenomx-LIMS interfacing module (including barcode labelling) and a QC control system (to test accuracy).

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Wednesday, September 25th
2:30 PM - 4:00 PM

Determination of Stereochemistry by NMR

Chair: Ricardo Riguera

Speakers:

Philippe Lesot
Université Paris-Sud 11, France

Armando Navarro-Vazquez
University of Vigo, Spain

Míriam Pérez-Trujillo*
Universitat Autònoma de Barcelona, Bellaterra, Spain

Richard J. Lewis*
AstraZeneca R&D Mölndal, Mölndal, Sweden

* Upgraded Poster

Recent Achievements of NMR in Chiral Anisotropic Media: NMR Methodology and Applications

Philippe Lesot

Laboratoire de RMN en Milieu Orienté, ICMMO, CNRS UMR 8182, University of Paris Sud, F-91405 Orsay cedex, France

NMR spectroscopy in weakly orienting chiral systems is a powerful, original and emerging methodology for analyzing chirality/stereochemistry of guest solutes as well as providing numerous useful molecular information such as the natural site-specific (D/H) isotopic fractionation [1].

The growing success of this analytical approach in the community of (bio)chemists clearly originates from three additional aspects: i) the continuous development of new NMR experiments dedicated to the analysis of anisotropic spectra; ii) the discovering of new chiral liquid crystals possessing efficient enantiodiscrimination properties; iii) and the multi-domain application of this technique to solve specific problems or provide alternative analytical strategies.

In this presentation, two aspects of these developments are proposed. In a first part, we describe the first experimental detections of ^{2}H-^{13}C isotopomers at natural abundance level (1.7×10^{-4}%, namely, one molecule over 580 000) in oriented systems by combining cryoprobe, high magnetic field (21 T) and optimised ^{2}H-^{13}C heteronuclear 2D experiments [2]. Interestingly, we show that ^{2}H-^{13}C enantio-isotopomers can be distinguished using 2D NMR in polypeptide, chiral aligned media, thus providing a new tool for the chiral analysis.

In a second part, we present and discuss the spectral enantiodiscrimination properties of water compatible, DNA-based chiral mesophases using ^{2}H-{^{1}H} NMR to discriminate between enantiomers or enantiotopic directions. The case of alpha-amino acids will be especially examined [3]. Combining ^{2}H-{^{1}H} NMR and DNA mesophases, we experimentally demonstrate that the spectral monitoring of the enzymatic transformation of (*L*)-alanine-d_3 into its enantiomer (and *vice-versa*) by the alanine racemase (AlaR) is possible. The rate constants k_{catL} and k_{catD} of the enzymatic reaction determined by this method are similar to those observed by circular dichroism under slightly different conditions. The first results are very encouraging, and could provide shortly a promising alternative to classical, existing methods (CD, UV spectroscopy or chiral capillary electrophoresis) [4].

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NMR in Aligned Media, Computation and Chiroptics: from Constitution to Absolute Configuration

Armando Navarro-Vázquez[1], Roberto R. Gil[2], Clemens Anklin[3], and E. Troche-Pesqueira[1,2]

1. Organic Chemistry Department, Facultade de Química, Universidade de Vigo, Vigo, Spain
2. Department of Chemistry, Carnegie Mellon University, PA, USA
3. Bruker BioSpin Corp., Billerica, MA, USA

NMR-based computer-assisted-structural elucidation tools (CASE)[1] do nowadays an excellent job in the automatic determination of constitution of molecules of medium complexity. Modern programs usually combine molecular formula from accurate mass mass spectrometry, a set of multinuclear and multidimensional (mainly 2D) NMR experiments and chemical shift predictions to furnish a set of scored 2D structures (planar molecular constitution).[2] In the other hand, Residual Dipolar Couplings (RDCs) obtained by weakly aligning small molecules have shown to be a powerful tool for the determination of the relative spatial orientation of all atoms in a small molecules.[3] We present here how selected 2D structures from CASE programs can be fed to molecular modelling packages in order to generate conformational 3D ensembles which are later supplied to RDC analysis programs. In this way not only the 2D structure but also the correct configuration and conformational space of moderately flexible molecules can be determined. In a last step the absolute configuration can be obtained by computation of population averaged chiroptical properties such as electronic circular dichroism (ECD) or optical rotation using the previously obtained configurational and conformational information. Hence, all necessary steps for complete structural elucidation can be automated with a minimum of human intervention. This procedure has been tested in a series of known alkaloids such as strychnine, yombine, and others.

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Differentiation of Enantiomers in Complex Systems by NMR Spectroscopy and Chiral Solvating Agents: Applications and Methodology

Míriam Pérez-Trujillo[1,2] and Teodor Parella[1,2]

1. Servei de Ressonància Magnètica Nuclear, Facultat de Ciències i Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain
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Chiral molecules are present in nature (natural chiral compounds) and in the world of synthetic compounds (synthetic chiral compounds). Most of the endogenous metabolites, such as sugars, amino acids, hydroxy acids, polyphenols, terpens, etc. are chiral compounds and so are many drugs and reactants. The different character of two enantiomeric molecules is manifested when they are within a chiral environment, what happens to be intrinsic in nature. Nature is stereospecific, and so are most of the biological processes that take place in the living systems. Enantiomers are distinguished by nature and they play different roles. This feature means showing different biological activities or functionalities in the case of endogenous metabolites (such as D- and L-Serine), or manifesting different pharmacological activities or toxicities in the case of pharmaceuticals and exogenous metabolites (such as Thalidomide, Ethambutol, Naproxen or Ibuprofen and their degradation products). There is an important interest in distinguishing enantiomers in different areas, such as the pharmaceutical industry (drugs and their degradation products) but also in areas working with natural chiral compounds such as metabonomics.

At present, metabolic profiling (metabonomics/metabolomics) is conducted in a way that is largely unable to capture stereochemical details of molecular structure, and therefore a layer of complexity awaits exploration. Here we show how NMR spectroscopy used in combination with chiral solvating agents (CSAs) is a simple, rapid and robust method to differentiate enantiomers in complex mixtures such as body fluids; involving minimal sample manipulation and without requiring derivatization or purification of the sample. We show how this methodology can be applied to metabonomics, Chiral Metabonomics [1]. Furthermore, we present how the typical problem of overlapping when differentiating enantiomers in complex systems by ^{1}H NMR spectroscopy (such as pure molecules or mixtures, with complex ^{1}H NMR spectra), can be overcome with ^{13}C NMR spectroscopy. An evaluation of ^{1}H and ^{13}C NMR spectroscopy for the differentiation of enantiomeric molecules in complex systems using CSAs is shown. Parameters such as sensitivity, resolution, quantification and signal overlapping are assessed.

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Chiral Solvation Revisited: Developing a CSA Method with a Single Enantiomer

Richard J Lewis[1] Michael A Bernstein[2] Hui-Fang Chang[1], David Chapman[1], and Nils Pemberton[1]

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Chiral molecules are important in chemistry and no less in the pharmaceutical industry where many drugs and intermediates are chiral. We therefore require analytical methods to determine the enantiomeric purity of such molecules. Frequently chiral HPLC will be chosen as the assay method of choice, but we present a case that analysis by NMR using a chiral solvation reagent[1-2] method may frequently by more time efficient. We also present an enhancement that enables a CSA method to be devloped using only a single enantiomer of the analyte. This enables us to confidently determine whether or not a sample from synthetic chemistry has *or has not* be suject to racemisation during synthesis. We can also prove the enantiomeric purity of a final product without requiring synthesis of the other enantiomer.

1. Parker, D. Chem. Rev. 1991, 91, 1441
2. Wenzel, T.J.; Chisholm, C.D. Prog. Nucl. Magn. Reson. Spectrosc. 2011, 59, 1

Wednesday, September 25th
4:30 PM - 6:00 PM

Advances in Conformational Analysis and Dynamics by NMR

Chair: Teo Parella

Speakers:

Göran Widmalm
Stockholm University, Sweden

Burkhard Luy
Karlsruher Institut für Technologie (KIT), Germany

Matthias Köck*
Alfred-Wegener-Institut, Bremerhaven, Germany

Charles D. Blundell*
Conformetrix Ltd., Manchester, UK

* Upgraded Poster

Structure and Conformation of Oligosaccharides by NMR Spectroscopy

Göran Widmalm

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Glycans, as they are known in biochemical systems or just carbohydrates are molecules in which even the monomers display highly different stereochemical arrangements and they may also carry many functional groups and substituents. When the building blocks are linked together to give oligosaccharides or glycoconjugates such as glycopeptides or glycolipids, the structures may become highly complex. The study of all the sugar molecules and glycan structures in a cell is often referred to as glycomics with the aim to understand their function [1].

Knowing the primary structure of an oligosaccharide is a prerequisite for interaction studies and understanding of function. The CASPER program [2,3], based on ^{1}H and ^{13}C chemical shifts of the constituent monosaccharides and glycosylation shifts, that is, the differences between chemical shifts in di- or trisaccharides and those of the respective monomers, is able to determine the primary structure (sugar, absolute configuration, linkage position, ring form, sequence and substituents) of many glycans in an automatic fashion based solely on unassigned NMR spectra.

The three-dimensional structure in solution of biologically active oligosaccharides can be elucidated by NMR spectroscopy in combination with molecular dynamics simulations. A powerful approach in these analyses is to make use of transglycosidic three-bond spin-spin coupling constants through Karplus-type relationships [4], both heteronuclear $^{3}J_{C,H}$ and homonuclear $^{3}J_{C,C}$ couplings, where the latter may be readily obtained from site-specifically labeled compounds made by organic synthesis [5]. The molecular simulations are used for interpretation of experimental data and can shed light on dynamic processes that are difficult to resolve by experimental techniques.

1. Gabius, H.-J.; André, S.; Jiménez-Barbero, J.; Romero, A.; Solís, D. *Trends Biochem. Soc. 36*, 298-313, **2011**
2. Lundborg, M.; Widmalm, G. *Anal. Chem. 83*, 1514-1517, **2011**
3. http://www.casper.organ.su.se/casper/
4. Säwén, E.; Massad, T.; Landersjö, C.; Damberg, P.; Widmalm, G. *Org. Biomol. Chem. 8*, 3684-3695, **2010**
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Anisotropic NMR Parameters for the Structural Analysis of Small Molecules

Burkhard Luy

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Anisotropic NMR parameters like residual dipolar couplings (RDCs), residual quadrupolar couplings (RQCs), and residual chemical shift anisotropies (RCSAs) are very powerful structual parameters that allow the conformational, configurational, and constitutional analysis of small molecules [1]. If chiral alignment media are used, even the distinction of enantiomers is possible with the approach.

Novel technical developments, like the introduction of useful alignment media, imaging of alignment, useful pulse sequences, and a method for the identification of meso compounds compared to a racemic mixture of corresponding enantiomers will be presented. A special focus will be on a fast HSQC scheme, termed ASAP-HSQC, and the measurement of homonuclear decoupled CLIP-HSQC spectra for accurate one-bond coupling determination.

1. for a review see e.g.: G. Kummerlöwe, B. Luy, *Annu. Rep. NMR Spectrosc.* **68**, 193-232 (2009).

Configurational Analysis with a Small Number of NOEs – a Test Case

Julie Muñoz[1], Alexis Krupp[2], Stefan Immel[2], Michael Reggelin[2], Gerald Culioli[3], and **Matthias Köck**[1]

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2. Clemens-Schöpf-Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Petersenstraße 22, D-64287 Darmstadt, Germany
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The determination of the relative and absolute configuration of natural products is essential to understand their biological activity and to allow their procurement through total synthesis. But so far, there is no general method for the secure assignment of the relative or even the absolute configuration of non-crystallizable natural products. The method of choice for small molecules with several stereogenic centers is the combination of distance geometry (DG) and distance bounds driven dynamics (DDD) calculations using NOE/ROE-derived distance restraints (r) [1]. The most important aspect of the NOE/ROE-restrained DG/DDD method (fc-rDG/DDD) is the possibility for the configurations to change during the simulation (floating chirality, fc) [2] and therefore to determine the conformation and the relative configuration of small organic molecules simultaneously.

One of the most investigated complex natural products over the past decade was palau'amine. Since none of the palau'amine congeners have been crystallized, there was a special demand on NMR spectroscopy for this structurally very complex class of natural products. Applications on palau'amine derivatives as well as other dimeric pyrrole-imidazole alkaloids will be presented.

Brown algae of the genus *Cystoseira* are known to produce various linear diterpenes and meroditerpenes. The isolation of several new meroditerpenes from the brown alga *Cystoceira baccata* led to a strong indication for a general revision of the bicyclo[4.3.0]nonane system for compounds of this family. Since the number of NOEs was not sufficient for an unambiguous assignment of the relative configuration by fc-rDG/DDD, residual dipolar couplings (RDC) were used to refine the structures. The absolute configuration of the new compounds was assessed by comparison of the circular dichroism (CD) spectra to the calculated spectra.

1. a) R. Kaptein, R. Boelens, R. M. Scheek, W. F. van Gunsteren, *Biochemistry* **1988**, *27*, 5389-5395; b) R. M. Scheek, W. F. van Gunsteren, R. Kaptein, *Methods Enzymol.* **1989**, *177*, 204-218; c) M. Reggelin, M. Köck, K. Conde-Frieboes, D. F. Mierke, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 753-755; d) A. Grube, M. Köck, *Angew. Chem. Int. Ed.* **2007**, *46*, 2320-2324; e) M. Köck, G. Schmidt, I. B. Seiple, P. S. Baran, *J. Nat. Prod.* **2012**, *75*, 127-130.
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Quantification of Free Ligand Conformational Preferences by NMR and Their Relationship to the Bioactive Conformation

Charles D. Blundell[1], Martin J. Packer[2], and Andrew Almond[1]

1. Conformetrix Ltd., Manchester, UK
2. AstraZeneca, Alderley Park, Cheshire, UK

Accurate unbound solution 3D-structures of ligands provide unique opportunities for medicinal chemistry and, in particular, a context to understand binding thermodynamics and kinetics. Previous methods of deriving these 3D-structures have had neither the accuracy nor resolution needed for drug design and have not yet realized their potential. Here, we describe and apply a NMR methodology to the aminoglycoside streptomycin that can accurately quantify accessible 3D-space and rank the occupancy of observed conformers to a resolution that enables medicinal chemistry understanding and design. Importantly, it is based upon conventional small molecule NMR techniques and can be performed in physiologically-relevant solvents. The methodology uses multiple datasets, an order of magnitude more experimental data than previous NMR approaches and a dynamic model during refinement, is independent of computational chemistry and avoids the problem of virtual conformations. The refined set of solution 3D-shapes for streptomycin can be grouped into two major families, of which the most populated is almost identical to the 30S ribosomal subunit bioactive shape. We therefore propose that accurate unbound ligand solution conformations may, in some cases, provide a subsidiary route to bioactive shape without crystallography. This experimental technique opens up new opportunities for drug design and more so when complemented with protein co-crystal structures, SAR data and pharmacophore modeling.

Poster Sessions

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Tuesday, September 24th
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Co-Chairs: Michael Hammer and Krish Krishnamurthy

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1 ^{1}H S-Filter: Quantitative Edition of Singlets in 1D ^{1}H NMR Spectra for the Study of Complex Mixtures

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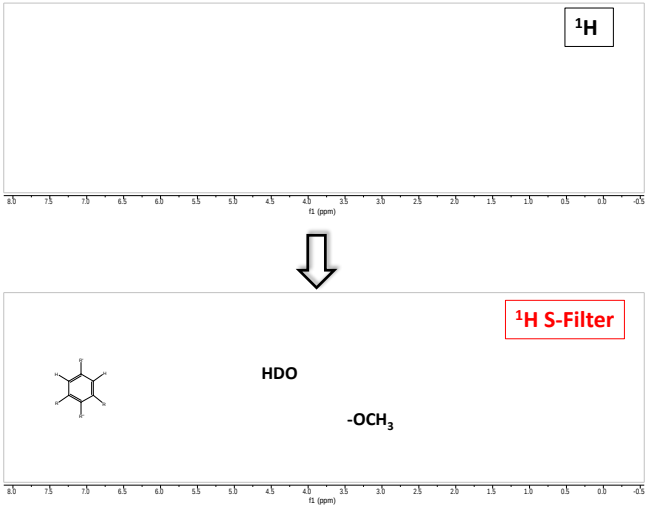
NMR is a useful tool for the characterization of complex mixtures of compounds or metabolites. The applications cover a range that includes the analysis of food, biological fluids, tissues and cell extracts [1-2]. The multi-variable statistical analysis of the signals in the spectra with chemometrics methods [3-4] permits to detect patterns in the NMR signals that can be related with a property of interest, e.g. disease [1].

One dimensional NMR experiments relying on ^{1}H are the fastest and more sensitive NMR spectra. Nevertheless, in complex mixtures severe signal overlap often hampers the identification of key metabolites and/or quantitation of their concentration. Several types of NMR experiments have been developed for the edition/filtering of signals in the 1D proton spectrum based in properties such as signal relaxation or translational diffusion [5-7]. However, these methods do not apply too well for complex mixtures of metabolites that typically have similar relaxation and diffusion properties.



In this work, a new experiment called ^{1}H S-Filter is presented for selective edition of singlet peaks in 1D proton spectra. The ^{1}H S-Filter has been tested with a variety of food samples having a complex ^{1}H spectrum, and the results show a clean and robust edition of singlet peaks. Moreover, the spectra retain most of its sensitivity and quantitativity. Future developments of the method could find application in the area of in-vivo localized MR spectroscopy.

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2 CRAFT (Complete Reduction to Amplitude Frequency Table) – Principles and Application to Food Sciences

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The regulatory guidelines for dietary supplements stipulate that manufacturers provide verification that all products meet established identity, purity, strength, and composition requirements. The evaluation of botanical extracts and dietary supplements using NMR spectroscopy provides researchers with a flexible and powerful tool to comply with regulatory requirements. The intrinsic quantitative nature of NMR is increasingly exploited in areas ranging from complex mixture analysis as in food extracts. Complex NMR spectra are more common than not, and therefore, extraction of quantitative information generally involves significant prior knowledge and/or operator interaction to deconvolute peaks of interest.

We present an algorithm that achieves a Complete Reduction to Amplitude Frequency Table (CRAFT) in an automated and highly time-efficient fashion – thus converting the FID to a frequency-amplitude table. CRAFT tables can be used further for data mining of quantitative information using fingerprint chemical shifts of compounds of interest and/or statistical analysis of modulation of chemical quantity in a biological study (metabolomics). CRAFT approach is quantitative to very low concentrations even in complex mixtures.

Three commercially available dietary supplements containing soybean extract were investigated using ^{1}H, ^{13}C and Pure-shift 1D NMR spectroscopy. The spectra were analyzed using CRAFT. The basic principle behind the CRAFT approach, its application to the soy supplements and its potential for high throughput targeted and untargeted metabolomics will be presented.

3 Engagement of CF3 Group in N−H•••F−C Hydrogen Bond in the Solution State: NMR Spectroscopy and MD Simulation Studies

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Unambiguous evidence for the engagement of CF_3 group in N−H···F−C hydrogen bond in a low polarity solvent, the first observation of its kind, is reported. The presence of such weak molecular interactions in the solution state is convincingly established by one and two-dimensional ^{1}H, ^{19}F, and natural abundant ^{15}N NMR spectroscopic studies. The strong and direct evidence is derived by the observation of through-space couplings, such as, $^{1h}J_{FH}$, $^{1h}J_{FN}$, and $^{2h}J_{FF}$, where the spin polarization is transmitted through hydrogen bond. In an interesting example of a molecule containing two CF_3 groups getting simultaneously involved in hydrogen bond, where hydrogen bond mediated couplings are not reflected in the NMR spectrum, $^{19}F–^{19}F$ NOESY experiment yielded confirmatory evidence. Significant deviations in the strengths of $^{1}J_{NH}$, variable temperature, and the solvent induced perturbations yielded additional support. The NMR results are corroborated by both DFT calculations and MD simulations, where the quantitative information on different ways of involvement of fluorine in two and three centered hydrogen bonds, their percentage of occurrences, and geometries have been obtained. The hydrogen bond interaction energies have also been calculated.

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4 Versatile Incorporation of Drugs into Highly Aqueous Physical Hydrogels, Evaluated by NMR Techniques

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The clinical use of sparingly water soluble drugs is hampered by the wide range of administration problems that they pose, including the technological difficulties regarding their incorporation into adequate pharmaceutical vehicles. [1,2] In this work, we present the utilization of a new highly aqueous physical hydrogel as a pharmaceutical delivery vehicle of small hydrophobic drugs.[3] These hidrogels also serve as vehicle of small hydrophilic drugs. The high water content and large pore sizes typical of hydrogels often provoke the rapid release of this type of drugs, constituing another important difficulty from a farmacological point of view.[4, 5] The endogen protein albumin was incorporated into the composition of this pharmaceutical system, on the basis of its intrinsic capacity of binding and carrying amphiphilic molecules throughout systemic circulation [6,7], which offers chances for conveniently tuning the release of the drug from the hydrogel.

The binding interaction between albumin and model therapeutic molecules incorporated in the hydrogel system was characterized by nuclear magnetic resonance spectroscopy (NMR). The drugs incorporated were the hydrophobic drugs prednisolone and ketoconazole and the hydrophilic drug cloxacillin. A combination of the well-known saturation transferred difference (STD) spectra [8,9] and a novel Double Titration assay followed by NMR was applied to study all the possible binding modes, from weak to strong affinity, between albumin and each one of the drugs. The ability of the hydrogel system to release each drug was also measured in-vitro as a function of the amount of albumin loaded in the hydrogel. The results of the release studies obtained for each drug had a remarkable correlation with the binding capacity derived from the NMR binding studies, thus confirming the potential of the NMR approach proposed as a predictive technique in the design of new pharmaceutical dosage forms.

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5 Structural and Mechanistic Studies on Enamine and Dienamine Intermediates

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The catalysis of carbonyl compounds through enamine and dienamine intermediates gains rising interest since almost a decade.[1-3] Alpha-functionalization of various carbonyl compounds is achieved by enamine and gamma-functionalization through dienamine catalysis, several domino reactions and a variety of other transformations have also been reported.[4,5] Although the synthetic applications of enamine and dienamine are almost countless, the underlying mechanisms remain hidden so far.[1,3]

We investigated the aldol reaction of propionaldehyd catalyzed by proline computationally and could show that the conformation of the intermediate and starting material structures are highly important to the outcome of the reaction. The reaction path is also highly dependent on the used co-catalyst, be it water of dmso. IRC-reaction path calculations could show the transformation of iminium ions into the corresponding oxazolidinones and the corresponding enamines.

The formation of dienamine intermediates of a variety of alpha,beta-unsaturated aldehydes was investigated by react-NMR. The formation of 2E, and 2Z-dienamines were monitored and the impact of solvent, substitution pattern of the aldehyde and catalyst structure was screened on several structural features was investigated. A surprising influence of acidic co-catalysts was found, that shows a large impact on the formation of the 2Z-dienamine but not on the 2E-dienamine of 2-pentenal and a Jorgensen-Hayashi type organocatalyst. A quite rigid structure of all dienamine intermediates was found in the catalyst substructure whereas for the aldehyde derived part of the intermediate, depending on the catalyst, structural diversity was identified.

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6 Observation of GBV-C E1 Peptide Direct Binding to HIV-1 Fusion Peptide by STD-NMR

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The GB virus C (GBV-C) has been investigated in the context of HIV infection and it is associated with improved survival or improved prognosis in HIV-infected individuals. However, the mechanism responsible for the beneficial effect that the GBV-C virus exerts on the course of infection caused by HIV has yet to be fully defined. Haro and coworkers demonstrated in biophysical assays that the peptide from amino acids 269 to 286 (from E2 glycoprotein of GBV-C virus) interacts with the fusion peptide of HIV-1 gp41 (1-23) and modifies its conformation [1]. These and posterior studies identified several peptides that were able to mediate HIV-1 inhibition *in vitro [2]*. Also, in our previous studies, a scan of the GBV-C E1 protein was carried out by the semiautomated syntheses of multiple 18-mer peptides overlapped by fifteen residues and the resulting 58 linear peptides were synthesized and screened for their ability to inhibit the gp41 fusion peptide [3,4]. Five out of the 58 peptide sequences corresponding to the E1 protein of GBV-C were capable of interfering with the HIV-1 fusion peptide (HIV-1 FP)–vesicle interaction. Peptide E1P8 (APEDIGFCLEGGCLVALGP) was selected as one the two best candidates for future studies because its ability to inhibit membrane fusion and the interaction of HIV-1 FP with lipid bilayers. Unfortunately, equilibrium dyalisis and ITC experiments did not confirmed the peptides interaction in solution.

Our next objective was to confirm the direct interaction at molecular level between FP gp41(1-23) and the identified peptide inhibitor E1P8. The study of the interaction between the two peptides in solution by 1H NMR spectrocopy and the formation of a complex is hampered by the similar amino acid composition (rich in Leu, Ala and Glycine amino acids) and their length (23 and 18 amino acids). One solution is to use isotopic labeling by synthesis or recombinant expression, but we were interested in a more high-throughput methodology that would allow to screen several peptides and to optimize their structure so these peptides could be potentially used in future anti-HIV-1 therapies.

Saturation transfer difference (STD)-NMR is a method that allows to screen the binding of compounds to a receptor and to characterize the binding epitope. This methodology requires that the macromolecule has a molecular weight >10 kDa to havea fast rapid cross-relaxation of 1H spins and intramolecular spin diffusion. In our case, the studied system comprises two low molecular weight peptides. But, in an alternative approach, we investigated whether the interaction between the two peptides could be studied by STD-NMR if one of the peptides is attached covalently to a PLGA-PVA nanoparticles [5] so that it acted as the high molecular receptor in the STD experiments.

PLGA-PVA nanoparticles were functionalized by HIV-1 FP gp41(1-23) using the methodolody previously described [6]. After NPs preparation, we characterized NPs functionalization level (w/w %,

mg of conjugated peptide/mg NPs %) by 1H NMR spectroscopy. Next, we used STD-NMR to confirm the interaction at molecular level between FP functionalized NPs and a constrained version of peptide E1P8 (by intramolecular disulfide bond) and to obtain information about the binding epitope.

Using this methodology, we have confirmed the interaction between peptide E1P8 and the fusion peptide of HIV-1 gp41. The experimental results that we have obtained will be presented in this communication.

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7 Pure Shift HSQC Measurements with perfectBIRD Decoupling – a Method to Decouple Diastereotopic Protons

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The discrimination of different compounds which exhibit similar structural features, such as configurational isomers, can easily be prevented by signal overlap even when using modern high field NMR instruments. In such cases the use of signal dispersion in multiple indirect spectral dimensions is likely to be inefficient in achieving signal separation as the differences in chemical shifts observed can be quite small, possibly smaller than either the resolution of the indirect dimension or the multiplet width imposed by J-coupling.

To diminish these problems pure shift techniques hold great potential, as they can provide both high resolution and J-coupling suppression in the *direct* dimension.[1] HSQC experiments with homonuclear decoupling in the direct dimension have recently been presented using both pseudo-3D acquisition and a real-time 2D acquisition scheme.[2] The BIRD decoupling element[3] employed by the two techniques leads to a significant simplification of the spectra obtained, making them well suited for the study of complex problems. However it fails to suppress geminal scalar couplings, resulting in an "irreducible" doublet appearance of signals from diastereotopic protons.

As presented here, full homonuclear decoupling is possible even in the case of diastereotopic protons if BIRD decoupling is combined with a perfect echo element.[4] The incorporation of the resulting perfectBIRD decoupling element into HSQC experiments, such as the F_2-coupled CLIP-HSQC experiment[5] which is suited for precise one-bond RDC-measurements, is presented.

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8 NMR in Combating Counterfeit Pharmaceuticals

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Counterfeit drug products are a real and increasing threat to both the consumer and the phamaceutical industry which spends an enormous effort in bringing efficacious and safe drugs to the public. Counterfeiters often introduce their products to the market with complete disregard to efficacy or safety. The counterfeit products generally contain none of the indicated active phamaceutical ingredient (API) and may contain materials that are hazardous to the patient's health.

When material suspected to be counterfeit is discovered, identification of the active and non-active ingredients within the product become part of the investigative process. Counterfeit products are often manufactured from common materials that are locally available and that makes NMR a fast and convenient method for the identification and also quantitation of the organic components. Structural information from NMR data combined with molecular weight information from mass spectrometry (MS) leads to definitive identification of the unknown components. Quantitation by NMR ensures no significant constituent has been missed. Application of NMR supported by LC-MS in two such cases will be described.

9 ^{15}N NMR Spectroscopy for the Characterization of Disubstituted 1,2,3-Triazoles

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1,2,3-Triazoles (TAs) are highly appealing heterocycles with important applications in medicinal chemistry, chemical biology, molecular recognition, polymer chemistry and catalysis [1]. Their physicochemical properties make them very versatile for controlling conformational properties, for implementing non-covalent interactions or bio-conjugation processes. Currently, there are robust synthetic methodologies for the preparation of the three possible isomeric forms of TAs, using metal-catalyzed dipolar cycloaddition reactions [2].

In spite of the good-to-excellent regioselective methods reported for the preparation of disubstituted TAs, the unambiguous assignation of the correct isomer is not always an obvious task. Several NMR-based solutions have been recently reported, mainly based on NOE or ^{13}C NMR techniques. Although these have shown to be useful and valuable methods for the 1,4- and 1,5-isomers [3], they are less applicable for the third 2,4-TA derivative or when the residues attached to the TA core contain ^{1}H and/or ^{13}C signals overlapping with those of the heterocycle. During an undergoing project [4], we deemed to prepare a family of differently substituted TAs to be introduced as amide isosters in constrained peptoids and, in many cases, we found that the unambiguous assignation of the correct isomer was not so evident by comparing ^{1}H and/or ^{13}C NMR signals. Accordingly, we decided to use ^{15}N NMR techniques, since the ^{15}N chemical shift window extends over a wide range of values, being a suitable diagnostic probe for the chemical environment and thus for the substitution pattern.

Herein, the three isomeric forms of disubstituted 1,2,3-triazoles (1,4- or 1,5- or 2,4-disubstituted derivatives) have been unambiguously distinguished by routine ^{1}H/^{15}N HMBC experiments at ^{15}N natural abundance. Besides, the experimental ^{15}N NMR chemical shifts have been successfully correlated with the theoretical GIAO-DFT calculated values.

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10 Differentiation of Enantiomers in Complex Systems by NMR Spectroscopy and Chiral Solvating Agents: Applications and Methodology

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Chiral molecules are present in nature (natural chiral compounds) and in the world of synthetic compounds (synthetic chiral compounds). Most of the endogenous metabolites, such as sugars, amino acids, hydroxy acids, polyphenols, terpens, etc. are chiral compounds and so are many drugs and reactants. The different character of two enantiomeric molecules is manifested when they are within a chiral environment, what happens to be intrinsic in nature. Nature is stereospecific, and so are most of the biological processes that take place in the living systems. Enantiomers are distinguished by nature and they play different roles. This feature means showing different biological activities or functionalities in the case of endogenous metabolites (such as D- and L-Serine), or manifesting different pharmacological activities or toxicities in the case of pharmaceuticals and exogenous metabolites (such as Thalidomide, Ethambutol, Naproxen or Ibuprofen and their degradation products). There is an important interest in distinguishing enantiomers in different areas, such as the pharmaceutical industry (drugs and their degradation products) but also in areas working with natural chiral compounds such as metabonomics.

At present, metabolic profiling (metabonomics/metabolomics) is conducted in a way that is largely unable to capture stereochemical details of molecular structure, and therefore a layer of complexity awaits exploration. Here we show how NMR spectroscopy used in combination with chiral solvating agents (CSAs) is a simple, rapid and robust method to differentiate enantiomers in complex mixtures such as body fluids; involving minimal sample manipulation and without requiring derivatization or purification of the sample. We show how this methodology can be applied to metabonomics, Chiral Metabonomics [1]. Furthermore, we present how the typical problem of overlapping when differentiating enantiomers in complex systems by ^{1}H NMR spectroscopy (such as pure molecules or mixtures, with complex ^{1}H NMR spectra), can be overcome with ^{13}C NMR spectroscopy. An evaluation of ^{1}H and ^{13}C NMR spectroscopy for the differentiation of enantiomeric molecules in complex systems using CSAs is shown. Parameters such as sensitivity, resolution, quantification and signal overlapping are assessed.

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11 A Microfluidic NMR-Chip for Swift and Thorough Kinetic Studies

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An interesting approach in NMR to overcome its intrinsically low sensitivity is the miniaturization of the detection radiofrequency, since the signal to noise ratio is inversely proportional to the coil diameter [1]. Different microcoil geometries, planar, solenoidal and stripline, can be used, allowing the analysis of mass-limited and volume-limited samples with an improvement of sensitivity compared to the conventional NMR probe. Planar microcoils are easier to integrate in microfabrication processes using photolithography techniques allowing a precisely controlled geometry and an accurate coil sample positioning. These microcoils can be implemented in a microfluidic system, resulting in an NMR-chip that can be incorporated in a hollow tube with the corresponding electronic circuits to define a flow NMR (micro)probe which can be hyphenated to other techniques.

Previously in our group, the NMR (micro)probe was hyphenated to a continuous-flow microwave reactor (Resonance Instrument Inc.,) for on-line monitoring and rapid optimization of a Diels-alder cycloaddition. The NMR-chip, for having a smaller active volume than the reaction volume provides several data points corresponding to different portions of the latter, what accelerates the optimization process due to the wide amount of information provided from a single constant-flow experiment [2].

We here report the use of a hyphenated system consisting of a commercial, standardized, “plug and play” continuous flow microreactor (Labtrix® Start) hyphenated with a Nanoliter NMR set-up comprising a less than 19.5 µl reaction volume for the glass microreactor and a 20 nL detection volume for the microfluidic NMR-chip for the preparation and kinetic analysis of a small library of isoxazole and pyrazole derivatives, interesting motifs present in numerous natural products of medicinal value.

Usually, to carry out a very elementary kinetic study, the next step after the rate law is known, is the analysis of the reaction over as wide a range of temperature as possible, extracting the rate constant at each temperature. Finally, the activation energy and the frequency factor are estimated when the rate constants at different temperature values are fitted to the Arrhenius equation [3]. Herewith, the intrinsic features of the hyphenated Labtrix® Start-Nanolitre NMR microfluidic chip setup brings an advantage in the acquisition and treatment of data due to the possibility of analyzing small reaction volume portions, different in temperature, initial concentration and residence time values among each other’s, allowing the determination of the rate law, kinetic constant (k), activation energy (Ea) and pre-exponential factor (A)

within a single constant-flow experiment of around 600 seconds, when the data are fitted with a theoretical model developed using the Maple software.

Moreover, the use of the activation energy value extracted from our methodology, in a Vapourtec R series reactor enables a more efficient scale-up synthesis of 3,5-dimethylisoxazole, one of the compounds of the small library, since the integration of this Ea value into the Vapourtec provides the most effective reaction conditions for the scale up process, which could be different to the ones obtained from a microreactor scale. It results in a gaining of 4.8 g/day in the production of that heterocycle ring.

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12 Two-Dimensional NMR Spectroscopy on a Desktop Spectrometer

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Two-dimensional (2D) NMR spectroscopy is a general concept that enables chemical information to be encoded into a second dimension making use of spin-spin interactions like the J-coupling. It is particularly useful to simplify 1D spectra with overlapping signals and to identify coupled chemical groups. While 2D techniques are routinely used at high field they are not exploited on benchtop NMR spectrometer. One of the main reasons for this is the high stability required to sample the data along the indirect dimension. Frequency or phase instabilities between scans result in stripes along the t1 direction also known as t1 noise. Furthermore, the acquisition of meaningful 2D spectra is only possible if the spectrometer provides enough resolution and high signal-to-noise, otherwise the acquisition time for a 2D spectrum can become excessively long.

In this work we demonstrate the performance of 2D NMR on a 1 Tesla permanent magnet benchtop spectrometer. The magnetic field homogeneity of the magnet can be finely shimmed to achieve sub Herz resolution by means of shim coils up to order three, and high field stability is achieved by means of an external lock system. From the large variety of available 2D pulse sequences we tested the performance of J-resolved spectroscopy, correlation spectroscopy (COSY), and double quantum filtered (DQ-COSY). The results presented here demonstrate that 2D NMR is of great assistance for benchtop NMR spectroscopy where the spectrum of small molecules may appear crowded due to the strong coupling limit.

13 NMR Studies of the Photocatalytic Coupling of N-arylamines and Nucleophiles

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The fast developing field of photochemistry and photocatalysis and thus the investigation of their reaction mechanisms became more and more important in recent years. Especially for the optimization of the reaction conditions a profound knowledge of the reaction mechanism is particularly important. However, most of the reaction mechanisms are still not fully understood.

For that reason the aim of our work is the investigation of the key steps of the photocatalytic coupling of 2-Phenyl-1,2,3,4-tetrahydroisoquinoline with nucleophiles (e.g. nitromethane or dialkyl phosphite), using ruthenium(II) polypyridine complexes or organic dyes like eosin Y as catalysts [1,2].

To enable the *in situ* detection of the elusive intermediates, an LED based illumination device was created, consisting of an optical fiber which is guiding the light of the LEDs into the NMR tube inside of the spectrometer. By switching the LEDs directly by the spectrometer (in continuous or pulsed operations), connecting the LEDs directly to the optical fiber without a shutter and using a mechanically roughened fiber tip, the device enables the detection of diamagnetic intermediates as well as millisecond time resolved Photo-CIDNP spectroscopy [3].

For the measurements presented here the samples were illuminated with blue LEDs with a center wavelength of 455 nm. We were able to detect and characterize the key intermediates of the reaction *in situ* by NMR spectroscopy. The intermediates tetrahydroisoquinolinium and hydrogen peroxide, the coupling product and the tetrahydroisoquinoline-dimer byproduct have been identified. Further insight in the reaction mechanism and the role of the intermediates was gained by recording the reaction profile of the diamagnetic species derived from rows of ^{1}H NMR spectra.

Using Photo-CIDNP spectroscopy [4,5] we were able to detect signals of radical precursors for the ruthenium(II) polypyridine complex as well as for the isoquinoline-dimer. The dimer is proposed to be formed out of isoquinoline radicals or isoquinoline radical cations, which is investigated in current studies.

The *in situ* illumination allows for the application of well established NMR techniques on photochemical problems. With this in hand we detected and characterized diamagnetic intermediates and radical precursor species of the photocatalytic coupling of tertiary amines with nucleophiles, giving detailed insight in the reaction mechanism.

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14 The Stability of N-Heterotetracenes Towards Light and Oxygen as Investigated by NMR Spectroscopy

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N-Heterotetracenes are promising compounds for various applications. Especially their stability towards light and/or oxygen in solution is of special interest.

There are two essential pathways possible for a decomposition of those compounds: Oxidation or dimerization. For some applications, oxidation must be avoided.

Both pathways lead to a variety of decomposition products and may even occur simultaneously. This leads to a lot of new signals in proton NMR spectra. To be certain about the reason for decomposition, the new signals have to be assigned to the possible products of these pathways. This poses a great challenge due to low solubility and little or no visible couplings in NMR spectra. The exclusion of the appearance of oxidation processes therefore can be complicated.

We studied the stability and the pathway of decomposition of two N-Heterotetracenes in solution. Using a comparison of experimental and calculated carbon chemical shifts, we were able to determine whether dimerization or oxidation or both takes place. With the help of 2D NMR experiments, we succeeded to assign the important proton signals of the decomposition products.

The poster will show the results of this study.

15 From the Food to the Pharmaceutical Industry: How to Apply TD-NMR in Formulated Drugs

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Time-domain Nuclear Magnetic Resonance (TD-NMR) has been extensively applied for over 45 years in the food industry for QC/QA in all production stages of final products to measure moisture, oil, and protein content. Those assays are based on relaxometry, by measuring the FID decay, T_1 and T_2 measurements, and population distributions from inverse Laplace transformation of T_1 and T_2. Examples on those applications are the hydration studies of wheat starch, amylopectin, amylose gels and bread [1], determination of ethanol in alcoholic beverages [2], water mobility and distribution in green coffee [3], and simultaneous qualification of fat and water content in cheese [4]. The food and the pharmaceutical industries share many common areas. Both are regulated by worldwide regulatory agencies such as the Food and Drug Administration (FDA) in the US but with some differences in their regulation laws and specifications. They also use the same technologies and analyses to measure the properties of their materials, being NMR as one of their common technologies. High resolution NMR has been widely applied in both industries. However, low resolution NMR in the time domain has mainly been applied in the food industry. There are few peer-reviewed manuscripts that applied TD-NMR in the pharmaceutical industry. Kuentz's group [5] have tested the softening of gelatin and HPMC capsule shells based on T_1 measurements but with empty capsules at 20 MHz. Denk and Posset [6] have demonstrated that TD-NMR can be applied as at-line PAT tool to determine the weight of parental prefilled syringes and capped vials at 20 MHz and 7.5 MHz respectively. Because of the small footprint and simplicity of its operations, TD-NMR appears to be an ideal instrument as a PAT tool to use in any laboratory and by analyst. In our laboratories, we have evaluated a 23 MHz MQC Oxford instrument to measure bulk properties of formulated drug products with the purpose to understand the feasibility of the instrument, its use by non-NMR spectroscopists, its portability as a PAT tool, and the type of assays based on relaxometry. We have tested several measurements in our drug development portfolio, such as moisture, amorphous content, tablet hardness, tablet coating, and others. In this poster we will discuss the evaluation of TD-NMR on those properties measured with recommendations on their practical use in our industry.

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16 Synthesis and Spectroscopy Characterization of New 6-Aminobenzimidazole Derivatives Useful as Biomarkers

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Benzimidazoles are heterocyclic aromatic organic compound that find application in the treatment of several diseases like epilepsy, diabetes, anti-fertility, etc. [1]

Recently several researches elucidated that biological profiles of benzimidazole analogs can suitably be modified by introduction of different heterocyclic moieties to exhibit a broad spectrum of further biological activities, as anti-cancer and antifungal,[2] antiviral,[3] antibacterial,[4] antihelmintic,[5] antiinflamatory,[6] antihistaminic and antioxidant.[7]

The synthesis of the 6-aminobenzimidazoles derivatives **1-5** were carried out by reacting commercially available 4-nitrophenylenediamine with a variety of aromatic aldehydes (1.1 eq.) in a mixture of DMSO:H_2O (1:1). The reaction was stirring at room temperature while was added slowly $Na_2S_2O_4$ (3 eq). The reaction mixture was refluxed for 4 hours to the completion of the reaction. Finally, the reaction was added to ice-bath resulted in precipitation of a solid which was then purified using DMSO:DCM. The structures of these compounds were determined by ^{1}H NMR, FT-IR, MASS, UV-VIS and Fluorescence. The yields of the reactions are between 82-96 %. The quantum yield of the derivatives are between 0.15-0.44.

All synthetic compounds **1-5** were prepared through the one-pot reaction in excellent yield and moderate quantum yield could be useful as biomarkers.

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17 The Effects of Fast Molecular Motions and Solvation on NMR Parameters

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It is well established that fast molecular motions, such as vibrations, conformational averaging, molecular aggregation, will average NMR parameters. Isotope shifts and the temperature dependence of NMR parameters are experimental manifestations of such dynamic averaging effects. Quantum chemical calculations are typically performed using static structures, i.e. at 0 K, and neglecting zero-point motion and dynamics can lead to significant discrepancies between computed and experimental data. In solutions, where the configurations of neighbours change dynamically, the intermolecular contributions to shielding also change with changing configurations. Therefore, dynamic averaging needs to be taken into account, even when there are no significant strong intermolecular interactions, such as hydrogen bonding. In the solid state, local dynamics will average the NMR tensor parameters, such as the chemical shift anisotropy (CSA), dipolar interactions and quadrupolar interactions, leading to discrepancies between calculated data and experimental measurements (which are usually performed at ambient temperature).

Solvent modeling has become a standard part of first principles computations of molecular properties. However, a universal solvent approach is particularly difficult for the NMR shielding and spin-spin coupling constants that in part result from collective delocalized properties of the solute and the environment. In this work, bulk and specific solvent effects are discussed on experimental and theoretical model systems comprising hydrated neutral (zwitterionic), cationic, and anionic forms of alanine, hydrated N-methyl acetamide, and chloroform molecules in a series of solvents. Classical molecular dynamics (MD) simulations are compared to Car-Parrinello MD (CPMD) simulations. The quantum mechanical CPMD approach revealed a more structured solvent and significant differences in the radial and angular distributions of the water molecules around the solute [1]. NMR chemical shifts were calculated based on the generated ensembles by density functional theory (DFT) and averaged. Obtained values were significantly closer to experimental parameters than those calculated by the conventional implicit dielectric solvent model [2]. The NMR results also quantitatively reflect the superiority of the CPMD over the MD explicit solvent treatment [1,3]. Differently positioned water molecules in the clusters cause an unexpectedly large variation of the NMR parameters. The NMR chemical shift was found to be much more sensitive to the molecular solvation than the coupling. The results thus indicate a large potential of the NMR spectroscopy and quantum simulations to probe not only the structure of molecules but also their interactions with the environment. The results also show that computationally efficient solvent modeling is possible and can reveal fine details of molecular structure, solvation, and dynamics.

The influence of fast molecular motions on NMR parameters in molecular organic solids is explored on a set of amino acids and nucleic acid bases. A combination of DFT molecular dynamics and calculations of

shielding and electric field gradient (EFG) tensors reveals the impact of vibrational motions on isotropic chemical shifts, chemical shift anisotropies and quadrupolar interactions. We demonstrate that molecular motion has a significant effect on average molecular structures, and that neglecting the effects of motion on crystal structures derived by diffraction methods may lead to significant errors of calculated isotropic chemical shifts. Re-orientation of the NMR tensors by molecular motion reduces the magnitudes of the NMR anisotropies, and inclusion of molecular dynamics can significantly improve the agreement between calculated quadrupolar couplings and experimental values [4].

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18 Free Ligand Conformational Populations in Solution – A Powerful Drug Discovery Tool

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The dynamic interchange of accessible low energy conformations for ligand molecules has traditionally been predicted computationally or extrapolated from small molecule crystal structure data. Here we present a new quantitative and precise NMR methodology that provides experimentally-derived detailed conformational information for free ligands in aqueous solution. These '4D-structures' give unprecedented insight in how to exploit and control ligand conformational preferences in drug design.

The detailed conformational information on ligand molecules contained in 4D-structures can be applied to all stages and settings of the drug discovery process. In Hit identification, accurate 3D-pharmcophore information derived from reference compounds permits the rapid identification of chemical equity independently of other screening approaches (e.g., VS, HTS). In Lead-Generation and Lead-Optimisation, 4D-structures can significantly impact on compound design separately or in conjunction with traditional structure-based drug design approaches. Specific examples for a range targets are presented, demonstrating how 4D-structures can be been used to great effect in drug discovery.

19 Quantification of Free Ligand Conformational Preferences by NMR and Their Relationship to the Bioactive Conformation

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Accurate unbound solution 3D-structures of ligands provide unique opportunities for medicinal chemistry and, in particular, a context to understand binding thermodynamics and kinetics. Previous methods of deriving these 3D-structures have had neither the accuracy nor resolution needed for drug design and have not yet realized their potential. Here, we describe and apply a NMR methodology to the aminoglycoside streptomycin that can accurately quantify accessible 3D-space and rank the occupancy of observed conformers to a resolution that enables medicinal chemistry understanding and design. Importantly, it is based upon conventional small molecule NMR techniques and can be performed in physiologically-relevant solvents. The methodology uses multiple datasets, an order of magnitude more experimental data than previous NMR approaches and a dynamic model during refinement, is independent of computational chemistry and avoids the problem of virtual conformations. The refined set of solution 3D-shapes for streptomycin can be grouped into two major families, of which the most populated is almost identical to the 30S ribosomal subunit bioactive shape. We therefore propose that accurate unbound ligand solution conformations may, in some cases, provide a subsidiary route to bioactive shape without crystallography. This experimental technique opens up new opportunities for drug design and more so when complemented with protein co-crystal structures, SAR data and pharmacophore modeling.

20 Chiral Solvation Revisited: Developing a CSA Method with a Single Enantiomer

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Chiral molecules are important in chemistry and no less in the pharmaceutical industry where many drugs and intermediates are chiral. We therefore require analytical methods to determine the enantiomeric purity of such molecules. Frequently chiral HPLC will be chosen as the assay method of choice, but we present a case that analysis by NMR using a chiral solvation reagent[1-2] method may frequently by more time efficient. We also present an enhancement that enables a CSA method to be devloped using only a single enantiomer of the analyte. This enables us to confidently determine whether or not a sample from synthetic chemistry has *or has not* be suject to racemisation during synthesis. We can also prove the enantiomeric purity of a final product without requiring synthesis of the other enantiomer.

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21 A Spin Diffusion Based Method to Determine Protein-Ligand Complex Structures Demonstrated on Enzymes of Pharmaceutical Interest

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Structure-based drug design mainly relies on high-resolution crystal structures of the receptor-ligand complex to obtain the required information for optimizing target binding of small molecules. However, obtaining crystals and structures of sufficient quality cannot be achieved for approximately 40% of pharmaceutically relevant protein targets. For those target proteins that cannot be crystallized, NMR spectroscopy is an alternative. We developed a fast and reliable methodology, termed STI, to use experimental, spin diffusion based NMR data as a scoring function for molecular docking structures: 1) The INPHARMA method [1] derives the relative binding mode of two ligands, given that they target the same binding site 2) Saturation Transfer Difference (STD) [3] yields information on the protein-buried and water-exposed part of the ligand and 3) Transfer-NOE reveals the bound ligand conformation. For every docking model, the NMR peak volumes of all three data sets are back-calculated with our software SpINPHARMA and then correlated with the experimental data. We show on the example of Protein Kinase A (PKA) ligand complexes, that cross validation of docking results against NMR restraints leads to the same binding modes as X-ray crystallography and performs clearly better than docking scoring functions. In the following, the methodology is demonstrated on two enzymes of on-going pharmaceutical research, sEH and DXS.

Soluble epoxide hydrolase (sEH) is an enzyme that hydrolyses the epoxide moiety formed on unsaturated fatty acids like arachidonic acid during metabolism. Inhibition of this reaction has been shown to have favourable effects on cardiovascular diseases [3], making sEH an interesting new drug target. Designing drugs for sEH inhibition is on-going and an early characterized ligand class consists of a central urea moiety positioned in the middle of the binding site, framed by two substituents on the left and right side. For drug optimization of these ligands it is crucial to know their orientation in the binding site, which is shaped like a tunnel with entries to both sides. Four ligands were chosen and binding to sEH was confirmed with STD spectra and INPHARMA peaks were observed between all of the ligand pairs in the presence of sEH. Yet, the application of our STI approach did not yield a clear proposal of the binding modes, as in the case of PKA. Instead, the data give the impression that the ligand substituents can flip around the urea moiety and every ligand is present as two distinct binding modes. To validate these observations, three ligands were successfully co-crystallized with sEH. The crystal structures underline strikingly the observation from NMR: the substituents of the ligands are always present in both binding modes. This interesting observation confirms that our STI approach can also deal with very complex

ligand binding behaviours. A new approach of population weighting was also applied to further quantify the occupancies of each binding modes, as it can be done with X-ray crystallography. The project now continues with stronger sEH binders from the pharmaceutical company Sanofi.

1-deoxy-D-xylulose-5-phosphate synthase (DXS) was chosen as the target of a structure-based drug design project. DXS catalyzes the first and rate-limiting step of the non-mevalonate pathway and employs thiamine pyrophosphate (TPP) as cofactor. Important pathogens such as *Mycobacterium tuberculosis* and *Plasmodium falciparum* use the non-mevalonate pathway for the biosynthesis of the universal precursors of the essential isoprenoids. Given that humans exclusively utilise the alternative mevalonate pathway, the enzymes of the non-mevalonate pathway have emerged as attractive targets for the development of new drugs against infectious diseases like tuberculosis and malaria [4].

To evaluate the applicability of our innovative methodology, we started studying the binding mode of deazathiamine, a derivative of the natural binder TPP. While the binding mode of TPP is known from X-ray crystallography (PDB: 201x), there is no co-crystal structure of deazathiamine with DXS available in the RCSB protein databank. Nevertheless, deazathiamine can be expected to have a very similar binding mode to DXS as TPP, due the high similarity. Yet the measured IC_{50} value of 430 µM for deazathiamine is much lower than that of TPP (14 nM). The molecule **1** was designed *de novo* as a TPP-competitive inhibitor but it is no more a derivative of TPP. **1** was found to possess a promising IC_{50} value (1.81 ± 0.48 mM) and it was the objective to further improve this molecule by better filling the space of the DXS binding pocket in a structure-based approach. In order to optimize or grow **1**, information about its binding mode is necessary, therefore we applied our NMR methodology to this specific case. In the first place, INPHARMA peaks were observed between deazathiamine and ligand **1.** By application of our STI method, the binding mode of both molecules was determined. Hereby the binding mode of deazathiamine is very close to that of TPP, so we were very confident with this binding mode. Based on this complex structure, ligand **1** was optimized. The methyoxy group of **1** was replaced by a trifluoromethoxy group and the right-hand side of 1 was further grown into a benzimidazole group. This new ligand **2** was found to have an IC_{50} of 0.595 mM. The inhibition potency of the original fragment **1** has been therefore improved by four times. A NOESY spectrum of ligand **2** and deazathiamine was recorded and INPHARMA peaks were clearly observed. Applying the STI method, the binding mode of **2** was determined and is similar to the binding mode of ligand **1**, as would be expected.

In conclusion, we have established a robust method to determine complex structures of small molecule ligands based on scoring of docking modes with the spin diffusion based NMR parameters INPHARMA, trNOE and STD. The method was amenable to be applied for pharmaceutical research and structure-based drug design, as demonstrated on the interesting drug targets sEH and DXS.

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22 Defective Glucose Metabolism in Polycystic Kidney Disease Identifies a New Therapeutic Strategy

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder characterized by a slow progression that leads to end -stage renal disease [1]. ADPKD is characterized by bilateral renal cysts formation, whose gradual expansion compresses and eventually replaces the normal tissue. Therapeutic interventions targeting cyst expansion is currently being tested in multiple clinical trials to delay renal disease progression. ADPKD is mainly caused by mutation in the *Pkd1* gene, encoding for polycystin-1, a large non tyrosine kinase receptor that acts as a mechanoreceptor and it is involved in a several of signaling pathways.

The hypothesis that polycystin-1 could act as a "metabolic regulator" arose from a serendipitous observation during routine culture revealing that $Pkd1^{-/-}$ cells acidified the medium faster than the $Pkd1^{+/+}$, while the opposite was observed in cells over-expressing *PKD1* (by Dr. Alessandra Boletta, Fondazione Centro San Raffaele). It was hypothesized that this metabolic change might be related to an enhanced activity of mammalian target of rapamycin (mTOR), that is negatively regulated by polycystin-1, through ERK pathway [2]. To determine which metabolic pathways were altered in these cells, we performed a metabolomic profiling of the conditioned extracellular medium of $Pkd1^{+/+}$ and $Pkd1^{-/-}$ cells using ^{1}H NMR spectroscopy. 22 metabolites were identified and quantified, allowing identification of some metabolic differences between $Pkd1^{-/-}$ and $Pkd1^{+/+}$ mouse embryonic fibroblasts (MEF) cells. In particular the exometabolome of the $Pkd1^{-/-}$ MEF cells displayed an increased content of lactate, glutamate and alanine, presenting features similar to tumor cell metabolism [3]. Conversely, the exometabolome of the $Pkd1^{+/+}$ MEF cells had an increased content of choline, formate, glucose, glycine and pyruvate [3]. An unsupervised statistical analysis revealed that the metabolomic profile of $Pkd1^{-/-}$ cells differs significantly from that of $Pkd1^{+/+}$ cells, the most prominent alteration being reduced glucose

and increased lactate concentrations. This finding suggests that *Pkd1*$^{-/-}$ MEF cells use aerobic glycolysis as a source of energy (the so called Warburg effect) [3], one of the most prominent metabolic hallmarks of tumor cells [4]. Indeed, glucose deprivation completely abrogated the increased ATP content of *Pkd1*$^{-/-}$ cells. In line with this, real-time PCR analysis revealed that *Pkd1*$^{-/-}$ cells display a transcriptional signature of glycolytic enzymes, showing an increase of the transcript of lactate dehydrogenase A (LDHA) and pyruvate kinase M2 (PKM2).

We next asked if the glycolytic switch observed in cells occurs *in vivo*, upon inactivation of the *Pkd1* gene in the kidney. To this aim we injected subcutaneously uniformly-labeled ^{13}C-glucose in *Ksp*-Cre*:Pkd1*$^{flox/-}$ or littermate controls (*Ksp*-Cre*:Pkd1*$^{flox/+}$) and followed the levels of ^{13}C-glucose or ^{13}C-lactate using ^{13}C-NMR spectroscopy. We found that 40 minutes post-injection the cystic kidneys displayed a significantly higher uptake of ^{13}C-glucose, which is more efficiently converted to ^{13}C-lactate as compared to non cystic control kidneys. These data demonstrate that the cystic kidneys are characterized by aerobic glycolysis *in vivo* [3].

We next asked if this is a general feature of the cystic kidneys from patients with ADPKD. Thus gene expression profile of both gluconeogenesis and glycolytic pathways were analyzed using a previously established microarray database derived from *PKD1* human renal cysts (compared to minimally cystic cortical tissues from the same kidneys as control). Many enzymes involved in gluconeogenesis/glycolysis were differentially expressed in the renal cysts, with the vast majority of genes being down-regulated. Most of the genes encoding enzymes involved in gluconeogenesis were down-regulated while several genes encoding enzymes involved in glycolysis were up-regulated in renal cysts. Overall, these data suggest that increased glucose consumption and enhanced glycolysis are likely features of human *PKD1* [3].

Based on the above data we hypothesized that therapeutic interventions interfering with glucose metabolism in *Pkd1* mutant cells/tissues might present a novel strategy to retard cyst expansion. In order to interfere with glucose metabolism mice were treated with 2-deoxy glucose (2DG), a glucose analogue that cannot be metabolized [5]. Importantly, using the uniformly-labelled ^{13}C-glucose strategy described above, we demonstrated that 2DG acts indeed by reducing glycolysis in *Ksp*-Cre*:Pkd1*$^{flox/-}$ kidneys. Accordingly PKD kidneys treated with 2DG showed a reduction of the cysts volume as compared to the non treated PKD mice [3]. Taken together, these data indicate that defective glucose metabolism is intimately involved in the pathobiology of ADPKD. Our finding of a metabolic switch to glycolysis in the absence of functional polycystin-1 signaling provides the rationale for a novel therapeutic approach in ADPKD.

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23 Do We Need High NMR Resolution for Small Molecules? – The Only Man Who Makes No Mistakes is the Man Who Never Does Anything

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With the continuous advancement of NMR spectrometers, along with the development of more powerful and more user-friendly NMR methodologies, there is a growing general notion that the structure elucidation of small organic molecules is becoming less dependent on specialized human skills, especially when the compound in question is available in pure form and in sufficient quantities and contains only a few heteroatoms. However, practice shows that our increasing technical capability of obtaining abundant high-quality experimental NMR and MS data does not necessarily make structure elucidation a mechanical process, and misinterpreting those data is always a possibility that one should take into account.

As an example, herein we disclose the intriguing story of the structural elucidation of an organic molecule that formed in a highly unexpected reaction and which had a completely different structure (with a different elementary composition) from that expected chemically. Due to the interplay of a multitude of misleading and distracting factors, including the fact that the compound could not be purified entirely, had broad NMR signals due to conformational exchange, and had the same nominal mass as the expected molecule, in a first (routine) approximation its structure seemed to correspond to the expected molecule. Only a deeper investigation, initiated by some further unexpected events in relation to this reaction scheme, revealed the true structure of the compound. This story demonstrates how easy it is to misinterpret experimental data when one subconsciously seeks to confirm expectations (one of the several “mental traps” that can lead to erroneous structural deductions), especially in a time-pressed industrial research environment. We wish to point out the importance of being aware of such mental traps in order to avoid falling into them, and we also show how state-of-the-art instruments can help circumvent those traps.

24 Crystal Effects on NMR Chemical Shift Tensors: Electron and Shielding Deformation Densities

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The relationship between the NMR observables and the supramolecular structure of any system is not straightforward. In this work we examine the influence of the crystal packing for several purine derivatives on the principal components of the NMR chemical shift tensors (CSTs). We employ density functional calculations to obtain various molecular properties (the ground-state electron density, the magnitudes and orientations of the components of NMR chemical shift tensor, and the spatial distribution of the isotropic magnetic shielding) for the isolated molecules and for the molecules embedded in supramolecular clusters modeling the crystal environment and evaluate their differences. The concept has enabled us to rationalize the effect of the crystal packing on the NMR CSTs in terms of the redistribution of the ground-state electron density induced by intermolecular interactions in the solid state [1,2].

Application of our approach employing the electron deformation density (EDD) and the shielding deformation density (SDD) will be demonstrated for the hydrogen bonding and stacking interactions in several pharmaceutical substances.

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25 Configurational and Conformational Analysis of Flexible Natural Compounds Using Residual Dipolar Couplings and Nuclear Overhauser Enhancements

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α,β-Unsaturated δ-lactones, well-known Michael acceptors, constitute the pharmacophoric group of a broad range of natural products. Many of these compounds display pharmacologically relevant properties, e.g., antimicrobial, cytotoxic, and antitumoral activities. These bioactive products, occurring in several members of the mint family (Lamiaceae), comprise polyacylated-6-heptenyl-5,6-dihydro-2H-pyran-2-ones particularly abundant in the plant genus *Hyptis*.[1, 2] They are structurally related to pironetin, an anticancer acetogenin of microbial origin that selectively targets Lys-352 of α-tubulin.[3]

Conformational and configurational analysis of flexible small organic molecules using one-bond CH residual dipolar couplings (RDCs) in NMR have not been implemented successfully as done for rigid molecules.[4, 5] This situation prompted us to undertake a study directed to obtain enough information about the structure, configuration, and preferred conformations of two 6-heptenyl-5, 6-dihydro-2H-pyran-2-ones isolated from *Hyptis spicigera*. These compounds were selected as representative small flexible molecules for applying a protocol based on the systematic comparison between experimental and theoretical RDCs.

Experimental one-bond CH RDCs ($^1D_{CH}$) data were collected for each compound using *J* scaled F1 proton-coupled BIRD HSQC (JSB HSQC) spectrum under isotropic and anisotropic conditions. The alignment media was a cross linked poly(methylmethacrylate) (PMMA) gel and the isotropic media was $CDCl_3$. The RDCs values were calculated by the difference from the data registered for each compound in the compressed gel and the data under isotropic conditions.[5d, 6] The measurement of interproton distances were determined by analysis of transient NOE intensities obtained from 1D selective transient NOESY spectra in $CDCl_3$ using the Peak Amplitude Normalization for Improved Cross-relaxation (PANIC) method originally proposed by Hu and Krishnamurthy [7] and further developed by Butts *et* al.[8] Determinations were based on NOE build-up slopes at mixing times 150, 250, 350, 500, and 700ms.

A conformational search was carried out for each configuration in order to compare theoretical and experimental data. The method included a broad conformational search using a molecular mechanics force field of selected diastereoisomers for each compound, followed by geometry refinement at the DFT B3LYP level. The matrix order analysis was performed using the single value decomposition method (SVD) incorporated in the MSpin software.[9] The SVD calculation led to the best-fitting alignment tensor. Each compound minimum energy conformers were used for the treatment of conformational

averaging through the use of single tensor approximation. Once the alignment tensor was fitted, RDCs and distances were obtained. The configuration was analyzed by application of the correlation between the theoretical and experimental NMR data and they were scored in terms of the Cornilescu's *Q* factor for RDCs. Population averaged interproton distances were scored in terms of root-mean-square deviation (RMSD) using in-house Python scripts. The remarkable correlation between the theoretical and experimental data demonstrated the predictive value of this approach for the stereochemical assignment of the tested compounds.

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26 Lanthanide-Chelating Conjugates as Tools for the Structural Elucidation of Small Molecules and the Study of Their Recognition by Receptors

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Paramagnetism offers a rich source of long-range structural restraints through the induction of residual dipolar couplings, paramagnetic relaxation enhancement (PRE) and pseudocontact shifts (PCS), which have been thoroughly employed for structural characterization of biological molecules. [1, 2]

Previously, we reported the synthesis of the first lanthanide-chelating linker attached to a sugar molecule, [3] which allowed us successfully measuring PCSs in the carbohydrate moiety. The use of paramagnetic restraints emerges as a very convenient approach for the structural elucidation of carbohydrate molecules, as there are often problems to obtain structural information on these systems by NOEs, due to signal overlapping, strong coupling, and/or the scarcity of key NOE information.

Importantly, we show that the so-produced paramagnetic ligands can also be used as tools for the study of structural aspects of their recognition by macromolecular receptors. In particular, we studied the recognition of paramagnetic mono- and disaccharides by a prototype lectin, i.e. the CRD of human galectin-3 (gal-3), demonstrating that paramagnetic effects can be transferred through the space to the protein from the non-covalently bound ligand, as shown in ^{1}H-^{15}N HSQC spectra.

Also, for those instances where recombinant production or isotopic labeling are not possible (e.g., proteins isolated from natural sources), we conceived a strategy suitable to detect binding events, involving the tagging of the protein by a fluorine-containing probe and the monitoring of paramagnetic perturbations, exerted by ligands, of the protein fluorine signals in 1D ^{19}F-NMR spectra.

Finally, we present additional studies on the molecular recognition of systems involving non-protein receptors, such as cyclodextrins. We show that paramagnetic tagging of hydrophobic guests of β-cyclodextrin increases the sensitivity of the characterization of their binding by NMR methods, and permits the extraction of topological information.

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27 Precise Prediction of NMR Chemical Shifts in Heavy Transition-Metal Complexes (Pt, Ir, Au, Pd)

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Predicting and reproducing the NMR chemical shifts is very important task in theoretical chemistry. Nowadays, a number of computational approaches (mainly based on DFT) are available and software packages are designed also for less experienced users and offer good precision in the NMR chemical shift calculations. However, when the heavy element is present in the molecule, the relativistic contributions of the heavy atom(s) to the NMR shielding constants of bonded light atoms must be taken into account. These relativistic effects can easily represent about one third (30%) of the total NMR shielding constant of the light atom. One may use exact four-component relativistic methods (difficult to use, time demanding) or one- or two-component relativistic approximations like two-component ZORA. However, the deviation from the experimental values in more user friendly two-component calculations can still represent about 10-15% of the total NMR shielding constant.

We present a simple method for correct and precise NMR chemical shift prediction, employing the calibrated amount of exact-exchange admixture in hybrid GGA functionals (e.g., PBE0), which is routinely available in programs like ADF. The amount of exact-exchange admixture was carefully calibrated relative to experiment for platinum(IV) and iridium(III) octahedral complexes with adenine-based ligands [1] and further tested on square-planar platinum(II), palladium(II), and gold(III) complexes with pyridine ligands. In all cases the increased amount of exact exchange improved the agreement with experiment significantly not only for atoms directly bonded to and influenced by the heavy element (deviation of ~15 ppm dropped down to ~1-2 ppm) but virtually for all ^{13}C and ^{15}N NMR chemical shifts. Using this approach we are able to predict the 13C and 15N NMR chemical shifts with RMSDs from experiment of about 1-2 ppm (compared to RMSDs about ~4-7 ppm for standard GGA functionals).

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28 Total FID Analysis via Complex Truncated Jacobi Operators

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Without a doubt, very few mathematical tools have had the impact of the Fourier Transform (FT) on science and engineering in general, and on NMR in particular. Since this technique was first applied in NMR by R. Ernst and W. Anderson almost 50 years ago [1], it has become the gold standard mathematical procedure for converting the encoded time signal (FID) into its familiar and analyzable spectral representation in the frequency domain. Whilst modern NMR cannot be understood without the FT, it is well known that it presents a number of limitations, ranging from its low resolution power (i.e. high resolution would require sampling at long times, and truncated FIDs show distorted lineshapes and sinc oscillations), to the fact that the FT yields an envelope or shape spectrum with no quantification on its own so that some additional analysis procedures such as peak picking, integration or line fitting (deconvolution) must be applied if any qualitative and quantitative information is sought. Other ubiquitous problems associated with the FT are baseline and phase distortions which cannot always be effectively compensated or automatically determined, and therefore make difficult the extraction of the spectral parameters of interest.

We have recently presented [2] a new parametric, time-domain based approach aimed at circumventing the essential limits of the FT by building and studying rational, Padé-like approximants, to obtain a reliable list of the fundamental spectral parameters of genuine NMR resonances directly from the recorded FID. We present here the evolution of our endeavors in this research area, which leverage a number of connections between rational approximants, Stieltjes transforms, Jacobi operators and functional analysis; all well known in the realm of mathematical physics.

This novel method employs a robust procedure to numerically dissect the available FID, however short it might be due to operational or experimental conditions, to determine the highest possible number of genuine resonances. Examples that show how this algorithm can resolve and quantify tightly overlapped signals and discriminate between real and spurious signals will be presented.

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29 Quantitative NMR For Reaction Understanding And Dissolution Monitoring - Tube v's Flow Applications

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The use of NMR Spectroscopy is widespread throughout pharmaceutical development providing a rich source of structural and quantitative information. NMR's inherent quantitative nature enables a wide variety of analyses to be performed; including bulk drug purity, drug susbstance assay, residual solvents, process related impurity levels and more recently genotoxic and cyctotoxic impurity analysis down to ppm limits of detection.

Here we describe the use of quantitative NMR for two additional applications of great importance to the drug development process; for reaction understanding and for tablet dissolution monitoring. For both applications, standard tube based approaches and the use of flow-systems will be discussed. The latter has been facilitiated by the exploitation of the "flow-tube" system developed by the University of Manchester [1].

NMR is commonly used for monitoring and understanding chemical reactions, however, this is normally achieved by sampling (and with the addition of deuterated solvents) to run in-process tests or checks (IPT's & IPC's) or in order to determine the end of reaction (EOR). We have a dedicated NMR instrument for reaction monitoring and understanding, operating in standard tube mode, with a $^{1}H\{^{13}C\}$ flow probe or using the flow-tube system with a broad band 5mm probe. The system allows for quantitative NMR data to be acquired directly on a reaction sample, either with the reaction taking place in the tube within the magnet, or externally in a reaction vessel with fully temperature controlled transfer lines to enable full kinetic profiles to be determined (the NMR experiments are routinely run unlocked). We will demonstrate the use of the system with case studies of both tube and flow applications, with the acquisition of ^{1}H & ^{31}P NMR data to generate reaction profiles and rate constants.

The measurement of *in-vitro* dissolution of active pharmaceutical ingredients and excipients is important to help understand the mechanisms that control the dissolution performance of solid dosage forms. Here we report the application of NMR combined with conventional UV analysis to give insight into the relationship between the dissolution rates of an excipient and three active ingredients in the commercially available combination product: Beechams All-in-One Tablets™. One of the excipients, lactose, is invisible to the conventional pharmacopoeial testing method using ultra-violet (UV) detection and the UV absorption spectra of the three active ingredients are severely overlapped. Conventional thinking suggests that NMR is unsuitable for dissolution testing of pharmaceutical tablets given its low sensitivity and the preference for deuterated solvents - we have show this can be achieved using a medium field NMR spectrometer fitted with a cryo-probe and the implementation of solvent suppression techniques on-flow.

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30 Study of Tn Antigen Mimetics and Their Interaction with Lectins Using NMR STD

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The Tn antigen is an *O*-glycan present in mucins that it is found to be overexpressed in a large number of human disorders including cancers such as lung cancer or breast cancer *[1]*. The Tn antigen consists in *N*-acetyl-D-galactosamine with a glycosidic alpha linkage to serine or threonine *[1]*. Thus, Tn antigen has been used as a pharmacological target for the development of new antitumoral drugs. We have developed a new synthetic scaffold (compound 1) as a potential mimic of an *O*-linked *N*-acetyl-D-galactosamine (figure) *[2]*. This scaffold can be easily inserted in a peptidic chain, thus generatating the corrresponding glycopeptidomimetic, such as compound 2. We have studied the recognition of these two molecules by different a-galactose specific binding lectins from different sources: *Erythrina crystagalli* lectin (ECL), *Helix pomatia* agglutinin (HPA) and a truncated form of Human Macrophage Lectin 2 (HML-2).

The binding mode (epitope and affinity constants) of compounds 1 and 2 has been characterized through NMR Saturation Transfer Difference (STD) experiments, including competition experiments with O-Methyl alpha D-galactoside or O-Methyl *N*-acetyl-D-galactosamine. These studies revealed that compound 1 is indeed able to bind to these three different galactose specific binding lectins. In particular, compound 1 binds better to ECL than O-Methyl alpha *N*-acetyl-D-galactosamine, and the corresponding glycopeptidomimetic, compound 2, is recognised by ECL through the saccharidic part exclusively.

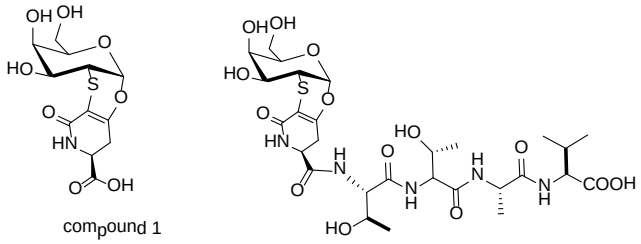

Figure 1.

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31 NMR Chemical Shifts in Nucleotides: Effect of Mg^{2+} Coordination at Various Sites

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In the last couple of decades, Nuclear Magnetic Resonance (NMR) spectroscopy has turned into an indispensable tool for the chemical analysis, structure determination and dynamical process analysis [1-3] in variety of systems, from simple solid phases to biological complexes. Knowledge of the structure of biomolecules is crucial in order to understand their function, and NMR is playing an ever larger role in it.

Here, we study the molecules of life, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). As we know, both DNA and RNA are made up of long polymers of nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. Attached to each sugar is one of four types of nucleobases: adenine, cytosine, guanine, thymine (only in DNA) and uracil (only in RNA). Also, it is well-known that the phosphate groups contains a negative charge, which is compensated by counter-ions (Na^{+}, Mg^{2+}) [4,5].

In particular, magnesium is one of the most abundant metal-ions in biochemistry and it is an essential cofactor for various enzymatic reactions involving nucleotides [6,7]. Therefore, we have computationally explored if the coordination site of magnesium ions at nucleotides can be uniquely inferred from NMR chemical shifts, especially for ^{13}C and ^{1}H but also for ^{31}P, ^{17}O, and ^{15}N nuclei, using DFT at the BP86 and SSB-D levels of theory. The latter functional has been proposed as a new all-round density functional [8,9] and was recently shown to perform excellently in computations of ^{13}C-NMR chemical shifts [10].

Our investigations focus on DNA and RNA complex with Mg^{2+} in aqueous solution, a situation about which little is known in structural terms, in contrast to the more abundant X-ray crystal structures of DNA and RNA complexes with Mg^{2+} [11,12]. We used for our computations the Amsterdam Density Functional (ADF) program in combination with the conductor-like screening model (COSMO) for simulating bulk solvation in water. Our model systems comprise the nucleotides of all five nucleic acids occurring in DNA and RNA. RNA was investigated with the C_3'-endo and DNA with the C_2'-endo conformation of the sugar ring, wich correspond to the pucker conformations in which the polymeric RNA/DNA species are mostly observed.

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32 Cromolyn: Study of its Aggregation Model and Enhanced Properties as Alignment Media for Water Soluble Molecules

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Chromonic phases are a family of lyotropic liquid crystals (LC) formed by ionic aromatic mesogens as disodium cromoglycate (cromolyn), sunset yellow and others. Despite being well-known that chromonic phases are oriented in the presence of external magnetic fields, leading to the observation of otherwise hidden NMR observables such as quadrupolar splittings or residual dipolar couplings,[1,2] they have been scarcely applied for practical applications in the field of aligned media NMR based structural elucidation in part due to the fact that they impart a too strong degree of alignment in guest molecules. However, the use of cromolyn/brine mixtures can lead to an optimum degree of alignment of small water-soluble molecules making possible to record ^{1}H-^{13}C RDCs with a good degree of accuracy. Also, cromolyn presents several enhanced properties with respect to other alignment media used for RDC extraction in small water molecules, like better homogeneity and signal to noise ratio, smaller line broadenning (LB=5-7 Hz) or easier sample preparation.

There is still debate about the microscopic structure of cromolyn, with J-ladders and columnar stack models being proposed. We have obtained alignment tensors for different guests in the cromolyn phases which help to discern between different proposed models for these phases. Besides, we will show that it is possible to obtain residual dipolar couplings in a single F1 coupled HSQC [3] experiment due to the coexistance of the isotropic and nematic phases.

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33 HSQMBC-TOCSY Experiment: A Complementary Tool for Structure Elucidation

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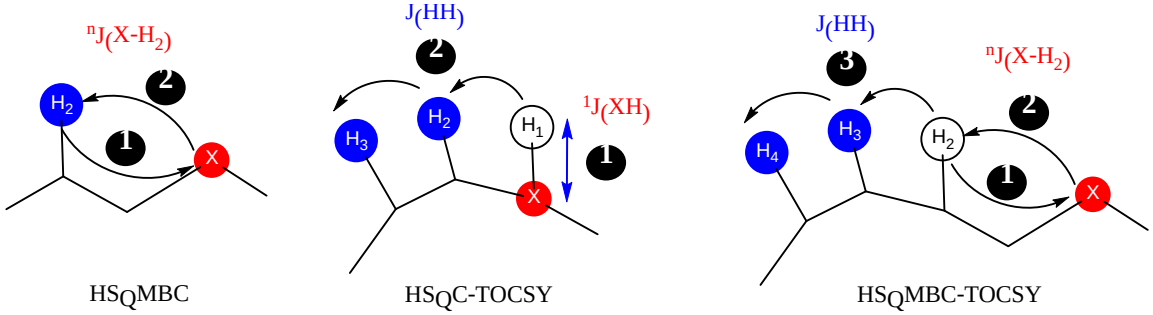
Long-range heteronuclear correlation experiments, typically based on the HMBC [1] or HSQMBC [2] pulse sequences, are key tools widely used in the structural characterization of small and medium-size molecules in solution because they provide connectivities between protons and heteronuclei separated by more than one bond. In these spectra, cross-peak intensities strongly depend of the magnitude of long-range proton-carbon coupling constants and therefore, sometimes some expected cross-peaks are missing or they present very low intensity. Alternatively, the HSQC-TOCSY experiment [3] also provides remote heteronuclear correlations between protons and carbons belonging to the same spin system but its application is only limited to protonated carbons.

In this work several versions of a complementary HSQMBC-TOCSY experiment for the success application on structural elucidation of small molecules in solution will be presented. We evaluate the possibility to obtain TOCSY information for a non-protonated carbon and we also extend this feature to other heteronuclei, such as ^{19}F, ^{31}P or ^{109}Ag among others. We compare and discuss experimental data obtained from the conventional HSQMBC and HSQC-TOCSY experiments with respect to this new HSQMBC-TOCSY experiment. Additionally, ^{1}H-selective versions will be also proposed for the accurate measurement of the magnitude of heteronuclear coupling constants [4], with special emphasis on the determination of the positive/negative sign of these couplings on non-protonated centers. We concentrate our studies on the determination of small J values in challenging conditions, such as complex multiplets, using the IPAP technique. We also evaluate the simultaneous implementation of multiplicity-edited information, where CH/CH_3 cross peaks can be clearly distinguished from CH_2/C correlations thanks to their opposite up/down relative phase. Discussion about the pulse schemes, details on experiment set-up and several examples applied for different heteronuclei will be provided for each case to illustrate the main features of these new valuable techniques.



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34 Metabolomic Responses of *Quercus ilex* Seedlings to Wounding Simulating Herbivory

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The study of plant metabolic changes and processes related with herbivore attack has been mostly limited to the identification of single compounds or families of metabolites. Ecometabolomics aims to analyze the metabolome of the plant, i.e. the total number of metabolites, and their shifts in response to environmental changes [1,2]. Plants defense themselves against herbivory at several levels. One of these levels is the synthesis of inducible chemical defenses. By using a NMR-based metabolomic approach we studied the metabolic changes of plant leaves [3] after a wounding treatment simulating herbivore attack in the Mediterranean sclerophyllous tree *Quercus ilex*. The results show an increase of glucose concentration linked to an increase of photosynthetic rates in wounded leaves. Besides, an increase of the concentrations of C-rich secondary metabolites such as quinic acid and quercitol, both related to the shikimic acid pathway and linked to the defense against biotic stress, was observed. Finally, a shift in N-storing amino acids as leucine, isoleucine, asparagine and choline was detected. The increase of the content of asparagine is related to the higher contents of choline through serine that has been proved to be the precursor of choline. Choline is a general antiherbivore and pathogens deterrent. The study shows the fast metabolic response of *Q. ilex* to defend its leaves based in a rapid increasing production of quinic acid, quercitol and choline. The results also confirm the suitability of ^{1}H NMR-based metabol¡c profiling studies to detect the global metabolome shifts after a biotic stress in tree leaves, and therefore its suitability in ecometabolomic studies.

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35 Ultrafast-*exo*NOESY: a New Tool for Monitoring Dipolar Coupling

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In recent years, UF-NMR has demonstrated itself to be an appropriate and effective technique to overcome the temporal drawbacks of traditional nD spectroscopy. Different UF-sequences based on the homonuclear and heteronuclear coupling have been described [1]. Studies using 2D Ultrafast TOCSY, HSQC and HMBC have provided the real-time monitoring of the breakage and formation of new covalent bonds with dynamic chemical systems, i.e. organic reactions [2,3,4].

Based on the EXSY sequence described by Lucio Frydman [5], we have developed a UF-NOESY sequence to detect the weak dipolar interactions between H nuclei. For this purpose, strychnine was chosen as the reference compound. Using soft techniques, the field of view of a 2D experiment is restricted to selected bands of both F1 and F2 frequency dimensions [6]. Applying this concept, we have developed a band-selective version of the UF-NOESY experiment, named UF-*exo*NOESY. Compared to standard UF-NMR, cleaner spectra with enhanced resolution and S/N ratio were obtained.

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36 Multi-Slice Spatially-Encoded NMR Experiments

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Recently, it has appeared a substantial interest in spatially-localized NMR spectroscopic techniques based on the incorporation of the traditional slice-selection concept. The most serious drawback of these experiments is their reduced sensitivity because the observed signal only arises from a discrete slice of the sample. Therefore, novel approaches should be strongly required to improve such low sensitivity in order that these types of experiments become attractive for practical use in moderate concentrated samples.

Experimentally, spatial frequency encoding is achieved easily by simultaneous application of a frequency-selective 180º ^{1}H pulse and a spatial-encoding gradient, G_s. Herein we exploit the benefits of applying a multiple-frequency modulated pulse to simultaneously excite different slices in a single NMR experiment. Our proposal is based on the careful setting of multiple offsets to avoid the excitation of mutually J-coupled protons into the same slice (Fig. 1) which would produce distorted multiplets due to J_{HH} evolution. A description and experimental details on the effectiveness of multiple-frequency pulses in slice-selective experiments will be provided.

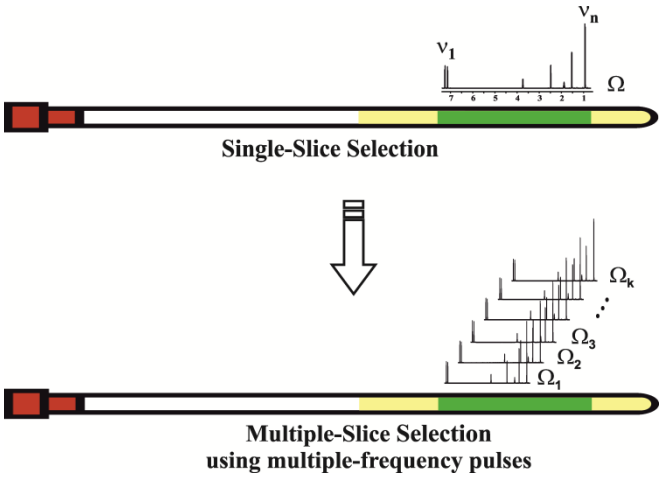

Figure 1: Schematic representation of conventional single-slice versus multi-slice selection by a multiple-frequency pulse modulated according to different offset frequencies (Ωk).

The successful application of the multi-slice selection concept to an unknown sample containing many different resonances will depend of the complexity of its ^{1}H spectrum. It will be shown that sensitivity improvements by a factor around 13.5 without observing phase distortions due to J_{HH} evolution can be obtained when applying a 15-site multiple-frequency pulse versus the conventional single-slice counterpart. In addition, it will be shown that multi-frequency pulses are easily implemented in existing NMR experiments. As an example, it will be shown how the signal-to-noise ratio in a broadband

homodecoupled ^{1}H NMR spectrum recorded using the pure-shift ZS technique [1-4] is enhanced by an experimental average factor of 6.7 when a 8-site multiple-frequency 180º pulse is applied instead of a conventional single-frequency one.

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37 Conformational Analysis of Small Organic Molecules Using NOE and RDC Data: A Discussion

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For the determination of three-dimensional structures of rigid molecules in solution by NMR spectroscopy, one usually uses dihedral angles determined from scalar coupling constants via the Karplus equation, distances from the NOE, and recently the angular information from RDC data.

The structure of flexible molecules is much more difficult to determine. In this case, only the average of the NMR parameters is accessible. This complicates the structure determination. If one succeeds though, important information about the structure and dynamics of the molecule under investigation can be obtained in this way.

Due to the different information content of NOE and RDC and the different behavior in conformational averaging, both interactions have advantages and disadvantages. Depending on the molecule, a consideration of only one of the two parameters therefore may be sufficient.

It was recently proposed that the observation of only one distance determined by NOE in strychnine leads to a distance value which can only be explained by conformational flexibility of strychnine [1]. One of the first examples that show that RDC data can yield information on the flexibility of a molecule is α-methylene-γ-butyrolactone [2].

In both cases, however, only either NOE or RDC data were considered. Therefore, the combined examination of NOE and RDC data shall be shown on selected example molecules, including a discussion of the strengths and weaknesses of both methods.

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38 Exploring Small Molecule Aggregation using NMR Spectroscopy and Small Molecule Probes

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Driven by non-covalent π-π interactions, planar aromatic molecules such as dyes [1], pigments [2] and drug compounds [3] are known to spontaneously self-aggregate. Moreover, as these associations are involved in phenomena such as DNA base-pair stacking [4], protein misfolding that leads to various neurodegenerative disorders [5], and metal-organic frameworks responsible for hydrogen storage [6], it is crucial to understand their interaction with other small molecules.

The aggregation process has been shown to play a critical role in various solute and solvent properties. Not only will increasingly larger assemblages result in lower diffusion coefficients [7] and increased viscosity [1], but the unique magnetic environments within each layer will be altered by an enhancement of the ring shielding effect in neighbouring molecules [8].

Nuclear Magnetic Resonance can be employed as an effective tool in such an investigation. Using a pulsed field gradient echo experiment to monitor diffusion coefficients or tracking concentration dependent changes in chemical shifts, it is possible to quantify the size and degree of aggregation. Here we aim to apply the already accepted NMR toolbox in a new way. By introducing a small reporter probe molecule, Fluorophenol (1 mol%), into a range of samples with varying amounts of the self-aggregating dye Sunset Yellow FCF, we will show that it becomes possible to interpret their association in terms of simple diffusion and thermodynamic models.

The hetero-association of our two chosen species can be viewed in various ways. With the aid of a comparative diffusion technique and an understanding of NMR at the fast exchange limit, it is possible to calculate the extent to which the probes have associated with the aggregates. Analysis of the variation in chemical shifts, on the other hand, further reveal an insight into equilibrium constants that would describe the process. From such findings we have been able to outline one possible mode of association, where these probes first associate at the stack ends, and are quickly thereafter buried deep within.

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39 Multinuclear Nanoliter 1D and 2D NMR Spectroscopy with a Single Planar Microcoil

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An intense effort has been devoted over the past decade to the development of microcoil NMR probes for the analysis of mass-limited and volume-limited samples [1]. We previously reported the study of supramolecular interactions using ^{1}H and ^{19}F NMR spectroscopy in a microfluidic chip equipped with a planar transceiver microcoil [2,3]. Recently, the on-line monitoring of a microwave-assisted cycloaddition was also described as a proof-of-concept for the hyphenation of small-volume NMR spectroscopy to other techniques [4].

Herein, we present the design of a second generation of microfluidic chips for nanoliter NMR spectroscopy. [5]. A planar transceiver microcoil integrated in a microfluidic chip (active volume 25 nL) enables the detection of different nuclides in a broadband range of Larmor frequencies (e.g. at 9.4 T from 61 to 400 MHz). All routine 1D and 2D, homo- and heteronuclear experiments can be carried out using our set-up. The possibility of detecting different nuclei combined with the advantages of working on-flow opens the path for many lab-on-a chip applications, including real time monitoring of chemical reactions using sub-microliter volumes of reagents.

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40 Can Urine Metabolic Profiling Help to Diagnose or Predict Disorders of the Mother, Fetus and Newborn?

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Several prenatal disorders have a significant impact on the health of the mother and fetus during pregnancy, as well as of the newborn. In spite of the remarkable advances in recognizing new markers of prenatal disorders [1], fetal malformations (FM) and chromosomal disorders (CD) diagnosis remains lengthy and, ultimately, very invasive, whilst for gestational diabetes mellitus (GDM), preterm delivery (PTD), preeclampsia (PE) and intrauterine growth restriction (IUGR) there are no adequate predictive methods. In this context, urine emerges as a valuable matrix to search for novel diagnostic and predictive signatures of maternal, fetal and newborn disorders due to its non-invasiveness and ease of collection.

In the first part of this presentation, ^{1}H NMR metabolomics of 2nd trimester maternal urine is explored as a possible diagnostic tool of fetal trisomy 21 (T21), FM and specifically malformations of the central nervous system (CNS). PLS-DA models of the ^{1}H NMR spectra were found to be characterised by classification rates (CR) ranging from 94% for T21, 84% for FM and 87% for CNS. These numbers are significantly higher than other non-invasive diagnostic methods used in clinical practice and typically based on biomarkers found in blood (55-75% for T21 [2] and 17-74% for FM [3]) [4].

Secondly, the attempted prediction of poor pregnancy outcomes (PTD, GDM, IUGR and PE) through metabolic profiling of 2nd trimester maternal urine is described, based on a previous report [4]. For this study, urine was collected several gestational weeks (g.w.) before the clinical onset of the disorders. A similar methodology produced PLS-DA NMR models with considerable predictive power, as shown CR ranging from 84% at 11-20 g.w. prior to PTD, 83% at 2-21 g.w. prior to GDM diagnosis, 94% at 2-22 g.w. prior to IUGR diagnosis and, finally, 94% at 18-24 g.w. prior to PE diagnosis). For further compreehensive understanding of GDM, the metabolic trajectory of the disease was traced considering three different stages: pre-diagnosis state, at the moment of diagnosis and during treatment, taking into account a previously established NMR-measured metabolic trajectory for healthy pregnancies [5]. Furthermore, prediction of treatment response (insulin requiring *vs*. non-insulin requiring) through urine metabolic profiling of GDM subjects was attempted, giving promising exploratory results, highlighting the possibility of *a priori* prediction of personalised treatment response.

Finally the impact of prenatal disorders in newborns´ health was explored by profiling urine of babies born from PTD and GDM-affected pregnancies, babies with disturbed fetal growth and neonatal asphixia.

The results hereby presented highlight the potential of urine metabolomics for prenatal diagnosis, still requiring larger cohorts and external validation so that clinical application may be achieved.

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41 Multinuclear NMR Investigation of GPTMS Condensation

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3-Glycidoxypropyltrimethoxysilane (GPTMS) is one of the most commonly used organic silanes for hybrid organic–inorganic material fabrication. Controlling the reaction condition (Ph, temperature) it is possible to modulate the behavior through the organic polymerization path (via epoxide hydrolisis) or through the inorganic condensation path (via Si-O-Si network formation)[1]

In this work we present a multinuclear NMR study (^{1}H , ^{13}C and ^{29}Si) of the Si-O-Si condensation process at room temperature and pH=7.

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42 NMR Approaches to Characterize the Structure and Self-assembly of Bacterial Cyclic Lipodepsipeptides

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Cyclic lipodepsipeptides (CLPs) are a very diverse group of secondary metabolites that are typically produced by soil-dwelling or plant-associated bacteria. They feature an oligopeptide cyclized via a lacton bond between the C-terminal carboxylgroup and a side chain hydroxylgroup, and a fatty acid moiety bound to the N-terminus [1,2]. These biosurfactants not only have drawn attention because of their potent antimicrobial activity (such as the clinical antibiotic daptomycin), they also play diverse roles in the lifestyle of their producers: protection against predators, bacterial swarming, biofilm formation, contribution to the virulence of plants or triggering the plant defence system. Their involvement in plant diseases is of great relevance in agriculture and crop protection [3]. Despite the broad interest in their biological function, an overall understanding of their modus operandi and the relation between their molecular diversity and function remains elusive. Our objective is to characterize both natural and synthetic non-natural CLPs in terms of their structural, dynamical and physicochemical properties, including the interaction with the cellular membrane, generally believed to be their target.

An often returning feature of CLPs is their ability to self-assemble to larger complexes in solution. In our study of a collection of *Pseudomonas* CLPs known as the viscosin group, we have found a self-assembly to supramolecular structures of unlimited size to take place in non-polar solvent solution, a process that is possibly related to their ability to form pores in cellular membranes [4,5]. The self-assembly in non-polor organic solvents has fuelled the development of novel NMR approaches to characterize this self-assembly and to gain both mechanistic and structural insight.

A first method we developed [6] involves an assessment of the anisotropic shape of the assembly by analysis of the heteronuclear T_1 and T_2 $^{13}C^{\alpha}$ NMR relaxation rate constants within the peptide. Their sensitivity to the orientation of the corresponding CH bond vector — available from the monomer conformation — within an anisotropicly tumbling object allows the anisotropy of the rotational Brownian motion to be assessed through the rotational diffusion coefficients, effectively revealing assembly shape information. In addition, the orientation of the monomer unit within the assembly vs. the direction of growth is obtained.

The second method is based on the measurement of translational diffusion coefficient data as a function of concentration, as obtained by PFG-NMR. As the self-assembly is occurring under fast exchange

conditions, the diffusion coefficient is found to be an ideal descriptor of the degree of self-assembly, allowing the comparison between various natural and synthetic non-natural CLPs. While the diffusion coefficient holds information concerning the assembly size distribution and self-assembly thermodynamics, the extraction of this information is complicated by the requirement of prior knowledge of the diffusion coefficient of each assembly size. As an alternative, we are working on a model-free approach, i.e. a formalism that does not assume an oligomer shape beforehand. The oligomer shape is in fact summarized in only one parameter that can be fitted from the data and may even be related to the fractal dimension of the oligomer. In addition, this analysis has the ability to reveal separate stages of the self-assembly process, thus revealing mechanistic information.

Both NMR methods thus allow structural information of the assembly to be obtained, even when there is little or no prior knowledge available on the mechanism driving the self-assembly. In this presentation, the focus will lie on the application of these methods within the context of CLP research.

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43 New Methods for the Rapid and Simple Measurement of the Magnitude and Sign of ^{1}H-^{19}F Coupling Constants

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In fluorinated molecules, structural information can be obtained from proton–fluorine coupling constants that are typically derived from the ^{1}H spectra. The multiplet of a proton coupled to fluorine shows an extra coupling and the ^{1}H-^{19}F coupling constant is measured as the difference in frequency between the relevant peaks. In the case of complex multiplets, however, determination of ^{1}H-^{19}F couplings is not as simple and proton decoupling at one or more frequencies is often applied to simplify the multiplet and to facilitate the measurement. However, when the proton signals are unresolved or the ^{1}H–^{19}F coupling constants are small in magnitude, extraction of the coupling constants from the proton spectrum is not as simple. In addition, no information about the sign of the ^{1}H-^{19}F coupling can be obtained from a ^{1}H spectrum. In this work, new approaches based on 1D- and 2D-HSQC and 1D- and 2D-TOCSY experiments are proposed for a rapid and accurate measurement of the magnitude and sign of ^{1}H-^{19}F coupling constants.[1,2]

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44 T_1 and T_2 Relaxation of Tungsten Stannyl Complexes

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Our group has been studying the structure and reactivity of tungstenocene stannyl complexes of the type $Cp_2WXSnYR_2$ (X=H,Cl; Y=Cl) to explore the potential of cooperative reactivity of these disparate metals [1,2,3]. Such cooperative reactivity, previously found in bimetallic species, sometimes allows reactions inaccessible to complexes containing only one metal [4,5]. With an eye to exploring the tungsten-tin interactions in the solution state we have turned our attention to a careful study of the relaxation parameters T_1 (spin-lattice) and T_2 (spin-spin) of the tungsten nucleus and tungsten hydride as they give important structural and dynamic information about a molecule [6]. However, pulse sequences that directly detect tungsten magnetization to determine relaxation values are difficult to carry out because of the low sensitivity of the ^{183}W nucleus.

INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) sequences have previously been used on proteins and other biological molecules to increase the sensitivity of nitrogen T_1 measurements [6]. However, as these types of inverse detection studies have been conducted less extensively on low frequency metals such as ^{183}W, we are currently exploring and will report on the utility of gradient selected inverse detected T_1 and T_2 pulse sequences for use on organometallic complexes. These sequences, based on previous sequences developed for use on proteins by Kay et al. [7], use a refocused INEPT to transfer magnetization from proton to tungsten, with and without suppression of dipolar/chemical shift anisotropy (CSA) cross correlation effects [7]. We will report on the utility of these sequences as applied to tungsten stannyl complexes such as $Cp_2WHSnCl^tBu_2$ over a range of temperatures.

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45 Symmetric N-X-N Halogen Bonds are Preferred in Solution

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Halogen bonding (XB) is a close to linear, noncovalent interaction between an electropositive halogen (X) and an electron donating species. Due to its strong resemblance with hydrogen bonding (HB), XB is now regarded as a potential complementary tool to HB in molecular recognition and crystal engineering. Whereas the symmetry of N-H-N and O-H-O H-bonds has been extensively investigated, the understanding of the behaviour of the analogous N-X-N halogen bonds in solution is still in its infancy.

By applying the NMR technique isotopic perturbation of equilibrium (IPE) using selective ^{2}H-labelling and ^{13}C NMR detection to two bispyridine-based N−X−N model systems, one freely adjustable and one conformationally restricted, we have successfully determined the symmetries of N−I−N and N−Br−N halogen bonds in solution [1,2]. The observation of the temperature dependence of the isotope shifts makes it possible to distinguish a static symmetric complex [N···X^{+}···N] with a single well energy potential from an asymmetric complex comprised of two rapidly equilibrating tautomers [N^{+}−X···N] ⇄ [N···X−N^{+}] with a double well energy potential. Preference for a symmetric arrangement was observed for both N−X−N (X = Br and I) model systems in CD_2Cl_2. Diffusion NMR experiments with ^{1}H and ^{19}F detection for determination of the degree of ion pairing of the [N···X^{+}···N] cation and triflate anion revealed a strong coordination. The fact that the symmetric arrangement of the [N···X^{+}···N] cation was not disturbed by a strongly attached counter ion, indicates a high energetic gain upon the formation of a symmetric halogen bond. Computational studies on the DFT level confirmed the preferred symmetric arrangement. Even in the more polar solvent CD_3CN, the N−X−N complexes remained static symmetric [3]. The ^{15}N coordination shifts indicate the higher strength of the iodine centred as compared to the bromine centred halogen bond [2].

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46 NMR and QSAR Studies of Purines and Pirimidines Antitumoral Agents

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Dichloroacetate (DCA) is a highly selective antitumoural drug. It is able to target the unique metabolic profile that characterizes most cancers: mitochondrial glucose oxidation. Its effectiveness is explained by a dual mechanism that induces apoptosis: first, by mitochondrial depolarization and efflux of proapoptotic mediators and second, by an increase in Kv channel expression/function [1]. We have reported (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purine as an important apoptotic inducer against the MCF-7 human breast cancer cell line [2]. We have decided herein to connect the double-ringed nitrogenous bases to the ethyl acetate (drastic molecular pruning) moiety.

We report the synthesis and anti-proliferative activity of two 5-FU derivatives (**1-2**, Figure 1), and six purinic scaffolds (**3-6**, Figure 1) against the human breast cancer cell line MCF-7, the human colon carcinoma cell line HCT-116, and two human melanoma cell lines such as A375 and G361.

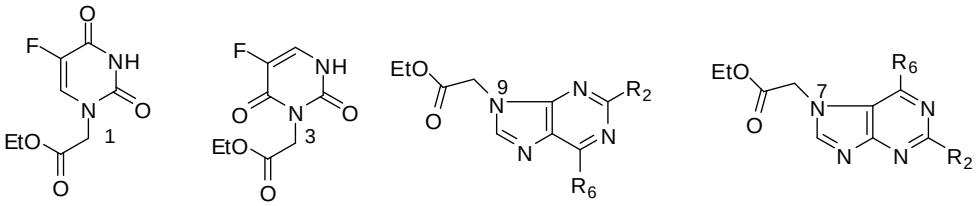

Figure 1. N-1 (1) and N-3 (2) 5-FU derivatives, N-9 (3-5) and N-7 (6-8) purine compounds.

The IC_{50} values suggest that there should be a correlation between the lipophilicity and the antipoliferative activity of the target compounds. We therefore decided to find the corresponding QSAR equations. Used in its logarithmic form (clog *P*) the octanol-water partition coefficient is the most widely accepted measure of lipophilicity. Fragmental methods make it possible to create data banks and to perform log *P* calculations by computer. We have obtained two QSARs between the antiproliferative IC_{50} values for compounds **1-8** and the clog *P* against the melanoma cell lines A375 and G361.

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47 Introducing Atomic Force Microscopy in Structure Elucidation – the Discovery of Breitfussin A and B, Highly Modified Halogenated Dipeptides from the Arctic Hydrozoan *Thuiaria Breitfussi*

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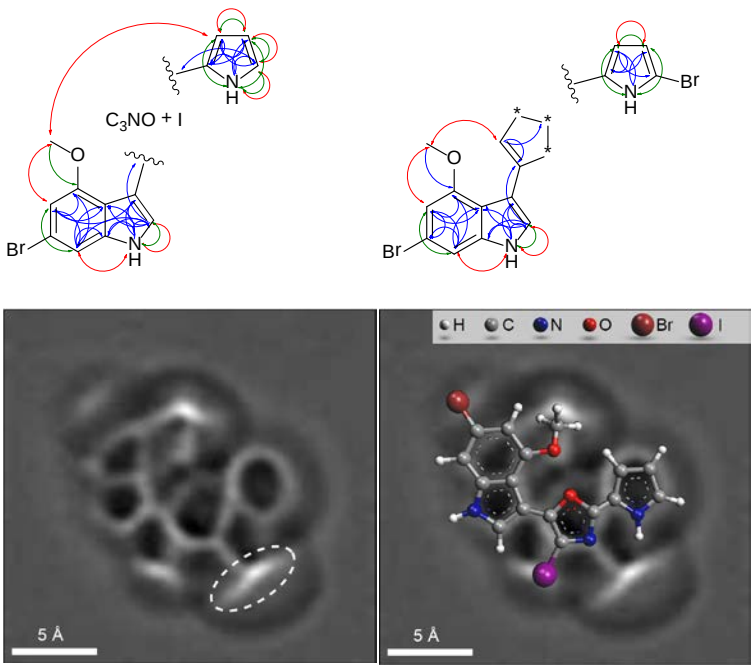
Atomic force microscopy (AFM) with atomic resolution has for the first time been used to aid in the elucidation of unknown natural products (Hanssen et al., *Angew.Chem.* 51, 12238, 2012). AFM shows great potential for the structural characterization of planar, proton-poor compounds, as these compounds are difficult to elucidate with spectroscopic methods and therefore prone to corrections. Given the limited quantity isolated, x-ray crystallography was not feasible and structure determination using spectroscopic data alone had difficulties proposing a single unequivocal structure consistent with all data. We show here the combined use of spectroscopic methods, AFM, computer assisted structure elucidation (CASE) and electronic structure calculations (DFT) to solve the structures of breitfussins A and B, isolated from the Arctic hydrozoan *Thuiaria breitfussi* (Family Sertulariidae), which could not be solved using either method alone.

This study presents the first compounds from the hydrozoan *Thuiria breitfussi*, revealing an unusual structural framework containing a combination of indole-oxazole-pyrrole. Remarkably, AFM could be used to determine all the connection positions of the cyclic systems as well as those of the substituent groups: MeO, Br, and I - information that can be difficult to obtain with other techniques.


C3NO + I
O
Br
N
H
Br
N
H
O
Br
N
H
H
C
N
O
Br
I
5 Å
5 Å

48 Application of Spatial Encoding in Relaxation Measurements and with Inhomogeneous Magnetic Fields

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Ultrafast methods in NMR have revolutionized the method of performing NMR experiments. One of the approaches for acquiring fast NMR data is the use of spatial encoding and the use of selective pulses. In single-scan 2D NMR, such spatial encoding is followed by a conventional mixing period and by a detection block based on echo planar imaging [1,2]. Ultrafast NMR has been successfully used for many applications for real time kinetic studies of protein, RNA folding elucidation and mechanistic details of organic reaction monitoring [3,4,5].

Single scan Relaxation Experiments: Utilising the concept of slice selective excitation, relaxation times can be measured by single scan experiment for samples having sufficient S/N ratio [6]. In this presentation, possibility of applying this powerful technique for measuring T_1 and T_2 relaxation times as one – and two-dimensional experiments will be discussed. Principle of 'single scan inversion recovery' involves applying 180^0 pulse to invert the magnetization of whole sample. As the magnetization is relaxing back towards equilibrium state we observe each T_1 encoded slice of the sample in a single shot. For the T_2 measurement, a series of slice selective excitation followed by a hard 180^0 degree pulse is proposed. Incorporating such measurements as part of single scan 2D experiments is being explored.

NMR in Inhomogeneous Magnetic Fields: Conventional NMR instrumentation is large and expensive however because superconducting magnets offer maximum sensitivity and resolution. Traditional NMR limits when arbitrarily large samples require nondestructive analysis and necessitates the development of mobile NMR systems. The inherent inhomogeneous magnetic fields of mobile NMR systems lead to signal overlapping and complete loss of spectral information. Nutation echo is one of the novel techniques where rf field gradients and static field gradients are matched to refocus static field inhomogeneities but retains the chemical shift and coupling information [8]. Multidimensional NMR experiments can be designed based on nutation echo to get structural information in inhomogeneous magnetic fields. Inherent multi scan nature of these experiments makes NMR experiments time consuming. Focus on the methods utilising high resolution feature of nutation echoes and ultrafast data acquisition feature of spatial encoding provide high resolution NMR spectra in inhomogeneous magnetic fields with in fraction of a second. Principles and our results based on these techniques will be presented and possible applications will be discussed.

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49 Exploration of the Structure-Activity Relationship of Natural, Self-assembling Cyclic Lipodepsipeptides

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Cyclic lipodepsipeptides are non-ribosomal peptides produced by bacteria, mainly *Pseudomonas* and *Bacillus* spp. They consist of a short sequence of D- and L-amino acids, which forms a cyclic structure by means of an esterbond between the C-terminal and a side chain alcohol function. They exhibit antagonistic activity for several bacterial and fungal species. We are mostly interested in the CLPs of the viscosin group. These consist of 9 amino acids, of which 7 are contained in the cyclic part of the molecule. Previously, the structure and conformation of pseudodesmin A has been extensively investigated using X-ray analysis and elaborate NMR relaxation measurements [1,2].

The mechanism by which these compounds exert their antagonistic activity is as of yet unknown. One hypothesis is that they form ion pores in the bacterial cellular membrane, causing an ionic imbalance and eventually cell lysis. We propose that in hydrophobic environments such as a lipid bilayer, the lipodepsipeptides of the viscosin class self-assemble to larger supramolecular structures, only limited by the confines of the membrane. In this structure, the hydrophylic residues are directed inward and the hydrophobic residues outward. It is possible to observe this self-assembly process in non-polar organic solvent solutions such as chloroform, previously reported extensively for pseudodesmin A [2,3].

An interesting feature of the viscosin group is the unique variability of the steregenic center at the 5-position in the peptide sequence, either featuring a D-Leu or L-Leu residue. Currently, conformations have only been reported of CLPs featuring a D-Leu5 residue. In this study, the structure and self-assembly properties of the viscosinamide (containing L-Leu5) has been investigated by NMR spectroscopy, allowing us to make a comparison with pseudodesmin A (containing D-Leu5). Using rotating frame nOe-derived distance restraints and homo- and heteronuclear coupling constants, the conformation of viscosinamide could be determined. Using PFG-NMR, the self-assembly capacity can then be characterized and compared to pseudodesmin A by observing the diffusion coefficient at various concentrations.

The analysis of the conformation of viscosinamide is an essential first step for understanding the self-assembling behavior of this molecule. Using NMR heteronuclear relaxation experiments, the diameter and length of the supramolecular structure can be determined at different concentrations. Additionally, the orientation of the monomeric units within the supramolecular structure can be calculated.

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50 A Close-up of New Coumarine Derivatives Inhibitors-AChE Interactions: Observation by Direct NMR Spectroscopy in Coordination with Docking Simulation

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Alzheimer disease (AD), a major and most common threat to the elderly population in West directly linked to the acetylcholine (ACh) deficiency in the brain [1]. Electric impulses are transferred from one nerve cell to another though ACh; a major neurotransmitter found in vertebrates as well as in arthropods [2] The Acetylcolinesterase (AChE) catalyzes the hydrolysis of acetylcholine (ACh) into the choline and acetate resulting termination of the impulse transmitted by ACh through cholinergic synapses [3]. Thus, the most useful treatment that could possibly lead to elimination of the Alzheimer disease may exist in AChE inhibition according to cholinergic hypothesis [4]. In this context, NMR spectroscopic inhibitor-AChE binding studies have been carried out by taking help of most widely used interaction techniques like STD and Tr-NOESY. To accomplish our purpose, we have used a set of four coumarin derivatives AChE inhibitor with potent to moderate inhibition activity. Furthermore, the comparisons of dissociation constant as calculated by STD NMR titration were also made with already used drug (tacrine). The NMR findings exposed that, our potent inhibitor provided tight binding even better than tacrine. While, in structure based affinity as obtained through STD NMR saturation time-dependent experiments clearly showed the pipridinyl based side chains are responsible for tight bindings. Moreover, to further consolidation to these NMR results, docking simulation were also performed, where docking results ranked tacrine on the top with less inhibitor-AChE complex energy. Nevertheless, the docking results revealed that these inhibitor compounds showed binding with peripheral binding sites, anionic sub-site, bottom of gorge and quaternary binding site as well. As reported earlier [5], the inhibitors that bind simultaneously with two sites like, PAS and catalytic sub-site, besides stopping ACh hydrolysis may also help to muffle the pre-aggregation of beta-amyloid. On the basis of these NMR and docking results we could suggest our potent inhibitor as a possible alternate to tacrine, and a new drug candidate for Alzheimer´s disease that demands clinical trial.

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51 From an Extract to Lead Candidate; A Faster and Ample Natural Product Extract Screening Through 1D & 2D STD NMR, 2D Tr-NOESY Together with a Hyphenation System

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The rapid screening of compounds that bind to the target of interest (some specific protein) plays a vital role in the drug discovery [1]. Most frequently, the identification of biologically active compounds is done from the library of structurally known compounds. However, we successfully illustrate here, that NMR techniques including saturation transfer difference (STD), transfer nuclear overhauser spectroscopy (TrNOESY) and STD-TOCSY (total correlation spectroscopy) in coordination with separation methods not only enable the rapid and comprehensive screening of active components, but their unequivocal structural characterization as well. Furthermore, a time cutback for the recognition of leads can also be possible with this application. To probe the binding studies, a hydroethanolic fraction of crude extract-(1mg) from natural product (*Rauia resinous*) was used for the initial assessment with BSA protein. Following that, the docking simulation using an earlier published protocol [2] was performed with BSA in the region of Thr190, Arg198, Arg217, Trp213, Arg256, Ala290 and Tyr451 to further refine the active compound towards the leads. Docking results mimic binding as identified by the STD, Tr-NOESY and STD-TOCSY. From our recognized library, Isovetexine-2-rhamnosoide was found to be most active through group epitope mapping results as well as the docking simulation with -7.2770 free energy[3]. This experiment provided magnificent results through this direct NMR screening method. Using Bovine Serum Albumin as a reference, we illustrate that this approach offers an excellent way for the first hand detection of the active constituents/inhibitors from the natural remedies that are being used in folk medicinal treatments.

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52 Enhancing qNMR with Automated Multiplet Selection

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Quantitative NMR (qNMR) is widely recognised as an invaluable tool in very diverse applications, and is routinely relied upon for critical measurements of compound concentration and purity. Much has been written on these applications[1], and how the experiment should be performed.[2]

A bottleneck and inadequacy exist, however, in data handling in that users are typically required to select one or more compound multiplets to be used for quantitation. Whilst this may not be difficult for a skilled spectroscopist, the universality and possible reliability of the method is compromised when one considers its use by relatively unskilled hands. Since the final result is based on these (currently) subjective selections, it stands to reason that a method based on objective multiplet selection will have significant benefit. Having this method will also facilitate the reliable adaption of the process to automated workflows.

We have designed such an expert system that attempts to suggest to the user which multiplets will provide the most accurate qNMR concentration estimation.[3] The workflow is based on a spectrum that has had the multiplets defined or Automatically Assigned. The user may then choose from a number of "scenarios" to lead the algorithm towards selection of those multiplets whose peak areas are most likely to uniquely reflect analyte alone: typically this process is performed once for a series of spectra. The functionality is tightly integrated with Mnova software, and this allows full use of complementary algorithms where needed, such as spectrum deconvolution (GSD).

In this poster we will describe the functionality, and illustrate its application to a number if widely-used experiments, including concentration determination, purity verification, salt ratio determination, and impurity solvent quantitation. We show a comparison of results for the fully automated process compared with results obtained by an expert, and the close correlation even at low mM concentrations.

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53 A Versatile Software Tool for Mixtures Analysis

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Quantitative NMR (qNMR) is widely recognised as an invaluable tool in very diverse applications, and is widely relied upon for critical measurements of compound concentration and purity. Much has been written on these applications[1], and how the experiment should be performed.[2]

The discipline can, broadly speaking, be divided into the analysis of pure compounds, and mixtures. Again, very generally, one form of mixtures analysis is the cases where a complex and complete analysis is performed on all componenets of the mixture, even when there is considerable spectral complexity and peak overlap.

With many metabonomics approaches that are applied to body fluids such as plasma and urine, the study will rely on a Chemometric approach to data analysis and sample clustering. This approach is linked to differences in specific resonances, revealed in a "loadings plot", but these particular biomarkers may not be understood or known. Alternately, known biomarkers may be detected an the mixture and quantified using sophisticated alignment and line fitting algorithms.

A second form of mixtures analysis covers a large number of simpler cases as required, e.g., for the analysis of foods – hence, "foodomics". In a single experiment, established markers of identity, adulterants, preservatives, additives, and degradation products can be identified. This emerging technology has already shown considerable utility, and we can confidently forecast expanding interest. For example, NMR can very effectively show the source and state of products from *Aloe vera*, which are important consumer products.[3] The distinction we make between these mixtures' analyses is that component characterisation is largely accessible through knowledge and understanding of signature peaks/multiplets from constituent components of interest. It follows that a relatively small number of measurements of isolated components is, in principle, all that is required to define an analytical protocol.

We shall describe a software solution that runs in the Mestrelab environment as part of the qNMR Suite. This allows the user full flexibility in establishing "rules" to describe a component, and automatically perform the analyses. The algorithmic capability is complemented by a flexible reporting regime that can be customised to accommodate all needs.

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54 A Comprehensive NMR Analysis of the Interaction between DKP-RGD Ligands and Membrane Proteins on Intact Cells

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NMR experiments, transferred NOE and Saturation Transfer Difference, were used to shed light on the binding epitope of a small library of DKP - RGD peptidomimetics with integrins alphavbeta3, expressed on the membrane of ECV304 bladder cancer cells, and alpha5beta1, overexpressed on the membrane of MDA-MB-231 breast adenocarcinoma cells.

Integrins are a large family of transmembrane heterodimeric glycoprotein receptors, composed of two non-covalently associated alpha and beta subunits. Once bound to extracellular matrix proteins, they regulate a variety of cellular processes.[1] As a consequence of their role in important physiological phenomena, integrin defects have been implicated in many common diseases. In particular, the observation that alphavbeta3 and alpha5beta1 integrin subtypes are essential for tumor angiogenesis and can be successfully inhibited by small-molecule ligands containing the Arg-Gly-Asp (RGD) motif have turned them into attractive targets for cancer research.[2]

The DKP-RGD compounds studied are characterized by a diketopiperazine (DKP) scaffold introduced into cyclic peptidomimetics containing the Arg-Gly-Asp (RGD) sequence and differ for the configuration of the two stereocenters of the DKP and for the substitution at the diketopiperazinic nitrogens.[3]

The understanding of the interaction of a protein with its ligands requires precise knowledge about the underlying recognition events at a molecular level. A variety of biophysical methods have been developed for this purpose and several NMR spectroscopic techniques have emerged as powerful tools to identify and characterize the binding epitope of ligands to receptor proteins.

In particular, transferred NOE (tr-NOE) has gained momentum, as it provides the basis for a variety of experimental protocols that are designed to detect and characterize binding activity. The observation of tr-NOEs relies on the different behaviour of a small ligand molecule free in solution rather than bound to a receptor protein. In fact, a ligand bound to a large-molecular weight protein behaves as a part of the large molecule and adopts the corresponding NOE behaviour, showing strong negative NOEs, so called tr-NOEs. These tr-NOEs reflect the bound conformation of the ligand. Binding of a ligand to a receptor protein can thus easily be distinguished by looking at the sign and size of the observed NOEs. A NMR spectroscopic technique, complementary to tr-NOE, is Saturation Transfer Difference (STD). This sequence helps identifying the group epitope, revealing which moieties of the ligand molecule are closest

to the receptor in the bound state. The method is based on the transfer of saturation from the protein to the bound ligand which, by exchange, is released into solution where it is detected. The degree of saturation of individual ligand protons (expressed as absolute-STD percent) reflects their proximity to the protein surface and can be used to describe the ligand-target interactions.

Tr-NOE and STD experiments conducted with living cells have showed that the primary interaction between the ligand and the receptor is performed by the guanidine residue of the arginine side chain, the extended conformation of the ligands ensures the formation of the electrostatic clamp (STD effects on protons of the Arg and Asp residues), the opposite configuration at stereogenic centres of the DKP scaffold induces a different binding epitope for the non-RGD moiety of the ligands (diketopiperazine and benzyl substituent).

The NMR results were supported by docking calculations of these ligands in the active sites of alphavbeta3 and alpha5beta1 integrins receptor and were compared to the results of competitive alphavbeta3 receptor binding assays and competitive ECV304 and MDA-MB-231 cell adhesion experiments. The different stereochemistry and the different substitution at the nitrogen atoms of the scaffold strongly influences the conformations adopted in the free-state and the binding mode of these ligands in the active site of the integrin receptor.

Previous conformational studies of these cyclic RGD-peptidomimetics revealed that the highest affinity ligands display well-defined preferred conformations featuring intramolecular hydrogen-bonded turn motifs and an extended arrangement of the RGD sequence [Cbeta(Arg)-Cbeta(Asp) average distance $\geq$ 8.8 Å].

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55 Ionic Liquids Polarity Effects on the Keto-enol Tautomerism Studied by NMR

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The concept of polarity of ionic liquids (ILs) has been a theme for several studies. Yet, it is far from being a well understood subject. The study of solvent-dependent equilibria is an insightful approach to this issue and has been widely conducted for molecular solvents.

In this work, acetylacetone (acac), namely its keto-enol tautomerism was used to describe polarity and the overall behavior of ILs. This equilibrium was already studied for the case of molecular solvents, and shown to have a significant correlation to the polarity of the solvent [1]. This was also sparsely addressed for the case of ILs[2]. A further understanding of the effect of ILs, bearing 1-alkyl-3-methylimidazolium cations, on this equilbrium has been sought. A variety of ILs with different cation/anion combinations was used. A variation of the size of the side chain of the cation was used, with different anions: tetrafluoroborate, hexafluorophosphate, bis(trifluromethanesulfonyl)imide, methylsulfate, ethylsulfate, and dicyanamide.

1D NMR was used to characterize the equilibrium of the system, namely the equilibrium constant of enolisation (K_{eq}), Fig. 1. It was attempted to correlate these data to several physical and chemical properties of ILs, through both continuum and molecular approaches. This has shown a signifficant component of ion-dipole interaction forces between these solvents and the solute, in agreement with other characterizations of ILs [3]. A good correlation was also found with the the volume of the ion pair, determined as the sum of the volume of the two ionic species, as suggested before [4]. This comes to show that, when considering solvation, ionic liquids can be considered more as ion pairs than what was thought before.

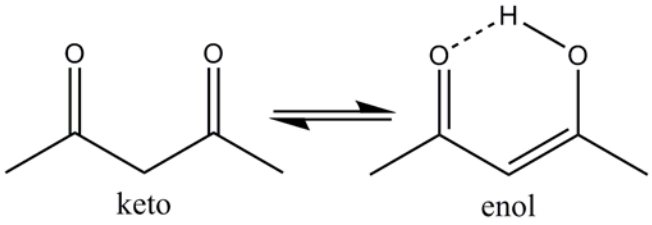

Fig. 1 Keto-enol tautomerization of acetylacetone (acac)

Further NMR studies were conducted to assess the occurrence of specific interactions between acac and ILs. This was done using NOE (homo- and heteronuclear) and DOSY experiments, which allowed to probe interactions of the keto and enol form of acac with the cation and anion of the ILs. These showed that the most relevant interactions are established between the anion and the keto form of acac.

It was also observed that such interactions can be related to the ion pair volume, and they are no longer detectable when the cation enlarges (growth of the alkyl side chain of the imidazolium cation). Alongside with this, the ratio of keto form decreases. This suggests that the most relevant interactions within ILs are provided by the anion. The NMR data are coherent with what is described above, concerning the effect of the volume of the ion pair.

In conclusion, the polarity and solvation of ILs is dependent on the size and nature of the ion pair, namely the ability to establish specific interactions.

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56 Solution and Solid State NMR Spectroscopic Characterization of Efavirenz

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Nowadays, in order to evaluate a drug in the solid state it's very important to ensure its quality. Also, much attention has been focused on the predisposition of pharmaceutical solids to crystallize in several crystalline forms. The different crystalline forms of a drug can exhibit distinct chemical and physical properties, the important ones being stability, dissolution and bioavailability. An extremely powerful tool, which has been increasing their importance and applicability for the characterization of pharmaceutical formulations, is the solid state nuclear magnetic resonance spectroscopy (ssNMR).

A study about the nature of the Efavirenz (EFZ) structure – an important antiretroviral used in the National AIDS Control Programme in Brazil –, has been conducted in solution and in the solid state by using ^{1}H and ^{13}C NMR spectroscopy. Solution NMR and ^{13}C CPMAS NMR spectra were obtained and compared with X-ray diffraction data[1] and molecular modeling.

The solution NMR spectra was obtained in a Bruker Avance 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) using $CDCl_3$ as solvent (zg30, d1 = 1s). ^{13}C CPMAS solid state NMR spectra were recorded on a Bruker Avance III 400 (9.4 T) spectrometer, operating at 100.64 MHz. The contact time was optimized to 10,000 μs. The recycle delay was 4s, and π/2 pulse length was 4 μs.

The ^{13}C NMR spectra of EFZ, showed to be very interesting due the differences in the chemical shifts $\Delta\delta = \delta_{liquid} - \delta_{solid.}$ These values are indicative of rigid and conformationally flexible fragments in the molecule (the latter are expected to undergo larger changes) and also could be used as probe for ascertain intermolecular interactions as H-bond[2]. Compared to the averaged value in the liquid state, the signals of 2-cyclopropylethynyl substituent and C-14 (CF_3) are shifted remarkably (0.3 to 2.8 ppm) and are splitted in the solid-state NMR spectra.

The C-11 carbon shows large differences in chemical shifts between the solid and solution states ($CDCl_3$) $\Delta\delta$=2.8 ppm and its signal is also splitted into a duplet at -0.7 and -1.5 ppm. Additionally, the peaks corresponding to C-10, C-12, C-13 carbons are splitted into a triplet. The splitting pattern indicates a different level of deshielding produced by different chemical environments and larger changes on chemical shift due to the conformationally flexible fragment. This also suggests that dissimilar molecular conformations or asymmetric units are present in the unit cell of EFZ. However, the differential scanning calorimetry (DSC) curve presented only a sharp endothermic peak corresponding to its melting point ruling out the possibility of polymorphism in these samples.

The EFZ structure was fully optimized with density functional theory – DFT (B3LYP/6-311+G(2d,p)) methodology. The conformational analysis was carried out on the optimized structure by using an adaptation of the method described by Mahapatra and coworkers[3]. In this way, a conformation was sampled each 60 around the C(14)–C(15) bond. The calculation showed that benzoxazin-2-one ring is conformationally restricted, and the 2-cyclopropylethynyl substituent prefers the equatorial position of the ring. It was observed that all six conformer present closed energy values and the most stable conformer is that one with has the dihedral angle defined by C(9) - C(10) - C(11) - C(12) equal to 29.77º. Crystallography data[1] showed that EFZ unity cell consist in two different molecules, associated by two hydrogen bonds forming we also carried out the molecular modeling of dimeric form. The X-ray geometry of EFZ dimeric form was used as a starting point for calculations. There are no significant differences between the crystal and optimized structure. The results has shown that the dimeric form is much more stable than monomer (- 9.44 x 10^5 kcal/mol). All the tests and measurements that were carried out with EFZ indicate that the structure exists in solution in a very similar way as it does in the solid state. In according to the crystallographic data, EFZ cell's consist in two different molecules, which is associated by two hydrogen bonds forming a cyclic via the eight membered […HNC (O)]$_2$ amide synthon.

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57 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, $C_8H_{10}N_4O_2$) is a well-known alkaloid, which was characterized 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 cheallenge for NMR.

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. The only major difference between the two molecules is found in the predicted ^{1}H spectrum, which has a CH_3 signal at 2.3 ppm, compared to 3.7 ppm in caffeine.

Overall the theoretical analysis of caffeine and a proposed structural alternative shows only a small difference in the predicted proton and carbon NMR spectra of the alternative. Additionally, the the predicted ^{15}N chemical shifts for both molecules are also very close, so that an easy distinction seems to be very unlikely.

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. Until now the only possibility seems to be that alt-caffeine should have one 1,1,-ADEQUATE correlation, whereas caffeine does not have any of them.

The ongoing synthesis of the alternative and acquisition of the NMR spectra hopefully will improve our understanding of the problem and possible solutions. Alternatively we are looking into further NMR methods e.a. correlating carbons and nitrogen in natural abundance, for the distinction.

58 Polymer Networks for the Measurement of Anisotropic NMR Parameters

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Anisotropic NMR parameters have gained more and more importance in many fields of analytical organic chemistry and a variety of different alignment media is nowadays available for that purpose.[1] For very reactive compounds like organolithiums, strain induced alignment in a gel (SAG) is the most promising approach. Polystyrene networks represent a rather chemically inert environment and are straightforwardly applicable after only few changes to the original procedure [2] (no initiator, thermal polymerization). Thereby, we were able to prove residual quadrupolar couplings (RQCs) measured for ^{7}Li (I = 3/2) to be a very powerful tool for the discrimination between aggregates with high and low symmetry. The latter show a quadrupolar triplet inside the polymer gel, while ^{7}Li NMR spectra of the former still display a singlet.[3]

While this method works very smoothly for organolithiums and is also applicable to other quadrupolar nuclei, the swelling times required for polystyrene gels are still considerable long (roughly seven days for toluene). Furthermore, organometallic compounds often are part of equilibria[4] or display structural flexibility. Therefore, the temperature range in which gels can be successfully applied comes into focus. We addressed these aspects in different ways:

The temperatures at which quadrupolar splittings can be observed were investigated for polystyrene (PS) and polybutylacrylate (PBA) networks. Furthermore, slice-selective excitation (SSE) was employed to monitor the swelling behaviour of different gels. This is a very efficient technique and enables resolving of the swelling process with spatial resolution. Consequently, the swelling properties of four different polymers sdticks (PS, PS-RAFT, PBA, PBA-RAFT) were studied. The presence of a RAFT (reversible addition fragmentation chain transfer) agent sould result in a more homogeneous and uniform network.[5] First measurements showed interesting and promising results.

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59 Computer Assisted Structure Elucidation: Faster and More Reliable Solutions Through Enhanced Data Preparation

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Computer Assisted Structure Elucidation (CASE) is coming to be a broadly accepted method for the derivation of novel or difficult chemical structures, especially those of natural products, drug metabolites, and synthetic impurities[1,2]. A key impediment to the employment of these techniques has been the preparation of NMR data in a way that is suitable for computer analysis. A novel data entry modality has been developed to prepare NMR data for entry into CASE algorithms. This methodology vastly simplifies the processing required for experimental data, while accommodating multiple different data types and processing strategies. Additionally, the method can readily support data that has overlapping impurities, experimental artifacts, or less-than-optimal signal-to-noise ratios. This presentation illustrates certain capabilities of the software that make such a step forward in the solution of specific problems.

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60 qNMR Targeted Profiling of Food Compounds

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NMR has been widely used for the quantitative analysis of common food components. However, accurate quantifications in complex NMR with numerous overlapping signals is still challenging. Even though various computational procedures have been proposed they generally show limitations in analytical accuracy and/or abilities for integrating in (semi-)automated routines[1-3]. Among the different procedures available, the use of interactive targeted profiling using Chenomx[1,4] is often advantageous. Despite the analysis requires critical inspection and close supervision by the NMR researcher, the procedure generally leads to good analytical precision and satisfactory sample throughput. Since the spectral database in Chenomx is specifically optimized for biofluids, the targeted profiling methodology can however not directly be transferred to food systems[5]. Additional calibration is needed to translate the excising response factors into accurate quantitative food models. We demonstrated that the calibrated quantitative food models agreed with established and certified analytical techniques. Agreement (precision) between the different methods was tested by comparing the estimated concentration levels of common food compounds (sugars, organic acids, amino acids, nucleotides etc.) in different food products and food ingredients. In this test the concentrations were unequally distributed in the range 0-3.5%w/w with 95% confidence interval 1.43%w/w to 1.85%w/w at midpoint of the concentration interval (1.64%w/w). We also demonstrated that accuracy levels reached the 5% acceptance limits of two certified reference materials TraceCERT® Maleic Acid (Fluka) and Standard Reference Material SRM3287 (Blueberry Fruit, NIST). Based on this validation study it was concluded that NMR targeted profiling and the traditional analytical methods can be used interchangeably. To make this targeted profiling approach operational in the analytical laboratory the targeted profiling approach was therefore further adjusted. The method now provides a fully automatic calculation module (including the calibration factors), a Chenomx-LIMS interfacing module (including barcode labelling) and a QC control system (to test accuracy).

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61 Solvent Dynamics: Counting Water Partners in a Protonation-Deprotonation Equilibrium

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The short-timescale rigidity of water around a solute, termed the solvent cage, limits the availability of molecules to undergo chemical reactions and modulates its electron transfer. The properties of the solvent cage have mainly been investigated using femtosecond resolved spectroscopy in the context of electron transfer. There still exists a gap, however, on the effect of this dynamic structure for thermal reactions, which occur on a slower timescale. In this study, we investigate this problem with NMR by focusing on one of the simplest examples of a chemical reaction, a protonation-deprotonation equilibrium. We count the rate at which an O-H bond is broken by doing linewidth analysis on the OH resonance, and then consider what fraction of those bond breaking events lead to exchange by carrying out diffusion experiments, thus being able to calculate the probability of exchange per bond-breaking event. We show different regime, discuss possible scenarios using DFT calculations and illustrate the role of protonation in TiO_2-dopamine hybrids. We believe that this study will serve as a platform for understanding the behavior and role of the solvent in more complex chemical and biological processes.

62 qNMR Approaches for Polar Low Molecular Weight Compounds in Complex Food Samples, a Case Study

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qNMR is a comprehensive application area of NMR that focuses on the quantitative compositional analysis of targeted compounds in solution. Due to the large variety and complexity of food sample compositions, the use of established 1D ^{1}H signal integration of NMR spectra is not directly feasible [1,2], due to severe signal overlap. Several different qNMR approaches are currently available to analyse these food systems. For large sample series accurate, selective, sensitive and automatic quantification is, however, not trivial. To gain selectivity, other NMR techniques such as 2D HSQC [3] or targeted profiling using Chenomx [4] or PERCH [5] are required.

As a typical example, we investigated common polar low molecular weight compounds present in commercially available (tomato based) pasta sauces. In this case study we explored and compared the performance of qNMR techniques in terms of accuracy, selectivity and sensitivity. The 1D ^{1}H NMR spectra of these types of samples consist of many overlapping signals and 1D ^{1}H signal integration leads to incorrect quantification results. To be able to compare the results that we obtained with the different qNMR approaches, calibration models of single compounds were developed for absolute quantification. We demonstrated that 2D HSQC as well as 1D targeted profiling are all selective qNMR approaches, because we can distinguish the different compounds present in the sample, without signal overlap. We concluded that the 2D HSQC approach is less favourable than 1D targeted profiling, because it is less sensitive for measurements at low concentration levels. Targeted profiling using Chenomx or PERCH allowed us to identify and accurately quantify up to 30 different polar low molecular weight compounds (sugars, amino acids, organic acids, nucleotides, alkaloids) typically present in a (tomato based) pasta sauce. The results demonstrated that 1D targeted profiling outperforms the 2D HSQC and 1D ^{1}H NMR quantification in terms of accuracy, selectivity and sensitivity. When analysing large batches of samples with a similar composition, an automatic quantification procedure (e.g. PERCH) is highly preferable. Chenomx is favourable when working with small data sets comprising mixtures with large variations in composition and concentrations, because it benefits from the flexible, interactive fitting procedure. Both Chenomx and PERCH should be used as complementary qNMR techniques to cover the wide range of different food systems and quantification problems.

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63 Configurational Analysis with a Small Number of NOEs – a Test Case

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The determination of the relative and absolute configuration of natural products is essential to understand their biological activity and to allow their procurement through total synthesis. But so far, there is no general method for the secure assignment of the relative or even the absolute configuration of non-crystallizable natural products. The method of choice for small molecules with several stereogenic centers is the combination of distance geometry (DG) and distance bounds driven dynamics (DDD) calculations using NOE/ROE-derived distance restraints (r) [1]. The most important aspect of the NOE/ROE-restrained DG/DDD method (fc-rDG/DDD) is the possibility for the configurations to change during the simulation (floating chirality, fc) [2] and therefore to determine the conformation and the relative configuration of small organic molecules simultaneously.

One of the most investigated complex natural products over the past decade was palau'amine. Since none of the palau'amine congeners have been crystallized, there was a special demand on NMR spectroscopy for this structurally very complex class of natural products. Applications on palau'amine derivatives as well as other dimeric pyrrole-imidazole alkaloids will be presented.

Brown algae of the genus *Cystoseira* are known to produce various linear diterpenes and meroditerpenes. The isolation of several new meroditerpenes from the brown alga *Cystoceira baccata* led to a strong indication for a general revision of the bicyclo[4.3.0]nonane system for compounds of this family. Since the number of NOEs was not sufficient for an unambiguous assignment of the relative configuration by fc-rDG/DDD, residual dipolar couplings (RDC) were used to refine the structures. The absolute configuration of the new compounds was assessed by comparison of the circular dichroism (CD) spectra to the calculated spectra.

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64 Automated Analysis of Polymers using NMR Spectroscopy

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NMR is an established method of analyzing polymers where information such as chain branching, end group analysis and determination of monomer ratios for copolymers is obtained. In most cases, spectroscopists become familiar with identifying resonances, such as distinguishing end groups from the repeating monomer units, and apply their knowledge to evaluate materials produced within their company and for competitor analysis. Analysis is traditionally done by manual interpretation of the NMR spectra. In this study, we worked with a skilled polymer expert to train software to analyze a polymer spectrum in full automation and report the important spectral features that characterize the material. Automated acquisition, processing, analysis and reporting steps will be discussed.

65 Automated ^{1}H NMR Screening of Energy Drinks

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Energy drinks (ED) represent a $9 billion dollar industry in the United states in 2011. Many EDs contain natural product extracts (ginseng, ginkgo, milk thistle, etc.) and vitamins (niacin, pantothenic acid, etc) in their ingredient list and therefore are classifed as dietary supplements. With the 2007 FDA cGMP ruling, manufacturers must ensure identity, purity, strength, and composition of their dietary supplement products. As a result, a demand for new analytical tools to evaluate dietary supplements has become a priority. Here, an automated analytical tool using Nuclear Magnetic Resonance (NMR) spectroscopy called Assure – Raw Material Screening (RMS) is shown. This method conducts a chemical screening analysis of the dietary supplement which involves: 1.) Identification of components through spectral matches, 2.) screening check for any potential unknowns in the mixture, and 3.) absolute quantification using a calibrated NMR spectrometer. An ED method using Assure-RMS is currently being developed. Various chemical compounds in the complex beverage mixture such as sugars, caffeine and herbal extracts such as ginseng using ^{1}H NMR analysis is presented. The results from this study show the potential of using Assure-RMS for qualitative and quantitative assessment of dietary supplements such as EDs.

66 $^{1}H,^{31}P$ UF-HMBC: a Useful Tool for Real Time Monitoring of the Michaelis-Arbuzov Reaction

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The use of the UF-NMR technique, developed by Lucio Frydman and coworkers [1], has revealed a number of new possibilities. Among the most promising of these is the ability to monitor dynamic systems, such as chemical reactions, in real time.

UF-NMR allows the collection of a complete 2D NMR data set within a single continuous acquisition. It is compatible with existing standard pulse sequences (TOCSY, HMQC, HMBC) and a conventional hardware. Recently we have applied this interesting technique to the study of organic reactions in real time [2,3,4].

The Michaelis-Arbuzov rearrangement is one of the most versatile procedures for the formation of C-P bonds and is the traditional method of obtaining phosphonates [5,6]. These are key functional groups in biological and synthetic organic chemistry, especially in C-C bond formation [7,8]. Some mechanistic possibilities have been described, but there is a lack of knowledge about the formation and nature of the intermediates participating in the reaction. Once again, UF-NMR offers the possibility of studying the details of the process. According to this, the Michaelis-Arbuzov reaction between benzyl bromide and triethylphosphite in the presence of zinc bromide was monitored in real time by heteronuclear ultrafast HMBC ^{1}H, ^{31}P. We have proven the formation of a phosphonium salt intermediate as has been proposed in the literature. For the first time a dynamic study with ^{31}P was carried out, extending the possibilities for monitoring organic reactions.

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67 Solvation of Carbohydrates Isomers as Studied by DOSY NMR Spectroscopy

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The "shape" of the molecules depends, among other factors, on their interactions with surrounding molecules such as solvent and/or other solute molecules. In this context, carbohydrates are a very interesting group of biomolecules with a characteristic pattern of hydroxyl groups along their skeleton that are involved in both intra- and intermolecular hydrogen bonding and, as consequence, they might exhibit a larger "apparent" hydrodynamic radius than the expected from their plain structure. [1,2]

COSY-NMR spectroscopy is best known as a very well suited NMR technique to achieve "virtual" separation of the components of a mixture when the molecular weight difference between the species in solution is significant. However, molecules with identical molecular weight but different shape may exhibit also different diffusion coefficients. [1] Recently, the separation of isomers by using either a specific solvent matrix or solvent mixtures has been described. [3]

We have investigated the solvation properties of a set of carbohydrates isomers in different solvents by acquiring DOSY NMR spectra of selected binary isomers mixtures and comparing the resulting diffusion coefficients of the components of the mixture. Despite the structural and chemical similarities of the studied carbohydrates isomers, we could observe, in some cases, an unexpected but clear separation of the isomers what seems to correlate with a distinctive mode of interaction between the individual solutes and the solvent.

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68 UGM Enzymatic Inhibitors: Enhanced Affinity Through Polyfluorination Studied by NMR

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The introduction of fluorine atoms in organic molecules is a strategy followed by the pharmaceutical industry in the development of new bioactive compounds [1]. The presence of fluorine brings about unexpected properties [2] that have been the subject of different controversies such as its hydrogen bond acceptor ability [3] or the so called polar hydrophobicity [4].

In this work, we have studied the effect of the tetrafluorination of a UDP-galactopyranose mutase (UGM) inhibitor through NMR.

UGM is the enzyme in charge of catalyzing the isomerization of UPD-Galacto*pyranose* into UDP-Galacto*furanose*, which it is later used in the formation of the mycobacterial cell wall of different pathogens, including *M. tuberculosis* [5]. Thus, the development of UGM inhibitors is a topic of major interest [6].

We have developed a new inhibitor by introducing CF_2 groups in positions 2 and 3 of the galactofuranose ring of the natural enzymatic product (UDPGal*f*). STD-NMR experiments have allowed to map the epitope for the interaction with the enzyme of the new polifluorinated ligand, showing an increased STD effect on the protons of the galactofuranose ring with respect to the non-fluorinated one. Besides, STD-NMR competition experiments demonstrate the better binding affinity of the polifluorinated analogue with respect to the non-fluorinated one. These results could be a consequence of the polar hydrophobicity effect generated by the *gem*-polifluorination.

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69 Differential Metabolic Response to Salinity and Silicon of Tomato Varieties

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Increasing salinity levels affect negatively tomato plant growth and fruit yield and the incidence of blossom end rot is much higher. On the contrary, salinity improves inner quality of tomato characterized by taste and health-promoting compounds. Among the several strategies used to overcome the deleterious effects of salinity on plants the relevance of silicon has been recently recognized [1].

In this work, the approach of metabolic profile using HRMAS NMR was applied to compare the effect of salinity and silicon treatments separately and combined on the chemical composition of three tomato varieties. Tomatoes were grown under natural greenhouse conditions in Almería (Spain) and the varieties chosen (Raf, Delizia and Tigre) belong to the so called "flavor varieties". An important characteristic for this type of tomatoes is their relative high salt-tolerance. Thus, whereas for most of the conventional varieties irrigation with an electrical conductivity (EC) higher than 3.5 dSm^{-1} is hardly tolerable, the standard EC for flavor varieties is 4 dSm^{-1}. The experimental design consisted of a control (***C***) and three treatments: ***EC*** (tomatoes grown under electrical conductivity of 5.5 dSm^{-1}. The increase in conductivity was achieved by addition of potassium sulfate); ***Si*** (plants treated with SILIFORCE®) and ***ECSi*** (nutrition with 5.5 dSm^{-1} plus addition of SILIFORCE®).

A total of 480 fruits (ten fruits per day/variety/treatment) were hand-picked at the stage commonly marketed during the months of December of 2010 (2^{sd} and 16^{th}) and January of 2011 (17^{th} and 31^{th}). ^1H HRMAS NMR analysis was performed on tomato purée samples that were prepared form fresh tomatoes (one fruit per sample). Spectra were recorded on a Bruker Avance500 spectrometer working at a frequency of 500.13 MHz. The MAS rate used was 4600 Hz and128 scans of 36 K data points were acquired at 298 K. The strong water signal was suppressed by using a 1D gNOESY presaturation scheme.

Evaluation of the relative amount of individual metabolites through the integration of selected signals showed both the effect of the harvest day and treatments which were, in turn, function of variety and harvest day. For all the studied varieties, tomatoes harvest in January showed a higher (low) amount of sugars (citric acid) than those harvest in December. Salinity increases the sugar content for fruits of *cv*. Delizia and particularly *cv*. Raf but only for tomatoes harvest in December. On the contrary, no differences were found between control and salt-treated fruits belong to Tigre variety. This variety was, however, the most affected by silicon treatment. Independently of the harvest date, silicon originated a significant increase in the relative content of citric acid of fruits belonging to *cv*. Tigre. Concerning the

effect of the combined treatment ECSi, the observed changes did not differ significantly from those found when the two treatments (EC and Si) were applied separately.

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70 Rapid Pulsing Artefacts in Pulsed-Field Gradient DQF-COSY Spectra

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Double Quantum Filtered Correlation Spectroscopy (DQF-COSY) has significant advantages over conventional Correlation Spectroscopy (COSY) for proton-proton correlation. However, DQF-COSY spectra are prone to artefacts, even when pulse-field gradients (PFGs) are used for coherence selection, so COSY is still commonly used whenever experiment duration needs to be minimized. Over 20 years ago, Derome and Williamson [1] showed that significant artefacts in phase-cycled DQF-COSY resulted from retention of magnetization between transients ("rapid pulsing" artefacts). These artefacts make it impractical to acquire DQF-COSY spectra with very short relaxation delays. This paper demonstrates that a similar mechanism generates artefacts in PFG DQF-COSY spectra, and that the artefacts can be suppressed by modifying the intensities of z-axis PFGs [2] or by modifying the acquisition order in the indirect evolution domain [3]. These are preferable to purge pulses [4] which destroy signal. Using these methods, high-resolution DQF-COSY spectra can be obtained in less than 3 minutes.

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71 Magnetization Transfer Study on the Exchange Reaction of [Rh(N,O-Bid)(CO)(PPh_3)] Complexes with PPh_3

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Chemical exchange reactions between free ligands and ligands coordinated to a metal centre fulfil a fundamental role in quantifying catalytic processes and synthetic routes. Magnetization transfer is an NMR experiment in which one signal (e.g. the metal complex signal) in an equilibrium mixture is selectively "labeled" by selective excitation at that single frequency, without the disturbance of the neighbouring signals. While this spin relaxes during a mixing time, a chemical exchange process converts the labeled spin to a signal at different chemical shift (e.g. that of the free ligand). By following the rate at which the magnetic equilibrium is restored, the kinetics of the exchange reaction can be determined [1,2].

Considering the lack of information regarding phosphine exchange in rhodium complexes, the exchange of free triphenylphosphine with triphenylphosphine coordinated in complexes of the type [Rh(N,O-Bid)(CO)(PPh_3)] was studied. Two complexes were chosen as study subjects: [Rh(2,6-Cl_2-Phony)(CO)(PPh_3)] and [Rh(2,6-Me_2-Phony)(CO)(PPh_3)]. This allowed for the comparison of the electronic effect of the substituents on the phenyl rings, since the two complexes display similar steric properties while chloride substituents are electron withdrawing and methyl groups are electron donating.

The magnetization transfer experiments were done at varying free triphenylphospine concentrations and at a range of temperatures, while the concentration of the Rh complex was kept konstant. It was found that the rate of reaction, k_1, for the phosphine exchange in [Rh(2,6-Cl_2-Phony)(CO)(PPh_3)] is approximately three times faster than that of [Rh(2,6-Me_2-Phony)(CO)(PPh_3)]. This can be explained by the electron donating properties of the methyl substituents, which increases the electron density of the *d*-orbitals of the rhodium atom and is therefore stabilizing the five-coordinate trigonal bipiramidal intermediate. The decreased electron density surrounding the rhodium atom in [Rh(2,6-Cl_2-Phony)(CO)(PPh_3)] also allows for the apparent more efficient reversal of the reaction as indicated by k_{-1} values which was also calculated from the exchange reactions. This value is virtually absent in the reaction of the [Rh(2,6-Me_2-Phony)(CO)(PPh_3)] complex.

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72 NMR, Infrared and Theoretical Studies on Conformational Preferences of L-Aspartic Acid Dimethyl Ester

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The L-aspartic acid (Asp) has been extensively studied by both experimental and theoretical approaches due to its importance to the human beings [1]. Nevertheless, as it exhibits a zwitterionic form in solution, its study has been almost limited to the gas phase [2,3]. An alternative approach to supersede this problem is to analyse its ester derivative, not explored yet. Thus, this study is aimed to investigate the conformational behavior of L-aspartic acid dimethyl ester ($AspOCH_3$) in the isolated phase as in several solvents to understand the interactions that rule its behavior by NMR and infrared spectroscopies combined with theoretical calculations.

Experimental hydrogen vicinal coupling constants $^3J_{HH}$ were obtained in solvents of different polarities, but they showed no significant changes. These constants were also calculated for each conformer in solution using the IEFPCM model at the ωB97XD/aug-cc-pVTZ and B3LYP/EPR-III levels for N and O atoms and for H and C atoms, respectively, presenting excellent agreement with experimental values and confirming that there are no changes in conformer populations on changing the solvent. The infrared (IR) carbonyl stretching band in CH_2Cl_2, $CHCl_3$ and CH_3CN also confirmed those results.

In order to determine the $AspOCH_3$ conformers, tridimensional potential energy surfaces were built by scanning the (O)C-C-C-C(O) and O=C-C-C dihedral angles which revealed 17 minima. However, a selection of the lower energy geometries (E_{rel}<1 kcal mol^{-1}) led to 8 conformers at ωB97XD/aug-cc-pVTZ, whose the most stable (31%) is the *gauche* taking into account the N-C-C=O dihedral angle of main chain. Its greater stability can be explained by the Quantum Theory of Atoms in Molecules (QTAIM) and Natural Bond Orbital (NBO) calculations. They showed that a balance between steric and hyperconjugative interactions as well as the presence of an intramolecular hydrogen bond are the main effects responsible for its lower energy. These results show how powerful is the NMR spectroscopy for the study of aminoesters conformational equilibria, giving results in excellent agreement with IR data and theoretical calculations.

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73 Solid State Fluorine NMR Studies on Bioactive Compounds

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Since the biological activity of a compound appears to be enhanced due to fluorine substitution [1], in the last few years ^{19}F NMR in solution has proved to be a very valuable tool to run structural studies in pharmaceutical and agrochemical research [2]. However many fewer examples are encountered in the literature devoted to ^{19}F solid state NMR [3], as wide-bore NMR spectrometers with a probe that will allow observation of fluorine nuclei are uncommon.

The recent implementation of our Bruker WB 400 spectrometer with a 2.5 X/F/H probe, which allows observation of fluorine nuclei in systems dilute in ^{19}F giving the possibility to adquire on ^{19}F at fast MAS while simultaneously applying ^{1}H decoupling, permitted us to approach the solid state study of several phenomena on a variety of fluorinated compounds ranging from curcumin related analogues [4] to benzimidazoles and benzodiazepinones for treatment of several pathologies [5], with satisfactory results.

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74 Constant Time Gradient CRISIS HMBC (CTgc2HMBC) – Quantifying Improvements in SNR and Resolution

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Since its introduction, the two-dimensional heteronuclear multiple bond correlation (HMBC) experiment has rapidly become the standard way of detecting the presence of long-range (two- and three bond), proton-carbon couplings in small- to medium- size molecules.[1,2] Though widely used, the HMBC experiment suffers from ^{1}H-^{1}H *J*-modulation during t_1, which lowers sensitivity (SNR) and resolution in F1 - limiting the amount of information which can be extracted from these spectra in some cases.[2]

In order to quantify improvements to the HMBC experiment we designed a constant time gradient HMBC experiment with adiabatic pulses to remove ^{1}H-^{1}H modulation. We compare its performance against comparable non-constant time experiments. This experiment termed CTgc2HMBC gave better peak separation, signal-to-noise ratio and resolution than normal HMBC experiment at both low and high F1 resolution.

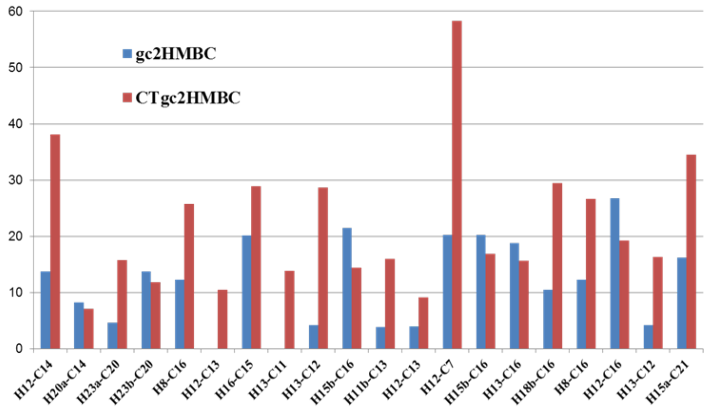

Figure 1: SNR for the weakest 20 gc2HMBC correlations (blue) for strychnine compared to their SNR in CTgc2HMBC (red)

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75 Understanding the Diastereoselectivity of the Directed *Ortho*-Lithiation of Phosphinic Amides

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During the last years we have been developping synthetic applications based on phosphorus-bearing lithium organyls. The reactive intermediates involved in the transformations include $C_{\square}$, $NC_{\square}$, C_{ortho} *and* $C_{\square}C_{ortho}$ phosphinic acid derivatives [1,2]. Electrophilic trapping of these species proceed more often than not with high diastereo- or enantioselectivities and provide easy access to multidentate hemilabile ligands which are being explored in asymmetric catalysis. The stereocontrol observed in these processes find the roots in the lithiation step. Understanding why they occur with such topicities is not always easy to ascertain. Here we report one successful example of this issue, the identification of the origin of the diastereoselectivity in the *ortho*-lithiation of (*S*)-*N*-(3,3-dimethylbutan-2-yl)-*P,P*-diphenylphosphinic amide using multinuclear magnetic resonance techniques.

The powerful combination of standard NMR methods together with bidimensional nOe, ^{7}Li,^{31}P- and ^{7}Li,^{15}N-HMQC experiments helped us to unravel the structure of the lithium organyls formed by treating the substrate with one equiv of ^{n}BuLi in THF-d_8 and explains the results obtained in the laboratory scale reactions. ^{1}H, ^{7}Li, ^{7}Li{^{31}P} and ^{31}P NMR spectra as a function of temperature and concentration were also acquired in order to obtain critical coupling constants and determining the aggregation state of the existing species. We found that the lithiated species exist in a monomer-dimer equilibrium. Furthermore, incomplete lithiation leads to the formation of a mixed aggregate between the dimer and the starting material as deduced from pattern of signals observed in both ^{7}Li- and ^{31}P-NMR spectra.

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76 Structural Characterization of Carbazeran 3-N-Glucuronide

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Carbazeran (CBZ) is a phthalazine based phosphodiesterase inhibitor that was developed in the 1980s as a potential treatment for congestive heart failure (1, 2). During preclinical trials with rat and dog the compound exhibited both promising pharmacology and pharmacokinetic properties. However during clinical trials, human exposure to the compound was significantly lower than expected. Further work demonstrated this lack of exposure was due to aldehyde oxidase (AO) metabolism. More recently CBZ has been used as part of a "yard stick" model to evaluate the activity of AO toward discovery compounds (3). As part of an ongoing study to understand the metabolic rate and fate of CBZ in various species and matrixes, a new human metabolite with a molecular weight consistent with an N-glucuronide was observed in in vivo incubations (4). CBZ contains four nitrogen atoms, three of which may be biologically susceptible to AO metabolism. LC/MS and LC/MS/MS data could not provide insight to the position of conjugation. In order to establish the site of metabolism an isolate of the glucuronide was prepared from human liver microsomes augmented with CBZ and uridine 5'-diphospho-glucuronic acid. The resulting metabolite was isolated via preparative chromatography and analyzed by 1D (1H and NOE) and 2D NMR (COSY, HSQC, HMBC) techniques. All data sets that were acquired are consistent with the structure of the metabolite being the carbazeran 3-N-glucuronide.

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77 Unusual 2(*1H*)-Pyrazinones from a Brazilian *Streptomyces sp*. Revealed by LC-UV-SPE-NMR

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For new secondary metabolites were isolated from ethyl acetate extract of a culture of a brazilian marine-derived *Streptomyces* strain designated SS99BA-2. This extract presented biological activity against *Escherichia coli*, *Candida albicans* and also glyoblastome SF295 and human colon HCT-8 tumor cells and could therefore represent a source of potential candidates for new drugs. Analysis of this extract revealed the presence of four novel metabolites, whose structures were fully elucidated by NMR spectroscopy and ultrahigh-resolution mass spectrometry. Chemical analysis was completely conducted in a coupled automated LC-UV-SPE system with the use of a cryogenic NMR probehead (Bruker Avance III instrument, 14.1 Tesla / 600 MHz, equipped with an automatic sample changer and a TCI 5 mm triple resonance ($^{1}H/^{13}C/^{15}N$) z-field gradient cryo-probe) and HRMS. The structural feature of these new compounds is the presence of a completely substituted 2(1*H*)-pyrazinone ring skeleton, which is relatively rare in nature. Others reseachers have shown how these microrganisms produce this class of secondary metabolites by coupling two diferent amino acids[**1**, **2**, **3**, **4**]. When it comes to the compounds found in the present work, it seems that they follow the same amino acid coupling, but followed by condensation with malonyl-CoA at C-5 position of the ring system.

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78 Dilithiated 1,1'-Methylene-Bis-Imidazolethiones. Polymer or Discrete Species in Solution?

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Methylene bridge imidazole-2-thiones are interesting building blocks for the synthesis of multidentate ligands towards metal complexation. These metal-based complexes have shown catalytic activity in for instance polymerization of norbornene. Very recently, we have been interested in their reactivity towards organometallic reagents and main group element compounds in order to disclose not only structure and bonding modes but also to understand how these species react against different nature electrophiles.

We report herein the characterization of dilithiated methylene-bis-(imidazolyl-2-thione) (**2**) in solution and in the solid state. The powerful combination of standard NMR methods together with PGSE NMR, homo and heteronuclear nOe experiments helped us to unravel its structure. ^{1}H, ^{7}Li, and ^{13}C NMR spectra as a function of temperature and concentration were also acquired in order to obtain critical coupling constants, determining the aggregation state and the strength of ion pairing of species **2** in solution. These studies showed that in THF solution the dianion exists as a monomeric contact ion pair (CIP) with a $J(^{13}C,^{7}Li)$ of 35.9 Hz. On the contrary, X-ray diffraction analysis revealed a very different structure not comparable to the one found in solution.

79 Automated Structure Verification: How Does it Compare to a Chemist?

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Automated structure verification (ASV) software [1,2] has the potential to revolutionise the use of NMR by automating some of the more rouine aspects of NMR interpretation. Software has been in development for a number of years, but progress has been slow and ASV has yet to gain wide acceptance.

We report the results of a company-wide evaluation designed more to assess the potential of ASV as a general technique than to compare individual software. In particular, we have baselined the results of ASV with what a typical chemist can achieve given the same set of (limited) data. We find that on average, taken across a range of molecules, that ASV can perform about as well as a human. Considering individual molecules, in some cases ASV could solve a problem where the human failed, and in others the ASV failed on molecules that the chemist found straightforward.

Assuming vendors improve the software to solve the “easy” problems which ASV currently finds difficult, we can forsee a future where ASV could perform better than a human given the same data. Nevertheless, philisophical and practical issues remain that affect the use of ASV in a high-throughput environment, and some of these will be discussed.

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80 Reducing Convection Effects in DOSY with Triax Gradients

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Convection affects many solution NMR experiments, but is often neglected because it does not influence interpretation, just resulting in some loss of sensitivity. However Diffusion-Ordered SpectroscopY (DOSY) is very sensitive to convection, which can easily cause severe problems. A wide variety of pulse sequences have been developed to compensate for the effects of convection [1,2], but these methods can be very time-consuming due to their long phase cycles and reduced sensitivity compared to normal DOSY experiments. An alternative solution is presented here that relies on diffusion encoding/decoding in the transverse plane, using the X- and/or Y- instead of the Z-gradient coils of a triple axis gradient probe. Advantages and disadvantages of using the XY-gradient coils are discussed in detail, based on experimental comparison with currently used Z-gradient encoding methods.

Convection is reduced in a capillary because the smaller diameter of the tube greatly increases the threshold vertical temperature gradient for convection, and decreases transverse temperature gradients. Experiments show that convection in typical NMR experiments is often instigated by the latter, not the former as is usually assumed, but may be driven by both types of temperature gradient, leading predominantly to vertical motion in the active sample volume, with the result that Z-encoded experiments are much more vulnerable to convection effects than X/Y. A set of small molecules were chosen to compare some DOSY methods systematically, with and without convection compensation, using diffusion encoding/decoding along any of the three directions. The results suggest that DOSY experiments can take advantage of triple gradient probes by using XY-gradients for diffusion encoding/decoding, with the stronger Z-gradient only used for coherence transfer pathway selection and homospoil purposes.

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81 Interaction Studies of Human Laforin with Oligosaccharides by NMR, MST and ITC

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Laforin is a unique human dual-specificity phosphatase as it contains an amino terminal carbohydrate binding module (CBM) and a carboxyl terminal phosphatase domain containing a HCXXGXXR(S/T) catalytic active site motif [1]. Laforin mutations have been associated with Lafora disease, an early onset fatal progressive myoclonus epilepsy with autosomal recessive inheritance. The laforin CBM carbohydrate binding activity has been poorly characterized. We describe here our studies of the interaction of recombinant human Laforin [2] with a series of oligosaccharides using a variety of techniques. The Laforin oligosaccharide binding preferences were first screened by Microscale Thermophoresis (MST) using soluble oligosaccharides of increasing length, which has shown an increased preference of laforin for longer oligosaccharides (both linear and circular), with the highest affinity at pH 7.5. The affinity constant of Laforin to linear oligosaccharides decreased systematically from K_d = 2700 μM for maltotriose to K_d = 173 μM, while for the cyclodextrins K_d varied from 841 μM for α-CD to 114 μM for γ-CD. The Laforin affinity constants obtained for γ-CD and maltoheptaose) by Isothermal Titration Calorimetry (ITC) titrations compare favorably with the above results. The thermodynamic parameters determined by ITC were also consistent with other protein-carbohydrate interactions. Finally we assessed ligand binding of γ-CD and maltoheptose using STD [3] and CPMG NMR techniques [4]. Both compounds were detected unambiguously on the two ligand-based NMR experiments. Although various resonance overlaps are observed for this kind of molecules, a group epitope mapping (GEM) from the STD-NMR experiment discloses the most likely binding profile for these sugars.

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82 RDC Based Conformational Analysis of Melohenine-B and O-Ethyl-14-epimelohenine-B

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The alkaloids melohenine B (**1**) and O-ethyl-14-epimelohenine B (**2**) have been isolated from *Melodinus henryi*,[1,2] a cane used to treat meningitis and bone injuries in Burma, Thailand and China. O-Ethyl-14-epimelohenine B is reported to display moderate cytotoxic activity.[2] Both compounds were synthetised and subjected to thorough structural analysis.

According to single crystal diffraction **1** and **2** adopt identical conformations in the solid state. Nevertheless considerable differences in ^{1}H chemical shifts of some methylene protons are observed in $CDCl_3$ solution. Since DFT calculations imply that the effect of C-14 substitution and stereochemistry on ^{1}H chemical shifts of these methylene protons is negligible we assume that **1** and **2** have certain degree of conformational flexibility and therefore they might adopt different conformations in solution then in the solid state.

In order to confirm this hypothesis, samples of **1** and **2** were oriented in a lyotropic liquid crystalline matrix of poly-benzyl-L-glutamate (PBLG) and two sets of 10 and 12 $^{1}D_{CH}$ RDCs were obtained using ^{1}H,^{13}C-CLIP-GSQC and ^{1}H,^{13}C-G-BIRD-HSQC experiments [3,4]. Conformational flexibility was assessed by semi-empirical RM1 and B3LYP/6-31G** methods and for each compound four conformations with relative energies below the threshold of 10 kJ/mol were found and subjected to RDC analysis based on aligment tensor computation using SVD methodology.[5,6]

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83 Differences on the NMR Parameters of the 1,2-Dihaloethenes Isomers

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Coupling constants and chemical shifts are strongly dependents on the electronic structure and stereoelectronic interactions. These interactions may explain many behaviors observed in NMR, mainly on the components of shielding tensor and indirect spin-spin coupling [1]. An example is the heavy atom effect on ^{13}C chemical shift, where the stereoelectronic interactions may affect the Spin-Orbit (SO) component [2]. In this work we studied how these interactions affect the ^{13}C chemical shift and coupling constants $^{2}J_{CH}$ in *cis*- and *trans*-1,2-dihaloethenes. The experimental $^{2}J_{CH}$ coupling constant and ^{13}C chemical shifts were obtained for a solution of 10 mg of solutes in 0.7 mL of $CDCl_3$, chemical shifts were referenced to TMS. Theoretical calculations of chemical shifts and NBO electronic interactions were performed with ADF package at KT2/TZ2P level of theory and ZORA-SC and ZORA-SO Hamiltonian, while theoretical calculations of the coupling constants were achieved using Dalton program at the SOPPA(CCSD) level and EPR-III basis set for C, F and H atoms, cc-pVTZ basis set for Cl and Br atoms and 6-311G* for I atom.

The shielding effect was more pronounced in *trans* than *cis* isomer for bromine and iodine compounds. The chemical shifts in 1,2-diiodoethene were 80.4 ppm for *trans* and 97.8 ppm for the *cis* isomers. The SO and paramagnetic components of shielding tensor were used to assess the chemical shift differences between the two isomers. The NBO results show that there are a lower lone pairs occupancy of iodine derivatives and higher occupancy in $\pi^*_{C=C}$ orbital, indicating that the hyperconjugation is greater for *cis* isomer, leading to lower SO term (25.7 ppm) in *cis* than *trans* isomer (35.9 ppm). This behavior could be due to steric repulsions between halogen atoms in the *cis* isomer. The $^{2}J_{CH}$ coupling constants increase as electronegative of the substituents increase as was observed for 1-haloethenes [3,4]. However, for 1,2-dihaloethenes the Fermi Contact (FC) is twice higher in comparison to 1-haloethenes, showing addictive effect on coupling constants in the *cis* isomers (for chlorine $^{2}J_{cis}$=+15.78 Hz, bromide $^{2}J_{cis}$=+14.76 Hz and iodine $^{2}J_{cis}$=+10.53 Hz), while for *trans* isomers $^{2}J_{CH}$ coupling are near to 0.0 Hz.

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84 Quality Control of Compound Libraries

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Partly due to the current outsourcing efforts in pharma industry, there is a significantly growing demand for a more accurate characterization of the contents of lead discovery substance libraries of small molecules.

In order to reduce the ratio of false positive screening results already very early in the drug discovery process, often the quality of the compounds validated prior to their distribution to the biological screening. We show a setup where the quality assurance process is based on automated NMR techniques which may be combined with LC-MS to confirm the sample identity, purity, and compound concentration along with the water content of the H-DMSO screening solutions.

NMR has traditionally been the preferred tool for sample quality control with the highest information content. The NMR spectrum provides the analyst with information on the structural integrity of the compound. But NMR also provides inherent information on the concentration of the compound in solution and it supplies additional information on the purity, orthogonal to other methods such as optical HPLC detectors. In the same way, NMR can also be used to determine the water content on a sample in a solvent, which serves as an important information for a quality factor in pharma industry compound management facilities.

NMR has not been used until very recently as the standard quality assurance tool for all these analytical tasks since the data analysis had to be done from scratch and completely manually on each dataset. We present an integrated and flexible workflow to automatically setup, measure and analyze even large batches of compounds.

We also present the latest developments to support the spectroscopists with the analytical tasks associated with this increased amount of data. We will show details on the workflow implementation in a typical industry environment which can be seamlessly combined with information derived from different analytical tools such as LC-MS data-analysis, for maximum confidence on the resulting quality factors.

85 Guanidine Derivatives – H-Bond Networks and Conformational Preferences with Artificial Receptors Elucidated by NMR

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Guanidine derivatives, like acyl-, carbamoyl-, sulfonylguanidines, which act as less basic isosters of guanidines, have numerous applications in the field of organic and medicinal chemistry. [1, 2, 3, 4] Their ability to form strong fork-like hydrogen bonds enables them to take part in various molecular recognition processes. [5] Especially in the field of G-protein coupled receptors (GPCRs) guanidine derivatives are among the most pronounced ligands. [1, 2, 3, 4] Despite the important role of hydrogen bonding networks in molecular binding events of these membrane receptors, the detailed understanding of hydrogen bonding on a molecular level is often restricted, because direct studies of ligand-receptor interactions, via high resolution NMR spectroscopy in solution, are either very time consuming or very challenging.[6, 7, 8]

Very successful and significant regarding the elucidation of binding modes and conformational preferences of the ligand class acylguanidines has been the approach of our group employing trans hydrogen bond scalar couplings between artificial receptors and therefore low temperature measurements in aprotic solvents. These investigations gave insight into the H-bonding networks and the binding geometry of these pharmacologically important guanidine derivatives. [9, 10] Now we have extended our approach to carbamoyl- and sulfonylguanidines and studied their interplay with anions and artificial receptors in aprotic solvents, including freons and furthermore investigated the dependence of their conformational preferences on these interaction patterns.

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86 NMR Assignment of Neomycin and Differentiation of Neomycin B and C Forms

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Aminoglycosides are important antibiotics used in the treatment of many serious bacterial infections. Paromomycin (PA) or Amynosidin is an aminoglycoside which has not only antibacterial activity but also anti-protozoan. It's a powerful agent for the treatment of infections, for example, *Leishmaniosis* and *Tuberculosis*. Neomycin shows antibacterial activity, especially with mycobacteria, but not against fungi. It is used in the treatment of skin sores, but if applied in excess, it can cause allergic dermatitis [1,2]. PA is produced by *Streptomyces riomosus var. Paramomycinus* and Neomycin is produced by *Streptomyces fradiae*, and both aminoglycosides inhibit gram-positive and gram-negative bacteria [1,3].

As all family members of Neomycin, this molecule is chemically composed of a cyclohexane, which is linked to a pyran ring and a furan ring by the anomeric hydroxyl, and in turn, the furan ring is linked to the anomeric hydroxyl of the pyran ring. PA does not have a defined NMR assignment or x-ray structure. But, REID & GAJJAR (1987) have published an assignment for Neomycin B, a molecule very similar to PA.

Due to the pharmacological potential, this study aims to use ^{1}H and ^{13}C 1-D and 2-D techniques of Nuclear Magnetic Resonance spectroscopy (NMR) for the complete assignment of Neomycin, in an attempt to perform a 3D structure elucidation in the end, differentiating the axial and equatorial proton positions of the furan ring, which is responsible for the differentiation of the B and C form of Neomycin. Our assignment of Neomycin B will be assisted by a revisited assignment published in the literature in 1987. We have decided to revisit that assignment because of bogus carbon assignments in the original publication. Following the assignment we can do the determination of the stereochemistry using Residual Dipolar Couplings (RDCs), in which case we use stretched gel samples and different methods of interpretation [4,5].

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87 Serine and Threonine Methyl Esters: Conformational Analysis and Relative Stabilities

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The conformational equilibrium of some amino acids and their derivatives have been recently investigated [1-3]. It was found that ubiquitous steric and hyperconjugative interactions, and not H-bonding, are responsible for conformational preferences of these compounds.

The side chain is of the special importance for the conformational equilibrium of amino acids. Serine an threonine exhibit complex side chains due the presence of polar functional group –OH that dramatically increases the number of low-energy conformers. Indeed, the presence of the methyl group in the substitution of the one hydrogen atom present in serine, may increase the contribution of the steric effect on threonine and thus, these systems can be used as probe for investigate intramolecular interactions in biomolecules. However, it is well-known that amino acids in the gaseous phase adopt the nonionic form far from *in situ* conditions of the condensed medium in which biological reactions take place. Moreover, in aqueous solution and in cristals, these compounds present zwitterionic structure, i.e, a bipolar form of the type (H_3N^+-CHRCOO$^-$), which does not occur in the polipeptide chain. In this work, we propose the conformational analysis of serine (Ser-OMe) and threonine (Thr-OMe) methyl esters, that do not show zwitterionic forms in solution, by ^{1}H NMR and theoretical calculation. The minima of energy of these systems were obtained by three-dimensional potencial energy surfaces built at the B3LYP/cc-pVDZ theoretical level by scannning both the χ_1 [N-C-C-O(H)] and χ_2 [C-C-O-H] dihedral angles while the other angles were previously optimized for the alanine methyl ester [3]. There were obtained 27 and 41 conformers for Ser-OMe and Thr-OMe, respectively. Subsequently, each conformer was optimized (B3LYP/aug-cc-pVDZ) including solvent effects (acetonitrile, pyridine, methanol and DMSO) through the CPCM solvatation model. Then, $^3J_{H\alpha H\beta}$ spin-spin coupling constant calculations were carried out at the B3LYP method and EPR-III(for C and H) and aug-cc-pVDZ (for O and N) basis sets. The calculated values are in agreement with the experimental data and show that $^3J_{H\alpha H\beta}$ obtained in diferent solvents do not show changes with their polarity indicating that there are no changes in the conformer populations in the equilibrium relative to the side chain. It was found that the presence of methyl group have marked importance on the conformational preference of relatively stable conformers. In addition, in order to understand the effefts that rule the conformational equilibrium of these systems, NBO (Natural Bond Orbital) and QTAIM (Quantum theory Atoms In Molecules) were employed. The results show that steric and hyperconjugative effects together with H-bonding are the interactions responsible for conformational preferences of Ser-OMe and Thr-OMe.

This work has been carried out with financial aid from Fapesp

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88 A Comparative Study of Some NMR Experiments Used for Measuring Heteronuclear Coupling Constants in Small Molecules

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A significant number of NMR experiments have been proposed for measuring heteronuclear coupling constants in molecules. Long–range coupling constants ($^{n}J_{XH}$, where n > 1) are invaluable in structural and conformational analysis of molecules because of their relatively high abundance compared to that of proton – proton coupling of the same molecule as well as the relationship between three bond coupling constants ($^{3}J_{XH}$ in this case) and dihedral angles (Karplus relation). Some of these experiments are limited by requiring laborious post–processing line fitting procedures and/or give coupling constants between protons and protonated centres only (e. g. CPMG – HSQMBC, HSQC – TOCSY, and 1D – TOCSY). However, some recent experimental methods (namely EXSIDE and IPAP-HSQMBC) circumvent these issues and allow straight-forward readout of $^{n}J_{XH}$ directly from in-phase 2D spectra. This study will compare these latter methods for relative ease of use, speed and sensitivity. It is noted, that the purpose of this investigation is not to establish superiority of one experiment over the other, but to provide adequate data/information that will enable the user make appropriate choice of experiment to use based on the range of values of coupling sought and/or the quantity/amount of sample available.

89 Application of a 60 MHz Permanent Magnet NMR System to Online NMR Reaction Development in the Pharmaceutical Industry

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The use of online NMR technology in the development of organic reaction processes provides information rich data which can be used to gain a deeper understanding of the process under investigation. This technique has been employed in process development at Pfizer for a number of years, predominantly in the development stage of pharmaceutical research. Much of this work has been conducted at high field (400 MHz). The utilization of a compact and portable 60 MHz instrument provides increased flexibility and cost benefits over traditional cryogenically-cooled super-conducting magnets. These advantages allow the analysis to be performed at the location where the chemistry is being conducted, rather than bringing the chemistry to the lab space specifically designed for online NMR.

The utility of the instrument will be demonstrated with comparative examples of reaction processes that have been monitored by the 60 MHz permanent magnet NMR in parallel with our high field reaction monitoring system.

90 Application of DOSY for Organogel Observation

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Low-molecular-weight gelators (LMWG) are due to many potential applications like photovoltaics, templates for making nanoparticles and nanoclusters, controlled drug release and others very popular in recent years. Gelator molecules can self-assemble to form a 3D supramolecular network structures in which the solvent is trapped via various noncovalent interactions as hydrogen bonding, dipole-dipole interaction, host-guest interaction and other. The general rules for gels are: a) continuous structure with macroscopic dimensions that is permanent and b) is solid like in the rheological behavior. Our long term interest to find alternative compounds to the toxic and persistent insecticides has been aimed to the synthesis of molecules with low toxicity and easy biodegradation. Generally structure assumed conjugates of steroids, phytosterols or triterpenes with carboxylic acids or their derivatives [1,2,3].

LMWGs have been studied by UV-VIS, SEM, TEM, XRD, AFM or IR so far. The NMR spectroscopy has been applied only as alternative method mainly for structure confirmation and the effect of line broadening was used as another evidence for gel formation. We are presenting a new application of NMR spectroscopy in study of gels. Different concentration/temperature dependence of diffusion coefficient measured by DOSY (**D**iffusion **O**rdered **S**pectroscop**Y**) in case of molecules with and without gelation properties was observed. As comparative method to NMR spectroscopy the AFM was used. Several aspects of this approach be discussed and presented.

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91 COSMOS Program: A Promising RDC Evaluation Tool from Rigid to Flexible Organic Molecules

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The program COSMOS uses molecular dynamics simulations with orientational constraints (MDOC) [1]. Such constraints are based on residual dipolar couplings (RDCs). MDOC simulations are performed in vacuum and allow the orientation and reorientation of the molecule to take place, so that the motional time averages would represent the tensorial NMR properties of the experimentally measured parameters. The full tensorial calculation performed allows improved characterisation of the orientation of the system. This feature is in contrast to other approaches for RDC analysis where an alignment tensor is calculated for a sterically fixed orientational model, therefore this makes the COSMOS approach in principle suitable for flexible molecules.

We have performed an evaluation of the application of the COSMOS program for a small number of organic molecules with varying flexibility: from rigid models as strychnine to a flexible organic compound with e.g. five-membered rings. The run is monitored with two parameters such as a quality factor for the correspondence of experimental and calculated data and the overall temperature of the MD simulation. The quality measure for the RDCs is defined as the summed squared difference of the experimental and calculated RDC divided by the respective error. High quality measure and low overall temperature during the simulation serve as an indication for the correct structure. Here, we present preliminary results obtained within the evaluation process.

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92 Effect of a β-Phenyl Substituent on the Puckering Modes of Proline

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The high significance of proline in peptide conformation and biology stimulates the development of proline analogues with tailored properties. The attachment of substituents to the five-membered ring is particularly atractive in this regard. Such substituents may serve to incorporate additional functionality as well as to modulate the conformational properties of proline.

In this context, we have explored [1] the effect produced by a phenyl substituent attached to the β-carbon atom of proline on the puckering modes adopted by the pyrrolidine ring. Both the *cis* and *trans* configurations of the β-phenyl group relative to the carbonyl moiety have been considered.

The puckering modes of such proline analogues, cis- and trans-β-phenylproline, have been examined by NMR when incorporated into model dipeptides. We observed that the pyrrolidine conformation is significantly affected by the presence of the β-phenyl group. Those conformations that alleviate most the steric hindrance introduced by the bulky phenyl substituent are preferred. The orientation adopted by the aromatic moiety is much more restricted in the cis isomer. Thus, cis-β-phenylproline shows a highly marked propensity to adopt a Cγ-endo arrangement. In contrast, the trans derivative exhibits a higher flexibility, with Cγ-endo and Cγ-exo pyrrolidine shapes being accessible, as for proline itself.

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93 The Geographical Region Influence on ^{1}H-NMR Urine Metabolomics Based Diagnosis

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The urine metabolomics is increasingly used for medical diagnosis. Most of the discriminating regions/buckets in the spectrum are based on various metabolic markers associated with the studied condition.

There is also an increasing awareness that the NMR urine profile bears a signature of the geographical region where the subject lives.

In this paper we present a comparison between concentrations for various metabolites in a control and a diabetes group. Both groups belong to a population located in Romania (Eastern Europe).

Our data are in good agreement with some previously reported data but they are not identical. Possible explanations for the small variations are discussed in terms of NMR experimental parameters and lifestyle differences. The present study indicates that when both a control and a pathologic group are chosen from the same geographical region, the tendencies can be interpreted with confidence due to the fact that the nutrition and metabolism particularities are averaging well for the two groups.

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94 Anticancer Chloroquine-Metal Complexes: Characterization and Biological Activities

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Nowadays, the drug discovery and/or drug development that can fight well against some specific target disease is a main purpose and perhaps the most challenging work for the inorganic chemist and for pharmacist. Since the discovery of cisplatin, various studies involving investigation, based on drug-metal complexes of ruthenium, platinum, iridium, cobalt, nickel, iron and others are attracting an increasing attention as chemotherapeutic agents against a variety of diseases [1]. Within this framework, many complexes of chloroquine (CQ) have been studied and mainly applied against antimalarial, and several cancer cell lines [2, 3, 4]. In the present study, the structure of free ligands and the nature of bonding in the metal-CQ complexes [Pt(dppf)(CQ)Cl] and [Ru(arene)(phenylphen)(CQ)] were characterized mainly by using 1D and 2D NMR spectroscopy. Literature data describe the chloroquine binds to Ruthenium through the quinolone nitrogen [5]; this event was possible to be demonstrated successfully by using the ^{15}N-HMBC NMR in both metal-CQ complexes. An initial screening to assess the biological activity in these cancer cell lines: L929 (healthy cells of mice - Fibroblast), MDA-MB-231 (breast cancer cells – invasive) and MCF-7 (breast cancer cells – non-invasive); were carried out for the [Pt(dppf)(CQ)Cl] and [Ru(arene)(phenylphen)(CQ)]. Comparison of the initial screening from biological activity values with the control experimental outcome (cisplatine and CQ) indicated an excellent activity results for complex [Ru(arene)(phenylphen)(CQ)] in cell lines MDA-MB-231, MCF-7, and a lower activity in the L929. The complex [Pt(dppf)(CQ)Cl] was unable to show activity in cell line MDA-MB-231, but afforded a lower activity in the cell lines MCF-7 and L929.

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95 Resolving Component Spectra in Diffusion NMR Data: OUTSCORE

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Diffusion-ordered spectroscopy (DOSY) combines pulsed field gradient (PFG) NMR with post-processing to allow mixture analysis based on differences in molecular diffusion. The commonest approach (HRDOSY [1]) uses univariate fitting of the PFG NMR data to extract diffusion coefficients (D) on a peak-by-peak basis; however, this regularly fails where there is spectral overlap between mixture components. Multivariate decomposition (e.g. SCORE [2]) attacks the problem of overlap by using the full DOSY dataset to build the model set of diffusion decays and component spectra that "best" describes the original data. Typically, the "best" fit minimises the residual between model and original data. Where components have similar Ds (<30% difference), however, a continuum of similar solutions will fit the data: resulting in cross-talk between the contributions from different components, making it difficult or impossible to obtain true component spectra. We show that supplementing residual minimization with a different criterion, maximization of the difference between the component spectra, (i.e. minimisation of cross-talk) in the OUTSCORE (Optimised Unmixing of True Spectra for COmponent REsolution) can allow true component spectra and diffusion coefficients to be extracted.

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96 From Separation to Structure - NMR Hyphenation and Automated Structure Generation

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LC-NMR hyphenation has developped over the last decade to a powerful tool for the efficient isolation of individual constituents of complex mixtures and subsequent spectroscopic investigation by nuclear magnetic resonance spectroscopy (NMR) and (high resolution) mass spectrometry (MS).

Advances in NMR hardware and probe technology allows the acquisition of all required NMR data, i.e. ^{1}H, ^{13}C, COSY, HSQC, HMBC, ^{15}N HMBC with only a few tens of micrograms injected on column, while high resolution mass spectrometry provides the molecular formula of the unknown which is the pre-requisite for structure elucidation.

Modern software allows to generate possible structures from the raw data which can be further validated by ranking based on ^{13}C chemical shift prediction.

These advances provide us a powerful tool for fast dereplication of already known and characterisation of new unknown natural products or other proton carrying compounds [1].

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97 Structure Verification – Computer Assisted NMR Data Analysis

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Today's generation of NMR instruments makes data acquisition more efficient than ever – without requiring expert knowledge. However, the analysis of NMR data can be a time consuming task even for experienced spectroscopists. In order to support chemists and spectroscopists in the complex task of structure verification, Bruker developed powerful software tools for interactive and assisted NMR data analysis.

At the Fraunhofer Institute for Toxicology and Experimental Medicine in Hannover, Germany, organic chemists routinely use NMR spectroscopy for structural characterization of any synthesized compound. As successive synthesis steps usually show only minor changes, chemists typically control their synthesis by verifying each intermediate compound based on a 1D ^{1}H NMR spectrum. With the aid of Bruker's software CMC-assist, these 1D ^{1}H spectra can be processed and analyzed at the push of a button. Furthermore, this spectra interpretation can be directly linked to the data acquisition, providing the chemist with a fully analyzed spectrum within seconds after the acquisition. In some cases verification of an organic compound might require a full set of 1D and 2D NMR spectra. This more extensive way of structure verification can also be streamlined and largely automated. Bruker's software CMC-se analyzes these spectra automatically and proposes assignments that fit all the experimental correlations. Based on NMR data of the Fraunhofer Institute, the different approaches and applications of these two software packages will be explained in more detail.

98 Kinetics News: From Multiple Receivers to Dynamics Center

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NMR has proven to be a very useful technique to monitor reactions and processes [1,2]. It is information rich, quantitative by default and highly complementary to other techniques such as vibrational spectroscopy.

We show *i*) the use of multiple receivers to monitor reactions and *ii*) new tools for the analysis of kinetic data.

The Diels-Alder reaction between maleic anhydride and 1,3-cyclohexandiene was monitored by simultaneously acquiring 1D ^{1}H and ^{13}C NMR experiments [3]. The methodology can be extended to other nuclei, making it highly versatile and relevant for the study of fast reactions.

The data obtained were analysed with the new Kinetics Method (Dynamics Center) [4,5], a fit for purpose, easy-to-use package for the extraction of kinetic information from NMR data. The new kinetics tools include powerful algorithms for peak tracking, capacity for handling high number of spectra, profiling, fitting and reporting. Furthermore different data sets can be analysed in parallel and displayed side by side as it is shown for the Diels-Alder ^{1}H and ^{13}C data.

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99 Application of Theoretical Calculations to the Unequivocal Assignment of all the Solid State ^{13}C NMR Signals of the Lamivudine Biftalate Cocrystal

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Cycle of HIV virus replication presents various events, and proteins play an important role on it. Thus, proteins become targets of pharmacological antivirals agents like lamivudine, which has an important role in the treatment of AIDS.[1] However, some of these drugs have limited solubility and the search for cocrystals represents a way of trying to overcoming this limitation. An example of this is the lamivudine biftalate that has higher solubility in water than the pure lamivudine.[2]

The characterization of these drugs and derivatives in solid state is very important to evaluate the quality of them, and one technique used for it is the Solid-State Nuclear Magnetic Resonance (SS-NMR). In this context, the computational chemistry is a powerful tool on the structural elucidation of organic compounds, especially in cases in which the unequivocal assignment is not a trivial task,[3,4] as for example in some ^{13}C SS-NMR spectra. The present study aims to apply the theoretical calculations of NMR properties as an auxiliary tool to an unequivocal assignment of all the ^{13}C SS-NMR signals of the lamivudine biftalate cocrystal.

The SS-NMR experimental data of lamivudine biftalate were obtained on a Bruker Avance III 500 MHz spectrometer, by ^{13}C CPTOSS experiments. Theoretical calculations were performed in Gaussian 09 program using B3LYP/cc-pVTZ model and GIAO method to compute the NMR shielding tensors.

In a previous work, the theoretical and experimental data of pure lamivudine showed an excellent correlation with the linear correlation coefficient (R) of 0.99. In the present case of the lamivudine biftalate cocrystal, the corroboration between experimental and theoretical data showed a lower degree of correlation compared to the pure lamivudine, with a correlation coefficient equals to 0.94. One possible factor that could contribute to this decrease of the coefficient value is the fact that lamivudine biftalate has intermolecular hydrogens bonds between the asymmetric units, not present on pure lamivudine, and the calculation method used for compute ^{13}C NMR properties did not take into account these bonds. Calculations with plane wave methodology (GIPAW) for better mimic solid state are currently in study.

Theoretical calculations of the NMR properties confirmed that experimental assignment of the ^{13}C SS-NMR data was correct for all the signals. It was also possible to note that ^{13}C SS-NMR data indicated that there is only one lamivudine biftalate in the asymmetric unit, confirming a previous x-ray analysis.

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100 Preparation and Characterization of a Binary Complex of Albendazole-Secnidazole by ssNMR

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The antiparasitics are some of the most used drugs in Brazil because of its large rural area and poor hygienic conditions in many urban centers. Within this class albendazole (ABZ) and secnidazole (SCZ) are two of the most used. ABZ is cheaper but exhibits problems related to its solubility, mainly in water. In contrast, SCZ is completely soluble, even in higher concentration. Thus, the purpose of this work is to evaluate the possibility of using the solubility properties of the SCZ to develop a co-formulation that possibly improves the solubility of ABZ. Furthermore, it should be possible to administrate them in a combined therapy by using a single tablet, since these drugs act against different classes of parasites. For characterization ssNMR (Solid State Nuclear Magnetic Resonance) was used once its use has been increasing for studying the formation of co-crystals or binary complexes [1]. Certainly, its use has been done together with other techniques, not only to characterize the products but also as an analytical tool in order to assess information about the composition of commercial formulations.

Hence, both powdered drugs were sonicated in acetonitrile and recrystallized by simple solvent evaporation. The ssNMR analyses were performed on a Bruker Avance III-400 equipped with a MAS probehead. The samples were packed into a zirconia rotor. Spectra were obtained employing ^{13}C-CPTOSS (cross polarization with total sideband suppression), using adamantane as an external reference. The contact time was 2 ms, the recycle delay of 3s, and spinning speed on magic angle of 5KHz. For ^{15}N-CPMAS (cross polarization under magic angle spinning) spectra, glycine was used as external reference, the contact time was 3 ms, the recycle delay was 5s and the spinning speed under magic angle of 10KHz, to avoid sidebands. Two-dimensional maps ^{1}Hx^{13}C-FSLG-HETCOR were also obtained using a contact time of 500μs and spinning speed of 10KHz. The product of the mixture is a yellow powder, in contrast to isolated drugs, which are white. Fourier Transform Infrared Spectroscopy (FTIR) obtained from isolated drugs recrystallized in acetonitrile did not reveal changes in comparison to initial product [2]. However, the spectra obtained is different from that one obtained from the complex ABZ-SCZ, being an additional evidence of the complex formation.

Significant differences were observed in the carbon-13 spectrum of the binary complex when compared to those ones from the isolated drugs. For ABZ signals it was observed a considerable change in the carbons with chemical shift of 152.6 (C2) to 145.4 ppm after complex formation.

In the region of aromatic carbons of the complex ABZ-SCZ spectrum it was observed the overlap of several signals, being not possible to obtain interesting information. The analyses of SCZ signals in the spectrum of the complex revealed the presence of twofold signals in 67.1 (C7') to 67.4 / 66.45 ppm, providing evidence of a possible point of coordination in the of hydroxyl group surroundings.

The ^{15}N spectrum of the binary complex also showed significant variations in the chemical shifts. The main difference lies in the variation in the chemical shift related to the nitrogen atoms in 242.7 (N3') in SCZ and 127.5 (N10) ppm in ABZ, to 187.2 and 80.5 ppm, respectively, in complex ABZ-SCZ. Due to changes in chemical shift of the aromatic carbons of the complex are likely to occur in different conformations of one or both chains, attached to the bicyclic ring system. The FSLG-HETCOR map of the complex ABZ-SCZ showed signals close to 14 ppm, which comes from the correlation between the carbonylic carbon (C11) of ABZ and the acid hydrogen of ABZ (H10), indicating that this hydrogen involved in a weak hydrogen bond with other nitrogen atom, probably N3'. These changes occurred in 1D and 2D spectra and are indicative of the occurrence of an intermolecular interaction through hydrogen bond between more basic nitrogen from SCZ (N3') with acid hydrogen of ABZ (H10). As for the complex ABZ-SCZ it was observed a broad band of OH stretching in 3343 cm^{-1} feature with hydrogen bonding.

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101 JAMFIS: A New Approach for Conformational Studies of C-disaccharides

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The major advantage of the conformational studies of C-glycosides with respect to O-glycosides is that four proton–proton coupling constants across the pseudo-glycosidic linkage can be measured and converted into dihedral angles through the well-established Karplus–Altona equation [1]. Because the four proton–proton coupling constants report on the conformational behavior around the pseudoglycosidic bonds, we have developed a new approach, called JAMFIS (J-based Analysis of Molecular Flexibility in Solution), based on Cicero's methodology [2], to perform a quantitative analysis of the conformational equilibrium in water solution.

This method was validated by the comparison of JAMFIS results with those obtained with the combination of NOEs and coupling constants for three compounds. Finally, JAMFIS method was applied to 12 compounds previously examined by a qualitative coupling constants analysis [3-11].

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102 Contribution of NMR Spectrometry within a Pharmaceutical Development Resulting from Extract of Plants

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For a pharmaceutical application, the Laboratories Pierre Fabre developed an original extract of Eucalyptus globulus sheets. The phase impossible to circumvent so that a new drug can succeed is obtaining an AMM (Autorisation de Mise sur le Marché) implying a qualitative knowledge of a maximum of chemical components from this extract.

The most active compounds of the extract are known thanks to previous works. In order to perfect the chemical knowledge of the extract, a total analytical methodology was developed within the ISA. To start this work, an important interest was carried to the compounds of the family of the acylphloroglucinols and flavonoïds specific to the eucalyptus kind. Semi-preparative and preparative chromatography enabled the purification of these compounds obtained from the extract and/or from enriched fractions of low content products. 1 to 5 mg of products are collected and characterized with Nuclear Magnetic Resonance, High resolution mass spectroscopy, Ultra-violet and Infra-red spectroscopies. Among the 9 identified compounds, 3 had never been identified before in the vegetable kingdom (diformylphloroglucinol, macrocarpals P and E') and 1 (known) never mentioned in the sheets of E.globulus (grandinol). The 5 others compounds were previously identified in the E. globulus, they correspond to acylphloroglucinols which account for approximately 44% of the extract.

This work shows the interest of NMR spectroscopy which allowed, thanks to 2D NMR experiments (HMBC and ROESY) to establish chemical structures never identified even on small quantities. Moreover, where the mass spectrometry indicated the same empirical formula NMR spectrometry highlighted for the macrocarpal E (already known in plane structure) the existence of macrocarpal E' (epimer on C9').

Thanks to this study, the knowledge of the extract of Eucalyptus Globulus sheets at least doubled. At the end of this work, we estimate the knowledge of extract at about 57%.

103 NMR Diffusion and Chemometric Methods to Analyze Complex Mixtures of Surfactants

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Study of complex mixture is an analytical challenge. Here, we used NMR spectroscopy which combines many advantages: structural and quantitative information of simple mixtures, minimum sample preparation and no degradation of sample. Our method is developed on surfactant mixtures. A sample database has been created composed of anionic, non ionic and amphoteric surfactants. 1D ^{1}H NMR spectra of each sample have been recorded. NMR spectra were divided into 0.04 ppm buckets over a range of 11 ppm (Amix, Bruker). No scaling was used for buckets. Combined to Principal Components Analysis, NMR spectroscopy enables the discrimination of the different family and to distinguish the different sub-structures inside a same family.

In the case of complex mixtures, peaks superposition is the most important problem. To check the efficiency of NMR for resolving such problem, we prepared test complex mixtures. NMR diffusion experiment was chosen. In particular, DOSY experiment using pulsed field gradient allows solving peaks superposition [1, 2]. Using inverse Laplace transform (ILT), all chemical shifts in the direct dimension are dispersed along the second dimension in function of diffusion coefficient. This approach does not make any assumption about the number of exponential components. Diffusion coefficients are correlated to molecular mass through the Flory's law [3-5]: $D = KM^{-\alpha}$, $\alpha > 0$. However, this relation between molecular mass and diffusion coefficient is dependent of the family of samples and solvent. Samples were prepared in DMSO-d_6 to limit the formation of micelles. A calibration curve has been fitted for pure surfactant samples and we extracted $K = 0.76$ and $\alpha = -0.50$. Our fitted equation was then tested on a complex mixture composed of three compounds (TAM12002 + TAN12007 + TNI12018). Four diffusion coefficients were discriminated: $D_{DMSO} = 886 \pm 89$ µm².s^{-1}, $D_{TAM12002} = 497 \pm 50$ µm².s^{-1}, $D_{TAN12007} = 269 \pm 27$ µm².s^{-1} and $D_{TNI12018} = 145 \pm 15$ µm².s^{-1}. Those results lead to: $M_{TAM12002} = 143 \pm 25$ g.mol^{-1}, $M_{TAN12007} = 488 \pm 90$ g.mol^{-1} and $M_{TNI12018} = 1680 \pm 350$ g.mol^{-1}. We can confirm the efficiency of NMR of resolving peaks superposition in case of surfactants mixtures.

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104 Quantitation of Plasma Metabolites Using Prep HPLC and NMR

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Regulatory guideline point out the importance of identifying and characterizing drug metabolites as early as possible in drug discovery. The guidelines suggests that metabolites identified in human plasma should be present at equal or greater levels in at least one of the preclinical species used in safety assessment studies.[1]. As NMR is a quantitative, non-destructive technique the combination of plasma pooling, preparative HPLC and NMR[2] can be used to get early information about quantity and identity of plasma metabolites

Here we show the results of a pilot study performed at our lab to setup and evaluate the method using plasma pooling, preparative HPLC and quantitative NMR to get relative concentrations and identity of circulating metabolites.

The study showed that we were able to detect four metabolites in this study of which the major one was quantified as 2.7% of the parent. Structural studies reveals that this metabolite was hydrolized in comparison to the parent.

Furthermore the study showed that the mimimal amount of the parent in the plasma pool should be at least 5ug to be able to detect and quantify metabolites at 10% relative to the parent.

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105 Comparative Analysis to Evaluate Metabolic Differences in Healthy and Pathologic Human Pancreatic Tissues Based on 1D HR-MAS NMR

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Cancer is known as genetic disorder disease which evolves through multi-step process [1]. Periampolar region and pancreatic cancer represents a challenge on diagnostic and therapy in modern medical science. Current data shows that only 30% of patients diagnosed with pancreatic cancer have the possibility of surgical treatment with curative intent and overall median survival at 5 years is approximately 4%. In addition, adenocarcinome and pancreatic cancer can occur concomitant with others diseases such as diabetes mellitus and chronical pancreatitis turning even more challenging a precise diagnostic without a surgical intervention. Previous attempts to determine specific genes expressed in pancreatic cancer have been hampered by similarities between adenocarcinoma and chronic pancreatitis [2]. Thus, medical science has been seeking alternatives that can help investigate pathologies and establish new biomarkers with high accuracy than those frequently used CA-19.9 and p53. Comprehensive analysis of small molecules had emerged as a powerful strategy to differentiate normal from abnormal cellular process. For this reason, metabolomics have been considering a more accurate representation of cellular phenotype being particularly important for clinical research. In this study, we present a ^{1}H HRMAS NMR pancreatic tissue profiling based on metabolomics approach.

A case of study involving 11 healthy tissues (H), 4 pancreatic ductal adenocarninome cancer (PDA), 3 moderated differenciatiate adenocarcinome (MDA), 11 pancreatic cancer (PC), 5 chronical pancreatitis (CP) and 4 cases with adenocarcinome ductal plus chronical pancreatitis (PC/CP) tissues, were developed to investigate cellular physiology alterations even between pathologic tissues. All biopsy tissues samples were furnished by Digestive Surgical Division at Hospital das Clínicas from University of São Paulo (Ribeirão Preto-SP). The 1D and 2D HRMAS experiments were carried out on Bruker Avance-III 400 spectrometer equipped with 9.4 T Oxford narrow bore magnet and 4 mm with *Z* gradient HRMAS probehead. All the analyses were conducted at 6°C (BCU-Bruker) and the samples were spun at 4000Hz of spinning speed. The processing and semi-quantitative analyses were performed through Topspin 3.0 software (Bruker). The chemometrics and Pearson correlation analysis were performed at Matlab 7.6.0 (R2008a) and PLS Toolbox 6.0.1 (Eingenvectors Research).

A first principal component analysis (PCA) model was performed using all sample tissues. Three principal components were used explaining 94.0% of total variance of mean centered of data and through two first's principal components (85.8%) a clear separation between pathologic tissues and healthy ones was reached. Reviewing the loading plot for PC1 X PC2, the characteristic resonances for polyunsaturated fatty acids (PUFA-δ 5.33 ppm), monounsaturated fatty acids (MUFA-δ 5.24 ppm), overlapped resonances of phospholipids (δ 3.21-3.24 ppm), threonine and -CH_2 from lipids (δ 1.33 ppm) and –CH3 from lipids (δ 0.92 ppm) appeared preferable in healthy tissues. A significant decrease on lipid levels in pancreatic cancer types and chronical pancreatitis confirm a changing on cell energy achievement through FA β-oxidation to produce ATP as alternative pathway in mitochondria. Moreover, high levels of acetate (Ac-δ 1.97 ppm), glutamate (Glu-δ 2.34 ppm), creatine (Cre-δ 3.02 ppm) and taurine (Tau-δ 3.46 e 3.24 ppm) could be observed in pathological tissues. High levels of lactate was also notice in cancer cells indicating a over expression of PKM2 enzyme in which promotes regulation of glucose metabolism due to increasing lactate production and decreasing oxygen consumption. Glutamate is an end product of amino acid degradation and it plays an important function in cells as nitrogen supplier either as substrate for TCA cycle.

Excluding H tissues samples a second PCA model, was performed using five principal components (98.3%). Score plot (PC1X PC2) shown discrimination between PDA/CP from pancreatic cancer types (PC, MDA and PDA). Lactate resonance (Lac-δ 4.10 and 1.34 ppm) were the main contributor to discriminate PDA/CP tissues. In contrast, Tau, Cre and phospholipids displayed high loading values to describe pancreatic cancer types. Especially, Tau have been investigated as possible biomarker for pancreatic cancer. Through PC3 X PC4 score plot (8.36%) CP could be separated from others oncologic tissues. Based on loading plot, α-glucose (α-Glc-δ 5.23 ppm), phospholipids (δ 3.21-3.24 ppm), Glu, glutamine (Glm), alanine (Ala), Cre, glycine (Gly) and inositol (Ino) appears with high levels in CP tissues. α-Glc presence in CP tissue reveals that both glycolysis and gluconeogeneses are active for energy production, while in PC, MDP, PDA, PDA/CP preferable glycolises is the dominant mechanism.

Pearson correlation analysis ($p > 0.05$) was applied for deconvoluted region correspondent to the discriminatory metabolites and a strong positive correlation (≈1) could be observed among Tau, Lac, Cre, Glu and negative with lipids (PUFA, MUFA and phospholipids). One hypothesis for negative correlation between Tau and lipids is a tentative of cancer cell to stabilize the oxidative environment in the mitochondrial acting as pH buffering agent and try to reduce release of reactive oxygen species (ROS), and perhaps even more important stabilize the very pH-dependent beta-oxidation of the fatty acids by the acylCoA dehydrogenase enzymes [3].

As conclusion, metabonomics analysis based on ^{1}H HRMAS spectra could provide significant information about metabolic differences among pancreatic diseases and healthy tissues. For pathologic tissues an intense glycolytic activity could be observed and enhance of gluthaminase (GLS) due to high levels of glutamate. One important discriminative remark for CP from oncologic tissues was the active presence of gluconeogeneses for energy production.

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106 ss-NMR Studies of Phase Transitions Under Temperature Variation for Two Drugs

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Nowadays, the literature reports numerous examples of molecules with phase transitions under temperature variation in solid state. The most appropriate technique for this type of study is the single crystal X-Ray diffraction and also X-Ray diffraction. However, the solid state NMR technique has been applied as an important tool in order to corroborate the X-Ray data. With this purpose, we used ssNMR to study phase transitions of diethylcarbamazine citrate and estradiol 17β valerate, already studied by X-Ray technique. Diethylcarbamazine (DEC) is a powerful anthelmintic used to eliminate lymphatic filariasis (elephantiasis), a neglected tropical disease caused by *Wuchereria bancrofti* adult worms [1]. Citrate form is used because it reaches better solubility in water. In 2010, an X-Ray study using single crystals showed four structures corresponding with the three solid-solid phase transformations of DEC citrate at different temperatures [2]. Estradiol 17β valerate (E2βV) is an esterified form of natural hormone estradiol. It is used for several treatments [3] including sleep problems, prevention of osteoporosis and atrophic changes in the genital tract, etc.

The ^{13}C-NMR experiments were conducted in an Avance-III-400 Bruker machine, equipped with a 9.4T Oxford narrow bore magnet and a variable temperature probehead. Samples were packed in a 4mm cylindrical zirconia rotor. DEC citrate sample spun at 4-6KHz and it was performed ^{13}C-CPMAS experiments with 2ms of contact time, 5s of recovering time and acquisition time of 34ms. For E2βV sample ^{13}C-CPTOSS experiments were performed with 3ms of contact time, 3s of recovering time, acquisition time of 34ms and spinning speed of 5 kHz.

For DEC citrate, the experiments were performed in a temperature range between 293 and 223K and it was possible to observe differences in the methyl group chemical shift (~14 ppm) as the temperature decreases. This result is in agreement with the X-ray diffraction, which demonstrated that DEC citrate crystallized in a ionic pair in the asymmetric unit, $(DEC)^+$ $(citrate)^-$, at room temperature, also observed in 2D-NMR-HETCOR experiment. Two carbons of one of the two ethyl groups of $(DEC)^+$ showed occupancy sites disordered over two positions and the other ethyl group is not disordered, because its methyl is bonded to the carbonyl moiety of one of the two carboxyl groups of $(citrate)^-$, through a nonclassical CH---O hydrogen bonding. At 235 K were found two $(DEC)^+$ and two $(citrate)^-$ fragments in the asymmetric unit (phase II) and these two $(DEC)^+$ molecules are those found in the asymmetric units determined at 293K (phase I) and 150K (phase III). The X-ray data showed too that there is a nonclassical intermolecular CH---N hydrogen bonding between the CH_2 methylene group of ethyl and a nitrogen of piperazine, connecting $(DEC)^+$ molecules and hence, ordering of the ethyl tail because this group is fixed through an intermolecular interaction. Thus, the ethyl groups are more organized at a lower temperature.

The chemical shift at approximately 177 ppm, corresponding to two carboxyl groups from citrate branch also changes as the temperature decreases. It is possible to verify that this signal is duplicated probably because, at lower temperature, some molecules do not exhibit a nonclassical CH---O hydrogen bonding, between $CH_{3(DEC)}$ and carbonyl $moiety_{(citrate)}$-. The minimum temperature allowed to conduct experiments in NMR equipment used in this work is 213K, hence it was possible to observe only the first phase transition of DEC citrate, from phase I to phase II.

Preliminary studies using single crystal X-Ray diffraction showed that at room temperature E2βV crystallizes in two independent molecules per asymmetric unit, with a high level of dynamic disorder in the valerate terminal chain, in both molecules this group is almost perpendicular to the plane of the rings. The packing proposed by X-Ray analysis exhibits strong intermolecular hydrogen bond between carboxyl and hydroxyl group (C=O---H—O) and this interaction affects mainly carboxyl carbon and the phenolic carbon. The ^{13}C solid state NMR spectrum at 298K exhibits duplicated signals for these carbons (carboxyl - 178.6; 177.7 ppm and phenolic 155.8; 155.5 ppm). This behavior is consistent with the existence of two different molecules at the same asymmetric unit. According to the thermal analysis data, the range between 251 and 257K can be considered as an interface of a phase transition, and a crepitation event is observed at 251K. At this range a fast and intense loss of crystallinity was observed and, due to this behavior, the XRD failed to study the events. However, solid state NMR is completely useful for this purpose, once it does not depend on the crystallinity of the material in order to obtain fine information about changes. With this objective, the experiments were performed at several temperatures: 298, 283, 268, 258, 251, 243, 298, 323 K. No differences in the spectra were observed in temperatures above 251K, but very significant changes were observed at 243K, once all the signals suffered changes in their chemical shift and/or in their shape. An example of this behavior occurs with the signals attributed to carbons from aromatic ring, all of them became broader or duplicated, which characterizes loss of crystallinity, which leads to several possibilities of orientations, resulting in the increase of the chemical shift anisotropy. Most of the signals related to the carbons from rings C and D and those ones from valerate chain suffered significant variation in their chemical shift. After reaching the temperature up to 243K we started to rise it up until 323K, and it was possible to observe a tendency to restore the initial conformation.

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107 Effect of a Hexanuclear Ruthenium Complex on the Metabolic Profile of Cancer Cells Studied by ^{1}H HR-MAS NMR Spectroscopy

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Multinuclear Ruthenium complexes play an increasing role as potential anticancer drugs owing to their favorable properties such as an enhanced permeability and retention effect, water solubility and high selectivity. However, the mechanisms of the cytotoxic activity for this class of compounds are not yet fully understood. Here, the results obtained for a water soluble hexanuclear ruthenium complex, synthesized and biophysically characterized in our group [1], are presented. The effect of the complex on the metabolic profile of A2780 cancer cells was studied by High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy. HR-MAS NMR allows obtaining well resolved ^{1}H NMR spectra from living cell suspensions [2] well suited for chemometric analyses.

Cultured A2780 ovarian carcinoma cells were incubated for 24h and 72h either with the drug dissolved in DMSO or with DMSO only. A total of 36 samples, 16 control and 20 drug treated, were submitted to 1H HR-MAS NMR. The spectra were analysed by means of uni- and multivariate analysis.

About 25 metabolites including amino acids, lipid components and nucleotides, could be assigned to the proton resonances of the HR-MAS cell spectra with the aid of ^{1}H^{1}H TOCSY and literature data. Control and drug treated samples were well discriminated based on PCA and PLS analysis of the corresponding ^{1}H NMR spectra. Moreover, there was also a clear separation between the two sets of control samples obtained from different incubation times emphasising the physiological processes the cells undergo during incubation. Lipid components and choline containing compounds strongly contributed to the separation between drug treated and control samples indicating potential drug-induced membrane breakdown. The single components responsible for the discrimination between all four groups are discussed in more detail in this presentation.

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108 Scalar and Spin-Orbit Relativistic DFT Calculations of Nuclear Spin-spin Coupling in Cp_2WXSnR_2 Complexes

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Our group is interested in the synthesis, structure and reactivity of heterobimetallic complexes containing metal-metal bonds between metals from different parts of the periodic table. Our goal is to harness inherent differences in electronegativity and bonding to enable cooperative reactivity for the activation of small molecules. Former and current studies have included solution-state (NMR) and solid-state (X-ray crystallography) structural studies to investigate the nature of the metal-metal bond.[1]

Several years ago we reported a simple inverse correlation between the solid-state bond lengths in a group of related tungsten-tin complexes and the relevant one-bond ^{183}W-^{119}Sn scalar coupling constants.[1] While this initial report was accompanied by structural calculations utilizing density functional theory (DFT) at the B3LYP/LANL2DZ level of theory, significant advances have been made in the intervening time that allow for a substantially better understanding of the observed effect. In particular, advances in the calculation of scalar coupling constants involving heavy metals that are subject to relativistic effects (in this case tin and especially tungsten) have made substantial advances.[2] Similarly, progress has been made in the accuracy of DFT calculations with respect to the structure of heavy metal organometallic complexes.[3]

In this poster we will present our findings with respect to advances in the understanding of the relationship between the W-Sn bond lengths and $^1J_{WSn}$ in complexes of the type $Cp_2WXSnClR_2$ (X=H,Cl; R=Ph,^{t}Bu). This will include an analysis of the influence of the level of theory (exchange and correlation functional, basis sets, as well scalar and spin-orbit relativistic contributions utilizing the ZORA approximation). We will compare the coupling constants calculated utilizing experimental (solid-state X-ray crystallographic) structures and the idealized geometries resulting from DFT structural optimizations at the previous level of theory and those based upon recent suggestions from the literature.[3] The primary premise of our previous paper, that there is an inverse relationship between the bond length and the nuclear spin-spin coupling constant, will be discussed vis-à-vis the individual terms contributing to the coupling constant and an NBO analysis[4] of the molecular orbital contributions to those various terms.

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109 PLS Regression Model Comparison of 60 and 300 MHz ^{1}H qNMR of EPA and DHA Omega-3 Fatty Acids Obtained at Different Points in a Fish Oil Nutritional Supplement Manufacturing Process

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^{1}H NMR of a series of samples taken from different points in the manufacturing process of a fish oil nutritional supplement were obtained on a 300 MHz superconducting NMR system and a cryogen-free bench-top 60 MHz NMR system. The resulting spectra were utilized to develop single wide-range partial least-squares regression models of the EPA and DHA omega-3 fatty acid content of the fish oils at the various sampling points on the process. The process involves initial concentration of the fatty acids by solvent precipitation, molecular distillation, ethyl ester esterification, clathration, and centrifugation. For each of the fatty acids a single full range PLS model was obtained utilizing the entire integral binned/normalized spectrum or utilizing a series of normalized peak integrations rather than the entire spectrum. It was demonstrated that 60 MHz NMR spectra yielded identical model performance to the higher resolution 300 MHz spectra. The 60 MHz system is compact enough that it can be placed in the manufacturing plant environment for at-line utilization. Alternatively it can be packaged to provide on-line/in-line process control.

110 Symmetry Investigation of Three-Center Halogen Bonds in Solution

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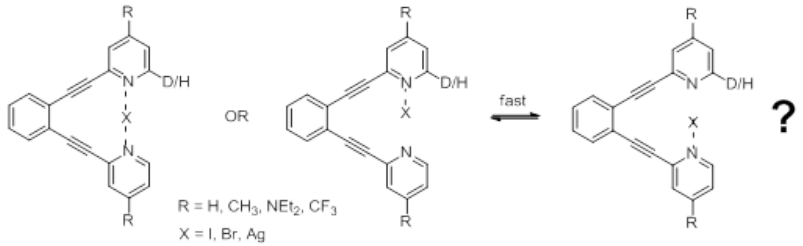
Three-center-four-electron interactions are common in Nature and have been the matter of discussions since decades[1]. While hydrogen bond symmetry in N-H-N type systems have been studied extensively[2], the analogous $[N\text{-}X\text{-}N]^+$ type halogen bond complexes have yet to be explored. It is especially relevant as such systems are in use as powerful halogenating agents[3] and in addition, possess the strongest known halogen bond to date.[4]

Previously, we have successfully characterized the symmetries of N−I−N and N−Br−N halogen bonds in solution by applying the sensitive NMR methodology of Isotopic Perturbation of Equilibrium (IPE)[5,6]. Isotope labeling was performed by regioselective deuteration of substituted pyridine derivatives, enabling us to distinguish between a static symmetric structure from a pair of rapidly equilibriating tautomers. Making use of the temperature dependendence of equilibrium processes, the variation of isotope effects upon altering temperature was detected.

Recently, we ventured towards the synthesis of a bis-pyridine chloronium complex where an electropositive chlorine(I) is incorporated between two nitrogenous electron donors for the first time in solution. Due to the marginal stability of such a highly reactive cation, the observed symmetric arrangement of the $[N\cdots Cl^+\cdots N]$ system concluded from NMR measurements were confirmed by DFT calculations optimized for N-X-N complexes (X=I or Br). Moreover, the corresponding $[N^+\text{–}F\cdots N]$ system appears to be asymmetric and also confirmed by computational methods.



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111 Characterization of Relative Configurations of Small Molecules using Residual Dipolar Couplings

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The three dimensional (3D) structures of the molecules play a critical role in drug development, as the compound shape and 3D rearrangement in solution have an important feature for its bioactivity. 3D structures can be obtained via X-ray or NMR, the second being able to characterize the molecule state in solution. Classical NMR approaches based on NOEs and coupling constants can be insufficient to solve all ambiguities in the structure. Residual Dipolar Couplings (RDCs) can provide a solution for this problem. They can be an useful tool for the analysis of the constitution, configuration and conformation. RDC measurements are considered as the key to enable the three dimensional structure determination of many unsolved molecules, therefore their analysis has been implemented in Novartis.

Here, we present a rigid structure with 7 chiral carbon atoms, which is analysed using RDCs recorded in a weak anisotropic alignment medium, PAN (Poly(acrylonitrile)) [1] with a stretching apparatus [2]. Experimental one-bond residual dipolar couplings $^{1}D_{C\text{-}H}$ are extracted from the CLIP-HSQC [3] and the geminal $^{2}D_{H\text{-}H}$ RDCs from the P.E.HSQC [4]. The experimental data is used to calculate RDCs with the programs COSMOS [5], PALES [6] and Mspin [7] and validated against the known absolute stereochemistry of the molecule as determined by X-ray studies. Permutations of the stereogenic centres, leading to wrong structures, are calculated the above programs and the results are compared with the ones for the correct stereochemistry.

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112 Hyphenated LC-MS and NMR Techniques to Identify Metabolites of Bioactive Compounds

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PT-31, 3-(2-chloro-6-fluorobenzyl)-imidazolidine-2,4-dione, is a new heterocyclic pentagonal compound with analgesic and sedative properties, chemically designed to interact with alpha-2 adrenoceptor.[1] During the development of a new drug it is very important to know the biotransformation products formed during the metabolism of the target bioactive compound. Studies of *in vitro* biotransformation offer many advantages when compared to *in vivo* studies and it was selected for this study. The aim of this work was to apply LC-MS^n and NMR techniques for the identification of *in vitro* metabolites obtained when liver microsomes from rats were used for the biotransformation of PT-31.

LC separation was performed on a phenyl-hexyl column (150 mm x 2.1 mm; 10 μm) using a mobile phase consisted of methanol/ammonium acetate buffer (5 mmol L^{-1}; pH 5.5) (gradient elution) at a flow rate of 0.4 mL min^{-1}. For the LC-MS^n analysis, electrospray ionization (ESI) interface was selected and used in the positive ion mode. The ion trap (IT, Esquire 6000 from Bruker Daltonics) was operated under the following conditions: nebulizer gas 30 psi, dry gas flow 7.0 $L.min^{-1}$, dry gas temperature 325°C and capillary potential 4500 V. Data acquisition consisted of full MS scan acquired for 100 ms accumulation time, target of 40000 and acquisition range from 100 - 400 *m/z* in conjunction with data-dependent MS/MS acquisitions on the most intense ions selected from MS-scan spectrum. Post-acquisition of the full-scan MS data was based on the generation of extracted ions chromatograms for expected metabolites. Using the optimized chromatographic conditions, PT-31 eluted at 10 min and, after microsomal incubation using rat liver microsomes, its profile was also monitored by extracted ion chromatogram (*m/z* 243). Identification of an unknown metabolite in rat liver microsome was achieved successfully by combination of LC-MS^n and NMR. It was shown that PT-31 was metabolized to yield 3-(2-chloro-6-fluorobenzyl)-imidazolidine-4-hydroxi-2,4-dione. The identity of this metabolite was established by comparing the LC-MS fragmentation profile and NMR data (^1H and ^1H-^{13}C-gHSQC). The NMR experiments were conducted in a Bruker Avance III-600, equipped with a ^1H {^{13}C, ^{15}N} TCI triple resonance cryogenically-cooled probehead. The ^1H NMR experiment was based on the 1D version of the NOESY sequence, using water and methanol suppression during the relaxation delay (2.40 s). The spectrum was acquired using 64k data points in a 12,019.23 Hz spectral width, giving an acquisition time of 2.73 s. 256 scans were run to acquire a spectrum with satisfactory signal to noise ratio. Processing was performed using exponential multiplication, applying a line broadening factor (lb) of 0.3 Hz.

LC-MS fragmentation profile revealed the presence of an ion peak at m/z 259, which is consistent with the introduction of a hydroxyl group into the substrate (PT-31). The literature describes several possible

pathways for hydroxylation, including aromatic rings and pro-chiral carbons. The MS fragmentation was proposed; however it was not enough to identify the precise position of the hydroxyl group. For this purpose, NMR was the best technique to be applied. A ^{1}H-NMR spectrum of the hydroxylated metabolite generated from PT-31 was obtained and compared to the ^{1}H-NMR spectrum of PT-31. In comparison to the substrate no significant differences in the chemical shifts of the benzyl group were observed. However, three main changes were observed in the imidazolidine ring, including deshielding of ^{1}H from N-H (1^{st}position) from 6.09 to 6.71ppm and the disappearance of CH_2 signal (5^{th} position). It was also observed a new singlet signal in 4.64 ppm, which did not exist before the biotransformation process. This signal integrates for 1 H and exhibits a chemical shift consistent with a proton attached to a C-OH group. Due to very low concentration of the obtained metabolite, 2D heteronuclear correlation experiments were not properly acquired. However, only 1D-^{1}H spectra provided data to conclude that the hydroxylation occurred in the 5^{th} position of the imidazolidine ring.

Acknowledgements: Fapesp, Capes and CNPq

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113 Determination of 3D Structures Combining NMR, MS, and X-ray

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The results of a study combining different structure elucidation/determination methods are presented. Structure elucidation was carried out with data from 1D and 2D nuclear magnetic resonance spectroscopy (NMR) and accurate mass spectrometry (MS and MS/MS). The data were analysed using Bruker CMC-SE software for computer-assisted structure elucidation [1,2]. Fully automatic 3D structure determination was carried out using SMART X2S single crystal diffractometer [3].

Neither x-ray nor NMR data in isolation were sufficient to automatically determine the structure. However the combination of results from x-ray, NMR and MS provided a unique structure very rapidly.

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114 NMR Study on the Dynamics of Chiral Dirhodium (II) Complexes

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During recent years, the potential of chiral dirhodium tetracarboxylate complexes as highly selective asymmetric catalysts and as NMR auxiliaries for chiral recognition has been studied extensively.[1] These complexes - mostly with amino acid-based tetracarboxylates - form high symmetry homochiral paddlewheel formed catalysts. Due to the ligands flexibility the overall symmetry is not fixed. This flexibility is important for the complexes ability for chiral discrimination and asymmetric catalysis.[2]

The unique paddlewheel structure of dirhodium complexes is responsible for their complex stereo-electronic properties. In this contribution, we investigate the conformational behavior of the carboxylate residues within the dirhodium complex and the mechanism by which a ligand molecule can nest into the cavity upon adduct formation. So far X-ray crystallography studies supported a “chiral crown” conformation (αααα) in which the catalyst has a reactive chiral face and an unreactive achiral face. Our NMR data and NOE analysis for two phthalimide derived complexes show that arrangements are favored in solution, in which the four tetracarboxylate ligands rest on different sides of the complexes side (ααββ with C_2 symmetry and αβαβ with D_2 symmetry). The “chiral crown” seems not to be predominant in solution.

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115 Fluxional C-H Activation in Sterically Encumbered Iridium Bis(N-Heteocyclic Carbene) Complexes

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The selective functionalization of unactivated C-H bonds remains one of the primary synthetic challenges of organometallic chemistry. Agostic systems represent a convenient platform to study parameters relating to the activation of alkyl C-H bonds. Protonation of sterically encumbered Iridium benzyl complexes with N-heterocyclic carbene ligands leads to species containing both agostic C-H and cis alkyl/hydride ligands [1]. Reversible σ-bond activation leads to degenerate exchange within the agnostic alkyl C(sp3)-H bond.

Of interest from the perspective of CH activation are the barriers associated with the fluxional processes occurring. In each case, degenerate ground-state structures interconvert by exchange between the agostic CH bond and the cis benzyl/hydride ligands. A combination of variable temperature proton NMR; line shape analysis and selective saturation transfer experiments were used to measure the observed exchange rates.

The resultant values of ΔH‡ and ΔS‡ derived from the Eyring plots are compared to the results of DFT simulations to show that the exchange process most likely occurs via a σ-CAM mechanism.

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116 Dereplication of Natural Products in Mixtures Using PFG Diffusion NMR Combined with an In-house ^{1}H NMR Spectra Database

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Natural products continue to play an important role in the discovery and development of new chemical entities as drug-lead compounds. The rapid identification of already known natural products present mixtures, a process known as dereplication, is strategically important for scientists involved in screening for novel bioactive compounds from natural sources. Efficient dereplication at the earliest possible stage in the discovery process is essential to avoid expensive and time consuming isolation and structure elucidation resources. Dereplication processes typically combine chromatographic and spectroscopic methods with searching in-house created databases. Strategies based on HPLC-DAD-MS (both low and high resolution) [1] or UHPLC-DAD-HRMS-MS/MS [2] have been shown to provide efficient dereplication of microbial natural product extracts. On the other hand, one-dimensional ^{1}H NMR spectra provide much richer structural information not only for the identification of known compounds but also for the identification of structure classes of novel compounds. For this reason, LC-NMR and HPLC-SPE-NMR have been recently developed as novel dereplication tools when dealing with natural product mixtures [3]. Unfortunately, these techniques require additional special equipment (flow probes, robotic SPE trapping modules, etc.) apart from the NMR spectrometer. Among the NMR techniques for mixture analysis, PFG diffusion-encoded experiments have a great potential for the “virtual separation” of the species allowing their characterization without the need for purification [4]. Thus, it represents an appropriate alternative to the previously mentioned hyphenated NMR approaches. However, despite its potential, it seems that diffusion NMR spectroscopy is clearly still undervalued in the field of natural products research [5]. Herein we propose a new dereplication approach of natural products in mixtures based on the combination of PFG diffusion NMR with an in-house database of ^{1}H NMR spectra. The strategy has been tested with model artificial mixtures of secondary metabolites. Both DOSY (diffusion-ordered spectroscopy) and multivariate (DECRA) processing of the diffusion-encoded NMR raw data of these mixtures have been used to extract the spectra of the different components before database matching. For creating and mining the in-house database, we have employed ACD/Labs software. A discussion on the capabilities and actual limitations of this approach based on the preliminary results obtained will be presented.

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117 A New Approach to Separate Chemical Shift and Scalar Coupling of 1D Proton Spectra

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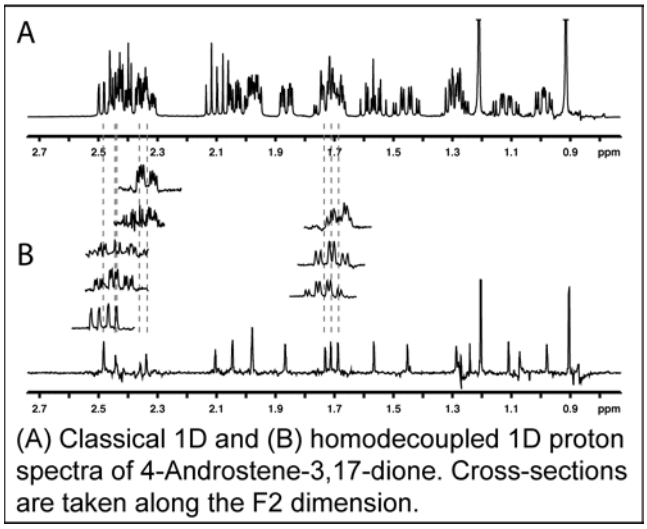
In ^{1}H spectra, the multiplet structure due to homonuclear scalar coupling makes it difficult to properly analyze 1D spectra in the case of complex samples with severe signal overlap. Obtaining 1D pure shift proton spectra is therefore a quite interesting decades-old challenge [1-4]. We propose here a new approach based on spacial encoding to quickly obtain high-resolution 2D spectra leading to homodecoupled 1D spectra after a simple processing. The experiment is based on the Zangger-Sterk element [5] and combined with spectral aliasing to increase the resolution in the indirect F1 dimension. The resulting 2D spectra show only diagonal signals with a multiplet structure only along the F2 dimension. A simple manipulation of the spectrum cleanly separates chemical shift and scalar coupling.



(A) Classical 1D and (B) homodecoupled 1D proton spectra of 4-Androstene-3,17-dione. Cross-sections are taken along the F2 dimension.

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118 Six-Membered Saturated Heterocycles (N,P,S,Se) and Their Oxidation Products. Configuration Determination Using Exp. and Calc. NMR Chemical Shifts

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The interest in determining the absolute configuration of chiral compounds stems from the widely recognized fact that the stereochemistry often determines important chemical and physical properties, biological activities and pharmaceutical use of these compounds [1,2]. Classical NMR methods for configuration determination using vicinal coupling constants and/or NOE are not always applicable. Therefore we decided to develop a method combining experimental and theoretically calculated NMR data for determination of the configuration at heteroatoms in *N*-oxides, phosphinoxides, sulfoxides and selenoxides.

The six-membered saturated heterocycles (4-*tert*-butyl-1-methylpiperidine (**1**), 4-*tert*-butyl-1-methylphosphinane (**2**), 4-*tert*-butyl-tetrahydro-*2H*-thiopyran (**3**) and 4-*tert*-butyl-tetrahydro-*2H*-selenopyran (**4**)) were prepared as suitable model compounds with well-defined geometry for an NMR study of their oxidation products. The corresponding epimeric N-oxides, phosphinoxides, sulfoxides and selenoxides were obtained by standard chemical preparation (oxidation with hydrogen peroxide) and also by *in situ* oxidation of **1 – 4** with *meta*-chloroperbenzoic acid directly in the NMR tube.

The experimental ^{1}H and ^{13}C chemical shifts were compared with corresponding calculated data obtained by GIAO approach with DFT and HF methods and various basis sets. The correlation of experimental *vs* calculated data showed the possibility to determine the stereochemistry of the epimeric oxidation products using fast DFT B3LYP/6-31G* method for both geometry optimization and NMR chemical shifts calculation. The application of this approach for configuration assignment in the series of sulfoxides and *N*-oxides is described in our recent papers [3-7].

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119 Conformation Analysis of Se and Te Containing Nucleoside Analogues

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The furanose five-membered ring conformation plays an important role in chemical and biological properties of nucleosides and nucleotides. In natural nucleosides and nucleotides, it usually adopts C3'-*endo* or C2'-*endo* arrangement with fast transition between these two conformers. Therefore, all NMR parameters are averaged according Boltzman distribution. To the best of our knowledge, an individual conformers of five-membered ring have not been yet distinguished in NMR spectrum.

Our goal is therefore to synthesise nucleoside analogues capable of the observation of individual conformers by low-temperature NMR. Inspired by the fact, that changing O atom for S in tetrahydrofurane ring leads to increasing of pseudorotation barrier[1] we have synthesized thymine and adenine nucleoside analogues containing Se and Te within five-membered ring. The barrier of pseudorotation and conformation preferences was then examined by DFT methods. The replacement of Se for Te results in 1 kcal /mol increase of the interconversion barrier. The results of conformation analysis via molecular modelling were compared with results from the NMR experiment. We have found that conformation preferences manifested by averaged ^{3}J(H,H) were significantly influenced by the presence of Se or Te in five-memebered ring. While Se-containing analogues exist as about 1:1 (adenine) to 3:2 (thymine) mixture of two conformers, the analogue containing Te prefers mostly (~90%) one conformation in the solution. The more stable conformer in solution for all studied compounds was north conformer according ribose/deoxyribose notation. Contrary, in solution less populated south conformer was found in crystal structure for thymine Se-containing nucleoside analogue. In addition to that, comparison of experimental and theoretical ^{13}C chemical shifts enabled the estimation of *syn*/*anti* orientation of the nucleobase.

Theoretically predicted barrier of interconversion between two conformers, which is >4 kcal/mol, has encouraged us to conduct low-temperature NMR study that should provide experimental evidence of conformers equilibrium and the pseudorotation barrier. Similar study was recently done for six-membered 1,4,2-oxazasilinanes[2] where barrier to ring inversion was 4.8 kcal/mol. The low-temperature NMR experiments are currently under way.

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120 Finding the Balance: Optimum Reprocessing and Reporting Strategies for the Automatic Analysis of FBLD NMR Data

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Fragment Based Lead Discovery (FBLD) has become a powerful tool in the drug discovery arena and it has been directly responsible for identifying dozens of drug candidates over the last decade.

The identification of said candidates is performed through the careful analysis of hundreds, sometimes thousands, of experiments, the analysis and reporting steps do tend to be long and tedious and frequently performed several times. Given how slow and at the same time important is this the analysis, an automatic analysis tool in order to automate and make these steps more nimble was developed[1].

Over the past decades a considerable amount of effort has been spent in identifying any potential pitfalls at each one of the experimental steps of FBLD, certain aspects of these have been summarised[2]. Less attention has been placed on the reprocessing and analysis strategies, in the following work the methodologies and approaches which have been proven to provide better results will be enumerated and explained.

The dependence of the results with certain reprocessing and analysis parameters, such as apodisation function and values, phase correction and baseline deviations were analysed and the results are detailed in order to bring attention to the importance of said parameters in not only the automatic analysis but also in the manual interpretation of results.

Special emphasis will be placed on nobel automatic phasing (developed specially for FBLD experiments) and optimum baseline correction methods, as well as in specific strategies depending on the data to be analysed: T1rho, CPMG, wLOGSY, STD and solvent suppresed samples, detailed conclusions will be presented for each type of experiments class.

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121 NMR Ivestigation of Strong Intramolecular Hydrogen Bonds in 5-Nitrosopyrimidines

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Hydrogen bonds are very important non-covalent interactions. Intramolecular hydrogen bonds can have an influence on the polarity of the molecule, which may lead to better membrane permeability. Natural pyrimidine derivatives play a key role in nucleic acids and modified pyrimidines are pharmacologically important; they are used as cytostatics, antivirotics or antimicrobial agents.

In this work, we prepared a series of 31 polysubstituted 5-nitrosopyrimidine derivatives. Substituted amino groups (cycloalkylamino, alkylamino, amino, acetylamino) and *para*-substituted phenylamino groups were attached to positions 4 and 6, respectively. The nitroso group can form intramolecular hydrogen bonds with both NH hydrogens in the neighboring positions (4 and 6), which leads to an equilibrium of two rotamers. Both rotamers are observable in ^{1}H and ^{13}C NMR spectra acquired at room temperature. The hydrogen bonds are so strong that even at 140 °C no signal averaging was observed. The rotamer ratio in equilibrium depends significantly on the nature of substituents in the positions 4 and 6 and could be finely tuned in the whole range (32-84 %) of A form (the nitroso group heading towards the 6-NH group). Electron-donating *para*-substituents of the 6-phenylamino group lead to the higher amount of rotamer A. The observed rotamer ratio correlates well with Hammett constants of the substituents. The orientation of the nitroso group has also a big influence on chemical shifts of carbon atoms of the pyrimidine ring, especially on C-4 and C-6. In the rotamer A, the C-4 atom is less shielded than the carbon atom C-6. The rotamer ratios and NMR parametres were also calculated using DFT method with a good agreement with the experimental data.

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122 Metabolomics of Mouse Models of Obesity and Lipoatrophy

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Type 2 diabetes mellitus (T2DM) is caused by a complex set of interactions among genetic and environmental factors and results from combination of insulin resistance and insufficient insulin secretion. Paradoxically, severe insulin resistance typically occurs not only in subjects with obesity but also in those with lack of adipose tissue. Insulin resistance is now accepted as a common basis of both T2DM and lipoatrophic diabetes [1]. NMR-based metabolomics represents an effective approach how to study such complex mechanisms because every change in metabolic status of an organism is reflected in NMR spectral profiles of biofluids and even complex samples can be measured and evaluated without any previous separation step [2]. Performing metabolomic studies in inbred rodents possesses significant advantages compared with human studies as the genetic background as well as environmental and experimental conditions can be better controlled.

Our project is focused on study of metabolic changes resulting from insulin resistance. Three different mouse models were applied, e.g. mice C57BL/6J fed by high fat diet as model of diet-induced obesity, NMRI mice with monosodium glutamate induced obesity, and A-ZIP mice as a model of lipoatrophy. We now present our preliminary results of an initial metabolic characterization of models under study. They indicate that groups of control and insulin-resistant mice can be distinguished using multivariate analysis of NMR spectra of urine samples but the careful evaluation of urine collection protocol is necessary.

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123 NMR spectroscopic Characterization of Unsymmetrical Cube-Octameric Silsesquioxanes (COSS)

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Polyhedral oligosilsesquioxanes (POSS) have long been studied as models for silica surfaces and other silica bound catalyst systems. Due to the availability of cheap precursor materials and the flexibility in the choice of the side chains, a wide range of applications as e.g. nanocomposite materials or homogeneous catalysts were developed.[1] Furthermore, they also show promising features as scaffolds for bio-logical applications.[2,3]

We present a series of molecular cube-octameric silsesquioxanes (COSS) with the general formula $(SiO_{1.5})8R'_nR_{8-n}$, used as fluorescence tags. Following the unsymmetric substitution, the silicon sites of the central silica polyhedron are chemically inequivalent and the (electronic) structure of these nanoparticles is readily studied by NMR spectroscopy. The R' side chain carries a fluorescence marker, while the other corner substituents are varied to modulate stability, solubility and chemical behavior.

Using ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{29}Si HMBC measurements a full assignment of all resonances, including the non-isochronous Si sites, is established. This not only confirms the integrity of the cage-like central structure, but also enables the investigation of side chain influences and of possible hydrolysis and decomposition pathways.[4]

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124 Combination of qNMR and DART-MS for the Fast Analysis of Suppositories without a Preliminary Extraction of Hydrophilic Substances

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Pharmacopoeia monographs of many countries refer to chromatographic and spectroscopic methods for component detection and quantitation after their extraction from pharmaceuticals. Such procedures requires using reference materials for quantitative determination and are time-consuming. Also the integral inaccuracies of the determination of the analytes increase with the number of analysis steps.

According to the definition of the CCQM (Comite Consultatif pour la Quantite de Matiere), NMR spectroscopy is a primary method for quantitative measurements [1,2]. It was used in our laboratory for the systematic analysis of drugs in the form of suppositories, containing hydrophilic (active pharmaceutical substances) and lipophilic (mainly triacylglycerides, TAGs) components.

We propose the following algorithm for determining their composition:

1. Selection of co-solvents to completely dissolving the drug.
2. Identification and semi-quantitative determination of active substance by DART-MS.
3. Registration of ^{1}H NMR spectra of solutions using appropriate deuterated co-solvents with the known amount of residual non-deuterated solvents.
4. Identification of the signals of target components by 2D DOSY experiments and choice of the signals for quantitative determination.
5. Development of the simplified procedure of analysis without using DART-MS and 2D DOSY.
6. Adaptation of the procedure for low-frequency magnetic fields NMR-spectrometers (45 MHz and more).

The algorithm has been employed for the determination of drug authenticity and for submitting the monographs to Russian Pharmacopoeia for suppositories, containing such active pharmaceutical substances as "Imunofan" (hexapeptide Arg-Asp-Lys-Val-Tyr-Arg) and

"Arbidol" (1-methyl-2-((phenylthio)methyl)-3-carbethoxy-4-((dimethylamino)methyl)-5-hydroxy-6-bromindole). These experiments were carried out using DART mass spectrometer (JEOL), NMR ECA-600 spectrometer (JEOL) and Picospin-45 spectrometer (Picospin). D_2O and TFAA-d_1 were used as co-solvents. At a weight ratio of lipophilic (hard fat) and hydrophilic (active pharmaceutical substance) parts of the drug of 50:1, the determination inaccuracies did not exceed 5%, which satisfies the requirements for pharmaceutical analysis.

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125 qNMR 1H: Aromatic Carbon Content (Car) and Fractional Composition of Petroleum and its Products Determination

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In accordance to REACH [1] reglament petroleum products imported to EU must be characterized by Car (aromaticity). Official method for aromaticity fossil fuel products determining is test method ASTM D5292 (reapproved 2009) based on liquid state ^{13}C qNMR. Industrial applications of ASTM connected with some problems: needs additional measurements of ^{1}H NMR spectra for verification absence of olefinic carbons, need to use a stationary high sensitivity NMR spectrometer, much time (several hours) for a single measurement. Earlier for light petroleum fraction next semi – empirical relationship between aromatics from ^{1}H and ^{13}C spectra has been found by Cookson [2]: $Car=[k(((Har)^{-1}-0.01)+0.01]^{-1}$. This equation can be used for Car in gas oils, kerosenes and some other petroleum and coal distillates but not for petroleum and height boiling materials.

We have found [3] that petroleum values Car are correlated with content ^{1}H atom of different molecular fragments ($Har+H\alpha + H\beta+ H\gamma =\Sigma H$). These fragments can be easily measured from the ^{1}H NMR spectra by using any high-resolution NMR spectrometer with an operating frequency of 30 MHz and higher.

Analysis of the results of quantitative ^{13}C (150 MHz) and ^{1}H (600 MHz and 45 MHz) NMR spectra of more than 40 petroleums and its products (from gasoline to bitums) allow us to get new universal relationship for prediction Car across ^{1}H NMR results:

$$Car=-9.962(\pm1.991)+3.499(\pm0.274)*Har+0.557(\pm0.129)*H\alpha+0.157(\pm0.032)*H\beta$$

$$R^2=0.969, \quad \sigma=0.93$$

Validation of this relationship was applied to our previously published results of ^{1}H and ^{13}C for 41 petroleum [4]. Average difference of Car measured and calculated from ^{1}H NMR spectra was found lower than 1%.

^{1}H NMR spectra which were registered by compact spectrometer showed adequacy of the results for the 45 and 600 MHz. These results allow us to recommend quantitative ^{1}H NMR spectra for the estimation of Car petroleums, using industrial commercial spectrometer with frequencies of 30 MHz and more. The limits of applicability of the new approach will be determined later.

Petroleum (merchantable oil) imported to EU is limited by fractional composition (FC), BP–200^{o}C (F1) and 200^{o}C – 300°C (F2). There are different procedures for determining the FC using its separation into individual temperature fractions by distillation in standard equipment under specified conditions.

The objects used to develop the procedure formed a set of 16 reference merchantable oils with different density (from 0.697 to 0.870 kg/dm3) and considerable variations in other characteristics (viscosity and sulfur, resin, asphaltene, and wax contents).

Calculation of the coefficients for multiple linear regression equations gave the following results:

$$F1 = -84 + 3.05\ (\pm 0.26)Hm + 0.44\ (\pm 0.14)Ham + 12.54\ (\pm 1.71)\ Harm - 21.41$$

$$(\pm 5.28)\ Harf;\ R^2 = 0.977$$

$$F2 = -29 + 2.24\ (\pm 0.28)Hm + 0.52\ (\pm 0.17)Ham + 12.30\ (\pm 1.80)Harm - 33.74\ (\pm 5.54)Harf;\ R^2 = 0.969$$

We successfully used for measurement these characteristics low-frequency NMR spectrometer (45 MHz).

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