SMASH 2007 NMR Conference

Chamonix, France

September 16th - 19th, 2007

SMASH 2007 NMR Conference

Dear SMASH 2007 Attendees,

Welcome to Chamonix and the 2007 SMASH NMR Conference!!!

This year's program contains an exciting array of oral sessions and workshops, all of which are to be held in the Majestic Conference Center. Oral session topics include: Metabolomics, Detection and quantification at the limits of sensitivity, Latest movements in solids, Spectral interpretation and quantification, Advances in the application of diffusion/dynamics, NMR in the food sciences, Novel developments in heteronuclear NMR spectroscopy, and the Student/Post-Doctoral session.

As in the past, the program also includes four workshops. Monday's workshop topics include the latest applications and developments in pulse programming, and the latest advances in methods and tools for Metabolomics. The workshops for Tuesday deal with Classical and novel approaches for elucidation of structure and Enhancing sensitivity.

In addition, we will be treated to an after-dinner talk on Monday night entitled 'Adapting to a Vertical (and rapidly changing) Environment' by keynote speaker, John Shockcor.

This year for the first time SMASH is not being held solely in one hotel and is taking advantage of the variety of hotels and restaurants within Chamonix. As you will find from the enclosed map, all are within close proximity to one another and offer the compensation of the picturesque Mont Blanc scenery.

On behalf of the entire SMASH Organizational Committee, we wish to thank you for your continued interest in, and support of, the SMASH NMR Conference.

We hope you enjoy this year's conference!!!

Sincerely,

Andy Nicholls and Eric Munson
Co-Chairs, SMASH 2007 NMR Conference

SMASH 2007 NMR Conference
Program

Sunday September 16th

4:30 PM - 6:00 PM **Registration, Majestic Conference Centre**
6:00 PM - 8:00 PM **Dinner, Restaurant Panoramique Le Vista, Hotel Alpina**
8:00 PM -11:00 PM **Mixer, Restaurant Panoramique Le Vista, Hotel Alpina**

Monday September 17th

8:15 AM - 8:30 AM **Opening Remarks**
8:30 AM - 10:00 AM **Metabolomic**
Chair: Jules Griffin, University of Cambridge

- **Advances in Metabolomics in Pharmaceutical R&D; From Mouse to Man**
  Susan C Connor, GlaxoSmithKline, UK
- **^{1}H NMR Metabonomics of Serum in the Risk Assessment and Diagnosis of Atherothrombotic Diseases – The Synergy Between Lipoprotein Lipids and Small Molecules**
  Mika Ala-Korpela, Helsinki University of Technology, Finland
- **Principles of High Resolution Magic Angle Spinning NMR: Applications in a Medical Environment**
  Martial Piotto, Bruker, France

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Detection and Quantification at the Limits of Sensitivity** Chair: Andrew Webb, Penn State University, USA

- **High Sensitivity NMR: Natural Product Discovery in Insects and Worms**
  Art Edison, University of Florida, Gainesville, USA
- **Potential of CapNMR Combined with MS-directed Microfrationation for Biomarker Identification in Plant Metabolomic Studies**
  Jean-Luc Wolfender, University of Geneva, Geneva, Switzerland
- **Into Thin Air: Approaches to NMR Analysis of Limited Quantities of Small Organic Molecules**
  Dan Sørensen, Merck Frosst, Canada

12:00 PM - 1:30 PM **Lunch, Restaurant Panoramique Le Vista, Hotel Alpina**
1:30 PM - 3:00 PM **Latest Movements in Solids**
Chair: Eric Munson, University of Kansas, USA

- **NMR Study of Structure and Dynamics of Amino Acids and Small Peptides in the Solid State**
  Marek Potrzebowski, Polish Academy of Sciences, Poland
- **High-Resolution Proton Solid-State NMR for Small Molecule Crystallography**
  Benedicte Elena, Ecole Normale Superlieure de Lyon, France
- **Solid-state Structure of a Plueromutlin and Succinic Acid: Lessons Learned from Solid-state NMR**
  Jacalyn Clawson, GlaxoSmithKline, USA

3:00 PM - 3:30 PM **Break**
3:30 PM - 5:00 PM **Workshops (Concurrent)**

- **I. Pulse Programming : Latest Applications and Developments**
  Krish Krishnamurthy, Lilly, USA
  Andrew Gibbs, Bruker Biospin, UK
  Peter Sandor, Varian, Germany
- **II. Metabolomics: Latest Advances in Methods and Tools**
  Andy Craig, BlueGnome, UK
  Oliver Cloerec, Servier, France
  Steve Bruce, Nestle Switzerland

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 6:30 PM **Social Gathering, Hotel Prieuré**
6:30 PM - 9:00 PM **Dinner, Restaurant Hotel Prieuré**
After Dinner Speaker: John Shockcor
9:00 PM - 11:00 PM **Mixer, Hotel Prieuré**

Tuesday September 18th

8:30 AM - 10:00 AM **Spectral Interpretation and Quantification**
Chair: Mike Bernstein, AstraZeneca, UK

- **Advances in Computer-Assisted Evaluation of NMR Spectra**
  Stan Sýkora, Extrabyte, Italy
- **^{19}F/1H-^{15}N Correlation Techniques for Structure Elucidation of Heterocycles**
  Steve Cheatham, Dupont, USA
- **Quantitative NMR in Research Chemistry**
  Richard Upton, GlaxoSmithKline, UK

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Advances in the Application of Diffusion/Dynamics**
Chair: Cynthia Larive, UC Riverside, USA

- **Pure Shift DOSY: Simplifying and Separating the Spectra of Mixture Components**
  Gareth Morris, University of Manchester, UK
- **Industrial Applications of Diffusion NMR; Applications to Mixture Analysis**
  Betsy McCord, Dupont, USA
- **NMR of Small Stuff: A Pharmaceutical Perspective on Dynamics**
  Edward Zartler, Merck & Co., USA

12:00 PM - 1:30 PM **Lunch, Hotel Prieuré**
1:30 PM - 3:00 PM **NMR in Food Science**
Chair: Ana Gil, University of Aveiro, Portugal

- **NMR in Human Nutrition**
  Ian J. Colquhoun, Institute of Food Research, UK
- **NMR-based Nutri-metabonomics for Optimized Nutrition and Health**
  Serge Rezzi, Nestle, Switzerland
- **NMR-based Hyphenation in Metabolomics**
  Manfred Spraul, Bruker Biospin, Germany

3:00 PM - 3:30 PM **Break**

3:30 PM - 5:00 PM **Workshops (Concurrent)**

- **I. Elucidation of Structure: Classical and Novel Approaches**
  Martin Will, sanofi-aventis, Germany
- **II. Enhancing Sensitivity**
  Jerzy Jaroszewski, University of Copenhagen, Denmark
  Dan Staerk, University of Copenhagen, Denmark
  Manfred Spraul, Bruker Biospin, Germany

5:00 PM - 6:00 PM **Free Time**
6:00 PM - 7:30 PM **Dinner, Restaurant La Calèche**
8:00 PM - 10:00 PM **Poster Session & Mixer, Majestic Conference Centre**
10:00 PM - 11:00 PM **Mixer, Majestic Conference Centre**

Wednesday September 19th

8:30 AM - 10:00 AM **Novel Developments in Heteronuclear NMR Spectroscopy**
Chair: Stefano Provera, GlaxoSmithKline, Italy

- **Solid-state NMR Investigations of Inorganic and Bioinorganic Model Systems in Ultra-high Magnetic Fields**
  David Bryce, University of Ottawa, Ontario, Canada
- **Development and Applications of Natural Abundance Deuterium NMR in Chiral Oriented Solvents: an Original Analytical Tool for (Bio)Chemists**
  Philippe Lesot, Université de Paris-Sud, France
- **Big Signals Without Big Magnets: An Early Assessment of the Utility of Dynamic Nuclear Polarisation for Liquid State 13C and 15N NMR Spectroscopy**
  Adrian Davis, Pfizer, UK

10:00 AM - 10:30 AM **Break**
10:30 AM - 12:00 PM **Student and Post-Doctoral Session**
Chair: Jake Bundy, Imperial College London, UK

- **Automated Quantification of Complex 1H NMR Spectra**
  Geoff Gipson, Drexel University, USA
- **New NMR Methods for Small Molecules**
  Christian Ludwig, University of Birmingham, Birmingham, UK
- **Sensitivity Enhanced ^{13}C and ^{15}N NMR Based Metabolomics Using a Chemical Derivatization Approach**
  Murthy Shanaiah, Purdue University, USA

12:00 PM - 12:15 PM **Closing Remarks**
12:15 PM - **Box Lunches, Majestic Conference Centre**

SMASH 2007 NMR Conference
Acknowledgements

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Advanced Chemistry Development, Inc., (ACD/Labs)
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Poster Session

Julian Griffin
University of Cambridge, UK

Monday, September 17th
8:30 AM - 10:00 AM

Metabolomics
Chair: Jules Griffin

Speakers:

Susan Connor
GlaxoSmithKline UK

Mika Ala-Korpela
Helsinki University of Technology, Finland

Martial Piotto
Bruker, France

Advances in Metabolomics in Pharmaceutical R&D; From Mouse to Man

S. C Connor

Metabolomics Group, GlaxoSmithKline, Park Road, Ware, Herts UK

Metabolomics (metabolic profiling/metabonomics) continues to thrive and is beginning to provide more than mere promises. The scope of metabolomics applications within GSK is described with reference to recent diabetes studies in mouse and man. Key results are put in context of both of known aspects of the disease and in terms of their usefulness in assessing drug development in the preclinical and clinical areas. The correlations of disease related effects between the mouse model and clinical disease will be described, with particular reference to the activity of 3 antidiabetic drugs. The ability to predict the response of a subject to treatment in baseline samples will also be described.

^{1}H NMR Metabonomics of Serum in the Risk Assessment and Diagnosis of Atherothrombotic Diseases – The Synergy Between Lipoprotein Lipids and Small Molecules

M. Ala-Korpela

Helsinki University of Technology, Laboratory of Computational Engineering, Systems Biology and Bioinformation Technology, Espoo, Finland

The central role of lipoprotein subclasses in the risk assessment of coronary heart disease (CHD), diabetes, and other diseases is currently well-established. ^{1}H NMR spectroscopy as a method to analyse lipoprotein subclasses has received wide clinical interest since the experimental part is a fast, routine procedure [1,2]. It is notable that ^{1}H NMR of serum can also provide data on other, potentially relevant biomolecules [2,3]. Thus, ^{1}H NMR offers two concomitant approaches – to quantify individual metabolites and also to consider the spectra of all the NMR-detectable compounds as metabolic profiles that relate, e.g., to the risk of CHD. The latter holistic approach – metabonomics – suggests that we do not need to quantify each metabolite if there are means to classify the profiles in an appropriate manner. The quantitative approaches and the metabonomic methodologies are complementary. However, in extensive clinical studies aiming for risk profiling, the metabonomic approach may well be more appropriate to start [4]. We have recently applied two molecular windows, LIPO and LMWM, in metabonomic ^{1}H NMR studies of serum [3]. The LIPO window represents a typical spectrum of serum showing broad overlapping resonances arising mainly from lipoprotein lipids [2]. The LMWM window takes the advantage of T2-relaxation to modify the detectable molecular information, thus enabling improved detection of low-molecular-weight metabolites [2,3]. In the talk, results that demonstrate the inherent suitability of ^{1}H NMR metabonomics to identify subtle changes in lipoprotein subclass-related metabolism are shown [1,4]. Also, we illustrate how the multi-metabolite data via ^{1}H NMR reveal the continuum of diabetic complications in a cohort of over 700 type 1 diabetic patients [5]. We also show preliminary results from a ^{1}H NMR metabonomics study of the metabolic characteristics in cognitive impairment [6]. This study also includes a third molecular window, namely data on extracted serum lipids. All these collaborative studies show the value of combining the macromolecular information with data from small molecules in clinical ^{1}H NMR metabonomics.

1. M. la-Korpela, et al. Atherosclerosis. 2007;190:352.
2. M. Ala-Korpela. Progr Nucl Magn Reson Spectr. 1995;27:475.
3. V.P. Mäkinen, et al. Magn Reson Mater Phy. 2006;19:281.
4. T. Suna, et al. NMR Biomed. 2007; in press.
5. V.P. Mäkinen, et al. 2007; submitted.
6. T. Tukiainen, et al. 2007; in preparation.

Principles of High Resolution Magic Angle Spinning NMR: Applications in a Medical Environment

M. Piotto[1,2], G. Erb[1,7], K. Elbayed[1], J. Raya[1], A. Neuville[3], M. Mohr[3], D. Maitrot[4], P. Kehrli[4] , M. Martínez-Bisbal[6], D. Monleon[5], O. Assemat[2], Bernardo Celda[6] and I. Namer[7]

1. ULP/CNRS LC3-UMR 7177, Strasbourg, France
2. Bruker Biospin, France
3. Department of Pathology, Strasbourg University Hospitals, France
4. Department of Neurosurgery, Strasbourg University Hospitals, France
5. FIHCUV, Hospital Clínico Research Fundation, University of Valencia, Spain
6. Department of Physical Chemistry, University of Valencia, Spain
7. Department of Biophysics and Nuclear Medicine, Strasbourg Hospitals, France

High Resolution Magic Angle Spinning (HRMAS) NMR [1] is a technique that allows the study of relatively mobile compounds contained within heterogeneous substances. The domain of application of HRMAS is extremely diverse and includes the analysis of molecules issued from solid phase synthesis, molecules in a membrane environment, swollen polymers, cells and more recently biopsies. These compounds are characterized by a strong heterogeneity and a complex distribution of magnetic susceptibilities that precludes obtaining high resolution NMR spectra under static conditions. An efficient solution to reduce the linewidth of these samples is to study them in rapid rotation at the magic angle (54.7°) [2].

A particularly promising field of application for HRMAS is the medical analysis. Using HRMAS, it becomes possible to identify a large fraction of the metabolites contained in an intact human biopsy [3]. Analyzing these metabolic data using multivariate statistical analysis techniques, it becomes possible to differentiate different types of tumors based solely on their metabolic content. This possibility is of particular value for the medical community since tumor typing and grading is a key element in the decision-making process that will lead to prognosis and therapeutic treatment. This classification relies today essentially on morphological criteria obtained through histopathological study of the biopsy. However, for certain types of human brain tumors, like oligodendrogliomas, this classification is not sufficiently reliable. The use by the neurosurgeon of histopathological data associated with metabolic data could lead to a better prognosis and better therapeutic management of the patient [4].

1. G. Lippens, M. Bourdonneau, C. Dhalluin, R. Warras, T. Richert, C. Seetharaman, C. Boutillon and M. Piotto, Curr. Org. Chem., 1999, 3, 147-169.
2. D. Doskocilova , D. Duc Tao ,B. Schneider, Czech. J. Phys. B., 1975, 25, 202.
3. M. Carmen Martínez-Bisbal, et. al, NMR in Biomed., 2004, 17, 191-205.
4. G. Erb, K. Elbayed, M. Piotto, J. Raya, A. Neuville, M. Mohr, D. Maitrot, P. Kehrli, I. Namer, Magn. Reson. Med., submitted.

Monday, September 17th
10:30 AM - 12:00 PM

Detection and Quantification at the Limits of Sensitivity
Chair: Andrew Webb

Speakers:

Art Edison
University of Florida, USA

Jean-Luc Wolfender
University of Geneva, Geneva, Switzerland

Dan Sørensen
Merck Frosst, Canada

High Sensitivity NMR: Natural Product Discovery in Insects and Worms

Arthur S. Edison[1,2,3], Ramazan Ajredini[1], Aaron T. Dossey[1], Fatma Kaplan[1], James R. Rocca[2], Cherian Zachariahy

1. Department of Biochemistry & Molecular Biology, University of Florida, Gainesville FL
2. McKnight Brain Institute, University of Florida;
3. National High Magnetic Field Laboratory, University of Florida

Nuclear magnetic resonance is a critical tool in natural product identification. Unfortunately, NMR is inherently a very low-sensitivity technique that traditionally requires relatively large amounts of sample. This limitation places great demands on natural product identification, because sample preparation is often tedious.

We have developed a new 600 MHz 1-mm NMR probe made from high temperature superconducting (HTS) material [1]. This probe has triple resonance capabilities (^{1}H, ^{13}C, ^{15}N, and ^{2}H lock) and a z-axis gradient. The total sample volume required ranges from about 5 to 10 μL, depending on the wall thickness of the tubes. The signal to noise per mass of sample is approximately 25x greater than a conventional room temperature probe or over 6x greater than a commercial 5-mm cryoprobe. Using this probe, we have been able to analyze less than 1 μL of the secreted venom of a single walkingstick insect [2]. We found that the stereoisomeric composition of the venom's monoterpene components changes between individuals and also over time within a single individual animal. More recently, we have found that the observed isomeric variation changes as a function of regional distribution and life stage development, suggesting additional roles for these secretions.

Using the 1-mm HTS probe and other analytical techniques, we have also discovered new natural product compounds from very small quantities of starting material. Using just a few insects we have identified a novel monoterpene in the defensive secretion of Parectatosoma mocquerysi, a walkingstick insect from Madagascar [3]. We are also utilizing this probe to identify secreted metabolites from the soil nematode, Caenorhabditis elegans.

The ability to examine very small amounts of material by NMR has allowed new questions to be addressed about chemical biodiversity and opens up numerous opportunities for drug discovery using natural products.

1. W. Brey, A.S. Edison, R.E. Nast, J. Rocca, S. Saha, & R. Withers, J. Magn. Reson. 179, 290-3 (2006).
2. A.T. Dossey, S.S. Walse, J.R. Rocca, & A.S. Edison, ACS Chem. Biol., 1 (8), 511–514 (2006).
3. A.T. Dossey, S.S. Walse, O.V. Conle, A.S. Edison, In Press J. Nat. Prod. (2007).

Potential of CapNMR Combined with MS-directed Microfrationation for Biomarker Identification in Plant Metabolomic Studies

G. Glauser[1,2], E. Grata[1,2], D. Guillarme[2], J. Boccard[3], P.A. Carrupt[3], S. Rudaz[2] and **J.L. Wolfender**[1]

1. LPP, 2. LCAP, 3. LCT
School of Pharmaceutical Sciences, University of Geneva,
University of Lausanne, Geneva, Switzerland

Plant metabolomics has gone, in a short time, from being just an ambitious concept to a rapidly growing, valuable technology which gives a global picture of plant molecular organisation at the metabolite level. For the detection of minor key stress biomarkers, UPLC-TOF-MS methods have proven to provide, with the help of advanced data mining, an efficient way to localise interesting biomarkers [1]. Often however, the structure determination of the biomarkers cannot rely only on mass spectral information. Thus in our approach, high resolution LC-MS triggered isolation of the biomarkers was conducted for a further complete structure determination at the microgram scale with capillary NMR (CapNMR) [2].

In the selected example, significant metabolome variations related to stress caused by wounding in the model plant Arabidopsis thaliana (Brassicaceae) were studied by UPLC-TOF-MS. Extensive research on the wound-response has indeed brought important information on the plant defence mechanism against herbivores and different bioactive oxygenated fatty acids belonging to the jasmonate family were found responsible for the expression of defence genes [3]. UPLC-TOF-MS permitted the detection of significant minor wound-biomarkers peaks among major constitutive metabolites. Besides known signalling molecules, original oxylipins and related products were highlighted. A targeted LC-MS triggered microfractionation of these metabolites of interest at the semi-preparative level, based on computed LC conditions from UPLC gradients, enabled the successful purification of the biomarkers. Based on this approach, a complete structural determination of the unknowns with at-line CAP-NMR was made possible thank to extensive 1D and 2D NMR experiments. Because of the convoluted nature of plant extracts and the important dynamic range in both polarity and concentration domains, this task was found very challenging for at-line LC-NMR studies.

Acknowledgements: SNSF is thanked (grant 205320-107735 to JLW and SR).

1. J. Boccard, et al., Chem. Intel. Lab. Syst. (2007) 86, 189-197
2. D.L. Olson, et al. Anal. Chem. 76 (10), 2966, 2004
3. E.E. Farmer, et al., Curr. Opin. Plant Biol. (2003) 6, 1-7

Into Thin Air: Approaches to NMR Analysis of Limited Quantities of Small Organic Molecules

Dan Sørensen and Laird A. Trimble

Merck Frosst Centre for Therapeutic Research, Merck Frosst Canada Ltd., Kirkland, Québec, Canada.

Modern technology has enabled the development of NMR into a truly analytical tool. High-field magnets coupled with cryogenic or capillary NMR probes, sophisticated pulse sequences, and computing power provide unprecedented sensitivity and access to structural and quantitative data from microgram amounts of small organic molecules. This 1000-fold change in scope, from milligrams to micrograms, is changing the way we employ NMR as a research tool.

LC-MS-SPE is a convenient method to rapidly isolate microgram quantities of compounds from complex mixtures (i.e., natural products, drug metabolites, etc.). Analytical HPLC columns afford optimal separation and resolution, MS provides confidence in the targeted isolation, and the integrated system minimizes contamination and handling issues. As illustrated by a case study [1], isolated material can be subjected to exhaustive structural analysis and quantitation by the use of traditional NMR tubes and NMR spectrometers equipped with cryogenic probes.

The small volume and high mass sensitivity of a capillary flow probe makes it ideal for automated acquisition of NMR data. A customized Protasis One-Minute NMR system is used for routine quality control and analysis of screening "hits" from the Merck sample collection as well as for chemical libraries of lead compounds in development. The NMR analysis consumes only 8 μL of a 10 mM DMSO-d6 solution (~ 20-60 μg) and provides additional quantitation by the application of an electronic internal reference signal (ERETIC).

1. Sørensen et al. J. Nat. Prod., 2007, 70, 121-123.

Monday, September 17th
1:30 PM - 3:00 PM

Latest Movements in Solids
Chair: Eric Munson

Speakers:

Marek Potrzebowski
Polish Academy of Sciences, Poland

Benedicte Elena
Ecole Normale Superlieure de Lyon, France

Jacalyn Clawson
GlaxoSmithKline, USA

NMR Study of Structure and Dynamics of Amino Acids and Small Peptides in the Solid State

J. Gajda, A. Jeziorna, S. Olejniczak, K. Trzeciak and **M. J. Potrzebowski**

NMR Laboratory and Department of Structural Studies of the Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, Łódź (Poland).

In recent years, high-resolution solid-state NMR spectroscopy has become an attractive and powerful tool for investigation of all kinds of condensed matter including crystalline, polycrystalline, amorphous samples, and glasses. New developments in methodology and instrumentation have extended the applicability of the technique. Today, solid-state NMR (SS NMR) spectroscopy is an indispensable method for characterization of biological samples. In this work we wish to present some recent applications of SS NMR in structural studies of amino acids and small peptides employing three models;

1) Tyr-D-Ala-Phe is the message sequence of dermorphin and deltorphins, natural opioid peptides that have been isolated from frogs (Phyllomedusa bicolor, Phyllomedusa sauVagei) that live on the border of Brazil and Peru. Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2) is the first known peptide produced by a eukaryote that contains D-amino acid. Opioid peptides with L-alanine are biologically inactive. Comparative structural analysis of tripeptide with D-alanine and L-alanine and new results showing interaction of peptides with phospholipid bilayers will be presented.[1]
2) L-selenomethionine (L-SeMet) is important amino acids commonly used in structure refinement of proteins. L-SeMet proteins now account for about two thirds of all new protein crystal structures phased by Multiwavelength Anomalous Diffraction (MAD). Systematic study of the thermal processes in L-SeMet crystals using Differential Scanning Calorimetry (DSC), SS NMR techniques, and X-ray diffraction will be shown.[2]

3) N-Benzoyl-L-Phenylalanine (N-BzPhe) is an interesting example of the polymorphism phenomenon in the solid state. Methodology of analysis of polymorphs based on ^{13}C-^{13}C recoupling strategy and 2D ^{1}H-^{13}C and ^{15}N-^{13}C HETCOR correlations will be discussed. [3]

1. Slabicki MM, Potrzebowski MJ, Bujacz G, Olejniczak S, Olczak J., J. Phys. Chem B 108 4535-4545, 2004
2. Gajda J, Pacholczyk J, Bujacz A, Bartoszak-Adamska E, Bujacz G, Ciesielski W, Potrzebowski MJ, J. Phys. Chem. B 110, 25692-25701, 2006
3. Hughes CE, Olejniczak S, Helinski J, Ciesielski W, Repisky M, Andronesi OC, Potrzebowski MJ, Baldus M. J. Phys. Chem. B, 109, 23175-23182, 2005

High-Resolution Proton Solid-State NMR for Small Molecule Crystallography

Bénédicte Elena[1], Elodie Salager [1], Anne Lesage[1], Guido Pintacuda[1], Chris Pickard[2] and Lyndon Emsley[1].

1. Laboratoire de Chimie, UMR 5182 CNRS/ENS Lyon, Ecole Normale Supérieure de Lyon, Lyon, France.
2. School of Physics & Astronomy, University of St Andrews, St Andrews, Scotland.

Proton spectroscopy of powdered organic molecules represents a potentially rich source of structural and dynamic information. With the recent improvement in homonuclear dipolar decoupling sequences used under Combined Rotation And Multiple Pulse (CRAMPS) conditions, ^{1}H resolution has been significantly increased to access dipolar couplings and thus structural information. Proton line-widths less than 0.2 ppm can be obtained at 700 MHz for crystalline powdered solids, using standard equipment and moderate magic-angle spinning frequencies using the eDUMBO family of decoupling sequences.

Here we present the refinement of the three-dimensional structure of organic compounds, in powder form and at natural isotopic abundance, obtained by an approach that in the first step combines molecular modeling (MM) in the Xplor-NIH program with experimental proton spin-diffusion data (PSD) obtained from high-resolution solid-state NMR of protons. This approach enables us to refine the molecular structure of β-Asp-Ala at natural abundance and in powder form to obtain a group of structures with an average rmsd of 0.1Å, and which deviates from the known structure by only ~0.6Å. Additionally, the conformation of thymol in its crystalline arrangement is investigated following the same MM-PSD optimization scheme. Thymol is a monoterpene phenol found in oil of thyme, with strong antiseptic properties. Due to the ease of obtaining large crystals, it was among the first systems studied by crystallographers, even before the advent of X-Ray methods.

In the second step we show how the PSD-MM structures can be used as a starting point for further refinement based on plane wave DFT geometry optimization and chemical shift calculations. This procedure results in structures that are identical to the known X-ray structure to within <0.2Å, and its validity is confirmed by comparing the DFT calculated chemical shifts for ^{1}H and ^{13}C with the experimental shifts. We observe a substantial improvement in the agreement between the calculations and experiments after DFT structure optimization.

Solid-state Structure of a Plueromutlin and Succinic Acid: Lessons Learned from Solid-state NMR

Jacalyn S. Clawson and Frederick G. Vogt

Chemical Development, GlaxoSmithKline, Plc, King of Prussia, PA

The solid-state structure of a drug can have an affect on the physical properties of the compound, such as density, hardness, solubility, and dissolution rate. These physical properties influence the bioavailability and manufacturability of the API and drug product. Hence it is desirable to understand the nature and to characterize the solid form of the drug. These solid forms can consist of polymorphs, solvates as well as salts or co-crystals when the API is combined with a second component. This presentation will illustrate how solid-state NMR can provide valuable structural information when single crystal X-ray data is not available. Specifically, the interaction between a pleuromutilin-derived antibiotic with succinic acid and water will be discussed. A hydrate salt prepared from the pleuromutilin derivative and succinic acid was discovered to have unusual properties during the course of drug development involving variability in the content of succinic acid. A variety of one and two-dimensional solid-state NMR experiments were used to understand the salt proton transfer and the hydrogen bonding network in this system, including ^{1}H MAS (at 35 kHz MAS rates), ^{1}H-^{13}C HETCOR, ^{13}C PASS and ^{15}N CPMAS. Variable humidity ^{13}C CP-TOSS and ^{2}H MAS and static experiments were utilized to understand how water interacts with the solid form. Finally, the solid-form of the API as is goes through the formulation process to a final drug product (an orally delivered tablet) will be discussed and studied by SSNMR.

Tuesday, September 18th
8:30 AM - 10:00 AM

Spectral Interpretation and Quantification
Chair: Mike Bernstein

Speakers:

Stan Sýkora
Extrabyte, Italy

Steve Cheatham
Dupont, USA

Richard Upton
GlaxoSmithKline, UK

Advances in Computer-Assisted Evaluation of NMR Spectra

Stanislav Sýkora[1] and Carlos Cobas[2]

1. Extra Byte, Castano Primo, Italy
2. Mestrelab Research, Santiago de Compostela, Spain

Though NMR spectra contain a lot of information about molecular structure, the link between an NMR spectrum and all compatible molecular structures is neither simple nor unique. It is still often mandatory to make use of a chemist's prior knowledge to reduce the number of 'admissible' molecules. With increasing sizes of the molecules of interest, we now encounter ever more complex spectra characterized by (i) incomplete spectral information, (ii) strong coupling, (iii) multiple matching spin systems and (iv) multiple molecules matching each spin system. The result is that interpretation of NMR spectra is again becoming an issue. nD and ^{13}C spectra can help to resolve some of these problems but, due to longer acquisition times, they are less fit for high-throughput operations.

The standard approach calls for an expert equipped with tools such as spectral prediction, simulation and fitting programs. The art of predicting spectral parameters from a molecular structure has finally reached practical usefulness, but its natural partner, the approximation-free spectral simulation, though it has reached maturity many decades ago, is still limited to a dismally small number of coupled spins (~12). Consequently, simulation nowadays calls for approximate algorithms involving novel spin-system fragmentation techniques. Parameters fitting methods are also in evolution since, due to the enormous number of transitions involved in present-day problems, the old Laocoon-like approaches are ruled out in favor of algorithms based on interval functions.

The time-honored style of proceeding from a molecule to the spectrum is also becoming inadequate because it relies on expert guessing. We must face the fact that the starting point is not the molecule but the spectrum - plus a set of a-priori ideas about what molecules might be acceptable. An artificial intelligence should therefore proceed from the spectrum to a molecule, taking into account any prior knowledge, and come up with a set of probable solutions. Such a perspective will profoundly affect future algorithms for computer-aided evaluation and interpretation of NMR spectra. It calls for two novel categories of data-evaluation techniques which will complement prediction, simulation and fitting. One is a multiplet spin-coupling analysis aiming at the enumeration of spin-systems which are compatible with the given spectrum. The other task is finding out all molecules compatible with each of the spin systems. Together, these two items represent the deductive path of spectrum evaluation, while prediction-simulation-fitting embodies the more traditional verification path.

We will discuss the inroads accomplished by our group along both of these paths.

$^{19}F/^{1}H$-^{15}N Correlation Techniques for Structure Elucidation of Heterocycles.

Steve Cheatham and Mike Kline

DuPont Crop Protection

Substitution of fluorine for proton is a common theme in the development of pharmaceutical and agrochemical compounds. Evaluation of ^{19}F-^{15}N and ^{19}F-^{13}C long range correlations can be of great benefit in structure confirmation especially in proton poor heterocyclic systems. Practically, we have found that a combination of data from $^{19}F/^{1}H$-^{15}N HMBC-type and phase sensitive HSQMBC experiments are most effective. While measured long range ^{19}F-^{15}N couplings are often small (2-3Hz) they are readily observed. This fact coupled with the unique character of the nitrogen lone pair effect on coupling means that regioisomers can be easily and unambiguously assigned. This is also the case for ^{1}H-^{15}N correlations as considerable literature is available regarding ^{1}H-^{15}N long range coupling constants. Examples from a number of heterocyclic types will be given to demonstrate the generality of the approach. In addition, techniques for speeding up data acquisition via multiple nuclei acquisition will be discussed.

Quantitative NMR in Research Chemistry

Richard Upton

GSK

Quantitative NMR (QNMR) is a powerful analytical technique due to the ability of NMR to act as a universal detector. In medicinal chemistry the introduction of parallel synthesis together with high throughput screening of compound libraries in DMSO has greatly increased the demand for easily assessing sample concentrations. In the past, QNMR had usually been limited by the requirement to add suitable internal standards to each sample, until the introduction of the ERETIC (Electronic Reference to Access In vivo Concentrations) method. We have used ERETIC successfully for many years, however implementation can be problematic. Hence, we discuss our development of alternative external standard methods.

Tuesday, September 18th
10:30 AM - 12:00 PM

Advances in the Application of Diffusion/Dynamics
Chair: Cynthia Larive

Speakers:

Gareth Morris
University of Manchester, UK

Betsy McCord
Dupont, USA

Edward Zartler
Merck & Co., USA

Pure Shift DOSY: Simplifying and Separating the Spectra of Mixture Components

Gareth A. Morris and Mathias Nilsson

School of Chemistry, University of Manchester, Manchester, UK

Proton NMR spectroscopy is a great tool for determining chemical structures, but its resolution is limited by multiplet structure, leading to frequent signal overlap. In conventional spectra this complicates, but does not necessarily prevent, analysis. In DOSY the effects of overlap are more serious, because it is extremely difficult to separate the individual contributions to the diffusional decay of overlapped signals. High resolution DOSY therefore relies heavily on the individual NMR signals of different species being well resolved.

We have used a minor variation on a technique that was published some years ago by Zangger and Sterk [1] to produce proton spectra in which almost all the effects of scalar coupling have been removed, leaving a single sharp signal for each chemically-distinct site in a molecule. Such "broadband homonuclear decoupling" trades sensitivity for resolving power in a very effective way, giving typically a tenfold improvement in chemical shift resolution. Applied to DOSY [2] this gives a 2D spectrum in which chemical shift is plotted against diffusion coefficient, with a resolution in the chemical shift domain that is unaffected by scalar couplings for first-order spectra. As a result the scale of complexity of system that can usefully be addressed with proton 2D DOSY is increased by about an order of magnitude, at the expense of a typical sensitivity reduction of around a factor of 50.

James Keeler has recently demonstrated a beautiful experiment based on a completely different principle, anti z-COSY, that can yield very similar results [3]. In both cases the core of the method is to invert all but a small proportion of the available spins, and then to focus just on the signal from these spins. In anti z-COSY this is done by using a mixing pulse of close to 180°, in the Zangger-Sterk method a simultaneously slice- and chemical shift-selective 180° pulse is used. Both methods require special data processing but can readily be implemented on standard instrumentation.

1. K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
2. M. Nilsson and G.A. Morris, Chem. Commun. 2007, 933-935.
3. A.J. Pell, R.A.E. Edden and J. Keeler, Magn. Reson. Chem. 2007, 45, 296-316.

Industrial Applications of Diffusion NMR; Applications to Mixture Analysis

Elizabeth F. McCord, Steve F. Cheatham, and Elizabeth U. Lozada

E.I. du Pont de Nemours and Co.

NMR can be used to measure diffusion coefficients (and thus the size/shape of molecules) through the use of Fourier-Transform Pulsed-Gradient Spin Echo (FT-PGSE) NMR. Further, via spectral editing, through 2D DOSY (Diffusion Ordered SpectroscopY), mathematical discrimination, or via 3D DOSY sequences, diffusion NMR can be used to spectroscopically separate and characterize components of mixtures. A variety of examples will be discussed in this presentation including characterization of complex mixtures of small molecules, electrolytes, and gas/liquid systems. Various DOSY pulse sequences and their ability to resolve test mixtures will be compared and evaluated. Results and experimental difficulties will be contrasted and compared with those from LC-NMR-MS. In many cases diffusion NMR can provide information that is difficult to obtain in any other way.

NMR of Small Stuff: A Pharmaceutical Perspective on Dynamics

Edward R. Zartler

Merck & Co.

All molecules move. This movement is inherent in the nature of the chemical bonds that holds them together. For many molecules, these dynamic processes are the key to their proper functioning. NMR is the only biophysical method that gives direct data on both structural and dynamic parameters for molecules in solution. NMR has provided very detailed pictures of the motions in proteins, leading to many insights into enzymatic function, yet this knowledge has not yet successfully been translated into the pharmaceutical milieu.

Drugs come in many shapes and sizes: small organic molecules (MW <400Da), peptides and similar molecules, small to large proteins (50 to 1000s of amino acids), polysaccharides, and virus-like particles and whole viruses. The molecular motions of these drugs can affect their therapeutic effect. Knowledge of these motions, in conjunction with structural information, can mean the difference between a successful and unsuccessful drug. In fact, considerable effort is expended to make sure that these motions are minimized. NMR can play a crucial role by describing these motions and help guide in process decisions. Typically, labeled molecules are not available, restricting the type of experiments that can be run. In many cases, the data takes on the simplest form, the linewidths of the molecules (indicative of the T_2 of the molecule). In the best cases, residual dipolar coupling data can be obtained. The most important aspect of the data is that the NMR results need to be connected to specific answers addressing concrete problems in a timely manner. Applications in this area have been demonstrated widely from early lead generation to very late stage development. Applications of both simple and complex data to problems encountered in the pharmaceutical industry will be discussed.

Tuesday, September 18th
1:30 PM - 3:00 PM

NMR in Food Science
Chair: Ana Gil

Speakers:

Ian J. Colquhoun
Institute of Food Research, UK

Serge Rezzi
Nestle, Switzerland

Manfred Spraul
Bruker Biospin, Germany

NMR in Human Nutrition

Ian Colquhoun, Gwénaëlle Le Gall, Kate Kemsley and Birgit Teucher

Institute of Food Research, Norwich Research Park, Colney, Norwich, UK

Metabolomics studies on long term dietary interventions in humans are scarce. We present a general approach to uncovering consistent but possibly small effects of nutritional interventions in the presence of much greater variation between subjects. The approach is illustrated for an NMR-based metabolomic study in which 16 postmenopausal volunteers were followed over a period of about 9 months (OSTEODIET [1]). OSTEODIET was conducted to investigate whether a high salt diet increases the risk of osteoporosis through sodium-induced increases in obligatory urinary calcium loss (calciuria) but the rationale for metabolomics was to identify potential markers of calcium and sodium intake.

The randomised cross-over trial consisted of four successive periods of controlled dietary intervention of 40 days each, followed by a minimum 4 week washout. Eleven subjects completed all four dietary interventions. Subjects were provided with all food consumed during the interventions from menus which were the same in each intervention except for the adjustments to Ca and Na content. Up to fifty 24-hour urine samples were collected from each volunteer: collection was made on the last 11 days of each intervention (controlled diet) and on the last two days of each washout preceding the start of an intervention (habitual diet).

PCA of the bucketed NMR spectra showed that inter-individual differences were by far the greatest source of variation. PLS-DA models were able to discriminate two classes (with diminishing rates of success) for (a) habitual diet vs controlled diet days (b) high Na vs low Na (c) high Ca vs low Ca interventions but little interpretation was possible when all subjects were included. We examined the high Na: low Na case by carrying out PLS-DA for each subject individually. Discrimination was usually evident on the 1st PLS factor but the loadings were not consistent. We carried out ANOVA on all buckets for each subject and listed for each one the 20 most significant buckets. For the high Na: low Na comparison, very few buckets occurred for four or more subjects. The most frequently recurring buckets corresponded to glycine, alanine and glutamine which all had higher mean levels in the high Na treatment although the difference between treatments was small. Other metabolites (acetate) showed larger but not consistent effects. This metabolomics study shows that systematic univariate analysis of all variables can detect dietary effects which are smaller than those from inter-individual variation. The ability to discern such effects is aided, as here, by close control of the diet and by availability of repeat samples from each subject.

1. http://osteodiet.ucc.ie/index.htm

NMR-based Nutri-metabonomics for Optimized Nutrition and Health

Serge Rezzi, Françoise-Pierre Martin, Ziad Ramadan and Sunil Kochhar

BioAnalytical Sciences, Nestlé Research Center, Lausanne, Switzerland

Increasingly genomics tools are being used to investigate health at the gene, protein or metabolite level using the corresponding "omics" technologies, namely transcriptomics, proteomics and metabonomics. Nutrigenomics, i.e. comprehensive mapping of changes in gene expression following nutritional interventions is very promising but from nutritional perspective, gene expression represents only a part of the metabolic picture. On the other hand, metabonomics-the holistic measurement of all metabolites is closer to the real outcome of potential changes suggested by genomics and proteomics. Metabonomics is also minimally invasive, and profiling of biological fluids by NMR ensures a simultaneous analysis of a wide range of metabolites that are the endpoints of molecular regulatory processes, diet and gut microflora metabolism and environmental factors. By opening a direct biochemical window into the metabolome in a holistic fashion, metabonomics is uniquely suited in developing new generations of biomarkers that are capable of providing better understanding of complex metabolic phenomena as well as assessing intra- and inter-individual differences. This property makes metabonomics very efficient for the generation of biomarker patterns for the comprehensive characterization of metabolic health, the prognostics and the diagnostics of diseases, and the generation of new insights in the understanding of the interactions between diet and metabolism.

Defining the metabolic phenotype or "metabotype" of human populations will offer a great opportunity to evaluate the metabolic response and the degree of this response to specific dietary modulations at the individual level. Similar to the "pharmaco-metabonomic" concept, pre-dietary intervention metabolite profiling could be used to model and predict the responses of individual subjects to special foods. Nutritional metabonomics can then be foreseen as a promising approach towards personalized nutrition. The presentation includes application of the minimally invasive metabonomics approach employing ^{1}H NMR and MS of biological fluids (plasma, urine, saliva) for comprehensive biochemical profiles in general human population to understand the metabolic diffrences due to phenotypic (gender, age and BMI) and lifestyle (stress, sports, smoking etc.) specific patterns.

NMR-based Hyphenation in Metabolomics

Manfred Spraul, Markus Godejohann, Silke Keller and Hartmut Schaefer

Bruker BioSpin GmbH Silberstreifen, Rheinstetten

NMR is a major analytical technique used in Metabolomics. It can be be applied in high throughput screening using flow injection NMR. To allow rapid input a preparation robot has to be integrated, running under full automation. The second analytical method used regularely is mass spectroscopy. Optimum correspondance between NMR and MS can be achieved running samples n a synchronized mode in an integrated set up also including preparation. Such a system produces for example high quality heteroanalytical covariance analysis.

Due to the lower sensitivity of NMR, many metabolites in a biofluid or extract will not be visible directly. A method to improve visibility in NMR is the combination with SPE to achieve a so called SPE-NMR, where still mixtures are investigated, however a reproducable prefractionation and enrichment for example due to polarity can be achieved.

Spectra obtained after elution can be investigated again with statistical methods and match algorythms. In addition the information from a LC-MS run can be available at the same time.

A third method to apply NMR in Metabolomics involves isolation of new biomarkers or drug metabolites, this can be achieved running LC-NMR/MS, preferably combined with post column solid phase extraction and a cryogenic NMR probe. Such it is possible to investigate very low concentration metabolites with NMR and get important strcutural information.

Examples are demonstrated for all applications mentioned.

Wednesday, September 19th
8:30 AM - 10:00 AM

Novel developments in Heteronuclear NMR Spectroscopy

Chair: Stefano Provera

Speakers:

David Bryce
University of Ottawa, Ontario, Canada

Philippe Lesot
Université de Paris-Sud, France

Adrian Davis
Pfizer, UK

Solid-state NMR Investigations of Inorganic and Bioinorganic Model Systems in Ultra-high Magnetic Fields

David L. Bryce

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

The increasing availability of ultrahigh-field NMR spectrometers continues to create new opportunities for solid-state NMR spectroscopists. In particular, in the case of half-integer spin quadrupolar nuclei one benefits not only from the expected increase in signal-to-noise, but also from the marked reduction in the second-order quadrupolar broadening of the central transition. Under magic-angle spinning conditions, this results in increased site resolution. In the case of stationary powdered samples, the effect of chemical shift anisotropy (CSA) is amplified at high field, thereby rendering feasible the measurement of complete CS tensors for quadrupolar nuclei, even in cases where the CSA may be quite small. In this presentation, highlights from our recent work on characterizing the binding enviroments of quadrupolar nuclei in small inorganic and bioinorganic model systems will be presented. For example, we have been developing $^{35}Cl/^{37}Cl$ SSNMR in amino acid hydrochloride salts as part of an effort to understand how this methodology might be used to describe chloride ion binding sites in larger biochemically-relevant systems. Another application of chlorine NMR is the identification of pseudopolymorphs of inorganic chloride materials. We have also begun to develop ^{23}Na and ^{39}K SSNMR to study cation-pi binding environments for sodium and potassium cations in model lariat ether receptor complexes; cation-pi interactions are known to play structural roles in proteins, nucleic acids, and supramolecular materials. Finally, prospects for the future of ^{43}Ca (spin-7/2) solid-state NMR at natural abundance (0.1%) in ultrahigh magnetic fields will be discussed.

All of our 21.1 T NMR data were acquired at the National Ultrahigh-Field NMR Facility for Solids in Ottawa, Canada (www.nmr900.ca).

Development and Applications of Natural Abundance Deuterium NMR in Chiral Oriented Solvents: An Original Analytical Tool for (Bio)Chemists

Philippe Lesot

Laboratoire de Chimie Structurale Organique, Equipe de RMN en Milieu Orienté, ICMMO, CNRS UMR 8182, Bat. 410, Université de Paris-Sud, Orsay, France

NMR spectroscopy in weakly ordering, chiral liquid crystals, made of organic solutions of polypeptide (commercially available), such as the poly-gamma-benzyl-L-glutamate (PBLG) or poly-epsilon-carbobenzyloxy-L-lysine (PCBLL) dissolved in chloroform or DMF, is an emerging and original approach for the study of chiral and prochiral compounds.

For chiral (or prochiral) molecules, the difference in the enantioselective interactions between the polypeptide helices and the S/R isomers (or the pro-S /pro-R directions) generates a sufficient differential ordering effect in the mesophase leading to their spectral discrimination through a difference of carbon-13 chemical shift anisotropies, or proton-carbon-13 dipolar couplings (^{13}C-NMR) or deuterium quadrupolar splittings (^{2}H-NMR), for instance [1,2].

In this talk, we mainly focus our attention on the methodological developments of natural abundance deuterium NMR (NAD NMR) in chiral oriented solvents as well as the most recent applications of this analytical tool. The advantages of recording NAD 2D spectra using selective 5-mm ^{2}H cryogenic probes operating at 14.1 T will be presented. Various examples of applications will be reported to illustrate how the method can provide elegant solutions to solve various practical problems encountered by (bio)chemists [3]. In particular, we will examine the potentialities of NAD NMR for investigating chiral hydrocarbons [2], for empirically determining the absolute configuration of small chiral molecules, or measuring the natural isotopic ratios (D/H) in biomolecules such as unsaturated fatty acids [4,5].

1. M. Sarfati, P. Lesot, D. Merlet, J. Courtieu, Chem. Commun., 2000, 2069.
2. P. Lesot, M. Sarfati, J. Courtieu, Chem. Eur. J. 2003, 9, 1724.
3. A. Parenty, J.-M. Campagne, A. Aroulanda, P. Lesot, Org. Lett., 2002, 10, 1663.
4. P. Lesot, A. Aroulanda, I. Billault, Anal. Chem. 2004, 76, 2827.
5. V. Baillif, R. Robins, I. Billault, P. Lesot, J. Am. Chem. Soc. 2006, 128, 11180

Big Signals without Big Magnets: An Early Assessment of the Utility of Dynamic Nuclear Polarisation for Liquid State ^{13}C and ^{15}N NMR Spectroscopy

Iain J. Day[1,2], John C. Mitchell[2], Martin J. Snowden[2] and **Adrian L. Davis**[1]

1. Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, Kent, UK
2. Medway Sciences, University of Greenwich, Medway University Campus, Chatham Maritime, Chatham, Kent,UK

Although NMR is a ubiquitous analytical technique, a significant limitation is the poor sensitivity for low-frequency, dilute spins such as carbon-13 and nitrogen-15. For example, the prevalence of non-protonated nitrogen atoms in pharmaceutical compounds, coupled with typically small sample sizes, means that detection of all nitrogen atoms is frequently not achieved, even though this would offer considerable advantages for structure elucidation.

Recently, the use of DNP-NMR to generate hyperpolarisation of carbon-13 and nitrogen-15 in the solution state, and thereby enhance sensitivity, has been demonstrated by Ardenkjaer-Larsen et al. Enhancements of 10^2-10^4 can readily be achieved. We will discuss the results we have obtained using a commercial implementation of the Ardenkjaer-Larsen technique, for a range of pharmaceutically relevant compounds, illustrating how we can use DNP to boost NMR sensitivity by orders of magnitude. We will describe rapid experimental methods to determine signal enhancements and NMR relaxation times , which enable us to predict, with some confidence, how effective DNP might be for a given compound. This work gives indications for how to improve the overall design of the Ardenkjaer-Larsen experiment.

Wednesday, September 19th
10:30 AM - 12:00 PM

Student and Post-Doctoral Session
Chair: Jake Bundy

Speakers:

Geoff Gipson
Drexel University, USA

Christian Ludwig
University of Birmingham, Birmingham, UK

Murthy Shanaiah
Purdue University, USA

Automated Quantification of Complex ^{1}H NMR Spectra

Geoffrey T. Gipson[1,2]

1. School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, Pennsylvania
2. Safety Assessment, GlaxoSmithKline, Collegeville, Pennsylvania

A common challenge for metabolomics investigators is quantification of individual metabolite levels in complex biological samples. Metabolic profiling using ^{1}H NMR (NMR) has the specific advantage of being a quantitative technique, capable of simultaneously measuring large number of co-occuring metabolites. Along with other "omics" fields, metabolomics is faced with the need to process and interpret large datasets. Specifically, analysis steps such as spectral preprocessing (e.g. alignment, normalization) and statistical analyses (e.g. univariate, multivariate, multiple test correction) are time-consuming tasks which can be streamlined into an automated process pipeline. In addition to the challenges posed by the magnitude of the collected datasets, the interpretation of metabolomics datasets is further complicated by the fact that NMR is an "open platform." This means that the data features measured are not implicitly associated with any particular physical interpretation. Typically, this challenge is approached through manual visual inspection of spectral regions of interest (e.g. statistically significant), with the aim of identifying characteristic features of known reference spectra.

Here, an approach is presented in which metabolite identification and quantitative levels are explicitly assigned [1]. Metabolite level estimates are calculated based on a constrained linear fit of reference spectra to a complex, biologically derived NMR spectra. In this model, spectra are discretized into arbitrary resolution (precise or coarse, heterogenous or homogenous) representations which maximally capture discriminative spectral information. The "best fit" metabolite levels are then estimated using quadratic programming to minimize the residual least-squares subject to investigator selected parameters such as non-negativity and/or upper-limit constraints, or non-uniform weighting. This method allows for the application of statistical methods to estimated levels of explicitly assigned metabolites, instead of non-descript spectral regions, providing a more intuive interpretation of the data.

1. G.T. Gipson, K.S. Tatsuoka, B.C. Sweatman, S.C. Connor. (2006) "*Weighted least-squares deconvolution method for discovery of group differences between complex biofluid* ^{1}H *NMR spectra.*" Journal of Magnetic Resonance 183:269-277.

New NMR Methods for Small Molecules

C. Ludwig[1], P.J. Michiels[2], U.L. Günther[1]

1. HWB-NMR, CR UK Institute of Cancer Studies, University of Birmingham, Edgbaston, Birmingham
2. Solvay Pharmaceuticals BV, Weesp (NL)

NMR has been a key technology in the study of small molecules for various pharmaceutical applications, including metabolomics and the investigation of protein-ligand interactions for the design of new drugs. The reasons using NMR in metabolomics and protein/ligand-binding studies are the excellent reproducibility of NMR spectra and the possibility to translate NMR measurements from in vitro to in vivo measurements.

Dynamic Nuclear Polarisation (DNP) represents an important method to overcome sensitivity limitations of NMR. This is achieved by transferring high spin polarization of unpaired electrons to coupled nuclear spins. In such experiments enhancements of >10,000 were achieved [1] for NMR analysis. However, it is very difficult to obtain these enhancements in complex metabolite mixtures like blood serum. We have optimised this implementation of DNP for applications in the context of metabolomics. The use of co-polarisation agents substantially broadens the applicability of DNP-NMR for metabolomics because many additional metabolites can be polarised which were previously not accessible to DNP-NMR. We have evaluated the suitability of this implementation of DNP to study various metabolic pathways.

NMR Method Development for Drug Design. To develop new drugs, knowledge about the binding orientation of a protein ligand or inhibitor is vital. We have developed a NMR method (SALMON) which can be used to map solvent accessibility epitopes from which the orientation of a ligand with respect to the protein can be determined. Small molecules such as DMSO or acetonitrile added to the solution can be used as alternative starting points for the magnetisation transfer. The solvent accessibility epitope can directly be translated into the orientation of a ligand with respect to the protein. The new experimental design has been explored for dehydrogenases and reductases (QO2, AKR1C3, HSD17b1) with very different binding pockets, including proteins where the ligand is bound close to the surface and others where it is buried inside the protein. SALMON has also been used to differentiate between inhibitors for different binding pockets based on their pH dependence for the NOE transfer which can be used to determine the nature of inhibition.

1. J.H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin, M. Thaning, and K. Golman, Proc Nat Acad Sci USA, 100, 10158-10163 (2003).

Sensitivity Enhanced ^{13}C and ^{15}N NMR Based Metabolomics Using a Chemical Derivatization Approach

Narasimhamurthy Shanaiah, M.Aruni DeSilva, Tao Ye, Yuliana Suryani, G.A. Nagana Gowda and Daniel Raftery.

Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

^{1}H NMR spectroscopy has been widely used in body fluid analysis owing to its high sensitivity and abundance of proton containing metabolites. NMR spectra of untreated samples represent an overview of all metabolites present in a body fluid and can be used as a finger print, while statistical methods can be employed for data reduction and screening purposes1. With the advances made in NMR spectrometer technology, ^{13}C and ^{15}N NMR is becoming more feasible. Despite the low inherent sensitivity and natural abundance, ^{13}C and ^{15}N can also provide a wealth of additional information on metabolites. This is mainly due to the large range of observed chemical shifts, resulting in many resolved signals. While standard methods of observing ^{13}C or ^{15}N NMR is still very challenging, we have developed methods to isotopically label metabolites of interest by chemical derivatization. This approach enhances sensitivity especially when combined with HSQC (Heteronuclear Single Quantum Coherence) experiments. The result is much more sensitive than a carbon-detected experiment and can simplify the study of metabolites in biofluids2. We present here simple and robust chemical derivatization methods with labeled ^{13}C and ^{15}N reagents in aqueous solution for detecting different classes of metabolites at low concentration in complex mixtures such as urine, serum and tissue extracts. Application of enhanced metabolites detection in biofluids and pathological tissue extracts provides a convenient and sensitive method for the qualitative and quantitative analysis of various classes of metabolites that may have high utility for diagnosis and for the study of pathophysiology in various diseases.

1. J.K. Nicholson, J.C. Lindon and E. Holmes, Xenobiotica 1999, 29, 1181-1189.
2. N. Shanaiah, M.A. Desilva, G.A. Nagana Gowda, M.A. Raftery, B.E. Hainline and D. Rafterym, Proc. Natl. Acad. Sci. (USA), 2007; 104, 11540-11544.

Tuesday, September 18th 8:00 PM - 10:00 PM

Poster Session

Sponsored by Varian Inc.

Chair: Julian Griffin

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22. A new Biomarker for Inhibition of the Farnesyl Pathway Identified by NMR Metabolic Profiling
23. Pushing the Limits of Metabolite Biomarker Identification from 1D NMR-Based Metabolomics Data
24. Selective J-resolved Spectra as a Tool for Measuring Homonuclear Scalar Couplings
25. Adaptive Spectral Library of Amino Acid ^{13}C Isotopomers
26. Complete ^{1}H NMR Spectral Analysis of Large Spin Networks
27. Rapid Identification of Monosaccharide Units in Polysaccharides Using a TOCSY Pattern Matching Approach
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32. Computerized Structure Verification
33. Structure Elucidation of Novel Alkaloids by 1- and 2-D NMR Experiments
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35. A Metabolomic Comparison of Mouse Models of Neuronal Ceroid Lipofuscinoses
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51. NMR as a Tool for Determination of Chiral Stability of 2,4-Thiazolidinediones Drug Substances

52. Automated Data Processing: How Post-Run Macros can Facilitate the Analysis of Illicit Drug Samples

53. ^{19}F HSQC

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56. The Metabolism Study of ZD4054. The Mystery of the Co-eluting Peak

57. NMR Peak Height Quantitation: Accurate, Precise, Easy

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59. Conformation and Dynamics of Heparan Sulfate Domains Investigated by NMR Relaxation of ^{15}N Enriched Oligosaccharides

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62. Latest Developments on the Study of N,N',N"-Triacetylchitotriose Binding Elderberry Lectin. What Does DOSY say about the Binding Stoichiometry?

63. Examining the Polymorphism of Aspirin by Solid-state NMR

64. Small Volume NMR in Natural Product Research

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1 Medium Effect on the Rotational Barriers of Carbamates and Their Sulfur Congeners

Ernani A. Basso, Rodrigo M. Pontes and Francisco P. dos Santos

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The potential of NMR for detecting nuclear exchange processes was recognized fro its early development through the spectra of amides[1]. Since then, a number of exchanging chemical systems have been studied with special interest into those resembling peptide bonds, among them, carbamates. To date, it is known that carbamates behave characteristically for most "amide-like" systems in the presence of polar solvents [2,3], but almost nothing had been reported about the medium effect on the rotational barriers of thiocarbamates and dithiocarbamates. In the present work [4], four compounds were submitted to experimental and theoretical investigation, namely: methyl N,N-dimethylcarbamate (1), S-methyl N,N-dimethylthiocarbamate (2), O-methyl N,N-dimethylthiocarbamate (3) and Methyl N,N-dimethyldithiocarbamate (4). The barrier to rotation around the conjugated C-N bond was measured for these compounds through dynamic nuclear magnetic resonance (DNMR). By line shape fitting, the following values were obtained for 1, 2, 3 and 4, respectively: 14.3±0.5 (CS_2), 14.0±1.1 (CS_2), 17.5±0.4 (CCl_4), and 14.6±0.5 (CCl_4). Compounds 2 and 4 presented the greatest variations upon changing the solvent polarity, contrasting with 1 and 2 for which no effect was measured. These observations could be explained with the help of computational calculations, using both quantum and classical approaches (molecular dynamics). In this way, we used the isodensity polarizable continuum model (IPCM) to mediate the effect of bulk solvent polarity (at B3LYP/6-311+G(2d,p) and HF/6-311+G(2d,p) levels) while hydrogen bonds were reproduced using molecular dynamics (MD) simulations. When combined, IPCM and MD brought into agreement theoretical calculations and DNMR measurements and revealed an interesting behavior regarding hydrogen bonding, namely, that it causes the rotational barrier to decrease for compounds 1, 3 and 4, contrasting with most of the molecular systems studied to date.

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2 The Dihedral Angle Dependence of $^3J_{CH}$ in Norcamphor

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Since the pioneering work of Karplus on the angular dependence of the 3JXY coupling constant in a X-C-C-Y fragment, of enormous importance in stereochemical assignments, several changes were introduced in the original equation to include other types of coupled nuclei, eletronegativity effects, torsional angles, etc. These have been recently reviewed [1,2]. In 4-tert-butyl-cyclohexanone, the $^3J_{C6H2eq}$ is only 3.1 Hz, although the dihedral angle is 173.9º. In contrast, the $^3J_{C1H3eq}$ is 8.4 Hz for a similar dihedral angle (176.9º). The $^3JC_{6H2eq}$ coupling across the carbonyl carbon atom has that unusual value because in the coupling pathway there is a strong nO/σ*(C1C6) hyperconjugative interaction and the C6-C1-C2 bond angle is close to 120º(sp2). Thus, the sum of these effects produce this unusual small coupling. The 3JC1H3eq is only affected by the nO/σ*(C1C2) interaction, since C1-C2-C3 angle is close to sp3 hybridization [1]. In the present work, an experimental and theoretical study has been performed to evaluate the correlation between $^3J_{CH}$ values and the Karplus relationship for norcamphor. The experimental $^3J_{C1H4}$ is only 3.8 Hz, which is very small for a coupling pathway with a C1-C7-C4-H4 dihedral angle of 180º. On the other hand, there is another coupling, the $^3J_{C4H1}$ = 8.8 Hz, with opposite coupling pathway and similar dihedral angle (C4-C7-C1-H1=176º). This $^3J_{C4H1}$ coupling is similar to 9.4 Hz, obtained for an anti conformation by Aydin and Günther [3], using a Karplus-like equation. However, the unusual small value of $^3J_{C1H4}$ is an indicator of hyperconjugative effects on the $^3J_{CH}$ coupling pathway in norcamphor. The NBO analysis showed the C1-C7 bond is involved in two hyperconjugative interactions, that is, the sigma(C1C7)/σ*(C2-O) and sigma(C1C7)/π*(C2-O) interactions, which yield a decrease in $^3J_{C1H4}$ coupling, since there is a charge transference from the C1 carbon, involved in the coupling, to two antibonding orbitals. These interactions do not affect 3JC4H1, which shows the normal behavior for $^3J_{CH}$ couplings with a dihedral angle close to 176º. Theoretically calculated $^3J_{CH}$ values are in close agreement to these experimental values. The full assignment of its spectra will also be reported, as well as the $^3J_{CH}$ data for the corresponding α-chloro- and α-bromonorcamphor, which showed a similar behaviour. These results show that it is important to keep in mind that the application of $^3J_{CH}$ coupling constant to determine stereochemical relationships may lead to erroneous conclusions.

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3 NMR Toolbox for Rational Drug Design – From Hit Identification to Lead Optimization

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NMR spectroscopy has an important role to play in rational drug design, particularly when combined with other data derived from structure-activity relationships, computational chemistry, X-ray crystallography, and pharmacological DMPK properties. In this report, successful NMR-based examples from our HIV and HCV programs will be shown to highlight various stages along the drug discovery pathway. At the hit-to-lead stage, NMR has been valuable as an aid for distinguishing compounds that specifically bind to the target macromolecule versus those that are non-specific or simply false-positive. An interesting example will be shown for a complex system where competition experiments were effectively employed to determine whether or not inhibitors attach to the desired binding site of a multi-pocketed target. Beside ^{1}H NMR methods, these binding and competition strategies also employed ^{19}F NMR which had the additional advantage of providing binding stoichiometry, etc. At the lead optimization stage, the elucidation of the binding modes, bound-state conformations and roles of the substituents of ligands were exploited for designing inhibitors with improved potency. For example, knowledge of the relative flexibility of crucial substituents were exploited by chemically rigidifying the free-state conformation to resemble the bound-state. The ensemble of data were also used to redesign alternate series of compounds with improved DMPK properties via scaffold replacements. Finally, as compounds advanced closer to pre-development status, knowledge of the metabolic liabilities became crucial. NMR-based metabolite identification was shown to be valuable, especially for cases where there were MS limitations. Overall, these and other examples demonstrate the importance of having available a full “toolbox of NMR strategies” to respond to critical questions that arise along the rational drug design pathway.

4 ^{1}H-^{1}H Biselective Excitation Applied to HSQC and HMBC Spectra

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The simultaneous application of multiplet selective pulses to two mutually coupled spins is known to produce effects that are different from those observed with non coupled spins. These effects have been studied in double inversion and double excitation experiments [1 – 3]. Later, the inclusion of simultaneous selective pulses in pulsed field gradient spin echo (DPFGSE) experiments [4] led to doubly selective experiments. They allowed the extraction of two signals from a crowded ^{1}H NMR spectrum if, and only if, they originate from two scalarly coupled spins. Further spin manipulation led to doubly selective TOCSY and TOCSY–COSY experiments [5].

An in-depth theoretical study of biselectivity was recently at the origin of an improved SERF pulse sequence, an experiment that is involved in the measurement of residual dipolar couplings [6]. The selective excitation of mutually coupled proton pairs is now used as a way to simplify the interpretation of HSQC and HMBC spectra of complexe molecules.

The poster reports the adjustements that were needed to adapt the initial ^{1}H – ^{1}H biselective pulse sequence to heteronuclear experiments. The peak intensities depend on an evolution delay whose optimal value may be predicted by numerical simulation or by a purposely written computer program [7].

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5 NMR Chemical Shifts and Theoretical Chemical Shifts Tensors in Substituted Quinolines

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Quinolines and their derivatives display a wide spectrum of biological activities such as antimalarial, antibacterial, cytotoxic, antimycobacterial, and anti-inflammatory behavior. Due to the interesting and important biological properties and practical applications of these quinoline derivatives (such as pharmaceuticals, polymers, cyanine dyes, antioxidants in the rubber industry and fungicides), there has been substantial interest in the study of their electronic properties.

In this work we have collected ^{1}H and ^{13}C NMR data of a family of 58 quinoline derivatives, and in conjunction with quantum chemical computations used this data to calculate chemical shifts tensors. Density Functional Theory using Becke hybrid functional (B3LYP), and 6-311G(2d,p) basis set were employed to carry out the geometry optimization and evaluate energy. Gauge-Independent Atomic Orbital method was used to calculate the CST´s using the same level of theory and basis set described previously. Statistical analysis showed a good agreement between experimental and calculated theoretical chemical shifts. Over estimated values of theoretical CST´s were corrected according to the average deviation between theory and experiment. Under these conditions new statistical analysis was carried out and non significant differences were obtained in the quality results. In all cases correlation coefficients were around 0.998.

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6 NMR Chemical Shifts Parameterization in Substituted Quinolines

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Because of the increased interest in quinolines and their derivatives as potential pharmacological agents such as antifungal, antitumoral, antileishmanial and others, new synthetic routes to produce chemicals with different physicochemical, spectroscopic properties and biologically activity have beendesigned. NMR chemical shifts are considered to result from the effect of local and non local contributions: local electron density on a specific nucleusof interest, non local factors include localized electron donor and electron attractorgroups, and spatial interactions with others intra or inter molecular fragments viainduced local magnetic fields.

In this work ^{1}H and ^{13}C NMR spectroscopic data of a group of 60 quinoline derivatives have been collected from previous studies [1,2] and other scientific reports [3-6]. We have analyzed the shielding factors to a wide range of side chain groups attached to the basic structure of the quinoline ring. For example: alkyl groups such as methyl, ethyl, isopropyl and others like aromatic and heteroaromatic substituents, and others including halogens, hydroxy, methoxy, amino, nitro, and carbonyl have been examined. The shielding factors are the result of an average value in the analysis of all studied cases.Using procedures to parameterize shielding factors of side chain groups demonstrates the possibility to generalization of the obtained values, and provide a general mathematical approach to evaluate the chemical shifts of any new substituted quinoline.

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7 Leakadine: Stability in Aqueous Media Studied by NMR

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Leakadine (2-carbamoylaziridine) is an antitumor immunomodulator, created at the Latvian Institute of Organic Synthesis. It is believed, that leakadine reacts with thiol groups of proteins of cancer cell membranes, making them easier to be eliminated by means of the immunoprotection system. Although different aziridines are well known as alkylating antineoplastic agents, it is believed not to be the case with leakadine [1]. In the presentation we will outline the results obtained from our research concerning the stability of leakadine and its reactivity with different nucleophiles in aqueous media.

The chemical shifts and coupling constants of leakadine are very pH dependent. A titration experiment showed that the geminal proton coupling constant changes its sign from positive to negative while pH increases. The pKa of leakadine's imino group estimated from NMR chemical shift and coupling constant titration curves was approx. 5, so that conclusions about its protonated or non-protonated state at a given pH value could be made.

Our time course experiments showed that leakadine alkylates itself at basic and neutral conditions to give diastereomers of addition at α- and β-carbons. The products of aziridine-ring opening were obtained only at highly acidic conditions.

The reactivity of leakadine towards different nucleophiles was estimated. In particular the reactivity with guanine 7-N in various oligonucleotides was examined.

8 A Multiple Field ^{35}Cl NMR Investigation of Solid Hydrochloride Salts of Several Common Drugs

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The availability of NMR spectrometers with applied magnetic field strengths exceeding 21 T has lead to an increase in research of half-integer spin quadrupolar nuclei in solid powdered samples. One of the main goals of this research is the characterization of chemical shift (CS) and electric field gradient (EFG) tensors, including their relative orientations. This information is critical for developing an understanding of the relationship between NMR observables and the local molecular and electronic structure for a variety of chemical species. High fields facilitate such studies due to the inverse dependence of the second-order quadrupolar interaction on B0 and the linear scaling of the nuclear magnetic shielding interaction with B0.

Chlorine NMR parameters for hydrochloride salts demonstrate particular sensitivity to the local hydrogen bonding environment of the chloride ion (e.g., see Bryce, D. L., Sward, D. G. J. Phys. Chem. B. 2006, 110, 26461). To better understand the connection between chlorine NMR parameters, chemical structure, and ultimately biological function, an array of organic hydrochloride salts need to be characterized. The properties of the two naturally-occurring chlorine isotopes, ^{35}Cl (I = 3/2; N.A. = 75.78%; Ξ = 9.798 MHz; Q = -8.165 fm2) and ^{37}Cl (I = 3/2; N.A. = 24.22%; Ξ = 8.156 MHz; Q = -6.435 fm2) necessitate the use of high external magnetic fields. However, the high natural abundance of ^{35}Cl and rapid spin lattice relaxation rates generally allow for short experimental times.

In the present research the solid hydrochloride salts of some common drugs (e.g., antidepressants, antihypertensives, etc.) have been investigated using ^{35}Cl NMR spectroscopy at moderate and high applied magnetic fields (11.75 and 17.63 T). Rapid acquisition of the mI = ½ ↔ mI = -½ central-transition ^{35}Cl NMR spectra of stationary samples was facilitated by the quadrupolar Carr-Purcell Meiboom-Gill (QCPMG) experiment. The Hahn-echo experiment was also used under both stationary and magic-angle spinning, MAS, (up to 35 kHz at 11.75 T) conditions. The combination of two applied magnetic fields with stationary and MAS sample experimental techniques were essential to afford an accurate analysis of our spectra. Particularly, the MAS experiments enabled separation of the spectral broadening resulting from the second-order quadrupolar and magnetic shielding interactions. For the compounds investigated, the measured ^{35}Cl quadrupolar coupling constants, CQ, range from ~3.3 to 5.8 MHz, and anisotropy in the CS tensors is <100 ppm. In addition, the chlorine NMR parameters are discussed in conjunction with available X-ray crystal structure data, and should prove useful for refinement of the hydrogen-bonded proton positions.

9 High Resolution 2D NMR Spectroscopy in the Earth's Field

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Throughout the development of NMR, there has been a marked trend to move to ever increasing magnetic field strengths in order to optimise both sensitivity and resolution. However, such strong, homogeneous magnetic fields can only be achieved at the expense of instrument size, weight and cost. There exist many applications for which these restrictions prohibit the effective use of NMR and so there are compelling reasons to push the limits of what can be achieved using small low-field NMR systems.

In a sense, the simplest low-field system is one that employs the ubiquitous and highly homogeneous Earth's magnetic field for signal detection. Until recently it was generally accepted that due to the vanishingly small chemical shifts of most nuclei in ultra-low fields, the only observable molecular interaction at Earth's field was hetero-nuclear J coupling. However, Appelt et al. [1] have now demonstrated that homo-nuclear couplings can be observed if the magnetic equivalence of the coupled nuclei is broken by hetero-nuclear J couplings. Therefore it is apparent that the highly homogeneous nature of the Earth's magnetic field coupled with the very long T_2 times of many organic compounds at ultra-low fields provides an unique opportunity to accurately measure small, long-range homo- and hetero-nuclear J couplings.

It is anticipated that 2D NMR techniques will be required to elucidate the coupling network for most systems of practical interest. The viability of 2D NMR spectroscopy in the Earth's field has been demonstrated by Robinson et al. [2]; however there still exist a number of technical challenges which must be overcome before high-resolution (sub-hertz) 2D NMR spectra can be realised.

In this work we present an NMR apparatus which addresses several of the technical challenges of high-resolution 2D NMR at Earth's field and yields sufficient homogeneity, frequency stability and sensitivity for sub-hertz resolution to be obtained. Our latest 2D NMR spectra will be presented and compared to simulated spectra.

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10 Implementation of RDCs as Parameters for Precise Structure Determination in Solution

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NMR spectroscopy of oriented molecules was considered to hold great promise and potential for extremely precise structure determination. However, despite the high intrinsic precision of the experimentally determined couplings in some cases abnormal and highly diverging values of structural parameters were obtained under various experimental conditions in different liquid crystals. In particular, even for simple model molecules with known structure the spread of data exceeded the standard error of individual measurement by 10-103.

One of the principal assumptions in NMR spectroscopy of aligned molecules is the independence of the indirect spin-spin couplings J on the phase state of the medium. We show that in some cases this approach is incorrect. The difference between isotropic J values in isotropic and anisotropic states of the media we have called “J-jump”. It depends on the physical properties of particular media. To clearly demonstrate this hypothesis the series of special experiments with acetonitrile as a probe in lyotropic sodium dodecyl sulfate (SDS) based media have been done.

If we use isotropic values of $^{1}J_{CH}$ measured (or estimated) in anisotropic state, the ratios of 1DCH/2DHH become constant and equal to the expected one. In cases of highly oriented molecules, when the measured dipolar couplings are 2-3 degree of order greater than J-couplings, we can ignore J-jump. The relative large effect it can have on weakly aligned molecules are when RDCs are comparable or smaller than J-couplings. In this case the neglect of possible difference of J-couplings in isotropic and anisotropic environments can lead to systematic errors.

11 Process Development: Application of ^{15}N NMR to Determine Acyl Migration

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NMR is is a very effective tool in the identification of both major products and by-products in support of chemical process development for pharmaceuticals. Complete idenfication is carried out by the application of homo- and heteronuclear correlation experiments. ^{1}H-^{15}N correlations are extremely helpful in the structural elucidation of pharmaceuticals and related compounds especially because they are generally nitrogen rich molecules. ^{1}H-^{15}N correlation experiments find more applications nowadays in structural elucidation due to the enhancement of the sensitivity provided by CryoProbes.

The reaction product in the example contained two compounds as indicated by HPLC. When the major compound was isolated and reanalyzed, the same HPLC behaviour was observed. The observation of the same molecular weight for both compounds indicated that product 1 rearranged under HPLC conditions to form 2. The structure of this unexpected intermediate had to be identified to understand this rearrangement. The structural elucidation of the two products were achieved using the analysis of 1-D and 2-D NMR data. ^{1}H-^{13}C long range correlations were not useful in differentiating the two molecules, however, they were differentiated using ^{1}H-^{15}N long range correlations. The structures indicate that the rearrangement of 1 to 2 occurs as a result of an acyl migration.

12 Characterization of Chiral Impurities by Solid-State NMR Spectroscopy

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The marketing of enantiopure pharmaceuticals has become more common due to the increased regulation of chiral active pharmaceutical ingredients. However, achieving the desired enantiopurity can be a challenge, and low levels of the undesired enantiomer (chiral impurity) may be present in the final product. Although the impact of chiral impurities on pharmaceutically relevant bulk properties such as dissolution rate and heats of fusion have been studied in the laboratory of Dr. David J. W. Grant, characterization of the location and local environment of chiral impurities themselves has been limited.

Solid-state NMR spectroscopy (SSNMR) is a valuable tool for investigating chiral impurities. They can be studied at low concentrations when isotopically labeled, and spectral subtractions provide information about the environment into which the impurity has been incorporated. Additionally, ^{1}H T_1 times indicate the degree of mixing intimacy within the sample, as well as sample mobility.

The amino acid proline was used as a model chiral molecule in this study. The L-enantiomer was the chiral impurity, and samples were prepared by lyophilizing solutions of 2-25% natural abundance or L-[1-^{13}C]proline with D-proline. The resulting crystalline materials were studied using ^{13}C CP/MAS solid-state NMR spectroscopy and thermal analysis.

^{13}C SSNMR spectral subtractions of lyophilized products showed several different peaks in the carbonyl region, indicating incorporation of the chiral impurity into multiple environments, including within an unpublished DL-proline polymorph and as a defect in the homochiral system. ^{1}H T_1 measurements as well as thermal analysis support the presence of multiple crystal environments within lyophilized enantiomeric mixtures of proline. As the concentration of chiral impurity increased, the impurity became incorporated to a greater extent in the new DL-proline polymorph than in the other species, and a solid solution solubility limit of approximately 1% was observed for chiral defects in the homochiral system.

13 Separation of Overlapped Proton NMR spectra of Enantiomers: Spin State Selective Detection of Active and Passive Couplings by Spin Selective Higher Quantum

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Anisotropic NMR spectral parameters like chemical shift anisotropy, dipolar couplings and quadrupolar couplings differ for differentially ordered enantiomers. The chiral polypeptide liquid crystal serves as a weakly aligned media not only to extract these parameters but also as the indicators for chiral discrimination. Proton detected one dimensional spectra fail to provide such a discrimination due to severe overlap of transitions arising out of too many short and long range couplings (which implies crowding of too many spin states in a single dimension) and also due to doubling of transitions from the two enantiomers. Spin selective excitation of higher quantum coherence of a chemically isolated group of protons enabled the spin state selective detection of proton single quantum transitions using passive spin states and thereby reducing the number of couplings and complexity of the spectrum in a single dimension. This overcomes the lack of resolution and permits the detection of the transitions for the components of the multiplet based on the spin state of the passive proton. The differential scaling of the chemical shift anisotropy and the passive couplings of each enantiomer by a factor n, achieved by detecting nth quantum coherence, provides enhanced separation of enantiomer peaks resulting in higher dispersion of the spectrum. The two dimensional spectra with scaled up passive coupling in the higher quantum dimension retains only the dipolar coupling between active protons in each cross section taken along the single quantum dimension. This results in the separation of short and long range couplings, remove the overlap of spectra of enantiomers, enable unambiguous visualization and the precise determination of spectral parameters. The developed methodology has been applied on three chiral molecules.

14 Antiparallel Double-Helical Structure in Peptides Containing a Mannose Derived Furanoid Sugar Amino Acid

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Sugar amino acids represent an important class of conformationally constrained scaffolds used extensively in the area of peptidomimetic research.[1] The multifunctional characteristics of sugar amino acids can be exploited to develop successful peptidic drugs by mimicking the three dimensional structures of natural peptides. In this respect, we have studied in our laboratory various sugar amino acid building blocks, e.g., tetrahydrofuran-based sugar amino acids, furan-based sugar amino acids, pyrrole-based sugar amino acids etc. These hybrid templates have been further used by us to mimic many natural bio-active peptides. In addition, various synthetic designer molecular scaffolds with interesting secondary structures have been synthesized and characterized. In continuation of our creation of nature-like, yet unnatural molecular architectures, we have recently carried out detailed NMR studies of peptides containing a mannose derived furanoid sugar amino acid which dimerise into novel anti-parallel double-helical motif in solution, resembling that of naturally occurring Gramicidin A. Detailed structural studies will be presented.

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15 Deuterium Isotope Effects on ^{1}H, ^{13}C and ^{15}N Chemical Shifts in Compounds with Intramolecular C=O···H-N- Hydrogen Bonds

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Deuterium isotope effects on NMR chemical shifts have proven to be useful for spectral assignment and structure determination of small and large organic molecules. The magnitude of the isotope effect on nuclear magnetic shielding depends on several factors: the number of bonds between the observed nucleus, the position of the isotopic substitution, tautomeric equilibrium, conformation, hybridization of nuclei involved, substituent properties and hydrogen bonding.

In the present investigation we studied the influence of hydrogen bond strength on the values of isotope effects in 2-aminomethylene-1,3-diketones. It was estimated previously that these systems are trans-fixed aminovinylketones without any tautomeric equilibrium, stabilized with an intramolecular hydrogen bond of the type N-H•••O=C, δ(1HN)~11-15 ppm. ^{15}N CS, ranging from 220 to 290 ppm, move downfield with H-bond strengthening. The experimental results are compared with quantum-chemical calculated electron densities, diatomic bonding energies, and geometric characteristics.

Our results show that $1\Delta^{15}N(H/D)$, $n\Delta^{13}C(H/D)$, $n\Delta^{1}H(H/D)$ isotope shifts strongly depend on the intramolecular H-bond strength, increasing or decreasing in accordance with δ(1HN).

Most of $n\Delta^{13}C(H/D)$ are positive, but negative ones were also observed. (H/D substitution of the intramolecularly H-bonded proton in compounds with R'=H results in greater values of the isotope effects).

The signs of the isotope effects on the nuclei forming the H-chelate cycle confirm one-minimum potential energy function for these systems.

16 A NMR Direct Evidence of the Formation of a Ternary Complex between Methyl benzoylformate, Mg^{2+} and a β-Lactam NADH Model

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2. SGIker-Servicio General de RMN Gipuzkoa

The enantioselectivity of the NADH-mediated reductions is governed by the formation of a ternary complex substrate/metal/NADH. Herein we wish to report the first direct detection of a complex between methyl benzoylformate, $Mg2^{+}$, and a beta-lactam NADH model using NMR techniques. For the design of the NADH model the reducing capability of 1,4-dihydronicotinamide was combined with a chiral amino acid core and a NMR sensitive "label" - the $–NCH(SiMe_3)_2$ group.

Reduction of methyl benzoylformate with the NADH models 1-2 was performed in CD_3CN-d3 in the presence of $Mg(ClO_4)_2$. A series of mono- and bidimensional NMR experiments were performed in order to study the complexation between the different species. We found practically no changes in the chemical shifts of both carbonyl groups of the methyl benzoylformate when $Mg(ClO_4)_2$ was added; in contrast, both amide and beta-lactam carbonyls of model 2 shifted downfield on complexation [1]. In the ternary system $3/Mg^{2+}/2$ the chemical shifts of the NADH carbonyls are intermediates between the free and Mg-complexed form, consistent with the formation of a new coordinated species. Difference ROE and ROESY experiments were performed with a 1/1/1 mixture of $3/Mg^{2+}/2$. Irradiation of the OMe signal of the keto-ester resulted in both intra- and intermolecular enhancements; also, intermolecular cross-peaks were observed in the ROESY spectrum.

These results represent the first direct observation of a ternary α-keto ester/Mg^{2+}/NADH entity in solution by NMR techniques. Theoretical calculations in order to propose a structure for the ternary complex, in agreement with NMR data are undergoing in our laboratory.

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17 Metabonomics of *Caenorhabditis elegans* Mutants by ^{1}H High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy.

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The nematode worm *Caenorhabditis elegans* was the first animal model whose genome was sequenced. The interest in this model organism is to monitor a wide range of biological process and to characterize genetic mutations responsible for human disease. However, many genetic mutations do not display any obvious morphological or behavioral phenotype under classical observations.

We show that ^{1}H high-resolution magic angle spinning (HR-MAS) nuclear magnetic resonance spectroscopy is able to identify and quantify a large number of *C. elegans* metabolites. In association with biostatistics / chemometrics, we characterize metabolic fingerprints associated to genetic mutations. Here in this study, we develop, validate and apply a metabonomic protocol based on ^{1}H HRMAS NMR spectroscopy of intact *C. elegans* worms, in order to investigate the metabolic signature induced by mutations of oxidative stress enzymes. To address the reproducibility and robustness issue of *C. elegans* metabolic profiling using ^{1}H HRMAS NMR, we have tested and controlled several biological and technological factors, namely the effects of strain, age, sample preparation and ^{1}H HRMAS NMR analysis itself.

A metabonomic analysis, i.e. the hypothesis-free interpretation of biological NMR data by multivariate statistics, is particularly suited to understand pathophysiological perturbations in *C. elegans* mutants. Supervised multivariate statistics reveal a remarkable discrimination between the N2 strain (WT) and mutants of oxidative stress: SOD-1 mutants sod-1(tm776), and Catalase-1 mutants ctl-1(ok1242). We identify a metabolic phenotype (metabotype) significantly associated with these mutations: a general reduction of fatty acyl resonances from triglycerides, corresponding to a compensative strategy for regulation of oxidative stress. This work opens perspectives for the use of ^{1}H NMR HRMAS as a suitable molecular phenotyping device for *C. elegans* functional genomics.

18 Fast Structure Elucidation by Hadamard NMR Spectroscopy

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A set of basic NMR experiments, such as COSY, DQ COSY, TOCSY, HSQC, NOESY, ROESY and HSQC-TOCSY that are typically used for structure determination in small organic molecules, have been modified to incorporate Hadamard encoding. Provided sensitivity is adequate, all the essential information that is required for structure determination of small organic molecules can be recorded in about three minutes. Examples of pharmaceutical interest are presented. Fully automated data collection and processing allows streamlining screening and analysis of new compounds.

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19 Methyl Iodide and Methane in Liquid Crystals Confined to Porous Materials as Studied by ^{13}C NMR Spectroscopy

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The behavior of thermotropic liquid crystals (LCs) Merck Phase 4 (eutectic mixture of p-methoxy-p'-butylazoxy-benzenes) and ZLI 1115 (trans-4-(4-heptyl-cyclohexyl)-benzonitrile) confined to mesoporous controlled pore glass (CPG) materials was investigated using one-dimensional ^{13}C NMR spectroscopy of methyl iodine and methane gas dissolved in the LCs. CPG materials consist of roughly spherical particles with randomly oriented and connected pore network inside. These materials, furthermore, possess a relatively narrow pore size distribution. In the present case the average pore diameters of the materials varied from 81 to 375 Å, and the temperature series measurements were performed on solid, nematic and isotropic phases of bulk LCs. Chemical shift, intensity and line shape analysis of the signals of the spectra contain lots of information about the effect of confinement on the phase of the LCs. Line shape of the ^{13}C signal originating from CH_3I molecules confined inside the pores was observed to be very sensitive to the LC orientation distribution. Effect of the magnetic field to the orientation of LC molecules inside the pores was examined in four different magnetic field strengths varied from 4.70 to 11.74 T. Magnetic field was found to have significant effect to the orientation of LC molecules in the largest pores close to nematic-isotropic phase transition temperature.

20 Assignment of Absolute Configuration on the Basis of the Conformational Effects Induced by Chiral Derivatizing Agents: The 2-Arylpyrrolidine Case

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A novel approach for determining the absolute configuration of a chiral compound is proposed. The methodology is based on the distinct conformational effects imposed on a chiral substrate by each enantiomer of a chiral derivatizing agent. As a proof of concept, the methodology has been applied to the configurational assignment of 2-arylpyrrolidines using MTPA[1] as the chiral auxiliary. It is shown that the absolute configuration of this type of compound can easily be determined by inspection of the multiplicity of the NMR signal corresponding to the methine proton of the pyrrolidine ring in the Mosher's amides[2,3].

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21 ^{19}F and ^{29}Si NMR Shielding and Spin-Spin Coupling Constants in Tetrafluorosilane and Hexafluorosilane in the Gaseous State

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The present work reports the gas-phase measurements of ^{19}F and ^{29}Si chemical shifts of tetrafluorosilane SiF_4 and hexafluorodisiloxane $(SiF_3)_2O$. The density-dependence of chemical shifts δ (or nuclear shieldings σ) [1] is strictly linear for silicon and fluorine nuclei in both compounds. An extrapolation of gaseous data to the zero-density limit permitted the measurements of the ^{19}F and ^{29}Si absolute nuclear magnetic shieldings of an isolated molecules at 300 K. They are as follows: $\sigma 0(SiF_4)$=362.87 ppm, $\sigma 0(SiF_4)$=488.15 ppm, $\sigma 0((SiF_3)_2O)$=356.50 ppm, and $\sigma 0((SiF_3)_2O)$=487.95 ppm. The silicon absolute shielding values were calculated on the basis of new established shielding scale [2]. Careful investigation of ^{19}F spectra shows the isotope effects related to the presence of three naturally occurring silicon isotopes: ^{28}Si (92.19%), ^{29}Si (4.70%) and ^{30}Si (3.09%). All of the isotope shifts measured for fluorine nuclei are upfield, indicating greater shielding of the fluorine on the heavier silicon nucleus than on the lighter one. They are not greater than 0.01 ppm in either compounds. These new experimental results were used for verification of theoretical evaluations reported in the literature [3,4].

Similarly the scalar spin-spin coupling constants $^1J(Si,F)$ were measured in the gas phase as a density function. They are not constant because they are modified by intermolecular interactions [5]. The extrapolation to zero-pressure limit values are $^1J(Si,F)$=168.85 Hz in tetrafluorosilane and $^1J(Si,F)$=166.83 Hz in hexafluorodisiloxane. Both results are slightly smaller in gas than in liquid solutions.

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22 A new Biomarker for Inhibition of the Farnesyl Pathway Identified by NMR Metabolic Profiling

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We present the results of NMR metabolic profiling of urine from a pre-clinical comparative profiling study and subsequent structure elucidation of an unknown molecule by application of LC-SPE, MS and NMR [1].

Sprague Dawley rats were treated with the two biphosphonates Ibandronate and Zoledronate. Toxicological assessment showed very different effects for the two compounds. Urinary metabolite profiles of rats treated with Ibandronate did not cause signs of toxicity. In contrast the drug-induced changes in the NMR urinary metabolite patterns of rats treated with Zoledronate suggest hepatotoxicity and nephrotoxicity: First, increased levels of urinary glucose and decreased levels of urinary creatinine indicated nephrotoxicity. Second, increased urinary levels of creatine and taurine points to hepatotoxicity. Both suggested organ toxicities were later confirmed by histopathology.

In addition, the benefit of metabonomics as an open approach compared to targeted methods was demonstrated by the identification of an unknown molecule in the urine of rats treated with zoledronate. Structure elucidation revealed this molecule as N-acetylfelinine. Analysis of the pathways proposed for the biochemical synthesis of this molecule showed that the synthesis and excretion of N-acetylfelinine could easily be explained by drug-induced inhibition of farnesyl diphosphate synthase. This is the reported mode of action of bisphosphonates. Until now, N-acetylfelinine was exclusively observed in the urine of felidae species where it is believed to be a precursor to a pheromone.

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23 Pushing the Limits of Metabolite Biomarker Identification from 1D NMR-Based Metabolomics Data

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NMR has become one of the primary tools used for metabolite biomarker discovery. Although transforming NMR spectra into multivariate distributions is relatively straightforward, deciphering biological meaning from these data is one of the most challenging tasks facing automated metabolomics analyses. We will describe two general strategies for identifying potential biomarkers in 1D ^{1}H NMR spectra and explain how metabolic changes can be linked to perturbations in metabolic pathways using an integrated informatics system.

The first strategy is to match statistical signatures from Principal Components Analysis (PCA) and Automated Filtering of NMR Spectra (AFNS) against a database of over 270 metabolite standards. In our case studies, AFNS is used to convert the large spectral datasets (normally 32K or 64K points per spectrum) into a few hundreds of statistically significant points with original digital resolution. The loadings plots generated by the PCA of such filtered data are used to search the metabolite database using a sophisticated peak search algorithm, which takes into account the chemical shift deviations and the counts of matched peaks. The resulting hit list can be further evaluated using the following second approach.

The second approach measures the correlation between the loadings plots and the candidate metabolite spectra by projecting the latter into the scores space of PCA, and observes their location along the factor dimension where the samples are classified. Residuals between the reconstructed and original spectra are also used to filter the matches. The identified metabolites are used to search the KEGG database for relevant metabolic pathways.

The scope and limits of these methods are demonstrated using two 1D ^{1}H NMR datasets, one of the extracts from Arabidopsis thaliana, and the other of a synthetic mixtures of metabolite standards with systematically controlled metabolite concentrations

24 Selective J-resolved Spectra as a Tool for Measuring Homonuclear Scalar Couplings

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The ever growing refinement of modern quantum-chemistry methods has brought an impressive level of accuracy in the prediction of NMR parameters such as chemical shifts and scalar coupling constants [1], all of which need ultimate validation against reliable experimental data. In particular, the accurate measurement of homo- or hetero-nuclear coupling constants is a quite demanding task that can be accomplished by means of different approaches.

In this context we propose a simple method to obtain a selective version of homonuclear J-resolved spectra [2], which relies on the refocusing properties of double pulsed field gradient spin-echoes [3, 4] and provides unambiguous assignment of the measured coupling constants. The method is of general applicability, easy implementation and requires no more than a simple optimization strategy to produce clean spectra.

As examples of possible applications, we have determined some long-range coupling constants in strychnine and trans-retinal, as well as Hα couplings in a tripeptide [5]. Possible issues connected to the different relaxation rates of in-phase and anti-phase magnetization are also discussed.

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25 Adaptive Spectral Library of Amino Acid ^{13}C Isotopomers

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Amino acid ^{13}C isotopomer ratios carry invaluable information on intracellular metabolic flux distribution used in metabolic flux analysis (MFA). Isotopomer mixtures are obtained, for example, from hydrolysis of partially ^{13}C labeled proteins. In this work, the natural abundance ^{1}H coupled ^{13}C NMR spectra of all the natural amino acids were measured in D2O at pH* 1. The accurate $^{1}H,^{13}C$ spin-spin coupling constants were analyzed using PERCH's total-line-shape fitting method. The obtained spectral parameters establish an adaptive spectral library of amino acid ^{13}C isotopomers, adaptive because using this library the spectra of isotopomers can be simulated at any field, line width and line shape obtained from the observed spectra. The calculated spectra can be then used as base spectra for quantifying ^{13}C-isotopomer mixtures of the amino acids and, thus, for exploring metabolic pathways. In this poster, we present a protocol for ^{13}C isotopomer analysis based on the adaptive spectral library of ^{13}C isotopomers.

26 Complete ^{1}H NMR Spectral Analysis of Large Spin Networks

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Due to signal overlap and complex second-order effects complete ^{1}H NMR spectral analysis of large spin networks, such as steroids, is a challenging task. In principle, the classical computational spectral analysis is the only way to obtain very accurate spectral parameters in presence of strong second-order effects. In this poster, complete ^{1}H NMR spectral analyses of some steroids are presented. To our knowledge, this is the very first time such a large continuous spin networks have been analyzed completely using classical computational spectral analysis. The results of the present analyses were used to improve the 4D coupling constant prediction, taking the molecular flexibility (the 4th dimension) into account.

27 Rapid Identification of Monosaccharide Units in Polysaccharides Using a TOCSY Pattern Matching Approach

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Solution NMR spectroscopy is a well established technique for the structural and conformational characterization of complex oligo- and polysaccharides. The particular approach will generally always involve 2D TOCSY spectra recorded at one or several mixing times. Indeed, each monosaccharide unit generally contributes a single spin system, isolated from its neighbours, which can be traced using the usually well resolved anomeric ^{1}H resonance. As the architecture of the monosaccharide spin system is mostly linear, the transfer of magnetisation during the spin lock from the anomeric ^{1}H to the opposite end will depend on the intervening $^{3}J_{HH}$ scalar coupling constants. These are strongly dependent on the associated torsion angle that for monosacharides are narrowly constrained due to the cyclic structure. A large coupling constant, involving two neighbouring axial C–H bonds, is expected to allow a fast transfer of magnetization, whereas a small coupling constant, typically for an axial-equatorial and equatorial-equatorial C–H bond pair will considerably reduce transfer efficiency. Since the most common aldohexoses differ from each other in the axial/equatorial disposition of the C–H bonds, each will be characterised by a different sequence of scalar coupling constants. Therefore, we can expect different patterns for the intensity build up of cross-peaks linking the anomeric ^{1}H to the other ^{1}H spins, providing an opportunity to obtain additional information on the monosaccharide type at the start of the assignment proces.

To investigate this, we have studied systematically the rate of the magnetization transfer through the monosaccharide spinsystem starting from the resolved anomer 1H resonance in a variety of model monosaccharides, using both MLEV17 and DIPSI-2 spin locking sequences. To avoid lengthy 2D experiments, selective 1D TOCSY experiments were used. We demonstrate that using a mixing time of 100 ms, a high level of distinction between glucose, galactose, mannose and rhamnose can be obtained, including differentiation between the α and β diastereomers. Using a commercially available complex N-linked oligosaccharide, we show that we can easily distinguish between α-D-mannose and β-D-mannose, that are notoriously difficult to discriminate. The general applicability is further demonstrated using a bacterial polysaccharide.

28 Follow-up Screening by NMR

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Over the last few years, screening by NMR has emerged as a potent method for the identification of small molecules that bind to macromolecules.[1,2] Although these techniques suffer from intrinsic low sensitivity compared to other screening methods, it allows the monitoring of low affinity ligands in a wide range of systems, and is complimentary to other biophysical techniques available. As opposed to lead identification, we have used STD[3] experiments for secondary screening; confirmation of hits from high concentration bioassays, SAR on lead material, mode-of-action studies and competition STD to provide binding site information.

NMR screening was carried out on a series of truncated ligands, based on the lead for an anti-infectives project. From the NMR results, in conjunction with ITC, we were able to identify which aromatic groups were essential for binding. The biophysical data was invaluable for project progression, as all of the analogues were out of the activity range of the bio-assay, and the NMR screen had a wider activity range than other biophysical techniques such as ITC and SPR. In a second example, NMR was used for confirmation of fragment binding from hits generated in a high concentration bio-assay. To further support crystallographic studies, competition STD was used to identify non-specific binders. These examples highlight that while the reliable evaluation of weak hits poses a formidable challenge for bioassay-based screening, NMR methods are well-suited to provide accurate data on low affinity binders.

Using NMR for follow-up screening has also highlighted the advantages of being able to acquire reliable, good quality data using ligand stocks previously prepared in protio-solvents. Recent improvements in NMR hardware and modifications to solvent suppression schemes are also discussed during the poster.

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29 Use of Broadband Proton Decoupled Proton Spectra to Detect Impurities and Solvents within an API

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Recent experiments developed by Keeler et al. (Magn. Reson. Chem., 45, 296-316 (2007)) have shown how it is possible to acquire broadband proton decoupled proton spectra with absorption mode lineshapes and reliable integrals. This poster will describe the application of this experiment to an API showing how it can be used alongside other experiments (for example selective TOCSYs and homonuclear decoupling experiments) to detect and quantify low level impurites or solvents that are otherwise obscured.

30 NMR Chemical Shift Prediction by Atomic Increment Based Algorithms

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Here, we would like to report on a new implementation of the Atomic Increments algorithm for NMR chemical shift prediction. Predictions were performed using both linear regression with a partial least squares (PLS) algorithm and neural networks under different conditions for ^{13}C nuclei and the results were compared.

The focus of the work was on strategies used to encode a chemical structure into a numerical representation; a key step required by the neural network or linear regression approaches. It was quickly discovered that a careful balance must be found. On the one hand, a detailed numerical description leads to more precise results for structures included in the training set and their structural relatives. On the other hand, however, a very detailed description inhibits the ability of the network to make generalizations, and the predictions for structures outside of the training set are very poor. The most important decision is how many different types of atoms should be distinguished, and how many spheres around the central atom should be taken into account. The best chemical shift predictions for ^{13}C resulted when all of the atoms were divided into 66 different classes according to their chemical nature, stereochemistry, and formal charge; and the information associated with atoms up to 5 bonds away was used to influence the prediction. Interaction between atoms and the effects of this were taken into account by using cross-increments. As it turns out, without cross-increments, neural networks performed better than the regression scheme. Remarkably, with cross-increments added (only atoms separated by one or two chemical bonds were considered), both linear regression and also neural networks performed similarly and much better than without cross-increments. As before, when too many cross-increments were introduced, over-fitting and poor predictions resulted.

With a training dataset of approximately 2 million individual ^{13}C shifts, the most efficient calculations had a mean error of prediction of 1.5 ppm (an independent test dataset of 150,000 chemical shifts was used).

Our conclusion is that the most important factor influencing the precision of the chemical shift prediction (and, probably, other structure-activity relationship studies) is the scheme used for encoding a chemical structure into a numerical input. With an appropriate incremental scheme, both the linear regression and neural networks method are highly effective, and suitable for most situations that require chemical shift prediction.

31 Validating Automated Structure Confirmation in a Blind Study

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In a previous publication[1], the results of an automated structure verification process for 1D proton NMR data was presented. Since that time, combined verification, an approach that confirms a structure by using 1D proton and 2D HSQC data, was developed and presented[2]. Based on the first results, many improvements have been made to the process that has enabled an even greater level of accuracy. Recently, a validation of this performance was made by putting the software through a blind test.

The blind test consisted of two data sets. One that was provided ahead of time for adjustment of processing settings and options (19 spectra sets), and one that was "blind" (10 spectra sets), and therefore not provided to the software or software operators. In this way, the true performance of the process could be evaluated without any bias of the result towards particular data sets by customization of the software settings.

The results of this study reveal how a completely automated system can reduce 85% of the datasets a spectroscopist has to evaluate manually. Presented here will be an analysis of these results and a detailed review of the structures that could not be automatically confirmed and why they failed.

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32 Computerized Structure Verification

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To make sense, computerized structure verification based on NMR-spectral data has to meet the same standards with respect to the ratio of correct and false positive/negative classifications as a manual evaluation. This ratio is essentially affected by:

1. The quality and variety of the available spectral data
2. The quality and reliability of the algorithm extracting the spectral parameters
3. The quality and statistical robustness of the algorithm comparing the extracted spectral parameters with those expected from the given structure

In a high-throughput environment the variety of available spectral data is often limited to 1D proton NMR. Therefore the performance of the subsequent steps of the analysis is even more crucial. This means the spectral parameters need to be extracted as complete and as accurate as possible. This can only be achieved by computational classical spectral analysis especially when dealing with considerable signal overlap and effects of higher order. Only this ensures that all spectral parameters are consistent throughout the complete spectrum and also reproduce the higher order effects. Together with an accurate prediction of the spectral parameters (based on the given structure and the relevant sample properties like solvent, concentration, pH, etc.) and well founded estimates of their reliability this allows highly selective computerized structure verification with minimal user intervention. The poster presents a typical workflow and some case studies.

33 Structure Elucidation of novel alkaloids by 1- and 2-D NMR Experiments

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There are four *Aconitum* species growing wild in Turkey, *A. orientale Mill., A. nasutum Fisch. Et Reichb., A. anthora L.* and *A. cochleare Woroschin*. The first three plants have been already studied [1]. In the present study the last Turkish species namely *A. cochleare* was investiged mainly in terms of the structure of its metabolites with the assistance of 1- and 2-D NMR techniques. The plant was collected from Eastern Turkey (Van). A literatue survey has revealed that *Aconitum* preparations have been used as cardiotonics, febrifugics and sedatives for many centures [2]. However, in Turkey they are not included in folk medicine and are used only as pain releavers under physicians control [3].

The powdered plant material (526g) was macerated with EtOH and subsequently separated by centrifugally accelarated radial TLC (Chromatotron).

Two new diterpenoid alkaloids, cochleareine and acoleareine together with the known alkoloids 14-acetyltalatisamine[4] and talatisamine [4] have been isolated and identified from the aerial parts of *Aconitum cochleare Woroschin* The structures of above mentioned compounds were deduced by extensive ^{1}H and ^{13}C studies. The unambiguous chemical shift assignments of cochleareine and acoleareine were achieved through a study of their ^{1}H detected by 2D NMR experiments ^{1}H-^{1}H COSY, HMQC, HMBC and stereochemisty by NOESY as well as 1D ^{13}C (APT,DEPT) experiments. In addition to the NMR the molecular formulas were determined by HRMS.

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34 Using Flow NMR Monitoring to Better Understand Reactions in Process R&D

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The pharmaceutical industry, particularly chemical process research and development, faces ever increasing pressure to develop synthetic process reactions for intermediate and final products that exhibit higher throughput, are more rugged and safe, have higher yields, and produce lower levels of byproducts (impurities). This can be achieved, in part, by intelligent acquisition of spectroscopic data that then leads to a better understanding and control of the reactions. The goal of this work was to monitor chemical reactions in real time by coupling a small scale (<0.5 L) temperature controlled reactor with a 500 MHz flow NMR and ReactIR(TM). This system is used to study several reactions that are deceptively simple on the surface, but upon closer examination using this system, are more complex. This understanding is then translated into changes in the chemical synthesis that reduces impurities and increase yields.

35 A Metabolomic Comparison of Mouse Models of Neuronal Ceroid Lipofuscinoses

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The Neuronal Ceroid Lipofuscinoses (NCL) are a group of fatal inherited neurodegenerative diseases in humans characterised by a common clinical pathology, storage body accumulation in cells and gross brain atrophy. In this metabolomic study we have investigated changes in 3 mouse models of the NCLs to examine common pathways correlated with neurodegeneration. The first model, a naturally occurring mouse mutant, termed motor neuron degeneration (mnd) mouse contains a one base pair insertion in the orthologous mouse Cln8 gene (82% identical to human), exhibiting abnormalities akin to those in human NCL patients. The second mouse variant models late infantile NCL and is termed the neuronal ceroid lipofuscinosis (nclf) mouse. The nclf mouse contains a one base pair insertion in the orthologous mouse Cln6 gene (90 % identical to human gene) resulting in a frameshift defect. These models were compared to the previously studied cln3, juvenile NCL (Batten disease) mouse model on a 129S6/Sv background (Pears et al. J Biol Chem, 2005. 280(52): p. 42508-14).

Brain tissues metabolites from mnd, nclf and controls (1 to 6 months) were extracted using a chloroform/methanol based technique. The ^{1}H-NMR spectra were recorded on a 500 MHz Bruker Avance spectrometer, processed using ACD NMR Manager and analyzed with SIMCA-P+ 11.5. The NMR profiles derived from mnd and nclf mice were distinguished according to disease/wild type status in both the cortex and cerebellum. The most discriminatory metabolite was glutamate, increasing in the cortices and cerebellum of mnd and nclf mice relative to wild type for all ages, similar to the cln3 mice, while GABA was increased in the nclf mice, unlike the cln3 model, and decreased in the mnd mice model when compared to the control. Phosphocholine and myo-inositol were increased and NAA decreased in the cortex of mnd mice; whereas in nclf mice the contrary was observed for myo-inositol and Phosphocholine. Our results allowed identification of glutamate, GABA and NAA metabolic traits being common to all NCL subtypes suggestive of impaired neurotransmitter cycling.

36 Defining the Changing Role of PPAR-α in Systemic Metabolism During Ageing

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Insulin resistance is a major feature of obesity and type II diabetes mellitus and is commonly accompanied by an increase in circulating free fatty acids. Fatty acids are able to stimulate their own metabolism through direct binding to Peroxisome Proliferator Activated Receptors (PPAR)-α[1]. In the present study the interactions between organs, ageing and a failure to express PPAR-α has been investigated using both metabolomic and transcriptomic approaches.

The use of COSY, TOCSY and J-RES experiments has confirmed the identification of approximately 50 metabolites per sample, which was increased to 150-200 metabolites per sample when used in combination with gas chromatography- mass spectrometry (GC-MS). Using multivariate statistics this data was combined with results from RT-PCR experiments used to define transcriptional changes.

The changes seen in the liver are consistent with perturbed glucose metabolism and an attenuated response to insulin. There is a dramatic loss of glucose and glycogen with age. This is likely to be exacerbated by a reduction in gluconeogenesis as we observed a 2.7 fold reduction in the transcription of G6pc, and a 5.0 fold reduction in Pck1, the latter being the rate limiting enzyme of the gluconeogenic pathway. Hearts of the PPAR-α null mouse also exhibit signs of perturbed energy homeostasis with decreased ATP and creatine concentrations. Furthermore, loss of beta-hydroxybutyrate at 5 and 9 months supports the well documented decline in ketogenesis in this mouse [2]. Metabolic and genetic alterations in the skeletal muscle and adipose tissue were also consistent with perturbed fatty acid metabolism and energy homeostasis.

In conclusion, our study provides evidence of perturbed fatty acid oxidation and glucose metabolism and identifies a number of markers of insulin resistance in the fed PPAR-α null mouse during the process of ageing and across systemic metabolism.

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37 Rapid Assessment of Grape Extract Compositions by ^{1}H NMR

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Natural product extracts contain large numbers of different chemical constituents which may be responsible for beneficial effects on health. Grape extracts, and in particular their polyphenolic constituents, have gained considerable interest since they have been associated with improvement of the vascular function, reduction of platelet aggregation, blood pressure and LDL levels. When marketing functional food ingredients with positive health effects it must be ensured that the active ingredients are present, regardless whether their exact identity is known. Thus we deployed NMR profiling during different stages of product development, i.e. sourcing and selection of commercial grape extracts and their shelf-life stability in a formulated product format.

^{1}H-NMR fingerprinting in tandem with Multivariate Data Analysis (MVDA) was used to survey the large range of commercial grape extracts being offered on the market, besides the compositional screening of known components was performed. Cluster analysis revealed unexpected similarities and differences in between extracts and with claimed content specifications. Furthermore, spectral similarity between NMR spectra could be used to design mixes of grape extracts based correlation coefficients and the association variances. In order to obtain a rapid assessment of overall composition of the grape extracts in terms of phenolic and sugar content, as well as their conjugation, DOSY was deployed. The diffusion constants of reference standards were compared with the diffusion constants (D) of the polyphenols from the grape extracts. The distribution of polyphenols on basis of diffusion constant showed that the major polyphenolic fraction of the extracts have Mw's between 400 and 800 g/mol. The conclusion is that the major polyphenolic compounds in extracts are glucose-conjugated polyphenols. Good mass balances could be calculated by quantification of polyphenolic groups against an internal standard, taken into account the presence of glucose conjugated polyphenols.

Typically, natural product extracts are formulated in attractive food formats, such as dairy drinks. It is critical to establish stability of formulated grape extract constituents during shelf-life. Thus we developed and applied a method that was able to provide a quantitative profile of the polyphenolic content in dairy formats during storage. When no adequate precautions were taken, the NMR profiles showed clear changes during shelf-life.

38 Integration of NMR Metabolomic and Transcriptomic Data to Identify Changes Associated with Phenobarbital Exposure

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Non-genotoxic carcinogens are a class of compounds which cause cancer but do not damage DNA. The mechanisms of their action are generally poorly understood. These compounds are difficult to screen for as they are not detected in standard, short term, in vitro mutagenesis assays such as the Ames assay, and usually are only identified by two year rodent bioassays, which are costly and time consuming [1].

In the first stage of our study, the effect of the well characterised rodent non-genotoxic carcinogen phenobarbital was investigated in a two week study. Phenobarbital causes an increase in the incidence of liver tumours in rats during long term exposure and importantly, changes, including increases in liver weight and cell replication, are detected after two weeks of exposure [2]. The study used a range of doses: a low dose which does not cause an increase in liver weight and is not carcinogenic, a dose which causes an increase in liver weight but is not carcinogenic and a high dose which causes increased liver weight and is carcinogenic during chronic exposure. Liver extracts, plasma and urine were profiled using 1D ^{1}H NMR spectroscopy. Multivariate statistical analysis was used to identify changes to metabolism in animals given a carcinogenic dose. In addition 2D ^{1}H NMR spectroscopy was used to aid identification of metabolites. Using these techniques, perturbations to amino acid and glucose metabolism were identified after only one day of exposure. Decreases in branched chain amino acids, glucose and glycogen and increases in glutamine, glycine, succinate and glutathione were observed in animals exposed to phenobarbital relative to control animals. Gene expression data acquired using Affymetrix microarrays has been integrated with the metabolomic data to aid identification of altered pathways. Using graphical representations, changes in glutamine, glutamate and glutathione metabolism have been revealed. A multivariate regression approach based on partial least squares identified correlations between glycogen and hexokinase D expression and between succinate and 5-aminolevulinate synthase expression.

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39 Conformation and Self-association of a Cyclic Lipodepsipeptide Investigated by NMR Spectroscopy

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1D and 2D NMR techniques have allowed to elucidate the structure of a new natural product with antibacterial activity recently isolated from Pseudomonas species. The molecule, named KMCP-1 for the time being, is shown to be a cyclic lipodepsipeptide, consisting of 9 amino acids, seven of which form the cyclic structure closed via a lactone bond between the C-terminus and the alcohol group of the Threonine side chain. The exocyclic N-terminus is attached to a β-hydroxydecanoic acid moiety. Using total hydrolysis combined with derivatisation for LC-MS analysis, and a recently obtained X-Ray structure, the stereochemistry could be unambiguously assigned. KMCP-1 appears to be a new member of the Viscosin family, a group of biologically active cyclic lipodepsipeptides produced by a variety of Pseudomonas species[1]. Results from the NMR conformational analysis in acetonitrille of KMCP-1 will be presented and compared to the X-Ray structure obtained from a saturated solution in the same solvent. The properties of KMCP-1 change dramatically when switching from a highly polar and/or hydrogen bonding (CD_3CN, DMSO) to a non-hydrogen bonding solvent (chloroform): in the latter it effectively behaves as a molecule of much greater dimensions than expected. We propose that KMCP-1 undergoes some form of self-association, where the molecules stack onto each other to form tube-like structures. Such behaviour could be the mode of action against other micro-organisms: tubes inserted into the apolar cellular membrane disturbe the osmotic balance by acting as small pores. This phenomenon has previously been observed for similar lipodepsipeptides[2]. The self-association in chloroform was verified and studied in more detail by diffusion NMR spectroscopy. By introducing different models that describe the self-association, we hope to unravel the thermodynamics of this process, and estimate the size of these tubes, thereby gaining more insight in the nature of the self-assembly on a molecular scale.

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40 Time-domain Bayesian Detection and Estimation of Noisy NMR Signals for Spectroscopy Based Metabolomics

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The problem of detection and parameter estimation for noisy signals arises in different areas of science and engineering such as audio processing, seismology, electrical engineering and nuclear magnetic resonance spectroscopy (NMR). For the latter application the free induction decay (FID) detected in an NMR experiment can be considered as a sum of exponentially damped sinusoidal components, and this model has proven to be adequate in a number of applications, including the analysis of solution-state biofluid NMR spectroscopy. In this case model detection reduces to the estimation of the number of components. The detection and estimation problem for the damped sinusoidal model has received considerable attention over the past two decades. However, it remains generally unsolved for difficult cases when SNR is low and the frequency of signal components are closely spaced (co-resonant) as occurs in many ^{1}H NMR spectra of complex mixtures. In this work we offer a joint detection-estimation algorithm for the complex-valued case with exponential damping following the reversible jump approach developed by Andrieu & Doucet [1].

The proposed algorithm has been tested on synthetic data and biofluid spectra (blood plasma and urine). The detection and estimation performance was compared with Akaike information criterion (AIC) and Minimum Description Length (MDL) and the matrix pencil method [2-3]. The results show the Bayesian algorithm to be superior in performance, especially in the difficult cases of detecting low-amplitude and closely-spaced resonances in noisy signals. The proposed approach allows both increases in sensitivity and resolution of the analysis while demanding very little user intervention.

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41 Solid State DNP of Heteronuclei

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Dynamic Nuclear Polarisation (DNP) is a hyperpolarisation technique that offers an increase of over 10,000 times [1] in the signal-to-noise ratio (SNR) for many spin=1/2 NMR nuclei in solution-state NMR spectroscopy. The sample of interest is doped with a trityl radical and dissolved in a mixture of solvent that will form a glass when frozen. It is then exposed to a very low temperature (~1.4 K) in the presence of a strong magnetic field (3.35 T). Under these conditions, the unpaired electrons on the trityl radical attain a high degree of Boltzmann polarisation(>90%). By applying microwave irradiation at the appropriate frequency (ca. 94 GHz) polarisation is transferrred to atomic nuclei. Once a sufficient level of hyperpolarisation has been reached, the sample is rapidly, <1 s, thawed using a dissolution solvent (eg water, methanol, toluene) and rapidly (~1 s) introduced into a high-resolution NMR spectrometer where the hyperpolarised spectrum is acquired [2].In the frozen state the NMR nuclei can become polarised via a number of mechanisms, broadly described as solid effect or thermal mixing. The dominant mechanism has been previously investigated [3] by monitoring the level of polarisation at discrete frequencies across the line width of the EPR resonance. These results suggest that thermal mixing dominates the polarisation for ^{13}C and ^{15}N. Using a high sensitivity broadband probe that can be inserted into the VTI of the polariser, we extend this work to monitoring polarisation for ^{2}H, ^{29}Si and 31P during a microwave sweep and polarisation build up. We also investigate the effect of radicals on the polarisation of these nuclei.

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42 New Developments in An Integrated System for NMR-Based Metabolomics Analysis

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Data processing and analysis in metabolomics studies involves many steps to transform, correct, analyze and interpret the data. The process also demands multi-disciplinary experience and knowledge in NMR spectroscopy, chemometrics, chemistry, and biology. To improve the efficiency of NMR-based metabolomics data analysis, we have developed an integrated software environment to cover the entire process from processing raw NMR data to biomarker identification, including the following functions:

- Batch processing of raw FIDs and batch import of processed spectra
- Visualization of spectral collections as an Overlap Density Heatmap (ODH) [1] a novel method to quantitatively evaluate the (dis)similarity among multiple overlaid spectra.
- Multiple optional binning/bucketing methods: fixed-width, variable-width, AFNS, and use of full-resolution spectra without binning.
- Wizard page guided principal component analysis (PCA).
- 2-way communication with advanced chemometrics tools for generating and applying predictive classification or regression models (KNN, SIMCA, PLS-DA with optional OSC, etc.)
- ^{1}H and ^{13}C spectral libraries of over 270 standard metabolites [2] provided for the identification of changed metabolites by searching the PCA loadings plots against standard/reference spectral peaks or projecting standard spectra into scores space [3].

This presentation demonstrates the scope and limits of these tools by using several real metabolomics datasets−from samples such as human serum, plant extracts and animal urine−with an emphasis on the newly developed methods for quantification, binning/bucketing, and identification of changed metabolites.

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43 Probing the metabolic response of *Arabidopsis thaliana* to hypoxic stress using ^{1}H-NMR

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The emerging field of systems biology, specifically metabolomics, has been used to study the response of *Arabidopsis thaliana* to hypoxic stress. Eighteen small-molecule metabolites have been quantified after plant tissue was subjected to liquid extraction and proton nuclear magnetic resonance (^{1}H-NMR) analysis. Integral regions within plant extract spectra were created to reflect resonances of more than eighty metabolites involved in central carbon and amino acid metabolism. Peak areas were normalized to total integral intensity measured for each sample. Principal components analysis (PCA) was conducted to determine the most significant variances in the dataset. The PCA scores plots revealed good separation among the sample different treatments. Fold change between growth conditions and signal-log ratios were determined and a t-test was used to establish confidence. Good correlations were observed between metabolic changes and those predicted from changes in gene expression levels measured using total and polyribosome-associated mRNAs.

44 Monitoring Solid-modified Apparent Molecular Mobility: A Tool for the Analysis of Mixtures and the Study of Liquid/Solid Interaction in Porous Systems.

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The advent of high-resolution MAS technology, integrating solid-state (sample spinning) and liquid-state (PFG and optimized proton linewidths) NMR tools allowed the introduction of methods tailored to exploit and/or to reveal solid/liquid interactions relevant of related to chromatography.

Among those, one introduced by us capitalizes on the affinity of small molecules for a solid or immobilized phase to induce up to manifolds deviations in the apparent molecular mobility with respect to the one measured in solution. When a solid phase (typically a silica gel) is added to a solution containing a mixture of small molecules, the corresponding molecular dynamics varies with the affinity of each moiety for the solid, in analogy to a typical LC behavior. We showed that this solid-induced effect can be used to simplify the separation of the spectra of the mixture, for instance in a DOSY experiment, whence the acronym Chromatographic NMR. Surprisingly, this method has been shown to outperforms LC in favorable cases.

In this work, we investigate by PFG methods some of the physical basis of the observed induced variations in the DOSY spectra. This information can be used directly in Chromatographic NMR, to ameliorate the sought spectral separation or to understand its limits, but also to peek into the intimacy of other technologically relevant areas involving liquid/solid phase contacting, such as LC, catalysis or nanofluidics processes.

The ratio solid-to-liquid phase is shown to have various effects, as it influences the position of the attached/free molecule equilibrium, but also the impact that molecules in the vapor phase can have on the average observed displacement.
Similarly, the structure of the solid phase, and more specifically its porosity, determine some of its adsorbing properties, as it is linked to the specific surface and it may induce size exclusion effects. In this perspective, the role of silanols as the major adsorbant site of silica has also been explored in this poster.

45 High-Resolution MAS Metabolomics of Solid Foodstuff

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Highly recognizable food products are associated to strict fabrication protocols, based on selected biotechnology processes and starting materials of certified origin. Such an elaborate selection of specific metabolic pathways and composition of the starting materials, are likely to produce clear metabolic signatures. The determination of metabolic biomarkers linked to a highly valued quality in food products has the double advantage of providing an independent labeling of a product, useful to counter fraud attempts and to suggest leads to single out the most relevant biological pathways determining the specialty of the final product.
Solid samples present the additional difficulty of being heterogeneous in composition, which can be hampering a metabolomic study, by providing an additional source of variance in the metabolite concentration.
In this context, we show that HRMAS NMR has a strong potential for the metabolomic study of foodstuff as:

- the sample can be analyzed essentially as such, with a minimal pre-treatment;
- the signal of light metabolites, the most sensitive items to investigate active metabolic pathways, is enhanced in HRMAS.

This poster presents a critical analysis of the HRMAS metabolomic work performed in our laboratories on different types of solid foodstuff. Through the examples of cheese and meat specimens, we discuss some of the potential and limitations of HRMAS in determining, by metabolomics, the age of ripening and the geographical origin of specialty foodstuff.

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46 Hyphenated Technology – Not Always the Best Choice

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Identification and characterization of low level impurities in pharmaceutical samples is a demanding task requiring an interdisciplinary approach. Thus disciplines like isolation of impurities; MS and NMR analysis (hyphenated or stand-alone) are most often required. In most cases examination of samples by LC-MS and LC-MS/MS analysis will be sufficient to describe the impurities present. However, in some cases more information is needed, most often NMR data in order to distinguish impurities like isomers. At present time the hyphenated techniques LC-NMR/MS and LC-SPE-NMR/MS are solving many problems concerning complex biological or chemical mixtures. However a major draw back when working with low-level impurities is the limited loading on the analytical column in the HPLC system coupled to the NMR instrument. The sensitivity of the NMR technique has partly been solved with the incorporation of a SPE (solid-phase extraction) module between the HPLC system and the NMR instrument. With this SPE module incorporated it is possible to accumulate an impurity of interest on a SPE cartridge. However other factors such as break through volumes and loading capacities need to be considered when working with the LC-SPE-NMR technique. Therefore classical preparative purification can be the most efficient way to gain material of a purified impurity and thereby acquire the necessary NMR information for the structural elucidation work. Today's preparative column material and packing technologies allow the direct up-scaling of analytical HPLC conditions to large scale preparative conditions with a high degree of preservation of the chromatography. A few runs on preparative scale can provide sufficient quantities of the impurities for identification, especially when using a 1 mm micro probe for the NMR experiments. This "preparative purification-micro-scale NMR" strategy has been applied to a case implying identification of a series of low-level isobaric isomers observed in a new drug substance. In the present work a traditional purification strategy was applied. The isolated impurities were subsequently studied by NMR spectroscopy using a 1 mm microprobe thereby reducing the acquisition time significantly. The identified impurities were quantified using qHNMR spectroscopy by recording a proton NMR spectrum of the original material. The applied strategy was found to compete on an equal basis with the hyphenated technology when looking in a time frame.

47 A Perfluoroelastomer-based Gel Stretching Apparatus for Efficiently Measuring RDCs in all NMR Solvents

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Residual dipolar couplings (RDCs) contain valuable structural information for defining and verifying the conformation and configuration of compounds. For obtaining RDCs it is necessary to partially orient the solute moleculesvia a so-called alignment medium.

We will present several gel-based alignment media for the most common NMR solvents (see e.g. [1-3] and references therein) that can be applied in combination with a mechanical stretching device which allows rapid and arbitrary adjustment of alignment strength. The device was originally designed for molecules dissolved in gelatine gels as the corresponding alignment medium. Here, a redesigned and optimized gel stretching apparatus is introduced for the measurement of RDCs on small as well as larger compounds in basically any kind of NMR solvent.

The core of the apparatus consists of a specially new designed tube made out of highly stretchable Kalrez*Perfluorelastomers with excellent resistance to practically all chemicals. Since the polymer tube is perfluorinated, it does not contribute in any way to the proton NMR spectrum. First examples for RDC-measurements will be shown and advantages/disadvantages of the novel approach will be discussed.

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48 Online-NMR Spectroscopy in Process Analytics – A Kinetic Study of the Formation of Urea-formaldehyde Resins

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NMR spectroscopy is one of the most information rich analytical techniques available and hence can provide a wealth of information on complex reactions that are of interest in process design and development. Due to its cost and complexity so far it has been rarely used in the field of chemical engineering, where mainly the acquisition of reliable quantitative data is of interest. Making use of hyphenated NMR techniques, reaction mixtures can be analyzed directly without sample preparation under industrially relevant conditions. No deuterated solvents are used. The method is well suited for quantitative measurements, i.e., for the determination of reaction kinetics.

In this poster process monitoring of the reaction of urea with formaldehyde by quantitative online NMR spectroscopy is discussed as an example for the application of the method. Urea-formaldehyde resins are the most important binding agent in the production of fiber boards. But despite extensive research for decades the reaction network leading to these resins is still not adequately understood. Achieving low formaldehyde emissions while maintaining excellent physical properties is the major goal. The main reason for the limited knowledge is the number and complexity of parallel reactions leading to many different intermediates, which, to the largest part, cannot be isolated from the reaction mixture and therefore have to be analyzed in situ. Only NMR spectroscopy allows distinguishing starting materials, intermediates and byproducts from each other in the constantly reacting system. The usefulness of NMR spectroscopy for elucidating complex technical reaction systems and the development of a reaction kinetic model of the reaction system are described.

For a kinetic data set spectra are acquired over the course of the reaction. Special software is used for deconvoluting and integrating the peaks for each spectrum. Reaction kinetics are modeled using an equation based process simulation environment. Prior to these kinetic studies, systematic experiments were carried out to allow peak assignment in the complex spectra. These include qualitative and quantitative 1D- and 2D-^{15}N spectroscopy with isotopically labeled urea using a virtual reference standard. Also natural abundance 2D-^{1}H-^{15}N spectroscopy in the flow regime on the technical reaction mixtures was found to be useful.

49 Protonation and Dynamics in Building Blocks of Benzothiadiazine Analogues of Anticancer Alkaloids

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2,3-Polymethylene-1,2,4-benzothiadiazine S,S-dioxides are bioisosters of natural quinazoline alkaloids desoxyvasicinone and tetrahydropyridoquinazolones, which are also pharmacophoric moiety of polycyclic anticancer alkaloids (luotonin, rutecarpine). NMR spectroscopy, an excellent tool to investigate acid-base equilibria [1] and chemical exchange processes [2], is applied to four small-molecule intermediates of novel tricyclic heterocondensed benzothiadiazines.

The tautomeric state of 2-(3,4-dihydro-2H-pyrrol-5-ylamino)benzenesulfonic acid (1) and 2-(3,4,5,6-tetrahydropyridin-2-ylamino)benzenesulfonic acid (2) is established from single and multiple bond $^{1}H,^{15}N$ correlation experiments. The single-tube ^{1}H NMR titration with in situ pH indication [3] characterize the basicity of the endocyclic imine group: log K = 9.57 and 10.41 in 1 and 2, resp. The rotamers of 1 around the exocyclic C-N bond exhibit slow exchange at room temperature in DMSO on both the ^{1}H and ^{13}C timescales. Inversion transfer experiments [4] are performed to determine the exchange rate constants. Variable temperature ^{1}H spectra reveal a coalescence at 50°C. Molecular modeling calculations are performed to support the conformational preferences.

Cyclisation of 1 and 2 with phosphoryl chloride leads to the corresponding tricyclic [1,2,4]benzothiadiazine 1,1-dioxides 3 and 4. These compounds undergo ring-opening upon protonation of their imine group (log K = 2.14 and 3.10, resp.), but the cyclic structure is restored upon further acidification. This unique kinetics of decomposition is studied by pH-dependent kinetic ^{1}H NMR spectral series.

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50 The DOSY Toolbox: Free Software for Processing PFGNMR Diffusion Data

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The importance of high resolution PFGNMR data for mixture analysis is steadily increasing, but there is no single best way to process such data. The commonest family of processing methods is known as DOSY (diffusion-ordered spectroscopy), and therefore it has become customary to refer to these data as DOSY data. The three major NMR manufacturers each offer different limited implementations of DOSY processing in their current software. The DOSY Toolbox is a free programme that allows users of all three instrument families access to the same wide range of processing schemes.

The DOSY Toolbox has a graphical user interface for easy access to the main processing schemes, and a command line mode for more advanced options. It is written in MATLAB, but is also available as free-standing compiled version that does not require any MATLAB installation. The MATLAB version runs on any platform, the compiled version only under Windows.

Basic features include:

- import of Varian, Bruker and JEOL data
- weighting, phasing, baseline correction and referencing
- and Reference deconvolution

DOSY data processing includes:

- DOSY (mono-, bi-, and multiexponential)
- correction for non-uniform field gradients
- DECRA
- MCR
- and CORE

The DOSY Toolbox is currently fully functioning, but requires more work to improve the user interface. It is therefore now being made available to volunteer end-users for beta testing. Our present intention is to continue releasing it as freeware under the GNU public licence.

51 NMR as a Tool for Determination of Chiral Stability of 2,4-Thiazolidinediones Drug Substances

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The most commonly used oral hypoglycemics for the Type 2 diabetes disease are sulfonylureas. These agents, however, induce serious hypoglycemia and exhibit primary or secondary failure, which is presumably due to their characteristics as insulin secretagogues. During studies of 2,4-thiazolidinediones - highly potent chiral antidiabetic compounds- it was, because of various patent and regulatory issues, of interest to develop a method to determine their chiral stabilities under gastric conditions. For the development of a general method a well-known anti-diabetic drug substance -namely pioglitazone - was chosen for our studies. The compound contains one chiral centre in the 2,4-thizolinedione ring system and is produced synthetic as a racemate. By means of ^{1}H-NMR measurements the chiral lability of pioglitazone could be determined indirectly by measuring the rate of disappearance of the proton attached to the chiral centre. From the measurements we could conclude that the half life time for the racemization is 49.7 days at 37°C at pH 2 (gastric conditions). Cetrimide was added to the sample solutions to improve the solubility of the drug substance and thereby facilitate the NMR measurements. The studies given herein documents, that the 2,4-thiazolidinediones measured can not be developed as pure enantiomers, but only as racemic mixtures. However, chemical changes in the substituent in the 5-position in the ring system has a significant impact on the chiral stability of this chemical class of compounds

52 Automated Data Processing: How Post-Run Macros can Facilitate the Analysis of Illicit Drug Samples

Robert A. Thompson and Patrick A. Hays

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In-depth sample analysis in direct support of law enforcement and strategic intelligence is the primary mission of the Drug Enforcement Administration's (DEA) laboratory system. Accurate identification and quantitation of both controlled and non-controlled substances found in complex drug mixtures are key steps in this process. Under most circumstances, NMR spectroscopy offers a fast, reliable, non-destructive method capable of identifying and quantifying nearly all components in a single experiment. Incorporation of post-run macros simplifies this process, allowing less experienced users immediate access to meaningful results. While NMR manufacturers supply a variety of macros to accomplish many routine tasks, custom macros are often required to fulfill the unique needs of individual users. Therefore, the DEA has developed several post-run macros focused on illicit drug analysis, including ones for quantitation of common components, as well as, identification of atypical components through custom library searches.

53 ^{19}F HSQC

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"Broadband" HSQC has historically meant wide spectral widths in F1 [1], but when the observed nucleus is F19 the bandwidth issue concerns F2. Utilizing the double spin echo [2] in the HSQC sequence allows use of broadband 180º pulses [3], greatly increasing the useful F2 bandwidth of the experiment. A second advantage of the double-echo sequence is that it allows independent control homo- and heteronuclear couplings, thereby making the experiment useful in the presence of homonuclear couplings that are the same size (~300 Hz) as $^{1}J_{C\text{-}F}$.

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54 An Improved Open-Access NMR Service

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The NMR team at AstraZeneca Charnwood has a long-standing interest in the customisation of vendor-supplied software to better integrate the systems into the company IT framework, and assist the open-access users in their experiment submission. Such an assisted system then allowed for robust data archival and referencing by virtue of disciplined use of unique identiers. Further software development has targetted spectral viewing and data extraction.[1]

As our NMR service grew we needed to refine the experiment submission process in a way that kept it easy for users and provided tools to assist the staff in manipulating samples and the instruments in use. At the same time it became necessary to accommodate a multi-vendor environment. We have devised a Java programme that assists with this task, providing an identical user experience on Bruker and Varian instruments.

Our data archival came under scrutiny and we took the opportunity to make this more robust, and use “commodity storage” supplied by our IT department. This led to a set of Perl scripts that facilitate the movement of data on the spectrometers to an upload area. A server retieves this, performs the necessary archival steps, and updates databases. Again, this accommodates data derived both from Varian and Bruker instruments.

These elements fit into a broader picture that meets the requirements of the end users’ ELNs. We describe here the systems.

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55 Directly Coupled HPLC-1H NMR – and its Limitations. The Metabolism Study of ZD4054

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HPLC-NMR is a well established analytical technique for the identification of drug metabolites, however, in some cases, it is unsuitable due to the nature of the analyte itself.

This poster presents the difficulties encountered with ZD4054 due to solvent effects. The analyte gave best peak shape in aqueous solution but severe signal broadening or even signal disappearance was encountered with 'typical HPLC conditions' or in organic solvents.

In order to avoid spectral misinterpretation, the metabolites were isolated and collected 'off line', and subsequently analysed by 2.5mm NMR tubes in D2O. From the 3 metabolites investigated, 2 could be positively identified and verified by MS and bacterial synthesis (Novacta Biosystems Ltd.).

56 The Metabolism Study of ZD4054. The Mystery of the Co-eluting Peak

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AstraZeneca ZD4054 is a specific endothelin A receptor (ETA) antagonist, which is being developed by AstraZeneca for the treatment of prostate cancer. When [14C]-ZD4054 was dosed to rat, dog and man, it was extensively metabolized. Metabolite P6 was of particular interest as it was a circulating metabolite in humans.

Whilst isolating the metabolite P6, an additional component was observed during the NMR analysis, which initially appeared to be ZD4054 related. However, further investigations by HPLC-MS indicated that only one component was ZD4054 related, whilst the co-eluting component could be identified as an endogenous compound.

In summary, the metabolite P6 was characterised as hydroxy-ZD4054. The co-eluting component was identified as the flavenoid, Daidzein. Flavenoids are common components of soy products, which in turn are a major constituent of animal diets.

57 NMR Peak Height Quantitation: Accurate, Precise, Easy

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Peak height quantitation is seldom used in NMR due to its inaccuracy and lack of precision when peak widths, measured at half-height, vary from sample to sample. Applying fixed line broadening improves peak width agreement, but is still inaccurate. By iteratively adjusting line broadening until the internal standard peak width reaches a specific value (e.g., 3.000 Hz), the majority of analyte peaks will conform to a consistent width and shape. If the analyte width is not in the "ideal" range, mathematic adjustments can be made. Several hundred illicit methamphetamine, cocaine, and heroin peak height quantitations were compared to peak area quantitations with excellent agreement.

58 Improvement of Multivariate Analysis and Metabolite Profiling by Advanced Data Pre-Processing

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Multivariate analysis (MVA) and metabolic profiling of complex mixtures based on 1D NMR has been successfully applied in many areas. Both techniques are inherently related and yield global trends as well as individually identified and quantified compounds. A major problem is that variables extracted from the data do not exactly relate to individual signals or individual compounds but rather to a superposition of signals. This superposition if often further influenced by broader background signals not originating from low molecular weight compounds but larger molecules such as proteins.

We demonstrate a data pre-processing technique called BaseTRIM, which can be used to filter out background signals to a wanted extent. This method is based on the separation of high and low-frequent components of the 1D spectra. Both the filtered data and the removed background can be separately used for further analysis. We demonstrate the method on 180 lupine samples. In these samples also the content of lupanine was determinated by different NMR quantification methods. MVA calculations and metabolic profiling are carried out with original and pre-processed data and are compared. It will be shown that PCA results are better suited for interpretation and quantification results of lupanin are more reliable, especially in samples which only contain a low amount.

59 Conformation and Dynamics of Heparan Sulfate Domains Investigated by NMR Relaxation of ^{15}N Enriched Oligosaccharides

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^{1}H-NMR spectroscopy is a convenient method for investigating molecular conformation in solution, but in carbohydrates (particularly those with repetitive structures) such studies are hampered by extreme signal overlap. This problem can be overcome to some extent by isotopic enrichment with a heteronucleus, e.g. ^{15}N, which has greater chemical dispersion (1). We have used enrichment protocols, similar to those applied to proteins, to produce bacterial polysaccharides with uniform ^{15}N-labeling that have a chemical structure similar to heparan sulfate (HS), found in the mammalian extracellular matrix. These polysaccharides were degraded enzymatically and purified into defined homogeneous oligosaccharide fractions. Using these materials, we present the first study of the dynamics of the non-sulfated region of heparan sulfate polymer (NA-domains). In particular, relaxation studies were performed on three lengths of the polymer (tetra, hexa and octa-saccharide) and at three different fields (proton frequency of 600, 800 and 900MHz). Using this approach, the problem of signal overlap is completely resolved at all fields, giving high quality data on internal dynamics and rotational diffusion. These data proved an excellent starting point for investigating local and overall molecular motions via the Lipari-Szabo model-free analysis. In concert with these measurements, the translational diffusion was probed using pulse field gradients in the DOSY experiment, providing information on general molecular shape, greatly aiding our conformational analyses. Relaxation measurements suggest that an anisotropic model is appropriate for octasaccharide and above. Furthermore, we derived anisotropic model-free equations that could accurately define the axis of motion, allowing accurate characterization of dynamics. The analyses revealed significantly higher flexibility in the NA domains compared with literature data (2) on sulfated HS fragments (S-domains). It is proposed that this flexibility has two functional roles. First, to give the tethered molecule a greater radius of interaction from its anchoring point on the core protein. Secondly, to allow multiple interactions along the same HS chain that are dynamically independent of each other.

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60 Sensitivity-Enhanced HSQC with Frequency-Swept Pulses

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Frequency-swept pulses have been demonstrated to offer a number of advantages, including near-perfect inversion and refocusing, "J compensation," and improved signal-to-noise. It is a relatively straightforward matter to incorporate 180° frequency-swept pulses in sequences, but 90° frequency-swept pulses present a greater challenge. Aside from the matter of contending with the severe phase roll produced by the pulses, there is also the question of whether a 90° frequency-swept pulse is a suitable replacement for a 90° hard pulse. In this poster we look at a version of the sensitivity-enhanced HSQC sequence. The sensitivity gain of the sequence arises from the recovery of magnetization along two simultaneous pathways. In the standard sequence the 90° carbon hard pulse at the end of the evolution period serves two purposes: to convert one component of the transverse carbon magnetization to z magnetization and to leave the remaining transverse carbon magnetization unchanged so that it can be recovered. When the 90° pulse is a frequency swept pulse, two questions arise: can the swept pulse produce carbon z magnetization efficiently, and what happens to the remaining carbon magnetization?

61 Residual-Based Binning: Combining Targeted And Global 1D NMR Spectral Profiling Techniques

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Targeted profiling is a technique for directly recovering quantitative compound information from complex mixtures acquired via 1D NMR experiments. This approach uses a predefined library of individual compound signatures that are scaled quantitatively and summed together using a linear combination model to create a reconstructed NMR spectrum. In a wide range of metabolomics experiments, this reconstructed NMR spectrum will not fully account for the spectral area in the experimental spectrum due to incomplete compound libraries or intentionally selective profiling of specific compounds.

In this work we introduce the concept of residual-based binning, where the reconstructed spectrum is subtracted from the experimental spectrum, and conventional global profiling techniques, such as spectral binning or full spectrum analysis, are applied to the residual spectrum. Statistical models can then be built upon the residual-based binning data and interpreted.

We apply residual-based binning to both real-world and synthetic datasets for each of these scenarios, and demonstrate how targeted and global profiling techniques can be effectively combined to build informative and comprehensive models of NMR-based metabolomics data.

62 Latest Developments on the Study of N,N',N"-Triacetylchitotriose Binding Elderberry Lectin. What Does DOSY say about the Binding Stoichiometry?

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4. Vakgroep Moleculaire Biologie, Universiteit Gent, Belgium

The overall 3D structure of elderberry lectin (EL), a 44 amino acid chitin binding lectin, is quite similar to that of hevein. The carbohydrate binding behaviour of EL was investigated using N,N',N"-triacetylchitotriose (NAG3) as model ligand. The chitin-binding site (W23, F25, Y32, and S21) is similar to other hevein domains, but EL contains four additional aromatic residues (W3, H15, F22, Y30). Protein-carbohydrate nOe contacts were found between aromatic resonances of Trp23, Phe25 and Tyr32 and proton resonances of the chitotriose. However, no major changes in the protein nOe's were observed after the addition of sugar, indicating small carbohydrate-induced conformational changes of the protein.

The affinity constant Ka was determined at six different temperatures ranging from 298 to 323°K in 5 degrees steps. The binding kinetics is intermediate on the NMR time scale at room temperature and evolves to fast exchange conditions as temperature increases. The interaction enthalpy and entropy were determined from van't Hoff analysis of the Ka and independently from microcalorimetry. ITC revealed a 2:1 EL:NAG3 binding stoichiometry, unexpected for a single hevein domain binding NAG3. Surprisingly, the NMR data could be fitted both to a 1:1 and to a 2:1 (protein : sugar) model and the association remained in the millimolar range. However, recent DOSY experiments bring more insight about the behavior of EL in the presence of NAG3 and seem to confirm the formation of a 1:1 EL:NAG3 complex. Further research is presently being done.

63 Examining the Polymorphism of Aspirin by Solid-state NMR

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Analysis of polymorphs typically relies on having pure forms, as there are few analytical techniques with the ability to characterize complex mixtures. Therefore, problems arise when pure forms cannot be generated. Our hypothesis is that Solid-state NMR (SSNMR) will allow us to detect and characterize individual polymorphs present in a mixture of forms.

The question of whether or not aspirin exists in more than one crystal form continues to be heavily debated in the literature [1-5]. The existence of other polymorphs is supported by theoretical calculations, but it has been difficult to generate pure forms experimentally. Therefore, characterization has been complicated and inconclusive. We hypothesize that SSNMR will provide the physical evidence that is necessary to show that other aspirin polymorphs exist. Experiments will involve the creation of polymorphs and/or mixtures of polymorphs through various processing and crystallization techniques. The samples will be analyzed by 13C SSNMR using cross polarization (CP) and magic-angle spinning (MAS), as well as relaxation analysis techniques.

The ^{13}C CPMAS NMR spectrum of lyophilized aspirin shows a splitting of the methyl peak as well as a shoulder on a peak in the aromatic region. These new peaks are not observed in recrystallized aspirin. Recrystallized aspirin has a ^{1}H T_1 of 57 s, while the lyophilized aspirin has a relaxation time of 11 s. Synchrotron analysis indicates that the lyophilized aspirin is a mixture of two forms. Aspirin with a ^{13}C labeled methyl group was synthesized and lyophilized. The two methyl peaks in the lyophilized labeled sample have similar chemical shift tensor values. Two-dimensional exchange SSNMR experiments are being performed on this sample to obtain better insight into the methyl group interactions. We are also analyzing aspirin rapidly crystallized from a solution of acetonitrile in order to address a recent report of polymorphs obtained in this matter [6].

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64 Small Volume NMR in Natural Product Research

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We will present experimental NMR data which has been run on a few micrograms of HPLC fractions from extracts of Morinda citrifolia - commonly known as noni - and which led to the identification of 4 completely new trisaccaride fatty acid esters - named noniosides. The NMR structure elucidation on these small sample quantities could only be achieved with the use of small volume/high sensitivity MicroProbes together with the utilization of modern structural elucidation experiments such as the H2BC experiment.

Like this example shows, miniaturization has become very important in all areas of modern research and development. Consequently, the advent of smallest volume NMR probes – such as Bruker's 1mm MicroProbe with a total sample volume of 5ul – has been very well accepted. But even though inverse experiments can yield indirect 13C chemical shift information, it is often also desirable to acquire 13C direct observe spectra. In many cases however it is not feasible to dissolve the required sample amount for these experiments in volumes of 5µL.

Here we show the results of a new room temperature NMR probe that matches the highest mass sensitivity on two channels – ^{1}H and ^{13}C – with a total sample volume of 30µL measured with 1.7mm tubes.
With this probe we show that it is now possible to acquire all NMR experiments for structural elucidation on less than 0.5mg of sample in an overnight run – including 13C direct observe expriments!

We will also show spectroscopic results achieved on the new 1.7mm MicroCryoProbe. This latest development in CryoProbe technology is a three channel ^{1}H, ^{13}C, ^{15}N probe which is equipped with z-gradient and automatic matching and tuning facility. It reaches the highest mass sensitivity commercially available. The probe accepts 1.7mm sample tubes with a total volume of 30µL.

The probe excels with superior mass sensitivity on two channels: ^{1}H and ^{13}C. The ^{1}H cannel mass sensitivity is about 3 times as much as on a 5mm cryogenically cooled inverse probe or a high sensitivity 1mm inverse room temperature MicroProbe. In other words, it is about 12 times more than on a 5mm room temperature inverse probe.

65 First Commercial Small Volume CryoProbe 30 µL $^{1}H/^{13}C$ Optimized Inverse CryoProbe

T. Kühn, D. Moskau, R. Kümmerle, N. Freitag

Bruker BioSpin AG, Fällanden

We present spectroscopic results achieved on a completely new cryogenically cooled triple resonance inverse probe, fully equipped with z-gradient and automatic matching and tuning facility, which reaches the highest mass sensitivity commercially available. The probe accepts 1.7mm sample tubes with a total volume of 30µl and thus is ideal for mass- or volume limited NMR samples - even in full automation.

The probe excels with superior mass sensitivity on two channels: ^{1}H and ^{13}C. The ^{1}H cannel mass sensitivity is about 3 times as much as on a 5mm cryogenically cooled inverse probe or a high sensitivity 1mm inverse MicroProbe. In other words, it is about 12 times more than on a 5mm room temperature inverse probe. The ^{13}C mass sensitivity for direct observe experiments on this small volume CryoProbe exceeds the respective value of a 5mm RT observe probe by more than 3 times.

The sample tube design for this 1.7mm CryoProbe is based on the extremely successful 1mm MicroProbe technology. Sample handling and automation of these 1.7mm sample tubes is equivalent to the proven 1mm sample handling concept. This enables the probe also to blend seamlessly into an existing automation environment by using all the established automation tools for these 30µl tubes as well – such as liquid handlers for automated tube filling and commercially available sample changing devices.

All this makes the probe ideal for any application where sample mass is limited. The fields of applications include structural elucidation work in natural product research, metabolic profiling with statistical data analysis of volume limited body fluids (e.g. rodent cerebrospinal fluid, plasma or urine) or mass limited bio NMR such as protein ligand screening. This is especially interesting since the mass sensitivity of this probe is virtually not influenced by the salt content of the NMR samples. The fact that all modern NMR automation devices can be linked up with this system also allows true high throughput NMR applications in only 30µl solvent.

We present first experimental results for these fields of research which would not have been possible without the use of this ultimate sensitivity probe. This will be illustrated by experiments where we directly compare results achieved on current state of the art high sensitivity NMR probes with experiments run on this 1.7mm CryoProbe.

66 Quality Assurance by NMR: a Reliable and Automated Tool for Compound Quantification

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The quality of compound depositories, as used in pharmaceutical companies in drug discovery today often is very poor and efforts have been made to improve their quality.

Here we show new approaches to use NMR methods to address three mayor issues in this respect:

1. Concentration determination of the screening solution is important since high throughput screening (HTS) is based on affinity tests, where the accurate knowledge of the concentration is crucial to determine reliable ID50 affinity values.
2. Identity control: the identity of the compounds have to be assured in order to define a lead structure from a screening hit.
3. Purity control of the compound: it has to be assured that the compound is pure and not a mixture that might contain other products which might lead to false positives.

Here we present the integration of an automated NMR spectroscopic method that gives rise to accurate quantification results along with the NMR spectrum. This method integrates well into the discovery workflow using only a few microliters of solution.

The purity and identity of the compounds in the depository is important, since these compounds are used for lead finding and might produce bio-activity hits for a given target. Many false positive hits result from wrong assignments and bad quality control in these compound depositories. Therefore hit validation is needed and this typically is a very time consuming and cost intensive step.

We also present a new NMR laboratory setup, which addresses the hit validation directly. Only a few micro-litres of compound depository stock solution are needed for the NMR based structure verification. The NMR experiments typically can be run in less than three minutes in normal (protonated) DMSO, even on a 400MHz instrument. The whole process such as sample preparation, sample handling and the NMR experiment itself is automated for high throughput.