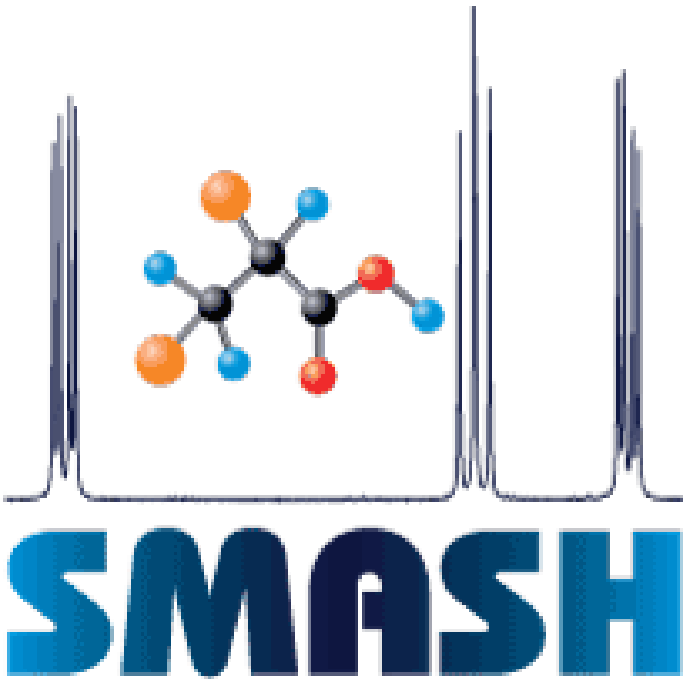


2022

Conference Program

August 28th –31st 2022

La Jolla, California

Dear SMASH 2022 Attendees,

We warmly welcome you to the 2022 ***Small Molecules Are Still Hot*** conference, which is returning to the Torrey Pines Hilton in La Jolla, California. From the enormous possibilities of San Diego to the stunning Torrey Pines State Reserve just a short walk away, La Jolla is the perfect home base for great science and great excursions.

SMASH 2022 pushes boundaries and comprehensively covers advances throughout small molecule NMR science. As always, SMASH has no concurrent sessions and provides an immersive community experience, so you never have to worry about missing out on a topic or presentation.

The SMASH program begins Sunday evening with registration, a new early-career symposium, mixer, and dinner. The first oral session Monday morning encompasses machine learning and computation, and is followed by a comprehensive workshop on computation. The sessions also feature the transformative role of NMR in pharma, solid-state NMR, guest-host and intermolecular interactions, the latest feats of spin manipulations, advances in metabolomics, and the expanding realm of benchtop NMR. And don't miss the second workshop, which pulls back the veil on qNMR.

Poster sessions are Monday afternoon (even numbers) and Wednesday morning (odd numbers). By the way, you will not have to look far to find other hot topics like hyperpolarization, data science, and more.

Be sure to see the latest advances in hardware and software from the sponsors, and take advantage of the opportunities for close discussions with colleagues, experts, and sponsors that SMASH enables throughout the sessions, workshops, posters, booths, meals, and mixers. We find it hard not to mention also that one of the excitements of SMASH is bumping into many of our field's luminaries at every turn.

On behalf of the Organizing Committee, we want to extend a warm welcome and our thanks to you for attending SMASH 2022.

Remember no ties, put on those think caps (or toques), get ready for some serious precession, and we'll see you soon!

Sincerely,

David Rovnyak and Ken Skidmore
Co-Chairs, SMASH 2022 NMR Conference

SMASH NMR Conference 2022

Event Schedule

Sun, Aug 28, 2022

9:00 AM

User Meeting: Bruker
9:00 AM - 12:00 PM, Aug 28
Fairway I (Pavilion)

12:00 PM

Vendor sponsored box lunch
12:00 PM - 1:00 PM, Aug 28
Fairway Garden

Provided for those attneding the vendor user meetings

1:00 PM

User Meetings: MestreLab and JEOL
1:00 PM - 4:00 PM, Aug 28
JEOL: Fairway VI (Pavilion) / MestreLab: Fairway I (Pavilion)

4:00 PM

Registration
4:00 PM - 5:00 PM, Aug 28
Grand Ballroom Foyer

4:30 PM

Magnetic Resonance in Chemistry Early Career Researchers Symposium
4:30 PM - 6:00 PM, Aug 28
Fairway II (Pavilion) then ERC mixer in Fairway Gardens

Magnetic Resonance in Chemistry and **Wiley** are pleased to announce the organization and sponsorship of this Symposium event at the world's premiere small molecule NMR conference.

The event will feature the presentation of a series of flash-talks (about 2-3 min each) by undergraduate students, graduate students or post-doctoral fellows from either academia or industry, as well as researchers working in industry within 5 years of the receipt of their Ph.D.

Topics might cover any field of the NMR research provided that the work is small molecule - based. Details the Symposium and format of the presentations will be provided here soon.

Register to present a flash talk
Do not miss the chance to present your work and expand your network! Register by sending an email to smashnmrorg@gmail.com.

Following the symposium, an MRC mixer event will take place to gather all in a friendly environment to allow participants to continue to discuss great NMR science before the opening conference mixer and dinner.

For questions regarding the event, please contact SMASH registrations (smashnmrorg@gmail.com).

5:00 PM

Welcome Mixer
5:00 PM - 7:00 PM, Aug 28
Parterre Terrace

7:00 PM

Dinner
7:00 PM - 9:00 PM, Aug 28
Parterre Gardens

Mon, Aug 29, 2022

8:50 AM

Welcome, Announcements and Opening Remarks
8:50 AM - 9:00 AM, Aug 29

Grande Ballroom ABC

9:00 AM

Two heads are better than one: How humans and machines learn NMR

9:00 AM - 10:30 AM, Aug 29

Grande Ballroom ABC

Session Moderator: Andreas Kaerner

This session highlights the growing coevolution of advances in the understanding both of how machine learning and user-driven approaches to analyzing spectral data can be improved. Foreshadowing a growing practice of researchers interacting with ML-enabled NMR data analysis, this session includes latest developments in ML technology in chemistry as well as research in the practice of NMR spectral interpretation.

Lead Speaker

Michelle Gill
Senior Deep Learning Scientist in Life Sciences
NVIDIA

Invited Speaker

Megan Connor
Postdoctoral Research Associate
University of South Florida

Speakers

Craig Butts
Professor of Structural and Mechanistic Chemistry
University of Bristol

Emily Crull
University of North Carolina – Wilmington, Wilmington, NC, US

---

4 Subsessions

- **Exploring Molecular Space and Accelerating Drug Discovery with MegaMolBART and Clara Discovery**
  9:00 AM - 9:25 AM, Aug 29
- **Teaching and learning the practice of NMR spectral interpretation: current outcomes and future reform**
  9:25 AM - 9:50 AM, Aug 29
- **Machines Learning NMR Better than Quantum Mechanics…. Nah…. Really?**
  9:50 AM - 10:10 AM, Aug 29
- **Combining NMR and Computational Methods to Interrogate Rapamycin's Mysterious Minor Conformer**
  10:10 AM - 10:30 AM, Aug 29

10:30 AM

Coffee Break

10:30 AM - 11:00 AM, Aug 29

Parterre Terrace

11:00 AM

Workshop 1: Computation of Chemical Shifts and Couplings

11:00 AM - 12:30 PM, Aug 29

Grande Ballroom ABC

Computational chemistry has always been a key part of the NMR toolbox. Predicting NMR parameters provides a molecular interpretation of the spectral data, assists in analyzing NMR spectra, and helps to guide the process of structure elucidation. This workshop on computational advances will be separated into solid and liquid phase discussions to address their particular features.

The growth in applications of NMR Crystallography, which can elucidate solid phase structure when traditional diffraction methods fall short and is finding wide utility in scientific fields today, has spawned an increased interest in accurate prediction of the chemical shielding tensor. Inclusion of the lattice structure is pertinent to the reliability of such calculations and common methodologies to incorporate the lattice structure, periodic boundary conditions and extended cluster models, will be central to this workshop. Due to the efficiencies of Density Functional Theory (DFT) to handle larger systems accurately, DFT has become the electronic method of choice. Strategies for efficient DFT approaches that incorporate the lattice environment will be emphasized.

The solid phase component will cover topics such as

1. Promoting and handling anisotropy of the chemical shift tensor
2. Removing systematic errors through least squares regression
3. Reliable geometry input parameters
4. Consideration of DFT planewave parameters
5. Using cluster models to climb Jacob's Ladder of DFT functionals

The solution phase component will focus on DFT methods augmented by parametric corrections of computed data to expedite computations and improve the accuracy. The most recent machine learning-augmented DFT method, DU8ML, will be highlighted.

1. Computations of nuclear spin-spin coupling constants
2. Computations of chemical shifts
3. Challenging structure elements and features of PES (intramolecular H-bonding, shallow PES, etc.)
4. PCM and solvents

Speakers

Andrei Kutateladze
University of Denver

Robbie Iuliucci
Professor of Chemistry
Washington & Jefferson College

12:30 PM

Lunch

12:30 PM - 2:00 PM, Aug 29
Parterre Gardens

---

1 Subsessions

On-Resonance networking event

12:30 PM - 2:00 PM, Aug 29
Parterre Gardens

2:00 PM

Poster Session I (Even Numbered Posters)

2:00 PM - 3:30 PM, Aug 29
Grande Ballroom DE

3:30 PM

Coffee Break

3:30 PM - 4:00 PM, Aug 29
Parterre Terrace

4:00 PM

NMR to the rescue in Pharma and Industry

4:00 PM - 5:30 PM, Aug 29
Grande Ballroom ABC

Session Moderator: Amy Freund

NMR is a powerful tool for addressing a whole host of topics, whether these involve reaction mechanisms, structure, quantitation, and more. Seemingly surrounded, at times, by a sea of HPLCs and mass spectrometers, NMR methods can often be the most efficient or practical approach for a variety of challenges. Join us for this session to see NMR come to the rescue in pharma and industry.

Lead Speaker

Alexei Buevich
Principal Scientist
Merck

Invited Speaker

Martin Koos
Senior Scientist, NMR Spectroscopist
Pfizer

Speakers

Purnima Khandelwal
BMS

Gregory Walker
Associate Research Fellow
Pfizer

---

4 Subsessions

● Driving to a Better Understanding of Acyl Glucuronide Transformations Using NMR and Molecular Modeling
4:00 PM - 4:25 PM, Aug 29

● RDC-Based Analysis and a Novel Water Gel in Rational Drug Design
4:25 PM - 4:50 PM, Aug 29

● NMR Structure Determination of Peptidomimetic Drug Leads
4:50 PM - 5:10 PM, Aug 29

● 19F qNMR in Human Absorption, Distribution, Metabolism and Excretion Studies of Nirmatrelvir: A Faster Route to Emergency Use Authorization
5:10 PM - 5:30 PM, Aug 29

7:00 PM

Dinner
7:00 PM - 9:00 PM, Aug 29
Parterre Gardens

Tue, Aug 30, 2022

9:00 AM

When harder is smarter: Solid-state NMR of small molecules
9:00 AM - 10:30 AM, Aug 30
Grande Ballroom ABC

Session Moderator: David Rovnyak

Solid-state NMR has largely come of age, finding widespread applications across a variety of disciplines. Protons are no longer inaccessible, quadrupoles are both widely excitable and interpretable. Crystallographic information from NMR is more routine than novelty. Solid pharmaceuticals are extensively characterized using a wide variety of solid-state NMR techniques and nuclei. Come hang out for this session to see how modern solid-state NMR is making waves in materials science.

Lead Speaker

Joe Lubach
Principal Scientist
Genentech, Inc.

Invited Speaker

Robert Schurko
FSU/MHMFL

Speakers

Kevin Chalek
Postdoctoral Research Scholar
San Diego State University

J

Jim Harper
Associate professor
Brigham Young University

4 Subsessions

- **Insights into Fluorinated Drug Substance and Drug Product via 19F Solid-State NMR Spectroscopy**
  9:00 AM - 9:25 AM, Aug 30
- **QNMRX-CSP: Crystal Structure Prediction Aided by Quadrupolar NMR Crystallography**
  9:25 AM - 9:50 AM, Aug 30
- **NMR Crystallography: Bridging Atomic-Level Structural Rearrangement and Macroscopic Motion in Molecular Organic Crystals**
  9:50 AM - 10:10 AM, Aug 30
- **Refining crystal structures using NMR chemical shift tensors as a target function**
  10:10 AM - 10:30 AM, Aug 30

10:30 AM

Coffee Break

10:30 AM - 11:00 AM, Aug 30
Parterre Terrace

11:00 AM

Stuck on you: Guest-host and binding interactions

11:00 AM - 12:30 PM, Aug 30
Grande Ballroom ABC

Session Moderator: Tim Claridge

Small molecules are even hotter when they interact with other molecules and host complexes. The exquisite sensitivity of NMR to local structure and environment has long made it a premier tool for detecting and characterizing noncovalent interactions, which often involves bringing powerful new tech to bear, from clever pulse sequences to hyperpolarization. Stick with us and see frontiers in the development and application of cutting edge NMR techniques to the study of noncovalent interactions.

Lead Speaker

Leah Casabianca
Associate Professor
Clemson University

Invited Speaker

Russell Bowers
Professor
Department of Chemistry, University of Florida

Speakers

Theresa Zorn
PhD student
Institute of Organic Chemistry, University of Würzburg, GER

Ran Wei
University of Edinburgh

4 Subsessions

● NMR Experiments and Simulations Reveal Interactions Between Small Molecules and Plastic Nanoparticles
11:00 AM - 11:25 AM, Aug 30

● Instrumentation, Advanced Heterogeneous Catalysts, and New Approaches to Spin order Transfer for Hyperpolarization of Metabolites from Parahydrogen
11:25 AM - 11:50 AM, Aug 30

● Am I still liquid? – Insights into hydrogel formation by a colourful NMR toolbox
11:50 AM - 12:10 PM, Aug 30

● High-Way or Bi-Way – New Stopped-flow NMR Methods
12:10 PM - 12:30 PM, Aug 30

12:30 PM

Lunch
12:30 PM - 2:00 PM, Aug 30
Parterre Gardens

1 Subsessions

● On-Resonance networking event
12:30 PM - 2:00 PM, Aug 30
Parterre Gardens

2:00 PM

The artful nutation of spin gymnastics: NMR methods
2:00 PM - 3:30 PM, Aug 30
Grande Ballroom ABC

Session Moderator: Dave Russell

Small molecule NMR continues to witness a rapid development of new experimental methods that uncover ever greater information on molecular structure and conformation, while cleverly dealing with the unique challenges of proton-poor spin networks and limited sample quantities. This session broadly encompasses the latest experimental developments, from manipulations of couplings to methods that study a broader range of nuclei.

Lead Speaker

Thomas Williamson
Distinguished Professor
University of North Carolina at Wilmington

Invited Speaker

Ralph Adams
Senior Research Fellow and Head of NMR
University of Manchester

Speakers

Robert Evans
Senior Lecturer in Physical Chemistry
Aston University, Chemical Engineering and Applied Chemistry, Birmingham, UK.

Justinas Sakas
EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK

---

4 Subsessions

- **Back to the Future: Exploration of new ADEQUATE Improvements and Applications**
  2:00 PM - 2:25 PM, Aug 30
- **Ultra-selective 1D NMR Spectroscopy**
  2:25 PM - 2:50 PM, Aug 30
- **Quantitative Interpretation of Molecular Diffusion Coefficients – From Big to Small**
  2:50 PM - 3:10 PM, Aug 30
- **NMR Methodology for Analysis of 15N-labelled Complex Mixtures**
  3:10 PM - 3:30 PM, Aug 30

3:30 PM

Free Time
3:30 PM - 3:30 PM, Aug 30

An opportunity for vendor discussions before a free evening.

Wed, Aug 31, 2022

9:00 AM

There's no place like 'omics: NMR advances in complex mixtures
9:00 AM - 10:30 AM, Aug 31
Grande Ballroom ABC

Session Moderator: Mark Dixon

NMR continues to contribute to and drive the growing applications of metabolomics. Advances in NMR methods have helped improve experimental outcomes, but more broadly have helped to enhance the biochemical knowledge of the metabolome. This session highlights new methods and developments in bringing NMR to bear on the study of metabolomes.

Lead Speaker

Elizabeth O'Day
Founder and CEO
Olaris, Inc.

Invited Speaker

Robert Powers
Professor Of Chemistry
University of Nebraska-Lincoln

Speakers

Nicolas Giraud
Professor
Université Paris Cité

John Cort
Scientist
Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland WA

4 Subsessions

- **Multidimensional NUS NMR-Based Metabolomics for In Vitro Diagnostic Discovery**
  9:00 AM - 9:25 AM, Aug 31
- **NMR Metabolomics Best Practices: What We Are Doing, What We Should Be Doing, and Some Recent Advancements**
  9:25 AM - 9:50 AM, Aug 31
- **Ultrahigh-Resolution NMR of Aqueous Biofluids: Towards A Better Quantitative Metabolic Profiling of Lymphoma Cells**
  9:50 AM - 10:10 AM, Aug 31
- **The Natural Products Magnetic Resonance Database (NP-MRD): Comprehensive Database and Repository for Natural Products NMR Data**
  10:10 AM - 10:30 AM, Aug 31

10:30 AM

Poster Session II (Odd Numbered Posters) with Coffee

10:30 AM - 12:00 PM, Aug 31
Grande Ballroom DE

12:00 PM

Lunch

12:00 PM - 1:30 PM, Aug 31
Parterre Gardens

1 Subsessions

- **On-Resonance networking event**
  12:00 PM - 1:30 PM, Aug 31

1:30 PM

Workshop 2: qNMR and the Shocking Truth About Integration

1:30 PM - 3:00 PM, Aug 31
Grande Ballroom ABC

The most common method of extracting quantitative information from NMR data relies on knowledge of peak integrals... but how reliable is that knowledge? We explore the effects of spectral processing on integration, including those of zero filling, random noise, and the mysterious scaling of the first point in the time domain. We also explore quantification in the time domain vs frequency domain, and the influence of non-uniform sampling for quantification of higher-dimensional data. We hope that by attending this workshop you will have a new-found appreciation for the subtleties and nuances involved in extracting accurate and precise quantitative information from NMR measurements, and that the shocking truth about integration helps avoid potential pitfalls in your quantitative workflows. To supplement our lessons, we will conduct the first SMASH QUANTIFICATION CHALLENGE (SMASHQUAC), where attendees will be supplied with raw data that they can quantify by any means they prefer

Speakers

Frank Delaglio
Principal Investigator
NIST IBBR

Gennady Khirich
Associate Principal Scientist
Merck

3:00 PM

Coffee Break

3:00 PM - 3:20 PM, Aug 31

Parterre Terrace

3:20 PM

Never leave the bench again: The power of low field NMR

3:20 PM - 4:50 PM, Aug 31

Grande Ballroom ABC

Session Moderator: Clark Ridge

Over the past decade, benchtop NMR has brought many novel applications to fruition. The accessibility and compactness of benchtop systems allow new uses for health care, teaching, reaction monitoring, quality control, real-time release, and more. Join us for an exciting session where we'll see why, in many cases, you never need to leave the bench again.

Lead Speaker

Katharine Briggs
Research Associate
University of Maryland, School of Pharmacy

Invited Speaker

Jason Hein
Associate Professor
University of British Columbia

Speakers

ikenna Ndukwe
Process Development Scientist
Amgen

Tristan Maschmeyer
PhD Candidate
The University of British Columbia

4 Subsessions

● wNMR for the Assessment of Complex Drug Products and Vaccines

3:20 PM - 3:45 PM, Aug 31

● Quantitative Reaction Monitoring using a Benchtop Stopped-Flow NMR

3:45 PM - 4:10 PM, Aug 31

● 400 MHz Cryofree NMR Technology - Leveraging High Field NMR in a Benchtop NMR Setting

4:10 PM - 4:30 PM, Aug 31

● Application of Benchtop NMR as a Chemical Detector for Pharmaceutical Development

4:30 PM - 4:50 PM, Aug 31

4:50 PM

Closing Remarks

4:50 PM - 5:00 PM, Aug 31

Grande Ballroom ABC

SMASH gratefully acknowledges the generous support from these companies

Elite Sponsor

MESTRELAB RESEARCH
Chemistry Software Solutions

Premier Sponsor

JEOL

Select Sponsor

BRUKER

ACD/Labs

AMGEN®

Genentech

magritek
www.magritek.com

Q.One Instruments

MRC

MERCK

nanalysis

NEWERA
The New Standard in NMR Sampling™

OXFORD INSTRUMENTS
The Business of Science®

Pfizer

ROYAL SOCIETY OF CHEMISTRY

SMASH 2022 NMR Conference

Program Chairs

David Rovnyak
Bucknell University

Ken Skidmore
Genentech

SMASH Organizing Committee

President	Secretary	Treasurer	Logistics
Maria Silva Elipe	Tim Claridge	Clark Ridge	Daneen Angwin-Kaerner
Amgen	University of Oxford	FDA	Loxo@Lilly

Vendor Liaisons

Margaret Levenberg
Stepan Company (Retired)

Craig Butts
University of Bristol

Poster Chairs

Krish Krishnamurthy
Chempacker LLC

Roberto Gil
Carnegie Mellon University

Carla Marchioro
Endura

Steven Coombes
AstraZeneca

Teo Parella
Universitat Autònoma de Barcelona

Laura Castanar Acedo
University of Manchester

Ann-Christin Poeppler
University of Wuerzburg

Amy Freund
Bruker Biospin

Andreas Kaerner
Eli Lilly & Co

Brian Marquez
Nalas Engineering

Armando Navarro-Vazquez
Universidade Federal de Pernambuco

Silvia Davalli
Aptuit

Mark Dixon
Mestrelab SL

Michael Hammer
Bruker Biospin GmbH

David Rovnyak
Bucknell University

David Russell
Genentech

Robert Espina
Magritek

SMASH 2022 Scholarship Winners

The following students received a scholarship to attend SMASH 2022.

Thanks to our meeting sponsors for their generous support.

- Alejandro Bara Estaun — Department of Chemistry, University of Bath, UK
- Asad Saib — Department of Chemistry, University of Bath, UK
- Bridget Tang — Aston Institute of Materials Research, Aston University, UK
- Chun Yee Calvin Yiu — Department of Chemistry, University of Bristol, UK
- Coral Mycroft — Department of Chemistry, University of Manchester, UK
- Emma Gates — Department of Chemistry, University of Manchester, UK
- George Peat — School of Chemistry, University of Edinburgh, UK
- Hao Lan — Department of Chemistry, University of Bristol, UK
- Johannes Eder — Chemistry Department, University of Regensburg, Germany
- Justinas Sakas — School of Chemistry, University of Edinburgh, UK
- Nele Reimets — National Institute of Chemical Physics and Biophysics, Tallin, Estonia
- Ran Wei — School of Chemistry, University of Edinburgh, UK
- Scott Wilcox — Department of Chemistry, Uppsala University, Sweden
- Stefan Peintner — Department of Chemistry, Uppsala University, Sweden
- Theresa Zorn — Institute of Organic Chemistry, Würzburg, Germany
- Zahra Al-Aasmi — Department of Chemistry, University of Bristol, UK

SMASH 2022

TALKS and WORKSHOPS

1 Exploring Molecular Space and Accelerating Drug Discovery with MegaMolBART and Clara Discovery

Michelle Gill

Senior AI Scientist - Deep Learning, Cheminformatics, and Proteomics Tech Lead, Clara Discovery NVIDIA

The development of therapeutics is being transformed by the increased availability of datasets, with the use of chemical databases containing billions of molecules becoming commonplace in lead discovery and optimization. However, the increased volume and complexity of data can be a bottleneck for modeling and analysis. To extract scientific insights, methods that can scale efficiently to these datasets are required. The high degree of parallelism afforded by GPUs has made them an ideal platform for the acceleration of high performance computing applications, including machine learning. GPU-accelerated analysis and visualizations can be combined with features derived from deep learning models to create analysis pipelines that are both faster and more accurate than the existing state of the art. This talk will cover: (1) the training and development of MegaMolBART, a transformer-based deep learning model for the prediction of cheminformatics tasks, such as forward and retrosynthesis, molecular properties, and molecule generation; (2) how MegaMolBART can be incorporated in GPU-accelerated cheminformatics clustering and visualization pipelines to enable analyses on a scale that was previously intractable.

2 Teaching and learning the practice of NMR spectral interpretation: current outcomes and future reform

Megan C. Connor

University of South Florida

Advancements in organic chemistry depend upon chemists' ability to interpret NMR spectra; however, research demonstrates that individuals complete the undergraduate chemistry curriculum lacking proficiency in this practice. Recent work investigating the development of expertise in ^{1}H NMR spectral interpretation points toward needed areas of instructional reform. To further refine NMR instruction and ensure that entry-level chemists can engage in this essential practice, insight into desired knowledge and skills is now needed from relevant stakeholders (e.g., industry employers). This talk will present current learning outcomes associated with interpreting NMR spectra in introductory organic chemistry courses, implications for instruction, and ongoing strategies to better prepare students for interpreting NMR spectra in the workforce.

3 Machines Learning NMR Better than Quantum Mechanics…. Nah…. Really?

Calvin Yiu, Ben Honore, Krystof Chrappova, Emiliano Orsini-Rottner, **Craig Butts**

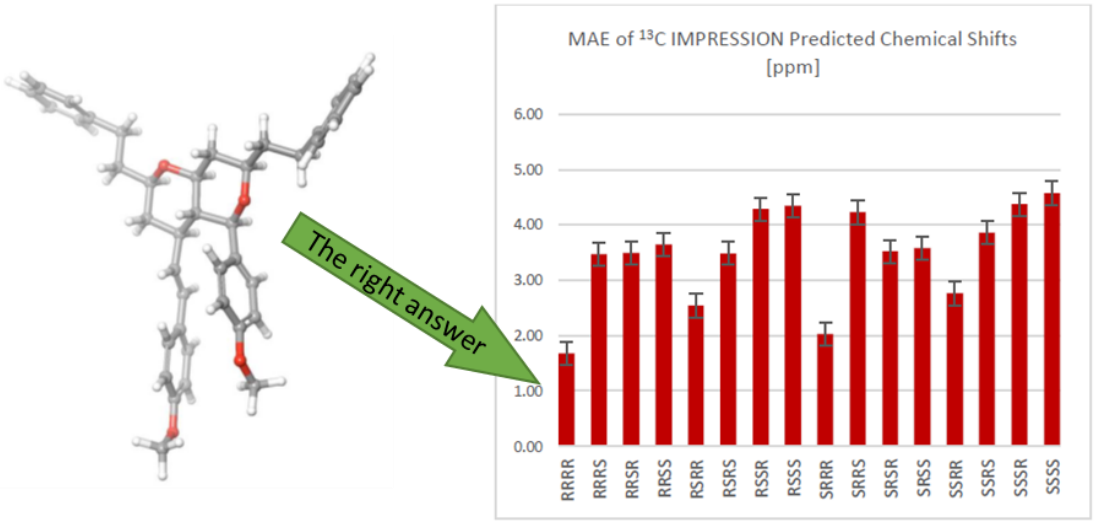
University of Bristol

We have developed a machine learning system that achieves quantum chemical accuracy for the prediction of NMR parameters *e.g.* chemical shifts, scalar couplings (and probably anisotropic stuff as well, but we haven't checked as I type this!). I'd like to tell you the story of how we have improved from a basic regression-based machine in 2019, to our attention-based graph neural network delta-machine learning system that we believe will be capable of better-than-DFT predictions in the near future.

Structure elucidation in solution by NMR is a key application, whether it's skeletal structure, regiochemistry, stereochemistry or conformation – NMR is the tool *par excellence*. Recent developments in determining the most challenging structures increasingly rely on quantum chemical calculation of chemical shifts, scalar couplings and residual anisotropic NMR parameters.[1] The problem, however, is that QM calculations are slow. Very slow. In ideal cases they take hours per structure, in challenging cases it can take months to calculate all the possible structures and conformers needed.

Here I will present developments beyond our 1st generation regression-based Intelligent Machine for PREdicting Shift and Scalar Information Of Nuclei (IMPRESSION)[2] that could only predict chemical shifts and 1-bond couplings for basic organic (C/H/N/O) molecules. We now have a cutting edge tool that can predict any NMR parameter, from low-cost molecular mechanics structures that covers the elements common in pharmaceutical/biological molecules.

With the addition of delta-machine learning tools we can make the predictions even more accurate and we demonstrate its ability to discriminate the correct diastereomers for natural products. In milliseconds.



1. Chhetri BK, Lavoie S, Sweeney-Jones AM, Kubanek J., *Nat Prod Rep.*, **2018**, 35(9), 514-531. doi:10.1039/c8np00011e
2. Gerrard W, Bratholm LA, Packer MJ, Mulholland AJ, Glowacki DR, Butts CP, Chemical Science, 11(2), 508–515, 2020; Gerrard W, Yiu C, Butts CP. Prediction of 15 N chemical shifts by machine learning. Magn Reson Chem. 2021 Aug 18. *in press* doi: 10.1002/mrc.5208.

4 Combining NMR and Computational Methods to Interrogate Rapamycin's Mysterious Minor Conformer

Emily B. Crull[1], Paul Hawkins[2], Ann E. Cleeves[3], Ajay N. Jain[3], Edmund I. Graziani[2], and R. Thomas Williamson[1]

1. University of North Carolina – Wilmington, Wilmington, NC, US
2. Apertor Pharmaceuticals Inc., South San Francisco, CA, US
3. Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA, US

Appearing in over 56,000 journal articles and more than 7,000 patents, rapamycin was originally isolated from an Easter Island soil sample in 1975.[1] Rapamycin is a potent immunosuppressant and antiproliferative, giving rise to its use for the treatment of organ transplants and cancer, respectively.[2] One of the first examples of a "molecular glue", the pipecolic acid region of rapamycin binds to FK-506 binding protein 12 (FKBP12), while the triene region binds to mammalian target of rapamycin (mTOR), bringing the two proteins together and disrupting the downstream signaling of the latter.[2] Inspired by the recent burgeoning interest in the application of rapamycin to the treatment of other therapeutic indications, there is renewed motivation to better understand the molecular dynamics of rapamycin in order to guide new drug discovery and design efforts.

Rapamycin has been shown by NMR studies to adopt two interconverting conformers, appearing in a 9:1 ratio in DMSO-d_6.[3,4] However, only the major conformer has been characterized, having been crystallized in both free and FKBP12-bound states as the *trans* amide.[3] It has been generally accepted that the minor conformer is the *cis* amide by comparison to a structurally related compound, FK506.[3] To our knowledge, no evidence has been published to conclusively identify the true nature of the minor conformer of rapamycin. This presentation will describe how our group has used NMR studies and computational chemistry to probe and unequivocally define the structural identity of this heretofore undercharacterized species.

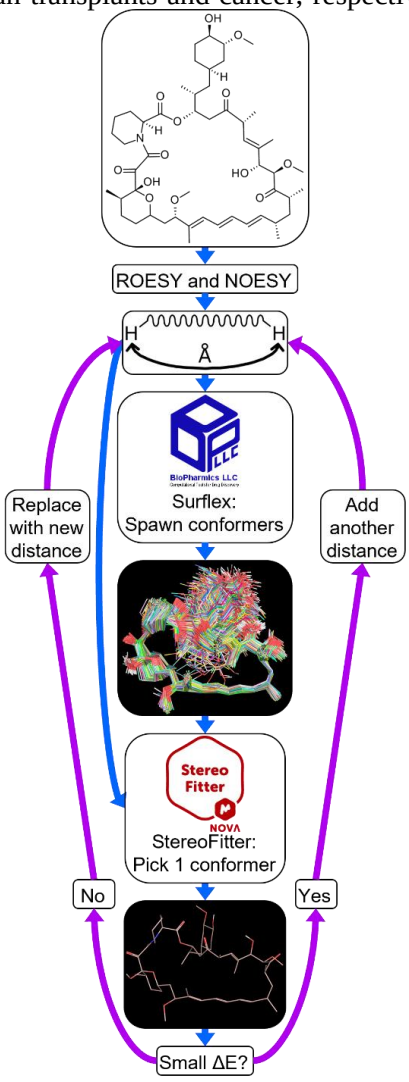


Figure 1 Flow chart of the developed method from starting structure to single 3D conformer

1. Vézina, C.; Kudelski, A., *J. Antibiot. (Tokyo).*, 28(10), 721–726, 1975.
2. Li, J.; Kim, S. G.; Blenis, *J. Cell Metab.*, 19(3), 373–379, 2014.
3. Kessler, H.; Haessner, R.; Schüler, W., *Helv. Chim. Acta*, 76(1), 117–130, 1993.
4. Pagano, T. G., *Magn. Reson. Chem.*, 43(2), 174–175, 2005.

5 Driving to a Better Understanding of Acyl Glucuronide Transformations Using NMR and Molecular Modeling

Alexei Buevich

Process and Analytical Chemistry, Merck & Co., Inc., 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Acyl glucuronide (AG) metabolites of carboxylic acid-containing drugs, and specifically the products of their transformations have long been implicated in drug-induced liver injury (DILI). To better understand DILI, the mechanism of formation of active AG intermediates is required. Given that a single AG metabolite can generate nine different species (via intramolecular acyl rearrangement, hydrolysis and epimerization reactions), the mechanistic studies of AG transformations are far from trivial. Analyses of full mechanism of AG transformations have rarely been attempted and only with partial success. A high degree of complexity and strong correlation between variables (kinetic rates) would usually prevent an independent determination of all rate constants or would cause a kinetic data overfitting. Here we present an example of detailed kinetic data analysis of AG transformations for ibufenac AG measured by *in situ* ^{1}H NMR and analyzed in approximation of eleven different kinetics models. A more comprehensive and robust level of analysis was achieved by applying 2D NMR spectroscopy for spectral analysis, measurements of kinetic isotope effects and theoretical DFT computations

6 RDC-Based Analysis and a Novel Water Gel in Rational Drug Design

Kathleen A. Farley[1], **Dr. Martin R. M. Koos**[1,2], Dr. Ye Che[1], Dr. Reto Horst[1], Dr. Chris Limberakis[1], Justin Bellenger[1], Dr. Ricardo Lira[1], Leandro F. Gil-Silva[3], Dr. Roberto R. Gil[2]

1. Medicinal Sciences, Pfizer, Groton, CT 06340, US
2. Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, US
3. 3729 Beechwood Blvd., Pittsburgh, PA, US

The activity of a pharmaceutical compound towards a protein target largely depends on its conformation. Any difference between the solution and bound conformation can reduce activity by an enthalpic or entropic penalty. The solution conformation of molecules of interest, in physiological or at least similar environment, therefore, is a key parameter to guide drug design.

NOE, chemical shift, and J-coupling can help answer some questions on conformation, especially for short distances, but RDC-analysis can inform about the overall conformation of a molecule. Such RDC measurements in medicinal chemistry are usually performed in $CDCl_3$ or DMSO-based gels, but a novel water-compatible gel we recently published can provide complimentary data from aqueous solution.[1]

1. Farley, K. A., Koos, M. R. M.,Che, Y., Horst, R., Limberakis, C., Bellenger, J., Lira, R., Gil-Silva, L. F., Gil, R. R., Angew. Chem. Int. Ed., 60, 26314, 2021.

7 NMR Structure Determination of Peptidomimetic Drug Leads

Purnima Khandelwal, Luciano Mueller, Christine Jorge and Janet Caceres Cortes

Discovery Chemistry Platforms, Small Molecule Drug Discovery,
Bristol Myers Squibb, Princeton, NJ, US

Conformationally constrained macrocyclic peptidomimetic compounds (millamolecules) offer an attractive venue for the design of orally bioavailable inhibitors of protein : protein interactions [1]. NMR spectroscopy has been used to help with structure activity relationship (SAR), characterize solution structure, select candidates for crystallization and study dynamics of millamolecules. Here, we will present recent results on structural characterization strategies that include (1) solution condition optimization, (2) conformational heterogeneity evaluation to triage compounds for X-ray crystallization, (3) 2D assignments using ACD Workbook, and (4) conformation ensemble generation using J-couplings, TALOS [2, 3] and NOE-distance restraints. This structural information helps to model docking of peptides into the target protein, and to delineate changes in peptide conformation on binding. Further, a comparison of solution vs X-ray crystal structure enables us to determine if the folds are similar in solution vs the solid state.

1. Horst Kessler, Angew. Chem. Int. Ed. Engl., 21, 512-523, 1982

2. Yang Shen, Frank Delaglio, Gabriel Cornilescu, and Ad Bax, J Biomol NMR, 44(4), 213–223, 2009

3. D. S. Wishart, B. D. Sykes and F. M. Richards, Biochemistry 31, 1647-1651, 1992

8 ^{19}F qNMR in Human Absorption, Distribution, Metabolism and Excretion Studies of Nirmatrelvir: A Faster Route to Emergency Use Authorization

Gregory S. Walker[1], Ravi Shankar P. Singh[2], Eugene P. Kadar[1], Loretta M. Cox[1], Heather Eng[1], Raman Sharma[1], Amit S. Kalgutkar[2], and R. Scott Obach[1]

1. Pfizer Worldwide Research, Development & Medical; Groton, CT 06340, USA,
2. Pfizer Worldwide Research, Development & Medical; Cambridge, MA 02139, USA
3.

PAXLOVID™ (nirmatrelvir[PF-07321332]/ritonavir - 300/100 mg) is the first oral SARS-CoV-2 main protease inhibitor to receive emergency use authorization (EUA) for the treatment of COVID-19 in patients with high risk of progression to severe disease. Because of the urgent need for new medicines to address the COVID pandemic, innovative approaches had to be employed to accelerate the development of PAXLOVID™. An overall understanding of the disposition of a compound in humans is a requirement for registration of any new medicine and is almost always done through an absorption, distribution, metabolism and excretion (ADME) study. Historically, ADME data has been obtained via the administration of a radiolabeled (typically 14C) analog of the parent compound with subsequent collection of excreta and plasma. The radiolabel is used because of the low inherent background and its universal analytical response factor. The synthesis of 14C analogs can often be costly and more importantly time consuming. Following the administration of 14C labeled drug, excreta and plasma samples are then analyzed for total drug content by liquid scintillation counting (LSC) to establish mass balance. Additionally, separately pooled samples of excreta and plasma are profiled for metabolites by liquid chromatography/mass spectrometry (LC/MS). In conjunction with the LC/MS analysis fractions are collected and analyzed by LSC to create a radiochromatogram.

To support the accelerated development of PAXLOVID™, synthesis of [14C]nirmatrelvir for a conventional human ADME study was not feasible. Hence, alternative detection methods for the ADME study were evaluated. Because 19F qNMR affords the same type of universal response, independent of chemotype, as radioactivity and that nirmatrelvir contains a trifluoroacetamide, 19F qNMR was used as a detection technique for the human ADME study. Using 19F qNMR we were able to assess the metabolism and excretion of nirmatrelvir within the first-in-human study following the administration of a single dose of unlabeled drug. Mass balance was achieved with approximately 84.9 ± 8.9% of the total administered dose recovered (urine 47.0% and feces 33.9%). Nirmatrelvir was the only drug-related entity observed in plasma. These data were accepted by the FDA for the EUA of PAXLOVID™. To the best of our knowledge, this is the first time a regulatory agency agreed to the use of 19F-qNMR data in lieu of a 14C study for ADME characterization. Notably, because of the 19F qNMR approach, the human ADME data was delivered much sooner to regulatory agencies and facilitated a rapid approval of a critical medicine that is helping address the worldwide COVID pandemic.

9 Insights into Fluorinated Drug Substance and Drug Product via ^{19}F Solid-State NMR Spectroscopy

Joe Lubach

Genentech, Inc.

High resolution characterization of pharmaceutical solid dosage forms represents an ever-challenging and ever-changing problem facing pharmaceutical scientists. Historically for pharmaceutical solids, ^{13}C is the most widely studied nucleus due to the information content available, its presence in nearly all pharmaceutical ingredients, and high resolution spectra that can be obtained. It is primarily accessed via cross polarization (CP) experiments due to its low natural abundance and relatively long relaxation times, which have hindered its use in quantitative analysis.

In many ways, ^{19}F is a friendlier nucleus from an NMR standpoint due to its relative sensitivity, and continues to become more widespread in pharmaceutical materials. It is highly useful when present in active ingredients and enables exquisitely selective analysis of the physical state of the active ingredient within a formulated product, even at very low drug loading. We will examine a few of the advantages, and drawbacks, of ^{19}F solid-state NMR, and a variety of ways it can be exploited in solid form analysis. These include simple crystal form identification, crystallographic inequivalency evaluation, water content determination of a hydrated API, and quantitative solid form measurements in complex drug products.

Deeper understanding of the state of drug particles in the presence of an excipient matrix offered by sensitive and selective ^{19}F NMR spectroscopy can provide valuable insight into dosage form design for more robust drug products and processes. It has become an indispensable tool in our toolbox for solid form characterization, and especially for solid dosage formulation characterization.

10 QNMRX-CSP: Crystal Structure Prediction Aided by Quadrupolar NMR Crystallography

Austin A. Peach,[1,2], Carl H. Fleischer,[1,2] Kirill Levin,[3] Sean T. Holmes,[1,2] Jasmin E. Sanchez,[1,2] and **R.W. Schurko**[1,2]

1. Department of Chemistry and Biochemistry, Florida State University, Tallahassee FL, USA
2. National High Magnetic Field Laboratory, Tallahassee, FL, USA
3. Département de Chimie, Université de Sherbrooke, Sherbrooke, QC, Canada

Crystal structure prediction (CSP) aided by NMR crystallography (NMRX) uses information from solid-state NMR (SSNMR), X-ray diffraction (XRD), and quantum chemical calculations for the prediction, solution, and/or refinement of crystal structures. To date, most NMRX studies rely upon the accurate determination of isotropic chemical shifts (CSs) for $I = ½$ nuclides, and in some cases, CS tensors or dipolar couplings.[1-4] However, the use of electric field gradient (EFG) tensors of quadrupolar nuclei (*i.e.*, $I > ½$; *e.g.*, ^{14}N, ^{17}O, ^{35}Cl, *etc*.), for NMRX has largely gone unexplored.[5,6]

The quadrupolar interaction, which is the interaction between the nuclear quadrupole moment and the EFG tensor at the quadrupolar nucleus, manifests in SSNMR spectra of quadrupolar nuclides in the form of distinct first- and second-order effects that influence powder pattern shapes. The measurement of the quadrupolar parameters, including the quadrupolar coupling constant, C_Q, and the asymmetry parameter, η_Q, allows for determination of the EFG tensor. EFG tensors act as excellent probes of local atomic environments, since they are extremely sensitive to even the most subtle differences or changes in structure (much moreso than CS tensors). Furthermore, first principles calculations of EFG tensors are computationally inexpensive in comparison to those of CS tensors. Hence, the combination of experimental and theoretical EFG tensors has great promise for numerous NMRX-CSP applications.

In this lecture, I will discuss a new QNMRX-CSP protocol, which focuses upon ^{35}Cl (I = 3/2) SSNMR for the prediction, solution, and refinement of crystal structures of organic HCl salts (more than 55% of solid active pharmacueitcal ingredients (APIs) are produced as HCl salts). Five organic HCl salts with known crystal structures are used as a training set, from which computational protocols are benchmarked and optimized. The protocol involves: (1) Monte-Carlo simulated annealing for generating thousands of candidate crystal structures; (2) truncated- and full-geometry optimizations for refining the structures using dispersion-corrected DFT (DFT-D2*);[7,8] and (3) filtering of candidate structures. Careful consideration is given to the benchmarking of several metrics and constraints, including: (i) unit cell parameters, (ii) static lattice energies, (iii) ^{35}Cl EFG tensors, (iv) atomic charges, and (v) starting molecular fragments and their motion groups. Then, I will discuss the application of this new protocol for the *de novo* structural prediction and refinement of several organic HCl salts, using only their experimentally determined pXRD patterns and ^{35}Cl quadrupolar parameters. Finally, I will discuss applications of the new QNMRX-CSP protocol to hydrates and stereoisomers of organic HCl salts, its expansion to include quadrupolar nuclei like ^{14}N and ^{17}O, and its general use for the prediction and refinement of crystal structures of APIs.

1. Hodgkinson, P. NMR Crystallography of Molecular Organics. *Prog. Nucl. Magn. Reson. Spectrosc.* **2020**, *118–119*, 10–53.
2. Elena, B.; Emsley, L. Powder Crystallography by Proton Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* **2005**, *127*, 9140–9146.
3. Salager, E. *et al*. Powder Crystallography by Combined Crystal Structure Prediction and High-Resolution 1H Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* **2010**, *132*, 2564–2566.
4. Salager, E. *et al*. Powder NMR Crystallography of Thymol. *Phys. Chem. Chem. Phys.* **2009**, *11*, 2610–2621.
5. Perras, F. A.; Bryce, D. L. Multinuclear Magnetic Resonance Crystallographic Structure Refinement and Cross-Validation Using Experimental and Computed Electric Field Gradients: Application to $Na_2Al_2B_2O_7$. *J. Phys. Chem. C* **2012**, *116*, 19472–19482.
6. Holmes, S. T. *et al*. A New NMR Crystallographic Approach to Reveal the Calcium Local Structure of Atorvastatin Calcium. *Phys. Chem. Chem. Phys.* **2019**, *21*, 6319–6326.
7. Grimme, S. Semiempirical GGA-Type Density Functional Constructed with a Long-Range Dispersion Correction. *J. Comput. Chem.* **2006**, *27*, 1787–1799.
8. Holmes, S. T.; Vojvodin, C. S.; Schurko, R. W. Dispersion-Corrected DFT Methods for Applications in Nuclear Magnetic Resonance Crystallography. *J. Phys. Chem. A* **2020**, *124*, 10312-10323.

11 NMR Crystallography: Bridging Atomic-Level Structural Rearrangement and Macroscopic Motion in Molecular Organic Crystals

Kevin Chalek, Xinning Dong, Fei Tong, Rabih Al-Kaysi, Joshua D. Hartman, Gregory J.O. Beran, Christopher J. Bardeen, and Leonard J. Mueller

Department of Chemistry, University of California, Riverside, California 92521

Crystals composed of photoreactive molecules represent a new class of photomechanical materials with the potential to generate large forces on fast timescales. An example is the photodimerization of 9-tertbutyl-anthracene ester (**9TBAE**) in molecular crystal nanorods that leads to an average elongation of 8%. This expansion results from the formation of a metastable crystalline intermediate termed the solid-state reacted dimer (SSRD). Photoreaction of bulk single crystal monomer invariably leads to strain that shatters the crystal, precluding direct characterization with single crystal X-ray diffraction. Here, the combination of powder X-ray diffraction, solid-state nuclear magnetic resonance, and first principles computational modeling is used to determine the crystal structure of the SSRD intermediate and establish a microscopic model for the macroscopic expansion. We find that the SSRD crystal unit cell and volume are quite similar to those of the monomer crystal, leading to the conclusion that gross changes in the volume or unit cell parameters of the SSRD are not responsible for the expansion. At the same time, solid-state NMR of the aligned monomer nanorods and the photoreacted product shows the generation of new lattice orientations within the nanorod. Based on our observations, the nanorods expand not due to a change in the volume of the unit cell, but rather due to an anisotropic rearrangement of the molecular contents. The ability to understand quantitatively how molecular-level photochemistry generates mechanical displacements allows us to predict that the expansion could be tuned from +9% to -9.5% by controlling the initial orientation of the unit cell with respect to the nanorod axis. This application of NMR-assisted crystallography provides a new tool capable of tying the atomic-level structural rearrangement of the reacting molecular species to the mechanical response of a nanostructured sample.

12 Refining crystal structures using NMR chemical shift tensors as a target function

Jim Harper

Brigham Young University

A two-step procedure is described for refining crystal structures from any source. This approach includes an initial lattice-including DFT relaxation step followed by a Monte Carlo sampling process to create new candidate positions for each atom within a structure. The candidate having the best agreement between experimental and computed ^{13}C NMR shift tensor principal values is selected for further refinement and the Monte Carlo process is repeated until convergence is achieved. This refinement can include all atoms within a structure or can be restricted to only poorly fit sites. This process is shown to improve ^{13}C NMR agreement from 6.1 ppm for a set of benchmark structures obtained from high quality diffraction data and not subjected to any refinement to 1.8 ppm after the two-step refinement. Although changes to atom positions from this refinement process are quite small (usually a few picometers), prior work is summarized to demonstrate that these changes can, in fact, yield new structural insights involving changes in hydrogen bonding, detection of hydrogen tunneling and new insights into protein backbone dynamics. Several non-NMR metrics, examined before and after refinement, indicate that this process does not introduce structural errors.

13 NMR Experiments and Simulations Reveal Interactions Between Small Molecules and Plastic Nanoparticles

Leah Casabianca

Department of Chemistry, Clemson University, Clemson, SC, USA

Plastic pollution in world waterways is a growing concern. The coronavirus pandemic has increased plastic waste through increased use and improper disposal of medical supplies and PPE, lifting of bans on single-use plastic bags, closing of recycling facilities, and increased demand for single-use plastic cutlery and plastic food packaging for restaurant take-out and delivery orders.[1] This plastic waste is broken down into smaller pieces, eventually forming micro-and nanoscale plastic particles.[2] These particles pose a danger to wildlife as they can be mistaken for food, leading to choking hazards or malnutrition.[3] Another concern is that plastic particles can concentrate other small molecules that are present in polluted waterways, such as carcinogenic polycyclic aromatic hydrocarbons, pharmaceuticals, and pesticides.[4] As plastic particles can move up the food chain, these toxic adsorbed molecules may be released in higher organisms or even humans.[5]

In this talk, we will present recent work we have done aimed at understanding the interactions that are responsible for sorption of small molecules on the surface of nanoscale plastic particles. From a solution-state NMR perspective, saturation-transfer difference (STD) NMR has been a useful tool to identify small molecules that interact with a plastic nanoparticle surface.[6] STD-NMR has also been used for epitope mapping, allowing us to determine the driving forces for binding between polystyrene nanoparticles and amino acids as model small molecules.[7] STD-NMR has also been used to examine binding between plastic nanoparticles and a selection of antibiotics, which are also expected to be present in polluted waterways.

Theoretical techniques including molecular dynamics simulations have also been used to gain insight into these interactions from the perspective of the plastic particle. These simulations complement the insight gained by STD-NMR, which is limited to being able to observe the small molecule. Results of this combined experimental and theoretical work could be useful to the design of biodegradable plastics that are resistant to sorption of small molecule toxins, and therefore pose less of a threat to environmental and human health as they decompose.

1. Prata, Joana C.; Silva, Ana L. P.; Walker, Tony R.; Duarte, Armando C.; Rocha-Santos, Teresa, Enviornmental Science & Technology, 54(13), 7760-7765, 2020.
2. Andrady, Anthony L., Marine Pollution Bulletin, 119(1), 12-22, 2017.; Andrady, Anthony L., Marine Pollution Bulletin, 62(8), 1596-1605, 2011.
3. Mattsson, Karin; Johnson, Elyse V.; Malmendal, Anders; Linse, Sara; Hansson, Lars-Anders; Cedervall, Tommy, Scientific Reports, 7, 11452, 2017.; Mattsson, Karin; Ekvall, Mikael T.; Hansson, Lars-Anders; Linse, Sara; Malmendal, Anders; Cedervall, Tommy, Environmental Science & Technology, 49(1), 553-561, 2015.
4. Rochman, Chelsea M.; Manzano, Carlos; Hentschel, Brian T.; Simonich, Staci L. Massey; Hoh, Eunha, Environmental Science & Technology, 47(24), 13976-13984, 2013.
5. Fred-Ahmadu, Omowunmi H.; Bhagwat, Geetika; Oluyoye, Idowu; Benson, Nsikak U.; Ayejuyo, Olusegun O.; Palanisami, Thavamani, Science of the Total Environment, 706, 135978, 2020.; Rochman, Chelsea M.; Hoh, Eunha; Kurobe, Tomofumi; Teh, Swee J., Scientific Reports, 3, 3263, 2013.; Rochman, Chelsea M.; Tahir, Akbar; Williams, Susan L.; Baxa, Dolores V.; Lam, Rosalyn; Miller, Jeffrey T.; Teh, Foo-Ching; Werorilangi, Shinta; Teh, Swee J., Scientific Reports, 5, 14340, 2015.

6. Zhang, Yunzhi; Xu, Hui, Parsons, Austin M.; Casabianca, Leah B., Journal of Physical Chemistry C, 121(44), 24678-24686, 2017.; Zhang, Yunzhi; Xu, Hui, Casabianca, Leah B., Magnetic Resonance in Chemistry, 56(11), 1054-1060, 2018.; Xu, Hui; Casabianca, Leah B., Langmuir, 37(1), 385-390, 2021.
7. Zhang, Yunzhi; Casabianca, Leah B., Journal of Physical Chemistry Letters, 9(23), 6921-6925, 2018.; Xu, Hui; Casabianca, Leah B., Scientific Reports, 10, 12351, 2020.

14 Instrumentation, Advanced Heterogeneous Catalysts, and New Approaches to Spin order Transfer for Hyperpolarization of Metabolites from Parahydrogen

C. Russell Bowers

Department of Chemistry, University of Florida and National High Magnetic Field Laboratory, Gainesville, Florida

Parahydrogen based hyperpolarization [1] has been extensively developed to the point where it is now poised to overtake dynamic nuclear polarization as the method of choice for preparing key metabolites for sensitivity-enhanced in-vivo magnetic resonance imaging [2]. Pyruvic acid is a key metabolite at the intersection of many biochemical pathways but unfortunately does not contain any unsaturated bonds that can be readily hydrogenated. In the side-arm hydrogenation (SAH) approach [3], the carboxylic acid is esterified with short side-arm moiety containing a C≡C or C=C bond (e.g., propargyl). After hydrogenation, the proton spin order is transferred to the carbonyl ^{13}C followed by cleavage by hydrolysis. This talk will present several new strategies for efficient spin order transfer based on adiabatic passage from the strong to weak coupling of the side-arm proton spin system. It will be shown that adiabatic passage through level anti-crossings (LACs) arising from certain combinations of homonuclear proton-proton couplings and chemical shifts can mediate spontaneous shuttling of parahydrogen spin order toward the carbonyl ^{13}C in allyl pyruvate. Furthermore, it will be shown how selective deuteration can significantly increase the absolute ^{13}C polarization level achieved by adiabatic passage through LACs in conjunction with selective coherence transfer.

A longstanding goal of the Bowers lab is to develop heterogeneous hydrogenation catalysts and reactor systems that afford continuous-flow production of contaminant-free hyperpolarized biomolecules with ^{13}C polarization levels rivaling those achieved by homogeneous catalysis. Recent work in our lab has demonstrated that the pairwise selectivity of hydrogenation can be significantly increased using certain intermetallic phases composed of an active (Pt, Rh) and an inert (Sn, In) metal [4]. The inert metal helps the parahydrogen ad-atom pair to "stick together" on the surface, thereby prolonging the singlet state lifetime on the catalyst surface.

Finally, the talk will review present two types of hydrogenation reactors that have been developed in our lab that are suitable for continuous-flow hyperpolarization experiments by homogeneous and heterogeneous catalysis with parahydrogen.

[1] C. R. Bowers and D. P. Weitekamp, *Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment*, J Am Chem Soc **109**, 5541 (1987).

[2] J. B. Hovener et al., *Parahydrogen-Based Hyperpolarization for Biomedicine*, Angewandte Chemie International Edition **57**, 11140 (2018).

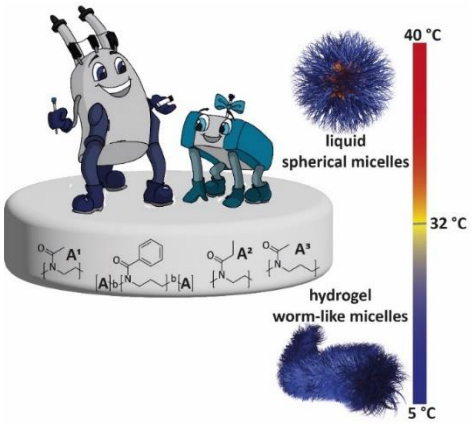
[3] F. Reineri, T. Boi, and S. Aime, *ParaHydrogen Induced Polarization of 13C Carboxylate Resonance in Acetate and Pyruvate*, Nature Communications **6**, 1 (2015).

[4] Y. Du, R. K. Behera, R. v. Maligal-Ganesh, M. Chen, T. Yunpu Zhao, W. Huang, and C. R. Bowers, *Mesoporous Silica Encapsulated Platinum–Tin Intermetallic Nanoparticles Catalyze Hydrogenation with an Unprecedented 20% Pairwise Selectivity for Parahydrogen Enhanced Nuclear Magnetic Resonance*, The Journal of Physical Chemistry Letters **13**, 4125 (2022).

15 Am I still liquid? – Insights into hydrogel formation by a colourful NMR toolbox

Theresa Zorn[1], Lukas Hahn[2], Kay, Saalwächter[3], Robert Luxenhofer[2,4], and Ann-Christin Pöppler[1]

1. Institute of Organic Chemistry, Würzburg, GER
2. Institute of Functional Materials and Biofabrication, Würzburg, GER
3. Institute of Physics, Halle, GER
4. Department of Chemistry, Helsinki, FIN



Hydrogels are increasingly used for biomedical applications. Their easily tunable physicochemical properties enable a rational design of the polymeric material for a specific application.[1] Prerequisite is a detailed structural under-standing on a molecular level. Recently, polyoxazoline gels gained interest due to their biocompatibility and stealth properties, thus being considered as an alternative to the widely used polyethylene glycols.[2] The herein investigated triblock copolymers comprising different hydrophilic A-blocks and hydrophobic 2-phenyl-2-oxazine repeat units form physically-linked, worm-like hydrogels, which dissipate into a micellar solution when heated to physiologically relevant temperatures (Figure 1).[3] Insights into this unusual sphere-to-worm, order-order transition could be especially achieved by using a variety of different NMR spectroscopic tools yielding the following key findings:

- **High-resolution NMR in solution:** ^{1}H NMR spectra as well as further ^{1}H-^{1}H NOE measure-ments indicated an overall decrease in signal intensity and therefore mobility upon gel formation with the phenyl groups of the hydrophobic polymer block being most affected.
- **Low-field NMR studies:** Mobile, intermediate, and rigid phase fractions within the polymer self-assemblies could be successfully quantified. Interestingly, the rigid phase fraction clearly exceeded the number of protons in the hydrophobic polymer blocks. These rigid protons appear invisible for NMR in solution, which complicates the interpretation of signal changes upon gelation. Further double-quantum NMR experiments confirmed a bottle-brush like appearance of the hydrophilic shell.
- **Solid-state NMR:** Both a mobile and a more rigid methyl group species were detected for the hydrophilic polymer blocks, with only the more rigid environment displaying through-space proximity to the phenyl groups.

Moreover, these effects of gelation directly follow the trend of macroscopic gel strength within the set of three polymers. Putting the pieces together, the sphere-to-worm, order-order transition must be critically affected by non-ionic, non-H-bonding interactions between the hydrophilic and hydrophobic moieties. These NMR-based findings now allow tuning of the hydrogel (bulk) properties by chemical variation of the hydrophilic blocks.

1. Cao H., Duan L., Zhang Y., Cao J., Zhang K., *Signal Transduct. Target. Ther.*, *6*, 426, **2021**.
2. Dargaville T. R., Park J.-R., Hoogenboom R., *Macromol. Biosci.*, *18*, 1800070, **2018**.
3. Hahn L., Maier M., Stahlhut P., Beudert M., Flegler V., Forster S., Altmann A., Töppke F., Fischer K., Seiffert S., *et al.*, *ACS Appl. Mater. Interfaces*, *12*, 12445-12456, **2020**.

16 High-Way or Bi-Way – New Stopped-flow NMR Methods

Ran Wei, Dušan Uhrín and Guy C. Lloyd-Jones

School of Chemistry, University of Edinburgh, UK

NMR spectroscopic analysis of chemical reactions remains an essential tool, as it offers a wealth of quantitative structural information and a high degree of spectral resolution [1]. Nevertheless, *in-situ* monitoring of rapid irreversible reactions that are initiated by mixing, poses challenges to traditional NMR experiments, therefore requires specialised techniques.

A new stopped-flow apparatus [2] was designed to allow the capture of the fleeting information from irreversible reactions at the millisecond timescale. Its adaption to standard NMR probes in obtaining qualitative and quantitatve kinetic data are discussed. With the aid of this technique, we are able to study evolving reactions that occur beyond the measurement deadtime of traditional NMR experiments. Here an irreversible reaction of $t_{1/2}$ = 45 ms was used as an example to demonstrate the monitoring of rapid irreversible reactions by interleaved ^{19}F stopped-flow NMR spectroscopy. Furthermore, by utilising the un-premagnetised channel of the stopped-flow instrument, chemical exchange studies can be carried out without the necessities of isotopic labelling; or when the exchanging species are too short-living to be analysed by traditional methods.

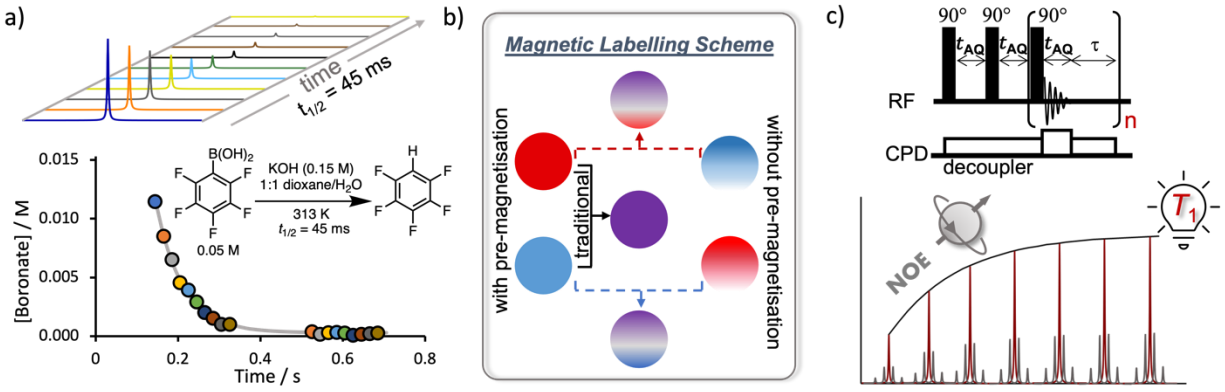


Figure 1. a) ^{19}F stopped-flow NMR spectroscopy to monitor the protodeboronation of penta-fluorophenyl boronic acid. b) chemical exchange studies by the un-premagnetised channel of the stopped-flow instrument. c) the FLOPS/FLAPS method for rapid T_1 measurement by utilising heteronuclear decoupling.

At the same time, rapid T_1 estimation methods, termed FLOPS/FLAPS (Fast Longitudinal relaxation by Ordered/Amplified Progressive Saturation) were developed following a previous method FLIPS [3]. By applying heteronuclear decoupling throughout the measurements, FLAPS offers an order of magnitude improvement in efficiency comparing to the inversion recovery method.

1. Lloyd-Jones, Guy C. *et al.*, *Prog. Nucl. Magn. Reson. Spectrosc.*, 129, 28-106, 2022.

2. Wei, Ran; Hall, Andrew M. R.; Behrens Richard; Pritchard, Mark S.; King, Edward J.; Lloyd-Jones, Guy C., *Eur. J. Org. Chem.,* 2021, 2331-2342, 2021.
3. Wei, Ran; Dickson, Claire L.; Uhrín, Dušan; Lloyd-Jones, Guy C., *J. Org. Chem.,* 2021, 9023-9029, 86.

17 Back to the Future: Exploration of new ADEQUATE Improvements and Applications

R. Thomas Williamson,[1] Ole W. Soresen,[2] Jeffrey G. Raab,[1] Gary E. Martin,[3] Marius Pelmus,[3] Ronald C. Crouch,[4] Mikhail Reibarkh,[5] Alexei Buevich,[5] Evgeny Tishchenko,[4] Michael Frey[4]

1. Department of Chemistry and Biochemistry, UNC Wilmington, Wilmington, NC USA
2. Copenhagen, Denmark
3. Department of Chemistry, Seton Hall University South Orange, NJ, USA
4. Analytical Instruments, JEOL USA Inc. Peabody, MA, USA
5. Analytical Research and Development, Merck and Co., Inc., Rahway, & Kenilworth NJ, USA

ADEQUATE and related experiments were first described in the mid-1990s and are a family of proton-detected NMR experiments used to establish carbon–carbon correlations at natural abundance. Applications of these experiments were initially limited by the statistical probability of two ^{13}C nuclides in the same molecule (~ 1:10,000) and the low intrinsic sensitivity of the NMR probe technology of the day. In the intervening two decades, there have been significant advances in NMR technology and the enhanced sensitivity offered by cryogenic NMR probes has reduced sample requirements for ADEQUATE experiments from the ~10 mg range to less than a milligram. In parallel with advances in NMR probe technology, progress in other areas like non-uniform sampling, and combining new variants of the ADEQUATE experiments have increased the intrinsic value of these experiments. In this talk, we will present a new family of related pulse sequence and describe the performance gains provided by these modifications. We will also share applications of these experiments utilizing ^{19}F detection, and applications to real structure elucidation problems. Finally, the observation and utility of isotope shifts in 1,1- and 1,n-ADEQUATE correlations will be discussed.

18 Ultra-selective 1D NMR Spectroscopy

Ralph W. Adams

NMR Methodology Group, Department of Chemistry, University of Manchester, Manchester, UK

The information provided by 2D NMR makes it an extremely powerful tool; correlations indicate connectivity and spatial proximity which enable the elucidation of molecular structure. While exceedingly useful, 2D spectra usually contain much more information than is required to solve a chemical problem. They can also take a long time to acquire. Selective 1D experiments target the required information, providing just what is needed in a fraction of the 2D experiment time.

Selective 1D experiments rely on radiofrequency pulses to excite individual signals; ambiguity is introduced when multiple signals overlap and cannot be selectively excited. As ^{1}H spectra are often extensively overlapped due to multiplets caused by homonuclear couplings, a different selection method is often required. The pulse sequence element, GEMSTONE (Gradient-Enhanced Multiplet-Selective Targeted-Observation NMR Experiment),[1,2] provides the required selectivity. It uses an approach derived from the chemical shift selective filter method [3,4] to select a specific proton signal from within an overlapping group of multiplets, but in a single scan.

GEMSTONE has the potential to significantly broaden 1D selective excitation applications. Examples using 1D selective NOESY,[1] TOCSY,[2] ROESY, and COSY show how the ultra-selectivity of the GEMSTONE element allows simple and rapid extraction of unambiguous information about spatial proximity and proton connectivity.

1. Kiraly P, Kern N, Plesniak MP, Nilsson M, Procter DJ, Morris GA, Adams RW, *Angew. Chem. Int. Ed.*, 60, 666-669, 2021.
2. Kiraly P, Nilsson M, Morris GA, Adams RW, *Chem. Commun.*, 57, 2368-2371, 2021.
3. Hall LD and Norwood TJ, *J. Magn. Reson.*, 76, 548-554, 1988.
4. Robinson PT, Pham TN, and Uhrín D, *J. Magn. Reson.*, 170, 97-103, 2004.

19 Quantitative Interpretation of Molecular Diffusion Coefficients – From Big to Small

Robert Evans

Aston University, Chemical Engineering and Applied Chemistry, Birmingham, UK.

Diffusion-ordered NMR spectroscopy (DOSY) has found increasing use as an analytical tool, capable of identifying the identity of components in a mixture [1]. Measuring accurate molecular self-diffusion coefficients, *D*, by nuclear magnetic resonance (NMR) techniques has become routine as hardware, software and experimental methodologies have all improved [2]. In principle, the values of diffusion coefficient obtained carry important information about the sizes of different species and on interactions between species, but the relationship between diffusion coefficient and molecular mass can be a complex one. The quantitative interpretation of such diffusion coefficient data remains difficult, particularly for small molecules on one hand and for molecules with disperse molecular mass distributions on the other.

The most basic relationship is that established in the Stokes-Einstein equation [3], which balances the energy of the system to the friction acting on the molecules. For larger species such as polymers and proteins, power laws can be used to relate the observed *D* to the molecular weight of the species present. Such power laws need to be calibrated for each distinct chemical species present and different methods for generating such calibration curves exist. Complications arising from the analysis of more disperse systems can be handled with careful choice of how the diffusion NMR data is acquired and processed.

For small molecules, the Stokes-Einstein equation becomes increasingly less valid as the size of the solute molecules approaches that of the solvent. A modification of the Stokes-Einstein equation that can successfully predict diffusion coefficients for small molecules has been developed [4]. The breakdown of the continuum model is corrected for by a variable friction term that depends on ratio of the solute to that of the solvent. The effects of shape, flexibility, solvation and composition cannot be treated analytically without prior chemical knowledge. However, by approximating small molecules as hard spheres with an average effective density ρ_{eff}, it is possible to successfully predict the diffusion coefficients of small molecules on the basis of their molecular weight. This new model has subsequently been validated over a wider range of literature small molecule diffusion coefficients, in deuteriated, protiated and some mixed solvents, at variable temperatures, and shown to predict diffusion coefficients to within 15 % of their experimental values [5].

1. Johnson, C. S., *Prog. Nucl. Magn. Reson. Spectrosc*., 34(3-4), 203-256, 1999.
2. Evans, R., *Prog. Nucl. Magn. Reson. Spectrosc*. 117, 33-69, 2020.
3. Einstein, A., *Ann. Phys*. 17, 549-560, 1905.
4. Evans, R., Deng, Z., Rogerson, A. K., McLachlan, A. S., Richards, J. J., Nilsson, M., and Morris, G. A., *Angew. Chemie Int. Ed.* 52(11), 3199-3202, 2013.
5. Evans, R., Dal Poggetto, G., Nilsson, M., and Morris, G. A., *Anal. Chem*., 90(6), 3987-3994, 2018.

20 NMR Methodology for Analysis of ^{15}N-labelled Complex Mixtures

Justinas Sakas and Dušan Uhrín

EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK

The analysis and structure elucidation of complex mixtures is an arduous task as they contain hundreds or even thousands of compounds that cannot be easily separated using chromatographic techniques. Methods that bypass the customary chromatographic step and use spectroscopic 'separation' instead have been proposed. These target specific moieties present in a subset of molecules. Recent examples include structure elucidation using NMR experiments designed for compounds tagged with $-O^{13}CH_3$ [1] and fluorinated compounds [2].

In this work we introduce a suite of NMR experiments designed for ^{15}N-labelled compounds. This includes ^{1}H, ^{15}N constant-time HMQC-COSY in which proton-proton correlations are obtained between adjacent CH and ^{15}NH groups. Long-range connectivity and correlations to ^{15}N atoms are then explored using ^{1}H, ^{15}N BIRD-HMBC in order to connect molecular fragments.

The new methodology was applied for structure elucidation of compounds produced during chloramination, a water disinfection method used around the world. The addition of chloramine kills pathogens, however it also reacts with naturally dissolved organic matter (DOM) and anthropogenic contaminants to produce a variety of disinfection by-products (DBPs) (Figure 1), which are known to be toxic or carcinogenic [3]; additionally, more than 70% of chloramination DBPs are unknown [4]. Therefore, it is necessary to elucidate their structures in order to find out if they pose any health risks.

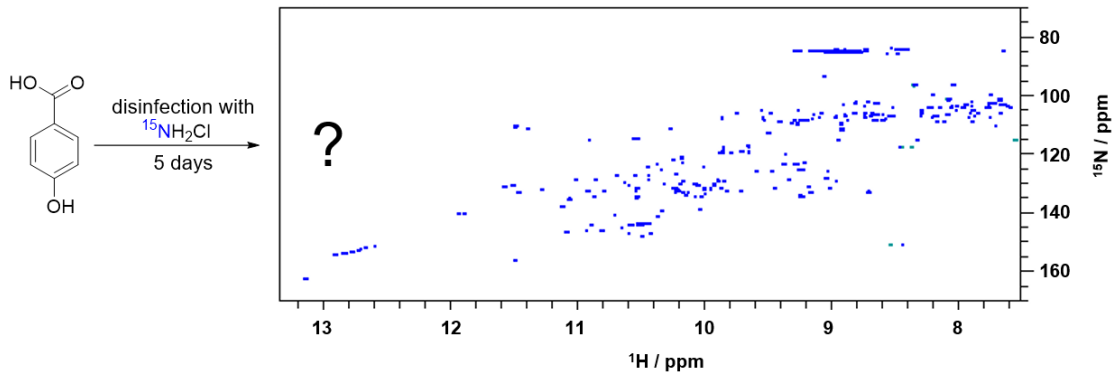


Figure 1. 2D ^{1}H, ^{15}N HSQC spectrum showing the complex mixture of nitrogen-containing compounds produced by chloramination of 4-hydroxybenzoic acid.

1. N. G. A. Bell, A. A. L. Michalchuk, J. W. T. Blackburn, M. C. Graham and D. Uhrín, *Angew. Chem. Int. Ed.*, 54, 8382–8385, 2015.
2. A. J. R. Smith, R. York, D. Uhrín and N. G. A. Bell, *Chem. Sci.*, 13, 3766–3774, 2022.
3. X. Li and W. Mitch, *Environ. Sci. Technol*., 52, 1681–1689, 2018.
4. G. Hua and D. Reckhow, *Water Res*., 41, 1667–1678, 2007.

21 Multidimensional NUS NMR-Based Metabolomics for In Vitro Diagnostic Discovery

Elizabeth O'Day

Olaris, Inc, Framingham MA 01702

The US Food and Drug Administration (FDA) classifies in vitro diagnostics (IVDs) as "those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae." In this era of "omic" technology we are witnessing a new wave of precision medicine IVDs aimed at getting the right drug to the right patient. The majority of currently available IVDs are genomic based and are limited to providing risk factor information. Metabolomics is the study of small molecule metabolites that provide energy and biomass for life to exist. As metabolites are influenced by the genome and the environment, they provide a more comprehensive readout of an individual. Indeed, numerous studies have identified altered metabolites and metabolism in diseases such as diabetes, neurodegeneration, cancer, obesity, cardiovascular, inflammation and even aging. Many of the most well-established diagnostic assays measure a single metabolite or small number of metabolites (glucose for diabetes and cholesterol and triglycerides for heart disease). By expanding our ability to detect and quantify the metabolome in a highly reproducible manner novel insights into disease will be uncovered. In this talk I will describe how we leverage an NUS NMR-based metabolomics platform, combined with machine learning for metabolite biomarker discover. Using case studies, I will showcase how metabolomic-based diagnosed are poised to transform how diseases are diagnosed and treated and detail the considerations needed for diagnostic development.

22 NMR Metabolomics Best Practices: What We Are Doing, What We Should Be Doing, and Some Recent Advancements

Robert Powers

Department of Chemistry and the Nebraska Center for Integrated Biomolecular Communication, University of Nebraska-Lincoln, Lincoln NE 68588-0304

NMR-based metabolomics has benefited a variety of fields from agriculture and environmental studies to drug discovery and disease diagnosis. Despite appearances, metabolomics is technically very challenging and requires expertise in a diversity of areas that range from analytical chemistry and cell biology to advanced statistical analysis and deep learning techniques. Accordingly, several challenges and problems exist that need to be overcome for the field to continue to succeed, which may be addressed by the adoption of a standard set of best practices by the community. This presentation will highlight common issues with sample preparation and handling, data collection and analysis, and statistical modeling and interpretation. This will include a summary of the results of a survey of NMR metabolomics papers published in 2010 and 2020 that describes widely employed protocols, and a meta-analysis of 24 pancreatic ductal adenocarcinoma (PDAC) clinical metabolomics studies from the literature that raises concerns about the reproducibility of results. Some recent advancements in NMR-based metabolomics techniques will also be presented.

23 Ultrahigh-Resolution NMR of Aqueous Biofluids: Towards A Better Quantitative Metabolic Profiling of Lymphoma Cells

Gildas Bertho[1], Xi Chen[1], Covadonga Lucas-Torres[1], Catherine Thieblemont[2,3], Véronique Baud[2] and **Nicolas Giraud**[1]

1. Université Paris Cité, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR CNRS 8601, Paris, France
2. Université Paris cité, NF-κB, Différenciation et Cancer, F-75006 Paris, France
3. AP-HP, Hôpital Saint-Louis, Service Hémato-Oncologie, F-75010 Paris, France

Lymphoma refers to the group of blood cancers developing from lymphocytes, which are key infection-fighting cells of our immune system. Diffuse Large B-Cell Lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma (40% of cases). Although DLBCL treatment outcome has significantly improved, to date 40% of patients will either be refractory or relapse and die.

Over the last years, metabolism has become a major topic in cancer research, unveiling new approaches to decipher the mechanism of the disease, as well as tools for developing new treatments.[1] In this context, we are carrying out a collaboration with Dr. V. Baud (INSERM) and Prof. C. Thieblemont (Hospital Saint-Louis, Paris) (i) to better describe the basal metabolism of Lymphoma cell lines and (ii) overcome drug resistance in DLBCL by using a combination of metabolic inhibitors.

We will show how Pure Shift ^{1}H NMR[2-3] can be used to decipher the metabolism of DLBCL and understand the mechanism of action of antimetabolic drugs. We will present the methodological developments carried out in our group to acquire Pure Shift spectra with efficient suppression of the water signal on these complex biofluids. We will present for the first time a workflow for quantifying accurately metabolites in extra-cellular media using Pure Shift data. We will describe how the analysis of the resulting metabolic profiles allows for getting a unique insight into the metabolic pathways that are key to Lymphoma Cells.[4]

1. Moses, C.; Garcia-Bloj, B.; Harvey, A. R.; Blancafort, P., *Eur. J. Cancer 93*, 10-18, 2018.
2. Zangger, K., *Prog. Nucl. Magn. Reson. Spectrosc. 86–87* (0), 1-20, 2015.
3. Foroozandeh, M.; Morris, G. A.; Nilsson, M., *Chemistry - A European Journal 24* (53), 13988-14000, 2018.
4. Bertho, G.; Lordello, L.; Chen, X.; Lucas-Torres, C.; Oumezziane, I. E.; Caradeuc, C.; Baudin, M.; Nuan-Aliman, S.; Thieblemont, C.; Baud, V.; Giraud, N., *J Proteome Res 21* (4), 1041-1051, 2022.

24 The Natural Products Magnetic Resonance Database (NP-MRD): Comprehensive Database and Repository for Natural Products NMR Data

John R. Cort[1,5], Amy M. Jystad[1], Niranjan Govind[1], Roger G. Linington[2], Lloyd W. Sumner[3], David S. Wishart[4]

[1]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland WA
[2]Department of Chemistry, Simon Fraser University, Burnaby BC
[3]Department of Biochemistry and MU Metabolomics Center, University of Missouri, Columbia MO
[4]Department of Biological Sciences and Department of Computing Science, University of Alberta, Edmonton AB
[5]Institute of Biological Chemistry, Washington State University, Pullman WA

NMR spectroscopy is essential to natural products and specialized metabolite research: for example, in novel structure determination, characterization of functions and interactions, or analysis of mixtures. However, progress in the field is hindered by the poor accessibility to NMR data for known natural products. Currently, chemical shift assignments are scattered throughout decades of published scientific literature and a few valuable, but incomplete, chemical shift databases. Furthermore, nearly all raw data (FIDs) used to determine structures of natural products is not archived and is likely unrecoverable. To address such inadequacies, the Natural Products Magnetic Resonance Database (NP-MRD, np-mrd.org) has been established with a goal to become a comprehensive, searchable, connected, and open database and repository for all natural products NMR data. The mission of NP-MRD is to benefit research through engagement and partnership with the worldwide natural products community. With derived (e.g. chemical shift assignments), raw (FID), and simulated NMR data, as well as tools and links to other databases, NP-MRD can facilitate dereplication, support correction of erroneous or missing chemical shift assignments, and enable structure validation or structure revision. Furthermore, NP-MRD can create opportunities for developing new artificial intelligence-based approaches for structure determination and chemical shift or spectral prediction, among other presently unforeseen applications of such a database resource.

25 *w*NMR for the Assessment of Complex Drug Products and Vaccines

Katherine Briggs

University of Maryland, School of Pharmacy

An intravenous drug approved for use as an anesthetic and sedative, propofol, or 2,6-diisopropylphenol, is a lipophilic molecule formulated as an emulsion in soybean oil under the brand name Diprivan. This product has a long-standing safety profile despite its susceptibility to both microbial growth and oxidation, and the inherent instability of emulsions. The brand, Diprivan, and generic, Propofol, contain different anti-microbial formulation components and were packaged under nitrogen. These two products were monitored over time by time-domain water proton NMR (*w*NMR) to determine whether *w*NMR is sensitive to product changes, such as oxidation. The transverse relaxation rate of water protons, $R_2(^1H_2O)$, indeed detects changes in propofol after exposure to oxygen.

Aluminum adjuvants are a component in many vaccines and have been widely used because of their high safety profile and effectiveness at enhancing the immune response to the vaccine. Adjuvant raw materials and vaccines were characterized by differences in suspension, sedimentation, and sedimentation rate, measured by $R_2(^1H_2O)$ over time. The sedimentation rate of two adjuvants, Alhydrogel® and Adju-Phos® shows a concentration dependence. Furthermore, vaccines with aluminum adjuvants are prone to damage due to freezing, which can accidentally occur during shipment or storage, and we found that the $R_2(^1H_2O)$ was sensitive to detect vaccine freezing.

Arguably, quality control practices need to evolve as drugs and vaccines become increasingly more complex. These results suggest that *w*NMR as a non-invasive assessment of product changes, has potential as a quality control method for manufacturing of complex drug products and vaccines, including at the point-of-care. At the level of drug products, *w*NMR has potential to bolster current quality control practices of statistical, batch-level sampling and visual inspection, and usher in vial-level quality control of complex drug products and vaccines.

26 Quantitative Reaction Monitoring using a Benchtop Stopped-Flow NMR

Jason Hein

University of British Columbia, Vancouver

Nuclear magnetic resonance (NMR) spectroscopy has the potential to serve as a widely applied reaction monitoring tool, particularly given the growth in commercially available benchtop NMR systems and accompanying flow cells. We herein present a stopped-flow benchtop NMR system devised of commercially available hardware components, all centrally controlled by an internally developed Python script. This system circumvents complications arising with continuous-flow NMR analyses and results in the ability to conveniently acquire quantitative reaction monitoring data. In our work, we first determine a set of 19F NMR acquisition parameters using benchtop NMR, allowing for quantitation using the absolute intensity method thereafter. This system and set of acquisition parameters are then applied to quantitatively monitor model reaction systems (via 19F NMR, 57 MHz) that are difficult to otherwise monitor due to gas evolution, use/formation of toxic reagents, and formation of species otherwise difficult to detect. These reactions include the activation of a carboxylic acid using sulfuryl fluoride (SO2F2) and the formation of a carbamate via modified Curtius rearrangement with diphenylphosphoryl azide (DPPA).

27 400 MHz Cryofree NMR Technology – Leveraging High Field NMR in a Benchtop NMR Setting

Ikenna E Ndukwe[1], Kyle Quasdorf[2], James Murray[2], Armando Navarro Vasquez[2] and Maria Victoria Silva Elipe[1]

1. Department of Attribute Sciences, Amgen Inc., Thousand Oaks, California 91320-1799, United States.
2. Pivotal and Commercial Synthetics, Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, California 91320-1799, United States
3. Departamento de Química Fundamental, Universidade Federal de Pernambuco, Avenida Professor Moraes Rego, 1235, Cidade Universitária, 50670-901 Recife, PE, Brazil.

Key drivers that facilitates the utilization of benchtop nuclear magnetic resonance (NMR) spectrometers for a wide-range of analytical applications in the pharmaceutical, agrochemical and battery industries, analogous to applications with mass spectrometry, high performance liquid chromatography and infrared/ultraviolet spectroscopy, include features such as compactness, mobility and low maintenance costs. Benchtop NMR spectrometers are especially useful in the chemistry laboratory for online or atline reaction monitoring processes and could potentially play a role in sterochemistry control strategy for some chemical reactions in biophamaceutical production plants. However, the ever-expanding complexities of molecular scaffolds being generated in the pharmaceutical industry that require high spectral resolution and/or complex NMR experiments, which are not currently available in the benchtop NMR software, may be limiting. The recently developed high temperature superconducting (HTS) magnet technology, which provides compact power-driven cryogen-free magnets, potentially bridges this gap [1-3]. The compact nature of the HTS magnet allowed the incoporation of a 400 MHz spectrometer into a chemistry laboratory fumehood, thus opening up a myriad of possibilities for both chemists and NMR spectroscopists [3-7]. The benefits derivable with this high field NMR spectrometer setup (in a benchtop setting), will be showcased with examples from reaction monitoring and structure elucidation projects. In particular, we will highlight our work on the application of anisotropic NMR data, acquired on the 400 MHz HTS magnet spectrometer, for stereochemical analysis of complex macrocycles, including AMG 176 [8].

1. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Magn. Reson. Chem*. 2018, 56 (9), 817-825. DOI: 10.1002/mrc.4740
2. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Instr. Sci. Technol.* 2019, 47 (2), 195-212. DOI: 10.1080/10739149.2018.1516226
3. Silva Elipe, MV, Cherney, A., Krull, R., Donovan, N. Tedrow, J., Pooke, D., Colson, K.L., *Org. Process Res. Dev.* 2020, 24 (8), 1428-1434. DOI: 10.1021/acs.oprd.0c00125

4. Murray J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Quasdorf, K., Caille, S., *Org. Process Res. Dev.* 2020, 24 (8), 1523-1530. DOI: 10.1021/acs.oprd.0c00256
5. Murray, J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Brown, D., Quasdorf, K., Caille, S., *J. Org. Chem.*, 2021. DOI: doi.org/10.1021/acs.joc.1c00886
6. Murray, J.I., Brown, D., Silva Elipe, M.V., Caille, S., *Org. Process Res. Dev.*, 2021. DOI: 10.1021/acs.oprd.1c00131
7. Quasdorf, K.W., Murray, J.I., Nguyen, H., Silva Elipe, M.V., Ericson, A., Kircher, E., Guan, L., Caille, S., *Org. Process Res. Dev.*, 26, 458-466, 2022. DOI: 10.1021/acs.oprd.1c00469
8. Caenepeel S *et. al.*, *Cancer Discov*. 2018 Dec;8(12):1582-1597. doi: 10.1158/2159-8290.CD-18-0387. Erratum in: Cancer Discov. 2019 Jul;9(7):980. PMID: 30254093.

28 Application of Benchtop NMR as a Chemical Detector for Pharmaceutical Development

Tristan Maschmeyer

Genentech Inc.

The evolution of benchtop NMR instrumentation has paved the way for the deployment of NMR systems in the chemical laboratory. Still, a wider adoption of benchtop NMR for routine reaction monitoring has been hampered by known limitations including low sensitivity and reduced signal dispersion. This presentation summarizes our efforts to normalize the use of benchtop NMR for reaction monitoring, with the direct comparison of monitoring such processes with high-field instrumentation. This includes monitoring relatively simple processes, such as the dechlorination of 1,3-dichloro-5,5-dimethyl hydantoin and the hydrolysis of 1,1'-carbonyldiimidazole (CDI), to synthetically relevant transformations, such as the activation of carboxylic acid with CDI and subsequent reaction with an amine to form the corresponding amide. Potential opportunities and challenges for the implementation of at-line NMR-based reaction monitoring will be discussed.

W1 Computation of Chemical Shifts and Couplings

Workshop coordinated by

Robbie Iuliucci, Washington and Jefferson College
Andrei Kutateladze, University of Denver

Computational chemistry has always been a key part of the NMR toolbox. Predicting NMR parameters provides a molecular interpretation of the spectral data, assists in analyzing NMR spectra, and helps to guide the process of structure elucidation. This workshop on computational advances will be separated into solid and liquid phase discussions to address their particular features.

The growth in applications of NMR Crystallography, which can elucidate solid phase structure when traditional diffraction methods fall short and is finding wide utility in scientific fields today, has spawned an increased interest in accurate prediction of the chemical shielding tensor. Inclusion of the lattice structure is pertinent to the reliability of such calculations and common methodologies to incorporate the lattice structure, periodic boundary conditions and extended cluster models, will be central to this workshop. Due to the efficiencies of Density Functional Theory (DFT) to handle larger systems accurately, DFT has become the electronic method of choice. Strategies for efficient DFT approaches that incorporate the lattice environment will be emphasized.

The solid phase component will cover topics such as
1. Promoting and handling anisotropy of the chemical shift tensor
2. Removing systematic errors through least squares regression
3. Reliable geometry input parameters
4. Consideration of DFT planewave parameters
5. Using cluster models to climb Jacob's Ladder of DFT functionals

The solution phase component will focus on DFT methods augmented by parametric corrections of computed data to expedite computations and improve the accuracy. The most recent machine learning-augmented DFT method, DU8ML, will be highlighted.

1. Computations of nuclear spin-spin coupling constants
2. Computations of chemical shifts
3. Challenging structure elements and features of PES (intramolecular H-bonding, shallow PES, etc.)
4. PCM and solvents

W2 qNMR and the Shocking Truth About Integration

Workshop coordinated by

Frank Delaglio: NIST IBBR, Principal Investigator
Gennady Khirich: Merck, Associate Principal Scientist

Abstract: The most common method of extracting quantitative information from NMR data relies on knowledge of peak integrals... but how reliable is that knowledge? We explore the effects of spectral processing on integration, including those of zero filling, random noise, and the mysterious scaling of the first point in the time domain. We also explore quantification in the time domain vs frequency domain, and the influence of non-uniform sampling for quantification of higher-dimensional data. We hope that by attending this workshop you will have a new-found appreciation for the subtleties and nuances involved in extracting accurate and precise quantitative information from NMR measurements, and that the shocking truth about integration helps avoid potential pitfalls in your quantitative workflows. To supplement our lessons, we will conduct the first SMASH QUANTIFICATION CHALLENGE (SMASHQUAC), where attendees will be supplied with raw data that they can quantify by any means they prefer.

SMASH 2022

POSTERS

1 Quantitative ^{19}F NMR Analysis of Carbonyl Groups in Bio-oils using Low-field (Benchtop) NMR

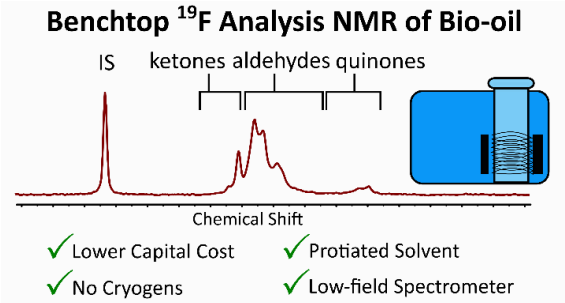
Bridget Tang[1], Katie Chong[2], and Robert Evans[1]

1. Aston Institute of Materials Research, Aston University, Birmingham, UK
2. European Bioenergy Research Institute (EBRI), Aston University, Birmingham, UK

Fast-pyrolysis bio-oil produced from lignocellulose biomass is believed to one of the most economically feasible carbon-neutral energy sources for replacing fossil fuel oils [1]. The main disadvantage of bio-oils, which makes them incompatible with current infrastructures, is their acidity [2]. Bio-oil is made up of hundreds of small organic molecules. Oxygen-containing compounds, such as aldehydes, ketones and quinones, are the main contributors to a fuel's acidity.

Nuclear Magnetic Resonance (NMR) spectroscopy is a versatile tool that has been widely used to analyse complex mixtures. However, 1H NMR analysis of bio-oil samples is limited by severe overlap of the signals of the many species present in the sample. The use of other nuclei, such as 19F, offers a promising alternative as their spectra are often much sparser. By derivatising the bio-oil sample with a fluorine-containing compound, e.g. 4-(trifluoromethyl)-phenylhydrazine, 19F NMR provides quantitative information about the carbonyl-containing compounds present [3].

The technique has been translated from traditional high-field NMR to benchtop, or low field, NMR. The aldehyde and ketone content of bio-oil produced from various biomass feedstocks has been successfully analysed using benchtop 19F NMR. These measurements were verified using both an oximation titration and elemental analysis. Benchtop NMR offers a low-maintenance, low-cost alternative to traditional NMR spectroscopy [4]. Compared to high-field spectrometers, benchtop equipment is cheaper, smaller, uses weaker magnets that require no cryogens and samples do not require deuterated solvents. This makes NMR methods more accessible for a wide range of users, from industry to academic institutions.



1. Park, J.Y., Kim, J.K., Oh, C.H., Park, J.W. and Kwon, E.E., Journal of Environmental Management, 234, pp.138-144. 2019
2. Bertero, M., de la Puente, G. and Sedran, U., Fuel, 95, pp.263-271. 2012
3. Huang, F., Pan, S., Pu, Y., Ben, H. and Ragauskas, A.J., Rsc Advances, 4(34), pp.17743-17747. 2014
4. Gracia-Vitoria, J., Rubens, M., Feghali, E., Adriaensens, P., Vanbroekhoven, K. and Vendamme, R., Industrial Crops and Products, 176, p.114405. 2022

2 Synergy of NMR, Computation and Synthesis for Novel PROTAC Linker Design & Hydrophobic Collapse Study

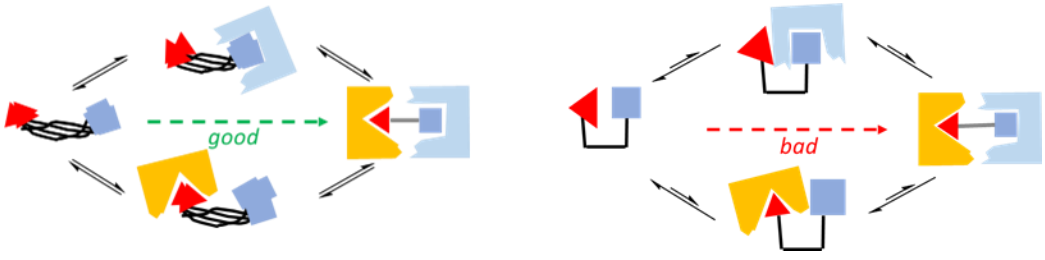
Hao Lan[1], Oliver Hsia[2], Alessio Ciulli[2], Craig Butts[1] and Varinder Aggarwal[1]

1. Chemical Synthesis CDT, School of Chemistry, University of Bristol, UK
2. CeTPD, School of Life Sciences, University of Dundee, Scotland, UK

The linker design is a core task in drug discovery for Proteolysis Targeting Chimera (PROTACs), which influences formation of ternary complex for selective protein poly-ubiquitination and degradation as well as physicochemical properties. Herein, we design, synthesize and characterize a novel linker chain under certain conformational control, which is applied for the development of VHL E3-ligase based Bromodomain degrader.

Through state-of-the-art solution state NMR experiment and multiscale molecular simulation, we elucidated chameleon behavior of few synthetic PROTAC molecules which effectively guided and predicted their membrane permabilities for intracellular protein degradation.[1] The severe problem of hydrophobic collapse was also revealed for a non-covalent PROTAC bearing rigid linker in both aqueous solution and binary binding states.[2]

All small-molecule NMR and computational studies were consistent with Bromodomain degradation activities observed from cellular assay so far. More biophysical assays and biological NMR experiments are in progress to correlate such aggregation behavior against required cooperativity of ternary complex formation. Our research has demonstrated that increase of linker rigidity does not necessarily improve degrader efficiency for BET-VHL system, which would enlighten future research for PROTAC linker design.



1. Yoseph Atilaw, Vasanthanathan Poongavanam, Caroline Svensson Nilsson, Duy Nguyen, Anja Giese, Daniel Meibom, Mate Erdelyi, and Jan Kihlberg, ACS Medicinal Chemistry Letters, 12(1), 107-114, 2021.
2. Dhanushka Weerakoon, Rodrigo J. Carbajo, Leonardo De Maria, Christian Tyrchan, and Hongtao Zhao, Journal of Chemical Information and Modeling, 62(2), 340-349, 2022.

3 Fast and Accurate Diffusion NMR Acquisition in Continuous Flow

Isabel A. Thomlinson,[1,2,3] Matthew G. Davidson,[1,3] **Catherine L. Lyall,**[2,3] John P. Lowe,[2,3] and Ulrich Hintermair[1,2,3]

1. Centre for Sustainable and Circular Technologies, University of Bath, Bath, UK.
2. Dynamic Reaction Monitoring Facility, University of Bath, Bath, UK.
3. Department of Chemistry, University of Bath, Bath, UK

Diffusion-ordered spectroscopy (DOSY) is a valuable technique for measuring diffusion coefficients of molecules in solution. It finds applications in solving many chemical problems including identifying individual components in a complex mixture,[1] observing solution-state aggregation of molecules,[2] and estimating molecular weights of small and large molecules.[3]

The FlowNMR technique makes use of a specially designed flow tube to continuously flow reaction mixture from an external reaction vessel through the spectrometer probe.[4] This allows various NMR experiments to be performed directly on the reaction mixture with high data density throughout the reaction, giving access to detailed and accurate chemical information under representative reaction conditions.

In the past, DOSY has always been performed on static samples rather than in a FlowNMR regime, likely due to the complicating effects of flow on sensitive diffusion measurements. In this work, effective techniques for obtaining accurate diffusion coefficients on flowing samples have been developed. The effects of acquisition parameters and hardware choices on the quality of DOSY data for a solution of five non-interacting small molecules in chloroform have been investigated. Based on these results, recommended settings for obtaining good quality FlowDOSY data have been derived. A convection compensated pulse sequence is essential for obtaining meaningful data, and is effective up to at least 4 mL min^{-1}. Flow corrections can be used to obtain static diffusion coefficients from FlowDOSY data. Most excitingly, the effect of flow on the measured diffusion coefficient has been almost entirely eliminated by reducing pump pulsation to <1% with the use of a rotary multi-piston pump. These results show flow effects in DOSY measurements of continuously moving samples are mostly a reflection of non-ideal flow such as turbulences and eddies, and convection compensation with suitable acquisition parameters can effectively deal with constant, linear sample displacement to directly yield accurate and precise molecular diffusion values at flow rates of at least up to 4 mL min^{-1}.

This work adds DOSY to the toolbox of FlowNMR techniques that can be used to obtain detailed information on reacting systems in real time. The use of FlowDOSY for reaction monitoring has been demonstrated for monitoring reactions including the ring-opening polymerisation (ROP) of lactide with a Zr amine trisphenolate initiator. Online DOSY measurements were shown to complement other reaction monitoring techniques to give rich information on the reacting system in real time.

1. Li, D., Hopson, R., Li, W., Liu, J., & Williard, *Org. Lett.*, **10**, 909–911, 2008.
2. Li, D., Keresztes, I., Hopson, R., & Williard, P. G., *Acc. Chem. Res.*, **42**, 270–280, 2009.
3. Evans, R., Dal Poggetto, G., Nilsson, M., & Morris, G. A., *Anal. Chem.*, **90**, 3987–3994, 2018.
4. Hall, A. M. R., Chouler, J. C., Codina, A., Gierth, P. T., Lowe, J. P., & Hintermair, U., *Catal. Sci. Technol.*, **6**, 8406–8417, 2016.

4 19F qNMR in Human Absorption, Distribution, Metabolism and Excretion Studies of Nirmatrelvir: A Faster Route to Emergency Use Authorization.

Gregory S. Walker[1], Ravi Shankar P. Singh[2], Eugene P. Kadar[1], Loretta M. Cox[1], Heather Eng[1], Raman Sharma[1], Amit S. Kalgutkar[2], and R. Scott Obach[1]

1. Pfizer Worldwide Research, Development & Medical; Groton, CT 06340, USA,
2. Pfizer Worldwide Research, Development & Medical; Cambridge, MA 02139, USA

PAXLOVID™ (nirmatrelvir[PF-07321332]/ritonavir - 300/100 mg) is the first oral SARS-CoV-2 main protease inhibitor to receive emergency use authorization (EUA) for the treatment of COVID-19 in patients with high risk of progression to severe disease. Because of the urgent need for new medicines to address the COVID pandemic, innovative approaches had to be employed to accelerate the development of PAXLOVID™. An overall understanding of the disposition of a compound in humans is a requirement for registration of any new medicine and is almost always done through an absorption, distribution, metabolism and excretion (ADME) study. Historically, ADME data has been obtained via the administration of a radiolabeled (typically 14C) analog of the parent compound with subsequent collection of excreta and plasma. The radiolabel is used because of the low inherent background and its universal analytical response factor. The synthesis of 14C analogs can often be costly and more importantly time consuming. Following the administration of 14C labeled drug, excreta and plasma samples are then analyzed for total drug content by liquid scintillation counting (LSC) to establish mass balance. Additionally, separately pooled samples of excreta and plasma are profiled for metabolites by liquid chromatography/mass spectrometry (LC/MS). In conjunction with the LC/MS analysis fractions are collected and analyzed by LSC to create a radiochromatogram.

To support the accelerated development of PAXLOVID™, synthesis of [14C]nirmatrelvir for a conventional human ADME study was not feasible. Hence, alternative detection methods for the ADME study were evaluated. Because 19F qNMR affords the same type of universal response, independent of chemotype, as radioactivity and that nirmatrelvir contains a trifluoroacetamide, 19F qNMR was used as a detection technique for the human ADME study. Using 19F qNMR we were able to assess the metabolism and excretion of nirmatrelvir within the first-in-human study following the administration of a single dose of unlabeled drug. Mass balance was achieved with approximately 84.9 ± 8.9% of the total administered dose recovered (urine 47.0% and feces 33.9%). Nirmatrelvir was the only drug-related entity observed in plasma. These data were accepted by the FDA for the EUA of PAXLOVID™. To the best of our knowledge, this is the first time a regulatory agency agreed to the use of 19F-qNMR data in lieu of a 14C study for ADME characterization. Notably, because of the 19F qNMR approach, the human ADME data was delivered much sooner to regulatory agencies and facilitated a rapid approval of a critical medicine that is helping address the worldwide COVID pandemic.

5 Combining NMR and Computational Methods to Interrogate Rapamycin's Mysterious Minor Conformer

Emily B. Crull[1], Paul Hawkins[2], Ann E. Cleeves[3], Ajay N. Jain[3], Edmund I. Graziani[2], and R. Thomas Williamson[1]

1. University of North Carolina – Wilmington, Wilmington, NC, US
2. Apertor Pharmaceuticals Inc., South San Francisco, CA, US
3. Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA, US

Appearing in over 56,000 journal articles and more than 7,000 patents, rapamycin was originally isolated from an Easter Island soil sample in 1975.[1] Rapamycin is a potent immunosuppressant and antiproliferative, giving rise to its use for the treatment of organ transplants and cancer, respectively.[2] One of the first examples of a "molecular glue", the pipecolic acid region of rapamycin binds to FK-506 binding protein 12 (FKBP12), while the triene region binds to mammalian target of rapamycin (mTOR), bringing the two proteins together and disrupting the downstream signaling of the latter.[2] Inspired by the recent burgeoning interest in the application of rapamycin to the treatment of other therapeutic indications, there is renewed motivation to better understand the molecular dynamics of rapamycin in order to guide new drug discovery and design efforts.

Rapamycin has been shown by NMR studies to adopt two interconverting conformers, appearing in a 9:1 ratio in DMSO-d_6.[3,4] However, only the major conformer has been characterized, having been crystallized in both free and FKBP12-bound states as the *trans* amide.[3] It has been generally accepted that the minor conformer is the *cis* amide by comparison to a structurally related compound, FK506.[3] To our knowledge, no evidence has been published to conclusively identify the true nature of the minor conformer of rapamycin. This presentation will describe how our group has used NMR studies and computational chemistry to probe and unequivocally define the structural identity of this heretofore under characterized species.

1. Vézina, C.; Kudelski, A., *J. Antibiot. (Tokyo).*, 28(10), 721–726, 1975.
2. Li, J.; Kim, S. G.; Blenis, *J. Cell Metab*., 19(3), 373–379, 2014.
3. Kessler, H.; Haessner, R.; Schüler, W., *Helv. Chim. Acta*, 76(1), 117–130, 1993.
4. Pagano, T. G., *Magn. Reson. Chem*., 43(2), 174–175, 2005.

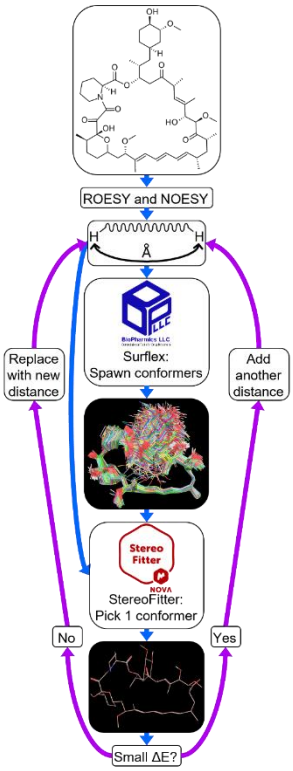


Figure 1 Flow chart of the developed method from starting structure to single 3D conformer

6 NMR-Based in situ Mixing Device: Unravelling the Mechanism of Phase-Transfer Catalysed Reactions

Yuan Gao,[1] Andrew M. R. Hall,[1] Francesco Ibba,[2] Véronique Gouverneur,[2] and Guy C. Lloyd-Jones[1]

1. School of Chemistry, University of Edinburgh, Edinburgh, Midlothian, U.K.
2. Chemistry Research Laboratory, University of Oxford, Oxfordshire, U.K.

Phase-transfer catalysis (PTC) enormously enhances the reaction rate between reagents located in immiscible phases. The main advantages of PTC are mild reaction conditions, inexpensive reagents, and simple work-up, which lead to the possibility of large-scale production, and make PTC appealing to industrial applications [1]. However, due to the inherent challenges associated with monitoring a heterogeneous system in situ, there is a lack of mechanistic investigation on PTC and most of the efficient phase-transfer catalysts have been developed by trial and error, which restricts their applications to a small range of substrates.

To address this, our group have designed and developed an NMR-based *in situ* mixing device, which is portable and can be easily coupled to any NMR spectrometer to enable *in situ* reaction monitoring of heterogeneous reaction that requires agitation (**Figure 1a**). To test the robustness of this device, we have applied it for monitoring of a phase-transfer catalysed asymmetric fluorination developed by the Gouverneur group [2] (**Figure 1b**). As shown in **Figure 1c**, the kinetic data collected from conventional *ex situ* monitoring method and with our *in situ* device are in excellent agreement. The concentration-time profiles, combined with kinetic modelling, $\{^1H\}^{19}F$ NMR, and ESI-MS, enables us to propose a reaction mechanism, which could potentially guide further improvement of the current catalyst. We speculate that further development of the mixing device will promote mechanistic investigations of PTC reactions in general.

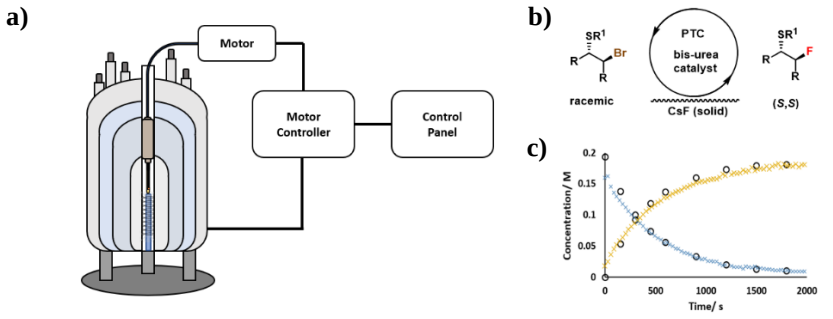


Figure 2: a) A schematic of the NMR-based in situ monitoring system. b) Phase-transfer catalyzed asymmetric fluorination. c) Representative concentration-time profiles from ex situ (dot) and in situ (cross) reaction monitoring.

1. Tan J. and Yasuda N., Org. Process Res. Dev., 19, 1731–1746, 2015.
2. Pupo G., Ibba F., Ascough D. M. H., Vicini A. C., Ricci P., Christensen K. E., Pfeifer L., Morphy J. R., Brown J. M., Paton R. S., and Gouverneur V., Science, 360, 638–642, 2018.

7 Ultra-high resolution NMR analysis of fluorine-containing systems

Coral Mycroft, Mathias Nilsson, Gareth A. Morris and Laura Castañar

Department of Chemistry, University of Manchester, Manchester, M13 9PL, United Kingdom

NMR measurements on compounds containing fluorine-19 are becoming increasingly widespread due to their importance in medicinal and biological chemistry. NMR provides crucial information on molecular structure and conformation, but in systems showing both homonuclear and heteronuclear couplings, this information is often difficult to extract due to signal overlap. Spectral resolution can be improved via the use of pure shift ^{1}H NMR[1], which suppresses the effects of homonuclear couplings, reducing spectral overlap by collapsing multiplet structures into singlets. However, in complex fluorinated systems, key structural information can still be difficult to extract as the effects of heteronuclear couplings remain. In these systems, FESTA[2–4], a class of heteronuclear spectral editing methods, can be used to alleviate signal overlap. FESTA methods provide ^{1}H subspectra for individual spin systems that contain a fluorine, aiding the extraction of valuable structural and conformational information. However, signal multiplicity can still hinder analysis in the presence of signal overlap.

Here, we present a collection of new NMR experiments that aid the structural analysis of complex fluorinated systems. To unambiguously extract chemical shift information, we present two general pure shift methods for the acquisition of ^{1}H and ^{19}F that simultaneously suppress both homonuclear and heteronuclear couplings, giving a fully pure shift ^{1}H NMR spectrum containing chemical shift information only (Figure 1c in red). These methods tolerate the broad chemical shift range of ^{19}F, and have been shown to suppress a wide range of J_{HX} couplings, with no increase in experiment time when compared to conventional pure shift methods. In complex spectra, even fully pure shift methods can suffer from signal overlap. We also show how combining the advantages of pure shift and FESTA methods allows further spectral simplification, giving an individual ^{1}H pure shift spectrum for each fluorine-containing spin system. The benefits of these new methods will be demonstrated in the analysis of complex fluorinated mixtures of chemical and biological interest, highlighting how these methods can be used to aid the analysis of even the most complex systems.

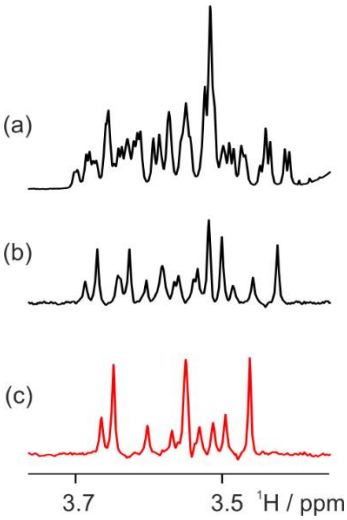


Figure 1: (a) Conventional, (b) pure shift, and (c) ^{19}F decoupled pure shift ^{1}H NMR spectra of a fluoroproline mixture, showing a region of severe overlap.

1. Castañar, L. *Magn. Reson. Chem.* 55 (1), 47–53, 2017.
2. Castañar, L.; Moutzouri, P.; Barbosa, T. M.; Tormena, C. F.; Rittner, R.; Phillips, A. R.; Coombes, S. R.; Nilsson, M.; Morris, G. A. *Anal. Chem.* 90 (8), 5445–5450, 2018.
3. Barbosa, M.; Castañar, L.; Moutzouri, P.; Nilsson, M.; Morris, G. A.; Rittner, R.; Tormena, C. F. *Anal. Chem.* 92 (92), 2224–2228, 2020.
4. Dal Poggetto, G.; Soares, J. V.; Tormena, C. F. *Anal. Chem.* 92 (20), 14047–14053, 2020.

8 IMPRESSION Generation 2 - Machine Learning for NMR Prediction

Chun Yee Calvin Yiu, Will Gerrard, Craig Butts

University of Bristol

NMR is a staple method in elucidating chemical structure, the application of machine learning prediction to this field could replace expensive quantum chemical NMR calculations. Our recently reported machine learning system, IMPRESSION[1,2] used kernel ridge regression to pioneer 3D NMR solution-state parameter prediction on molecules containing H/C/N/O atomic environments.

Herein we present IMPRESSION generation 2, a deep learning model utilising a graph transformer network. Capable of predicting multiple NMR parameters simulataneously (both chemical shifts and J-coupling values), and the capacity to handle a wider range of chemical environments (H/C/N/O/F/S/P/Si/Br atom containing molecules). Alongside an increase in training data, sampled from ChEMBL[3] & OTAVA, IMPRESSION-GEN2 shows a substantial improvement in prediction accuracy over its previous iteration, with mean absolute errors of 0.13 ppm for $\delta^{1}H$, 1.4 ppm for $\delta^{13}C$, 6.2 ppm for $\delta^{15}N$, 0.31 Hz for $^{3}J_{CH}$, 0.39 Hz for $^{3}J_{CC}$, and 0.21 Hz for $^{3}J_{HH}$. We believe the combination of improved accuracy, greater coverage of chemical space and incorporation of multiple bond coupling constants makes IMPRESSION the most versatile and accurate machine learning system available for 3D NMR parameter prediction.

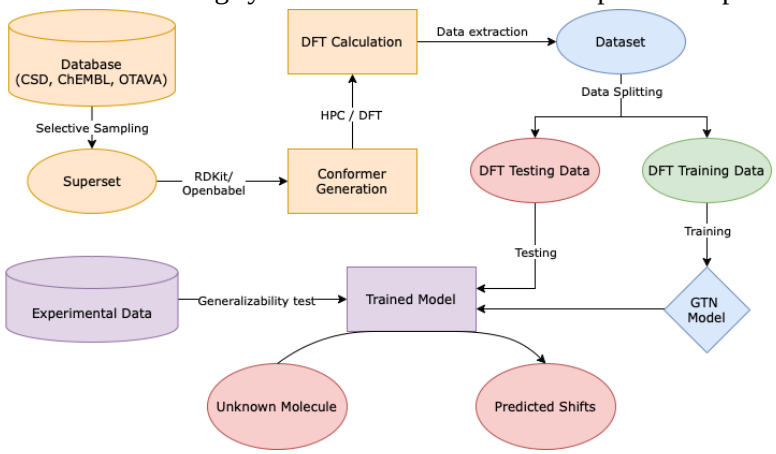


Figure 3 Pipeline of IMPRESSION generation 2

1. Gerrard Will, Bratholm Lars A, Packer Martin J, Mulholland Adrian J, Glowacki David R, Butts Craig P, *Chemical science*, 11(2), 508–515, 2020.
2. Gerrard, Will, Calvin Yiu, and Craig P. Butts. *Magnetic Resonance in Chemistry*, 2021.
3. Gaulton A, Hersey A, Nowotka M, Bento AP, Chambers J, Mendez D, Mutowo P, Atkinson F, Bellis LJ, Cibrián-Uhalte E, Davies M, Dedman N, Karlsson A, Magariños MP, Overington JP, Papadatos G, Smit I, Leach AR. *Nucleic Acids Res*., 45(D1) D945-D954, 2019.

9 High-Way or Bi-Way – New Stopped-flow NMR Methods

Ran Wei, Dušan Uhrín and Guy C. Lloyd-Jones

School of Chemistry, University of Edinburgh, UK

NMR spectroscopic analysis of chemical reactions remains an essential tool, as it offers a wealth of quantitative structural information and a high degree of spectral resolution [1]. Nevertheless, *in-situ* monitoring of rapid irreversible reactions that are initiated by mixing, poses challenges to traditional NMR experiments, therefore requires specialised techniques.

A new stopped-flow apparatus [2] was designed to allow the capture of the fleeting information from irreversible reactions at the millisecond timescale. Its adaption to standard NMR probes in obtaining qualitative and quantitatve kinetic data are discussed. With the aid of this technique, we are able to study evolving reactions that occur beyond the measurement deadtime of traditional NMR experiments. Here an irreversible reaction of $t_{1/2}$ = 45 ms was used as an example to demonstrate the monitoring of rapid irreversible reactions by interleaved ^{19}F stopped-flow NMR spectroscopy. Furthermore, by utilising the un-premagnetised channel of the stopped-flow instrument, chemical exchange studies can be carried out without the necessities of isotopic labelling; or when the exchanging species are too short-living to be analysed by traditional methods.

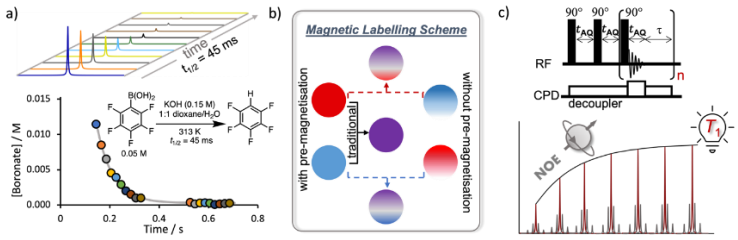


Figure 1. a) ^{19}F stopped-flow NMR spectroscopy to monitor the protodeboronation of penta-fluorophenyl boronic acid. b) chemical exchange studies by the un-premagnetised channel of the stopped-flow instrument. c) the FLOPS/FLAPS method for rapid T_1 measurement by utilising heteronuclear decoupling.

At the same time, rapid T_1 estimation methods, termed FLOPS/FLAPS (Fast Longitudinal relaxation by Ordered/Amplified Progressive Saturation) were developed following a previous method FLIPS [3]. By applying heteronuclear decoupling throughout the measurements, FLAPS offers an order of magnitude improvement in efficiency comparing to the inversion recovery method.

1. Lloyd-Jones, Guy C. *et al.*, *Prog. Nucl. Magn. Reson. Spectrosc.*, 129, 28-106, 2022.
2. Wei, Ran; Hall, Andrew M. R.; Behrens Richard; Pritchard, Mark S.; King, Edward J.; Lloyd-Jones, Guy C., *Eur. J. Org. Chem.*, 2021, 2331-2342, 2021.
3. Wei, Ran; Dickson, Claire L.; Uhrín, Dušan; Lloyd-Jones, Guy C., *J. Org. Chem.*, 2021, 9023-9029, 86.

10 Faster quantitative 13C and lower power in chemical shift scaling experiments with EXACT and Semi-Real Time acquisition methods

Zahra Al-Aasmi, 1 Alexandra Shchukina, 2 Craig P. Butts1

1. University of Bristol, School of Chemistry, Cantocks Close, Bristol, BS8 1TS
2. Centre of New Technologies, University of Warsaw, Banacha, 2C, 02-097, Warsaw, Poland

We demonstrate here the substantial acceleration of quantitative ^{13}C NMR experiments and reduced power demands in chemical shift scaling experiments as two new applications of the related EXACT (EXtended ACquisition Time) [1] and SRT [2] (Semi-Real Time) acquisition methods.
Firstly: $^{13}C\{1H\}$ NMR spectra can be acquired in roughly 50-60% of the time by reducing the need for long relaxation delays arising from Nuclear Overhauser Effect (NOE) build-up during the heteronuclear spin decoupling (Figure 1).

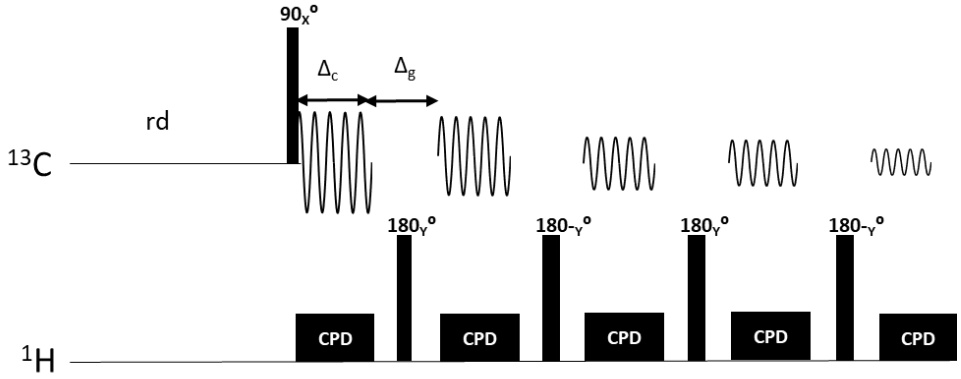


Figure1: EXACT acquisition on ^{13}C channel (WALTZ ^{1}H decoupling during the chunk acquisition and a single 180° refocusing pulse during the gaps).

Secondly: We approximately halve the high-power demands of real-time chemical shift scaling experiments which use high radiofrequency power from large numbers of 180° refocusing pulses being applied between every datapoint [3]. SRT and EXACT allow us to skip every second data point and thus spread the RF pulses over a longer period.

1. E. Ndukwe, A. Shchukina, K. Kazimierczuk, C. Cobas, and C. P. Butts, ChemPhysChem, 2016, 17, 2799-2803.
2. P. Kiraly, M. Nilsson, and G. A. Morris, 2018, J. Magn. Reson, 293, 19-27.
3. G.A. Morris, P. Niel, L. Lu-Yun, Angew., 2003, Chem. Int. Ed., 42, 823-825.

11 Solid-State DP4: A Tool for Spectral Assignment for Molecular Organic Solids

Caitlin L. Evans and Paul Hodgkinson

Durham University, Department of Chemistry, Durham, County Durham, UK

"NMR crystallography"[1] is now commonly used to denote the combined use of solid-state NMR data with first principles calculation to understand structure and dynamics in solid materials. An essential step of any NMR crystallographic study is the assignment of spectral features to crystallographic sites. It is then possible, for example, to validate structural models[2]. Assignment of solid-state NMR spectra, however, is not always straightforward, with overlap being a key limitation; in the literature, many assignments have had to be revisited[1]. Overlap also limits the usefulness of metrics, such as the root-mean-square deviation (RMSD) between calculated and experimental shifts, since the RMSD will not be significantly affected by mis-assignment of overlapped signals. The limitations of simple 1D spectra can be mitigated by spectral editing and 2D experiments, but it rapidly becomes difficult to combine this information in a coherent and reproducible manner. Hence, quantitative measures of the overall agreement of an assignment with all the available NMR data are desirable.

As part of efforts to establish robust protocols for spectral assignment of molecular organic solids, we have been developing a Bayesian probability-based framework, ssDP4, adapted from the seminal work of DP4 by Smith and Goodman[3]. As well as considering agreement between experimental and calculated chemical shifts, namely ^{13}C and ^{1}H, additional data from spectral editing and 2D experiments can be incorporated to assess the quality of a proposed experimental assignment. A similar approach has been proposed by Emlsey and co-workers for assignment of spectral data to computationally generated structures[4,5]. The proposed semi-automated approach is particularly helpful in regions of overlap, since the number of potential assignments becomes difficult to manage using more "manual" approaches. ssDP4 allows the information added by each experiment to be directly evaluated. We anticipate that ssDP4 and related approaches will help to deliver robust and reproducible assignments for solid-state NMR, especially in the context of NMR crystallography.

1. Hodgkinson, P., Prog. Nucl. Magn. Reson. Spectrosc., 118-119, 10-53, 2020
2. Evans, C. L., Evans, I. R., and Hodgkinson, P., Chem. Commun., 58, 4767-4770, 2022
3. Smith, S. G., and Goodman, J. M., J. Am. Chem. Soc., 132, 12946-12959, 2010
4. Engel, E. A., Anelli, A., Hofstetter, A., Paruzzo, F., Emsley L., and Ceriotti, M., Phys. Chem. Chem. Phys., 21, 23385-23400, 2019
5. Cordova, M., Balodis, M., Simões de Almeida, B., Ceriotti, M., and Emsley, L., Sci. Adv., 7, 1-10, 2021

12 A Faster Way to Interpret NMR in 3D Structure Elucidation

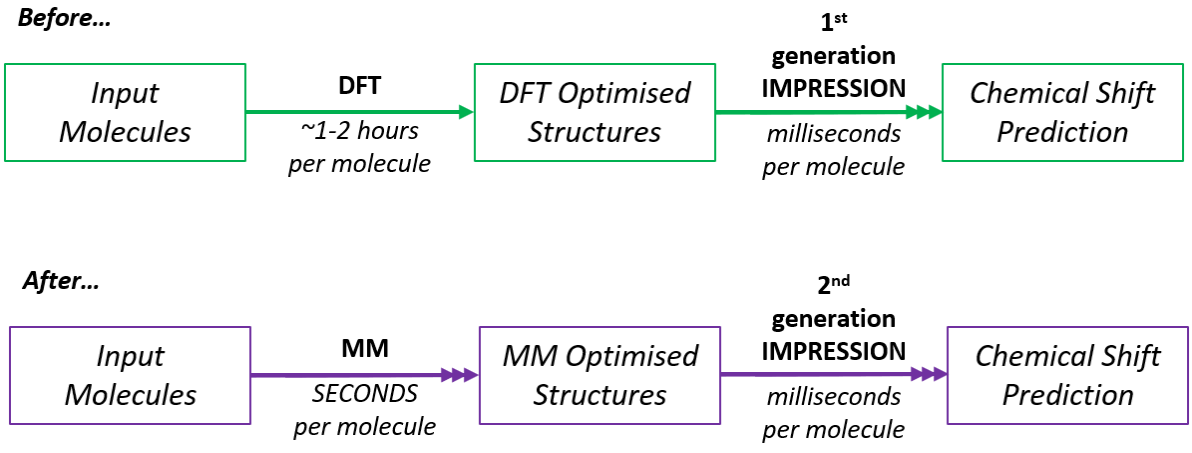
Ben Honoré

University of Bristol, School of Chemistry, Cantocks Close, Bristol, BS8 1TS

NMR spectra can be difficult to assign and ambiguous when it comes 3D structure, i.e., diastereomers or conformation. Therefore, it is useful to have an idea of how the spectrum of a target molecule ought to look beyond a chemist's rough estimation. Currently high-level density functional theory (DFT) calculations are the most accurate way to predict NMR parameters but are computationally intensive for large datasets.

We published IMPRESSION[1] in 2020 as a machine learning alternative to DFT calculations. By training a kernel ridge regression model on a dataset of DFT-calculated chemical shifts, IMPRESSION was able to predict shifts in milliseconds for new molecules to approaching DFT-level accuracy but required DFT-quality structures to do so, taking several hours to generate each input structure.

Here we present a 2nd generation of IMPRESSION which uses a neural network architecture, trains on much larger datasets and tests on molecular mechanics (MM) optimised structures. The resulting predictions are at least as good as 1st generation IMPRESSION and require only seconds per molecule.



1. W. Gerrard, L. A. Bratholm, M. Packer, A. J. Mulholland, D. R. Glowacki and C. P. Butts, Chem Sci, 2020, 11, 508-515.

13 Quantitative Interpretation of Molecular Diffusion Coefficients – From Big to Small

Robert Evans

Aston University, Chemical Engineering and Applied Chemistry, Birmingham, UK.

Diffusion-ordered NMR spectroscopy (DOSY) has found increasing use as an analytical tool, capable of identifying the identity of components in a mixture [1]. Measuring accurate molecular self-diffusion coefficients, *D*, by nuclear magnetic resonance (NMR) techniques has become routine as hardware, software and experimental methodologies have all improved [2]. In principle, the values of diffusion coefficient obtained carry important information about the sizes of different species and on interactions between species, but the relationship between diffusion coefficient and molecular mass can be a complex one. The quantitative interpretation of such diffusion coefficient data remains difficult, particularly for small molecules on one hand and for molecules with disperse molecular mass distributions on the other.

The most basic relationship is that established in the Stokes-Einstein equation [3], which balances the energy of the system to the friction acting on the molecules. For larger species such as polymers and proteins, power laws can be used to relate the observed *D* to the molecular weight of the species present. Such power laws need to be calibrated for each distinct chemical species present and different methods for generating such calibration curves exist. Complications arising from the analysis of more disperse systems can be handled with careful choice of how the diffusion NMR data is acquired and processed.

For small molecules, the Stokes-Einstein equation becomes increasingly less valid as the size of the solute molecules approaches that of the solvent. A modification of the Stokes-Einstein equation that can successfully predict diffusion coefficients for small molecules has been developed [4]. The breakdown of the continuum model is corrected for by a variable friction term that depends on ratio of the solute to that of the solvent. The effects of shape, flexibility, solvation and composition cannot be treated analytically without prior chemical knowledge. However, by approximating small molecules as hard spheres with an average effective density ρ_{eff}, it is possible to successfully predict the diffusion coefficients of small molecules on the basis of their molecular weight. This new model has subsequently been validated over a wider range of literature small molecule diffusion coefficients, in deuteriated, protiated and some mixed solvents, at variable temperatures, and shown to predict diffusion coefficients to within 15 % of their experimental values [5].

1. Johnson, C. S., *Prog. Nucl. Magn. Reson. Spectrosc.*, 34(3-4), 203-256, 1999.
2. Evans, R., *Prog. Nucl. Magn. Reson. Spectrosc.* 117, 33-69, 2020.
3. Einstein, A., *Ann. Phys.* 17, 549-560, 1905.
4. Evans, R., Deng, Z., Rogerson, A. K., McLachlan, A. S., Richards, J. J., Nilsson, M., and Morris, G. A., *Angew. Chemie Int. Ed.* 52(11), 3199-3202, 2013.
5. Evans, R., Dal Poggetto, G., Nilsson, M., and Morris, G. A., *Anal. Chem.*, 90(6), 3987-3994, 2018.

14 NMR Methodology for Analysis of ^{15}N-labelled Complex Mixtures

Justinas Sakas and Dušan Uhrín

EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK

The analysis and structure elucidation of complex mixtures is an arduous task as they contain hundreds or even thousands of compounds that cannot be easily separated using chromatographic techniques. Methods that bypass the customary chromatographic step and use spectroscopic 'separation' instead have been proposed. These target specific moieties present in a subset of molecules. Recent examples include structure elucidation using NMR experiments designed for compounds tagged with $-O^{13}CH_3$ [1] and fluorinated compounds [2].

In this work we introduce a suite of NMR experiments designed for ^{15}N-labelled compounds. This includes ^{1}H, ^{15}N constant-time HMQC-COSY in which proton-proton correlations are obtained between adjacent CH and ^{15}NH groups. Long-range connectivity and correlations to ^{15}N atoms are then explored using ^{1}H, ^{15}N BIRD-HMBC in order to connect molecular fragments.

The new methodology was applied for structure elucidation of compounds produced during chloramination, a water disinfection method used around the world. The addition of chloramine kills pathogens, however it also reacts with naturally dissolved organic matter (DOM) and anthropogenic contaminants to produce a variety of disinfection by-products (DBPs) (Figure 1), which are known to be toxic or carcinogenic [3]; additionally, more than 70% of chloramination DBPs are unknown [4]. Therefore, it is necessary to elucidate their structures in order to find out if they pose any health risks.

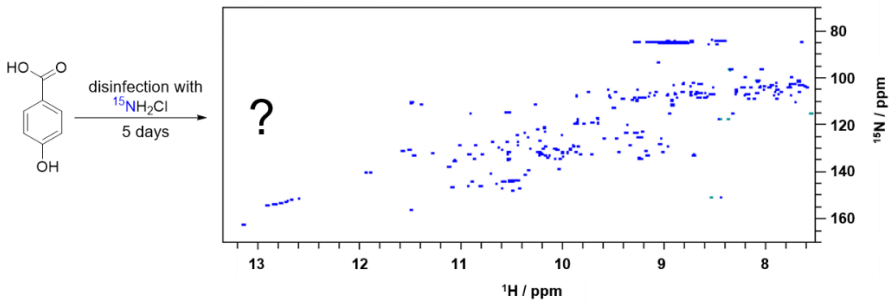


Figure 1. 2D ^{1}H, ^{15}N HSQC spectrum showing the complex mixture of nitrogen-containing compounds produced by chloramination of 4-hydroxybenzoic acid.

1. N. G. A. Bell, A. A. L. Michalchuk, J. W. T. Blackburn, M. C. Graham and D. Uhrín, *Angew. Chem. Int. Ed.*, 54, 8382–8385, 2015.
2. A. J. R. Smith, R. York, D. Uhrín and N. G. A. Bell, *Chem. Sci.*, 13, 3766–3774, 2022.
3. X. Li and W. Mitch, *Environ. Sci. Technol*., 52, 1681–1689, 2018.
4. G. Hua and D. Reckhow, *Water Res*., 41, 1667–1678, 2007.

15 Chemical Impurity Profiles of P-Fluorofentanyl using Stopped-Flow NMR and Online HPLC Analysis

Sara Guzman Castro[1], Dr. Jason Hein[1], and Richard Laing[2]

1. Chemistry Department, The University of British Columbia, Vancouver, BC, Canada.
2. Strategic Research and Science Development - Health Canada, Burnaby, BC, Canada.

In 2016, a public health emergency was declared in British Columbia (BC), Canada, due to the rapid increase in opioid-related overdose deaths [1]. Since then, the crisis has worsened due to the COVID-19 pandemic, a new record for the highest number of fatal illicit overdoses in BC per annum was set in 2021, with an alarming number of 2236 deaths, 85% of which contained fentanyl [2,3]. New fentanyl analogs, including halogenated synthetic opioids, continue to arise in the illicit drug market [4]. In January 2022, the Centers for Diseace Control and Prevention (CDC) reported an increased presence of para-fluorofentanyl in overdose deaths, and since December 2021, the BCCSU has reported an increase of street drug samples containing para-fluorofentanyl [5,6].

In the last five years, there has been an increasing interest in the identification of chemical attribution signatures of potent synthetic opioids, particularly fentanyl and its analogs, for chemical forensic purposes. Impurity profiling provides valuable information on the employed synthetic routes for illicit fentanyl production [7]. Several synthetic impurities associated with different synthetic methods of fentanyl and not-halogenated analogs have been identified. Multiple analytical technologies, alongside multivariate analysis, have been used to classify fentanyl and its main precursors, NPP and ANPP, based on the chemical impurity profiles (CIP) unique to each synthetic method [7-9]. Nevertheless, there is limited information on the impurity profiles of fluorinated fentanyl analogs such as para-fluorofentanyl and 4'-fluorofentanyl [10].

With the constantly changing illicit market, there is a need for a more targetted approach to clandestine sample analysis that takes into consideration the unfinished reactions at the point of seizure, as well as unexpected reaction conditions that lead to new impurities [11]. There is value in the quantitation of precursors and impurities during the course of a reaction for a direct comparison to illicit samples. Using Stopped-Flow benchtop NMR alongside online HPLC, a new method is proposed for the analysis of fluorinated synthetic opioids, allowing for real-time reaction monitoring and quantitation of analyte and impurities [12,13]. Potential applications of these combined methodologies include using known CIPs to determine the reaction(s) used during synthesis and determine the point at which a reaction was stopped based on impurity quantitation values.

1. Ibrahim, Mohamed, Child & Youth Services, 41 (3), 271–273, 2020
2. Sun, Yankun; Bao, Yanping; Kosten, Thomas; Strang, John; Shi, Jie; Lu, Lin, The American Journal on Addictions, 29 (3), 174–175, 2020
3. B.C. Coroners Service, 2022. Illicit Drug Toxicity Deaths in BC January 1, 2012–March 31, 2022, in: Service, B.C. (Ed.).
4. Rauf, Umaani; Ali, Majid; Dehele, Inderpal; Paudyal, Vibhu; Elnaem, Mohamed, H; Cheema, Ejaz, J Pain Res, 14, 2601–2614, 2021

5. British Columbia Centre on Substance Use. Drug Checking in British Columbia April 2022. www.drugcheckingbc.ca/dashboard/
6. Trecki, Jordan; Gerona, Roy, R; Ellison, Ross; Thomas, Chris; Mileusnic-Polchan, Darinka, MMWR Morb Mortal Wkly Rep, 71 (4), 153–155, 2022
7. Ovenden, Simon, P; McDowall, Lyndal, J; McKeown, Hugh, E; McGill, Nathan, W; Jones, Oliver, A; Pearson, James, R; Petricevic, Marija; Rogers, Michael, L; Rook, Trevor, J; Williams, Jilliarne; Webster, Renee, L; Zanatta, Shannon, D, Forensic Sci Int, 321, 110742, 2021
8. McKeown, Hugh, E; Rook, Trevor, J; Pearson, James, R; Jones, Oliver, A, Forensic Chemistry, 21, 100285, 2020
9. Casale, John, F; Hays, Patrick, A; Toske, Steven, G; Mallette, Jennifer, R, Forensic Chemistry, 17, 100203, 2020
10. Gilbert, Nicolas; Mewis, Ryan, E; Sutcliffe, Oliver, B, Forensic Chemistry, 23, 100321, 2021
11. Vandeputte, Marthe, M; Krotulski, Alex, J; Hulpia, Fabian; Van Calenbergh, Serge; Stove, Christophe, P, Journal of Analytical Toxicology, 46 (4), 350–357. 2022
12. Malig, Thomas, C; Koenig, Josh, D; Situ, Henry; Chehal, Navneet, K; Hultin, Philip, G; Hein, Jason, E, Reaction Chemistry & Engineering, 2 (3), 309–314, 2017
13. Maschmeyer, Tristan; Yunker, Lars, P; Hein, Jason, E, Reaction Chemistry & Engineering, 7 (5), 1061–1072, 2022

16 The effect of dimer formation on stereoselectivity in Brønsted acid catalysis

Maximilian Franta and Prof. Dr. Ruth M. Gschwind

University of Regensburg, Institute of Organic Chemistry, Regensburg, Bavaria, Germany

Over the last decades a huge amount of catalyst classes have been established in the field of Brønsted acid catalysis. Back in 2004, Akiyama *et al.* and Terada *et al.* have developed the BINOL (1,1´-bi-naphthol) derived chiral phosphoric acids (CPAs), which are nowadays known for their high stereoselectivity in a variety of reactions [1,2]. For example, the application of CPAs in transfer hydrogenations which was demonstrated by List *et al.*, Rueping *et al.* as well as MacMillan *et al.* [3,4,5]. Here, CPAs showcased that high yields as well as high enantioselectivities can be achieved. This reaction was later on established as a model system for theoretical calculations as well as mechanistic investigations using NMR spectroscopy by our group [6,7,8]. As catalytic transition state a ternary complex between the CPA, imine and Hantzsch ester was established. Besides this monomeric pathway, the working group of Niemeyer discvored with the transfer hydrogenation of quinolines that some CPAs can form a dimer which leads to a secondary pathway that can catalyze the reaction [9]. This pathway takes over the reaciton at increased catalyst concentrations and leads to an inversion of the enantioselectivity. Therefore, the aim of my work is to investigate, if the dimer formation also occurs with imines and if so the influence on enantioselectivity (see figure 1).

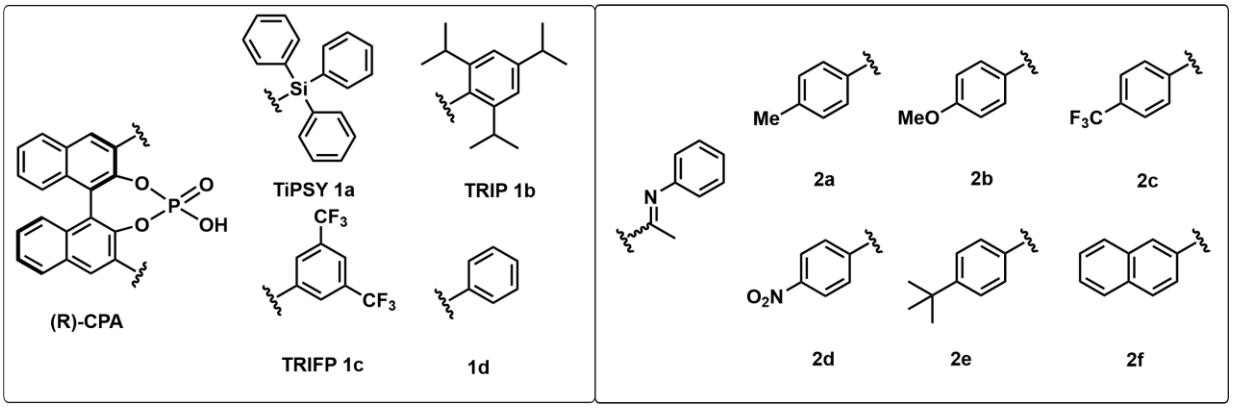


Figure 1 Structures of a few of the investigated CPAs (left) and imines (right).

First NMR investigations with a 2:1 stoichiometry of CPA to imine at 180K showed a similar hydrogen bond pattern in the ^{1}H-NMR as observed with the quinoline systems (see figure 2). During these investigations the binding pocket of the CPAs, which is determined by the size of the 3,3´-substituents on the BINOL backbone, turned out to be the key factor for dimer formation. Thereby, no dimer at all was observed with small binding pockets e.g. with TRIP, while with CPAs with a big binding pocket a variety of hydrogen bond species besides the dimer were observed. A clear preference of the dimer as the major

hydrogen bond species was observed with TRIFP based on ^{1}H-NMR and DOSY measurements. Furthermore, when combining electronic withdrawing properties on the 3,3´-substituent with electronic withdrawing substituents on the ketone-part of the imine or the other way around it was possible to favor the dimer formation over the formation of other species.

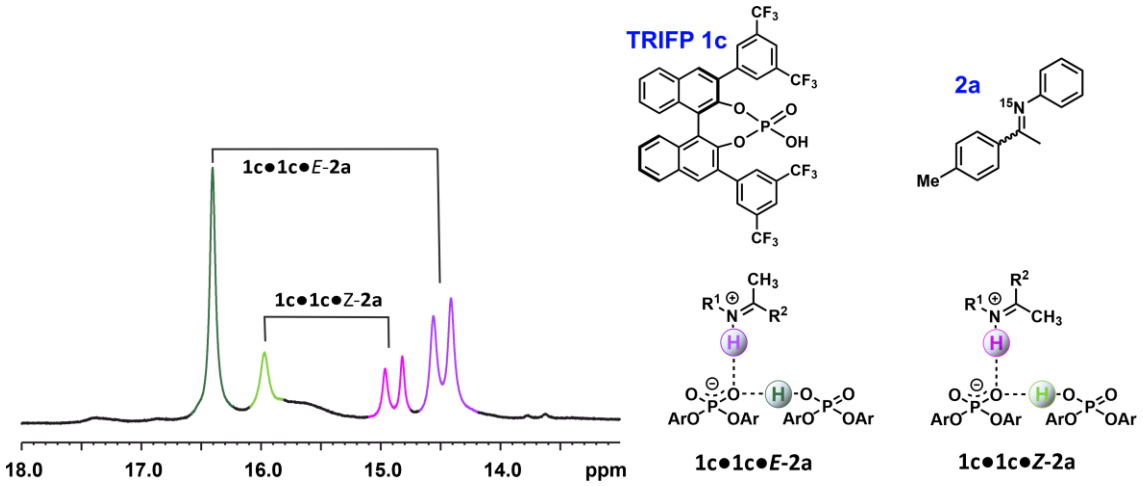


Figure 2 Hydrogen bond area of the ^{1}H-NMR spectrum of TRIFP + Me-imine with a 2:1 stoichiometry (50mM:25mM, CD2Cl2, 180K, 600MHz). The dimeric species is highlighted in green (CPA-CPA hydrogen bond) and pink (CPA-imine hydrogen bond).

To validate if the with NMR spectroscopy observed dimeric species also has an influence on the reaction and thereby the enantioselectivity, the transfer hydrogenation with Hantzsch 1,4-dihydropyridine ester was performed with various conditions and *ee* values were determined by chiral HPLC . Hereby, reaction conditions were crucial to force the reaction into the dimeric species and thereby into a dimeric pathway that was also observed by Niemeyer *et al.* [9]. In particular, decreasing the reaction temperature to -10 °C was essential to see the impact that this secondary pathway has on the enantioselectivity of the transfer hydrogenation. By doing so an inversion in enantiomeric excess was observed with all investigated CPA and imine combinations. Although no dimeric species was observed with CPAs with a small binding pocket like TRIP, the influence of the dimeric pathway was clearly reflected in the enantiomeric excess that was determined by HPLC. This demonstrated the significance of this pathway as already small traces of the dimeric species change the *ee* drastically and was also for the best of our knowledge the first time that this dimeric species was observed with CPAs with such huge substituents (TRIP,TiPSY). With these results, it was possible to establish guidelines for bypassing the influence of the dimeric pathway. Therefore, it is necessary to keep the temperature above 30 °C and use small amounts of CPA (<10 mol%). Increasing the concentration of the substrates also shifts the system slightly into the dimeric species and therefore leads to a small decrease in *ee*. Besides this guideline to circumvent the dimeric pathway it is also possible to use it to improve the *ee*. Therefore, it is necessary to further cool down the reaction to -30 °C. With this setup a complete inversion of the *ee* can be observed with most systems. By comparing the ee values of the (R)-CPA of the dimeric pathway with the *ee* values of the (S)-CPA of the monomeric pathway an improvement in enantioselectivity can be observed while using the dimeric pathway. Especially when using imines with electron withdrawing properties on the ketone-part of the imine a significant improvement can be achieved of up to 15 % in comparison with the monomeric pathway with the corresponding (S)-CPA.

In conclusion, the discovery of the dimeric species in the asymmetric transfer hydrogenation of imines with CPAs by NMR opened up two ways to further improve the enantioselectivity. On the one hand, general guidelines for the monomeric pathway were established. On the other hand, the dimeric pathway can also be exploited to increase the enantioselectivity which is especially useful for imines with electron withdrawing properties on the ketone-part.

1. T. Akiyama, J. Itoh, K. Yokota, K. Fuchibe, Angew. Chemie - Int. Ed., 43, 1566–1568, 2004.
2. D. Uraguchi, M. Terada, J. Am. Chem. Soc., 126, 5356–5357, 2004.
3. S. Hoffmann, A. M. Seayad, B. List, Angew. Chemie - Int. Ed., 44, 7424–7427, 2005.
4. M. Rueping, E. Sugiono, C. Azap, T. Theissmann, M. Bolte, Org. Lett., 7, 3781–3783, 2005.
5. R. I. Storer, D. E. Carrera, Y. Ni, D. W. C. MacMillan, J. Am. Chem. Soc., 128, 84–86, 2006.
6. K. Rothermel, M. Melikian, J. Hioe, J. Greindl, J. Gramüller, M. Žabka, N. Sorgenfrei, T. Hausler, F. Morana, R. M. Gschwind, Chem. Sci., 10, 10025–10034., 2019.
7. N. Sorgenfrei, J. Hioe, J. Greindl, K. Rothermel, F. Morana, N. Lokesh, R. M. Gschwind, J. Am. Chem. Soc., 138, 16345–16354, 2016.
8. M. Melikian, J. Gramüller, J. Hioe, J. Greindl, R. M. Gschwind, Chem. Sci., 10, 5226–5234, 2019.
9. D. Jansen, J. Gramüller, F. Niemeyer, T. Schaller, M. C. Letzel, S. Grimme, H. Zhu, R. M. Gschwind, J. Niemeyer, Chem. Sci., 11, 4381–4390, 2020.

Maximilian Franta is funded by Deutsche Forschungsgemeinschaft (RTG 2620 Ion Pair Effects in Molecular Reactivity Project 426795949).

17 SHARPER-DOSY: Measuring Diffusion Coefficients from Micromolar Concentrations

George Peat, Patrick J. Boaler, Claire L. Dickson and Dušan Uhrín

EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK

The SHARPER (Sensitive, Homogeneous And Resolved PEaks in Real time) NMR experiment, proposed originally in the context of reaction monitoring, removes all homonuclear and heteronuclear splittings from a selected signal by pulsing only on the observed nucleus. SHARPER compensates for magnetic field inhomogeneity, producing very narrow singlets [1]. A recent extension of the SHARPER technique allowed simultaneous monitoring of a reactant and a product [2]. Optimized acquisition and processing of SHARPER spectra further improved sensitivity as demonstrated by the implementation of SHARPER on benchtop NMR spectrometers [3].

Here, we present further developments that combine SHARPER with the chemical shift selective filter [4] and the DOSY experiment. This signal selection and subsequent collapse of a heavily overlapped multiplet allows determination of diffusion coefficients of compounds contained in low concentration mixtures. We also present a technique where it is possible to collapse parts or even an entire spectrum into a narrow singlet suitable for quantitative evaluation. Combined with a DOSY experiment, this approach allows determination of the diffusion coefficient of pure compounds at micromolar concentrations (Figure 1). The presented experiments move the applications of SHARPER beyond those of reaction monitoring and illustrate the versatility of the sequence as an efficient acquisition building block.

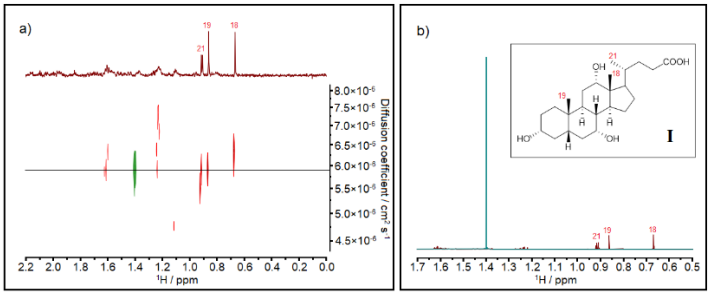


Figure 4. a) 1D 800 MHz ^{1}H NMR spectrum (maroon) of sodium cholate, **I** (1.4 μg, 8 scans), and its DOSY spectrum (red). D_2O impurities are visible as they show different diffusion coefficients. SHARPER-DOSY spectrum (green) obtained by subtracting the DOSY traces of D_2O alone from those of **I** and collapsing most of its signals into a singlet. b) First trace of the DOSY (maroon) and SHARPER-DOSY (turquoise) spectra showing 16-fold increase relative to the Me signals of **I** for the SHARPER spectrum.

1. A. B. Jones, G. C. Lloyd-Jones, D. Uhrín, *Anal. Chem*., 89, 10013-10021, 2017.
2. M. Davy, C. L. Dickson, R. Wei, D. Uhrín, C. P. Butts, *Analyst*, 147, 1702-1708, 2022.
3. C. L. Dickson, G. Peat, M. Rossetto, M. E. Halse, D. Uhrín, *Chem. Commun*., 58, 5534-5537, 2022.
4. P. T. Robinson, T. N. Pham, D. Uhrín, *J. Mag. Res*., 170, 97-103, 2004.

18 Quantitative and Convenient Real-time Reaction Monitoring using Stopped-flow Benchtop NMR

Tristan Maschmeyer, Lars P. E. Yunker, and Jason E. Hein

University of British Columbia, Department of Chemistry, Vancouver, BC, CA

Reaction monitoring plays a pivotal role in highlighting potential pathways for both process optimization and enhancing process safety. Many different analytical tools are employed, with the utilization of benchtop nuclear magnetic resonance (NMR) spectrometers attractive as they bring the power of NMR physically to a synthetic laboratory. However, solutions are commonly analyzed under continuous-flow conditions, lending to potential complications in terms of analyte homogeneity and pre-magnetization [1,2] - overall impacting ease of quantitative analyses.

We present a stopped-flow benchtop NMR system, with hardware components centrally controlled by an internally developed Python script. Application of this stopped-flow system circumvents complications arising with continuous-flow analyses and results in quantitative reaction monitoring performed both easily and dependably. With this system, a set of ^{19}F NMR acquisition parameters were determined allowing for quantitation using the absolute intensity method, without the introduction of an internal standard or electronic reference signal. This system and set of acquisition parameters were then applied to quantitatively monitor two model reactions (*via* ^{19}F NMR, 57 MHz) that are difficult to otherwise monitor due to gas evolution, use/formation of toxic reagents, and formation of difficult species to monitor. These reactions included the activation of a carboxylic acid using sulfuryl fluoride (SO_2F_2) and the formation of a carbamate *via* modified Curtius rearrangement with diphenylphosphoryl azide (DPPA).

1. Dalitz, Fraz; Maiwald, Michael; Guthausen, Gisela, *Chem Eng. Sci.*, 75, 318-326, 2012.
2. Maschmeyer, Tristan; Prieto, Paloma L.; Grunert, Shad; Hein, Jason E., *Magn. Reason. Chem.*, 58, 1234-1248, 2021.

19 Database Hit Quality Index (HQI) as Tool for Identifying Coffee Components using NMR and FTIR Data

Khalid AL Maqbali and Jioji Tabudravu

School of Natural Science, University of Central Lancashire, Preston, Lancashire, UK

Coffee discrimination has attracted global attention from researchers, especially on multivariate analysis[1,2]. Many studies have focused on chemical databasing to compare metabolites in biological samples and drug discovery[3]. In this study, we have designed a database showing that coffee beans and beverages can be discriminated against using ACD/Labs/DB software using the resulting percentage Hit Quality Index (HQI %). HQI % indicates the degree of matching between spectra of sample and known library compounds[4]. This method can identify, rank, and group the known components of the quarried sample. These can be used with other information like farming, geographic, country of origin, and roasting coffee practices to detect metabolite variation in coffee beans. To evaluate the performance of HQI %, about 605 molecular structures of known green and roasted coffee compounds were compiled and loaded to the ACD/Labs/DB platform. ^{1}H NMR and FTIR spectral data of 23 compounds were recorded, and 41 ^{1}H NMR raw data were obtained from the Biological Magnetic Resonance Data Bank website. In addition, 230 ^{1}H NMR spectra were calculated using the ACD/Labs NMR workbook suite. All ACD/Labs/DB parameters were set for optimal hits using ^{1}HNMR and FTIR. The discrimination process identified differences between Colombian and Rwandan green and coffee beans. The database provides good predictions for chemical composition, roasting profile, and sensorial bodies. The study concluded that Colombian coffee poses more chemical components than Rwandan beans. The overall results, HQI % indicates that it can be used to discriminate against coffee products.

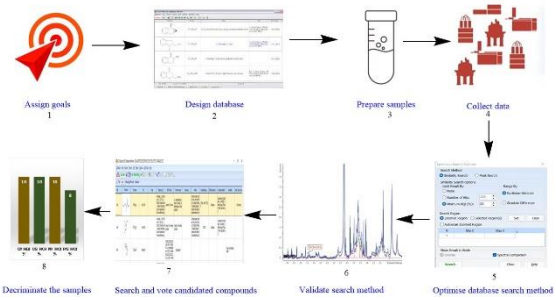


Figure 1. the overall stages for designing comparative coffee database.

1. Wei F, Furihata K, Koda M, Hu F, Miyakawa T, Tanokura M. Roasting process of coffee beans as studied by nuclear magnetic resonance: Time course of changes in composition. *Journal of Agricultural and Food Chemistry*. 2012;60(4):1005-1012. doi:10.1021/jf205315r
2. Wei F, Furihata K, Miyakawa T, Tanokura M. A pilot study of NMR-based sensory prediction of roasted coffee bean extracts. *Food Chemistry*. 2014;152:363-369. doi:10.1016/j.foodchem.2013.11.161
3. Charris-Molina A, Riquelme G, Burdisso P, Hoijemberg PA. Consecutive Queries to Assess Biological Correlation in NMR Metabolomics: Performance of Comprehensive Search of Multiplets over Typical 1D 1H NMR Database Search. *Journal of Proteome Research*. 2020;19(8):2977-2988. doi:10.1021/acs.jproteome.9b00872
4. Spectral Preprocessing for Raman Library Searching | American Pharmaceutical Review - The Review of American Pharmaceutical Business & Technology. Accessed January 28, 2022. https://www.americanpharmaceuticalreview.com/Featured-Articles/36904-Spectral-Preprocessing-for-Raman-Library-Searching

20 NMR Structure Determination of Peptidomimetic Drug Leads

Purnima Khandelwal, Luciano Mueller, Christine Jorge and Janet Caceres Cortes

Discovery Chemistry Platforms, Small Molecule Drug Discovery, Bristol Myers Squibb, Princeton, NJ, US

Conformationally constrained macrocyclic peptidomimetic compounds (millamolecules) offer an attractive venue for the design of orally bioavailable inhibitors of protein : protein interactions [1]. NMR spectroscopy has been used to help with structure activity relationship (SAR), characterize solution structure, select candidates for crystallization and study dynamics of millamolecules. Here, we will present recent results on structural characterization strategies that include (1) solution condition optimization, (2) conformational heterogeneity evaluation to triage compounds for X-ray crystallization, (3) 2D assignments using ACD Workbook, and (4) conformation ensemble generation using J-couplings, TALOS [2, 3] and NOE-distance restraints. This structural information helps to model docking of peptides into the target protein, and to delineate changes in peptide conformation on binding. Further, a comparison of solution vs X-ray crystal structure enables us to determine if the folds are similar in solution vs the solid state.

1. Horst Kessler, Angew. Chem. Int. Ed. Engl., 21, 512-523, 1982
2. Yang Shen, Frank Delaglio, Gabriel Cornilescu, and Ad Bax, J Biomol NMR, 44(4), 213–223, 2009
3. D. S. Wishart, B. D. Sykes and F. M. Richards, Biochemistry 31, 1647-1651, 1992

21 Developing applications for parahydrogen hyperpolarization

Nele Reimets, Kerti Ausmees, Sirje Vija, and Indrek Reile

National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

The detection limit of regular NMR restricts its usage in the analysis of dilute biological samples. Parahydrogen (pH2) hyperpolarization (HP) overcomes the sensitivity problem and enables detection in sub-micromolar concentrations. The non-hydrogenative chemoselective version of pH2 HP (nh-PHIP) [1] can detect hundreds of different compounds in a complex matrix (e.g. urine [2,3,4]) and therefore, could highly benefit different research fields as it is qualitative and quantitative.

We use nh-PHIP, a variant of parahydrogen-induced polarization (PHIP), to detect pH2 derived hydrides of short-lived complexes of analytes with iridium based HP catalysts. nh-PHIP does not require sample manipulation like labelling or sidearm addition and is therefore a relatively simple technique. It is a chemoselective method, which works quantitatively orders of magnitude below the usual NMR LOD. Furthermore, it allows to adopt NMR in drug quality control or drug detection (e.g. doping testing, pharmacokinetics [2]) and disease biomarker analysis [4].

We discuss here the possible applications of pH2 HP. We show that nh-PHIP can be used in pharmacokinetics for detecting and quantifying dilute analytes in biological mixtures [2]. We measured urine of humans exposed to nicotine and compared two common nicotine administration routes – smoking and absorption through skin. We followed urinary elimination of nicotine and cotinine, and determined their concentrations in urine during the onset and withdrawal from nicotine consumption.

Additionally, we demonstrate that nh-PHIP can be exploited for the detection of biologically important molecules like oligopeptides. It is possible to distinguish different oligopeptides in artificial as well as in biological mixtures without the need of extensive sample preparation. We show that oligopeptides exhibit similar binding modes to iridium catalysts as amino acids [5], and give rise to distinctive hydride resonance frequencies.

1. Hermkens, N. K. J., Eshuis, N., van Weerdenburg, B. J. A., Feiters, M. C., Rutjes, F. P. J. T., Wijmenga, S. S., Tessari, M., Anal. Chem., 88, 3406–3412, 2016
2. Reimets, N., Ausmees, K., Vija, S., Reile, I., Anal. Chem., 93, 9480−9485, 2021
3. Ausmees, K., Reimets, N., Reile, I., Mol., 27, 802, 2022
4. Ausmees, K., Reimets, N., Reile, I., Chem. Commun., 58, 463-466, 2022
5. Sellies, L., Aspers, R. L. E. G., Feiters, M. C., Rutjes, F. P. J. T., Tessari, M., Angew. Chem. Int. Ed., 60, 26954–26959, 2021

22 Modification of chiral phosphoric acid catalysts with ^{77}Se and NMR-spectroscopic hydrogen bond investigation

Johannes Eder and Ruth M. Gschwind

Chemistry Department, University of Regensburg, Germany

Natural chemical reactions utilize catalytic systems based on weak Lewis and Brønsted acids. Despite their weak interactions with substrates (commonly through hydrogen bonding), natural catalysts like enzymes provide exceptional selectivity and high efficiency [1]. Those intermolecular interactions served as inspirations for organocatalysis. It has been shown, that small chiral molecules can function like enzymes in organic reactions and alter the selectivity profiles exhibited by substrates. A breakthrough in this research field was the development of chiral phosphoric acid (CPA) with 1,1'-bi-2-naphthol (BINOL) backbones [2,3]. The success of chiral Brønsted acids originates from their high substrate tolerance, excellent stereoselectivity and high yields, thus ensuring their vast application in the field. These catalysts have proven themselves for multiple stereoselective reactions, such as inter- and intramolecular heterocyclizations, alkylation, reductive amination and many other reaction types [4-6].

Despite its enormous application field, rational mechanistic understanding is a major issue in organocatalysis. One of the most informative tools for this purpose are NMR-spectroscopic investigations of the hydrogen bonding with the respective substrate. This kind of research is well established in our group [7-9]. Since CPA catalysts exhibit bifunctionality with both Brønsted acid and basic groups in the active center, up to two substrates can be bound via hydrogen bonding. Furthermore, the axially chiral BINOL-backbone with its C_2-symmetry enables discrimination between enantiotopic faces [10,11]. The varying 3,3'-substituents can induce both steric and electronic effects and thus have tremendous impact on both yield and enantiomeric excess [12].

The specific structure of CPA catalysts allows reactions like transfer hydrogenations (see Figure 1). The reaction mechanism of the transfer hydrogenation of imines catalyzed by CPA with Hantzsch ester as hydrogen source was initially proposed by Rueping *et al.* [13]. The mechanism was later confirmed through computational studies [14,15]. Additionally, our working group did in-depth NMR spectroscopic, experimental and theoretical investigations corroborating the proposed mechanism (see Figure 1) [9].

In the previous work within our group, different chiral Brønsted acids were investigated in combination with imines regarding structure, hydrogen bond properties and reactivity. Thorough empirical observations, chemical shifts and coupling constants can be used to analyze the hydrogen bond area and thus help to determine transition state combinations (based on *ee* values and reactivities) and structures of intermediates.

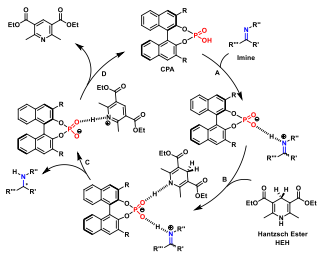


Figure 1: Asymmetric transfer hydrogenation of imines catalyzed by BINOL-based CPA utilizing Hantzsch ester (HEH) as hydrogen source. The reaction occurs via a ternary complex which is held together by hydrogen bonds.

However, these NMR-spectroscopic investigations generally rely on ^{1}H, ^{15}N-labeled and ^{31}P NMR measurements because of unfavorable properties of the ^{17}O isotope. Therefore, it has never been possible to address bifurcated binding modes or hydrogen bonds using the powerful information of ^{1}J scalar couplings to oxygen. Transition to selenium nuclei can provide desirable advantages for NMR studies. The ^{77}Se isotope (spin ½, 7.63% natural abundance, gyromagnetic ratio of 5.12) allows to observe $^{1}J_{\text{Se-H}}$ spin-spin coupling. Thus, substitution of oxygen with selenium in BINOL-based CPA can serve as a new and ideal handle to investigate the peculiarities of hydrogen bonding via NMR-spectroscopy.
In this work, a reproducible synthesis of a BINOL-based chiral selenophosphoric acid was achieved and first measurements in combination with imine substrates were performed (see Figure 2).

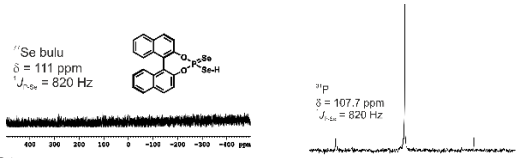


Figure 2: ^{77}Se bulu and ^{31}P spectra of the synthesized BINOL-based chiral selenophosphoric acid.

Since selenium as a chalcogen is expected to show similar binding properties as oxygen, all reactions that were performed with regular CPA could be investigated with chiral selenophosphoric acids (CSPA) instead. This novel chiral catalyst would then allow closer NMR-spectroscopic analysis of the hydrogen bonding situation and conclusions on reaction mechanisms.

1. Schreiner P., Chem. Soc. Rev., 32, 289-296, 2003.
2. Akiyama T., Itoh J., Yokota K., Fuchibe K., Angew Chemie - Int Ed., 43, 1566–1568, 2004.
3. Uraguchi D., Terada M., J. Am. Chem. Soc., 126, 5356–5357, 2004.
4. Wang Bin Y., Zheng S. C., Hu Y. M., Tan B., Nat Commun., 8, 1–9, 2017.
5. Schnitzer T., Budinská A., Wennemers H., Nat Catal., 3, 143-147, 2020.
6. Storer R. I., Carrera D. E., Ni Y., MacMillan D. W. C., J. Am. Chem. Soc., 128, 84–86, 2006.
7. Melikian M., Gramüller J., Hioe J., Greindl J., Gschwind R. M., Chem. Sci., 10, 5226-5234, 2019.
8. Rothermel K., Melikian M., Zabka M., Gschwind R., Chem. Sci., 10, 10025-10034, 2019.
9. Renzi P., Hioe J., Gschwind R. M., Acc. Chem. Res., 50, 2936–2948, 2017.
10. Terada M., Synthesis, 12, 1929-1982, 2010.
11. Sigman M. S., Jacobsen E. N., J. Am. Chem. Soc., 120, 4901-4902, 1998.
12. Saito K., Akiyama T., Chem. Commun., 48, 4573–4575, 2012.
13. Rueping M., Sugiono E., Azap C., Theissmann T., Bolte M., Org. Lett., 7, 3781–3783, 2005.
14. Simón L., Goodman J. M., J. Am. Chem. Soc., 130, 8741–8747, 2008.
15. Marcelli T., Hammar P., Himo F., Chem. Eur. J., 14, 8562–8571, 2008.

23 NMR Spectroscopic Investigations of Silicide Clusters in Liquid Ammonia

Franz F. Westermair, Paul Braun, Stefanie Gärtner, Franz Schmidt, Susanne Tiefenthaler, Nikolaus Korber and Ruth Gschwind*

Department of Chemistry and Pharmacy, University of Regensburg, Regensburg, Bavaria, Germany

Cluster anions of silicon (Zintl anions) have been known for a long time in the solid phase [1]. For decades, these species were assumed to be insoluble in any solvent, until *Sevov* and *Goicoechea* reported the first successful dissolution of $K_{12}Si_{17}$ and $Rb_{12}Si_{17}$ in liquid ammonia in the presence of 18-crown-6 or [2.2.2]-cryptand to obtain single crystals of (K-[2.2.2]-cryptand)$_3$(Si_9) • 8 NH_3 and (Rb-[2.2.2]-cryptand)$_2$(Si_5) • 4 NH_3 in 2004 [2]. The structures were determined *via* sc-X ray crystallography.

Although evidence for the solubility of silicide clusters was strongly suggested by these experiments, the direct detection in solution by means of ^{29}Si-NMR-spectroscopy was not achieved at the time and remained elusive for almost another decade [2]. Finally, the observation of such cluster anions being dissolved in liquid ammonia was reported by our group for the first time in 2013, when the tetrahedral $[Si_4]^{4-}$ (δ_{29Si} – 323 *ppm*) signal was measured in solutions of ^{29}Si-enriched $K_6Rb_6Si_{17}$ in the presence of [2.2.2]-cryptand [3].

Since this first publication and with ongoing optimization of solid-state synthesis, sample preparation and NMR-capabilities we were able to observe and characterize further functionalized and "naked" silicide clusters such as $[Si_5]^{2-}$ [4], protonated $[HSi_9]^{3-}$ [5] and $[HSi_4]^{3-}$ [4] as well as coinage metal functionalized $[NHC^{Dipp}Cu(Si_9)H]^{2-}$ [6] and $[(NHC^{tBu}Au)_6(Si_4)]Cl_2$ [7]. Despite these successes, such NMR-spectroscopic endeavors remain a technically challenging task, since the solubility of silicides is generally quite low, long longitudinal relaxation times (T_1) are assumed and regular, non-deuterated ammonia is used as solvent besides other obstacles. Potential solutions and experimental data will be presented and discussed.

Recently, we gained interest in the reactivity of low-oxidation state transition metal carbonyls with silicides in liquid ammonia since new, highly reduced metal species might be obtainable as well as novel functionalized silicide cluster anions. Especially the neutral complex $[Mo(CO)_4(TMEDA)]$ appeared to be a promising substrate, because ligand exchange of a distinct CO by ammonia is strongly suggested by ^{1}H– and ^{13}C-NMR data. Indeed, besides others, two new triplets at δ_{29Si} +381 *ppm* and –264 *ppm* (J_{SiSi} = 52 Hz, each) were detected in solutions of the molybdenum complex, enriched $K_6Rb_6Si_{17}$ and [2.2.2]-cryptand at 203 K. Since no corresponding signals were observable in a ^{1}H-^{29}Si-HMQC, a protonation is so far being ruled out; instead a mono– or bifunctionalized "butterfly" $[Si_4]^{x-}$ - core-moiety is tentatively assumed (in accordance with a C_2 symmetric species). The experimental data will be further accompanied by DFT-calculations of NMR-parameters of assumed species. Many more signals have been observed in ^{1}H-^{29}Si-HMQC/HMBC spectra of various samples, which are presumably downstream products of silicides with the solvent. These and their structural elucidation will also be addressed.

1. Schäfer, H.; Eisenmann B.; Müller W.; *Angew. Chem. Int. Ed. Engl.*, 12(9), 694–712, **1973**.
2. Goicoechea, J.M.; Sevov S.; *J. Am. Chem. Soc*., 126(22), 6860–6861, **2004**.
3. Neumeier, M.; Fendt, F.; Gärtner S.; Koch, C.; Gärtner T.; Korber N.; Gschwind R.M.; *Angew. Chem. Int. Ed.*, 52(16), 4483–4486, **2013**.
4. Hastreiter, F.; Lorenz, C.; Hioe, J; Gärtner, S.; Lokesh, N.; Korber, N.; Gschwind, R.M.; *Angew. Chem. Int. Ed.*, 58(10), 3133–3137, **2019**.
5. Lorenz, C.; Hastreiter, F.; Hioe, J.; Lokesh, N.; Gärtner, S.; Korber, N.; Gschwind, R.M., *Angew. Chem. Int. Ed.*, 57(39), 12956–12960, **2018**.
6. Streitferdt, V.; Tiefenthaler, S.M.; Shenderovich, I.G.; Gärtner, S.; Korber, N.; Gschwind R.M.; *Eur. J. Inorg. Chem.*, 2021(36), 3684–3690, **2021**.
7. Tiefenthaler, S.M.; Streitferdt, V.; Baumann, J.; Gärtner, S.; Gschwind, R.M.; Korber, N.; *Z. Anorg. Allg. Chem.*, 646(18), 1595–1602, **2020**.

24 GEMSTONE-ROESY: Ultra-selective, ultra-clean 1D rotating-frame Overhauser spectroscopy

Emma Gates[1], Marshall J. Smith[1], Jonathan Bradley[2], Myron Johnson[2], Göran Widmalm[3], Mathias Nilsson[1], Gareth A. Morris[1], Ralph W. Adams[1], and Laura Castañar[1]

1. Chemistry Department, University of Manchester, Manchester, UK.
2. Johnson Matthey, Johnson Matthey Technology Centre, Reading, UK.
3. Department of Organic Chemistry, Stockholm University, Sweden.

2D NMR methods provide extensive structural and conformational information on molecules, but are often time-consuming. Their 1D ^{1}H selective analogues allow key information required for analysis to be extracted in a much shorter time. However, in ^{1}H NMR the narrow range of chemical shifts and the typically high signal multiplicity often cause multiplets to overlap, so that traditional selective excitation methods cannot select a single chemical site. The chemical shift selective filter (CSSF)[1] allows selection of a single multiplet in an overlapped region, but is time-consuming because multiple increments are required to achieve selectivity. A new 1D ultra-selective approach has recently been developed, named GEMSTONE[2] (gradient-enhanced multiplet-selective targeted-observation NMR experiment). GEMSTONE requires only a single scan to achieve multiplet selectivity, saving significant time in comparison to the CSSF experiment. GEMSTONE has recently been shown to be compatible with both 1D NOESY[2] and 1D TOCSY[3] methods, providing unambiguous through-space and through-bond correlations, respectively, for each individually selected signal.

Here, the novel GEMSTONE analogue of the 1D ROESY[4] experiment is introduced. GEMSTONE-ROESY is shown to provide through-space interactions, aiding structural and conformational analysis where the NOE provides little or no intelligible data. The new method is demonstrated using cyclosporin, an oral immunosuppressant; multiplet overlap prevents the use of traditional 1D selective techniques which produce ROE correlations that cannot be unequivocally assigned (Figure 1b). Further benefits of the method will be shown in the analysis of a structurally complex oligosaccharide present in breast milk, lacto-*N*-difucohexaose I.

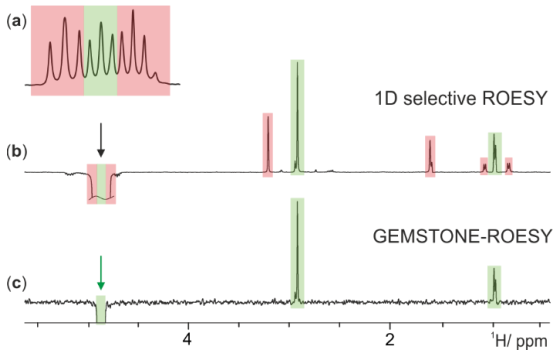


Figure 1: (a) Expansion of a conventional ^{1}H NMR spectrum, (b) traditional 1D selective-ROESY, and (c) GEMSTONE-ROESY spectra, of cyclosporin. (b and c) target the signal highlighted in green shown in (a). The ROEs highlighted in green and red are correlations from the targeted and the untargeted signals, respectively.

1. Hall, L. D.; Norwood, T. J. *J. Magn. Reson.* 78, 582–587, 1988.
2. Kiraly, P.; Kern, N.; Plesniak, M. P.; Nilsson, M.; Procter, D. J.; Morris, G. A.; Adams, R. W. *Angew. Chemie. Int. Ed.* 60 (2), 666–669, 2021.
3. Kiraly, P.; Nilsson, M.; Morris, G. A.; Adams, R. W. *Chem. Commun.* 57 (19), 2368–2371, 2021.
4. Thiele, C. M.; Petzold, K.; Schleucher, J. *Eur. J. Chem.* 15 (3), 585–588, 2009.

25 Characterisation of Acyl Intermediates in Rhodium/phosphine catalysed Hydroformylation by Multi-nuclear NMR spectroscopy

Alejandro Bara-Estaún,[1,2] Callum Haydon,[4] Rebekah Jeans,[4] Catherine L. Lyall,[1,2] John P. Lowe,[1,2] Paul G. Pringle,[4] Robert Franke,[5] Natalie Fey [4] and Ulrich Hintermair*[1,2,3]

1. Department of Chemistry, University of Bath, Claverton Down, BA2 7AY Bath, United Kingdom.
2. Dynamic Reaction Monitoring Facility, Material and Chemical Characterisation Facility, University of Bath, Claverton Down, BA2 7AY Bath, United Kingdom.
3. Centre for Sustainable & Circular Technologies, University of Bath, Bath BA2 7AY, United Kingdom.
4. School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom.
5. Evonik Performance Materials GmbH, Paul-Baumann-Straße 1, 45772 Marl, Germany.

On-line high resolution, multinuclear FlowNMR spectroscopy has recently been shown to be a powerful *operando* reaction monitoring technique for homogeneous catalytic systems under realistic conditions.[1] Understanding of reaction kinetics and identification of key intermediates has been possible by this technique in photocatalytic, polymerization, transfer hydrogenation reactions [2-5]. In 2020, we reported an investigation of the Rh/PPh_3 catalysed hydroformylation of 1-hexene by multi-nuclear, high pressure, *operando* FlowNMR spectroscopy [6]. ^1H NMR experiments together with selectively excited ^1H NMR and $^{31}P\{^1H\}$ NMR pulse sequences were interleaved to monitor the reaction. ^1H NMR data allowed the quantification of dissolved H_2 while tracking substrate consumption and product formation demonstrating real *operando* conditions. The isomeric *e,a* and *e,e* bisphosphine complexes $[RhH(CO)_2(PPh_3)_2]$ (**A/B**) were observed as the predominant hydrido phosphine Rh complex during turnover. They formed reversibly from the isomeric *e,e,e* and *e,e,a* trisphosphine complexes $[RhH(CO)(PPh_3)_3]$ (**C/D**) under CO pressure, directly from $[Rh(acac)(CO)_2]$ with excess PPh_3 under H_2 and CO. The monophosphine *trans*-acyl complex $[Rh(CO(CH_2)_5CH_3)(CO)_3(PPh_3)]$ (**Q**), in equilibrium with the *e,e* and *e,a* bisphosphine *trans*-acyl complexes $[Rh(CO(CH_2)_5CH_3)(CO)_2(PPh_3)_2]$ (**O**/**P**), was characterized for the first time. These acyl complexes could be obtained by mixing 1-hexene with **C/D** under an atmosphere of CO (or 99.99% enriched ^{13}CO) without H_2 present.

Here, we have synthesized a range of hydrido and acyl Rh complexes formed in the hydroformylation of 1-hexene (or ethylene/cyclooctene) with a variety of commonly used phosphorous ligands; PPh_3, P(m-tolyl)$_3$,P(OPh)$_3$, Alkanox, Dppe, Dppp, Dppf, BISBI, Xantphos and BIPHEPHOS. In the absence of H_2 this enables detailed NMR characterisation of rhodium acyl complexes of these substrates and ligands. ^1H NMR shows formation of aldehydes, and the observation of the CH and CH_2 from the acyl chain which provide useful information for the elucidation of the structure of the acyl complexes. Selective excitation ^1H NMR spectra are used to analyze the hydride peak from the hydrido Rh complexes whose multiplicity is caused

by $^{2}J_{P\text{-}H}$ and $^{2}J_{C\text{-}H,}$ which are sometimes obscured by fast exchange, and the magnitude depends on how the ^{13}CO and PR_3 ligands are coordinated. $^{31}P\{^{1}H\}$ NMR can be used to compare integrals of equatorial and axial coordinated P ligands (see **Figure 1** as example), and observe multiplicity caused by $^{2}J_{P\text{-}Rh}$ and $^{2}J_{P\text{-}C}$ with ^{1}H-^{31}P HMBC for two dimensional correlations.

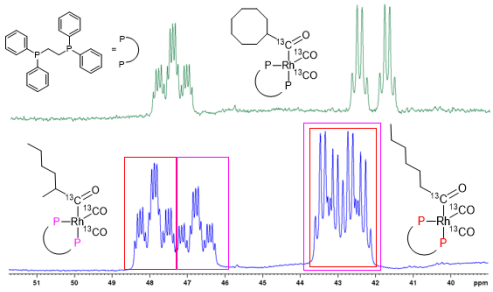


Figure 1. Stacked $^{31}P\{^{1}H\}$ spectra of the characterisation of the dppe acyl intermediates with cyclooctene (green) or 1-hexene (blue). There is one isomer for ethylene and two isomers for 1-hexene due regioselectivity of reaction.

$^{13}C\{^{1}H\}$ pulse sequences can be used to observe different carbon environments (in particular the terminal and acyl carbonyls), and their relative integrals and multiplicities be assessed from ^{1}H-^{13}C HMBC correlations (**Figure 2**).

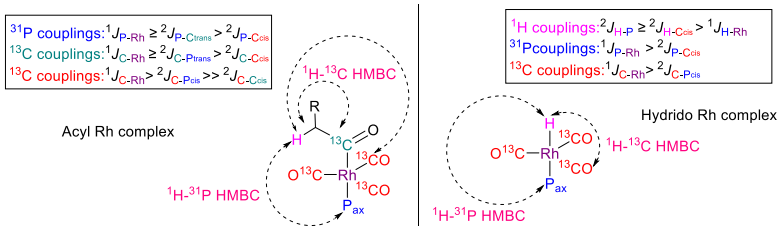


Figure 2. Characteristic couplings and experiments used to characterize acyl and hydrido Rh complexes.

Five-coordinate acyl mono-, di- and tricarbonyl complexes appear to be thermodynamically preferred over the four-coordinate species at low temperatures (-90 to 50 °C) and 1-5 bar of ^{13}CO. The number and position of coordinated ligands within the acyl complex varied between the types of ligands as dictated by their steric and electronic properties. Under non-catalytic reaction conditions (i.e., no H_2 present), NMR spectroscopy reveals the kinetic and thermodynamic selectivity of linear and branched acyl formation. Over the range of ligands investigated, the L/B regioselectivity observed in the acyl complexes at low temperatures with the regioselectivity observed for catalytic olefin hydroformylation at higher temperatures and pressures.

1 A. M. R. Hall, J. C. Chouler, A. Codina, P. T. Gierth, J. P. Lowe and U. Hintermair, *Catal. Sci. Technol.*, 2016, **6**, 8406–8417.

2 J. H. Vrijsen, I. A. Thomlinson, M. E. Levere, C. L. Lyall, M. G. Davidson, U. Hintermair and T. Junkers, *Polym. Chem.*, 2020, **11**, 3546–3550.

3 D. B. G. Berry, A. Codina, I. Clegg, C. L. Lyall, J. P. Lowe and U. Hintermair, *Faraday Discuss.*, 2019, **220**, 45–57.

4 A. M. R. Hall, P. Dong, A. Codina, J. P. Lowe and U. Hintermair, *ACS Catal.*, 2019, **9**, 2079–2090.

5 A. M. R. Hall, R. Broomfield-Tagg, M. Camilleri, D. R. Carbery, A. Codina, D. T. E. Whittaker, S. Coombes, J. P. Lowe and U. Hintermair, *Chem. Commun.*, 2018, **54**, 30–33.

6 A. Bara-Estaún, C. L. Lyall, J. P. Lowe, P. G. Pringle, P. C. J. Kamer, R. Franke and U. Hintermair, *Faraday Discuss.*, 2021, **229**, 422–442.

26 Illuminating Photoredox Reaction Mechanisms With LED-NMR

Yael Ben-Tal and Guy C. Lloyd-Jones

School of Chemistry, University of Edinburgh, Edinburgh, Scotland, UK

Photochemistry has become an increasingly important tool for synthetic chemists over the past few decades. One class of photochemical reactions that has proved of particular importance has been the dual photoredox/nickel catalysis pioneered in 2014 [1]. However, while there has been much development of the synthetic applications of these techniques, in both industrial and academic settings, the mechanisms of this type of reaction remain poorly understood and few mechanistic studies have been conducted.

NMR is well-established as a particularly powerful tool for reaction monitoring and mechanistic study [2]. Here we present the results of a detailed NMR kinetic study conducted on the silane-mediated sp^2-sp^3 cross-electrophile coupling developed by the MacMillan group in 2016 (figure 1.a) [3], which we take as a model reaction for dual photoredox/Ni catalysis more broadly. This was conducted via a bespoke *in-situ* illumination NMR spectroscopy system (LED-NMR) that we constructed in-house (figure 1.b).

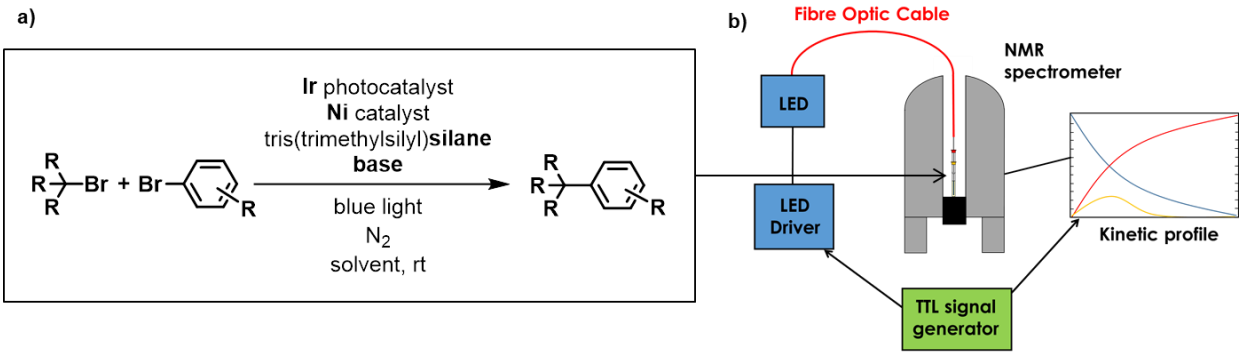


Figure 1. a) The Ni/photoredox cross-coupling studied in this work. b) A scheme of the LED-NMR setup.

The simultaneous direct monitoring of a large number of components in the reaction solution has enabled several important mechanistic observations. Systematic variation of the reaction conditions has allowed for the impact of each of the reaction components on the kinetics, and therefore mechanism, of the system to be observed. One particularly important outcome of this monitoring was the direct observation of a key intermediate that is the major resting state of the Ni catalyst throughout the cycle. The role of this intermediate species in the reaction was further elucidated through sophisticated isotope labelling studies, taking advantage of the unique

properties of NMR. A subsequent combination of control experiments and kinetic modelling has enabled mechanistic conclusions to be drawn [4].

1. Tellis, John C.; Primer, David N.; Molander, Gary A., Science, 345(6195), 433-436, 2014.; Zuo, Zhiwei; Ahneman, Derek T.; Chu, Lingling; Terret, Jack A.; Doyle, Abigail G.; MacMillan, David W. C., Science, 345(6195), 437-440, 2014.
2. Ben-Tal, Yael; Boaler, Patrick J; Dale, Harvey J. A.; Dooley, Ruth E.; Fohn, Nicole A.; Gao, Yuan; Garcia-Dominguez, Andres; Grant, Katie M.; Hall, Andrew, M. R.; Hayes, Hannah L. D.; Kucharski, Maciej; M. Wei, Ran; Lloyd-Jones, Guy C., Progress in Nuclear Magnetic Resonance Spectroscopy, 129, 28-106, 2022.
3. Zhang, Patricia; Le, Chi; MacMillan, David W. C., Journal of the American Chemical Society, 138 (26), 8084-8087, 2016.
4. Ben-Tal, Yael; Lloyd-Jones, Guy C., Manuscript in preparation.

27 When does Computer-Assisted Structure Elucidation (CASE) spare you from recording (IN)ADEQUATE?

Dimitris Argyropoulos and Mikhail Elyashberg

Advanced Chemistry Development (ACD/Labs) Inc., Toronto, Canada

The INADEQUATE [1] experiment was first reported in the literature in 1980 and was heralded as a unique means of establishing the C-C connectivity of organic molecules. However, it has found limited application mostly due to its inherently low sensitivity. Attempts to improve this pitfall were made in subsequent years. In 1996, the creation of the 1,1-ADEQUATE [2] experiment and its variants notably allowed the observation of the C-C bonds through their protons. Since these two experiments are the only means by which a scientist can directly observe the carbon skeleton of a molecule, they have been considered over the years to be the "holy grail" of NMR experiments for elucidation of the structures of organic compounds. Despite the emergence of new, more sensitive NMR hardware like cryogenically cooled probes, they nevertheless remain to be of very low sensitivity, requiring hours and days to run with the amounts of product normally isolated.

During the same period, computer-assisted structure elucidation (CASE) has evolved quite significantly [3-5] allowing scientists to elucidate large and complex structures using NMR data. The core of any CASE system is the structure generator engine, which will generate all the possible structures derived from the molecular formula and the imposed restrictions based on the observed NMR correlations. Modern structure generators are extremely efficient and can generate millions of isomeric structures within minutes. After the structures are generated, they are ranked, usually based on the agreement between the predicted 13C chemical shifts and those observed experimentally.

Taking the above into account, in this poster, we investigate how relevant experiments like INADEQUATE and ADEQUATE truly are, given the existence of powerful tools like CASE. To do this, we analysed a series of published examples in which (IN)ADEQUATE information was stated as being vitally necessary for unambiguous structure elucidation. We looked to determine whether using HMBC and COSY data within a CASE system could elucidate the structure(s) in a reasonable amount of time without using (IN)ADEQUATE spectra. We found that using only HMBC and COSY allowed us to get the correct solution in a reasonable time without utilizing time-consuming experiments in a series of examples. However, we also found that in most cases of large hydrogen deficient molecules, structure elucidation requires (IN)ADEQUATE spectra, and CASE only facilitates the structure elucidation.

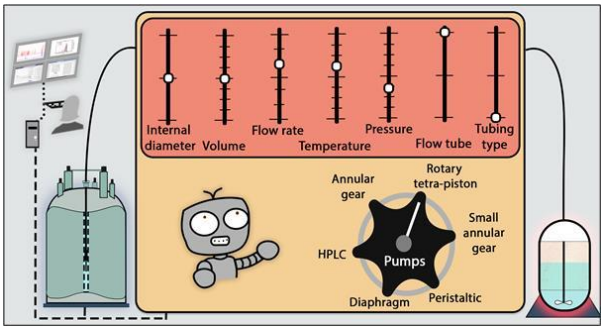
We will be presenting different examples to illustrate the observations. Moreover, we will be showing examples where even though the correct structure has been generated, it was not possible to clearly identify it as the correct structure because others existed that were ranked similarly. We will be discussing methods to resolve these ambiguities and get the correct result without necessarily involving low-sensitivity experiments.

1. Bax A, Freeman R, Kempsell SP., *J Am Chem Soc*. 1980;**102**:4849-4851.
2. B. Reif, M. Kock, R. Kerssebaum, H. Kang, W. Fenical, C. Griesinger, *JMR ser-A* 1996, **118**, 282-285
3. M. Elyashberg, D. Argyropoulos, *eMagRes*, 2019, **Vol 8**: 239–254.
4. M. Elyashberg, D. Argyropoulos, Mag. Reson. Chem., *Mag. Res. Chem*., 2021, **59(7)**, 669-690
5. M.E. Elyashberg, A.J. Williams. "Computer-based Structure Elucidation from Spectral Data. The Art of Solving Problems", Springer, Heidelberg, 2015, 454 p.

28 Engineering aspects of FlowNMR spectroscopy setups for online analysis of solution-phase processes

Asad Saib,[1,2] Alejandro Bara-Estaún,[1,2] Owen J. Harper, [1,2,3] Daniel B. G. Berry, Isabel A. Thomlinson, [1,2,3] Rachael Broomfield-Tagg, [1,2] John P. Lowe, [1,2] Catherine L. Lyall[1,2] and Ulrich Hintermair*[1,2,3]

1. Department of Chemistry, University of Bath, Claverton Down, BA2 7AY Bath
2. Dynamic Reaction Monitoring Facility, University of Bath, Claverton Down, BA2 7AY Bath, UK
3. Centre for Sustainable & Circular Technologies, University of Bath, Bath BA2 7AY, UK



Online analysis and monitoring of solution phase chemistry by way of nuclear magnetic resonance spectroscopy on a recirculating sample from an external reaction vessel (FlowNMR) has proven to be a valuable tool for understanding the dynamic behaviour of complex solution-phase systems in real time.[1] A variety of flow cells and setups have been developed that allow a continuous stream of sample solution to be pumped through the NMR active region of the spectrometer at both low and high magnetic field strengths for various applications, and the choice of materials, dimensions and components can have a profound impact on the quality and relevance of the data obtained.[2-10] Whatever the application, key to obtaining NMR data relevant to the process occurring within the external vessel is an appropriately designed flow system (Fig. 1) that fulfils the following criteria:

a) Full chemical compatibility – the system must not interact with the sample or disturb the system to be studied, and not cause any cross-contamination between different experiments. Conversely, the setup must withstand the conditions used and not be compromised by the application.
b) Provide reaction conditions with appropriate control over key process parameters – all physical parameters of importance to the system studied (light, temperature, pressure, atmosphere, etc.) must be sustained throughout the flow path to make the sample inside the NMR representative of the bulk.
c) Deliver a smooth, controlled sample flow throughout the system – minimise sample dispersion and risk of failures, and control any NMR flow effects that may influence signal quantification.
d) Safe to use – reliably safe to operate in a multi-user environment and without supervision with highly sensitive and expensive NMR equipment.

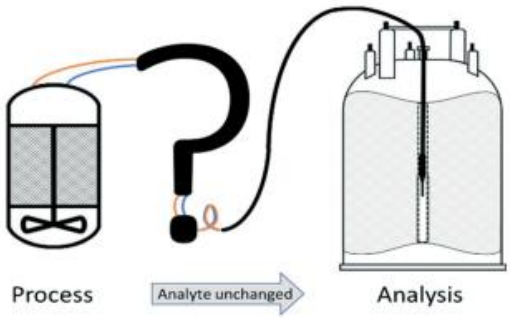


Fig. 1 Schematic illustration of the engineering challenges associated with efficiently interfacing a reaction vessel with an NMR spectrometer

With these considerations belonging more to the realm of chemical reaction engineering,[11] teams of molecular scientists and NMR spectroscopists seeking to use FlowNMR spectroscopy for a given application may lack the expertise and practical experience required for efficiently interfacing the reaction vessel with the spectrometer. Here we show the important and fundamental engineering considerations and selection of components of FlowNMR setups to help avoid common pitfalls and work towards establishing good practice quality guidelines (GxP) for FlowNMR investigations in academia and industry.

1. Hall, A. M. R.; Chouler, J. C.; Codina, A.; Gierth, P. T.; Lowe J. P.; Hintermair, U.; *Catal. Sci. Technol.*, 6, 8406–8417, 2016
2. Bernstein, M. A.; Štefinović M.; Sleigh, C. J.; *Magn. Reson. Chem*., 45, 564–571, 2007
3. Blanazs, A.; Bristow, T. W. T.; Coombes, S. R.; Corry, T.; Nunn, M.; Ray, A. D.; *Magn. Reson. Chem.*, 55, 274–282, 2017
4. Foley, D. A.; Doecke, C. W.; Buser, J. Y.; Merritt, J. M.; Murphy, L.; Kissane, M.; Collins, S. G.; Maguire, A. R.; Kaerner, A.; *J. Org. Chem.*, 76, 9630–9640, 2011
5. Fyfe, C. A.; Cocivera, M.; Damji, S. W. H.; Hostetter, T. A.; Sproat D.; O'Brien, J.; *J. Magn. Reson.*, 23, 377–384, 1976
6. Khajeh, M.; Bernstein M. A.; Morris, G. A.; *Magn. Reson. Chem.*, 48, 516–522, 2010
7. Maiwald, M.; Fischer, H. H.; Kim, Y. K.; Albert, K.; Hasse, H.; *J. Magn. Reson.*, 166, 135–146, 2004
8. Scheithauer, A.; Brächer, A.; Grützner, T.; Zollinger, D.; Thiel, W. R.; Von Harbou, E.; Hasse, H.; *Ind. Eng. Chem. Res.*, 53, 17589–17596, 2014
9. Bornemann, M.; Kern, S.; Jurtz, N.; Thiede, T.; Kraume, M.; Maiwald, M.; *Ind. Eng. Chem. Res.*, 58, 19562–19570, 2019
10. Giraudeau, P.; and Felpin, F. X.; *React. Chem. Eng.*, 3, 399–413, 2018
11. Simons, S.; Concepts of Chemical Engineering for Chemists, Royal Society of Chemistry, Cambridge, 2nd edn., 2016

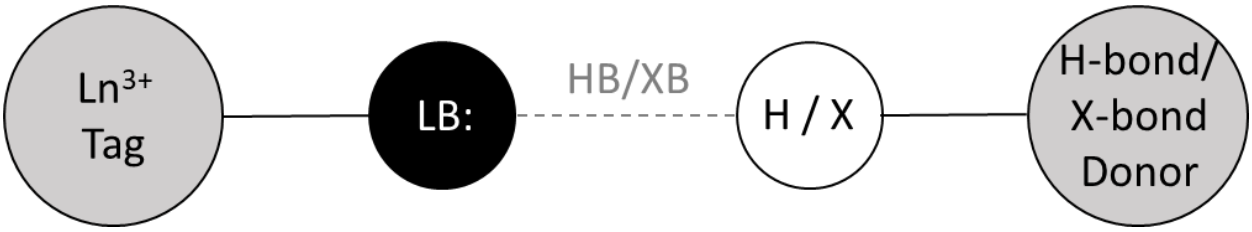
29 Characterising Weak Binding in Solution

Scott Wilcox and Mate Erdelyi

Uppsala University, Department of Chemistry - BMC, Uppsala, Sweden

Weak, non-covalent interactions are inherently difficult to detect in solution. Over the years, attempts have been made to characterise weak binding phenomena; however, these generally require optimised systems. When applied to NMR spectroscopy, this typically involves the preparation of concentrated samples of strong bond donors and acceptors, often being charged, multivalent or intramolecular, and dissolved in idealised solvents.[1] Until now, there has been no robust method to challenge any of these requirements.

We have designed a new approach that utilises paramagnetism,[2] to observe previously undetectable weak hydrogen bonds (HB) and halogen bonds (XB) at low concentrations in solution. Paramagnetic NMR exploits the large, long-range effects arising from the enormous magnetic moment (μ_e/μ_p = -658) that unpaired electrons posses. To harness this effect, we have designed a lanthanoid ion (Ln^{3+}) complexing agent and have functionalised it with a Lewis basic (LB:) entity. The degree of anisotropy on the paramagnetic centre that we use gives rise to pseudocontact shifts (PCS),[3,4] providing a vast increase in sensitivity of weak hydrogen bond- and halogen bond-donor interactions. NMR titrations were performed to detect these interactions in a semi-competitive environment (*d*-MeCN) and at low (<2 mM) concentrations. When referencing to a diamagnetic species – which shows negligible change in chemical shift during titration experiments – the weak interactions can be quantified. This work is expected to have further implications in elucidating the contribution of weak interactions in the fields of supramolecular, materials and medicinal chemistries, to name a few.



1. Wilcox, Scott; Herrebout, Wouter; Erdelyi, Mate; *Ch. 5* in *Halogen Bonding in Solution*, Ed. Huber, Stefan; Wiley-VCH Verlag GmbH & Co. KGaA., 153-194, 2021.
2. Softley, Charlotte A.; Bostock, Mark J.; Popowicz, Grzegorz M.; Sattler, Michael; *J. Biomol. NMR.*, 74, 287-309, 2020.
3. Müntener, Thomas; Joss, Daniel; Häussinger, Daniel; Hiller, Sebastian; *Chem. Rev.*, 122(10), 9422-9467, 2022.
4. Brath, Ulrika; Swamy, Shashikala I.; Veiga, Alberte X.; Tung, Ching-Chieh; Van Petegem, Filip; Erdelyi, Mate; *J. Am. Chem. Soc.*, 137, 11391-11398, 2015.

30 Spectroscopic investigations on organic anions as highly reducing photoredox catalysts

Lea Müller, Ruth M. Gschwind and Burkhard König

Chemistry Department, University of Regensburg, Germany

Photocatalysis has gained immense popularity over the last decades and led to a steep rise of newly reported methods for chemical activations and transformations in organic synthesis. Typically, polypyridyl transition metal complexes and redox-active organic dyes are employed as photocatalysts in these reactions.[1,2] When these compounds are irradiated with light of a certain wavelength, the energy of the photons can be harvested to subsequently drive reactions without the need of external heating or stoichiometric sacrificial compounds. Organic anions have been discovered as photocatalysts as early as 1989.[3] They exhibit unique photophysical properties over their neutral counterparts, like longer excited-state lifetimes, bathochromic shifts and reduced back electron transfer.[4] In addition, their superior electron donor ability makes them ideal candidates for highly reducing photocatalytic transformations, like the reduction of benzene (−3.42 V vs SCE).[5–7]

Despite the growing number of publications within the field of photoredox catalysis, in-depth mechanistic studies on chemical transformations with anionic photocatalysts remain scarce. Recently, a study from Nocera and coworkers sparked interest, as they showed that the often assumed radical anionic form of the excited state photocatalyst was indeed a anionic Meisenheimer complex that enabled diffusion controlled reactivity.[8] Often, a combination of different spectroscopical tools is used gain mechanistic insights in these reactions with NMR spectroscopy being one of the most informative methods. The investigation of hydrogen bonds, complex formations and chemical shifts throughout the course of the reactions can be utilized to determine active species and interaction between catalyst and substrates. This kind of research is already well established in our group.[9–11] As the photoinduced electron transfer of organic anions leads to radical species, EPR spectroscopy can be used as a complimentary approach to detect and study intermediates in the reaction mixture.

Organic anions exhibit immense reduction potentials upon irradiation, but the achieved chemical transformations often have shortcomings like a limited substrate scope, long reaction times or incomplete turnover of the redox neutral catalytic cycle.[5,7] A better understanding of the potential preassembly and interactions between photocatalyst and substrate could help in optimizing the reaction conditions to utilize the full potential of organic anions. Presumably, upon deprotonation of the neutral catalyst with an external base the organic anion is formed which can be excited upon irradiation and undergo a photoinduced electron transfer (PET) to the substrate. The so formed radical anion can react further in an inter- or intramolecular fashion. The catalytic cycle can now be closed either *via* a hydrogen atom abstraction (HAT) process or *via* a second PET step, which can generate the product in a redox neutral fashion (Figure 1). The challenge lies in the investigation of the two pathways which can sometimes occurr in parallel.[6]

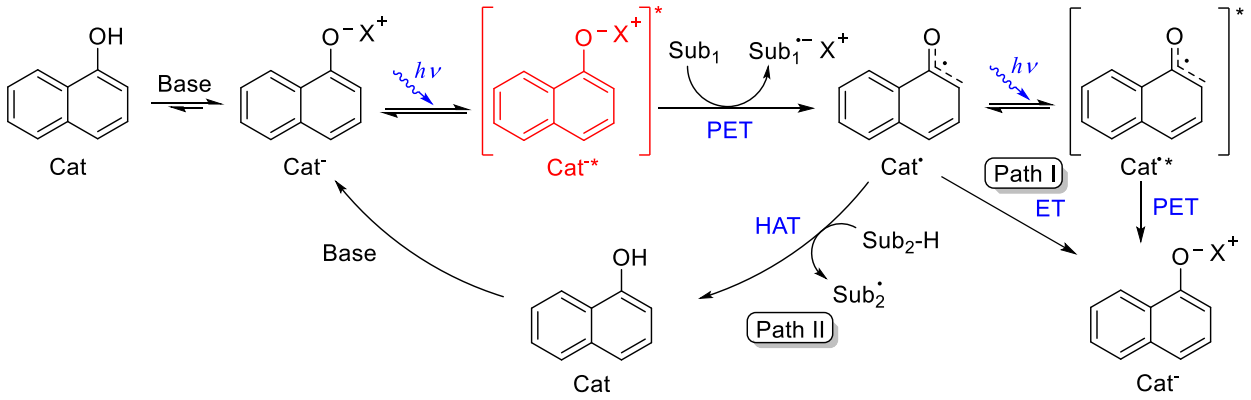


Figure 5: Reductive photocatalytic cycle which can be closed via a consecutive PET (Path I) or HAT process (Path II). Here exemplified by 1-naphthol as the photocatalyst (Cat).

In this work, a redox neutral photocatalytic reduction of benzothiophene (-2.84 V vs SCE) was achieved with anionic naphthalenediol derivatives as photocatalysts. Initial spectroscopical measurements were performed to decode the nature of the active catalyst in solution as well as the mechanism of the reaction. Due to the priviledged structure of 1,4-naphthalenediols, which can form stable radicals in solution, a complementary aproach of NMR and EPR spectroscopy was chosen for *ex situ* and *in situ* investigations. Insights on these catalyst structures can be utilized to tune the reactivity towards a photocatalytic two-electron-transfer process for dual functionalization of substrates.

1. Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C., Chem. Rev., 113, 5322–5363, 2013.
2. Romero, N. A.; Nicewicz, D. A. Chem. Rev., 116, 10075–10166, 2016.
3. Soumillion, J. P.; Vandereecken, P.; van der Auweraer, M.; Schryver, F. C. de; Schanck, A., J. Am. Chem. Soc., 111, 2217–2225, 1989.
4. Schmalzbauer, M.; Marcon, M.; König, B., Angew. Chem. Int. Ed., 60, 6270–6292, 2021.
5. Cole, J. P.; Chen, D.-F.; Kudisch, M.; Pearson, R. M.; Lim, C.-H.; Miyake, G. M., J. Am. Chem. Soc., 142, 13573–13581, 2020.
6. Schmalzbauer, M.; Svejstrup, T. D.; Fricke, F.; Brandt, P.; Johansson, M. J.; Bergonzini, G.; König, B., Chem, 6, 2658–2672, 2020.
7. Wei, D.; Li, X.; Shen, L.; Ding, Y.; Liang, K.; Xia, C., Org. Chem. Front., 8, 6364–6370, 2021.
8. Rieth, A. J.; Gonzalez, M. I.; Kudisch, B.; Nava, M.; Nocera, D. G., J. Am. Chem. Soc., 143, 14352–14359, 2021.
9. Wang, S.; Lokesh, N.; Hioe, J.; Gschwind, R. M.; König, B., Chem. Sci., 10, 4580–4587, 2019.
10. Renzi, P.; Hioe, J.; Gschwind, R. M., Acc. Chem. Res., 50, 2936–2948, 2017.
11. Nitschke, P.; Lokesh, N.; Gschwind, R. M., Prog. Nucl. Magn. Reson. Spectrosc., 114-115, 86–134, 2019.

31 The Natural Products Magnetic Resonance Database (NP-MRD): Comprehensive Database and Repository for Natural Products NMR Data

John R. Cort[1,5], Amy M. Jystad[1], Niranjan Govind[1] Roger G. Linington[2] Lloyd W. Sumner[3] and David S. Wishart[4]

1. Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland WA
2. Department of Chemistry, Simon Fraser University, Burnaby BC
3. Department of Biochemistry and MU Metabolomics Center, University of Missouri, Columbia MO
4. Department of Biological Sciences and Department of Computing Science, University of Alberta, Edmonton AB
5. Institute of Biological Chemistry, Washington State University, Pullman WA

NMR spectroscopy is essential to natural products and specialized metabolite research: for example, in novel structure determination, characterization of functions and interactions, or analysis of mixtures. However, progress in the field is hindered by the poor accessibility to NMR data for known natural products. Currently, chemical shift assignments are scattered throughout decades of published scientific literature and a few valuable, but incomplete, chemical shift databases. Furthermore, nearly all raw data (FIDs) used to determine structures of natural products is not archived and is likely unrecoverable. To address such inadequacies, the Natural Products Magnetic Resonance Database (NP-MRD, np-mrd.org) has been established with a goal to become a comprehensive, searchable, connected, and open database and repository for all natural products NMR data. The mission of NP-MRD is to benefit research through engagement and partnership with the worldwide natural products community. With derived (e.g. chemical shift assignments), raw (FID), and simulated NMR data, as well as tools and links to other databases, NP-MRD can facilitate dereplication, support correction of erroneous or missing chemical shift assignments, and enable structure validation or structure revision. Furthermore, NP-MRD can create opportunities for developing new artificial intelligence-based approaches for structure determination and chemical shift or spectral prediction, among other presently unforeseen applications of such a database resource.

32 RDC-Based Analysis and a Novel Water Gel in Rational Drug Design

Kathleen A. Farley[1], **Dr. Martin R. M. Koos**[1,2], Dr. Ye Che[1], Dr. Reto Horst[1], Dr. Chris Limberakis[1], Justin Bellenger[1], Dr. Ricardo Lira[1], Leandro F. Gil-Silva[3], Dr. Roberto R. Gil[2]

1. Medicinal Sciences, Pfizer, Groton, CT 06340, US
2. Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, US
3. 3729 Beechwood Blvd., Pittsburgh, PA, US

The activity of a pharmaceutical compound towards a protein target largely depends on its conformation. Any difference between the solution and bound conformation can reduce activity by an enthalpic or entropic penalty. The solution conformation of molecules of interest, in physiological or at least similar environment, therefore, is a key parameter to guide drug design.

NOE, chemical shift, and J-coupling can help answer some questions on conformation, especially for short distances, but RDC-analysis can inform about the overall conformation of a molecule. Such RDC measurements in medicinal chemistry are usually performed in $CDCl_3$ or DMSO-based gels, but a novel water-compatible gel we recently published can provide complimentary data from aqueous solution.[1]

1. Farley, K. A., Koos, M. R. M.,Che, Y., Horst, R., Limberakis, C., Bellenger, J., Lira, R., Gil-Silva, L. F., Gil, R. R., Angew. Chem. Int. Ed., 60, 26314, 2021.

33 Exploring weak interaction geometries in solution

Stefan Peintner and Máté Erdélyi

University Uppsala, Department of Chemistry, Uppsala, Sweden

Weak non-covalent interactions are inherently difficult to observe and quantify in solution. Despite the low abundance of the bound-form, weak interactions, i.e. hydrogen bonds, pi-pi, pi-NH interactions or halogen bonds, are vital for chemical reactions in metabolism, catalysis and ligand recognition.[1] Furthermore, the collaboration of weak interactions forming a cooperative network of structure stabilizing forces are substantial for the stabilization of secondary structures.

Here we disclose a method to analyse a single very weak (<2 kJ/mol) non-covalent interaction that is embedded into a cooperatively folding system.[2] The interaction under investigation is a halogen bond formed between a sp^2 carbon bound iodine and an ether oxygen that previously was impossible to detect by common titration techniques.[3] The model system conists of a peptide sequence that folds into a reversed beta-sheet structure held together by inter-strand hydrogen bonds. This preorganisation of the structures backbone promotes positioning of the halogen bond donor to acceptor on each side of the beta-strands. Conformational analysis of the model system and its reference (lacking this interaction) by NOESY buildup experiments paired with ensemble based data deconvolution revealed a 30% increase in folded beta-sheet content in the presence of a halogen bond as compared to the reference. To quantify the bond strength based on the detected increase in stability, we acquired the temperature dependent variation of selected chemical shifts and compared the melting curves of the folding-unfolding equilibria of both systems. Using the thermodynamic difference of the two compounds, we quantified the bond strength of the weak iodine-oxygen halogen bond to just below 1 kJ/mol. Entropy would make such a weak interaction undetectable in a standard intermolecular system. However, the peptidic system was designed to study the interaction of interest in an intramolecular setting in a cooperatively folding system, making detection of this weak interaction plausible. Further advantage of the preorganised sytem is its intermediate flexibilty that allows it to adopt a low degree of order in an alignment media, e.g. PBLG, to obtain RDCs for geometrical analysis directly at the halogen bond interaction site.[4]

The combination of NOE derived distances and RDC bond vectors to refine conformational ensembles to reveal the orientation of two flexible moieties that interact will be discussed. The presented method provides bond angle, interatomic distance as well as molar fractions of the conformers that possess a halogen bond, and hence is shown capable of the accurate description of the geometry and the dynamics of specific interaction sites of a flexible system at atomic resolution.

1. Alkorta I., Frontera A., *Crystals*, 10(3), 180, **2020**
2. Peintner S., Erdelyi E., *Eur. J. Chem.*, e202103559, **2022**
3. Beale T., Taylor M., *Chem. Soc. Rev.*, 42, 1667, **2013**
4. Navarro-Vázquez A., Williamson R. T., *Nat. Protoc.*, 14, 217-247, **2019**

34 Phosphate Form Spectroscopically Dark State Assembly Formation in Common Aqueous Solutions

Jiaqi Lu[1], Joshua Straub[2], Mesopotamia Nowotarski[2], Tanvi Sheth[2], Sally Jiao[2], Matthew P.A. Fisher[2], M. Scott Shell[2], Matthew E. Helgeson[2], Song-I Han[2], and Alexej Jerschow[1]

1. New York University, NYC, NY, US
2. University of California – Santa Barbara, CA, US

Phosphates and polyphosphates play ubiquitous roles in biology as integral structural components of cell membranes and bone, or as vehicles of energy storage via adenosine triphosphate and phosphocreatine. The solution phase space of phosphate species appears more complex than previously known. We present NMR based experiments that suggest phosphate species including orthophosphates, pyrophosphates and adenosine phosphates associate into dynamic assemblies in dilute solutions that are spectroscopically 'dark'.

^{31}P NMR is commonly used to characterize the composition, dynamics and structural properties of biomolecules and lipid interfaces. While performing ^{31}P NMR to investigate the native state of phosphate species as a function of temperature, we encountered peculiar line broadening and relaxation effects that cannot be explained by typical dynamical processes of small molecules (Fig. 1). We will present results showing that phosphate containing species reversibly assemble into unreported spectroscopically "dark" species that have much higher R_1 and R_2 rates and whose population is in exchange with the NMR-detectable phosphate species.

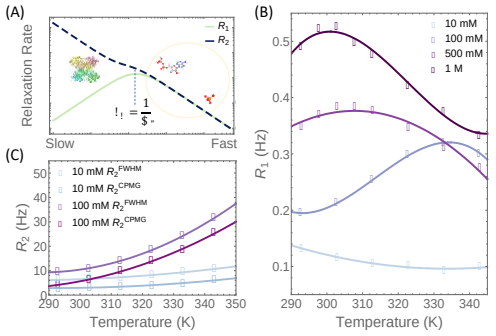


Figure 1. (A) Bloembergen-Purcell-Pound theory. (B) (C) R_1 and R_2 for various concentrations of potassium phosphate pH 4.5 as a function of temperature.

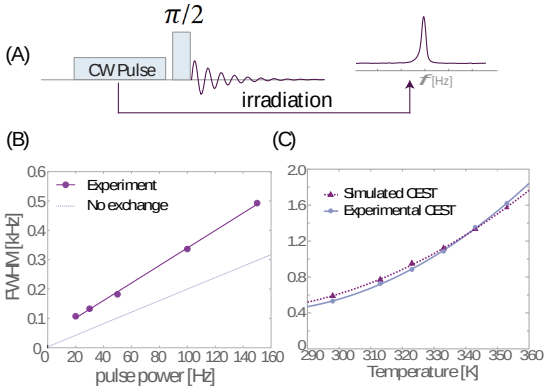


Figure 2. ^{31}P CEST results for 100mM orthophosphate (pH=4.5). (A) CEST pulse sequence (B) CEST z-spectra linewidth as a function of irradiation power at room temperature. (C) Experimental and Simulated CEST z-spectra linewidth as a function of temperature with 150Hz of irradiation power.

To further explore whether the phosphate species are in exchange with a spectroscopically dark population, we performed chemical exchange saturation transfer (CEST) experiments. CEST provides a means of identifying signatures of exchangeable species with distinct chemical shifts from the visible species, but far below NMR spectroscopic detection limits. This effect is achieved by saturating a selected region in the (spectroscopically invisible) spectrum, followed by the detection of the signal of a major species that is in exchange with the species below the NMR detection limit. Repeating these experiments with different saturation regions allows

scanning a whole spectrum for potentially exchanging species. This procedure can also be used to prove the existence of macromolecular pools with broad spectroscopic features, and has been employed, for example, to identify "dark" and weakly populated states in peptides and proteins.[1] Our CEST dip widths were found to be much wider than what one would expect from the spectral linewidth (by approximately a factor 2-3 larger than the rf saturation bandwidth) in the absence of exchange (Fig. 2B). The results indicate that exchange occurs with a population with a broad spectroscopic signature, invisible in direct spectroscopic measurements. This population appears to increase with temperature as shown by an increase in dip width (Fig. 2C), thereby supporting our hypothesis that orthophosphates assemble into assemblies, some of which are spectroscopically invisible but are in exchange with the detectable phosphate species.

The NMR-based observation is shown to be consistent with the formation of soft phosphate assemblies. The formation of spectroscopically dark states appears largely reversible (by cooling to room temperature), with a minor irreversible component. These findings suggest an entropically driven association mechanism, with enhanced populations and/or exchange rates at elevated temperatures. We show that assembly can be facilitated by the addition of depletants and modulated by changing counterion salt type, with trends found in line with the Hofmeister series. ^{31}P NMR Diffusion Oriented Spectroscopy (DOSY) revealed an increase in diffusion coefficients at elevated temperatures of 70 °C, indicative of dehydration.

This study presents the surprising discovery that phosphate-containing molecules ubiquitously present in the biological milieu can readily form dynamic assemblies largely invisible to NMR spectroscopy under a wide range of commonly used solution conditions, highlighting a hitherto unreported property of phosphate's native state in biological solutions.

1. Vallurupalli, P., Bouvignies, G. and Kay, L.E., Journal of the American Chemical Society 134, 8148-8161 (2012).

35 Evaluating Biosynthetic Engineering Campaigns with Atomic Sort

Brendan M. Duggan[1] and James J. La Clair[2]

1. Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, La Jolla, CA, USA
2. Chemistry Department, UC San Diego, La Jolla, CA, USA

As biosynthetic engineering has developed from concept to practice, it has become increasingly clear that companion tools and diagnostics are required to assess efforts to modify and direct cellular machinery to the desired outcome. Recently, we described the development of an atom based algorithm, Atomic Sort[1], to guide the discovery of novel materials within a microbial extract or fractionated extract. Modification of this algorithm to identify known compounds has allowed us to evaluate and direct several biosynthetic engineering campaigns. Notably, we were able to identify and quantify the desired products, as well as their precursors, fragments, and shunt products. Two case studies, explored for methods development and application, will be described.

Pladienolide B is a potent spliceosome inhibitor originally isolated from an engineered strain of *Streptomyces platensis*, Mer-11107 [2]. Since this strain was no longer available alternatives were sought. Three strains of *S. platensis* with different phenotypes, white, grey and black, were obtained and cultured as described previously [3]. Ethyl acetate extracts of whole cultures were used to record ^{1}H-^{13}C ASAP-HSQC spectra on a Bruker Avance III 600 MHz spectrometer fitted with a 1.7 mm triple resonance cryoprobe. Atomic Sort was able to identify Pladienolide B, and its olefinic precursor, and determine which strain was the most productive. Biosynthetic engineering efforts were then able to proceed using the strain producing the highest titer of Pladienolide B.

Didemnin B is a cyclic didepsipeptide well known for its anti-cancer properties [4]. Biosynthetic engineering is likely to afford easier access to it, and its analogues, than synthesis. To identify the optimal biosynthetic gene cluster, our team conducted a detailed analysis of the production of didemnin B within all known strains of the microbial producer, *Tistrella sp.* using the Atomic Sort approach. Didemnin B and only didemnin B (no congeners obtained) was identified in several strains along with peptide fragments of the target, and shunt products such as maculosin. Quantification directed further engineering efforts to maximise production.

Atomic Sort was able to not only identify entire molecules, but also, highlight areas of difference in closely related molecules. This is extremely useful when attempting to engineer modifications. We anticipate Atomic Sort will be a useful tool to evaluate biosynthetic engineering campaigns, and could also be applied to metabolomics studies and quality control of biologics.

1. Duggan, Brendan M., Reiko Cullum, William Fenical, Luis A. Amador, Abimael D. Rodríguez, and James J. La Clair. Angewandte Chemie International Edition 59, no. 3, 1144–1148, 2020.
2. Machida, Kazuhiro, Akira Arisawa, Susumu Takeda, Toshio Tsuchida, Yasuhide Aritoku, Masashi Yoshida, and Haruo Ikeda. Bioscience, Biotechnology, and Biochemistry 72, no. 11, 2946–2952, 2008

3. Sakai, Takashi, Tomohiro Sameshima, Motoko Matsufuji, Naoto Kawamura, Kazuyuki Dobashi, and Yoshiharu Mizui. The Journal of Antibiotics 57, no. 3, 173–179, 2004
4. Rinehart, Kenneth L., James B. Gloer, Robert G. Hughes, Harold E. Renis, J. Patrick McGovren, Everett B. Swynenberg, Dale A. Stringfellow, Sandra L. Kuentzel, and Li H. Li. Science 212, no. 4497, 933–935, 1981

36 Advances in liquid crystal and polymer gel weakly-aligning media. From organic solvents to ionic liquids

Armando Navarro-Vázquez,[1] Fernando Hallwass,[1] Daiane S. Carvalho,[1] Danilo G. B. da Silva,[1] José A. A. França, Gabriela P. Cavalcanti,[1] Higor D. F. Melo,[1] Juan Carlos Fuentes,[2] Andrei Leonov,[2] Christian Griesinger[2] and Ulrich Sternberg[3]

1. Departamento de Química Fundamental, CCEN,Universidade Federal de Pernambuco, Brazil
2. NMR based Structural Biology, MPI for Biophysical Chemistry, Germany.
3. Research Partner of Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

We will present recent advances from our group in the field or weakly aligning media applied to structural elucidation purposes incluing several examples of new lyotropic liquid crystals and polymer gels.

The preparation of new water compatible chromonic liquid crystals based on a bisperylene motif[1] and its application for RDC based conformational analysis of organic molecules as well as for enantiodiscrimination applications will be reported. Other new lyotropic systems prepared in our laboratory are graphene oxide systems with different types of polymers.[2]

Finally we will report new copolymeric gels compatible with polar organic solvents, such as acetonitrile and methanol as well as water and even ionic liquids. The use of fully deuterated gels for extraction of ^{1}H-RCSAs will be discussed as well as the application of these gels to the determination of molecular conformation and configuration. The extraction of RDC data in ionic liquids (ILs) swollen in polymer gels and their application to conformational analysis of IL cations, such as BMIM, using the tensor-free MDOC approach will be also discussed.[3]

1. da Silva, D. G. B.; Hallwass, F.; Navarro-Vázquez, A. Magn. Reson. Chem. 59(4), 408-413, 2021
2. Lei, X; Xu, Z.;Sun, H.; Wang, S.; Griesinger, C.; Peng, L.; Gao, C.; Tan, R. X. J. Am. Chem. Soc. 136(32), 11280-11283, 2014
3. Tvezkova, P.; Sternberg, U.; Gloge, T.; Navarro-Vázquez, A.; Luy, B. Chem. Sci. 10, 8874-8891, 2019.

37 LED-NMR insights into net photochemical carbon deletion of azaarenes

Samantha A. Burgess[1], Jisoo Woo[2], Mark Levin[2], Alec H. Christian[3], Yuan Jiang[1], and Umar Faruk Mansoor[3]

1. Analytical Research & Development, Merck & Co., Inc., Boston, MA, USA
2. Department of Chemistry, The University of Chicago, Chicago, IL, USA
3. Discovery Chemistry, Merck & Co., Inc., Boston, MA, USA

LED-NMR is an NMR technique that utilizes a light-emitting diode (LED) to deliver *in situ* illumination within an NMR spectrometer.[1,2] LED-NMR can provide structural insights into photochemical and photocatalytic reactions in addition to mechanistic and kinetic details. Furthermore, this technique allows for observation of reaction intermediates that only exist under irradiation conditions. *In situ* LED-NMR also provides access to a simple and convenient measurement of quantum yield using the NMR actinometers potassium ferrioxalate, *o*-nitrobenzaldehyde or 2,4-dinitrobenzaldehyde.[3]

We have recently reported a new synthetic method that yields a net carbon deletion of quinolines and related azaarenes through ring contraction; thus enabling chemists to hop directly between heteroaromatic scaffolds.[4] Specifically, this approach utilizes selective photochemical irradiation of quinoline *N*-oxide and related azaarenes with 390 nanometer light followed by acid rearrangement to yield *N*-acylindoles. This poster will highlight how *in situ* LED-NMR was utilized to monitor the transformation of 2-methyl-quinoline *N*-oxide to the corresponding benzoxazepiene. It will also describe how LED-NMR was used to measure a quantum yield of 0.096 for the conversion of 2-methyl-quinoline *N*-oxide.

1. Feldmeier, Christian, Bartling, Hanna, Riedle, Eberhard, Gschwind Ruth, M., *J. Magn. Reson.*(232), 39–44, 2013.
2. Ji, Yining, DiRocco, Daniel A., Kind, Jonas, Thiele, Christian M., Gschwind, Ruth M., Reibarkh, Mikhail, *ChemPhotoChem*(3), 984–992, 2019.
3. Ji, Yining, Hong, Cynthia, M., DiRocco, Daniel A., Wismer, Michael K., Reibarkh, Mikhail, *Org. Lett.*(20), 2156-2159, 2018.
4. Woo, Jisoo, Christian, Alec H., Burgess, Samantha A., Jiang, Yuan, Mansoor, Umar Faruk and Levin, Mark D., *Science*(376), 527–532, 2022.

38 Understanding Dynamic Exchange using Variable Field NMR

Jean-Paul Heeb, Craig Butts, Jonathan Clayden

Univeristy of Bristol

Variable temperature NMR (VTNMR) has long been an established method in small-molecule NMR to study the rate of exchange between nuclei exchanging conformationally or chemically. Despite the ability to quantify rates and activation barriers, several drawbacks limit the use of this technique across many fields. For example, it cannot easily be applied to the study of conformations of biological molecules with a relatively narrow window of temperature activity or stability. In contrast to VTNMR, chemical shift scaling (CSS) allows one to reach a coalescence point, and therefore extract a rate, by only changing the field strength.[1-3] In addition, application of CSS in concert with VTNMR enables several coalescence points (at different fields and temperatures) to be observed. This can then lead to Eyring plots with smaller errors and thereby more accurate estimations of enthalpies and entropies of activation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.

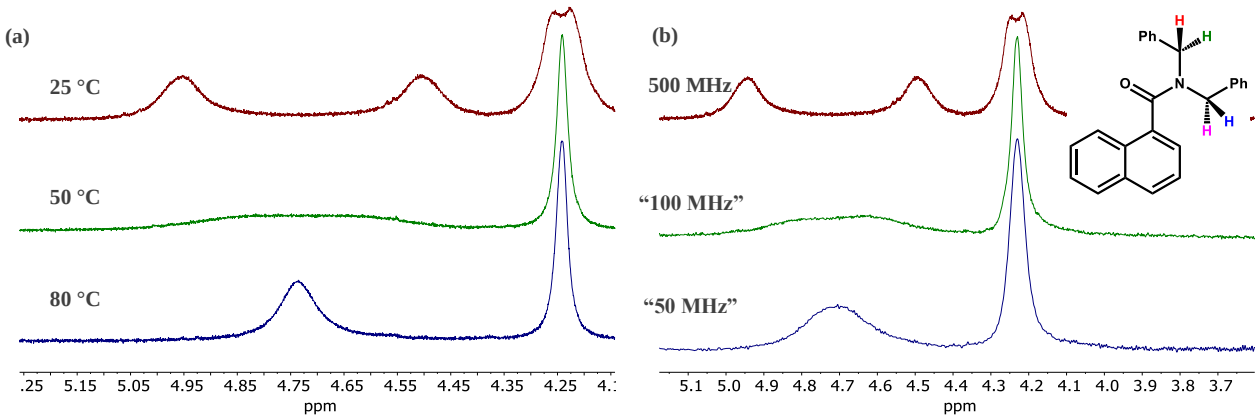


Figure 1. (a) Variable temperature NMR spectra of N,N-*dibenzylnaphthamide showing a coalescence point at 500 MHz and 50 °C. (b) Chemical shift scaled NMR spectra of the same compound, this time showing coalescence at "100 MHz" and 25 °C.*

Using *N*,*N*-dibenzylnaphthamide, we have demonstrated reliable shift scaling to 2% of the original frequency showing a coalescence point at 25 °C and 100 MHz (figure 1, compared to VTNMR alone at 50 °C and 500 MHz). By varying both the field and temperature as mentioned above, we have obtained several coalescence points and been able to extract activation energies matching that of the literature.[4] Current work is underway to apply CSS to more complex systems, such as biological molecules or dynamic foldamers, and extending the method to other nuclei.

1. Ellett, J. D.; et al. J. Chem. Phys. 51, 2851-2858, 1969.
2. DiVerdi, Joseph A.; et al. J. Chem. Phys. 75, 5594-5595, 1981.
3. Morris, Gareth A.; et al. Angew. Chem. Int. Ed. 42, 823-825, 2003.
4. Ahmed, A.; et al. Tetrahedron 54, 13277-13294, 1998.

39 FLIPS for Rapid T1 Estimation and its Application to Reaction Monitoring

Paul J. Bowyer[1], Iain J. Day[2], Tristan Maschmeyer[3], José Napolitano[3] and David Russell[3]

1. JEOL (UK) Ltd., JEOL House, 1-2 Silver Court, Watchmead, Welwyn Garden City, Herts AL7 1LT, UK
2. JASON Software Team, JEOL (UK) Ltd., 4 Bankside, Long Hanborough, Oxon, OX29 8SP, UK
3. Genentech Research and Early Development, 1 DNA Way, South San Francisco, CA 94080-4990

Reliable T_1 estimates are essential for a number of NMR applications, including quantitative NMR (qNMR), reaction monitoring, and other kinetics studies. Unfortunately, obtaining accurate T_1 estimates using conventional methods such as inversion-recovery (IR) can be time consuming, particularly when the T_1s are long and/or the signal intensities are low. This can be especially problematic for time-critical applications where monitoring of the sample needs to commence soon after the NMR sample has been introduced into the NMR spectrometer.

Recently, a method for T_1 estimation called FLIPS (Faster Longitudinal relaxation Investigated by Progressive Saturation)[1] has been published that provides around an order-magnitude reduction in experiment times compared to the IR method.

In this poster, we evaluate the FLIPS method by comparing T_1 values obtained using it and those obtained using IR. We demonstrate the application of FLIPS in the setting up and optimizing of ^{19}F-based reaction-monitoring experiments.

1. R. Wei, C. L. Dickson, D. Uhrín, G. C. Lloyd-Jones, *J. Org. Chem.* **2021**, 86, 9023−9029

40 Ranking mAb-Excipient Interactions in Biologics Formulations by NMR and Molecular Dynamics

Jonathan K. Williams[1], Chunting Zhang[1], Steven Gossert[1], Michael Little[1], Marilia Barros[1], Barton Dear[1], Bradley Falk[2], Ankit D. Kanthe[1], Luciano Mueller[2], Andrew Ilott[1] and Anuji Abraham[1]

1. Bristol Myers Squibb, Drug Product Development, New Brunswick, NJ, US
2. Bristol Myers Squibb, Discovery Chemistry Platforms, Lawrenceville, NJ, US

Small molecule excipients are added to biopharmaceutical formulations for enhancing protein stability and developing robust formulations which have acceptable physicochemical properties, but the mechanism by which they confer stability remains not fully understood[1]. NMR spectroscopy is a powerful and versatile tool within pharmaceutical product development which provides detailed information on molecular form and phase purity, molecular structure, and molecular interactions in both the solution and solid states. It is routinely applied to characterizing drug substance and drug product materials across the small molecule pipeline and is an increasingly routine characterization asset for large biologics projects. In this study, we aimed to elucidate the binding affinity of different small molecule excipients (sucrose, trehalose, mannitol, sorbitol, succinic acid and glycine) to a monoclonal antibody (BMS-mAb) by observing the NMR saturation transfer difference (STD) effect on the small molecule. Molecular dynamics (MD) simulations were used in parallel to determine the most probable binding interfaces of each excipient on the BMS-mAb. The results, along with measurements of thermal stability (Tm) and colloidal stability (B22), were used to determine a rank-order of the excipients to increase conformational stability of the BMS-mAb. Our STD-NMR results show that sucrose has specific binding and is the best stabilizer of the BMS-mAb, followed by trehalose, mannitol and sorbitol. The strong correlation between degree of binding by STD-NMR, Tm, and B22 for the different excipients suggests that strong excipient binding is the likely cause of improved protein stability. In general, close proximity of the excipient to the mAb may also provide better conformational stability to the mAb, as in the case of non-specific binding interactions, but the exact mechanism for this increase in stability needs to be examined further. Our hope is that by correlating STD-NMR experiments and MD simulations to traditional measurements of Tm and B22, we can help to aid and accelerate the excipient selection process of future biologic formulations by providing direct evidence of stabilizing mAb-excipient affinities before beginning the conventional large and time-consuming excipient screening studies.

41 Reaction Monitoring of Pressurized Systems in Real Time by NMR

Breanna Conklin, Jose Napolitano, Kenji Kurita, David Russell, and Jacob Timmerman

Genentech, South San Francisco, CA, US

High pressure NMR was used to investigate the mechanism of a enantioselective hydroformylation reaction. *In situ* reactions were analyzed using various pressures, ranging from 20psi to 450psi, to determine the reaction conditions required to produce the desired product. The simultaneous acquisition of ^{1}H and ^{31}P spectra during these reactions allowed to further identify and measure intermediates, and impurities including diastereomers and regio isomers of the reaction. These results clarified the variability and descrepencies from available offline results. Live reaction monitoring showed that catalyst scavenging after the reaction was essential to preserve the desired product. The data presented will show how this study aided in optimizing the scale up of this reaction.

42 400 MHz Cryofree NMR Technology – Leveraging High Field NMR in a Benchtop NMR Setting

Ikenna E Ndukwe[1], Kyle Quasdorf[2], James Murray[2], Armando Navarro Vasquez[2] and Maria Victoria Silva Elipe[1]

1. Department of Attribute Sciences, Amgen Inc., Thousand Oaks, California 91320-1799, United States.
2. Pivotal and Commercial Synthetics, Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, California 91320-1799, United States
3. Departamento de Química Fundamental, Universidade Federal de Pernambuco, Avenida Professor Moraes Rego, 1235, Cidade Universitária, 50670-901 Recife, PE, Brazil.

Key drivers that facilitates the utilization of benchtop nuclear magnetic resonance (NMR) spectrometers for a wide-range of analytical applications in the pharmaceutical, agrochemical and battery industries, analogous to applications with mass spectrometry, high performance liquid chromatography and infrared/ultraviolet spectroscopy, include features such as compactness, mobility and low maintenance costs. Benchtop NMR spectrometers are especially useful in the chemistry laboratory for online or atline reaction monitoring processes and could potentially play a role in sterochemistry control strategy for some chemical reactions in biophamaceutical production plants. However, the ever-expanding complexities of molecular scaffolds being generated in the pharmaceutical industry that require high spectral resolution and/or complex NMR experiments, which are not currently available in the benchtop NMR software, may be limiting. The recently developed high temperature superconducting (HTS) magnet technology, which provides compact power-driven cryogen-free magnets, potentially bridges this gap [1-3]. The compact nature of the HTS magnet allowed the incoporation of a 400 MHz spectrometer into a chemistry laboratory fumehood, thus opening up a myriad of possibilities for both chemists and NMR spectroscopists [3-7]. The benefits derivable with this high field NMR spectrometer setup (in a benchtop setting), will be showcased with examples from reaction monitoring and structure elucidation projects. In particular, we will highlight our work on the application of anisotropic NMR data, acquired on the 400 MHz HTS magnet spectrometer, for stereochemical analysis of complex macrocycles, including AMG 176 [8].

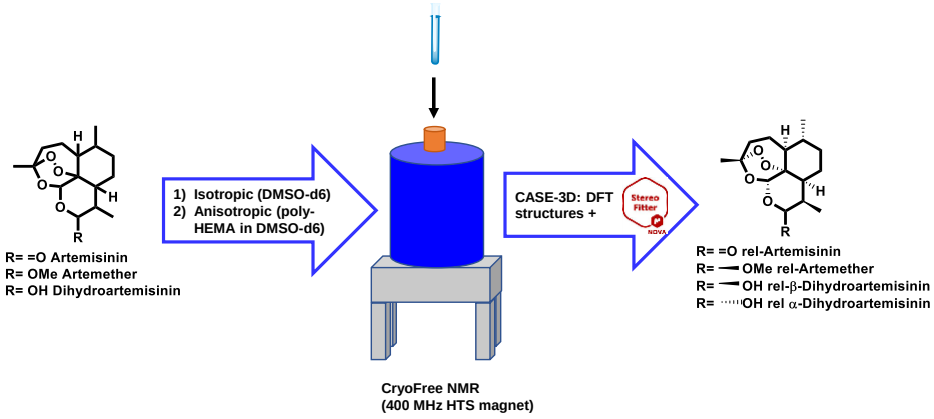
1. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Magn. Reson. Chem*. 2018, 56 (9), 817-825. DOI: 10.1002/mrc.4740
2. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Instr. Sci. Technol.* 2019, 47 (2), 195-212. DOI: 10.1080/10739149.2018.1516226
3. Silva Elipe, MV, Cherney, A., Krull, R., Donovan, N. Tedrow, J., Pooke, D., Colson, K.L., *Org. Process Res. Dev.* 2020, 24 (8), 1428-1434. DOI: 10.1021/acs.oprd.0c00125
4. Murray J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Quasdorf, K., Caille, S., *Org. Process Res. Dev.* 2020, 24 (8), 1523-1530. DOI: 10.1021/acs.oprd.0c00256
5. Murray, J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Brown, D., Quasdorf, K., Caille, S., *J. Org. Chem.*, 2021. DOI: doi.org/10.1021/acs.joc.1c00886
6. Murray, J.I., Brown, D., Silva Elipe, M.V., Caille, S., *Org. Process Res. Dev.*, 2021. DOI: 10.1021/acs.oprd.1c00131
7. Quasdorf, K.W., Murray, J.I., Nguyen, H., Silva Elipe, M.V., Ericson, A., Kircher, E., Guan, L., Caille, S., *Org. Process Res. Dev.*, 26, 458-466, 2022. DOI: 10.1021/acs.oprd.1c00469
8. Caenepeel S *et. al., Cancer Discov*. 2018 Dec;8(12):1582-1597. doi: 10.1158/2159-8290.CD-18-0387. Erratum in: Cancer Discov. 2019 Jul;9(7):980. PMID: 30254093

43 Evaluating the Performance of a 400 MHz Cryogen-free NMR Spectrometer for Measurement of Anisotropic NMR data

Ikenna E. Ndukwe[1], Armando Navarro Vasquez[2] and **Maria Victoria Silva Elipe[1]**

1. Department of Attribute Sciences, Amgen Inc., Thousand Oaks, California 91320-1799, United States
2. Departamento de Química Fundamental, Universidade Federal de Pernambuco, Avenida Professor Moraes Rego, 1235, Cidade Universitária, 50670-901 Recife, PE, Brazil

The recent discovery and development of the high temperature superconducting (HTS) magnet technology, which provides power-driven cryogen-free magnets, has enabled the successful incoporation of a 400 MHz nuclear magnetic resonance (NMR) spectrometer into a hood within our chemistry laboratory [1-3]. This spectrometer setup has improved NMR access to chemists who require NMR methodologies for a variety of tasks including structure identification and verification, and reaction monitoring [3-7]. The stability of the HTS magnets over time and their performance with complex NMR pulse sequence experiments are the main unknown factors of this new technology. It is therefore essential to determine the performace of the cryogen-free NMR spectrometer for acquisition of complex NMR data. In a previous study [2], the perfomance of the HTS magnets for acquisition of commonly used 1D and 2D NMR experiments was shown to be comparable to an equivalent conventional NMR spectrometer built with a low temperature superconducting magnet that uses liquid cryogens. In the present study, the performance of the HTS magnets for acquisition of NMR data in anisotropic media, using a poly-HEMA (poly-2-hydroxyethyl methacrylate) gel in a compression devise [8], will be evaluated with three commercially available natural products, artemisinin, artemether and dihydroartemisinin. The anisotropic NMR data acquired, residual dipolar coupling (RDC) in particular, is analysed with the computer-assisted structure elucidation (CASE-3D) protocol [9] implemented in Mestrenova software and tested against DFT (density functional theory) optimized conformers of the corresponding diasteromers of artemisinin, artemether and dihydroartemisinin,



respectively. In all cases, the correct diastereomer was consistently selected by the CASE-3D program as the best ranked structure when both RDCs and chemical shift values are combined in the input file, except

for the epimers of dihydroartemisinin that required two additional J_{HH} coupling constants to ensure satisfactory discrimination against the second ranked diastereomers.

1. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Magn. Reson. Chem*. 2018, 56 (9), 817-825. DOI: 10.1002/mrc.4740
2. Silva Elipe, M.V., Donovan, N., Krull, R., Pooke, D. Colson. K., *Instr. Sci. Technol.* 2019, 47 (2), 195-212. DOI: 10.1080/10739149.2018.1516226
3. Silva Elipe, MV, Cherney, A., Krull, R., Donovan, N. Tedrow, J., Pooke, D., Colson, K.L., *Org. Process Res. Dev.* 2020, 24 (8), 1428-1434. DOI: 10.1021/acs.oprd.0c00125
4. Murray J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Quasdorf, K., Caille, S., *Org. Process Res. Dev.* 2020, 24 (8), 1523-1530. DOI: 10.1021/acs.oprd.0c00256
5. Murray, J.I., Silva Elipe, M.V., Cosbie, A., Baucom, K., Brown, D., Quasdorf, K., Caille, S., *J. Org. Chem.*, 2021. DOI: doi.org/10.1021/acs.joc.1c00886
6. Murray, J.I., Brown, D., Silva Elipe, M.V., Caille, S., *Org. Process Res. Dev.*, 2021. DOI: 10.1021/acs.oprd.1c00131
7. Quasdorf, K.W., Murray, J.I., Nguyen, H., Silva Elipe, M.V., Ericson, A., Kircher, E., Guan, L., Caille, S., *Org. Process Res. Dev.*, 26, 458-466, 2022. DOI: 10.1021/acs.oprd.1c00469
8. Gayathri, C.; Tsarevsky, N. V.; Gil, R. R. Chem. - Eur. J. 2010, 16, 3622 DOI: 10.1002/chem.200903378
9. Navarro-Vaìzquez, A., Gil, R. R. and Blinov, K., *J. Nat. Prod.* 2018, 81, 203−210. DOI: 10.1021/acs.jnatprod.7b00926

44 Improvements in Flow-Injection NMR as a Tool for High-Throughput Sample Analysis

Paul Krolikowski[1], L. Steven Hollis[2], Roger Kautz[3], and David Strand[4]

1. Molecular Structure, Amgen Inc., 360 Binney St., Cambridge, MA 02142
2. Molecular Structure, Amgen Inc., 360 Binney St., Cambridge, MA 02142 (Retired)
3. Barnett Institute at Northeastern University, Boston, MA 02115
4. Protasis Corporation, Marlboro, MA 01752

Dramatic improvements in S/N have been obtained using customized flow-NMR instrumentation by incorporating small volume flow-cells (10-120 µL) with a high-sensitivity 600 MHz 5-mm cryoprobe and a commercially available sample delivery system. The large sensitivity gain provided by cryoprobe technology has dramatically reduced data collection times to a point where routine ^{1}H and g-HSQCdept spectra can be collected on small samples (25 µL at 20 mM) in under 10 minutes. These hardware improvements have allowed implementation of rapid quantitative-NMR analysis as part of our high throughput purification process (HTPP). In addition, new flow-cell inserts have been designed which easily can be interchanged to alter sample volume or switched from flow to tube-based operations in with minimal setup time using a standard pass-through cryoprobe. The sample delivery station and operating system provide a flexible and convenient platform for submitting samples in 96-well plate format, or as single samples in walk-up mode. Automated data processing provides a reliable solution for archiving and distributing the large amounts of processed data that are generated from plate-base HTPP submissions. In addition, segmented-flow NMR methods (SFA), using a susceptibility matched push-solvent (FC-43) to limit sample dispersion in the flow cell, are also being investigated in an effort to improve S/N with mass-limited samples. The results of these studies show up to a two-fold improvement in S/N compared to single-solvent push techniques. The culmination of these improvements and the addition of HSQC data, in a timely fashion, allows for more effective use of auto-structure verification for sample libraries.

45 SUN: Band-Selective Suppression of Unwanted Signals in Complex Mixtures

Elin Alexandersson, Corine Sandström, Lena Lundqvist, and Gustav Nestor

Department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Biological samples, e.g. plant extracts, blood, and urine, typically contain numerous different metabolites with large variations in concentration. When such samples are analyzed with NMR spectroscopy, the resulting spectra are often very complicated with severe spectral overlap and dynamic range problems. This may obstruct the identification and analysis of certain metabolites, in particular those of low concentration. To study low-abundant metabolites, sample pre-treatment such as chromatography is therefore often needed before the NMR analysis. However, this is undesirable as it is time-consuming, costly and disturbs the integrity of the sample.

We have developed an NMR experiment called SUN (Suppression of UNwanted signals) that can be applied to intact samples and allows signals from abundant or otherwise problematic compounds to be removed from selected regions of NMR spectra [1]. Other signals in the selected spectral region are retained, provided that they are *J*-coupled to at least one signal located outside of the targeted region. Thus, SUN is useful for identifying and characterizing low-abundant compounds in samples containing other compounds in high concentration. The approach has been successfully applied to various different samples containing large amounts of sugar, including artificial mixtures, orange juice, and plant samples.

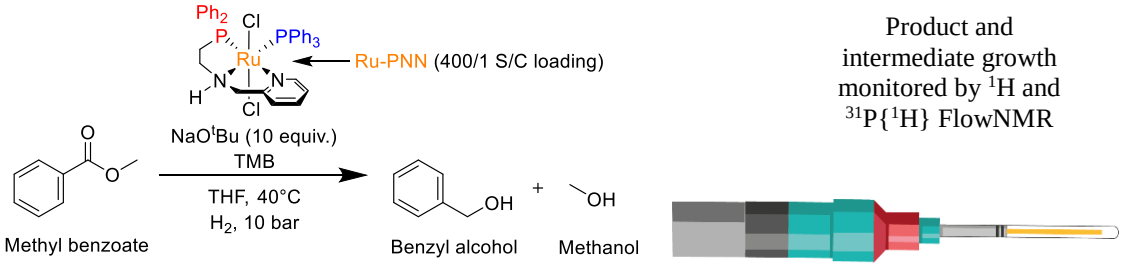
1. Alexandersson, Elin; Sandström, Corine; Lundqvist, Lena C.E.; Nestor, Gustav, RSC Advances, 10(54), 32511–32515, 2020

46 Insight into ester hydrogenation catalysis using multinuclear high field FlowNMR

Owen Harper,[1] Damian Grainger,[2] Antonio Zanotti-Gerosa,[2] Catherine Lyall,[3] John Lowe,[3] Ulrich Hintermair*[1,3]

1. Centre for Sustainable and Circular Technologies, University of Bath, Claverton Down, BA2 7AY, UK.
2. Johnson Matthey, 28 Cambridge Science Park, Cambridge, CB4 0FP, UK.
3. Dynamic Reaction Monitoring Facility, University of Bath, Claverton Down, BA2 7AY, UK.

The reduction of esters to alcohols is an important transformation in chemical manufacturing for pharmaceutical drug design, perfumes, surfactants, biofuels, and fine chemical intermediates.[1] Current methods employ use of stoichiometric hazardous metal hydrides ($LiAlH_4$)[2] or heterogeneous catalysts requiring harsh reaction conditions (>270 °C, 80 bar H_2)[3]. Pincer complexes such as Gusev's PNN catalyst have been recently designed which can perform reductive ester cleavage at low temperatures and pressures thus offering a milder and more sustainable alternative.[4] However, the mechanism behind these homogeneous catalysts remains largely unknown. We have utilized *operando* techniques such as multi nuclear FlowNMR[5] in order to gain insight into the mechanistic details of the hydrogenation of methyl benzoate to benzyl alcohol using the PNN catalyst, and have observed and characterized new derivatives that are key to understanding of the reduction process. Selective excitation of hydrides has also led to observation of multiple species of interest such as a dimer similar to studies on related ester hydrogenation pincer catalysts.[6]



1. J. Pritchard, G. Filonenko, R. Van Putten, E. Hensen and E. Pidko, *Chem. Soc. Rev.*, **2015**, 44, 3808.
2. L. Saudan, in *Sustainable Catalysis: Challenges and Practices for the Pharmaceutical and Fine Chemical Industries*, P. Dunn, K. Hii, M. Krische and M. Williams, John Wiley & Sons, **2013**, 37–61.
3. Y. Pouilloux, F. Autin and J. Barrault, *Catal. Today*, **2000**, 63, 87–100.
4. D. G. Gusev, *ACS Catal*. **2016**, 6, 10, 6967–6981.
5. A. M. R. Hall, J. C. Chouler, A. Codina, P. T. Gierth, J. P. Lowe and U. Hintermair, *Catal. Sci. Technol.*, **2016**, 6, 8406–8417.
6. L. Dawe, M. Karimzadeh-Younjali, Z. Dai, E. Khaskin, and D. Gusev, *J. Am. Chem. Soc.* **2020**, 142, 46, 19510–19522.

47 Searching Libraries of Known Structures for Dereplication: Benefits and Requirements

Karl Demmans, Dimitris Argyropoulos, Mary McKee, Rostislav Pol and Sergey Golotvin

Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada

A very important question that natural products chemists face is whether the newly isolated compound they have is truly novel or already known. This process, commonly referred to as dereplication, is also performed by people doing competitive product analysis, drug counterfeit analysis, reaction discovery, etc. There are several methods used to accomplish dereplication, including a comparison of the retention time and observed molecular ion from LC-MS, and a comparison of the NMR spectrum to spectral/structure libraries. When using NMR data in dereplication, ^{13}C NMR spectra are preferred [1] in most cases, as they provide a very clear fingerprint of the compound's carbon skeleton. We have previously explored the benefits of using databases with predicted spectra of compounds. Currently, there are several options in this respect, both commercial [2] and freely available [3-4]. In this poster, we explore the capabilities and requirements of such systems.

In order to establish a starting point, we selected 56 compounds from the Aldrich library of FT-NMR spectra, with a molecular weight (MW) range of 150-800, where most pharmaceutically active compounds are found. We then searched the library containing the predicted ^{13}C spectra of these compounds using the observed experimental peak frequencies together with the MW.

We explored the search options with respect to inclusion/exclusion of the MW information, together with the requirement to accept or reject hits with extra or missing peaks in the experimental spectrum. We saw that the MW information is essential as it provides a very clear starting point and deals effectively with symmetric compounds. We also saw that as the MW increases, the uncertainty and the number of missing or extra peaks increase as well. However, with careful adjustment of the search parameters, the correct result can be identified within a few seconds.

Detailed results will be presented, alongside an optimized workflow that allows one to unambiguously find the correct structures in the database in all cases.

1. Williams, R. B., O'Neill-Johnson, M., Williams, A. J., Wheeler, P., Pol, R., Moser, A., Org. Biomol. Chem., 13, 9957-9962, 2015
2. Argyropoulos, D., Golotvin, S., Pol, R., Moser, A., Ortel, N., Breinlinger, S., Chizuk, T., Niedermeyer, T., "Efficient Dereplication of Natural Poducts Using Predicted ^{13}C NMR Spectra" presented at 60th ENC, Poster 004, 2019
3. Kalchhauser, H., Robien, W., Chem. Inf. Comput. Sci. 25(2), 103-108, 1985
4. Reher, R., Kim, H. W., Zhang, C., et al., J. Am. Chem. Soc., 142(9), 4114-4120, 2020

48 NMR Crystallography: Bridging Atomic-Level Structural Rearrangement and Macroscopic Motion in Molecular Organic Crystals

Kevin Chalek, Xinning Dong, Fei Tong, Rabih Al-Kaysi, Joshua D. Hartman, Gregory J.O. Beran, Christopher J. Bardeen, and Leonard J. Mueller

Department of Chemistry, University of California, Riverside, California 92521

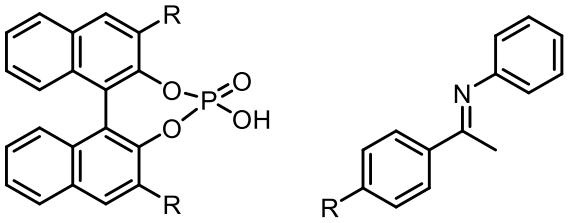
Crystals composed of photoreactive molecules represent a new class of photomechanical materials with the potential to generate large forces on fast timescales. An example is the photodimerization of 9-tertbutyl-anthracene ester (**9TBAE**) in molecular crystal nanorods that leads to an average elongation of 8%. This expansion results from the formation of a metastable crystalline intermediate termed the solid-state reacted dimer (SSRD). Photoreaction of bulk single crystal monomer invariably leads to strain that shatters the crystal, precluding direct characterization with single crystal X-ray diffraction. Here, the combination of powder X-ray diffraction, solid-state nuclear magnetic resonance, and first principles computational modeling is used to determine the crystal structure of the SSRD intermediate and establish a microscopic model for the macroscopic expansion. We find that the SSRD crystal unit cell and volume are quite similar to those of the monomer crystal, leading to the conclusion that gross changes in the volume or unit cell parameters of the SSRD are not responsible for the expansion. At the same time, solid-state NMR of the aligned monomer nanorods and the photoreacted product shows the generation of new lattice orientations within the nanorod. Based on our observations, the nanorods expand not due to a change in the volume of the unit cell, but rather due to an anisotropic rearrangement of the molecular contents. The ability to understand quantitatively how molecular-level photochemistry generates mechanical displacements allows us to predict that the expansion could be tuned from +9% to -9.5% by controlling the initial orientation of the unit cell with respect to the nanorod axis. This application of NMR-assisted crystallography provides a new tool capable of tying the atomic-level structural rearrangement of the reacting molecular species to the mechanical response of a nanostructured sample.

49 NMR Investigations on the photoisomerization of imines in presence of chiral phosphoric acids

Christian Scholtes and Ruth M. Gschwind

Universität Regensburg, Regensburg, Bavaria, Germany

Since their first introduction chiral phosphoric acids (CPAs) proofed to be widely applicable catalysts in asymmetric reductions [1]. Our group has been particularly interested in the asymmetric reduction of imines with Hantzsch ester in presence of CPAs [2].



CPA **Imine**

In the last years we gathered a bunch of insights on the concerning reaction mechanism and the corresponding transient states [3, 4]. Generally, inside of the CPA the Hantzsch ester can reduce the imine from above and from below. Additionally, using stated reaction conditions both the (*E*)- and the (*Z*)-isomer are present in the reaction. The combination of the circumstances provides four possible transient states, which affect the stereocenter of the resulting amine. Theoretically and experimentally, it has been shown that the most reactive transient states are Type I and II Z (Figure 6) [4, 5].

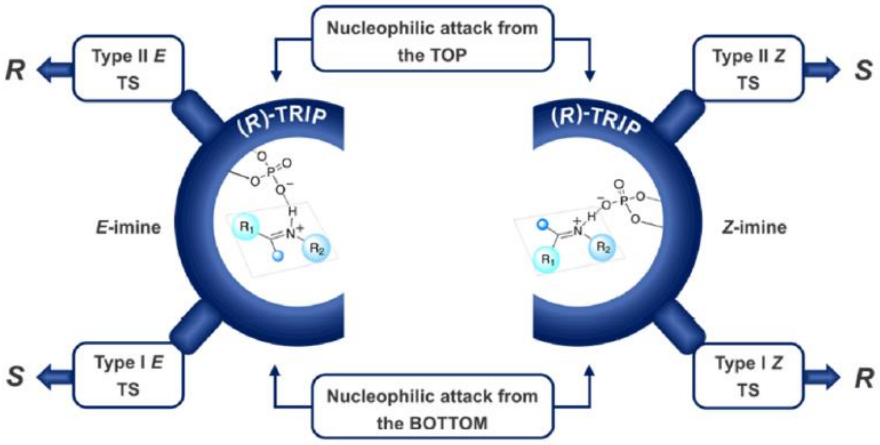


Figure 6: Theoretically possible transient states upon reduction of imines by Hantzsch ester in presence of a CPA. Both (E)- and (Z)-imine can be attacked by the Hantzsch ester from below and from above [4].

To gain insights on the active transient state via NMR our group developed DTS-hν (deciphering transient states by light) [4, 6]. Illuminating the reaction with our *in-situ* setup with 365 nm at 220 K increases the amount of (*Z*)-imine. Depending on the resulting ee and the reaction rate compared to the dark reaction we can conclude which transient states are mainly active. Having achieved this in accordance with theoretical data [4–6], we were curious about the isomerization itself. To date the suggested reaction mechanism assumed the possible photoisomerization of the imine inside of the complex, since both in absence and in presence of a CPA photoisomerization with 365 nm is observable. However, it is possible that the imine first needs to leave the sterically hindered complex, before isomerization is possible. Until now we gathered empirical data which suggest a strong influence of ion pairs and hydrogen bonds regarding the photoisomerization in presence of a CPA. These studies were carried out by NMR investigations using *in-situ* illumination and designing the experiment regarding our knowledge about strong hydrogen bonds between the imine and the CPA [2]. Additionally, we demonstrate that inside of the ternary complex formed by a CPA, unreactive Hantzsch ester and imine, no photoisomerization is observable.

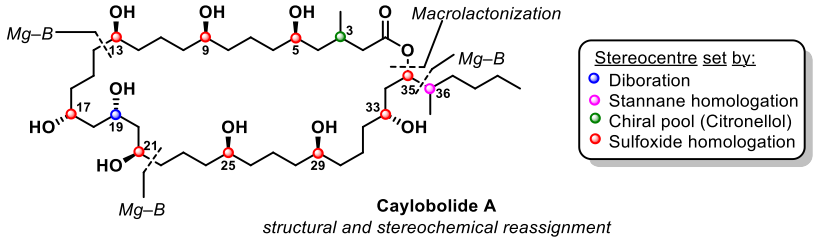
1. Terada, Masahiro, Synthesis(12), 1929–1982, 2010
2. Rothermel, Kerstin; Melikian, Maxime; Hioe, Johnny; Greindl, Julian; Gramüller, Johannes; Žabka, Matej; Sorgenfrei, Nils; Hausler, Thomas; Morana, Fabio; Gschwind, Ruth M., Chem Sci(43), 10025–10034, 2019
3. Melikian, Maxime; Gramüller, Johannes; Hioe, Johnny; Greindl, Julian; Gschwind, Ruth M., Chem Sci(20), 5226–5234, 2019
4. Renzi, Polyssena; Hioe, Johnny; Gschwind, Ruth M., J Am Chem Soc(19), 6752–6760, 2017
5. Simón, Luis; Goodman, Jonathan M., J Am Chem Soc(27), 8741–8747, 2008
6. Renzi, Polyssena; Hioe, Johnny; Gschwind, Ruth M., Acc Chem Res(12), 2936–2948, 2017

50 Ultra-high-resolution NMR and Total Synthesis; Uncovering the Stereochemistry of Caylobolide A

Malcolm R. P. George[1], Craig P. Butts[1] and Varinder K. Aggarwal

University of Bristol, Department of Chemistry, Bristol, UK

Caylobolide A is a 36 membered polyhydroxylated macrolide originally isolated from Bahamian cyanobacterium *Lyngyba Majuscula* by Molinski et al., later reisolated by Tidgewell et al.[1,2] The key challenge with Caylobolide A - and other structurally related macrolides - are their structural and stereochemical assignments, as a result of their distal 1,5-polyol moieties giving rise to near-degenerate frequencies in both ^{1}H and ^{13}C NMR.[3]



Caylobolide A
structural and stereochemical reassignment

Herein we present the structural and stereochemical reassignment of Caylobolide A, achieved through the utilization of ultra-high-resolution NMR techniques. Pureshift-HSQC and HSQC-TOCSY spectra enabled the unique, unambiguous determination of all proton and carbon chemical shifts of the molecule. Derivatization to the *(S)*- and *(R)*-Mosher esters was then performed, with subsequent analysis leading to the identification of absolute stereochemistry of all nine hydroxylated stereogenic centres.[4]

For the elucidation of stereocentres C3, C35 and C36 of Caylobolide A, a "mixture method" total synthesis was proposed, whereby two known diastereomeric mixtures will be synthesized, containing the eight remaining potential stereoisomers.[5] Direct NMR comparison to the genuine synthetic sample will reveal the absolute stereochemistry of Caylobolide A, through entirely NMR-based methods. This approach requires extremely high stereocontrol, which can be achieved through the repeated homologation of boronic esters with a homoallylic carbenoid followed by hydroboration to install 1,5-related stereocentres. Sequential stereospecific oxidation reveals the 1,5-polyol functionality, with virtually perfect stereocontrol.[6, 7]

1. J. B. MacMillan and T. F. Molinski, *Org. Lett.*, 2002, **4**, 1535-1538.
2. Solomon, K. (2020). Isolation, Structure Elucidation, and Synthesis of Natural Products from Marine Cyanobacteria (Master's thesis, Duquesne University). Retrieved from https://dsc.duq.edu/etd/1918
3. G. K. Friestad and G. Sreenilayam, *Pure Appl. Chem.*, 2011, **83**, 461-478.
4. J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512-519.
5. J. Wu, P. Lorenzo, S. Zhong, M. Ali, C. P. Butts, E. L. Myers and V. K. Aggarwal, *Nature*, 2017, **547**, 436-440.
6. G. Casoni, M. Kucukdisli, J. M. Fordham, M. Burns, E. L. Myers and V. K. Aggarwal, *J. Am. Chem. Soc.*, 2017, **139**, 11877-11886.
7. D. Fiorito, S. Keskin, J. M. Bateman, M. George, A. Noble and V. K. Aggarwal, *J. Am. Chem. Soc.*, 2022, **144**, 7995-8001.

51 Probing Structure and Dynamics of the Ipglycermide Ce-2d Analogue by NMR Spectroscopy

Samuel A. Kotler, Isabella Jacobsen, Liza Kanter, Christopher D. Collmus, James Inglese, and Christopher A. LeClair

National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, U.S.A.

Ipglycermide Ce-2, a lariat-like cyclic peptide, was discovered and identified by Yu *et al.* [1] as a potent inhibitor of cofactor-independent phosphoglycerate mutase (iPGM), which facilitates intramolecular transfer of the phosphoryl group through a phosphoserine intermediate. As the only PGM found in nematodes, this presents a highly selective anthelmintic drug target since the silencing of iPGM in *C. elegans* and *B. malayi* causes nematode death. The co-crystal structure of iPGM with Ce-2d, a truncated analogue of Ce-2 with retained potency, revealed the cyclic peptide structure was highly compact when bound to the hinge domain of the protein (PDB code 5KGN) [1]. Conformational studies using 2D NOESY experiments presented a near lack of NOE interactions between the macrocyclic core and the appendant linear chain suggesting the structure has an expected greater flexibility in solution as compared to its crystalline form. Additionally, an unexpected minor isomer was observed in a 4:1 ratio with the major isomer. These data compelled us to determine the 3D structure of free Ce-2d in solution for comparsion of the preferred solution conformations with the co-crystal structure confirmation. The Ce-2d analogue has a well dispersed 1D ^{1}H NMR spectrum possessing the requisite number of backbone amide-proton (NH) peaks expected for the major isomer. As such, a complete assignment of ^{1}H and ^{13}C NMR peaks of the major conformer was obtained using a suite of 1D and 2D NMR experiments confirming the proposed structure of Ce-2d. An observed second set of NH peaks with low signal intensity led to the hypothesis of a minor population of a Ce-2d conformer and prompted the collection of extensive NOE data for Ce-2d to calculate 3D structures by NMR using the Xplor-NIH program [2]. A 3D structural ensemble of Ce-2d was determined with the ten lowest-energy structures having a pairwise backbone RMSD of 1.5 Å. In addition, variable temperature (VT) NMR experiments were conducted with coalescence observed between corresponding peaks of the major and minor conformers resulting in a single set of broadened peaks at 60 °C. VT NMR was also used to determine amide temperature coefficients, a parameter sensitive to hydrogen bonding in peptides and proteins providing qualitative structural information. Importantly, temperature coefficients for NH peaks of Tyr-3 and Asp-6 are greater than -4.6 ppb/K, indicating hydrogen bond stabilization of the cyclic peptide. It is postulated that a stabilizing hydrogen bond between Tyr-3 and Asp-6 of Ce-2d exists, the disruption of which should preclude any observation of the crystal structure Ce-2d conformation in its free form. Collectively, these initial NMR studies suggest the structure of free Ce-2d primarily exists in an extended, dynamic conformational ensemble. Further studies are ongoing employing analogues of Ce-2 with rationally designed mutations and truncations to probe the stabilizing structural elements of key Ce-2d conformers.

1. Yu, H., Dranchak, P., Li, Z., MacArthur, R., Munson, M. S., Mehzabeen, N., Baird, N. J., Battalie, K. P., Ross, D., Lovell, S., Carlow, C. K. S., Suga, H., and Inglese, J. *Nat. Commun.* **8**, 14932, 2017.

2. Schwieters, C. D., Kuszewski, J. J., Tjandra, N., and Clore, G.M. *J. Magn. Reson.* **160**, 65–73, 2003.

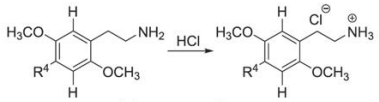
52 Benchtop NMR and its applications in Forensic Science

Juan F. Araneda, Alex F. G. Maier, and Susanne D. Riegel

Nanalysis Scientific Corp., Calgary, Alberta, Canada

Historically, law enforcement officers and the forensic community have relied primarily on the use of chemical tests, such as Nik Wipes, optical spectroscopy, such as handheld raman spectrometers, and/or gas chromatography-mass spectrometry (GC-MS) to identify illegal drugs. However, the drastic increase in designer drugs and new psychoactive substances (NPS) over the last decade has made the detection and identification of new classes of drugs extremely difficult. While not as common in drug detection, NMR Spectroscopy could provide value in identification and purity determination as it provides non-destructive, rapid analysis with a wide dynamic range that does not require blank runs, large amounts of flammable solvents nor the preparation of a calibration curve. One of the main reasons that high-field, superconducting NMR technology was overlooked in this regard, was the capital cost, the size and siting requirements and the expertise required to operate the traditional NMR instrumentation.

The emergence of permanent magnet-based benchtop NMR spectrometers promises a way to address affordability, maintenance, and ease-of-use concerns. These instruments provide an interesting NMR alternative to identify, quantify, and differentiate drug substances in a laboratory setting or directly at crime scenes, customs, border protection, etc. We are currently working on automated identification of new designer drugs via cloud-assisted database comparison of NMR data of seized unknowns. In this poster we will present the work that we have done in order to characterize 7 designer drugs commonly known as members of the 2C-X series by their 1D ^{1}H 60 MHz NMR spectra. We will also present some of the work that we have done to quantify MDMA in ecstasy tables employing ethylene carbonate as internal calibrant at 60 MHz.



R^4	trivial name	number
Br	2C-B	**1**
Cl	2C-C	**2**
Me	2C-D	**3**
Et	2C-E	**4**
^{n}Pr	2C-P	**5**
SEt	2C-T2	**6**
S^{n}Pr	2C-T7	**7**

53 Automatic Screening for Illicit Drugs and Their Analogues by NMR

Chen Peng[1], Wei Jia[2], Agustin Barba[1], Carlos Cobas[1] and Zhendong Hua[2]

1. Mestrelab Research SL., Santiago de Compostela, Spain
2. National Anti-Drug Laboratory, Drug Intelligence and Forensic Center, Ministry of Public Security, Beijing, China

This poster presents a collaborative development and application of NMR analysis and databasing software tools in the screening for illicit drugs and their analogues from large amounts of NMR spectra.

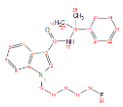
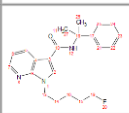
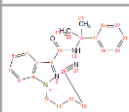
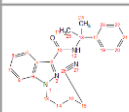
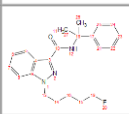
In order to prevent the synthesis of illegal substances, especially the fentanyl class in clandestine laboratories, it is important to routinely and efficiently screen the large amounts of H-1 and C-13 spectra acquired in third party NMR laboratories. While NMR spectra provide fingerprints for compounds, it is not always possible to rely on the exact spectral peak or shape match due to the potential synthesis of uncategorized, novel types of compounds with similar structures. Instead, reporting analogue structures with a similarity score is an attractive alternative for an efficient NMR surveilance system.

To implement such solution, we have carried out the following main tasks:

1. Collected around 1,500 H-1 and 1,500 C-13 NMR spectra for over 400 illicit drugs and precursors in different solvents (CDCl3, DMSO, D2O, MeOD). The spectra were collected on a 400 MHz instruments.
2. Built up a database with all the spectra and chemical structures saved as a library of standards. To speed up and facilitate this step, we have developed a batch processing workflow on top of the Mnova Gears and DB software tools, so that the whole process of spectral processing, peak and multiplet analysis, and databasing is done automatically in a batch mode.
3. Implemented an efficient database search algorithm that returns the match scores based on spectral similarities. A Mnova Gears/DB Search workflow was developed to batch process large quantitites of H-1 or C-13 spectra, search each spectrum against one or multiple databases using such an algorithm, and report the results in an overview spreadsheet. The user can then inspect the hits (e.g. those with a similarity score > 90%), pull out the individual reports where the query spectrum is stacked with the top hits from the database (See Fig. 1 for a real case), and confirm or reject a spectral match.

This system has been recently implemented in the National Anti-Drug Laboratory in Beijing. It is running on 5 Windows servers for screening NMR spectra collected from the third-party NMR laboratories in 24 provinces in China. So far we have screened about a million spectra and successfully identified and confirmed about 500 suspicious spectra for which legal actions were conducted. For those suspicious

spectra, most of the compounds were new psychoactive substances (NPS) that were not categorized and hence not regulated when they were acquired. No fentanyl class of substances have been identified so far.

#	Structure	Structure Label	TYPE	Solvent	Database	Score
Hit 1 - Record ID 1311		5F-CUMYL-PICA	1H	CDCl3	localhost - NNL-H	923
Hit 2 - Record ID 1299		5F-CUMYL-P7AICA	1H	CDCl3	localhost - NNL-H	830
Hit 3 - Record ID 1023		4CN-CUMYL-BUTINACA	1H	DMSO	localhost - NNL-H	818
Hit 4 - Record ID 941		4CN-CUMYL-BUTINACA	1H	CDCl3	localhost - NNL-H	811
Hit 5 - Record ID 1323		5F-CUMYL-PINACA	1H	CDCl3	localhost - NNL-H	805

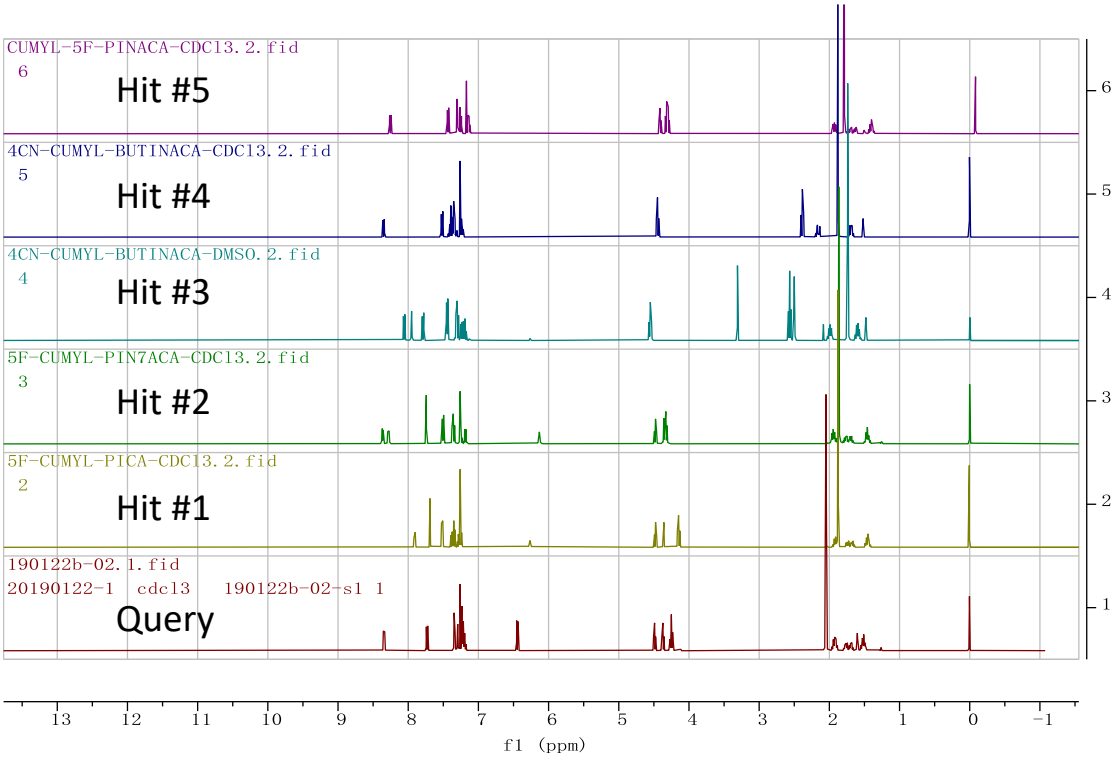


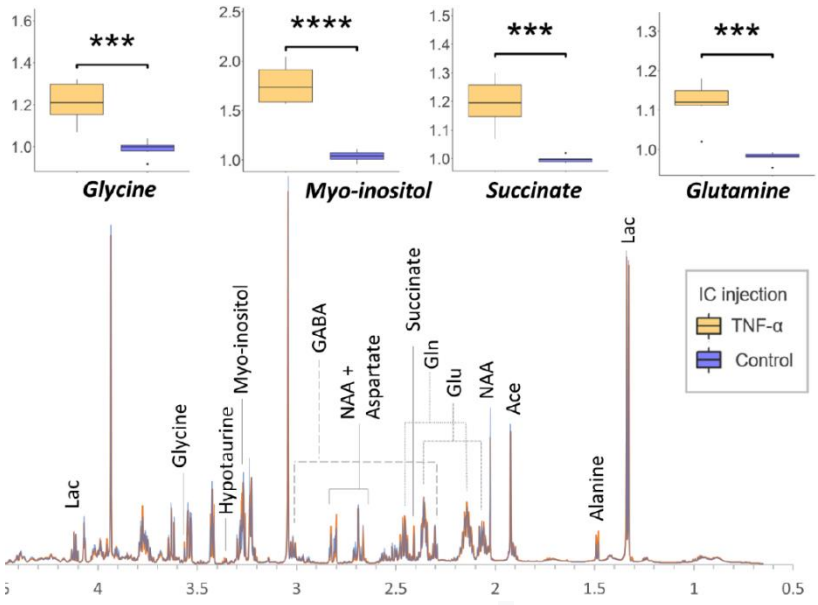
Figure 1. A real-case report from Mnova Gears/DB Search workflow leading to the finding of an uncategorized analogue, which was later identified as 5F-CUMYL-PeGaCLONE (not in the database). The table (top) shows the ID, structure, and score (out of 1000) of the top 5 hits.The spectra of the hits are stacked together with the query for manual inspection (bottom).

54 Ex-Vivo 13C NMR Spectroscopy of Rodent Brain Extracts: TNF Restricts Neuronal Utilisation of Astrocyte-Derived Metabolites

Tang Ng[1], Abi Yates[1,2], Daniel Radford-Smith[1,2], Daniel C. Anthony[2], Timothy D.W. Claridge[1], and **Fay Probert**[1]

1. Department of Chemistry, University of Oxford, Oxford, UK
2. Pharmacology Department, University of Oxford, Oxford, UK

Despite the clear role of tumor necrosis factor (TNF) in neuroinflammation, the precise effect of TNF on the biochemistry of brain cells remains poorly understood [1]. In the healthy brain, TNF is neuro-protective but, in inflamed brain, can activate reactive astrocytes leading to neurotoxicity [2]. Here, we sought to investigate the effect of TNF-α on astrocytic and neuronal metabolism (glycolysis, pentose phosphate pathway, citric acid cycle, pyruvate dehydrogenase, and pyruvate carboxylase pathways) using ex vivo ^{13}C NMR spectroscopy analysis of rodent brain extracts following infusion with [1,2-13C]-glucose, [2-13C]-acetate (to probe astrocyte-specific metabolites), or [3-13C]-lactate (to probe neuron-specific metabolites) [3].



A profound effect on the ^{1}H brain metabolome was observed as a result of TNF-α treatment. Significant increases in [4,5-13C]-glutamine and [2,3-13C]-lactate coupled with a significant decrease in [4,5-13C]-glutamate was observed in [1,2-13C]-glucose infused animals treated with TNF-α. As glutamine is produced from glutamate via an astrocyte specific enzyme (glutamine synthetase) this reflects increased astrocytic activity. As lactate is preferentially metabolised by neurons to produce glutamate, these results suggest a decreased neuron capacity to utilise lactate. This hypothesis was confirmed in the [2-13C]-acetate and [3-13C]-lactate infused rodents.

1. Abd-El-Basset, EM, AIMS Neurosci, 8(4), 558-584, 2021
2. Perry, SW, J Neurovirol, 8, 611-624, 2002
3. Rothman, DL, NMR Biomed, 24, 943-957, 2011

55 NMR Stability Analysis of Glutathione at Low pH for Bispecific Antibody Production

Kaitlyn Doolittle Catlin, Gennady Khirich, Ken Skidmore

Glutathione is a small molecule of key importance in the manufacturing of some bispecific antibodies (BsAbs), which have two different binding sites and are currently of significant interest across the biopharmaceutical industry. One way to produce BsAbs is to generate two half-antibodies separately, then connect those halves via formation of a disulfide bond. Reduced glutathione (GSH) catalyzes this disulfide bond formation. As such, the stability of a glutathione stock solution (composed of GSH and a small amount of oxidized glutathione, GSSG) is critical to the production process of BsAbs. The stock solution is kept at a low pH to inhibit the redox reaction of the glutathione in an attempt to lengthen viable hold time of the solution on the manufacturing floor. However, ^{1}H NMR observation of the low pH glutathione solution over time indicates that while the redox reaction is inhibited successfully, degradation of the glutathione by means of hydrolysis is also promoted under these conditions. The resulting mix of degradation products is surprisingly complex. A variety of NMR methods, including 1D TOCSY, HSQC, and HMBC were used, along with CRAFT processing, to identify and quantitate the GSH, GSSG, and associated degradation species. Analyzing the stock solution behavior by NMR ensures consistent manufacturing quality, and can be used to optimize the pH window for both the redox reaction and the hydrolysis degradation pathways to achieve the required process conditions.

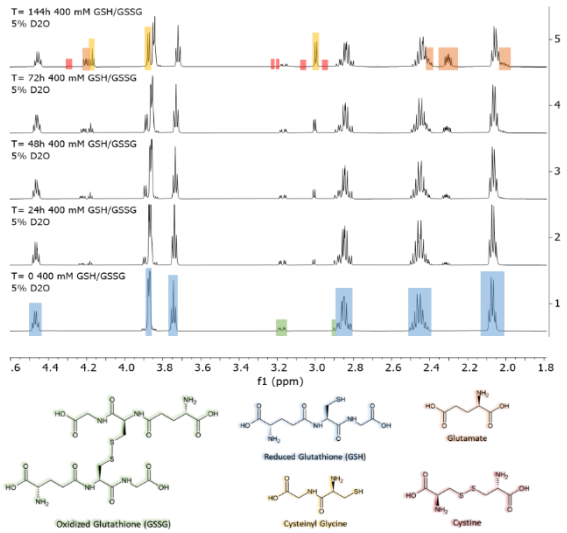


Figure 1. 600 MHz ^{1}H NMR spectra of the 400 mM GSH/GSSG stock solution time course, with signals corresponding to major species highlighted according to color. Green - Oxidized Glutathione (GSSG); Blue - Reduced Glutathione (GSH); Yellow - Cysteinyl Glycine; Orange - Glutamate; Red - Cystine.

56 Solid-State NMR investigation of Gamma Irradiation on a Clarifying Agent for Polypropylene Food Packaging

Clark D. Ridge[1], Sarah E. Donnelly[1], Mary Dawn Celiz[1], and Fu Chen[2]

1. Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD, US
2. Department of Chemistry & Biochemistry, University of Maryland, College Park, MD, US

Solid-state NMR can report on the structure and composition of materials as they are, without significant sample preparation, separation, chemical modification or even solvation. This property of ssNMR analysis was used in the investigation of gamma radiation on a clarifying agent used as an additive in polypropylene food packaging. Aluminum, hydroxybis[2,4,8,10-tetrakis(1,1-dimethylethyl)-6-hydroxy-12H-dibenzo [d,g][1,3,2]dioxaphosphocin 6-oxidato]- (CAS Reg. No. 151841-65-5) is used as a nucleating agent in polypropylene packaging. This packaging can be used for foods subjected to an irradiation process to reduce the presence of food borne pathogens. [1] A study was undertaken to understand the effects of irradiation on the additive and to search for possible radiolysis products. LCMS methods were used to analyze the agent in powdered resin samples containing the agent. They were irradiated with dosses of gamma radiation ranging from 1 to 20 kGy. These experiments showed no significant change to the additive but were not able to report on the whole additive in the plastic. The solvation and ionization processes separated the aluminum from the phosphates that linked it to the molecule. This made it difficult to determine if the irradiation separated the compound prior to analysis. To better understand the effects of gamma radiation on the additive both in plastic and as a pure compound, Phosphorous-31, carbon-13, and aluminum-27 SSNMR experiments were performed on irradiated and non-irradiated samples reporting on the structure of the additive in the resin. [2] Additional experiments on the pure additive were performed before and after irradiation to analyze for chemical or structural change of the additive for more sensitive results. Results of the NMR analysis will be presented with discussion of future applicability of ssNMR in the study of food packaging and food contact materials.

1. Ionizing radiation for the treatment of food. 2019. Code of Federal Regulations. Title 21, Part 179, Section 26.
2. Celiz, Mary D.; Morehouse, Kim M.; Ridge, Clark D.; Chen, Fu; deJager, Lowri S. and Begley Timothy H., Food Additives & Contaminants: Part A, 39(5), pg. 1009-1020, 2022

57 NMR Metabolomics Of Honey Bees: Current Methods And An Application To The Study of Colony Viral Loads

David Rovnyak[1], Jayne C. McDevitt[1], Joanna Raup-Collado[1], Amy Freund[2], Elizabeth A. Capaldi[3,4], and Marie C. Pizzorno[3]

1. Dept. of Chemistry, Bucknell University, Lewisburg PA, US
2. Bruker Biospin, 15 Fortune Drive, Billerica, MA 01821, USA
3. Dept. of Biology, Bucknell University, Lewisburg PA, US
4. Program in Animal Behavior, Bucknell University, Lewisburg PA, US

As losses of European honey bees in North America continue at a high rate, it is necessary to unravel the complex factors that are impacting honeybee health and behavior. Prominent among stressors are the complex viral loads that honey bee colonies in North America face. This work will summarize NMR metabolomic techniques that are employed in a collaboratory for characterizing bee health [1], including an overview of currently accessible organs that can be probed for targeted metabolomics as well as low field techniques (Figure 1). Next, building on prior work which identified strongly elevated brain proline levels in bees infected with deformed wing virus [2], we have sampled whole bees in multiple hives that exhibit distinct viral loads in order to identify broader metabolic changes of viral infections that would occur in managed hives.

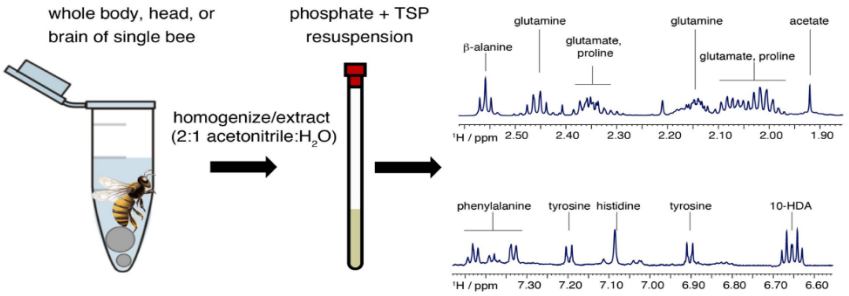


Figure 1. Acetonitrile is useful for extracting aqueous metabolites from whole bees, heads, bodies only, brains, and other parts and organs in order to be able to target metabolomic changes to specific physiologies as needed [1].

1. McDevitt, J.C., Gupta, R.A., Dickinson, S.G., Martin, P.L., Rieuthavorn, J., Freund, A., Pizzorno, M.C., Capaldi, E.A., Rovnyak,D., "Methodology for Single Bee and Bee Brain 1H-NMR Metabolomics", *Metabolites 11*(12), 864, 2021.
2. Pizzorno, M.C., Field, K., Kobokovich, A.L., Martin, P.L., Gupta, R.A., Mammone, R., Rovnyak, D., Capaldi, E.A., "Transcriptomic Responses of the Honey Bee Brain to Infection with Deformed Wing Virus", V*iruses 13*(2), 287, 2021.

58 Benchtop NMR Applications at BASF

Verena Streitferdt, Thomas D. Weiß, Stefan Emig, Dan-Tam Daniel Tang, Matthias Schmalzbauer, Fabian Schaaf

BASF SE, Ludwigshafen am Rhein, Germany

Benchtop NMR spectroscopy has gained increasing interest in recent years. Although these low field NMR spectrometers provide less sensitity and signal dispersion, they offer several significant benefits. Due to the installation of permanent magnets, no cooling by liquid nitrogen or helium is required minimizing maintenance and cost effort. The compact and lightweight design allows to set up the instruments almost anywhere. Easy user interfaces allow the devices to be operated by non-specialist personnel.[1] These properties amongst others make benchtop NMR attractive for use in industry such as at BASF. One of the major advantages of benchtop NMR over high-field NMR for BASF is that it can be used as analytical method on-site at production units, eliminating transportation distances and time delays. As such, several projects were initiated for on-site reaction monitoring and quality assessment. Therefore, data obtained by benchtop NMR (60 MHz) were validated by comparison with data obtained by high-field NMR or other methods such as HPLC. Chemometrics was consulted for further automated spectral processing and multivariate calibration. In this way, results can be obtained by non-specialist personnel. In another project, benchtop NMR shall be implemented as online-analytical method in an artificial intelligence coupled photo-reactor for automated reaction optimization. These benchtop NMR examples at BASF will be showcased and achievements as well as problems will be discussed.

1. Giberson, J., Scicluna, J., Legge, N., Longstaffe, J., Dev. benchtop NMR Spectrosc. 2015–2020, 153–246, 2021.

59 Application of Benchtop NMR as a Chemical Detector for Pharmaceutical Development

Tristan Maschmeyer,

Genentech Inc.

The evolution of benchtop NMR instrumentation has paved the way for the deployment of NMR systems in the chemical laboratory. Still, a wider adoption of benchtop NMR for routine reaction monitoring has been hampered by known limitations including low sensitivity and reduced signal dispersion. This presentation summarizes our efforts to normalize the use of benchtop NMR for reaction monitoring, with the direct comparison of monitoring such processes with high-field instrumentation. This includes monitoring relatively simple processes, such as the dechlorination of 1,3-dichloro-5,5-dimethyl hydantoin and the hydrolysis of 1,1'-carbonyldiimidazole (CDI), to synthetically relevant transformations, such as the activation of carboxylic acid with CDI and subsequent reaction with an amine to form the corresponding amide. Potential opportunities and challenges for the implementation of at-line NMR-based reaction monitoring will be discussed.