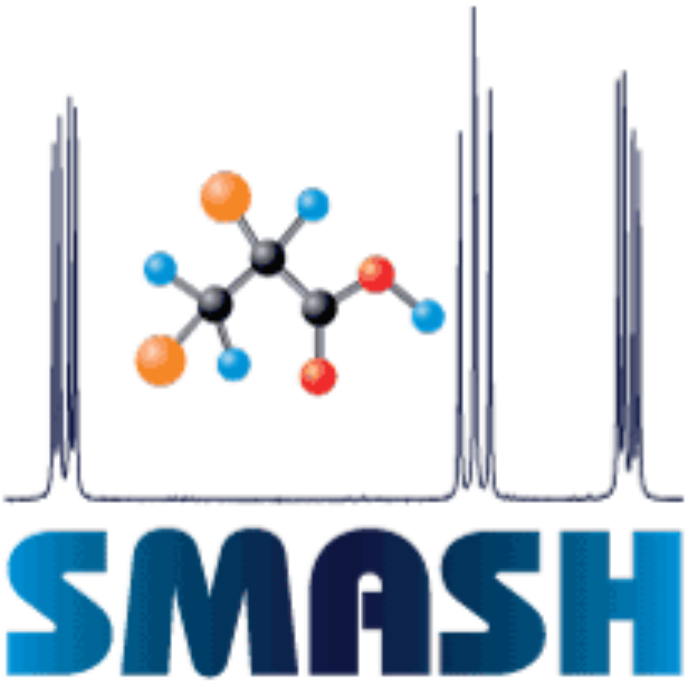


2024

Conference Program

September 15th –18th 2024

Burlington, Vermont

Dear SMASH 2024 Attendee,

Welcome to the SMASH 2024 conference, set against the stunning backdrop of Burlington, Vermont! We are delighted to have you join us at the Hotel Champlain Vermont, a venue that perfectly complements the engaging and collaborative spirit of our conference.

As Program Chairs, we are excited to offer you a rich scientific program free from parallel sessions, ensuring you won't miss any of the groundbreaking research and discussions. This year's agenda is packed with exciting sessions, workshops, and networking opportunities, all designed to foster knowledge exchange and collaboration in the field of small molecule NMR.

The conference kicks off on Sunday morning with coffee and user meetings hosted by our three main sponsors: JEOL, Bruker, and Mestrelab. This is followed by the **MRC Early Career Researchers Symposium** in the afternoon, and a welcoming mixer and dinner in the evening.

We then begin on Monday with a session on NMR Crystallography and the Solid State, followed by a workshop on **Multinuclear NMR Beyond C H N P F**, and a session on **Calculation and Data Analysis of Isotropic and Anisotropic NMR Parameters.**

On Tuesday, sessions will showcase applications and advancements in Benchtop NMR Spectroscopy and New Modalities in Pharma, with an afternoon workshop on **Preparation for Atypical Samples: Metabolism, Anisotropic Samples, Absolute Configuration**, followed by a free afternoon to relax and explore Burlington and its surroundings.

On Wednesday, we have sessions focused on Clinical Applications of NMR and Regulatory/Forensic NMR, culminating in a special session on Industrial Applications organized in collaboration with the PANIC committee.

Our poster sessions, spread across two days (Monday afternoon and Wednesday morning), will highlight a wide array of small molecule NMR applications and techniques. Meanwhile, the **On-Resonance Initiative** offers early-career attendees unique opportunities to interact with experts in the field during lunch sessions. We are also excited to host a **Women in NMR Mixer** on Monday afternoon after the poster session.

Don't forget to explore the vendor's exhibits, where leaders in NMR software and hardware will be showcasing the latest technological advancements. It's a great chance to engage with vendors and find solutions that could propel your research forward.

We encourage you to bring your enthusiasm, your questions, and your eagerness to connect—but leave your ties at home! We look forward to an unforgettable week of science and camaraderie.

On behalf of the organizing committee, we warmly welcome you to Burlington for SMASH 2024.

Kathleen Farley and Roberto R. Gil
Program Chairs, SMASH 2024 NMR Conference

Event Agenda

SMASH NMR Conference 2024

Sun, Sep 15, 2024

8:00 AM - 9:00 AM — Coffee
Location: Mezzanine

9:00 AM - 12:00 PM — JEOL User Meeting
Location: Vermont Conference Room

9:00 AM - 12:00 PM — Bruker User Meeting
Location: Lake Champlain Conference Room
Meeting agenda and registration information can be found at:
Bruker SMASH 2024 User Meeting

12:00 PM - 1:00 PM — Lunch provided by Mestrelab, JEOL and Bruker
Location: Mezzanine

1:00 PM - 4:00 PM — Mestrelab User Meeting
Location: Lake Champlain Conference Room
Meeting agenda and registration information can be found at:
Mestrelabs SMASH 2024 User Meeting

4:00 PM - 6:30 PM — Registration
Location: Prefunction Space

5:00 PM - 6:00 PM — MRC - Early Careers Researchers Symposium
Location: Lake Champlain Conference Room
Magnetic Resonance in Chemistry and Wiley are pleased to announce the organization and sponsorship of the 3rd edition of the SMASH/MRC Joint Early Career Researchers Symposium, which will take place at the SMASH NMR Conference 2024 (Burlington, Vermont, USA) – 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 - related.

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

6:30 PM - 7:30 PM **Welcome Mixer**
Location: Outside Patio

7:30 PM - 9:30 PM **Dinner**
Location: Adirondack Ballroom Salon D

Mon, Sep 16, 2024

7:00 AM - 8:45 AM **Breakfast**
Location: Adirondack Ballroom Salon D

8:00 AM - 8:45 AM **Registration**
Location: Prefunction Space

8:45 AM - 9:00 AM **Welcome & Introduction**
Location: Green Mountain Ballroom A-C

9:00 AM - 10:30 AM **NMR Crystallography and the Solid State**
Location: Green Mountain Ballroom A-C

Lead Speaker

Leonard Mueller
LEAD SPEAKER
Professor of Chemistry
UC Riverside

Nina Gonnella
LEAD SPEAKER
Senior Associate Director
Boehringer Ingelheim Pharmaceuticals Inc.

Speakers

Hironori Kaji
SPEAKER
Professor
Kyoto University

Jonathan Hau
SPEAKER
Scientist 4
Genentech, Inc.

Session Moderator: Ikenna Ndukwe

NMR crystallography – the synergistic application of solid-state NMR, X-ray diffraction, and first-principles computational chemistry – is developing as an atomic-resolution probe of solid-state structure across the molecular sciences. NMR crystallography addresses challenges that are often intractable through standalone techniques. NMR spectroscopy and diffraction are complementary; while diffraction excels in mapping long-range structural order, NMR is exquisitely sensitive to local chemical structure and dynamics. Their integration, often with the aid of first-principles computational chemistry, yields chemically-detailed, three-dimensional structures that offer insight into the relationship between structure, dynamics, and reactivity. This session will explore the integrative foundations of NMR crystallography and highlight applications in diverse fields such as biochemistry, pharmaceutical chemistry, and materials science

Lead Speaker

- **Leonard Mueller**, UC Riverside, Professor of Chemistry
- **Nina Gonnella**, Boehringer Ingelheim Pharmaceuticals Inc., Senior Associate Director

Speakers

- **Hironori Kaji**, Kyoto University, Professor
- **Jonathan Hau**, Genentech, Inc., Scientist 4

NMR Crystallography of Small Molecule Inhibitors at Work
9:00 AM - 9:25 AM
Speaker: Leonard Mueller

Development of QM/DFT Computational Tools to Accelerate Solution NMR Structure Elucidation and Solid Form Identification
9:25 AM - 9:50 AM
Speaker: Nina Gonnella

Structure Analysis by Solid-State NMR Contributing to Organic Light-Emitting Diodes
9:50 AM - 10:10 AM
Speaker: Hironori Kaji

Investigation of the State of Hydration of a Non-stoichiometric Hydrate in a Low Dose Formulation Using 19F Solid-State NMR
10:10 AM - 10:30 AM
Speaker: Jonathan Hau

10:30 AM - 11:00 AM Break
Location: Prefunction Space

11:00 AM - 12:30 PM Workshop - Multinuclear NMR Beyond C H N P F
Location: Green Mountain
Ballroom A-C
Workshop Chair: Clemens Anklin, Bruker Biospin, Head of NMR Applications

Workshop Chair

Clemens Anklin
WORKSHOP CHAIR
Head of NMR applications
Bruker BioSpin

Workshop Chair

- **Clemens Anklin**, Bruker BioSpin, Head of NMR applications

Why limit your NMR spectroscopy to the five isotopes when there are so many more. The many elements from Hydrogen to Uranium and beyond offer a large variety of NMR active isotopes that can be observed. Some are easy, some are hard, and others are impossible. Knowing which ones there are and how to get ready to measure them will be the main topic of this workshop. The important physical properties of these nuclei will be presented and how these affect the NMR experiment. Practical aspects of the preparation of the experiments such as pulse calibration, finding the signal and optimizing the conditions will then lead to the presentation of several practical examples of multinuclear NMR. The practical examples will include nuclei such as 195 Pt, 11/10B, 23Na, 119/117 Sn and a few more.

12:30 PM - 2:00 PM

Lunch

Location: Adirondack Ballroom Salon D

2:00 PM - 3:30 PM

Calculation and Data Analysis of Isotropic and Anisotropic NMR Parameters

Location: Green Mountain Ballroom A-C
Lead Speaker: Stefan Immel, Gianluigi Lauro ; Speakers: Jochen Junker, Ben Honoré

Lead Speaker

Stefan Immel
LEAD SPEAKER
Principal Research Scientist
Technical University of Darmstadt

Gianluigi Lauro
LEAD SPEAKER
Associate Professor
University of Salerno - Department of Pharmacy

Speakers

Jochen Junker
SPEAKER
Public Health Specialist
Oswaldo Cruz Foundation

Ben Honoré
SPEAKER
PhD Researcher
University of Bristol

Session Moderator: Tim Claridge
This session delves into the computational methods and analytical techniques used to determine as well as analyze both isotropic and anisotropic NMR parameters in small molecules. Isotropic parameters, such as chemical shifts, scalar coupling constants, and nuclear Overhauser effects (NOE), provide crucial information about molecular structure and dynamics. On the other hand, anisotropic parameters, including residual dipolar couplings (RDCs), residual chemical shift anisotropy (RCSAs), and residual quadrupolar couplings, offer insights into molecular orientation and interactions in anisotropic environments, leading to the determination of molecular configuration and preferred conformation/s in solution. Overall, this session aims to provide attendees with a comprehensive understanding of the computational and analytical tools available for studying isotropic and anisotropic NMR parameters in small molecules, and their applications in diverse fields such as chemistry, materials science, and biochemistry.

Lead Speaker

- **Stefan Immel**, Technical University of Darmstadt, Principal Research Scientist
- **Gianluigi Lauro**, University of Salerno - Department of Pharmacy, Associate Professor

Speakers

- **Jochen Junker**, Oswaldo Cruz Foundation, Public Health Specialist
- **Ben Honoré**, University of Bristol, PhD Researcher

Bayesian Inference Applied to NMR-Based Configurational Assignments of Natural Products by Floating Chirality Distance Geometry Calculations

2:00 PM - 2:25 PM

Speaker: Stefan Immel

Comparing Predicted and Experimental NMR Data for the Elucidation of the Stereochemical and Structural Features of Organic Compounds

2:25 PM - 2:50 PM
Speaker: Gianluigi Lauro

Sampling the Small Molecule Universe with NMR, WebCocon & ConArch+

2:50 PM - 3:10 PM
Speaker: Jochen Junker

The Intelligent Generation of Molecular Structures from Their NMR Spectra

3:10 PM - 3:30 PM
Speaker: Ben Honoré

3:30 PM - 4:00 PM — Break

Location: Prefunction Space

4:00 PM - 5:30 PM — Poster Session 1 - Even numbered posters

Location: Adirondack Salons A-C

6:00 PM - 6:30 PM — Women's Mixer

Location: Montpelier Salons B&C

Please join Sarah Robinson and Yuhui Zhou from Genentech for a women in NMR mixer on Monday, September 16th. A bar will be set up for the event, so feel free to get a drink and join at 6 PM. We look forward to connecting with you all in small group discussions to kick off the week. There is also an opportunity to get some swag and to join a whova app community for connecting and sharing invites for meals etc. throughout the conference.

6:30 PM - 7:30 PM — Mixer

Location: Prefunction Space

7:30 PM - 10:00 PM — Dinner

Location: Adirondack Ballroom Salon D

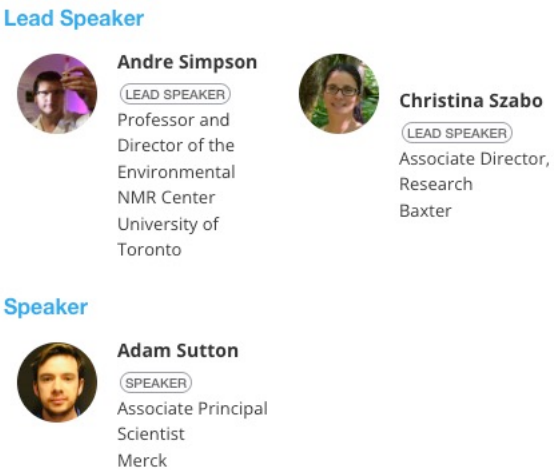
Tue, Sep 17, 2024

7:00 AM - 9:00 AM **Breakfast**

Location: Adirondack Ballroom Salon D

9:00 AM - 10:20 PM **Unveiling the Potential of Benchtop NMR Spectroscopy: Applications and Advancements**

Location: Green Mountain Ballroom A-C
Lead Speaker: Nichola Davies Andre Simpson, Christina Szabo ; Speaker: Adam Sutton



Session Moderator: Jose Napolitano
Benchtop nuclear magnetic resonance (NMR) spectroscopy has emerged as a powerful analytical tool, offering portability, affordability, and versatility for a wide range of applications. This session will showcase recent advancements and innovative applications of benchtop NMR across various fields, including chemistry, the environment, materials science, food science, and pharmaceuticals. In chemical analysis, it has demonstrated its utility in structural elucidation, quantification, and reaction monitoring in both organic and inorganic chemistry. In the characterization of materials, benchtop NMR has been utilized in the analysis of polymers, composites, porous materials, and nanomaterials, with a particular emphasis on quality control and process optimization. In food and beverage quality control, it has been instrumental in assessing authenticity, composition, and nutritional content, as well as in detecting adulteration and contaminants. In pharmaceutical analysis, it has played a significant role in drug discovery, formulation development, and quality assurance, including the rapid identification of the presence of impurities and degradation products.

Lead Speaker

- **Andre Simpson**, University of Toronto, Professor and Director of the Environmental NMR Center
- **Christina Szabo**, Baxter, Associate Director, Research

Speaker

- **Adam Sutton**, Merck, Associate Principal Scientist

The Vast Potential of Benchtop NMR for Environmental Research

9:00 AM - 9:30 AM
Speaker: Andre Simpson

Benchtop NMR Spectroscopy of an Aqueous Polysaccharide Product

9:30 AM - 10:00 AM
Speaker: Christina Szabo

Quantification of Small Molecules for Vaccine Development by Benchtop NMR

10:00 AM - 10:20 AM
Speaker: Adam Sutton

10:20 AM - 11:00 AM Break

Location: Prefunction Space

11:00 AM - 12:30 PM. New Modalities in Pharma

Location: Green Mountain Ballroom A-C
Lead Speaker: Nichola Davies, Marilisa Leone ; Speakers: Kaitlyn Doolittle Catlin, Nirmalya Pradhan

Lead Speaker

Nichola Davies
LEAD SPEAKER
Director, Analytical and Structural Chemistry
AstraZeneca

Marilisa Leone
LEAD SPEAKER
Senior Researcher
IBB-CNR Naples I taly

Speakers

Kaitlyn Doolittle Catlin
SPEAKER
Technical Development Senior Scientist
Genentech

Nirmalya Pradhan
SPEAKER
Postdoctoral Research associate
Texas A&M University

Session Moderator: Thomas Williamson
During the last two decades NMR spectroscopy has attracted a growing interest from both Academia and Pharma. Apart from representing a key analytical technique to assess identity and purity of compounds, it has assumed a pivotal role in the drug discovery field. Indeed, NMR spectroscopy can provide structure and interaction knowledge, that is essential for the development and evaluation of possible therapeutic agents and original molecular tools involved in the modulation of important pathological or physiological cellular pathways. NMR Spectroscopy surely underpins the discovery and development of traditional small molecule drugs. However, as we look to develop medicines for ever more challenging targets, there has been an emergence of new drug modalities such as targeted protein degraders, macrocycles, oligonucleotides, peptides, peptidomimetics and drug conjugates. These present new challenges for the NMR community, but also opportunities to positively impact research in the field of next generation therapeutics.

In this session we aim to highlight some of the innovative NMR approaches taken to support research in this growing field, such as screening, characterization, and conformational analysis of these small to medium sized molecules.

Lead Speaker

- **Nichola Davies**, AstraZeneca, Director, Analytical and Structural Chemistry
- **Marilisa Leone**, IBB-CNR Naples I taly, Senior Researcher

Speakers

- **Kaitlyn Doolittle Catlin**, Genentech, Technical Development Senior Scientist
- **Nirmalya Pradhan**, Texas A&M University, Postdoctoral Research associate

Potent and Bioavailable beyond Rule of Five Drugs: Insights from NMR

11:00 AM - 11:25 AM
Speaker: Nichola Davies

Hunting for original anticancer peptides: a key role for NMR within multidisciplinary approaches.

11:25 AM - 11:50 AM
Speaker: Marilisa Leone

Single-Use Technologies and Gamma Irradiation: Tracking Down a Biopharma Leachables Issue with NMR

11:50 AM - 12:10 PM
Speaker: Kaitlyn Doolittle Catlin

NMR Sensitivity Enhancement of Small Biomolecules using Hyperpolarized Water

12:10 PM - 12:30 PM
Speaker: Nirmalya Pradhan

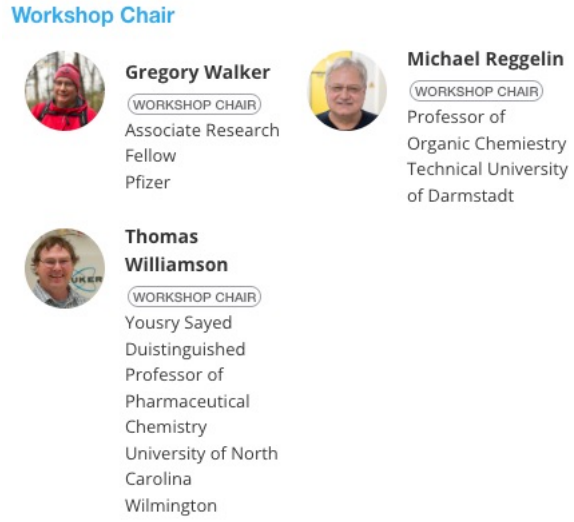
12:30 PM - 2:00 PM Lunch

Location: Adirondack Ballroom Salon D

2:00 PM - 3:30 PM

Workshop - Preparation for Atypical Samples: Metabolism, Anisotropic samples, Absolute Configuration

Location: Green Mountain Ballroom A-C
Workshop Chair: Gregory Walker, Michael Reggelin, Thomas Williamson



Workshop Chair

- **Gregory Walker**, Pfizer, Associate Research Fellow
- **Michael Reggelin**, Technical University of Darmstadt, Professor of Organic Chemiestry
- **Thomas Williamson**, University of North Carolina Wilmington, Yousry Sayed Duistinguished Professor of Pharmaceutical Chemistry

In many cases the preparation of an NMR sample is a straightforward process. However, there are times when sample preparation requires forethought and special considerations. This workshop contains three separate topics, all related to sample preparation for several types of NMR analysis: preparation of samples for anisotropic studies, the preparation of metabolite samples for structural elucidation and the preparation of mosher samples for the determination of absolute configuration.

The measurement of anisotropic NMR-parameters like residual quadrupolar couplings (RQCs), residual chemical shift anisotropy (RCSA) and especially residual dipolar couplings (RDCs) are of increasing importance not only in biomolecular NMR but also for the structural characterization of small molecules. In contrast to biomolecules, the focus here is mainly on the determination of relative configurations, although first results on the determination of absolute configurations are emerging.

A precondition for the measurement of anisotropic NMR data is to hinder the analyte from tumbling isotropically which can be done with uniaxially swollen gels or lyotropic liquid crystalline (LLC) phases. The talk will focus on the preparation and application of LLC-phases of helically chiral polyacetylenes and polyisonitriles for the (enantiodifferentiating) extraction of anisotropic NMR-parameters.

From the perspective of an NMR spectroscopist the structural elucidation of drug metabolites is particularly challenging for two reasons: the amount of material available for isolation can be extremely low, typically in the microgram range, and

the target compounds are contained in complicated matrices with a myriad of unrelated large and small molecules. The preparation of a successful metabolite sample can be divided into two parts, the separation of the metabolites out of its matrices and careful preparation of the samples for NMR analysis. Details and examples of these two processes will be discussed.

Time-honored approaches for the determination of absolute configuration include the application of the so-called “Mosher’s Method”. This technique involves derivatization of a stereocenter of unknown configuration with an agent of known chirality to form a diastereotopic system that can be analyzed by a variety of NMR techniques. The three-dimensional spatial configuration of the newly formed complex can be analyzed and interpreted based on comparison with known models or in some cases, through computational chemistry methodology. The activation barriers for the application of this powerful analytical method are often two-fold: 1. formation of the actual ester, amide, or other functionality, and 2. confident stereospecific NMR chemical shift assignment of all relevant protons or other applicable NMR nuclei. Solutions to these two problems and extension of this methodology to complex systems and alternate nuclei like ^{19}F will be discussed.

Wed, Sep 18, 2024

7:00 AM - 9:00 AM — Breakfast

Location: Adirondack Ballroom Salon D

9:00 AM - 10:30 AM — Clinician Impossible: the role of NMR in solving complex clinical problems.

Location: Green Mountain Ballroom A-C
Lead Speaker: Fay Probert, David Cistola ; Speakers: István Pelczer, Alessia Trimigno

Lead Speaker

Fay Probert
LEAD SPEAKER
Dorothy Hodgkin Fellow
University of Oxford

David Cistola
LEAD SPEAKER
Founder and Chief Scientific Officer, T2YourHealth
Professor, Diabetes & Metabolism, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso

Speakers

István Pelczer
SPEAKER
Sr. NMR Spectroscopist
Dept. of Chemistry, Princeton University

Alessia Trimigno
SPEAKER
Director of NMR Technology & Biomarkers
Olaris Inc

Session Moderator: David Rovnyak
The application of NMR to the analysis of complex biofluids is extremely powerful, providing a biochemical snapshot of an organism at a given point in time. This NMR-detectable molecular fingerprint can shed light on the chemical processes and biological pathways associated with disease states, facilitating the design of novel treatments and resulting in the identification of novel diagnostic and prognostic biomarkers.

NMR proves especially valuable in addressing heterogenous diseases which are particularly challenging to diagnose, diseases in which environmental and lifestyle factors are suspected to contribute and where conventional genomics and proteomics approaches fall short. The rapid, high-throughput, and non-destructive nature of NMR make it an ideally suited technology for integration into clinical pathways while recent advances in benchtop instruments are paving the way for NMR-based tests to become available in primary care settings, promising expanded access to its benefits in the future.

This session will explore innovative applications of NMR in tackling diagnostic and prognostic challenges, highlighting recent advances in methodology and

technology that have the potential to facilitate the integration of NMR into clinical practice.

Lead Speaker

- **Fay Probert**, University of Oxford, Dorothy Hodgkin Fellow
- **David Cistola**, Professor, Diabetes & Metabolism, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, Founder and Chief Scientific Officer, T2YourHealth

Speakers

- **István Pelczer**, Dept. of Chemistry, Princeton University, Sr. NMR Spectroscopist
- **Alessia Trimigno**, Olaris Inc, Director of NMR Technology & Biomarkers

NMR-based metabolomics to probe small molecule chemical pathways in the brain; towards novel treatments for neurodegenerative diseases

9:00 AM - 9:25 AM
Speaker: Fay Probert

Miniaturized NMR at Your Fingertip: Health Promotion and Diabetes Prevention

9:25 AM - 9:50 AM
Speaker: David Cistola

An Alternative, Craft-Based Approach to Extract Essential Inflammation Markers from Simple 1D Serum Samples

9:50 AM - 10:10 AM
Speaker: István Pelczer

Launching a NUS 1H-13C HSQC Clinical Diagnostic For Kidney Transplant Management

10:10 AM - 10:30 AM
Speaker: Alessia Trimigno

10:30 AM - 11:00 AM. Break

Location: Prefunction Space

12:30 PM - 2:00 PM Lunch

Location: Adirondack Ballroom Salon D

2:00 PM - 3:30 PM

Regulatory / Forensic

Location: Green Mountain Ballroom A-C
Lead Speaker: Charlotte Corbett, Marcos Batisttel ; Speakers: Jennifer Janovick, Ryan Cohen

Lead Speaker

Charlotte Corbett
LEAD SPEAKER
Senior Forensic Chemist
DEA

Marcos Batisttel
LEAD SPEAKER
Biologist
FDA

Speakers

Jennifer Janovick
SPEAKER
University of Maryland

Ryan Cohen
SPEAKER
Principal Scientist
Merck

Session Moderator: Amy Freund
It has been demonstrated time and time again that NMR is a versatile technique, used for the characterization of small molecules, the analysis of complex mixtures and metabolites, and the understanding of the structure and dynamics of large biomolecules. NMR can quantify with ease and accuracy every compound in a solution with one spectrum, even if a compound's structure is unknown. NMR can also reveal the structure of new compounds. Therefore, it should not come as a surprise that NMR has become a valuable analytical tool for research and development, quality control/quality assurance (QC/QA) in industry, testing of unknown substances in forensics, and playing a prominent role in academia and government agency research alike. This session aims to showcase the versatility of NMR, covering topics ranging from structure-function studies of bacterial polysaccharides, components of polysaccharide-protein conjugate vaccines, to structure elucidation and purity determination in forensic science.

Lead Speaker

- **Charlotte Corbett**, DEA, Senior Forensic Chemist
- **Marcos Batisttel**, FDA, Biologist

Speakers

- **Jennifer Janovick**, University of Maryland
- **Ryan Cohen**, Merck, Principal Scientist

Identity and Purity Deciphered Only via NMR

2:00 PM - 2:25 PM
Speaker: Charlotte Corbett

Glycan NMR: It is never too late but first hydrate the carbohydrate

2:25 PM - 2:50 PM
Speaker: Marcos Batisttel

Analysis of Polymers in Food Packaging Using Solid- and Liquid-State 19F NMR

2:50 PM - 3:10 PM
Speaker: Jennifer Janovick

Development and Application of a Universal, Selectively-Tunable 1H and 19F qNMR Calibrant

3:10 PM - 3:30 PM
Speaker: Ryan Cohen

3:30 PM - 4:00 PM **Break**

Location: Prefunction Space

4:00 PM - 5:30 PM **Industrial Applications (organized with the PANIC committee)**

Location: Green Mountain Ballroom A-C
Lead Speaker: Jacqueline Thomas, Debra Sysyn ; Speakers: Jose Napolitano, Kevin Robbins

Lead Speaker

Jacqueline Thomas
LEAD SPEAKER
Principal Scientist
Procter & Gamble

Debra Sysyn
LEAD SPEAKER
Research Associate
ExxonMobil Technology and Engineering

Speakers

Jose Napolitano
SPEAKER
Senior Principal Scientist
Genentech Inc.

Kevin Robbins
SPEAKER
Associate Principle Scientist
AstraZeneca

Session Moderator: Mark Dixon
NMR is a highly versatile measurement tool that is essential in applied research and development for industrial needs. NMR is key to bringing unique insights in the areas of complex mixture analysis, studying ingredient interactions, and determining mechanism of action for delivering industry needs. This session will showcase industrial examples of where the use of applied NMR methodologies has been used and leveraged.

Lead Speaker

- **Jacqueline Thomas**, Procter & Gamble, Principal Scientist
- **Debra Sysyn**, ExxonMobil Technology and Engineering , Research Associate

Speakers

- **Jose Napolitano**, Genentech Inc., Senior Principal Scientist
- **Kevin Robbins**, AstraZeneca, Associate Principle Scientist

Applied NMR Methodology to Aid Consumer Product Understanding
4:00 PM - 4:25 PM
Speaker: Jacqueline Thomas

Characterization of Complex Mixtures for Petroleum Applications.
4:25 PM - 4:50 PM
Speaker: Debra Sysyn

Deciphering the Chemical Complexity of Polysorbate Surfactants
4:50 PM - 5:10 PM
Speaker: Jose Napolitano

Automated Structure Verification (ASV) Tools
5:10 PM - 5:30 PM
Speaker: Kevin Robbins

5:30 PM - 5:45 PM **Closing Remarks**

Location: Green Mountain Ballroom A-C

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Pfizer, USA

Roberto Gil
Carnegie Mellon University, USA

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Andreas Kaerner Eli Lilly & Co	Brian Marquez Nalas Engineering	David Rovnyak Bucknell University
Nicola Davies AstraZeneca, UK	Mark Dixon Mestrelab SL	Robert Espina Magritek
Fay Probert University of Oxford	Ikenna Ndukwe Amgen	Paul Bowyer JEOL
	Charlotte Gotfredsen Danish Technical University	

SMASH 2024 Scholarship Winners

The following students received a scholarship to attend SMASH 2024.

Thanks to our meeting sponsors for their generous support.

Karl Schulz	Carnegie Mellon University
Pratiman De	National Institute of Science Education and Research, India
Bhawna Chaubey	University of Washington Seattle
Nirmalya Pradhan	Texas A&M University
Hugo Rocha	University of Manchester
Alisa Sannikova	Grinnell College
Zheqi Jin	University of Bristol
Hussain Al Zaindeen	University of Manchester
Jared Wood	University of North Carolina Wilmington
Peter Costa	University of Toronto
Calvin Yiu	University of Bristol
Ben Honoré	University of Bristol
Sakshi Bhagat	Indian Institute of Technology, Jodhpur
Ivan Kiganda	University of Nairobi

SMASH 2024

TALKS and WORKSHOPS

NMR Crystallography of Small Molecule Inhibitors at Work

Leonard Mueller[1]

1. UC Riverside, Professor of Chemistry

NMR crystallography – the synergistic application of solid-state NMR, X-ray diffraction, and first-principles computational chemistry – is developing as an atomic-resolution probe of structure and function across the molecular sciences. Here, I will discuss the integrative foundation of this approach and present an example of the insight it offers into the chemistry and dynamics of small molecule inhibitors bound within enzyme active sites.

Development of QM/DFT Computational Tools to Accelerate Solution NMR Structure Elucidation and Solid Form Identification

Nina Gonnella[1]

1. Boehringer Ingelheim Pharmaceuticals Inc., Senior Associate Director UC Riverside, Professor of Chemistry

Structure elucidation, in both the solution and solid state, plays a critical role in organic chemistry, medicinal chemistry, and pharmaceutical development. Because chemical structure and/or crystalline form can affect properties such stability, solubility, bioavailability, storage characteristics, drug efficacy, and drug delivery, understanding and determining a compound's chemical and crystal structure is essential in the development of new therapeutics. NMR is an excellent experimental technology for structure determination; however, experimental approaches can sometimes yield ambiguous or inconclusive results. To accelerate the structure elucidation process in solution NMR we developed two complementary computational programs: Holistic in-silico Prediction Application Software (HiPAS) [1] and Diastereomeric in Silico Chiral Elucidation (DiCE) [2]. HiPAS uses molecular modeling, quantum chemistry, and density functional theory (QM/DFT) calculations with solution NMR experimental data to accelerate chemical structure elucidation. This is further enhanced with DiCE which uses Bayesian probabilities from the differences between DFT-GIAO NMR calculations and experimental NMR data to accurately predict stereochemistry and regiochemistry. For solid form identification, Operant Probability Theory in Crystal Solutions (OPTICS) [3] was developed. OPTICS uses QM/DFT, and Bayesian probability theory combined with 13C ssNMR experimental data as a powerful means of polymorph identification. The presentation will describe these prediction tools and associated applications for rapid chemical structure elucidation and identification of the correct solid form.

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STRUCTURE ANALYSIS BY SOLID-STATE NMR CONTRIBUTING TO ORGANIC LIGHT-EMITTING DIODES

Hironori Kaji[1] and Katsuaki Suzuki

1. Kyoto University

Our research group is currently investigating organic light-emitting diodes (OLEDs) from the following four aspects: 1) *in-silico* molecular design for OLED emitters and devices fabrications [1,2], 2) quantum chemical calculations [3], 3) multiscale simulations [4], and 4) NMR analysis of OLEDs [5-11]. Here, we will focus on our studies on solid-state NMR analyses of materials for OLEDs.

OLEDs have now been widely applied in TVs and smartphones. However, the fundamental understanding, especially the detail at the molecular level, is still lacking. In this study, the relationship between structure and light-emitting properties is analyzed for a well-known light-emitting electron-transporting material, tris(8-hydroxyquinoline) aluminum(III) (Alq3). Alq3, which has been the most widely used material for OLEDs, possesses interesting features. Normally, Alq3 shows green-to-yellow fluorescence. However, blue emission is observed in some cases and the origin had not been clear. Dipolar-decoupled magic angle spinning (DD/MAS) 27Al NMR spectra (Fig. 1) provide broad spectral patterns for the green-to-yellow fluorescence Alq3s and sharp spectral patterns for the blue fluorescence Alq3s. From the axially-symmetric sharp 27Al NMR spectra, blue Alq3 are found to be composed of the facial isomers with *C*3 symmetry. It is also suggested that the green-to-yellow Alq3 are in the meridional forms. Our cross-polarization MAS (CP/MAS) 13C NMR experiments combined with the gauge-including projector augmented-wave (GIPAW) calculations support the conclusion. Two-dimensional double-quantum spectroscopy (2D DOQSY), developed by Schmidt-Rohr [12, 13], clearly concluded that the green-to-yellow Alq3 are composed of the meridional isomer, whereas blue Alq3 is composed of the facial isomer. The remaining time will be used to present our on-going studies.

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Investigation of the State of Hydration of a Non-stoichiometric Hydrate in a Low Dose Formulation Using ^{19}F Solid-State NMR

Jonathan Hau[1], Paroma Chakravarty[1], Joe Lubach[1]

1. Genentech, Inc.

GDC-4379 was developed as a non-stoichiometric hydrate into a formulated capsule with a 1% drug loading. The water content of this hydrate varied from 0-0.7 moles over the relative humidity (RH) range of 0-98% (25°C). Since a variable state of hydration coupled with rapid equilibration of lattice water with the environmental RH can lead to challenges in formulation development, an analytical method to directly and accurately determine the state of hydration of the active in such a low dose formulation was deemed necessary. Owing to its high selectivity and fast acquisition times, 19F solid-state NMR was effectively utilized to directly determine the lattice water content of the active in the formulated capsule. By correlating $\Delta\delta$, the chemical shift difference between the isotropic peaks, with the relative humidity and ultimately the lattice water content, the state of hydration of GDC-4379 in the formulated capsule was experimentally determined as 0.63 moles of water/mole of anhydrate.

Workshop – Multinuclear NMR

Beyond C H N P F

Clemens Anklin[1]

1. Bruker BioSpin

Why limit your NMR spectroscopy to the five isotopes when there are so many more. The many elements from Hydrogen to Uranium and beyond offer a large variety of NMR active isotopes that can be observed. Some are easy, some are hard, and others are impossible. Knowing which ones there are and how to get ready to measure them will be the main topic of this workshop. The important physical properties of these nuclei will be presented and how these affect the NMR experiment. Practical aspects of the preparation of the experiments such as pulse calibration, finding the signal and optimizing the conditions will then lead to the presentation of several practical examples of multinuclear NMR. The practical examples will include nuclei such as 195 Pt, 11/10B, 23Na, 119/117 Sn and a few more.

Bayesian Inference Applied to NMR-Based Configurational Assignments of Natural Products by Floating Chirality Distance Geometry Calculations

Stefan Immel[1]

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The NMR-based configurational analysis of complex molecular structures including natural products is still not a routine task. Different NMR parameters are used for the assignment of the relative configuration: NOE/ROE, homo- and heteronuclear J couplings as well as anisotropic NMR parameters. In contrast to well-established methods that evaluate possible structure models against the experimental NMR data, we persue a route that allows molecular structures (configuration and conformation) to directly evolve from NMR restraints without prior assumptions.

The combined restrained distance geometry (rDG)[1] and distance (and data) bounds driven dynamics (DDD)[2] method allows a model-free approach for the determination of the relative configuration that is invariant to the choice of an initial starting structure and does not rely on comparisons with (DFT) calculated structures. Moreover, there is also no physical force-field involved and therefore any bias that could potentially originate from missing or wrong force-field parameters is absent.

In the talk we present our program 'ConArch+' ('Configuration Architect+')[3] which allows to include isotropic (NOE, J) as well as anisotropic NMR data such as residual dipolar couplings (RDCs), residual quadrupolar couplings (RQCs) and residual chemical shift anisotropies (RCSAs) from multi-alignment data sets into distance geometry based configurational assignments.

The power of this new methodology is presented for some natural products,[4] including extensions towards the treatment of molecular flexibility in multi-conformer rDG/DDD simulations.

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Comparing Predicted and Experimental NMR Data for the Elucidation of the Stereochemical and Structural Features of Organic Compounds

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1. University of Salerno - Department of Pharmacy

Data from NMR experiments (e.g., chemical shifts, homonuclear and heteronuclear J coupling constants) are a useful source of information for elucidating the structural and stereochemical features of natural and synthetic compounds. In addition, the possibility to predict such NMR parameters at the density functional theory (DFT) and to compare them with the related experimental ones successfully aided the identification of the correct three-dimensional structure of organic molecules in a rapid and robust way. This approach, known as the DFT/NMR combined approach,[1,2] is based on a general workflow involving different steps: 1) building of the 3D input structure(s) (e.g., those related to the all the possible isomers of the molecule under investigation); 2) conformational search rounds; 3) geometry optimization of the sampled conformers; 4) computation of NMR data, considering the influence of each conformer on the total Boltzmann distribution and taking into account the relative energies computed at the DFT level; 5) comparison of experimental and predicted data.[1,2] Among them, step 4) usually features a high source of potential drawbacks that can in turn dramatically affect the final results. Indeed, DFT often shows limitations in correctly reproducing the conformational dynamics of organic compounds (especially, highly flexible and/or polyhydroxylated ones) by overestimating improper conformations (e.g., those showing intramolecular H-bonding). Different strategies have been proposed to solve this problem, e.g., combining several computed/experimental NMR parameters and redefining the Boltzmann distribution by stochastic approaches.[3] In order to deeply investigate this aspect, we have recently developed a computational tool based on gradually and systematically modulating the conformational ensemble of a case-study compound by modifying the relative weight of the conformers from the original Boltzmann distribution. Depending on the number of conformers to be considered and on the degree of variation from the original distribution, a variable number of "artificial" ensembles can be quickly built in a systematic way, each accounted for the subsequent comparison of experimental/predicted data.

In this contribution, several examples of successful implementation of the combined DFT/NMR approach are presented, also focusing on challenging cases that may make structural elucidation of organic compounds difficult or misleading.

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Sampling the Small Molecule Universe with NMR, WebCocon & ConArch+

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A molecular formula, as obtained by MS or elemental analysis, normally comes with a huge number of possible constitutions, configurations and conformations, a universe of possibilities. Nuclear Magnetic Resonance is one of the methods commonly used in order to reduce this universe to a single solution, or eventually a small set of solutions.

In a first step chemical shifts (mainly Carbon and Proton) and different correlation experiments (COSY, HSQC, ^{13}C-HMBC, 1,1-ADEQUATE, ^{15}N-HMBC, among others) are used to obtain connectivity information. As correlation data sets might be incomplete or just not enough to define all connectivities of all atoms, various constitutions might be compatible with the NMR correlation data, and various assignments might be observed for a single constitution. This first step is carried out by CoCon [1,2], which outputs all possible constitutions with all possible assignments for a given correlation data set.

WebCoCon uses different approaches to reduce the set of possible solutions created by CoCon.

1. A simple Molecular Dynamics (MD) is used to create 3D structures. The total energy is used for the ranking of the solution within the list. Sometimes, specially with complex ring systems, this approach fails. But, it is the computationally cheapest method [2].
2. A simple Distance Geometry (DG) is used to create 3D structures. DG is much more robust against complex molecular structures and generally provides better results, at slightly higher computational cost. The total energy is used for the ranking of the solutions.
3. A Simple DG with NOE restraints is used to create improved 3D structures. DG uses very coarsely defined distances for the structure calculation, at essentially no additional cost. The obtained solutions compare much better to reference X-Ray structures, different from the previous solutions [3].
4. A simple ORCA [4] ^{13}C chemical shift calculation. The calculation of chemical shifts based on previously defined conformations seems very promising, but the structures generated by WebCocon without NOEs are not good enough for high quality results.

The results obtained by DG with NOEs indicate that using more experimental data from NMR can improve the selection process for the correct constitution and assignment, and lead to better solutions overall. Thus, ConArch+ [5] was interfaced with CoCon, as it is a DG package that not only handles NOEs, but also *J*-couplings, RDC, RCSA and other anisotropic data. Initially, only NOEs and RDCs are supported. When tested with Brucine, one (of two) assignment of the correct constitution was clearly favored in the results, when compared to the other two possible constitutions (with two assignments each) that CoCon found with theoretical NMR correlation data. Additionally, the ^{13}C chemical shift calculations performed with ORCA also improved in quality for the correct assignment, showing considerably lower deviations from experimental data when compared to the results obtained with the other methods. We are now working on obtaining other examples for this application. Unfortunately the publication of such complete NMR data sets is not very frequent.

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THE INTELLIGENT GENERATION OF MOLECULAR STRUCTURES FROM THEIR NMR SPECTRA

Ben Honoré[1], Calvin Yiu[1], Craig Butts[1]

1. University of Bristol

At Bristol we have developed IMPRESSION [1], a machine learning tool for predicting solution state chemical shifts and coupling constants of 3D molecular structures. The gold standard of IMPRESSION is a multitask graph transformer network that calculates ^{1}H and ^{13}C chemical shifts to within a 0.5% error of high-level quantum mechanical (DFT) calculations in a matter of seconds.

IMPRESSION provides chemists with highly accurate simulated NMR data that can act as a comparison when analysing a spectrum to confirm whether a known structure has been successfully isolated. However, if the contents of an NMR sample are entirely unknown, a tool that could perform the opposite task to IMPRESSION, and from NMR data predict the molecular structure, would be very powerful. We describe this idea as solving the 'inverse problem'.

In such an inverse approach, IMPRESSION serves as a useful scoring function for rapidly evaluating structures by their NMR predictions. A Monte Carlo style method for iteratively generating molecules from their constituent atoms and ranking them with IMPRESSION was found to be effective for molecules of up to nine heavy atoms. For molecules any larger, however, this approach broke down due to the vast chemical space that the search needed to cover.

Hence, we have sought to develop an intelligent mode of molecule generation in an attempt to generalise to larger structures. Our approach is a reinforcement learning task where the model builds structures through stepwise addition of bonds, receiving a reward calculated by IMPRESSION at each step to indicate whether the bond created was a sensible one given the chemical shifts of the surrounding environment. Training the model on many examples of these actions then optimises a policy for building molecules that fit with a set of input NMR data.

We have seen some highly promising results in small molecule space that prove this relationship between NMR data and chemical structure can be learned. The challenge going forward will be designing datasets and training protocols to move to larger molecules and a broader chemical space within the bounds of reasonable computational demand. This is a rapidly developing and exciting area of the NMR field.

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The Vast Potential of Benchtop NMR for Environmental Research

Andre Simpson[1]

1. University of Toronto

NMR Spectroscopy is the only tool in modern science that can identify novel chemical structures from scratch in all forms (solids, gels, liquids) without the use of libraries. If environmental research is restricted to libraries of previously identified molecules, then, as a field, we are always looking for "what we already know" and critical highly toxic transformation products, or new classes of pollutants, can go undetected for decades. Two examples demonstrate this the best. Firstly, in 1976, Prof. Donald Taves performed ^{19}F NMR of his own blood and found "*widespread contamination of human tissues with organic fluorocompounds derived from commercial products*". But as NMR is underused for environmental discovery, they were 'rediscovered' 2 decades later using MS, at levels above that known to induce health issues in humans. Second, is 6PPD-quinone (the ozonation product of a major tire additive 6PPD) which was discovered by our group[1] in 2020. 6PPD-quinone has since been found to be ubiquitous in the environment, with enough chemical in a single tire to kill 50 billion salmon, and is now known to be closely related to numerous respiratory diseases in humans. The simple message is that without NMR 6PPD-quinone, one of the most toxic chemicals ever found, would still be ***undiscovered***.

Benchtop NMR spectrometers do not require cryogens, can be placed on a lab bench thus making NMR more accessible across the globe including countries, where cryogens are not available. While mobile systems will have reduced sensitivity and resolution vs high field NMR, this can be partially offset by developing new NMR experiments to overcome overlap and to boost sensitivity. In this presentation a wide range of techniques first at high field and then on a benchtop system will be discussed. Techniques to reduce overlap will include, spectral editing in 1D and 2D NMR and DREAMTIME[2], while focusing and steady-state free precession[3] will be used as a means to increase sensitivity per unit time.

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BENCHTOP NMR OF AN AQUEOUS POLYSACCHARIDE PRODUCT

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Various analytical techniques are conventionally employed to characterize polysaccharides: e.g., wet chemical methods are used for identification and number of dextrose equivalents (DE) determination and nuclear magnetic resonance (NMR) spectroscopy, including high resolution ^{13}C, is applied to determine the branching ratio (Figure 1).
With a well-developed NMR method, in one experiment, not only can the polysaccharide be specifically identified, the DE and branching ratio can also be calculated based on relative quantitation by NMR. Therefore, one NMR experiment has the potential to replace all three test methods.

Furthermore, benchtop NMR advancements in recent years have improved measurement resolution and solvent suppression, making benchtop NMR equipment amenable to characterizing polysaccharides in aqueous solutions. A robust NMR method could dramatically shorten the testing cycle, saving time and resources while reducing environmental impact.

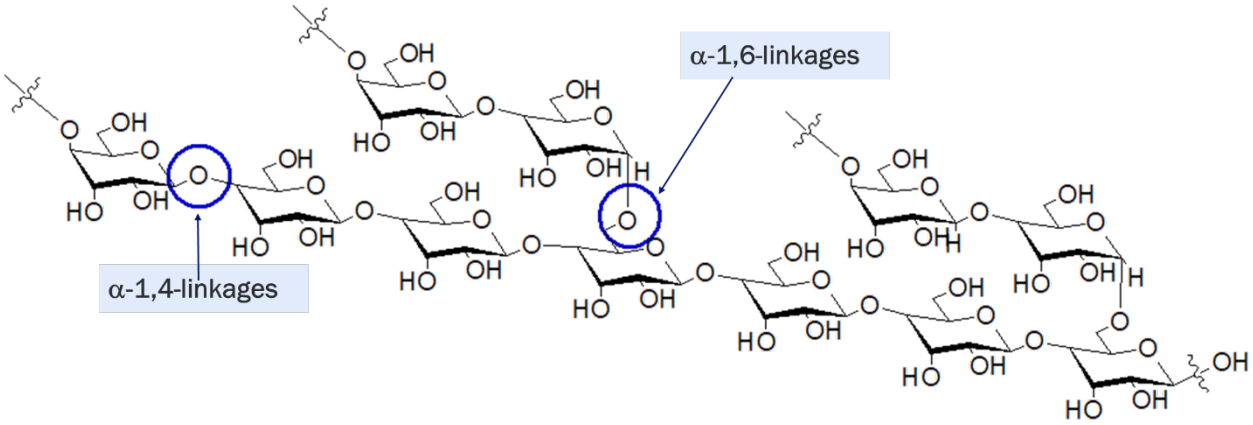

Figure 1. Representative structures of polysaccharide

The validity of the results of the developed must be assessed. There are multiple approaches to analytical procedure validation. These include traditional analytical method validation according to USP <1225> Validation of Compendial Methods (Ref 1) as well as the holistic life cycle approach described in the proposed general chapters USP <761> NMR Spectroscopy (Ref 2) and USP <1761> NMR Spectroscopy – Theory and Practice (Ref 3), which is aligned with USP <1220> Analytical Procedure Lifecycle (Ref 4). With a focus on the quantitative branching procedure, various approaches are compared and discussed including: traditional individual assessment of analytical procedure performance characteristics of accuracy, precision, intermediate precision, robustness, specificity, etc.; total analytical error; and total measurement uncertainty.

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Quantification of Small Molecules for Vaccine Development by Benchtop NMR

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Several different types of vaccines are regularly examined in the pharmaceutical industry. Vaccines can contain large molecules such as proteins and polysaccharides or larger particles such as attenuated viruses, lipid nanoparticles or virus like particles. All these vaccine types contain a complex mixture of small and macromolecules, therefore several analytical tools often need to be assessed in order to assist in the various stages of vaccine process development.

Benchtop NMR is an advancing technology that can be a versatile analytical tool that provides quantitative information about the multiple components that arise in the development of a vaccine. Benchtop NMR often involves minimal sample preparation since macromolecular species are usually not detectable or can be removed by T_2 filters. The software for benchtop NMR is easy to use and provides automatic data processing further making benchtop NMR an attractive analytical tool that can be quickly assessed when comparing different analytical approaches.

In this presentation the use of benchtop NMR as an alternative to chromatographic methods will be discussed. Its use for real-time monitoring and fast method development will be demonstrated. Examples of using benchtop NMR to quickly determine solvent compositions, to confirm excipients and study alum sedimentation in vaccine-related samples are some of the examples that will be covered, with some comparison to chromatography-based methods. Furthermore, we will discuss how benchtop NMR method development is possible even from those with no NMR experience, which are clear advantages for the implementation of benchtop NMR in the pharmaceutical industry.

Potent and Bioavailable beyond Rule of Five Drugs: Insights from NMR

Nichola Davies[1]

1. AstraZeneca

The Lipinski 'Rule of Five' and subsequent 'drug-likeness' variants have revolutionised medicinal chemistry design by providing guidelines for passive permeability of drugs. In their seminal paper in 1997, Lipinski and colleagues recognised that oral absorption of drugs can be related to their molecular structural features and intrinsic physical chemistry properties. Despite this, several oral drugs have reached clinical development and regulatory approval whilst violating of one or more of these guidelines.

To understand why these Ro5 violations are tolerated, we examined Structure-Property Relationships of clinical candidates and marketed drugs using experimental free ligand solution conformations. Using NMR techniques, we explored the experimental 'drug-likeness' space using experimental structural descriptors for hydrogen bond donor and acceptor counts, and number of rotatable bonds, in additional to chromatographic measure of lipophilicity (ChromlogD) and exposed polar surface area (ePSA). We observed that potent oral drugs preferentially preorganized into a bioactive conformation and adopt conformations which allow polarity masking. This analysis provides important insights leading to more accurate prediction of the likelihood of oral exposure for beyond Ro5 drugs.

Hunting for original anticancer peptides: a key role for NMR within multidisciplinary approaches.

Marilisa Leone [1]

1. IBB-CNR Naples, Italy

Most physiological and pathological processes within the cells are regulated by protein-protein interactions (PPIs). Peptides hold a certain interest in drug-discovery as possible therapeutic agents for their superior potential, with respect to other small organic compounds, to work as PPI modulators. PPIs often occur through large and flat interaction interfaces that can be better targeted by peptide conformational plasticity and ability to establish an increased number of intermolecular interactions with the target proteins, leading consequently, to better binding affinity and selectivity. Advances in peptide synthetic routes and discovery of original ways to enhance ADME properties have also contributed lately to the growing interest of both Academia and Pharma in peptide therapeutics. [1]

Studies in our laboratory are focused on a class of protein binding modules called Sam (Sterile alpha motif) domains and on the EphA2 receptor tyrosine kinase. [2,3] EphA2 is a well-known target in the anticancer drug discovery field as its levels are upregulated in several tumour-types and it governs a complex and controversial signalling route by balancing pro- and anti-cancer pathways. EphA2 contains, within its domain organization, a Sam domain (EphA2-Sam) that is engaged in heterotypic Sam-Sam interactions with other protein regulators of receptor stability. Peptide inhibitors of EphA2-mediated Sam-Sam interactions represent potential anticancer agents. [2] To identify anti-cancer peptides targeting EphA2-Sam and its interactome, we are employing a multidisciplinary research plan where solution NMR techniques play a pivotal role. Structure-based virtual screening is first conducted to select putative peptide hits. Next, NMR screenings by diverse 1D and 2D experiments are performed to validate in silico predicted peptide ligands and rank them according to binding affinities through direct interaction assays and with the support of ancillary biophysical techniques. NMR-based displacement experiments are later employed to verify peptide ability to hamper Sam-Sam associations. Moreover, NMR is engaged to evaluate peptide selectivity for a certain Sam domain and to gain crucial structural insights for optimization routes. We will describe applications related to several kinds of Sam-targeting peptides (linear, stapled and with a variety of cyclic arrangements). [2,3]

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Acknowledgement: The research leading to these results has received funding from AIRC under IG 2021 – ID. 26121 – P.I. Leone Marilisa

Single-Use Technologies and Gamma Irradiation: Tracking Down a Biopharma Leachables Issue with NMR

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The implementation of single-use technologies (SUT) in biopharmaceutical manufacturing continues to grow throughout the industry in support of new modalities. Despite the upsides to SUT, there is concern about small molecule impurities from the single-use plastic and polymeric components. These impurities pose risks for product quality, as there is potential risk for particle formation and inhibited cell growth in protein-containing and biological material exposed to gamma-irradiated SUT components. To meet regulatory requirements, NMR spectroscopy is utilized as an essential tool for quality control and assessing product purity. In this work, NMR found high levels of leachables in a SUT set up designed for a possible drug vial filling process. Further investigation by NMR was used to determine that the contaminants were leaching out of silicone tubing in gamma-irradiated fill assemblies. Finally, this work shows that the underlying source of these leachable species is the gamma irradiation-induced degradation of plastic packaging surrounding the tubing in the assemblies.

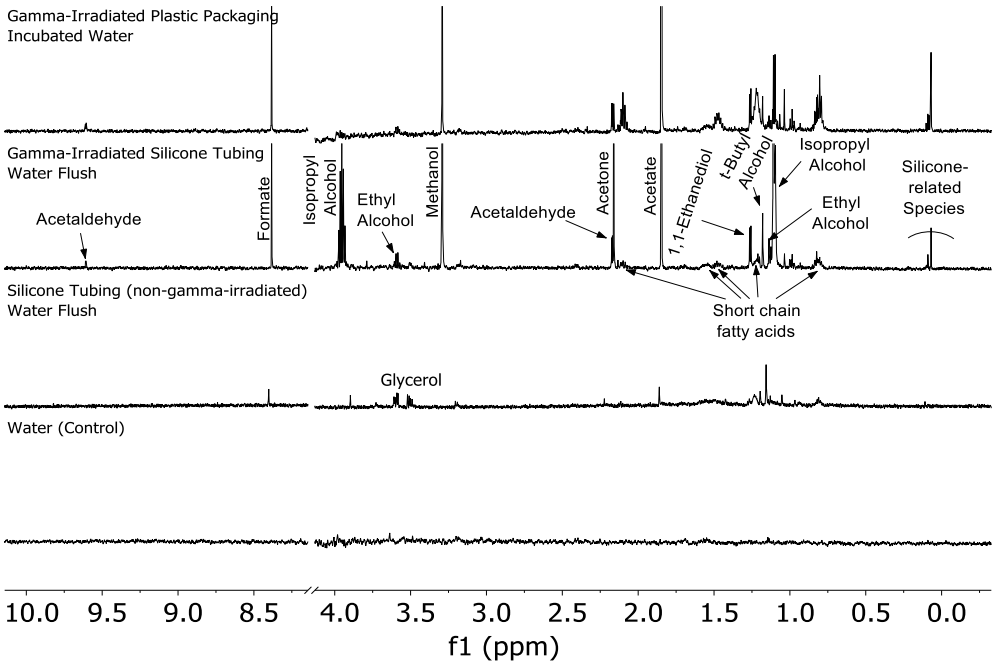

Figure 1. Stacked overlay of 600MHz ^1H spectra of a water control (bottom), a D_2O flush through silicone tubing from a non-gamma irradiated fill assembly (2nd from bottom), a water flush through silicone tubing from a gamma-irradiated fill assembly (2nd from top), and a water incubation of plastic packaging film (ultra low density polyethylene/ethylene vinyl alcohol/ethylene vinyl acetate) from a gamma-irradiated fill assembly (top).

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NMR Sensitivity Enhancement of Small Biomolecules using Hyperpolarized Water

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The low natural abundance and less sensitive NMR detection of nuclei such as ^{15}N limits applications at physiologically relevant concentrations. Dissolution dynamic nuclear polarization (D-DNP) readily polarizes spins of NMR active nuclei in most molecules.[1] Here, we demonstrate the use of hyperpolarization derived from water protons to enhance the NMR signal of ^{15}N in a small molecule. The highly concentrated proton spins in water allow hyperpolarization of target molecules through proton exchange and dipolar interactions.[2] This technique, alternative to the direct hyperpolarization of ^{15}N, removes a barrier preventing the repetition of polarization for ex-situ polarized spins. We employed a cross-polarization (*J*-CP) pulse sequence, which provides an efficient polarization transfer mechanism to ^{15}N near an exchangeable proton.[3] Hyperpolarization derived from water protons enhanced the NMR signal of ^{15}N in a small molecule, benzamidine, by 1480-fold. The polarization factor favorably compares to factors of 17 and 110-fold using INEPT and NOE based polarization transfer mechanisms, respectively. The polarization transfer from the exchangeable proton enables the accumulation of spin polarization on ^{15}N that is largely independent of the concentration of water protons, reducing the effect of the dilution of the hyperpolarized sample during dissolution with deuterium oxide. The thus produced hyperpolarization, facilitated the detection of binding of benzamidine at < 1 mM concentration to the target protein trypsin, in a single-scan measurement of ^{15}N R_2 relaxation. The binding of the ligand resulted in an increase of the R_2 relaxation rate from 31.3 ± 1.8 s^{-1} in the absence of protein to 41.1 ± 2.1 s^{-1}, in presence of protein. Further, the limit of detection that would correspond to an SNR of 3 is extrapolated to be ~50 μM of benzamidine. This concentration regime is compatible with the study of biological systems by observing the otherwise insensitive ^{15}N nucleus. Exchangeable protons bound to nitrogen nuclei are ubiquitous in biological molecules including proteins and nucleic acids, as well as small molecules involved in cellular processes, constituting a large pool of substrates hyperpolarizable by this method. In addition to protein-ligand binding studies, applications include the measurement of protein dynamics, protein-protein interactions, metabolomic research and others, in a native environment and in a short time frame.

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Workshop – Preparation for Atypical Samples: Metabolism, Anisotropic samples, Absolute Configuration

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Thomas Williamson[3]

1. Pfizer
2. Technical University of Darmstadt
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In many cases the preparation of an NMR sample is a straightforward process. However, there are times when sample preparation requires forethought and special considerations. This workshop contains three separate topics, all related to sample preparation for several types of NMR analysis: preparation of samples for anisotropic studies, the preparation of metabolite samples for structural elucidation and the preparation of mosher samples for the determination of absolute configuration.

The measurement of anisotropic NMR-parameters like residual quadrupolar couplings (RQCs), residual chemical shift anisotropy (RCSA) and especially residual dipolar couplings (RDCs) are of increasing importance not only in biomolecular NMR but also for the structural characterization of small molecules. In contrast to biomolecules, the focus here is mainly on the determination of relative configurations, although first results on the determination of absolute configurations are emerging.

A precondition for the measurement of anisotropic NMR data is to hinder the analyte from tumbling isotropically which can be done with uniaxially swollen gels or lyotropic liquid crystalline (LLC) phases. The talk will focus on the preparation and application of LLC-phases of helically chiral polyacetylenes and polyisonitriles for the (enantiodifferentiating) extraction of anisotropic NMR-parameters.

From the perspective of an NMR spectroscopist the structural elucidation of drug metabolites is particularly challenging for two reasons: the amount of material available for isolation can be extremely low, typically in the microgram range, and the target compounds are contained in complicated matrices with a myriad of unrelated large and small molecules. The preparation of a successful metabolite sample can be divided into two parts, the separation of the metabolites out of its matrices and careful preparation of the samples for NMR analysis. Details and examples of these two processes will be discussed.

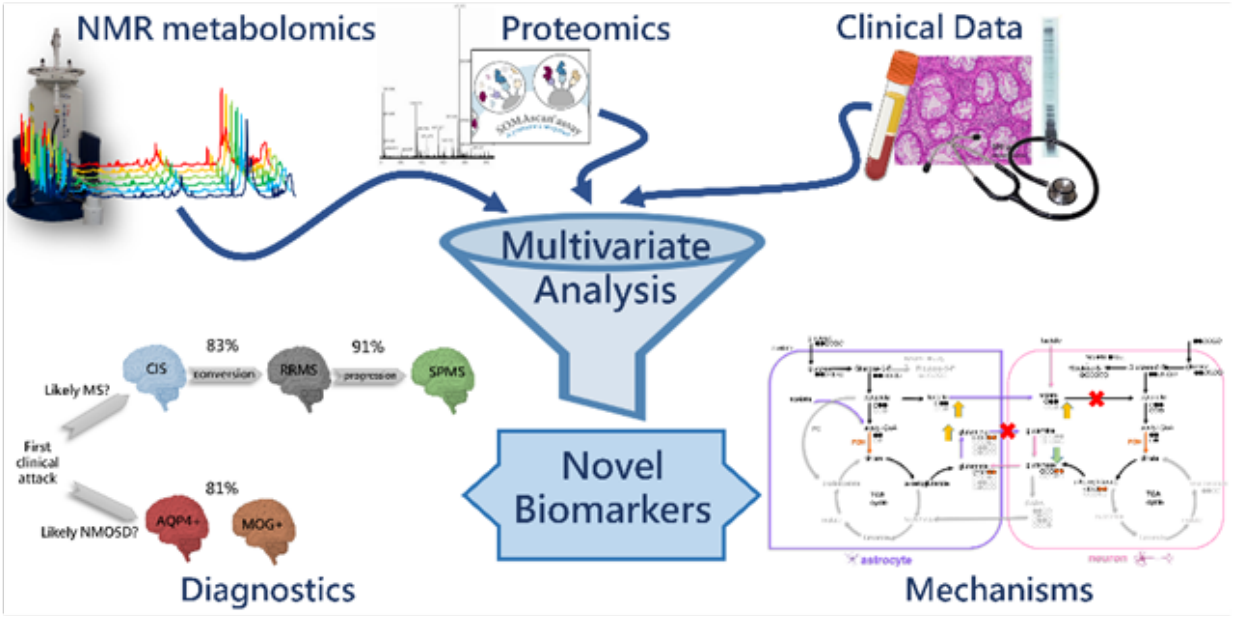
Time-honored approaches for the determination of absolute configuration include the application of the so-called “Mosher’s Method”. This technique involves derivatization of a stereocenter of unknown configuration with an agent of known chirality to form a diastereotopic system that can be analyzed by a variety of NMR techniques. The three-dimensional spatial configuration of the newly formed complex can be analyzed and interpreted based on comparison with known models or in some cases, through computational chemistry methodology. The activation barriers for the application of this powerful analytical method are often two-fold: 1. formation of the actual ester, amide, or other functionality, and 2. confident stereospecific NMR chemical shift assignment of all relevant protons or other applicable NMR nuclei. Solutions to these two problems and extension of this methodology to complex systems and alternate nuclei like ^{19}F will be discussed.
Acknowledgement: The research leading to these results has received funding from AIRC under IG 2021 – ID. 26121 – P.I. Leone Marilisa

NMR-based metabolomics to probe small molecule chemical pathways in the brain; towards novel treatments for neurodegenerative diseases

Fay Probert[1]

1. University of Oxford

Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system which affects 2.8 million people worldwide and is the leading cause of non-traumatic disability in young adults. While chronic inflammation is a hallmark of MS, the mechanisms by which inflammation contributes to cell death are not well understood and there remains no single pathognomonic clinical feature or diagnostic test. Consequently, diagnosing MS can be highly challenging, leading to difficulties in ensuring patients receive appropriate therapy promptly and potentially resulting in the incorrect selection of patients for inclusion in clinical trials.



We have shown, using a combination of NMR analysis of biofluids and machine learning methods, that inflammation in the brain generates profound NMR-detectable, small molecule changes in blood providing evidence that the development of an NMR-based blood test could be clinically useful for neurodegenerative diseases such as MS. Indeed, we have now applied NMR metabolomics to each stage of the MS diagnostic pathway and demonstrated that our methods can 1) distinguish between people with MS and other inflammatory neurodegenerative diseases (Accuracy)[1], 2) identify MS patients at the earliest signs of disease (Accuracy 83%[2]), 3) determine the stage of disease progression (Accuracy 91%)[3], and 4) identify clinical relapse[5].

The biomarkers identified by these studies reveal dysregulation of energy and neurotransmitter metabolism in people with MS which we have confirmed are associated with active neuroinflammation using *ex vivo* ^{13}C NMR spectroscopy analysis of MS model 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). Taken together, these data provide insight into novel neuroinflammatory chemical pathways which may prove to be useful drug targets in the future.

1) PMID: 29208041 2) PMID: 31659123 3) PMID: 32709911 4) PMID: 34755110

MINIATURIZED NMR AT YOUR FINGERTIP: HEALTH PROMOTION & DIABETES PREVENTION

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Diabetes mellitus places an enormous burden on individuals, families, and societies worldwide. The diabetes pandemic continues to worsen as prevention efforts have been only minimally effective. There is an urgent need for new approaches. This talk will highlight how miniaturized magnetic resonance (MR) technologies are revolutionizing the early detection of diabetes risk and the preservation of insulin-secreting beta cells. This breakthrough technology addresses the root cause at the right stage. It promotes cardiometabolic health, enabling individuals to avoid both prediabetes and diabetes.

Type 2 diabetes develops slowly (years) and in stages. The initial stage is early metabolic imbalance (EMI), a hidden state of compensated insulin resistance that eludes conventional medical screening. In 2017, we reported the serendipitous discovery that plasma and serum water T_2 detect EMI with high sensitivity and specificity. The initial discovery, from a study population of apparently healthy adults (n=72), was subsequently validated in an ancillary study of the PREMIER clinical trial (n=810). In those studies, T_2 was measured by time-domain MR relaxometry using a Bruker mq20 benchtop instrument. Current measurements utilize a miniaturized portable MR device developed by WaveGuide Corp. and a tiny finger-stick blood sample (15 microliters). Thus, plasma and serum water T_2 are remarkably practical, enabling health screening where people are: homes, workplaces, schools, fitness centers, stores, clinics, etc.

To streamline the procedure, we investigated whether similar health information could be detected using settled whole blood. It yields two T_2 values: T_{2S} detects water in the supernatant plasma, and T_{2P} detects water in the blood cell pellet. The latter monitors the functional status of hemoglobin inside red blood cells, as non-functional hemoglobin causes paramagnetic relaxation enhancement. To our surprise, whole blood T_{2P} was strongly associated with markers of insulin resistance, inflammation, and hypoxia. Thus, whole blood T_{2P} linked hemoglobin function to metabolic health. The missing link between insulin resistance and the slow beta-cell decline leading to diabetes appears to be an insidious, subclinical defect in hemoglobin's ability to deliver oxygen to cells and tissues. Pending further validation, this discovery provides the foundation for a new screening, monitoring, and treatment strategy to rescue beta cells and prevent diabetes.

To expand access to metabolic health testing, we developed a patented tabletop device and method to measure T_2 non-invasively in the living human fingertip. This novel MR platform monitors T_2 and lipid fluidity variations from the chemically diverse triacylglycerol molecules stored in adipose tissue. In turn, adipose tissue fluidity reflects diet (saturated/trans vs. mono- vs. poly-unsaturated fats – "you are what you eat") and *de novo* lipogenesis stimulated by insulin resistance. Thus, finger T_2 is an *in vivo* lipid fluidity meter, providing a unique window into metabolic health and disease risk. Research is ongoing.

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An alternative, CRAFT-based approach to extract essential inflammation markers from simple 1D serum samples

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There are a few very specific inflammation markers in blood, namely lipoprotein NMe signals of protein clusters (GlycA and GlycB) [1] and a composite resonance of phospholipids (SPC) [2,3]. The relative integrals of these resonances provide clear indication of inflammatory conditions, often related to serious diseases like cancer or COVID-19 infection.

Relatively complicated, yet very efficient experimental methods have been introduced recently (DIRE [2], JEDI [3]) to suppress the rest of the spectrum and thus allowing measurement of these integrals of interest.

We introduced a simple alternative processing method using CRAFT, a time-domain (FID) analysis tool [4], which can highlight selected subsets of the spectrum by choice for quantitative analysis. The output of this approach is direct, spreadsheet-based representation of the required peak amplitude (integral) values, ready for comparative analysis. The significant advantage of this alternative method is that it only needs a simple 1D spectrum with no experimental manipulation whatsoever reducing the experimental preparations to a minimum while saving time and avoiding specific hardware and software requirements for the data collection. In addition, there are no pre/post processing steps (such as baseline and/or phase correction) involved, thus further minimizing potential dependency on subjective decisions by the user and providing an opportunity to automate the entire process.

We applied this methodology to horse serum samples to follow inflammation for cohorts with OCD conditions. The CRAFT-based approach can be similarly applied to any other biofluid from any sources, including human samples. There is a potential to extend such a simple analysis to other, previously identified relevant markers as well.

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Launching a NUS 1H-13C HSQC Clinical Diagnostic For Kidney Transplant Management

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There is an unmet clinical need to develop better monitoring tools for kidney transplant recipients. The current blood-based rule out assays have limited utility and there is a need for rule-in assays that allow for early detection and course correction before irreversible damage is done to the graft. Here we will describe our process of discovery using the myOLARIS-Toolbox to elucidate a urine-based metabolite signature to detect graft injury. This includes use of Navigators or molecules that monitor sample quality and software that combines statistics, ML/AI and linking back to biology. Finally, we will discuss the analytical validation approach required to launch the assay as an LDT. To the best of our knowledge this is not only the world's first rule-in assay for transplant injury, but the first NUS HSQC-based clinical diagnostic.

Identity and Purity Deciphered Only via NMR

Charlotte Corbett[1]

1. DEA

In forensic drug chemistry, NMR is an ideal tool where the unexpected is the norm. Samples are mixtures that greatly vary. New compounds are continually created in the ever-changing world of circumventing the controlled substance laws. When neither reference material nor reference spectra exist, NMR is the only instrumental technique capable of identifying and quantifying. NMR can do this even when other compounds are present without chromatographic separation.

Some counterfeit fentanyl injectable solutions contain other controlled substances, with or without fentanyl. Analysis by liquid chromatography may be blind to the existence of other components. The low concentrations of active ingredients and the sample's aqueous nature does not lend itself well to other techniques, especially gas chromatography mass spectrometry, the drug chemist's workhorse. One NMR spectrum identifies and quantifies every constituent, such as cocaine, methampehtamine and heroin, which have been found in these solutions.

Some new compounds are too fragile for mass spectrometry, even with soft ionization techniques. When the molecular ion is unknown, and the molecular formula is in question, NMR can still elucidate the molecular structure with some confidence.

When reference material is unavailable, purity determination can only be performed via quantitative NMR (qNMR), which is fast, accurate, and reliable. Besides fentanyl, nitazenes have also caused overdoses. Are overdoses caused by tablets of varying concentrations, or is there a small span between the desired effects and an overdose? Without substantial method development, qNMR provides the information needed.
Forensic science applies the fundamentals of NMR to answer questions in the simplest way possible to the dynamic world created by the clandestine synthetic chemists. With one spectrum, NMR can identify the substances and determine their concentrations, without prior knowledge. This session demonstrates NMR's versatility and importance.

Analysis of Polymers in Food Packaging Using Solid- and Liquid- State ^{19}F NMR

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The presence and identification of compounds or polymers containing per- and polyfluoroalkyl substances (PFAS) in food packaging materials has been of interest to the FDA for many years. [1] Polymers containing fluorinated alkyl moieties are applied to food packaging specifically for grease and water resistance. In 2020, the FDA obtained commitments from the food contact substance (FCS) manufacturers marketing FCS containing certain PFAS to phase-out select grease proofing agents containing the 6:2 fluorotelomer alcohol. [2] As the FDA recently announced the completion of this phase-out, [3] this raises the need for analytical methods as the FDA monitors the market disappearance of these substances in products. Examining the structures and identities of these polymers can be challenging, however NMR offers numerous advantages compared to other techniques that allows us to better analyze these materials. [4] Specifically, fluorine-19 NMR (^{19}F NMR) has been shown to be useful when examining fluorine content due to favorable properties of the fluorine nucleus. For example, fluorine has a 100% isotope abundance, a high gyromagnetic ratio, and a large chemical shift range. Additionally, it generally provides relatively clean spectra in complex samples as there are often very few fluorine-containing compounds present. [5] In this study we aim to examine the fluorine content present in food packaging market samples using NMR. To assess the fluorine in the paper samples a base hydrolysis was performed, then NMR was used to examine the samples. Liquid-state ^{19}F and ^{1}H NMR were used to identify the presence of the fluorotelomer alcohol, a compound known to be present in samples containing certain fluorinated polymers. Additionally, solid-state ^{19}F NMR with magic-angle-spinning was utilized to examine the samples before and after the hydrolysis to assess the amount of fluorine removed. Finally, we will discuss experimental parameters that should be considered for these experiments, which are especially important for our efforts at quantification of the fluorine.

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Development and Application of a Universal, Selectively-Tunable ^{1}H and ^{19}F qNMR Calibrant

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Quantitative NMR (qNMR) is a robust technique used in a variety of chemical industries due to its numerous analytical advantages. It exhibits a universal response, a broad linear range, diverse compound applicability, and allows for facile method development. The most accurate qNMR results are obtained with an internal calibrant, but no truly universal calibrant currently is known that is stable, non-toxic, commercially available, exhibits a unique chemical shift, is highly soluble in both aqueous and non-polar solvents, and exists as a non-hygroscopic solid for accurate weighing. In this study, we investigated several potential qNMR calibrants and identified 2,2-difluoroacetamide (DFA), which meets all the aforementioned criteria. This calibrant is soluble in a wide polarity range of both protic and aprotic solvents from D_2O to CD_2Cl_2, and it exhibits selectively tunable ^{1}H and ^{19}F chemical shifts via decoupling that resonate in sparse regions of the typical NMR spectrum. We evaluated the broad utility of this standard by assaying 13 active pharmaceutical ingredients in 12 different deuterated solvents. Our results demonstrate excellent measured accuracies and precisions of 100 ± 1% and % RSD (n=3) less than 1%, respectively. We then applied this internal calibrant for several challenging quantitation problems in the pharmaceutical industry, such as purity analysis of complex, macrocyclic peptides, biocatalytic reaction monitoring, mass limited drug discovery samples, and analysis of low solubility zwitterionic reaction intermediates. Therefore, we recommend difluoroacetamide as a reliable and generally applicable internal calibrant for qNMR analysis.

Applied NMR Methodology to Aid Consumer Product Understanding

Jacqueline Thomas[1]

1. Procter & Gamble

This presentation will highlight the co-creation process of Analytical working with modelers to go after relevant parameters for building models. The seminar will cover the measurement of pKa's of SDS/CAPB by NMR. The use of pKa determination by NMR is often overlooked and sometimes long forgotten art. The methodology for pKa determination and best practices of the measurement will be discussed.

Characterization of Complex Mixtures for Petroleum Applications

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1. ExxonMobil Technology and Engineering Co.

NMR (Nuclear Magnetic Resonance Spectroscopy) is one of the best chemical diagnostic tools for the characterization of hydrocarbons. However, a major limitation of the NMR technique is its low sensitivity and difficulty resolving peaks in a mixture. Many advances have been made since its inception to overcome this limitation in both hardware and software; arguably the most important one in recent years is the development of cryogenically-cooled probes, giving up to a 4-fold increase in sensitivity. The increase in sensitivity (and, thus, 16x shorter acquisition time) and resolution at higher fields enables detailed characterization of complex hydrocarbon mixtures greatly enhancing the utility of two-dimensional (2D) NMR experiments.

Bio-based materials, such as lignocellulosic biomass from trees, are composed of complex polymeric structures of cellulose, hemicellulose, and lignin. Biomass pyrolysis is a thermal degradation process in the absence of air to break the polymeric molecules into smaller oxygenated species. Biomass pyrolysis oils are composed of water, acids, aldehydes, sugars, guaiacols, phenols, alcohols, and ketones. They could be potentially upgraded to make transportation fuels and other chemicals. Understanding the composition of pyrolysis oils from different sources and processes is important to guide their applications.

NMR provides quantitative information on oxygenated species essential to understanding the composition and utility of pyrolysis oils from Bio-based materials. Two-dimensional (2D) NMR techniques separate the signals in two dimensions for better resolution and assignment of the peaks. Multiple-bond Carbon – Proton correlated 2D NMR experiments allow for detailed assignment of oxygenate functional groups to its nearest neighbors.

Detailed molecular characterization is performed using a variety of homonuclear and heteronuclear 2D NMR experiments that significantly enhance the structural characterization of complex mixtures. In particular, the analysis of bio-based feeds containing complex mixtures of small, oxygenated molecules such as Phenols, sugars, aldehydes and ketones will be discussed.
An overview of 2D NMR techniques and their utility for characterizing complex petrochemical mixtures will be presented. The use of derivatization reactions to quantitate small molecule oxygenates in a complex mixture will also be discussed.

DECIPHERING THE CHEMICAL COMPLEXITY OF POLYSORBATE SURFACTANTS.

José G. Napolitano.[1]

1. Synthetic Molecule Pharmaceutical Sciences (SMPS), Genentech Inc.

Polysorbates are non-ionic surfactants used extensively over the past few decades in the development and manufacturing of pharmaceutical products.[1] Despite their widespread use, evaluating the quality and composition of polysorbates is difficult due to their heterogeneity. This presentation will highlight the application of both experimental NMR approaches and computational tools to study the inherent chemical complexity of polysorbate surfactants at a molecular level. Characterization of headgroups, polyethylene glycol chains, and terminal fatty acids was carried out using a combination of qualitative and quantitative 1D/2D NMR experiments, quantum-mechanical spectral analysis,[2] and deuterium-induced differential isotope shift analysis.[3] In addition, chemometric approaches were implemented to categorize and differentiate four different types of polysorbate surfactants and to investigate lot-to-lot variability.

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Automated Structure Verification (ASV) Tools

Kevin Robbins[1]

2. AstraZeneca

Accurate structure verification is essential for advancing drug discovery research. This presentation introduces an Assisted Structure Verification (ASV) tool, developed at AstraZeneca in collaboration with ACD/Labs, designed to streamline structure verification for reaction products using NMR data.
The ASV tool features the generation of reaction products, automated peak picking and data analysis, along with a match factor score for each product. We will demonstrate its capabilities through case studies and discuss strategies for mitigating automation risks, ensuring dependable and efficient results.
By leveraging the ASV tool, chemists can achieve more reliable results in less time, accelerating the drug discovery process.

SMASH 2024

POSTERS

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Accurate ^{13}C Chemical Shift Anisotropies (RCSAs) of Water Soluble Small Molecules Measured in Stretched Gelatin Gels

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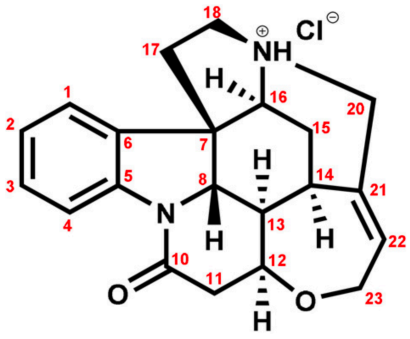
3. Institute of Chemistry, University of Campinas—UNICAMP, Campinas, Sao Paulo, Brazil

Using the CASE-3D protocol,[1] the correct configuration of strychnine-HCl (**1**) was unambiguously selected from a pool of 13 possible diastereoisomers by applying ^{13}C residual chemical shift anisotropies (^{13}C-RCSAs) measured on a sample of **1** aligned in stretched gelatin gels in D_2O. Kuchel's stretching device [2] was used to prevent the interference of isotropic chemical shifts on the measurement of the ^{13}C-RCSAs. Gelatin samples of different bloom strengths were used. Since the strength of the supramolecular structure of gelatin strongly depends on the sample's pH, the ^{2}H residual quadrupolar couplings (^{2}H-RQC) of D_2O in stretched gelatin were measured at different pH values.

Accurate ^{13}C-RCSAs for every carbon of **1** were measured using standard 1D $^{13}C\{^{1}H\}$-NMR experiments collected on an NMR instrument running at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Measurements and data analysis of RCSAs were performed according to a well-established protocol.[3] This is the first report on the measurement of RCSAs in stretched gelatin gels.



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2

Better Liver through NMR? Markers of Fatty Liver Disease Probe Biochemical Pathology and Surgical Intervention

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Metabolic associated fatty liver disease (MAFLD) is among the most widespread diseases in the world. An increasing indicator for liver transplants and leading to greater patient mortality, MAFLD is straining healthcare infrastructure and challenging clinicians and researchers who are in need of non-invasive tests to assess the complex progression of this disease. The gold standard for assessing the liver remains an invasive and costly liver biopsy, hindering its use in care and in evaluating potential therapeutics.

We previously demonstrated the ability to design potent studies of liver health with standard-of-care sera that may also help to better model realistic subjects in the general population.[1] We report here on the next and larger phase of this work, a suite of several NMR metabolomic studies assessing liver health, spanning about 200 subjects, that first evaluated reproducibility and then examined longitudinal biomarkers of response, as well as additional selected case studies, in order to analyze and develop the potential for NMR to assess liver health and inform the complex biochemical progression of MAFLD. While focusing on NMR-derived biomarkers of MAFLD, particularly its advanced stages relative to intervention, insights on working with lipemic sera and detecting exogenous compounds will be included.

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3

Drug- like small molecules binding to RNA: NMR structure and binding assessment of the complex

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Abstract: Here we present two projects under investigation in our group.
The first project reports the discovery of a small series of drug-like small molecules that satisfy all Lipinski rules of pharmacologically viable small molecules and bind to the precursor of the pro-oncogenic and pro-inflammatory non-coding RNA miRNA-21 with mid-nM affinity and specificity. These molecules target a local structure at the Dicer cleavage site and induce distinctive structural changes in the RNA which correlate with specific inhibition of miRNA processing. Among all the molecules investigated, compound 52 showed numerous intermolecular NOE connections with mir-21 along with closing its loop by forming G: U base pairs, suggesting that the entire 52 molecule is in direct contact with the RNA. Structurally conservative single nucleotide substitutions eliminate the conformational change induced by the small molecules, which is not observed in other miRNA precursors. The most potent one reduces cellular proliferation and miR-21 levels in cancer cell lines without inhibiting kinases or classical receptors, thereby providing an avenue towards therapeutic development in multiple diseases where miR-21 is abnormally expressed.

The second project reports NMR structure of a specific complex between Palbociclib and HIV-1 TAR RNA. Palbociclib binds to the TAR with nM affinity with specificity. Palbociclib recognizes a site spanning the UCU bulge and induces formation of a new G36-C29.C24+ base triple and a structure never observed before. The C24 base of UCU bulge is deeply inserted into the major groove of stem I and is in close contact with G36. The aromatic protons of Palbociclib depict gigantic chemical shift changes and exhibit intermolecular NOEs with G21, A22, G26, U40 and C41 residues. Furthermore, 2-OH and NH2 groups for all residues of Stem I are observed in NOESY and 1H-15N HSQC indicating the stabilization of the RNA structure.

4

On the possibility of 13C NMR based titration through AI-driven denoising

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A significant portion of research in the development of spectroscopic processing techniques focuses on developing methods to filter the useful, information-rich portion from the raw mixture of desired and undesired signal components. Nuclear Magnetic Resonance (NMR) spectroscopy is no exception. A prevalent issue in 13C NMR spectroscopy is the poor signal-to-noise (S/N) ratio, attributed to the very low natural abundance and low gyromagnetic ratio of the ^{13}C isotope.

Obtaining interpretable data for small molecules hence requires several measurement repetitions, which is highly time-consuming. When the concentration of the NMR sample is low, acquiring even a relatively good 1D spectrum can take up to 5-6 hours.

Recent advancements in machine learning have addressed numerous problems in the field of spectroscopy, such as image denoising and signal processing. Previously, Convolutional Neural Network (CNN) and U-Net (Wu et al.) have been used in denoising. However, most of the models are trained on a particular target molecule, and hence often fails during generalization.

We have successfully implemented Recurrent Neural Network (RNN) models, specifically Long Short-Term Memory (LSTM) and Gated Recurrent Unit (GRU) models, to denoise 13C NMR spectra. Our model can enhance the S/N ratio in 13C NMR spectra by a factor of up to 1000x compared to the input signal. For instance, after processing, a 16- scan input spectrum can achieve an S/N ratio equivalent to that of a 5000-scan spectrum. Besides enhancing 13C spectra, the model can also retain the phase information of the peaks. As a result, it can be used in NMR experiments like Distortionless Enhancement by Polarization Transfer (DEPT), where phase encodes useful information and hence is very important for spectral analysis. Additionally, we trained our model on both timedomain and frequency-domain data to see how denoising works and to understand why it's more effective in frequency-domain data.

One of the best features of our model is its remarkable generalization capabilities, allowing a single model to be applied to different molecules of comparable spectral complexity. This advancement drastically reduces the time required to obtain high-quality ^{13}C NMR spectra, thereby facilitating more efficient and accurate spectral analysis. We have also used other models like CNN and transformers to compare the results.

In conclusion, integrating AI-driven denoising techniques, specifically RNN models, represents a transformative advancement in 13C NMR spectroscopy. By effectively enhancing the S/N ratio, these methods enable more rapid and reliable spectral analysis, 1 promoting deeper insights and fostering significant scientific progress across various disciplines. Also, with slight modification, this type of model can be used to denoise spectra in different types of spectroscopic processing techniques.

5

GNAT FOR STATS: A COMPREHENSIVE MODULE FOR CHEMOMETRICS AND METABOLOMICS FOR NMR DATA

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The General NMR Analysis Toolbox (GNAT)[1] has established itself as a versatile software suite for processing and analyzing NMR data. Now the toolbox has expanded to encompass comprehensive metabolomics analysis. This expansion is driven by the inclusion of a new suite of powerful statistical tools designed for tasks like classification, discrimination, and correlation analysis, such as Principal Component Analysis (PCA), Partial Least Squares Discriminate Analysis (PLS-DA)[3], Orthogonal Projections for Latent Structure Discriminant Analysis (OPLS-DA)[4] and Total Statistical Correlation Spectroscopy (STOCSY)[5]. A set of preprocessing tools before metabolomics analysis for binning[2] and variable selection (iPLS and biPLS)[6] is also available, as well as graphical tools for outlier detection. Additionally, graphical tools for outlier detection are available, allowing researchers to identify and address potential data inconsistencies. Model validation and application to unknown samples are a straightforward process, accompanied by relevant analytical figures of merit. All analyzes done in the toolbox can be exported as reports in various formats (i.e. .txt, .xmls or .mat). The standard version is intended to be run with MATLAB. User can also use an available standalone compiled versions for Windows, Mac, and Linux operating systems. These new functionalities are demonstrated using a test data set for classification of edible oils.

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6

NMR Relaxation Studies of Chelated Diphosphine Platinum Dichlorides

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Platinum complexes with bidentate phosphine ligands Ph_2P-$(CH_2)_n$-PPh_2 exhibit varied regioselectivity and catalytic rates in olefin hydroformylation and other processes.[1-2] Previous research has shown that increasing the methylene chain length of diphosphine ligands from 1 to 3 carbons, when used with $SnCl_2$ as a co-catalyst, results in a more than 100-fold increase in the relative rate of hydroformylation of 1-pentene.[2] Gaining more in-depth knowledge about the structures of these catalysts in the solution state could enhance the understanding of their mechanisms. We decided to explore the environment around the platinum center by investigating the longitude relaxation constant (T_1) values for L_2PtCl_2 (L_2 = dppm, dppe, dppp) (Figure 1). Following the method developed in Claridge's group, we measured T_1 using exchange NMR spectroscopy (EXSY) (Figure 2).[3] The technique involves acquiring a series of EXSY NMR spectra for each molecule in the study while varying the mixing times (τ_{mix}) as an independent variable. To obtain T_1 values, rate values were determined from the relationship between $I_{cross}/I_{diagonal}$ (cross-peak intensities) and τ_{mix}. The experiments were conducted in two solvents, DMSO and DMF. This presentation will discuss our findings from these relaxation studies conducted at room temperature (Table 1) and initial studies of the temperature dependence of the relaxation behavior.

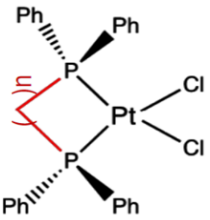

Figure 1.
$dppmPtCl_2$ (n=1)
$dppePtCl_2$ (n=2)
$dpppPtCl_2$ (n=3)

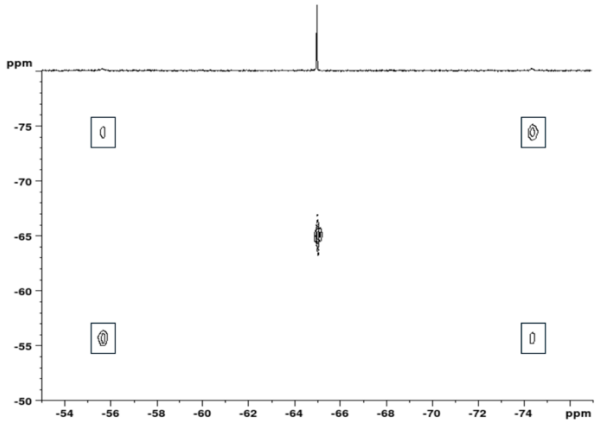

Figure 2. An example of P EXSY NMR spectrum that was analyzed to calculate T_1 for $dppmPtCl_2$ in DMF with integration regions.

Solvent / Compound	T_1 (ms) in DMF	T_1 (ms) in DMSO
$dppmPtCl_2$	11.9	5.68
$dppePtCl_2$	29.2	15.5
$dpppPtCl_2$	21.6	11.5

Table 1. Final T1 values in ms depending on the compound and the solvent.

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7

EXPANDING THE SCOPE OF HIGH-THROUGHPUT NMR: HANDLING PECULIAR 2D PEAKS IN AUTOMATED STRUCTURE VERIFICATION

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The synergy of modern NMR instruments, automated sample handling, and data interpretation through Automated Structure Verification (ASV) has ushered in an era of unparalleled high-throughput NMR capabilities. These automated systems are typically configured with standardized acquisition parameters[1] to cater to a diverse array of samples efficiently. Nevertheless, despite scientists' best efforts to optimize these parameters, instances arise where these parameters yield spectra with peaks deviating from their anticipated positions or phase.

For instance, employing an HSQC experiment with an F1 width set to 0-160 ppm for samples containing aldehyde or Si•CH3 groups may lead to peaks appearing at the periphery of the F1 window, if not entirely beyond it and aliased over[2]. Additionally, modern adiabatic HSQC-DEPT pulse sequences, designed to enhance experiment sensitivity[3], may yield inaccurate results in ASV systems for samples with cyclopropyl or acetylene protons. Peaks corresponding to these groups may manifest with the opposite phase in the resultant spectra.

Discrepancies between expected and observed peaks can introduce significant disruptions to high- throughput workflows, forcing chemists to manually review datasets and, in some cases, re-record spectra with parameters more suitable for that sample. However, by providing the ASV system prior knowledge of the proposed structure, and training it to recognize peaks with unexpected presentations, these disruptions can be avoided.

This poster examines such an automated solution that seamlessly addresses this challenge, demonstrating its effectiveness in high-throughput laboratories. A range of examples showcasing each case will be presented, underscoring the robustness and reliability of the proposed approach.

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8

Generative Machine Learning for Automating Structure Elucidation in Synthesis

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Accurately elucidating molecular structures from Nuclear magnetic resonance (NMR) spectroscopy is of pivotal importance in chemical synthesis and drug discovery. Current methods, such as Computer-Aided Structure Elucidation (CASE)[1], are based on 'library search' and cannot interpret unseen molecules, thus limiting the accuracy of elucidation. Therefore, we want to make use of machine learning methods, so that the relationship between NMR spectroscopy data and molecular structures can be learned, and the process of structure elucidation can be automated.

In this project, an architecture with combination of Graph Neural Network (GNN) and Recurrent Neural Network (RNN) is trained to generate molecules from their NMR chemical shifts alone (Fig. 1a). Starting from floating atoms with atom types and chemical shifts extracted from the NMR spectra, the model sequentially assigns bonds that best fulfil the environment of chemical shifts until valences are fully saturated and a complete molecule is generated (Fig. 1b).

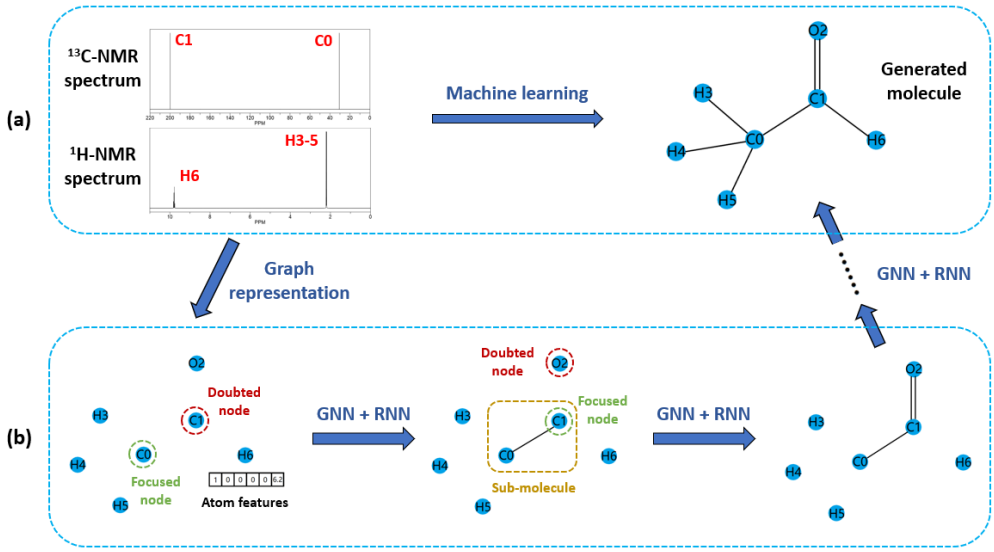

Fig. 1 (a) The target of this project. (b) The workflow of the machine learning model.

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REACTION MONITORING USING PSYCHE PURE SHIFT NMR SPECTROSCOPY

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NMR spectroscopy is a powerful method for process monitoring. Being both non-destructive and quantitative, is excellent for real-time measurement of reaction kinetics, especially when the reaction can be performed in a standard NMR tube. The commonest method to track a reaction by NMR spectroscopy is the ^{1}H pulse-acquire experiment due to its simplicity and sensitivity. However, ^{1}H spectra often suffer from severe overlap because of a narrow chemical shift range and an abundance of homonuclear couplings. This overlap can result in resolution problems, complicating or even making it impossible to measure concentration changes during a chemical reaction. One effective solution is to record pure shift spectra, where the effects of homonuclear couplings are supressed, producing only one signal per chemical environment. This approach significantly enhances the resolution, but it comes with drawbacks including lower sensitivity and longer acquisition time. Pure shift spectra are not directly internally quantitative within each spectrum – the relative integrals are not directly proportional to the number of protons. However, when T_1 and T_2 relaxation times do not change as the reaction progresses, they are quantitative between experiments. This is true for most multi-pulse experiments, such as HSQC, commonly used for reaction monitoring [1]. This study explores the potential and limitations of the PSYCHE pure shift method [2] for reaction monitoring, using a prototypical reaction: sugar hydrolysis. Saccharides are particularly prone to spectral overlap due to their limited chemical shift range and exist in the forms of tautomers in solutions, making excellent candidates for evaluating the use of pure shift NMR in reaction monitoring.

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10

Quantification of Ethanol Clearance in Liposomal Emulsion Vaccines Using Benchtop NMR

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The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) mandates a stringent limit of 5000 µg/mL residual ethanol content in pharmaceutical products[1]. Recently, mRNA-based vaccines that utilize Lipid Nanoparticle (LNP) formulations as the drug delivery system have been employed to reduce the COVID-19 pandemic[2]. To formulate LNPs or other emulsion systems, ethanol is utilized to dissolve essential lipid components for the targeted formulation [3,4]. Ethanol is removed through filtration methodologies during downstream procedures and residual ethanol is traditionally quantitated via Gas Chromatography (GC) [5]. However, this technique is associated with significant drawbacks such as reliance on external helium or hydrogen gas sources and vapor generation, instrument maintenance, disruption of the liposome, and significant user training for new analysts. The work described herein introduces the development of Benchtop Nuclear Magnetic Resonance (NMR) protocols for quantifying residual ethanol content in multiple LNP or complex lipid emulsion systems. Using a 60 MHz Benchtop NMR, we successfully developed methods capable of quantitating ethanol down to 12 ug/mL, over 400-fold below the required threshold. Suppression of solvent and lipid signals was achieved through utilization of built-in WET and T2 filters from the software. The method development conducted substantiates the viability of Benchtop NMR as a potential alternative to GC for ethanol quantification for process monitoring. Key advantages of benchtop NMR include simpler operation, decreased instrument maintenance, minimal sample preparation, and an absence of waste generation. The robustness of this environmentally friendly method was demonstrated through precise measurements of accuracy, linearity, repeatability, and sensitivity across multiple complex liposomal formulations, including LNPs and other liposomal emulsions for use in vaccine formulations.

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11

Advanced Structure Analysis Reveals a Transient Portimine B Hydrate

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Portimine B was isolated from an extract derived from the dinoflagellate *Vulcanodinium rugosum*, a known producer of the closely related portimine A. Initial molecular characterization studies of portimine B suggested an exact mass of 418.2230^{+}, consistent with an open tetrahydrofuran ring, contrary to the intact ring moiety found in portimine A.[1] Additionally, portimine B eluted well ahead of portimine A in reversed phase chromatography, despite the replacement of one acyclic hydroxy group found in the latter with a ketone. Further, an important $^{3}J_{CH}$ HMBC correlation from H10 to C7 was observed in portimine A, but not in portimine B, thus supporting an open ring structure. In 2023, the Baran lab synthesized both portimines A and B and provided powerful evidence that both macrocyclic analogs contained the intact tetrahydrofuran ring tautomer.[2] Their work also provided material that allowed deduction of the anti-cancer mechanism of action for both compounds. While Baran's efforts clearly established the correct structure of portimine B, key incongruities between the original and total synthesis reports remained unaddressed. In this work, we utilized recent advancements in density functional theory and NMR spectroscopy to define the origin of the structural divergence, which was concluded to ultimately be attributed to the presence of a transient hydrate moiety. In support of this structure, chemical shift predictions were carried out using the DELTA50 methodology, which yields extremely high accuracy for data acquired in $CDCl_3$.[3] Further, the transient hydrate structure was confirmed using the recently reported i-HMBC NMR experiment which allows differentiation of $^{2}J_{CH}$ vs $^{3,4}J_{CH}$ HMBC correlations.[4] These combined techniques enabled us to provide an orthogonal confirmation of structure and highlight modern molecular characterization approaches that could have helped to avoid the structural misassignment of portimine B.

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12

Assembling a small-molecule library for NMR drug discovery approaches

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It is well known that protein-protein interactions (PPIs) are the drivers of many physiological and pathological processes within the cell.[1] NMR spectroscopy represents one of the most appealing techniques in drug-discovery by providing a variety of direct and displacement-based interaction assays, along with protein-based or ligand-based screening approaches, to identify novel ligands of specific target proteins and PPIs.[2] In this context, small molecules can be considered optimal candidates as modulators of PPIs because they are often linked to advantageous ADME (Absorption, Distribution, Metabolism, and Excretion) profiles.[1] Since more complex compounds may undergo sub-optimal interactions and/or clashes, the development of small molecules targeting proteins can start from molecular fragments which usually overcome these issues.[3] The fragments, despite being characterized by low affinities, provide the possibility to explore greater chemical space and can be easily transformed into more efficient ligands.[3] Therefore, a small fragment library to be implemented in NMR screenings against disease-related proteins was recently ad hoc assembled in our laboratory. More in detail, the dataset "Maybridge fragment collection", that includes ~30000 fragments, was deeply analyzed and the largest attention was given to three of its compound subsets: "^{19}F", "Ro3 Diversity", "Ro3 Compliant for CherryPicking".[4] The purpose was the selection of fragments eligible for the applications of ^{19}F NMR spectroscopy in drug discovery, possessing the drug-like properties described in the so-called "Rule of Three" (molecular weight < 300 Da, number of H-bond donors ≤ 3, number of H-bond acceptors ≤ 3 and ClogP ≤ 3) and characterized by high levels of chemical diversity. [4] Based on these criteria, thus far 424 fragments have been selected, and, a few of them have been purchased. First, compound identities and purities have been evaluated by 1D [^{1}H] and 2D [^{1}H, ^{1}H] TOCSY and NOESY experiments. Next, fragments have been subdivided into mixtures (including 4-5 members) and are being evaluated against protein targets available in our laboratory, such as the Sam domains from the EphA2 receptor and its binding partners (e.g., Ship2-Sam and Odin-Sam1) that might play a role in cancer onset and progression.[5] To analyze interactions between the compound library and Sam domains, we are employing NMR approaches based on both protein- and ligand-observation. Our final goal is the discovery of "hit fragments" that can possibly be employed for future optimization steps.

Acknowledgement: The research leading to these results has received funding from AIRC under IG 2021 – ID. 26121 – P.I. Leone Marilisa.

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13

Unravelling the essence of 4-arginine in conopressin: synthesis, characterisation, in-silico exploration and behavioural impact of L and D-Arg conopressin

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Conopressins are single disulfide conopeptides with close sequence similarity to vasopressin and oxytocin and have shown "grooming" effects in animals similar to vasopressin and oxytocin. CFIRNCPKG* is identified from the Conus monile species from the southeastern coast of India. Arg residue is conserved in most of the conopressin sequences identified to date [1,2]. Hence, we aimed to understand the importance of L and D-Arg on the conformation of the cyclic peptide. The present study involves determining the 3D structures of synthesised conopressins, CFIRNCPKG* and CFI^{D}RNCPKG*, by solution NMR and the effects of L and D Arg on the conformation of the peptide backbone. We have elucidated the 3D structures of these peptides and investigated the conformational effects of L- and D-Arg residues. Our findings reveal distinct structural features, including type II beta turns and *trans* proline conformations, influenced by the stereochemistry of Arg. Furthermore, peptide-metal interaction studies uncover intriguing copper complex formation, shedding light on potential metal-mediated effects. Pharmacological assays involving intracerebral peptide injection in mice demonstrate dose-dependent alterations in grooming behaviour, with differential effects observed between L-Arg and D-Arg variants. Additionally, *in silico* binding studies elucidate the differential interactions of these peptides with oxytocin and vasopressin receptors, highlighting the importance of Arg residue stereochemistry. This study provides an understanding of the impact of Arg residue on structure and activity that can help to understand its role in conopressins and design novel and selective ligands.

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14

USING ON-LINE REACTION MONITORING BY FLOW NMR TO IMPACT DRUG DEVELOPMENT

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On-line reaction monitoring by flow NMR is an invaluable tool for chemists to monitor reactions "live" under flow conditions, yielding instantaneous information about starting materials, intermediates, products, and impurities simultaneously. Information gained from this type of analysis can be used to guide reagent addition, optimize reaction conditions, and study reaction mechanisms.[1] This is especially important when other methods of reaction monitoring (i.e. LCMS, GCMS, IR, SFC) are incompatible with the reaction components or conditions. Having the ability to quickly scale-up synthesis of a compound is critical in the pharmaceutical industry when a hit has been identified and further testing is required. We have developed a process utilizing on-line reaction monitoring by flow NMR with a Bruker Insight 3mm flow line, allowing analysis on as little as 2 mL solvent. Traditionally, flow NMR requires more than 20 mL solvent and significant quantities of reagents. Our system uses low volumes to minimize the use of precious starting materials and limiting the cost of using deuterated solvent to avoid the subsequent loss of signal(s) near solvent peaks due to solvent suppression techniques. Additionally, minimizing solvent and reagent requirements allows chemists to increase process sustainability and reduce unnecessary expenditures. We have established the ability to monitor reactions by 1D NMR (observing ^{1}H and/or ^{19}F nuclei quantitatively and ^{13}C nuclei qualitatively) or 2D NMR (fast, single-scan COSY and/or HSQC using NUS). These NMR experiments can be interleaved as needed to customize monitoring different compounds that are concurrently present in the reaction mixture.

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Background-Free Food for *Daphnia magna* – Towards Investigating *In-Vivo* Processes at Natural Abundance ^{13}C

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The application of NMR to environmental research is becoming increasingly important. More specifically, *in-vivo* NMR has potential to be instrumental in toxicity studies, explaining how chemical stressors impact organisms on a biochemical level.[1] Organisms act as the 'ultimate biosensor,' allowing researchers to study the biochemical pathways impacted and examine the toxic-mode-of-action of stressors.[2] *Daphnia magna*, commonly used in toxicity testing,[3] can be studied with *in-vivo* NMR, allowing for a thorough understanding of toxicity pathways at the molecular level.

2D HSQC experiments are often employed to overcome the broad lineshape and spectral overlap that arises during *in-vivo* experiments.[4] The reduced sensitivity of 2D HSQC experiments, compared to 1D ^{1}H, is typically offset by using ^{13}C-enrichment. During *in-vivo* experiments, organisms must be sustained with food to prevent extra stress. ^{13}C-enriched *D. magna* can be fed algae at natural abundance (NA) ^{13}C to avoid spectral interference, given the 100-fold lower signal from the algae. This, however, would produce significant spectral interference when analysing NA organisms, which is becoming more feasible with the improved sensitivity of novel NMR technologies. Studying organisms directly from the environment is subsequently inhibited, generating a need for a background-free food source for *in-vivo* NMR. To address this, we use algae grown using 99.99% ^{13}C-depleted CO_2 for use as a 'background-free' food source for NA *D. magna*.

^{13}C-depleted *Chlamydomonas reinhardtii* algae was grown in an airtight photobioreactor using 99.99% ^{12}C-CO_2. The ^{13}C-depleted algae shows a 99.87% reduction in signal intensity compared to NA algae. The signals from the algae itself are hardly detectable after 21 hours on a cryoprobe at 500 MHz.

The need for ^{13}C-depleted algae for *in-vivo* experiments with organisms at NA ^{13}C is demonstrated through the reduction in background signal from the algae. Figure 1b and 1d show an approximate 30 % reduction in signal from the *Daphnia* fed ^{13}C-depleted algae compared to NA algae, indicating that the NA algae contributes significant background signal. Feeding organisms is necessary for prolonged *in-vivo* experiments to avoid inducing the stress of starvation. Since there is overlap of the metabolites found in algae and *Daphnia* (Figure 1a,c), feeding the organisms ^{13}C-depleted algae is essential for studies involving organisms at NA. Future applications of ^{13}C-depleted algae may also include growth of ^{13}C-depleted *D. magna*, allowing for 'NMR invisible' organisms which could be applied to study the biotransformation pathways of complex molecules, when isotopic labelling is not feasible. Similarly, this *in-vivo* depletion scheme could be applied to other nuclei, broadening the scope of possible biological NMR experiments, to obtain a better understanding of chemical toxicity in the environment.

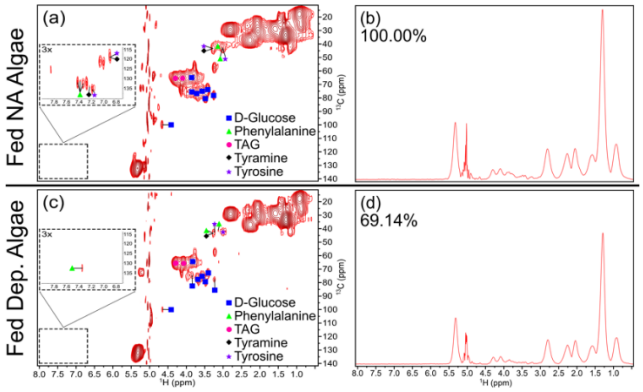

Figure 1. ^{1}H-^{13}C HSQC of *D. magna* sustained using an *in-vivo* flow system. The rows correspond to *D. magna*: (a,b) fed NA algae; and (c,d) fed ^{13}C-depleted algae. The leftmost column (a,c) displays the HSQC spectra with an expanded view of the aromatics (6.7–8.0 ppm) at a 3x contour level. The rightmost column (b,d) shows the horizontal projection of the HSQC displaying the normalized total signal intensity in a percentage.

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Unravelling In Vivo Processes - Heteronuclear NMR and ^{13}C Statistical TOCSY for Studying Organisms Without Enrichment

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In vivo NMR is a powerful non-invasive and non-destructive method that, in combination with flow systems, can maintain organisms indefinitely, allowing the capability to monitor and study biological processes such as metabolism, and stress.[1] While highly effective, in vivo NMR studies are limited due to magnetic susceptibility distortions typical of complex biological samples and a large broad water signal, which limits ^{1}H approaches.[1,2] Typically, ^{1}H-^{13}C HSQC experiments using ^{13}C enriched organisms are used to improve spectral dispersion and metabolic coverage, however, the rising costs of ^{13}C enriched food and organisms make long-term studies economically unfeasible.[2] This study aims to demonstrate the applications of heteronuclear NMR and ^{13}C Statistical Total Correlation Spectroscopy (STOCSY) as effective methods to study biological processes in organisms without enrichment. An oxygenation flow system was developed for larger 10 mm probes which allows a) larger organisms, or b) larger populations of smaller organisms to be studied. The increased biomass offers the potential to permit 1D ^{13}C NMR and/or ^{1}H-^{13}C Heteronuclear NMR without isotopic enrichment, increasing the general applicability of in vivo NMR.

Here, a ^{13}C STOCSY of 1D ^{13}C NMR follows the growth of unenriched brine shrimp for 48 hours after birth (Figure 1). The ^{13}C STOCSY correlates signals that co-vary across the data, where intramolecular correlations support metabolite identification while the intermolecular correlations are ideal for understanding the types of molecules that co-flux.[3] 1D projections can be obtained from the ^{13}C STOCSY to probe further insight into the overall metabolite systems in organisms. In Figure 1B, no normalization is applied so signals pointing down decrease over time and those pointing up increase. However, in Figure 1C the projection is normalized to the lipids at ~32 ppm which essentially removes the influence of the lipids such that they are suppressed. In this case, signals pointing up are created faster than lipids are consumed, while those pointing down are utilized faster than the lipids. The projections show that amino acids increase while lipids and carbohydrates decrease, which aligns with the brine shrimp's energy utilization for growth and development.

Overall, STOCSY is an effective technique for quickly visualizing temporal changes across a series of 1D in vivo data. The application of normalization methods can help to visualize specific trends and when used in combination with larger diameter probes provide a promising technique to unravel in vivo processes without enrichment.

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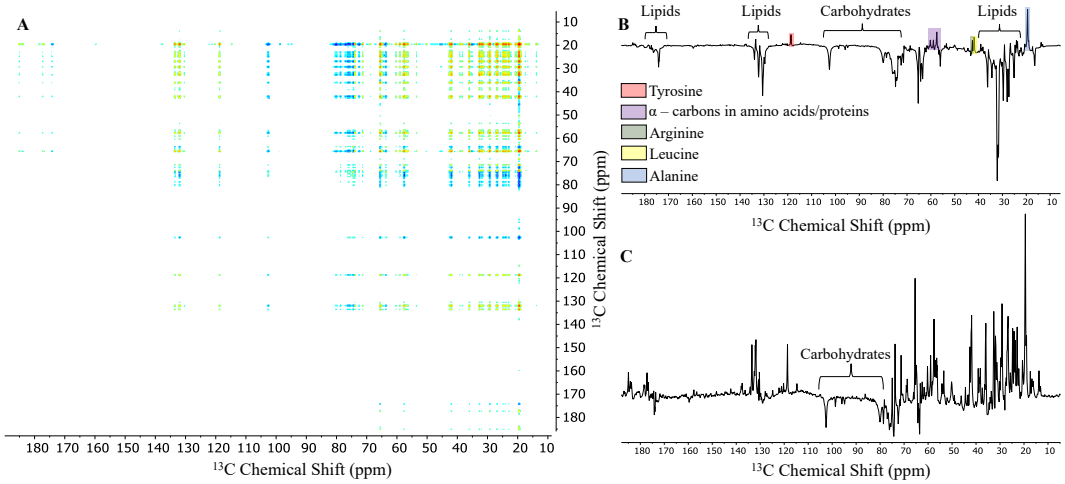

Figure 1. (A) STOCSY two-dimensional representation of ^{13}C NMR spectra of brine shrimp. The STOCSY highlights intra and intermolecular bonds among cross -peaks, with blue contours representing a strong negative correlation and red contours representing a strong positive correlation. (B) 1D projection of STOCSY with no normalization. (C) 1D projection of STOCSY with normalization to the largest lipid signal.

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IMPRESSION – Graph Transformer Network for Rapid and Accurate Prediction of NMR Parameters

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Density Functional Theory (DFT) is the most accurate approach for generating simulated NMR parameters. This capability is indispensable as an explorative tool in various fields such as natural product identification, structure reassignment, conformation identification, and structure verification in drug discovery pipelines. However, the utilization of DFT is not only hindered by the prerequisite knowledge and training required to operate specialized software, but more crucially, the significant hardware and computation time costs in the quantum chemical calculation associated with resolving NMR parameters.

Herein, we introduce a subtantial update to IMPRESSION[1,2], our graph transformer network designed for the simultaneous prediction of NMR parameters with state-of-the-art accuracy and speed. IMPRESSION offers a cost-effective solution by accurately predicting DFT NMR parameters at a fraction of the computational expense incurred by DFT calculation, reducing runtime from hours-days to the milliseconds-seconds per molecule. Moreover, it provides a user-friendly interface, thus overcoming the accessibility challenges associated with DFT methods.

IMPRESSION predicts $^{1}H/^{13}C/^{15}N/^{19}F$ chemical shifts and their associated 1-4 bond J-couplings for small molecules containing a range of nuclei including H, C, N, O, F, P, Br, S, and Si. With mean absolute errors below 0.1 and 1.0 ppm for ^{1}H and ^{13}C chemical shifts respectively, and 0.2-0.4 Hz for common J-couplings, IMPRESSION achieves accuracy levels well within the margin of error observed between DFT calculations and experimental results.

This breakthrough provides unprecedented access to conformationally aware NMR parameters, previously inaccessible through conventional means without significant compute resources. It has the potential to supplant DFT methods and facilitate the generation of extensive computed NMR databases. These can be utilized in various downstream tasks, including employing NMR shifts and couplings as features in molecular property prediction, or as training data in generative tasks aimed at producing spectra-to-molecule mappings. Thus, IMPRESSION paves the way for the integration of generative AI into NMR structure elucidation.

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Characterization of antiviral rotenoids from the leaves of *Millettia oblata* ssp. *teitensis*

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In Kenya, traditional medicine is key in the management of various ailments to fulfill its 2030 vision of adequate health care for all.1 Among the highly utilized species, the genus Milletia has been reported to treat, ulcers, menstrual disorders, inflammation, bronchitis, tuberculosis, hepatitis, and bruises.2-3 Specifically milletia oblate has been employed in the management of cough, bladder problems, and stomachache in Kenya.3 In this study we continued our study on the leaves of Millettia oblata ssp. Teitensis. From the leaves of M. oblata ssp. Teitensis, three new rotenoids (1-3) were isolated and characterized by NMR spectroscopic and mass spectrometric techniques. The skeleton of the structures was identified employing 1H, 13C, COSY, HSQC, HMBC, and NOESY experiments. The absolute configuration of these compounds was established by comparison of experimental electronic circular dichroism (ECD) and vibrational circular dichroism (VCD) with theoretical calculations. Compound 1 was revealed to contain an unusual substitution pattern on ring A making it distinct from the previously known isomeric structure (1a). Based on the combination of 13C NMR chemical shift, key NOESY correlations and 3JHH couplings provided crucial information for the elucidation and differentiating of compound 1 from region isomer 1a. The relative configuration of 1 was determined based on the NOESY correlation. However, the absolute configuration was established as 6aS,12aS-1 based on ECD and VCD spectroscopy. In addition, the compounds showed good antiviral activities towards virus Respiratory Syncytial Virus Infection (RSV) and Human Rhinoviruses (HRV).

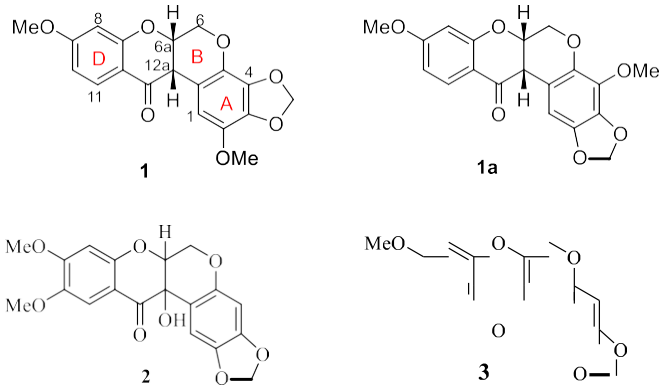

Figure 1: New rotenoids (**1-3**) isolated from the leaves of *M. oblata*

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Solvation dynamics of small molecules in Water-Dimethyl sulfoxide (DMSO) binary mixtures: Multinuclear 1D NMR Analysis

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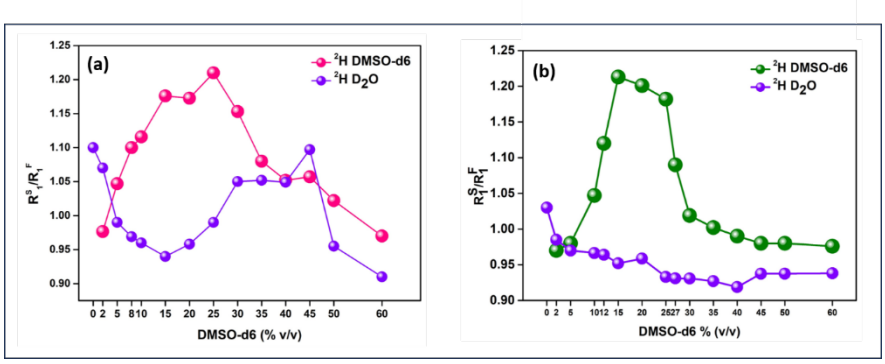
Dimethyl sulfoxide (DMSO), an aprotic polar solvent has attracted much attention both in the field of Chemistry and Biology due to its ability to dissolve a variety of molecules. Moreover, the binary mixture of DMSO with water is also widely used as a cryo-preservative due to its cell penetrating abilities. DMSO-water co-solvent systems are investigated both theoretically and experimentally unravelling solvent dynamics, solute-solvent interactions and solvation phenomena[1]. The present study majorly aims to establish solvent relaxation analysis as a straight forward method to probe conformation and structure alterations of solute as it interacts with the DMSO-water co-solvent system. Deuterium (^{2}H) spin-lattice relaxation rate (R_{1D}) of the solvent nuclei present in the co-solvent system has been used to investigate how the solvation shell of the solute molecules alters as a function of co-solvent composition. Relaxation rate ratio ($R_{1Dsolute}/R_{1Dfree}$) of the solvent molecules were plotted to analyse the behaviour of solvation shell of the solute. The diverse nature of R_1 ratio of D_2O and DMSO-d6 in presence ($R_{1Dsolute}$) and absence (R_{1Dfree}) of solute molecules clearly indicated that the solute molecules are undergoing preferential solvation by DMSO over a certain concentration range[2]. The figure below describes the alteration in relaxation rate of solvent molecule in presence of solute molecule and it will reflect the changes in the solvation shell in the vicinity of the solute molecules and in the bulk. Correlation time, $\tau_{C(D)}$ extracted for the solvent molecules using ^{2}H relaxation rates allowed us to decipher the changes in the mobility of solvent molecules in the solvation shell and in the bulk state. To corroborate our relaxation data, we further employed proton (^{1}H) 1D selective NOE based measurements for 25% (v/v) of DMSO-water co-solvent system containing the solutes of interest[3]. Cross-relaxation rates were quantified in order



to confirm solute-solvent dipolar interactions at a molecular level [2]. Findings from our 1D NOE data are consistent with our relaxation data.

Figure. *R_{1D} ratios for D_2O in the presence of (a) L-tryptophan and (b) AF Dipeptide to a free cosolvent mixture (without solute molecules) as a function of % (v/v) DMSO-d6 composition in D_2O.*

In conclusion, we demonstrated that solvent relaxation rate measurements are potential methods to understand solvation shell structure of a solute. Since ^2H relaxation is governed by quadrupole interaction, extraction of correlation time at high field is possible with a prior knowledge of quadrupole coupling constant. In particular, both the solutes exhibited preferential solvation by DMSO in the co-solvent composition range of 15-25%, however, the relaxation behaviour of D_2O was significantly different in both the cases over the complete composition range.

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Finger T_2 is a Non-invasive Fluidity Meter for Human Adipose Tissue: Implications for Metabolic Health

David P. Cistola[1,2], Shayan Pourjavaheri[1], Diana F. Sandoval[1], Valeria Diaz-Pacheco[1], Erin B. Campbell[1]

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Background: Dietary fatty acid profiles have been linked to cardiovascular risk. The fatty acid profile of human adipose tissue (AT) mirrors dietary intake and hepatic *de novo* lipogenesis. In turn, lipogenesis is amplified by insulin resistance, another risk factor for cardiovascular disease. Thus, AT composition and fluidity are potential biomarkers of cardiometabolic health and disease risk. To assess the fluidity and composition of AT noninvasively, our laboratory developed a device that measures the transverse relaxation times (T_2) of the living human finger.

Hypothesis: The T_2 profile of the human fingertip closely resembles that of edible oils and reflects variations in AT fluidity. Biologically relevant edible oils of varying fatty acid composition show a linear association between T_2 and triacylglycerol (TAG) fluidity [1].

Methods: Lipid ^{1}H CPMG T_2 relaxation decay curves were recorded at 37°C using a 0.47T Bruker mq20 benchtop magnetic resonance relaxometer and a custom-built 0.5T (23 MHz) prototype finger relaxometer from Resonance Systems GmbH. The decay curves were deconvoluted into 3-component T_2 profiles using the discrete components singular value decomposition algorithm in XPFit (Alango Ltd.) and the inverse Laplace transform algorithm in CONTIN [2]. Macroscopic fluidity was measured at 37°C using a ViscoLab 5000 viscometer.

Results: Each T_2 profile contained 3 peaks assigned to distinct mobility domains within each TAG molecule. For Peak 1, the mean T_2 values in msec increased with cis-double content: 275.3 (olive oil, high oleic acid), 373.2 (safflower oil, high linoleic), 388.0 (cod liver oil, mixed composition), 459.7 (flaxseed oil, high alpha-linolenic), 488.6 (fish oil 2, moderate eicosapentaenoic, EPA, and docosahexaenoic, DHA) and 691.5 (fish oil 3, high EPA and DHA). Across this series of edible oils, the T_2 values were linearly associated with fluidity (R^2= 0.83) and the average number of cis-double bonds per TAG molecule (R^2= 0.95), $p<0.001$. Similar linear associations were observed for Peaks 2 and 3.

Conclusions: The fluidity and *cis*-double bond content of biologically relevant TAGs can be monitored non-invasively using benchtop magnetic resonance. These results provide the framework for interpreting adipose tissue T_2 measurements in the living human finger.

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Whole Blood T_{2P} Links Poor Metabolic Health to an Impairment in Hemoglobin Oxygenation

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Background: Plasma water T_2 is an early, global, and practical biomarker of cardiometabolic health. It is measured by compact magnetic resonance relaxometry using separated human plasma. A prior goal was to determine whether plasma water T_2 could be estimated using unseparated whole blood. Settled, EDTA-anticoagulated whole blood yields two T_2 values: T_{2S} for the plasma supernatant and T_{2P} for the blood cell pellet. Whole blood T_{2P} measures the properties of water inside red blood cells and monitors the oxygenation and oxidation states of hemoglobin. Here, we investigated the independent and mediating role of T_{2P} with markers of cardiometabolic health.

Methods: This was Phase 2 of an observational, cross-sectional biomarker discovery study, enrolling 44 asymptomatic disease-free adults, ages 23-61, of varying race/ethnicity and 47% female. Each participant underwent a medical history & physical exam and was metabolically phenotyped using >130 biomarkers and clinical lab tests. Whole blood T_{2P} values were recorded using a Bruker mq20 benchtop relaxometer and resolved using the discrete components singular value decomposition algorithm in XPFit (SoftScientific, Alango, Ltd.). The association of T_{2P} with candidate predictors was explored using JMP Pro v16.2 (SAS Institute, Inc.). Machine-learning predictor screening was followed by multi-variable linear regression along with correlation, latent-factor, and mediation analyses. The effect size was a standardized beta coefficient with a 95% confidence interval (CI).

Results: The strongest predictor of T_{2P} was a latent variable incorporating markers of hyperinsulinemia, oxidative stress, inflammation, adiposity, dyslipidemia and hypoxia: β= -10.5, 95% CI: -15.4, -5.7, $p<0.0001$. Preliminary mediation analysis provided evidence that whole blood T_{2P} (hemoglobin deoxygenation and/or oxidation) is a full mediator for the association between fasting insulin and markers of hypoxia (lactate) or chronic inflammation (IgG).

Conclusions: A missing link between non-optimal cardiometabolic health and insidious cell/tissue damage may be a subclinical impairment in hemoglobin's ability to deliver oxygen. To pinpoint the impairment, whole blood T_{2P} and metabolic markers are being compared with hemoglobin parameters from blood gas analyses of asymptomatic disease-free adults. As whole blood T_{2P} can be measured using a fingerstick drop of blood and a miniaturized low-cost magnetic resonance device, it shows promise for metabolic health screening at the point of care.

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To the Point-of-Care…and Beyond: Plasma Water T_2 as a Powerful and Practical Screening Tool for Cardiometabolic Health

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Magnetic resonance (MR) technologies continue to revolutionize healthcare and diagnostics, exploiting the striking spatial resolution of imaging and the exquisite atomic resolution of spectroscopy. While high-end magnetic resonance technologies have successfully penetrated the world of hospitals, imaging centers, and clinical diagnostic laboratories, the next frontier lies at the point-of-care and point-of-service. To penetrate those worlds, MR technology needs to be not just clinically meaningful and analytically valid, but also simple, rapid, minimally invasive, inexpensive, and portable. Time-domain MR relaxometry, the lesser-known cousin of imaging and spectroscopy, can meet those criteria and holds unique promise for health screening and monitoring in community settings.

In 2017, we reported that human plasma water T_2, measured in the time domain using a benchtop relaxometer, is an early, global, and practical marker of metabolic syndrome [1]. Approximately half of the U.S. population has some degree of metabolic abnormality, from early metabolic imbalance to prediabetes and/or metabolic syndrome to overt type 2 diabetes and related conditions [2]. Early detection in young, apparently healthy individuals is vital to detect hidden abnormalities, preserve cell and tissue function, promote health, and prevent disease [2]. Because of its unique ability to detect hidden multi-component metabolic abnormalities in one simple measurement, plasma water T_2 is well suited for health screening & monitoring, especially for diabetes prevention [2].

This poster will present the latest findings from the analysis of plasma water T_2 in human study populations. In addition, we will introduce preliminary new results obtained with the miniaturized portable WaveGuide Formula device (www.waveguidecorp.com) and compare its performance characteristics with the well-established Bruker mq20 Minispec benchtop instrument (www.bruker.com).

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Comparison of the Structure of Two Small-Molecule Organic Electrode Materials, TAQ and TAPT, by Multinuclear Solid-State NMR

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Two insoluble tricyclic organic molecules that form promising hydrogen-bonded electrode materials, previously termed TAPT[1] for tetraamino-phenazine-1,4,6,9-tetrone and TAQ[2] are investigated. The structures of TAQ, $^{15}N_6$-TAQ, and TAPT (tetraamino-phenazine-1,4,6,9-tetrone), in addition to their precursor TABQ (tetraamino-benzoquinone), are compared by ^{13}C, ^{15}N, $^{13}C\{^1H\}$, $^{15}N\{^1H\}$, and 2D ^{15}N-^{15}N solid-state NMR. Similarities of spectra and of dipolar dephasing indicate that TAQ and TAPT share the same molecular structure: a fully conjugated pyrazine fused with 2,3-diamino-1,4-dibenzoquinones. Tautomeric structures previously proposed for TAQ are excluded by the observed chemical shifts and dipolar couplings. The pyrazine core is found in both samples, with a characteristic 308-ppm ^{15}N chemical shift and devoid of the proton previously proposed[2]. A splitting of the NH_2 and ^{13}C-NH_2 signals of TAQ is due to symmetry-lowering by packing effects, analogous to splittings documented here in detail for TABQ, which disappear in solution. In conclusion, no structural difference in terms of chemical bonding could be established between TAPT and TAQ, and both are to be described as tetraamino-phenazine-1,4,6,9-tetrone.

Acknowledgement

The authors thank Bowen Tan and Prof. Mircea Dincă of MIT for providing TAQ and TAPT samples for this NMR study.

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Small Molecule Formulation Component Testing in Protein-Based Therapeutics via Benchtop NMR

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Measuring the concentrations of formulation components is a critical process control and QC requirement for the production of protein-based therapeutics. Recently, there has been increased interest is leveraging benchtop NMR for this purpose [1].

Here, we show that a benchtop NMR system has the potential to enable fast, accurate, and precise multi-attribute monitoring of these small molecule components, even in the presence of relatively large concentrations of protein. Thus, a simple pre-calibrated benchtop NMR assay provides results equivalent to several resource intensive process control and release assays, and is capable of testing a single sample of drug product for buffer, sugar, surfactant, and antioxidant concentrations, as well as pH.

As an example, we show results from an 80 MHz benchtop system, using a CPMG-based method [2] to suppress 75 mg/mL protein drug resonances and measure the concentrations of histidine, sucrose, methionine, and polysorbate 20 in the formulation. At typical formulation concentrations (240 mM sucrose, 20 mM histidine, 10 mM methionine, and 0.4 mM polysorbate 20), accuracy is typically within a relative +/- 5%, well within the specifications needed for process control and product release. Total measurement time per sample is relatively short, varying from 10 to 30 minutes, depending on the particular formulation composition.

Further, we have found that a one-time calibration to a sealed tube of 1 mg/mL dimethyl sulfone allows for accurate measurements of these components without the need to prepare direct standards during each assay session. Thus, rather than a direct proton-to-proton quantitative strategy, we find that a pre-calibration of the "response factor" of each component relative to the DMS is the preferable and most efficient approach for assessing the formulation components in protein-based therapeutics, and compensates for the use of a CPMG filter and the relatively poor peak separation compared to high field NMR.

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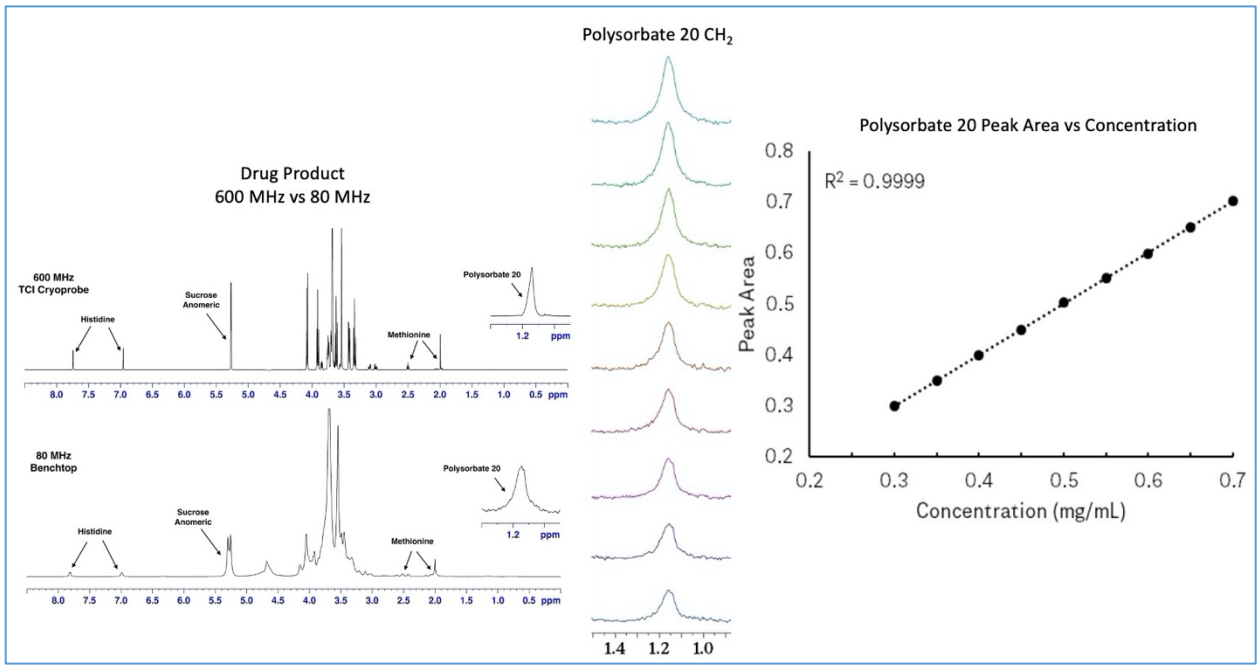
Drug Product
600 MHz vs 80 MHz
600 MHz
TCI Cryoprobe
Histidine
Sucrose
Anomeric
Methionine
Polysorbate 20
1.2
ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
80 MHz
Benchtop
Histidine
Sucrose
Anomeric
Methionine
Polysorbate 20
1.2
ppm
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
Polysorbate 20 CH2
1.4 1.2 1.0
Polysorbate 20 Peak Area vs Concentration
R2 = 0.9999
Peak Area
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.2 0.3 0.4 0.5 0.6 0.7
Concentration (mg/mL)

25

Study of *Escherichia coli* metabolism using high sensitivity ^{13}C NMR

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Antimicrobial resistance (AMR) is an important issue that has been studied in various organisms, however, studying AMR from a metabolic perspective in organisms like *Escherichia coli* during their growing phase could provide better insights into the biochemical mechanisms underlying such resistance. While many studies have biochemically profiled *E. coli* using NMR, recent advancements in probe design have enhanced sensitivity that enables a deeper look at *E. coli* metabolism by capturing low concentration metabolites or, potentially, unknown metabolites. We aim to develop a comprehensive approach to capture the dynamic nature of *E. coli* metabolism during growth and explore the peculiarities of antimicrobial resistant strains. By running 1D and 2D NMR experiments, we captured catabolism and anabolism of 15 timepoints from a ^{13}C labeled minimal media *E. coli* culture. We analyzed the evolution of metabolites inside the cells and in the media using STOCSY, PyINETA, SAND [1][2] and other inhouse algorithms. This is a first step in understanding the metabolic differences between sensitive and resistant strains, particularly when grown in D_2O media [3].

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26

Expanding the Utility of Triazabutadiene Chemical Probes to Target and Elucidate Protein Dynamics through ^{19}F NMR

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Protein dynamics are an important phenomenon to study as proteins are transient in nature and acquire multiple conformations that can be influenced by their environment or associated complexes, such as protein-protein interactions. One area that we are interested in studying is mitochondrial dynamics. Mitochondrial dysfunction, enhanced through a shift in the equilibrium processes of mitochondrial dynamics, has been implicated in a wide range of human pathologies; however, the interplay between its molecular machinery has yet to be fully unravelled.[1] Targeted delivery of small molecules into the mitochondria can be an advantageous approach to probe and study the protein players of these diseases. Aryl diazonium ions are a class of small molecules that can selectively modify tyrosine residues which are known interfaces of protein-protein interactions.[2] However, these molecules are always "on" and can react with nucleophilic residues within proximity. Triazabutadienes are functionalize able small organic molecules that can generate these aryl diazonium ions *in situ* under physiological pH and can be further modulated for targeted delivery intracellularly. The utility of this chemical moiety can be expanded to not only include covalent modification of intracellular proteins but can also be utilized to study the dynamics of protein structures and conformational changes during transient interactions via NMR. We have displayed the ability to utilize ^{19}F NMR to study the differences in rate of reactivity of the p-trifluoromethyl aryl diazonium ion conjugated macromolecules via kinetics and T_2 relaxation studies.

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27

NOAH-UTOPIA – Expanding the Scope of UTOPIA NMR to Include Multiple Homonuclear and Heteronuclear 2D Experiments

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The Unified Time-OPtimized Interleaved Acquisition (UTOPIA) NMR acquisition method allows the collection of both high-γ (e.g. 1H) and low-γ (13C) spectra by means of either fast switching of a single receiver, or by employing two receivers or transceivers.[1] UTOPIA NMR collects orthogonal information in a time-efficient method through the circumvention of one or more recycle delay periods, making it highly useful for small molecule characterisation using a workhorse spectrometer with a conventional BBFO probe. However, the methodology is currently limited to homonuclear (1H–1H) 2D experiments to avoid perturbing the low-γ magnetisation during nX detection.

In this work, the UTOPIA methodology is expanded to include:

INEPT- and DEPT-based polarisation transfer 1D experiments
DOSY and other 1H-1H homonuclear (pseudo)-2D experiments
HSQC and other 1H-nX indirect-detection experiments

To push the limits of small molecule characterisation, a NOAH supersequence[2–3] – containing 10 (pseudo)- 2D experiments – was extended with the UTOPIA method to provide a complete set of 1D and 2D characterisation information in a single pulse sequence and NMR experiment. Here, the DEPTQ-45 experiment is more effective at providing complementary 1D information for small molecule characterisation.

Pulse sequences based on the UTOPIA method are easy to prepare by appending existing 1D and 2D pulse sequences, and templates have been prepared based on refocused INEPT, DEPT, and DEPTQ. Optional features, contained within flags, include presaturation, purge element (before the low-gamma experiment), and a delay period for relaxation or power-gated decoupling.

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28

Efficient NMR Sample Preparation and Data Analysis through Innovative Automation Solutions

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The Analytical Chemistry Core (ACC) within the National Center for Advancing Translational Sciences (NCATS) aims to alleviate analytical bottlenecks through the development of innovative platforms and technologies in conjunction with optimization of processes and workflows to increase the efficiency, productivity, reproducibility, and integrity of translational research. These efforts are focused on helping fulfil the fundamental NCATS goal of providing more treatments to all patients more quickly. NMR spectroscopy is an essential component of the drug discovery and development process delivering vital data and analysis such as compound identity and purity, structural conformation, and target engagement. Despite this wealth of information, methods can often be antiquated and time-consuming with manual workflows struggling to meet the high demand for rapid NMR analyses in a reliable and efficient manner. As such, the ACC installed and optimized a custom MicroTasker liquid handling platform from Sirius Automation to facilitate automated NMR sample preparation with an expanded repertoire of twelve methods for increased throughput and reproducibility. Compounds are dissolved in a deuterated solvent to a known concentration, shaken to aid solubilization, aspirated from the source vial, and dispensed into a 5 mm NMR tube. Integrated into the sample preparation process is a 3D-printed device we designed that enables simultaneous capping of 48 NMR tubes. This innovation addresses the challenges of process time and scalability but also minimizes the manual capping issues of tube breakage and sample adulteration. The automated NMR sample preparation platform was used to expeditiously create individual reference samples of a Maybridge ^{19}F fragment library (385 fragments) in both DMSO-d6 and PBS buffer, 19 fragment pools (20-21 fragments per pool), and the fragment pools plus a protein target of interest. An Agilent 600 MHz NMR was utilized to acquire ^{1}H and ^{19}F NMR spectra for the reference samples and pools while STD and CPMG NMR experiments were employed for ligand-protein binding studies with the fragment pool samples containing protein. Implementation of Mestrelab Mnova software automation modules streamlined data processing and analysis while increasing throughput for large and complex NMR datasets. MixDesign was used to determine the fragment pools and MScreen performed automated processing of ligand-observed NMR screening data. MGears is for automated workflow design in the retrieval, processing, and analysis of NMR data. This successful pilot study represents a significant advancement in our efforts toward developing a vendor agnostic end-to-end automated NMR spectroscopy workflow.

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STUDYING THE MECHANISM OF SOLID-STATE CYCLOREVERSION OF DIANTHRACENES BY SOLID-STATE NMR

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Molecular solar thermal (MOST) energy storage uses light-responsive molecules to store solar energy in strained chemical bonds and release it as heat on demand. Recent developments of reversible topochemical cycloaddition reactions enable high-density MOST energy storage in solid state. Topochemical [4+4] photocycloaddition of anthracenes stores energy in strained cycloadducts and releases the stored energy through thermally activated cycloreversion, both via crystal-to-crystal transformations [1]. Differential scanning calorimetry (DSC) of the energy releasing process showed unusual non-Gaussian exothermic features.

In this study [2], the cycloreversion processes of several dianthracene-based MOST energy storage compounds were characterized by time-dependent solid-state ^{13}C NMR. Our results reveal that the chemical dissociation of dianthracenes leads to the formation of a mixed intermediate phase of monomer pairs in the dimer crystal, and eventually phase transition to the stable anthracene crystal. The solid-state kinetic analysis shows sigmoidal character due to auto-catalyzed cycloreversion facilitated by the partially cooperative transformation of molecules in the crystalline state.

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Advances in direct CRAFT analysis of selected inflammatory biomarker NMR resonances in horse serum samples

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Biofluids, such as blood serum or plasma, are natural targets for NMR-based metabolomics, including quantitative assessment of selected metabolic components in the mixture. There are a small number of such component resonances, namely $N^{+}Me$ signals of lipoproteins (GlycA and GlycB, [1]) and a composite signal of phospho-glycoproteins (SPC [2]), which are especially indicative of inflammatory conditions.

CRAFT [3] is a time-domain based method for processing NMR signals, producing a spreadsheet of data as opposed to a traditionally analyzed spectra. This technique eliminates the need for phase and baseline correction, therefore minimizing errors. Furthermore, CRAFT produces more reliable amplitudes of overlapping signals compared to frequency-domain analysis.

In the full spectrum there is significant overlap for all three signals of interest with other peaks – beta-glucose (SPC), broad resonances of unsaturated lipids (GlycA and GlycB), respectively. This overlap can be largely removed by experimental suppression of unwanted signals [4]. However, this suppression affects all signals on the spectrum at some level. Our approach uses the full 1D spectrum (only water suppression applied). Therefore, all resonances will be present with undisturbed intensity.

We have been exploring the various strategies of parametrization in CRAFT, tested diffusion filtered spectra to assess effect of experimental signal suppression and compared results of conventional integration (line fit) methods with CRAFT data.

We have applied the CRAFT technology to a cohort of 20 + 20 horses, control and those diagnosed with OCD (Osteochondritis Dissecans), respectively. We also used selected integral ratios for multivariate (O-PLS-DA) analysis with good results. Similar analyses of multiple datasets from earlier studies are in progress, too.

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Optimizing the single-scan selective excitation of overlapping multiplets using GEMSTONE

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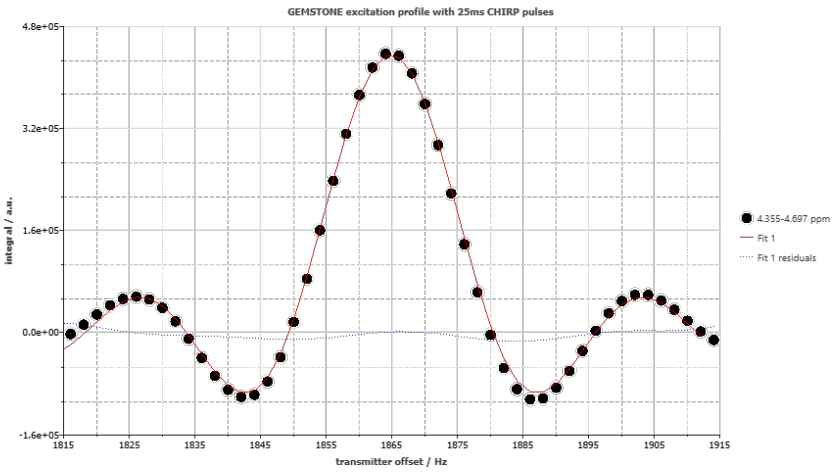
Signal overlap in ^{1}H NMR often requires utilization of multi-pulse sequences either based on mutli-dimensional data collection or spectral filtering by selective excitation. The former strategy routinely needs significant increase to the minimum experiment time (even when using state of the art NUS techniques to minimize this impact). Selective excitation is very efficient when ambiguity in assignment only needs a few correlations and the complete 2D spectrum contains extensively redundant information. However, classic selective excitation is limited because it requires at least one relevant proton outside of overlapping regions. The recently introduced GEMSTONE (Gradient-Enhanced Multiplet-Selective Targeted-Observation Nmr Experiment) technique enabled single-scan selective excitation of overlapping multiplets [1]. Its use for rapid acquisition of various correlations including NOESY [1], TOCSY [2], COSY [3] and ROESY [4] were demonstrated.

Here, we present a series of Spinach simulations to explore various factors that influence the success of GEMSTONE in practical reality. There is a common difference between theoretical limits and experimental results due to relaxation, diffusion (and convection), and B_0 homogeneity (i.e. 'shimming' quality).



Fit Name	Equation	Variable Name	Inital Value	Fitted Value	Standard Error
Fit 1	A*(Math.sin(x/bw*3.14-C/bw*3.14)/(x/bw*3.14-C/bw*3.14))	A	4.30e+05	4.355e+05	399.6106
		bw	20.00	15.550	0.0076
		C	1864.00	1864.904	0.0078

Figure 1 Experimental excitation profile mapped with a water sample. Data processing and fitting was performed by using JASON software [5].

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Real-Time Monitoring of Small Molecule Reactions Using the X-Pulse Benchtop NMR

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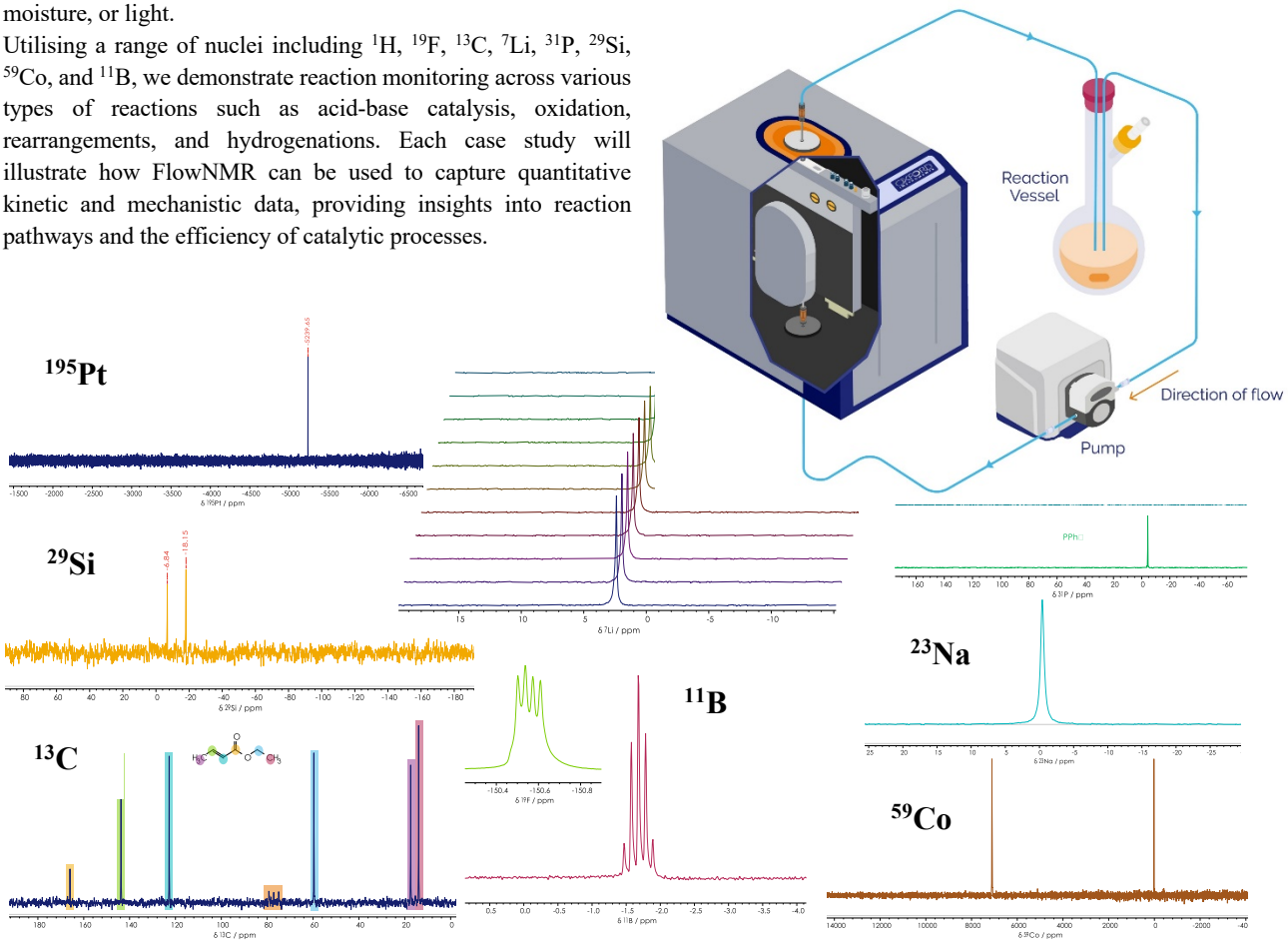
The X-Pulse benchtop FT-NMR spectrometer extends the accessibility of advanced NMR capabilities, traditionally available only in high-field systems, to a versatile and cost-effective benchtop format. With full broadband capability, variable temperature settings, and automation features, the X-Pulse offers flexibility and precision for a wide range of NMR applications.

This instrument is particularly well-suited for real-time monitoring of small molecule reactions, offering broad multi-nuclear capabilities essential for detailed chemical analysis. This work focuses on the application of online FlowNMR with the X-Pulse system to monitor dynamic chemical transformations in real-time. FlowNMR facilitates high-density data acquisition under realistic conditions and allows for the continuous monitoring of reactions by interfacing the NMR system with a flow setup. Such setups provide control over experimental conditions such as temperature and pressure, and in environments sensitive to air, moisture, or light.

Utilising a range of nuclei including ^{1}H, ^{19}F, ^{13}C, ^{7}Li, ^{31}P, ^{29}Si, ^{59}Co, and ^{11}B, we demonstrate reaction monitoring across various types of reactions such as acid-base catalysis, oxidation, rearrangements, and hydrogenations. Each case study will illustrate how FlowNMR can be used to capture quantitative kinetic and mechanistic data, providing insights into reaction pathways and the efficiency of catalytic processes.



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REDUCING THE COMPUTATIONAL BURDEN OF STRUCTURE GENERATION IN COMPUTER- ASSISTED STRUCTURE ELUCIDATION (CASE)

Alexander Bourque, Sergey Golotvin, Maxim Kisko, Rostislav Pol and Dimitris Argyropoulos

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NMR data is invaluable in determining the structures of new and/or unknown compounds using Computer- Assisted Structure Elucidation (CASE). [1] Starting with a molecular formula, most existing CASE systems will solve the problem by defining a set of constraints derived from the observed chemical shifts (usually at least ^{1}H and ^{13}C) and the analysis of the observed correlations in the various types of 2D spectra (HSQC, HMBC, COSY, and others). All possible chemically sensible structural isomers are generated using a mathematical procedure, including all possibilities by renumbering the atoms. The ones satisfying the previously implied constraints are selected to be ranked, usually by the deviation between the experimental and observed chemical shifts. Despite the huge advances in computing power over the past years, this structure generation step remains the bottleneck in CASE workflows. This becomes especially problematic as the overall number of atoms increases and heteroatoms are involved, which makes the computational task formidable.

To reduce the time required for this structure generation step, one must increase the number of constraints. This can be done either by recording additional spectra that would reveal more correlations (e.g., C-C correlation spectra like ADEQUATE and INADEQUATE) and/or identifying some known fragments of the structure using the existing data. While it is not always possible or feasible to record additional spectra, identifying known fragments can more easily be achieved using fragment libraries or the ability of NMR spectroscopists to recognize familiar spectral patterns, specific to particular fragments.

In this poster, we present an automated approach that mimics this process that the NMR expert would do and identifies spectral patterns characteristic of phenyl fragments based on the chemical shifts, observed connectivities, and symmetry. Depending on the resolution of the signals, this method would produce one or more Molecular Connectivity Diagrams (MCDs) that already include the phenyl group(s) thus, significantly reducing the complexity of the problem and shortening the elucidation time. We will compare the time taken to elucidate several structures with and without this approach to demonstrate its utility.

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Navigating Challenges Impacting Anisotropic NMR Analysis of Atropisomerism in Sotorasib

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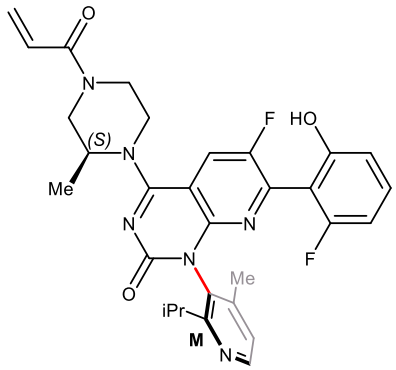
Sotorasib, a $KRAS^{G12C}$ covalent inhibitor used in the treatment of non-small cell lung cancer, feaures an axially chiral biaryl moiety that forms configurationally stable atropisomers arising from a restricted rotation about an axially chiral biaryl bond. The (**M**)-atropisomer, sotorasib, maximizes $KRAS^{G12C}$ activities and is developed as a single-atropisomer drug [1]. The proton deficiency and planar structure of the quinazolinone moiety of sotorasib, limit the structure elucidation of atropisomers by NMR. In this presentation, we showcase the application of anisotropic NMR [2] and CASE-3D [3] protocol for atropisomeric differentiation of sotorasib.



(M)-Atropisomer (sotorasib)

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Small molecule characterization of ^{13}C enriched duckweed *Spirodela polyrhiza*

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Attention has recently returned to the *Lemnaceae* family due to the high-quality genome mapping of *Spirodela polyrhiza* (*S. polyrhiza*), and 9 other duckweed species, which provides advantageous opportunities for complimentary experimental approaches aimed at better characterizing this rising model organism [1,2]. Defining the metabolite network of a complete plant at the systems level is of particular interest, which requires characterizing both the primary and secondary metabolites [1], a feat that has not yet been undertaken with *S. polyrhiza. S. polyrhiza* fronds were grown under no light conditions on Schenk & Hildebrandt liquid media supplemented with 0.5% ^{13}C glucose to achieve 96% ^{13}C enrichment, and both the small molecule metabolites and alcohol insoluble cell wall constituents were extracted. A suite of highly resolved NMR data was collected, including INADEQUATE (incredible natural abundance double-quantum transfer experiment) and ^{13}C-JRES (J-resolved) experiments. Using the Python-based INADEQUATE (PyINETA) network analysis, structural information and database matches of known molecules can be identified to lay the foundation of metabolites that can be found in *S. polyrhiza* [3]. PyINETA will allow for high confidence metabolite identification, and provide fragment information for which to probe unknown metabolites [3]. With a comprehensive metabolite reference data library, *S. polyrhiza* will be a robust tool that can be leveraged in future studies encompassing natural product discovery, enzymatic activity assays, biofuels, and other studies that rely on understanding the metabolite network of this humble aquatic monocot [2].

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Consumer Health Tracking Using an *In ViVo* MRS Scanner

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Magnetic Resonance Spectroscopy (MRS) is a technique with diverse healthcare applications, such as brain tumor characterization and prostate cancer detection. It is also a promising technology in the field of routine, in vivo quantification of metabolite concentrations. Such data acquired repeatedly over time may be leveraged to infer alterations in various user health states. Our collaboration has been developing the underlying technology that will form the basis of a benchtop health-tracking device and conducting ground- truth metabolomic benchmarking. The commercial aim of this research is the consumer health scanner DigiScan, being produced by ViBo Health. This utilizes MRS to measure key blood and tissue biomarkers akin to a blood test, but non-invasively and without the associated costs and inconveniences. DigiScan is suitable for public spaces like pharmacies, gyms, workplaces, and homes, as well as clinical settings. After insertion of a digit, metabolite spectra are read from multiple locations along a finger. The results are displayed on a health dashboard app, offering an overall health score and metrics for factors like pre- diabetes or diabetes, cholesterol, obesity, fitness and recovery, which are linked to variability in such metabolites as glucose, lactate, lipids, and CRP. Here we describe the project status, including the benchmarking of in vitro MRS experiments, the development of signal processing pipelines, and the use of simulations in developing novel equipment. We shall outline the challenges faced and describe future plans.

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Comparative NMR analyses of disaccharide structures of *Pseudohyphozyma bogoriensis* glycolipids.

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Sophorolipids are glycolipid biosurfactants that are composed of a glucose-derived disaccharide head-group (sophorose) linked to a hydroxy-fatty acid tail. Biobased sophorolipids and their hydroxy fatty acids derivatives have multiple applications including in the cosmetic and pharmaceutical industries. In this study, we compared the incorporation of glucose and cellobiose into glycolipids produced by the yeast *Pseudohyphozyma bogoriensis* to evaluate the effect of feedstock on the potential development of unique glycolipid structures with new properties, and to gain insight into the cellular mechanism involved in that incorporation. These disaccharide structures can be distinguished based on the linkages of their glucose, as sophorose has an unusual β-1,2 bond, whereas cellobiose has a β-1,4 bond. This report focuses on the use of two-dimensional nuclear magnetic resonance spectroscopy (NMR) to elucidate the structures of the resultant glycolipids. It was found that rather than directly incorporating the feedstock disaccharide, *Pseudohyphozyma bogoriensis* first converts cellobiose into glucose residues which are then used to make the sophorolipid.

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Metabolite Fraction Libraries

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One-dimensional ^{1}H NMR spectroscopy of complex biological mixtures suffers from extensive peak overlap. Two-dimensional NMR methods improve resolution and greatly help with the annotation of compounds in databases but require long acquisition times and have limitations for unknowns[1]. Finally, neither 1D nor 2D methods enable functional testing of compounds, which require additional tedious steps[2]. To address these issues and to build on the ideas presented by Whiley et al. (2019), we have developed an approach using preparative HILIC high-performance liquid chromatography to create metabolite libraries of 140 fractions for downstream analysis and long-term storage[3]. The chromatography separates overlapping peaks, and multiple injections concentrate low-abundance compounds for improved NMR signal-to-noise. We will incorporate time-domain modeling using Spectral Automated NMR Decomposition (SAND) and present new computational tools to analyze and annotate the NMR fraction library data[4]. Using our workflow, we have annotated known metabolites via automatic database matching and collected many "fingerprints" of unknown compounds. The fractions containing these unknown compounds can be measured by additional 2D NMR experiments, spiking studies, LC-MS/MS, or other analytical techniques. Fractions can also be functionally characterized for properties such as pheromone activity or protein binding.

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A rare pharmaceutical mesophase – avibactam tomilopil Form 1

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Avibactam tomilopil (AVT) is an orally available prodrug of the β-lactamase inhibitor avibactam. AVT Form 1, the commercially nominated solid form, has been identified as a thermotropic mesophase through a collaborative approach, including extensive solid-state characterization and computational modelling. NMR crystallography was employed as part of this comprehensive characterization effort, as an atomic-level understanding of intramolecular mobility is crucial when evaluating mesophases. ssNMR enabled a site-specific investigation into the local mobility of AVT Form 1 through analysis of spectral lineshapes/linewidths and relaxation measurements. Additionally, simulated ssNMR spectra calculated from the lowest energy predicted crystal structures were compared to the experimental ssNMR data to provide insight into the regions of the molecule with increased order/disorder. The NMR crystallographic analysis was supplemented by MD simulations of the predicted crystal structures, which show that the hydrophobic tails of the molecule exhibit an above average degree of dynamic disorder. This type of mobility is not typically observed in long-range ordered crystal structures due to the inherent rigidity and periodic potential of their lattices. The case of AVT Form 1 demonstrates how NMR crystallography is an important analytical tool that can aid in revealing the molecular-level structure of pharmaceutical solids, even in the more complex case of a mesophase.

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Universally quantitative band-selective pure shift NMR

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NMR spectroscopy is an intrinsically quantitative analytical technique, in that the absolute integral of a signal is proportional to the number of spins which give rise to that signal. However, typically only the simple pulse-acquire experiment is "universally" quantitative, meaning that the relative integrals of different signals directly reflect the relative numbers of spins which contribute to those signals. Unfortunately, the extraction of quantitative information from ^{1}H pulse-acquire spectra is often complicated by signal overlap, which arises from the limited chemical shift range and extensive signal multiplicity in ^{1}H NMR. Pure shift NMR techniques[1] represent one approach to reducing signal overlap, by suppressing the effects of homonuclear scalar coupling. Although these techniques improve the accessibility of signal integrals, they also introduce site-dependent signal losses and thus are not, in the general case, universally quantitative.

One way of obtaining universally quantitative data from multiple-pulse NMR experiments is to measure the degree to which the pulse sequence attenuates each signal integral. Provided that the pulse sequence elements that cause signal loss act independently, repeating each element a variable number of times before acquisition allows extrapolation back to the loss-free signal integral. Here, we apply this principle, which was first proposed in the time-zero extrapolated HSQC (HSQC0) experiment,[2] to band-selective pure shift NMR.[3,4] We suggest the name EXQUISITE (**ex**trapolating **qu**antitative **i**ntegrals by **s**uccessive **ite**ration) for the more general application of this principle.

Our initial implementation of the EXQUISITE method with band-selective pure shift NMR yielded relative signal integrals within ±0.5 % of those obtained from a pulse-acquire experiment for a three-component mixture.[5] However, in the initial implementation the overall experiment time required scales with the number of EXQUISITE iterations *n* performed, as the data for each iteration must be acquired separately. Here, we present a new acquisition mode for EXQUISITE pure shift NMR that measures the data for all iterations sequentially within a single acquisition. In applying this method to a three-component mixture with overlapping signals, we obtained relative integrals with a quantitative accuracy of better than ±1 %. This novel acquisition mode is therefore close to a "something for nothing" approach to quantitative band- selective pure shift NMR, as there is essentially no additional cost in experiment time compared to the conventional pure shift NMR experiment.

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AUTOMATIC qNMR DATA ANALYSIS APPROACH: PROTOTYPE OF qQMSA-BASED DIGITAL PRODUCT

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Keywords: Automatic data analysis, qNMR, qQMSA, Digital product

ABSTRACT: The present study introduces a novel methodology for the automated analysis of ^{1}H quantitative nuclear magnetic resonance (^{1}H qNMR) data using quantitative Quantum Mechanical Spectral Analysis (qQMSA). This digital approach aims to improve data analysis efficiency beyond the capabilities of traditional manual methods in NMR. The proposed digital product pipeline consists of several key steps: (i) calculating chemical information for molecules stored in a digital spectral file (dSF), (ii) developing a validated qNMR method to acquire high-resolution NMR data, (iii) creating a database of dSFs), and (iv) implementing automated NMR data analysis. This process significantly diminishes the need for manual intervention in qNMR data analysis and exhibits potential in spectral fitting by comparing the calculated spectrum derived from a dSF with experimental NMR data in a chemometric manner. The digital product's efficacy and adaptability have been evaluated through various test samples, with case studies demonstrating its effectiveness across many sectors. The results indicate that the digital product enhanced efficiency and precision in ^{1}H qNMR data analysis. This qQMSA approach provides trustworthy qualitative and quantitative assessments in basic 1D ^{1}H qNMR studies and paves the way for digital solutions in future compendial applications.

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SpinFit: A new approach to 1H NMR Spin System determination

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Quantum mechanical (QM) methods are essential for analyzing 1H NMR spectra, particularly in complex molecular systems where classical techniques often fall short [1]. Traditional approaches frequently fail to resolve overlapping resonances or accurately determine coupling constants in multispin systems, challenges that are exacerbated by second order or strong coupling effects. These issues are especially pronounced in benchtop NMR spectra, where the effect goes beyond common "roofing" effects that often appear even in high-field spectra. Such limitations complicate 1H-NMR interpretation, increasing susceptibility to errors and frequently obstructing a thorough analysis. This often prevents the accurate determination of coupling constants and their interrelationships, essential for mapping the complete spin system network.

QM approaches to 1H NMR analysis have been evolving since the 1960s, highlighting their complex and formidable nature [2]. Despite decades of development, the challenges of QM analysis persist, driving the need for continual methodological innovations. Recent advancements aim to enhance the accuracy and efficiency of spectral interpretation by tackling the nonlinear and computationally intensive nature of solving inverse problems, where multiple valid solutions and numerous local minima on the error hypersurface are common. These improvements necessitate sophisticated optimization algorithms and significant computational resources to achieve reliable data interpretation, as demonstrated in recent research (See [3] and references there in).

In this work, we present our approach to automating QM 1H NMR spectral analysis. We leverage all available information from Mnova software, including predictions and both manual and automatic assignments. We also utilize parameters from first-order systems wherever possible, alongside efficient optimization methods. Our poster will showcase results from both moderately and highly complex systems, emphasizing the strategies employed to enhance the precision and efficiency of NMR analysis. The primary goal is to determine the spin systems of a compound from a 1H NMR spectrum, laying the groundwork for various subsequent applications, including the identification and quantification of compounds within mixtures using, for instance, tools such as USP-ID [4]

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ElPulpo: An Advanced Algorithm for Enhanced Resolution of 1D NMR Spectra

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The resolution of overlapping peaks in 1D NMR spectra is a longstanding challenge, often limiting both qualitative and quantitative analysis [1]. We introduce ElPulpo, a novel algorithm that significantly enhances spectral resolution by selectively narrowing Lorentzian peaks without altering their integral values, ensuring compatibility with quantitative NMR (qNMR). This algorithm leverages the mathematical properties of complex Lorentzian derivatives combined with Savitzky-Golay convolution filters [2], enabling both peak sharpening and noise control. In addition to leaving peak integrals rigorously invariant, its performance is virtually independent of digitization density, provided that a minimum of about 7 data points per peak is respected. The algorithm adapts to various user-defined contexts by means of a multitude of resolution enhancement modes. Extensive validation using simulated as well as real data demonstrates ElPulpo's superiority in reducing linewidths while preserving integral accuracy and avoiding the introduction of artifacts, compared to traditional resolution enhancement methods.

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IDENTIFYING THE DIETARY SIGNATURES OF ARTHRITIS THROUGH METABOLOMICS

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We report results from the Versus Arthritis 'Designa' project, investigating the role of human biofluid metabolites, endogenous and exogenous (dietary), in the onset and progression of rheumatic diseases, specifically osteo- and rheumatoid arthritis (OA and RA). The technique of choice for examination of small molecules is high-resolution 600 MHz NMR spectroscopy, which has been used in our work to examine the polar fraction extracted from blood (serum). The initial results presented here are from 7 RA patients who have been monitored at multiple time points over a period 10 years, with timepoint = 0 corresponding to first onset/diagnosis of arthritic disease.

Two different routes were used to extract and quantify peak area information from the NMR spectra. The first approach used the Chenomx software package (Chenomx Inc.,Edmonton, Canada) which is based on using spectral reference libraries to quantify a collection of annotated metabolites. The manual implementation of this approach, as used here, yields precise concentration estimates but requires substantial human oversight, a recognised bottleneck in large-scale metabolomics studies. The second approach utilised global spectral deconvolution (GSD [1]) as implemented in Mnova (Mestrelab Research S.L., Santiago de Compostela, Spain) to annotate peaks at the chemical shift level and estimate their areas and intensities. This was followed by density-based clustering applied to the chemical shifts to extract peaks found to be present at different intensities in all samples. Apart from setting some hyper-parameters, this approach is fully automated.

Many of the metabolite concentrations were found to be significantly intercorrelated, forming multiple different, unconnected networks. Both data tables revealed interesting effects across the decade-long study, including some trends common to all study participants. Further, in unsupervised cluster analysis, both approaches show that a major source of systematic variance in the datasets is patient identity: metabolite profiles for individuals are distinct and persist over the decade study duration. Canonical correlation analaysis confirmed that significant information is common to both the manually and automatically prepared data tables.

We conclude that the use of two complementary peak extraction methods allows for mutual validation of findings from the respective peak information tables, and the commonality of information suggests that the fast, automated GSD-approach can provide a pilot data table for useful exploration in advance of tackling the more resource-intensive metabolite quantitation.

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CHEMICAL SHIFT PREDICTION USING MESSAGE-PASSING NEURAL NETWORKS

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We are using advanced artificial intelligence approaches - artificial neural networks, ensembles, and deep learning - to enhance chemical shift prediction, spectral assignment, and automated structural verification in NMR spectroscopy, collectively known as the 'forward' problems. Message Passing Neural Networks (MPNNs) have emerged as a promising architecture for this purpose. These networks naturally handle molecular structures as graphs, with atoms as nodes and bonds as edges. The key advantage of MPNNs is their simultaneous use of node feature information *and* their connectivity as described by the graph adjacency matrix.

Our ongoing work involves training MPNNs on large (>10,000s) collections of molecular structures, fully annotated with experimentally observed proton (^{1}H) and carbon (^{13}C) chemical shifts. The stochastic nature of the approach allows for improved performance by pooling predictions from ensembles of trained MPNNs for each target nucleus. This is conveniently executed in parallel on the multiple GPU nodes of an HPC facility. Initial results for both nuclei have yielded prediction errors that compare favourably with those reported in the literature. For example, from application to a large test set (n ~ 28,000 nodes) of previously unseen structures, the median absolute error in prediction is ~1.2 ppm for ^{13}C. For ^{1}H, the median absolute error is 0.09 ppm. The error distributions are fat-tailed compared to the normal distribution but are smooth, symmetric, and can be well-represented by a Gaussian kernel density method. This suggests a data-driven, probabilistic route to structural assignment and verification.

Key areas for further research include: investigating the balance of node subgraph representations in the training set and their impact on prediction performance; exploring alternative graph-theoretical representations of molecular structures to better characterize molecular diversity; and extending the capabilities of the model beyond diastereotopic protons to address stereoisomerism more widely.

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A gRED NMR Prediction Database:

Facilitate QC analysis and structure elucidation

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Solution-state NMR parameters, including chemical shifts and scalar coupling constants, play an important role in the structure confirmation and structure elucidation of small molecules. In collaboration with ACDLabs, we developed a NMR prediction database that utilizes experimental ^{1}H and ^{13}C NMR data collected and annotated over 10+ years at Genentech. The training of the NMR prediction database with in-house experimental data improves accuracy for related structures.

The prediction database is designed to support our human NMR data review for quality control (QC) of all synthetic small molecules and peptides synthesized in Discovery Chemistry. We analyse an average of 2500 – 8000 per year with LC-MS/MS-CAD and ^{1}H NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC data. Our internal LIMS system information and meta-data associated with every compound is associated with the compound's analytical data in the database. When verifying a structure to an NMR spectrum, a Match Factor is calculated. This helps us to identify any potential error in the structure. With our in-house data in the prediction database, the unmatched structure will stand out and help us quickly identify the structure difference. For instance, when a compound (MW ~600) from the compound class is not included in the prediction DB, the annotation score is ~70% accurate. After a compound from that compound class has been added to the database, we found that ~80% of annotations are correct.

Additionally, the database is easily searchable from raw spectra for similar spectra or from substructure for analogue MS/MS and NMR data. Collectively, our goal is to database all of our data, learn from this data to enhance our prediction database, and to complement our NMR prediction database with MS/MS learning database for more accurate MS/MS spectral annotation as well.

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Structure elucidation of unstable and isomeric impurities in bictegravir and sonidegib

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Bictegravir (BIC) is a USFDA-approved drug for HIV wild type. Sonidegib (SDG) is an anticancer drug developed for treating basal cell carcinoma [1,2]. Regulatory agencies such as USFDA, EMA and ICH guidelines mandate forced degradation studies to understand the intrinsic stability of the drugs, define storage conditions and help preformulation studies. These studies are leveraged into environmetal water treatment studies for effective depollution strategies [3]. BIC and SDG under various stress conditions revealed the formation of 21 degradation products (DPs) and were further identified using LC-PDA. All the DPs are characterized using LC-MS/MS studies, whereas B-4, B-5, and B-6 of BIC in hydrolytic conditions and S-11 and S-12 of SDG under oxidative stress posed difficulty in characterization due to their isomeric nature. Tandem mass sepctrometry data revealed no characteristic fragments to distinguish the DPs [2]. Enrichment and isolation studies were performed to get pure compounds for NMR. Analysis posed challenging owing to the unstable nature of the DPs by subsequent conversion to other forms. Using characteristic NOEs from 2D ROESY NMR, we have elucidated diastereomeric B-4/5 and isomeric B-6/BIC configurational structures. Characteristic chemical shift changes of ^{1}H and ^{13}C resonances in pyridine and morpholine moieties of S-11 and S-12 are discerned to label the unstable N-Oxide DPs. These studies helped in solving the DP structures, proposing the degradation pathways and *insilico* toxicity prediction.

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DOSY in probing weak binding

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The diffusion properties of small molecules can be observed by NMR to differentiate the different behaviors of ligands in free and bound states.[1] This NMR approach is not limited to characterizing protein-ligand interactions, but also applicable to observing ligand-ligand bindings regardless of the low binding affinity. The weak interactions (K_D ~M) between small molecules helps to form intermediates in reactions. However, since the formation of a bound complex from a pair of small molecule ligands induces tiny changes in the observed diffusion coefficient, a DOSY experiment with high consistency and reproducibility is required to accurately determine the dissociation constant (K_D) for weak binding events. In this study, the parameters of the DOSY experiment were optimized by sampling hundreds of measurements under various conditions, and the NMR raw data was automatically batch-processed by a Python script, which dramatically improved the accuracy and efficiency of data processing.

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Investigating the Molecular Assembly of the CTD of RNAP II during LLPS

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The intrinsically disordered carboxy terminal domain (CTD) of RNA polymerase II (RNAPII) may nucleate the formation of transcription factories through a mechanism of liquid-liquid phase separation (LLPS). Transcription factories are densely packed membraneless organelles (MLOs) that contain all the necessary components to perform the transcription cycle. The CTD in model organisms such as S.cerevisiae, S. pombe and C. albicans, is composed of ~26 copies of consensus repeats ($Y_1S_2P_3T_4S_5P_6S_7$). The CTD is highly conserved at its Ser-Pro motifs, which are targets of phosphorylation and cis-trans prolyl isomerization by Ess1. Ess1, an essential peptidyl-prolyl isomerase in yeast, has a highly conserved evolutionary relationship with the CTD. To study the biophysics of the assembly of the CTD during LLPS, we utilize a small peptide version of the S.cerevisiae CTD composed of five repeats. We hypothesize Ess1 and phosphorylation may modulate the phase behavior of the CTD. The objective of this study is to determine how linear amino acid sequence, phosphorylation, and cis-trans prolyl isomerization influences CTD phase behavior. To do so, we utilize a variety of biophysical characterization assays such as UV-Vis spectroscopy, DIC microscopy, NMR, and phase separation prediction algorithms. Results from this study will elucidate how post-translational modifications, conformational changes, and extrinsic conditions such as salt, temperature, and molecule concentration influence the LLPS or assembly of the CTD and Ess1.

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ENHANCING ANALYTICAL WORKFLOWS: AN NMR DIGITAL TWIN FOR AUTOMATED STRUCTURE VERIFICATION AND QUANTIFICATION

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In the last few decades, highly pure, physical reference materials have played a crucial role in analytical and pharmaceutical chemistry. These highly valuable materials have been used for the structure verification and quantitation of active compounds and excipients. These physical reference materials are purchased from a supplier and are limited by availability, as well as processing and shipping times. Once the physical material is received, the user must prepare the standard for analysis, perform the measurement, analyze the resulting data, and dispose of the material in a suitable way.

For the user, all that is needed is the corresponding analytical data to compare to that of their own sample. Therefore, if users can access high-quality, pre-processed data suitable for comparative analysis with their own sample, costly, error-prone steps can be eliminated from their workflows. Due to the repetitive nature of these workflows, time savings can be achieved.

We present the first steps towards the future of analytical testing with MilliporeSigma's (Merck KGaA) online platform, ChemisTwin, that provides analytical solutions encompassing different analytical techniques. The ChemisTwin portal contains an extensive database of digital reference materials (dRMs), serving as digital twins of the physical reference materials. These dRMs are based on a digital package of datasets that define a physical material, and are produced from high-quality physical materials, ensuring full traceability to the physical material.

ChemisTwin leverages ACD/Lab's NMR Workbook Suite spectral prediction, automated processing, and spectral comparison technologies to automatically compare the user's NMR spectrum with the dRM spectrum and provide a detailed report. This first-of-its-kind tool allows users to verify, identify, and/or quantify their analytes of interest directly from the corresponding raw NMR sample data.

ChemisTwin provides scientists with an efficient, sustainable, readily available, and more reliable alternative to manual comparative analysis using physical reference materials or literature references. In this poster, we present a case study to illustrate the benefits of using ChemisTwin to verify the identity of a target compound versus its closely related compounds using NMR data.

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Arrayed Spin Lock Durations in One Dimensional Total Correlation Spectra for Structural Characterization

Sara Maute[1], Alexander Marchione[1], Michael Davis[1], Elizabeth Diaz[1]

1. Chemours Leveraged Analytical

The use of arrays of 1D TOCSY and TOCSY-DEPT spectra for structural characterization is demonstrated. Spin magnetization is propagated via scalar coupling throughout a spin system, and a plot of spectral intensity v. spin lock duration for each resonance enables spectral assignment. This technique is especially applicable to the assignment of interior nuclei in long chains with minimal chemical shift resolution. A DEPT-type transfer from ^{1}H to ^{13}C permits propagation of magnetization along a ^{1}H spin system followed by detection and assignment with the improved resolution offered by ^{13}C. A novel processing routine for these spectra is presented, enabling simple visualization of the propagating magnetization. Examples with several long-chain alkyl and complex cyclic analytes are presented.

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Efficient detection approach for INADEQUATE correlations

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INADEQUATE is an NMR technique mainly used to obtain ^{13}C-^{13}C correlations. This method can directly observe carbon-carbon bonds, which form the basic framework of organic compounds, making the information obtained extremely useful for structural analysis. However, its sensitivity is very low, so it is usually measured using a highly sensitivity instrument. Here, we verified how efficiently INADEQUATE correlation signals can be confirmed by designing pulse sequences and data processing using common hardware, which is a combination of standard 400 MHz NMR system and a room temperature probe. In addition, we adopted the approach of using 1D INADEQUATE with selective excitation pulses to save increment data points for 2D experiments.

Specifically, three improvements were applied to general 1D selective INADEQUATE [1]: the addition of artifact reduction measures, the addition of simultaneous ^{1}H and ^{19}F decoupling, and the application of the PCW method [2], and the results were verified.

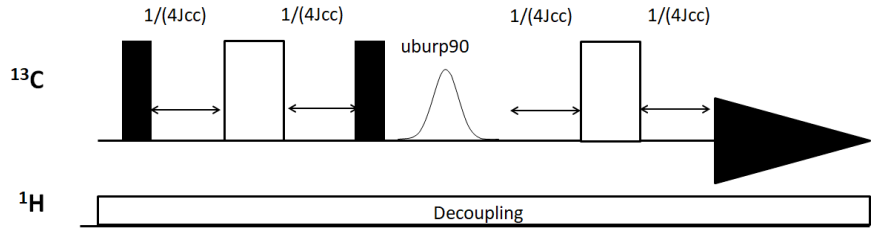

Fig.1 : General 1D selective INADEQUATE with refocusing.

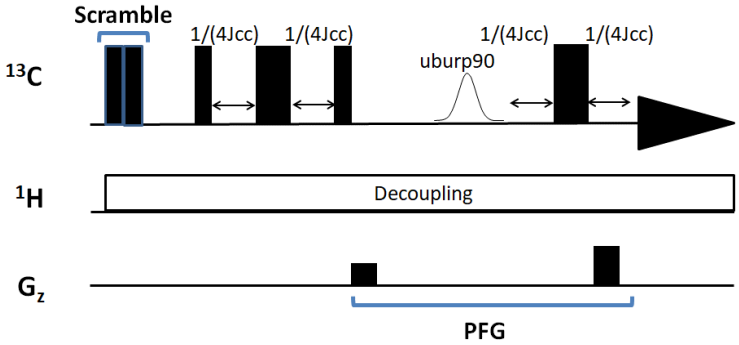

Fig.2 : Modified INADEQUATE pulse sequence that add a scramble pulse to reduce remanent magnetization and PFG for coherence selection.

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Utilizing NMR to Improve In-Process Control Testing During Pharmaceutical Manufacturing

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It is widely known that NMR is an inherently data rich analytical technique used for both structural elucidation and quantitative analyses. The pharmaceutical industry has commonly relied on this data to advance their pipeline due to the powerful and versatile nature of this technique. Studies in recent years have advanced the capabilities of quantitative NMR analyses, thus giving labs confidence in using NMR in lieu of standard chromatography methods.[1] Although NMR has been primarily utilized in R&D settings, the industry has started to increasingly implement the technique into GMP (Good Manufacturing Practices) facilities for material identification and release testing.[2] Integrating NMR into manufacturing gives chemists further control of their reactions by adding in-process control (IPC) tests that were previously unavailable.

The information presented in this poster will further demonstrate the advantages, both in terms of ease and time, of applying NMR to IPC analyses. As an example, traditional KF methods were unable to accurately quantitate the water content in an isolated intermediate due to sample polymerization and subsequent release of water molecules during the analysis. This resulted in an overestimation of the amount of water in the sample via KF analyses. On the other hand, NMR provides a means to avoid the polymerization reaction and determine the true water content in the sample (Table 1). Additional examples will highlight how the complexity of multiple IPC analyses during manufacturing campaigns that can be simplified by using qNMR methods in place of chromatography to measure multiple attributes simultaneously (see Figure 1).

Analytical Method	**Water Content (w/w%)**	**Time of Analysis**
Volumetric KF	9.3%	30 mins*
Headspace KF	9.5	45 mins*
qNMR	1.7	8 mins

Table 1: Comparison of water content results acquired by qNMR and KF methods.
*Duration depends on titration time.

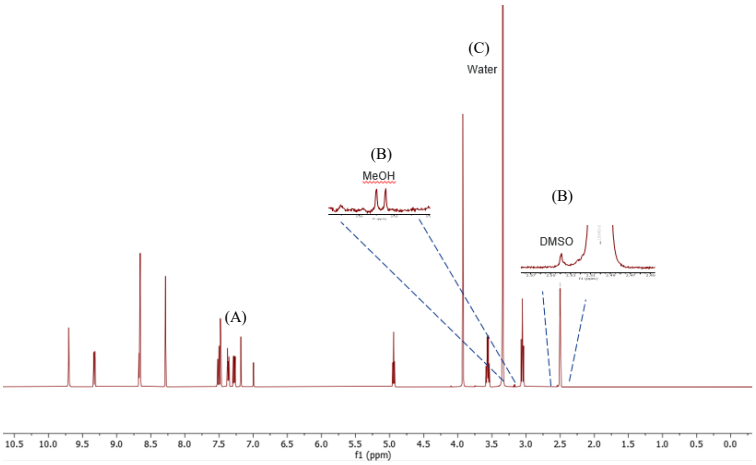

Figure 1: Quantitative ^{1}H NMR spectrum of a test sample demonstrating how the acquisition of one dataset can replace three IPC tests: (A) Analyte assay, (B) water content, and (C) residual solvent content.

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