

2023 CANMRDG Meeting

ORGANIZERS G. Joseph Ray, U. Illinois, Chicago, Chair • Catherine Eichhorn, University of Nebraska - Lincoln • Andrew Namanja, AbbVie • Josh Kurutz, University of Chicago • Milo Westler, University of Wisconsin, Madison • Cassie Yang, AbbVie • Tuo Wang, Michigan State University • Justin K. Thomas, Curia

Saturday, Sept 23, 2023

AbbVie, North Chicago, IL

Local Host: Cassie Yang, Andrew Namanja, Ian Marsden

Keynote speaker: José Napolitano

Senior Principal Scientist, Genentech

Meeting Program

TABLE OF CONTENTS

SPONSORS.....	Page 2
TALKS.....	Page 3
POSTERS.....	Page 9

CANMRDG 2023 PROGRAM

Thank you to our sponsors!!



Mestrelab Research



Cambridge Isotope
Laboratories, Inc.
isotope.com



**Millipore
Sigma**



NORELL®



CANMRDG 2023 PROGRAM

Registration, coffee & Poster Setup • 08:00-08:30 am

Welcoming Remarks • 08:30-08:55 am

Shuhong Zhang, Ph.D, Vice President of Development Sciences, AbbVie

Session I: Small molecules • 08:55-10:05

Tuo Wang presiding

08:55-09:25 NMR Metabolomics Best Practices: What We Are Doing, What We Should Be Doing, and Some Recent Advancements

Robert Powers, Charles Bessey Professor of Chemistry, University of Nebraska-Lincoln

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

09:25-09:45 Fragment Based Drug Discovery Accelerated by Protein NMR Fingerprint Analysis

Thomas Frederick, Senior Scientist II, AbbVie

NMR spectroscopy is an information-rich technology that provides critical insights for early phase drug discovery programs. Specifically, protein-detected NMR is widely used for fragment-based drug discovery (FBDD), and is a gold-standard for validating HTS, DEL and VLS hits. Of these, fragment hits present project teams with a unique challenge for progressing chemical matter especially when traditional biochemical assays are unavailable. NMR plays a key role in bridging that gap. To support FBDD efforts, we developed tools internally as well as through external collaborations to streamline our workflows. In this talk, I will discuss our NMR workflow that involves single-concentration affinity ranking of ligands coupled with quantitative affinity determination of fragments for driving SAR using NMR fingerprint analysis methods.

09:45-10:05 NMR Full Spin Analysis of *Mitragyna speciosa* Major Constituent, Mitragynine, and its Diastereomers

Daniel Zagal, Graduate student, University of Illinois Chicago

Kratom has recently gained popularity in the Western world due to its antinociceptive effects, which arise from its alkaloids binding to the mu and kappa opioid receptors. Currently, the value of the kratom market in the US is estimated at approximately \$1.3 billion. The major alkaloid in kratom, mitragynine, along with its diastereomers, were characterized roughly 100 years ago and have been recently reviewed using NMR spectroscopy. However, it remains challenging to fully understand their quintessential ¹H NMR spectra, particularly to determine the multiplicities of all signals, which encode the relative stereochemistry of the

CANMRDG 2023 PROGRAM

compounds. This can be addressed by quantum mechanical spin analysis (QMSA) using "Cosmic Truth." This application allows supervised and/or automated iterative fitting of experimental spectra with root mean square errors remaining below 0.10. From these simulations, full sets of ^1H spin parameters of the molecules can be determined. This approach, termed HifSA (^1H iterative functionalized SA), allows the determination of relative configurations in diastereomers and the study of how the configuration of chiral centers affects the chemical shifts and signal multiplicities of nearby resonances. Importantly, this process works regardless of the degree of signal overlap as the simulated spectrum is deconvoluted with high accuracy. The present work utilizes kratom compounds of global interest to showcase the workflow and some capabilities of "Cosmic Truth," emphasizing its utility in confirming the structure and relative stereochemistry of small molecules.

10:05-10:20 **Coffee Break**

Session II: Nucleic acids • 10:20-11:20

Catherine Eichhorn presiding

10:20-10:40 **Visualizing a two-state conformational ensemble in 7SK RNA central domain**

Momodou Camara, Graduate student, University of Nebraska-Lincoln

The 7SK ribonucleoprotein (RNP) regulates RNA polymerase II transcription elongation by binding and inactivating the kinase activity of the positive transcription elongation factor b (P-TEFb) protein. Release of P-TEFb from the 7SK RNP requires the assembly of additional proteins that bind to the central stem-loop 3 (SL3) of 7SK RNA. However, little high-resolution information is known regarding 7SK SL3 structure. Here, we combine solution NMR spectroscopy and dimethyl sulfate mutational profiling with sequencing (DMS-MaPseq) approaches to characterize the structural features of the 7SK RNA SL3 distal end. We find that SL3 undergoes two-state exchange modulated by changes in temperature. We rationally design constructs that lock SL3 into a single state, validating the observed two-state exchange. A survey of 7SK RNA sequences reveals that both states are conserved among vertebrates, suggesting functional importance for both states. These results provide novel insights into the structural dynamics of a 7SK RNA SL3 and emphasize the power of integrating NMR and chemical mapping to deconvolute multi-state systems.

10:40-11:00 **Solution structure of poly(UG) RNA**

Riley Petersen, Graduate Student, University of Wisconsin-Madison

Poly(UG) or pUG RNAs are UG or GU dinucleotide repeat sequences that are highly abundant in eukaryotes. Post-transcriptional addition of pUGs to RNA 3' ends marks mRNAs as vectors for gene silencing in *C. elegans*. We have determined the solution structure of the free pUG RNA (GU)₁₂ by nuclear magnetic resonance spectroscopy and small and wide-angle x-ray scattering (NMR-SAXS-WAXS). The low complexity sequence results in overlapped NMR signals that complicate assignment of chemical shifts. To overcome this problem we utilized single, site-specific deoxyribose modifications which did not perturb the structure and introduced well-resolved methylene signals that could be used to validate chemical shift assignments. Additionally, we developed optimized pulse sequences for RNA to assign all ^1H , ^{13}C , ^{15}N , and ^{31}P resonances and to efficiently measure magnetization transfer across hydrogen bonds. In total, 549 NOEs, 21 residual dipolar couplings, 72 hydrogen bonds and 143 dihedral angle restraints were used to refine the solution structure in conjunction with SAXS-WAXS data to a root mean squared deviation (RMSD) of 0.62 Å. The structure is an unusual left-handed parallel intramolecular

CANMRDG 2023 PROGRAM

quadruplex with a Z-form backbone. Hydrogen-deuterium exchange experiments indicate the structure is highly kinetically stable with quartet opening rates on the timescale of several days. Overall, the structure is similar to our previously determined crystal structure of (GU)₁₂ bound to the ligand N-methyl mesoporphyrin IX (NMM). These data indicate the pUG fold is unaltered by docking of the NMM ligand. The solution structure reveals conformational details that could not be resolved by the crystal structure and explains how the pUG fold can form within longer RNAs.

11:00-11:20 **Hydrogen bonding in duplex DNA probed by DNP enhanced solid-state NMR N-H bond length measurements**

Lakshmi Bhai N V, Postdoctoral Research Scientist, Columbia University

Numerous biological processes and mechanisms depend on the details of base pairing and hydrogen bonding in DNA. Hydrogen bonds are challenging to quantify directly by X-ray crystallography and cryoelectron microscopy due to difficulty of visualizing hydrogen atom locations but can be probed with site specificity by nuclear magnetic resonance (NMR) techniques in both solution and solid state with the latter particularly suited to large, slowly tumbling DNA complexes and assemblies. Recently, we have shown that low-temperature dynamic nuclear polarization (DNP) enhanced solid-state NMR is a valuable tool for observing and distinguishing Hoogsteen base pairs from canonical Watson-Crick base pairs in a broad range of DNA systems under native-like conditions. Here, using a 12-mer DNA duplex containing two central adenine-thymine (A-T) base pairs in either Watson-Crick or Hoogsteen confirmation as a model, we demonstrate DNP solid-state NMR based measurements of thymine N3-H3 bond lengths, which are highly sensitive to details of N-H-N hydrogen bonding and permit hydrogen bonds for the two A-T base pair conformers to be systematically compared within the same DNA sequence context. For the model 12-mer DNA duplex effectively identical thymine N-H bond lengths of 1.055 ± 0.011 Å and 1.060 ± 0.011 Å were found for Watson-Crick A-T and Hoogsteen A(syn)-T base pairs, respectively, relative to a reference amide bond length of 1.015 ± 0.010 Å determined for N-acetyl-valine under the same experimental conditions. These results indicate that there aren't significant differences in N-H-N hydrogen bonds between Watson-Crick and Hoogsteen conformers for this system. More generally, high precision measurements of N-H bond lengths by DNP solid-state NMR based methods are expected to facilitate detailed comparative analysis of hydrogen bonding for a range of DNA complexes and base pairing environments.

POSTER SESSION • 11:20-13:00

Poster listings on Page 9

LUNCH BREAK • 13:00-13:45

Session III: Solid State NMR • 13:45 - 14:55

Justin K. Thomas presiding

13:45-14:05 **Solid-State NMR Analysis of Fungal Cell Walls from *Cryptococcus neoformans* Species**

Ankur Ankur, Graduate student, Michigan State University

Cryptococcus neoformans is the etiological agent of cryptococcosis, a systematic fungal infection with dissemination to the central nervous system causing meningoencephalitis. Elucidating the cell wall structure, dynamics, and adaptive mechanisms of its cell wall is paramount for designing targeted

CANMRDG 2023 PROGRAM

therapeutics against this human pathogen. The presence of polysaccharide capsules anchored to the cell wall distinguishes *C. neoformans* from other fungal species, playing a crucial role in virulence. Notably, glucuronoxylomannan (GXM) and glucuronoxylomannogalactan (GXMGal) are the capsular components with GXM being a predominant constituent. In this study, we employed solid-state NMR spectroscopy to investigate the polysaccharide moieties presence in the wild-type *C. neoformans* and an acapsular mutant lacking the major capsular component (GXM). Our findings reveal a structurally rigid core encompassing beta-1,6-glucan, alpha-1,3-glucan, beta-1,3-glucan, GXM polymer, chitin, and chitosan. Additionally, mobile phase polymers including GXMGal and glucans were identified. This comprehensive characterization of intact cell polysaccharides offers valuable insight into the pathogenicity of *C. neoformans* and paves the way for the development of improved antifungal therapeutic agents with improved efficacy.

14:05-14:25

NMR for the membrane location of SE of HIV gp41 proves the role of the hairpin in HIV fusion

Md Rokunujjaman, Graduate student, Michigan State University

Human immunodeficiency virus (HIV) is the main culprit of AIDS which initially infect target white blood cells by joining (fusion) the HIV and host cell membranes, with consequent deposition of the viral nucleocapsid in the cytoplasm. Gp41 is a single-pass integral viral membrane protein in HIV responsible for fusion catalysis; that contains a ~150-residue soluble domain outside the virus [1,2]. This soluble ectodomain (SE) adopts a hairpin structure that contains N-helix-turn-C-helix, and two helices align themselves antiparallely and there is van der Waals contact between them. Moreover, three hairpins associate together as a six-helix bundle as the final fusion structure. The important role of the SE hairpin in fusion catalysis vs. no catalytic role in fusion is hypothesized by the association with binding to the membrane. This phenomenon is supported by the rapid membrane vesicle fusion induced by the hairpin under some conditions [1]. Moreover, a recent trans-dominance effect study through membrane vesicle fusion of V513E to wild-type ectodomain of gp41 shows the quantitative similarity of HIV gp160 V513E-dominant reduction of fusion and infection with fusion by the gp41 ectodomain hairpin supports an important fusion role for the final trimer-of-hairpins structure [3]. The position of the SE hairpin might help in a better understanding of membrane apposition (viral and host membrane) during fusion since deep insertion of SE predicts the ease of close membrane apposition whereas surface insertion predicts less ease of membrane apposition. Thus, this study is designed to determine the membrane location of the SE hairpin in the membrane. We expressed a large soluble ectodomain in minimal media using either ¹³C-glucose and ¹³C-glycerol, with a dilution of ¹³C labeling done by mixing with unlabeled glucose or by selectively labeled glycerol [4]. The proximity between ¹³C-labeled protein and ²H labels at the center of the membrane was done by Rotational-echo double-resonance (REDOR) solid-state NMR [5]. Peak assignment of different labeled carbon on the side chain of amino acids was observed in NMR due to the use of the different carbon sources for ¹³C protein expression. A significant REDOR dephasing for samples from different carbon sources indicates that the soluble ectodomain has a deep location in the membrane.

14:25-14:45

Exploring Surface Treatments of CsPbBr₃ Perovskite Quantum Dots Using Solid-State NMR

Yunhua Chen, Senior Scientist I, AbbVie

Cesium lead bromide (CsPbBr₃) quantum dots (QDs) are versatile semiconductors with low production costs, widely applied in lighting, displays, and various optoelectronic applications. The photoluminescent characteristics of CsPbBr₃ QDs heavily depend on their surface structure, where different surface defects are believed to lead to lower photoluminescence quantum yields (PLQY). Previous studies have shown that treating CsPbBr₃ QDs with didodecyldimethylammonium bromide (DDDMABr) in combination with

CANMRDG 2023 PROGRAM

CsBr or PbBr₂ after synthesis can enhance PLQY, likely by healing surface defects. To investigate the surface structure of post-synthetically treated CsPbBr₃ QDs, we employed optical spectroscopy and solution and solid-state NMR spectroscopy. The presence of a ¹³³Cs NMR signal with a distinct chemical shift in the surface-selective ¹³³Cs spectrum indicates that surface Cs sites are coordinated by oleate ligands. This coordination was previously undefined in the literature and is confirmed by DNP-enhanced ¹H ¹³C{¹³³Cs} CP D-HMQC experiments. Both solution and solid-state NMR spectroscopy reveal that post-synthetic surface treatments displace the native oleate ligands, with didodecyldimethylammonium strongly binding to the CsPbBr₃ QD surface. By comparing calculated and experimental ¹H{¹³³Cs} S-RESPDOR and ¹H{²⁰⁷Pb} S-REDOR dipolar dephasing curves, we establish that CsPbBr₃ QDs remain CsBr terminated after surface treatments, with quaternary DDDMABr ammonium cations occupying surface Cs vacancies. Notably, treatment with DDDMABr + PbBr₂ results in the most significant displacement of native oleate ligands, likely replaced with Br anions, ultimately leading to QDs with the highest PLQY. These findings underscore the significance of high-resolution solid-state NMR spectroscopy in unraveling the atomistic surface structure of nanomaterials and its impact on their optical properties.

14:45-15:00 **Coffee Break**

Session IV: Pushing the Frontiers of NMR • 15:00-16:40

Josh Kurutz presiding

15:00-15:30 **NMR and Computational Studies of Toxic Small Molecules Interacting with Plastic Nanoparticles and DNA**

Leah Casabianca, Associate Professor, Clemson University

Plastic pollution is a growing worldwide concern. In the environment, large plastic particles break down, eventually forming micro- and nano-scale plastic particles. These plastic nanoparticles have a large, reactive surface, and can sorb small molecules that are also present in polluted waterways. When plastic nanoparticles are ingested by aquatic life, which may eventually be consumed by humans, these toxic small molecules can be released into the body. We have used a variety of solution-state NMR methods to examine interactions between small molecules and polystyrene nanoparticles in solution. Similarly, ingredients used in cosmetics such as sunscreen and hair dyes have raised concern for their potential negative effects on human and environmental health. We have also examined how these small molecules may interact with DNA using computational and experimental NMR methods.

15:30-15:50 **High Resolution Structure of PET Ligand Bound Alpha-Synuclein Fibrils**

Collin Borcik, Postdoc, Department of Biochemistry, University of Wisconsin- Madison

Parkinson disease and related synucleinopathies are neurodegenerative conditions caused by aggregation of the Alpha-synuclein (Asyn) into fibrils in the brain. Rational drug design targeting these diseased fibrils has been historically hindered by the lack of high-resolution fibril structures and drug bound fibril structures. We present structural studies of a high affinity PET imaging ligand binding to in vitro Asyn fibrils using a combined SSNMR and Cryo-EM approach. We have determined a putative binding site of the PET imaging ligand to Asyn and determined several structural features of this binding site that can be used to guide rational drug design for early detection, disease phenotyping, and treatment of Parkinson disease.

CANMRDG 2023 PROGRAM

15:50-16:40 **NMR applications to advance small (and not so small) molecule pharmaceutical research**
Jose Napolitano, Senior Principal Scientist, Genentech

NMR is a mature analytical technique that provides unique insights into molecular structure and dynamics at atomic level. As a result, NMR is widely applied in pharmaceutical research to investigate chemical structure, content, as well as molecular interactions and transformations. This presentation highlights how Genentech's structure elucidation group (SEG) leverages decades of knowledge in NMR to support pharmaceutical R&D and to advance an increasingly complex and diverse pipeline. Collaborations with our analytical, process, and formulation teams will be highlighted. NMR applications in structure elucidation, quantitation, reaction monitoring, and molecular interactions will be discussed.

Closing Remarks • 16:40-16:50
G. Joseph Ray, Chair, CANMRDG

AWARD CEREMONY & SOCIAL MIXER • 16:50-17:30

ADJOURN

CANMRDG 2023 PROGRAM

POSTER ABSTRACTS

SESSION TIMES 11:45-13:00 (Atrium)

- Poster 1** **Solid-State NMR ^{13}C Sensitivity at High Magnetic Field**
Eric Han, Graduate student, University of Wisconsin - Madison
- Poster 2** **Control of Bruker NMR Spectrometers from Python with KovriginNMR**
Evgenii Kovrigin, Director, Magnetic Resonance Research Center, University of Notre Dame
- Poster 3** **Quality Control of Natural Amino acids with Benchtop qH and 2D NMR**
Daniela Rebollar Ramos, Postdoctoral Research Assistant, University of Illinois Chicago
- Poster 4** **Fragment-based screening by protein-detected NMR spectroscopy**
Paul Kerber, Research associate, Medical College of Wisconsin
- Poster 5** **Magnetic Resonance Imaging of Insects with Contrast Agent: An Undergraduate Experiment**
Charles Abrams, Professor Truman College - one of the City Colleges of Chicago
- Poster 6** **Distinguishing Near-identical Diastereomers with Digital NMR Reference Materials**
Aysegul Caskurlu, Researcher, University of Illinois Chicago
- Poster 7** **Solid State NMR Comparison of Cell Wall Polysaccharides in *A. fumigatus* and *A. nidulans***
Isha Gautam, Graduate Assistant, Michigan State University
- Poster 8** **Molecular Level Understanding of Candida Cell Walls Using SS-NMR**
Kalpana Singh, Graduate Assistant, Michigan State University
- Poster 9** **Introduction of NMRFAM, including the spectrometers, user program, and service.**
Songlin Wang, Scientist NMRFAM, University of Wisconsin-Madison
- Poster 10** **Quantitative Analysis of Selective Proanthocyanidin Enrichment by qNMR**
Shuxi Jing, postdoctoral researcher, UNIVERSITY OF ILLINOIS AT CHICAGO
- Poster 11** **Unveiling Fungal Cell Wall Complexity with Proton Detection Fast MAS NMR**
Jayasubba Reddy Yarava, Research Associate, Michigan State University
- Poster 12** **Structural Adaptation of Fungal Cell Wall in Hypersaline Environment**
Anand Jacob, PhD Student, Michigan State University
- Poster 13** **A Study of Fundamental Reaction of Lignin with Formaldehyde by Solid-State NMR**
Debkumar Debnath, Graduate Student, Michigan State University
- Poster 14** **Small multidrug transporter EmrE unaffected by lipid composition when bound to substrate**
Ashley Hiett, Graduate Student Research Assistant, University of Wisconsin-Madison
- Poster 15** **NAN & NMRbox: resources for NMR data collection, management, processing, and analysis**
Mark Maciejewski, Assistant Professor, UConn Health

CANMRDG 2023 PROGRAM

- Poster 16** **Poloxamer NMR Method Development for Covid-19 Vaccine**
Mikhail Gerasimov, Scientist, Sanofi
- Poster 17** **Objective Approaches to Acquire and Assess Multidimensional NMR Spectra of Biological Solids**
Benjamin Harding, Graduate Student, UW-Madison
- Poster 18** **Solid-state NMR structure of the A30P hereditary mutant alpha-synuclein fibril**
Moses Milchberg, PhD student, University of Wisconsin - Madison
- Poster 19** **SSNMR Fibril Structure Determination Using PASD and Strict Symmetry**
Owen Warmuth, PhD Student, University of Wisconsin-Madison
- Poster 20** **Substrate-dependent transport behavior of a Small Multidrug Resistance transporter from P. aeruginosa**
Juliet Cheng, Graduate Student, University of Wisconsin Madison
- Poster 21** **Structural analysis of a Pseudomonas aeruginosa second messenger binding protein**
Christopher Ptak, Assistant Director, University of Iowa
- Poster 22** **Customizable In-house 3D-printed Tool Kit for SSNMR**
Martin Olson, Undergraduate Research Assistant, NMRFAM
- Poster 23** **Benchtop NMR Spectroscopy of an Aqueous Polysaccharide Product in Manufacturing**
Christina Szabo, Associate Director, Research Baxter International

CANMRDG 2023 PROGRAM

Poster 1

Solid-State NMR ^{13}C Sensitivity at High Magnetic Field

Eric Han, Graduate student, University of Wisconsin - Madison

In the low B_0 field limit, SNR can increase in proportion to $B_0^{7/4}$. For modern MAS probes, this approximation holds for rotor sizes up to 3.2 mm at 14.1 T. To obtain the anticipated benefit of larger coils and/or higher B_0 fields requires a quantitative understanding of the contributions to SNR, utilizing samples and protocols that reproduce SNR measurements with high accuracy and precision. Here, we present such a systematic and comprehensive study of ^{13}C SNR under MAS over the range of 14.1 to 21.1 T. We evaluate a range of probe designs utilizing 1.6, 2.5 and, 3.2 mm rotors. These robust approaches and standard operating procedures provide an improved understanding of the contributions from probe efficiency, receiver NF, and B_0 .

Poster 2

Control of Bruker NMR Spectrometers from Python with KovriginNMR

Evgenii Kovrigin Director, Magnetic Resonance Research Center, University of Notre Dame

The object-oriented Python software package, KovriginNMR, was designed to enable Bruker spectrometer control from Topspin Python interface. KovriginNMR is intended to help NMR users construct workflows incorporating multiple samples, experiments, as well as variable-temperature operation. A user is able to automate as much or as little of their workflow as desired, and the software may be started from user's own account (no need for NMR superuser privileges). KovriginNMR automatically logs events taking place during its operation and includes time stamps, sample and experiment names, data paths, thus assisting accurate record-keeping. The KovriginNMR Workflows module supports development of complex experimental routines involving multiple samples and experiments while the KovriginNMR VT module enables automated NMR measurements in a broad temperature range.

Poster 3

Quality Control of Natural Amino acids with Benchtop ^1H and 2D NMR

Daniela Rebollar Ramos, Postdoctoral Research Assistant, University of Illinois Chicago

Natural amino acids (AAs) are widely used in healthcare as nutrients/infusions, dietary supplements, and as drug excipients. As dietary supplementation, infusion, and excipient usage goes along with relatively high oral or i.v. doses, rigorous quality control measures and purity requirements are paramount for these compounds. While LC methodology is widely used for these purposes, the lack of UV chromophores and unfavorable chromatographic properties produce intrinsic challenges. Herein we demonstrate how qualitative and quantitative 1D ^1H and 2D HSQC benchtop (60 MHz) NMR methodology can assess the identity and the purity of some of the most challenging AAs and is a viable alternative for quality control. Experimental parameters such as sample amount, number of scans, relaxation delay and acquisition time were modified to improve the overall quality of the spectra. Comparison of purity values with those from high-field (600 MHz) measurement instills confidence in the benchtop NMR approach.

Poster 4

Fragment-based screening by protein-detected NMR spectroscopy

Paul Kerber, Research associate, Medical College of Wisconsin

Fragment-based drug discovery (FBDD) identifies low molecular weight compounds that can be developed into ligands with high affinity and selectivity for therapeutic targets. Screening fragment libraries (<10,000 molecules) with biophysical techniques against macromolecules provides information about novel chemical spaces that bind the macromolecule and scaffolds that can be modified to increase potency. A fragment-screening pipeline requires a standardized protocol for target selection, library assembly and maintenance, library screening, and hit validation to ensure hit integrity. Herein, the fundamental aspects of a fragment screening pipeline focusing on protein-detected NMR data collection

CANMRDG 2023 PROGRAM

and analysis are discussed in detail for researchers to use as a resource in their FBDD projects. Selected screening targets must undergo rigorous stability and buffer testing by NMR spectroscopy to ensure the protein structure is stable for the entire screen. Biophysical instrumentation that rapidly measures protein thermostability is helpful in buffer screening. Molecules in fragment libraries are analyzed computationally and physically, stored at appropriate temperatures, and multiplexed in well plates for library conservation. The screening protocol is streamlined using liquid handling robotics for sample preparation and customized Python scripts for protein-detected NMR data analysis. Molecules identified from the screen are titrated to determine their binding site(s) and K_d values and confirmed with an orthogonal biophysical assay. This detailed FBDD screening pipeline developed by the Program in Chemical Biology at the Medical College of Wisconsin has successfully screened many unrelated target proteins to identified novel molecules that selectively bind to these target proteins.

Poster 5

Magnetic Resonance Imaging of Insects with Contrast Agent: An Undergraduate Experiment

Charles Abrams, Professor, Truman College - one of the City Colleges of Chicago

As part of our continuing effort to expand undergraduate experiments possible with a colony of the beneficial insect *Podisus maculiventris* (Spined Soldier Bug), we investigated magnetic resonance imaging (MRI) using a desktop instrument available from Alegre Science. This instrument has a sample size of approximately 1 cm³, ideal for the adult and instars of the species. Images obtained using the Flash 3D pulse sequence showed the general shape of the insect. In a published undergraduate experiment, students synthesized lanthanide contrast agents (CA) and measured T₁ values for different concentrations of CAs in water/D₂O in an NMR spectrometer, but did not use their product in MRI. Our research shows that solutions of the student synthesized contrast agent provide image contrast to tap water in desktop MRI, and when injected in dead or anesthetized insects. We attempted to feed contrast agent solution to the insects to create images of the digestive system, and will present our preliminary results.

Poster 6

Distinguishing Near-identical Diastereomers with Digital NMR Reference Materials

Aysegul Caskurlu, Researcher, University of Illinois Chicago

Angelic (AA) and tiglic acid (TA) esters are a critical pair of cis/trans isomers widely found as components of sesquiterpenes, such as humulenes and germacrenes, and are relevant for botanical dietary supplements from the Apiaceae and Asteraceae families. Due to the high similarity, it is often hard to distinguish if marker compounds possess an AA or TA ester. Accordingly, their identification requires identical reference materials for each naturally occurring ester, however this demand cannot practically be filled for such rare chemicals. The present work demonstrates that Quantum Mechanics (QM)-based NMR analysis can fulfill this unmet need by providing a qualitative and quantitative reference. The different geometry of the two isomers gives rise to small yet significant differences in the J-coupling patterns and chemical shifts, which can be determined reproducibly from ¹H-NMR spectra. Thus, we demonstrate these spectroscopic differences in methyl angelate (MA) and methyl tiglate (MT) esters in CDCl₃, acetone-d₆ and benzene-d₆ at two highly different magnetic field strengths (600 MHz and 60 MHz); DFT calculations of chemical shifts and coupling constants were also performed. Definitive knowledge of these QM numerical differences facilitates their identification and allows replacement of physical reference materials with digital data as high-level reference.

Poster 7

Solid State NMR Comparison of Cell Wall Polysaccharides in *A. fumigatus* and *A. nidulans*

Isha Gautam, Graduate Assistant, Michigan State University

Aspergillus fumigatus and *Aspergillus nidulans* are opportunistic fungal pathogens causing life-threatening infections in immunocompromised patients. Studies have been made using chemical treatment approaches to compare the cell wall composition and functions, but a comprehensive understanding of their differences still requires further investigations. As the cell wall is enriched with carbohydrates and represents a promising target for antifungal drugs, characterization of the structure and dynamics of cell wall biomolecules is crucial for advancing drug discovery. Here we present the ongoing studies using solid-state NMR and dynamic nuclear polarization (DNP) to delve into the cell wall structure of *A. fumigatus* and *A. nidulans*. Our high-resolution solid-state NMR (ssNMR) revealed that both fungal species have a similar composition of alpha-glucan, beta-glucan, and chitin in the rigid inner core of the cell wall. Nevertheless, notable alterations in the relative abundance of mobile molecules, such as galactosaminogalactan (GAG), galactomannan (GM), and other portions of alpha-glucans, changed significantly. Employing DNP measurements, we have identified short-range (2 ms) and long-range (20 ms), homonuclear (^{13}C - ^{13}C) and heteronuclear (^{15}N - ^{13}C), disclosing 44 intermolecular contacts through proton-assisted recoupling (PAR) and NCACX experiments. Moreover, we unveil the densely packed arrangements of glucans and chitins in *A. nidulans*. By shedding light on the intricate composition and the organization of the cell wall in these fungi we facilitate the valuable pathways for efficient future therapeutic advancements.

Poster 8

Molecular Level Understanding of Candida Cell Walls Using SS-NMR

Kalpana Singh, Graduate Assistant, Michigan State University

Candida species cause superficial and systematic candidiasis, with the latter posing a significant burden on healthcare systems and patients, especially among immunocompromised individuals. *C. albicans* and *C. auris* are both opportunistic fungi; they show multiple drug resistance over other species. In the establishment of a fungal immune response, the first step is the recognition of the fungal cell wall, which is a crucial step in the anti-fungal immune response. Chemical assays have been used to compare cell wall compositions and functions in these two fungal species, but we still lack a molecular-level understanding of the differences between these two pathogens. As the carbohydrate-rich cell wall is promising target for antifungal drugs, detailed knowledge of the structure and dynamics of cell wall biomolecules is essential to facilitate novel drug discoveries. Here we studied *Candida* species at molecular level using solid-state Nuclear Magnetic Resonance. First, we treated *Candida* with caspofungin: which inhibits beta-glucan synthase enzyme at minimum inhibitory concentration to characterize the structural response of cell wall. As expected we saw a 50% reduction of beta-glucan after drug treatment. Drug-treated cell walls are highly hydrophobic and stiff which could help the microbes to resist external stresses. Fungi also increased the content of chitin to compensate for the loss of beta-glucans due to the inhibition of the biosynthesis of the carbohydrates by caspofungin. Second, we revealed that *C. auris* contains 4 times more chitin than *C. albicans* which is a major difference between the two species. Based on the analysis of peak volume, CAS reduces the amount of beta-1,3-glucans in *C. albicans* and *C. auris* cell wall from 94% to 47% and from 80 to 41% respectively in the rigid part of the cell wall. The relative abundance of mobile molecules such as mannan and beta-1,6-glucans invade the mechanical core of the cell wall with the drug treatment. These findings provide the structural foundation for identifying conserved macromolecular structure as potential targets of new antifungal drug.

Poster 9

Introduction of NMRFAM, including the spectrometers, user program, and service.

CANMRDG 2023 PROGRAM

Songlin Wang, Scientist NMRFAM, University of Wisconsin-Madison

Poster 10

Quantitative Analysis of Selective Proanthocyanidin Enrichment by qNMR

Shuxi Jing, postdoctoral, researcher, UNIVERSITY OF ILLINOIS AT CHICAGO

Proanthocyanidins (PACs, syn. condensed tannins) are oligomers to polymers of flavan-3-ols, which constitute a group of plant constituents that impact humans via their health, nutritional, and agronomic value. To date, quantitative analysis of PACs depends on colorimetric assays or phloroglucinolysis/thiolysis combined with UV-HPLC analysis, which are methods with limited accuracy, extended sample preparation, degradation, and/or need for identical reference standards. Towards our broader goal of developing new plant-sourced biomaterials that chemically and mechanically modulate the properties of dental tissue for clinical interventions, we have characterized 10 different PAC DESIGNER (Depletion and Enrichment of Select Ingredients Generating Normalized Extract Resources) materials. The DESIGNER approach is carried out by using centrifugal partition chromatography (CPC) for the selective enrichment of trimeric and tetrameric PACs. The NMR analysis of intact PACs has been advanced in the past decade by the application of high-field NMR equipment, low temperature NMR, and precise assignments using computer-aided ^1H full spin analysis (HifSA). Here, a quantitative analysis of the total PAC content of the DESIGNER materials by quantitative NMR (qNMR) method using an internal calibrant combined with diol-HPLC was developed. The DESIGNER materials were analyzed by diol-HPLC to deduce the polymerization profile of the sample; then the total PAC content was determined to be in a range from 61% to 95% by qNMR. We highlight the complementarity of diol-HPLC and qNMR to accurately assess the amount of PACs across a range of concentrations and PAC stability in the DESIGNER materials. This quantitative methodology paves the way to generate standardized DESIGNER materials and perform rigorous quality control for dental (pre-)clinical study of PACs.

Poster 11

Unveiling Fungal Cell Wall Complexity with Proton Detection Fast MAS NMR

Jayasubba Reddy Yarava, Research Associate, Michigan State University

Cell walls are complex structures consisting of a variety of molecules, including lipids, proteins, glycoproteins, polysaccharides such as chitin, chitosan, glucan, mannans etc. These components exhibit dynamic distribution patterns across different regions of the cell wall. Due to this inherent dynamic heterogeneity, determining the structure of cell wall is challenging. However, solid-state NMR has emerged as a powerful technique for elucidating the cell wall structure. Nonetheless, the NMR spectrum becomes overcrowded due to the multitude of resonances originating from these various components. To address this issue, we applied a set of 2D and 3D proton detection fast magic angle spinning NMR methods on the fungal cell wall *Rhizopus delemer* as an example. We employed proton detection-based 3D $\text{hcoCH}_3\text{coNH}$ and 3D hC_2NH_3^+ correlation methods to specifically isolate resonances from chitin and chitosan. This distinction was accomplished by selectively transferring magnetization through the N-CO-CH₃ pathway for chitin and the C₂-NH₃⁺ for chitosan. Furthermore, we established the spatial distribution of rigid components using ^1H - ^1H correlation methods. The covalently bonded carbon network was determined utilizing dipolar and scalar 3D correlation methods. Moreover, we applied relaxation filters and dipolar dephasing 1D methods to distinctly trace resonances from components in both rigid and semi-rigid regions. Finally, we investigated the dynamics of *Rhizopus delemer* using ^{13}C R₁ and ^{13}C R₁ ρ rate measurements. We analyzed these relaxation data using simple, extended and collective model free formalisms. This experimental protocol holds potential for other cell wall studies including plant and bacterial cell walls.

Poster 12

Structural Adaptation of Fungal Cell Wall in Hypersaline Environment

CANMRDG 2023 PROGRAM

Anand Jacob, PhD Student, Michigan State University

Halophilic fungi, which can survive in hypersaline environments and are exposed to a variety of severe circumstances, have drawn a lot of interest because of their potential use in abrasive industrial processes like bioremediation and fermentation. Uncertainty persists regarding the function of the cell wall in surviving various environmental factors. In this study, we compare the cell wall architecture of *Aspergillus sydowii* with that of other halophilic and halotolerant fungus over salinity gradients using solid-state NMR spectroscopy. According to analyses of entire cells, *A. sydowii* cell walls have a stiff core made of chitin, -glucan, and chitosan, which is protected by a surface shell made of galactomannan and galactosaminogalactan. *A. sydowii* increases chitin production and integrates -glucan to produce thick, rigid, and hydrophobic cell walls when exposed to hypersaline environments. Such structural changes give the fungus a strong defense against external stress and allow it to adapt to both hypersaline and salt-deprived environments. These results about the halophilic strains can be helpful for biotechnology applications.

Poster 13

A Study of Fundamental Reaction of Lignin with Formaldehyde by Solid-State NMR

Debkumar Debnath, Graduate Student, Michigan State University

As a part of adhesive in the wood industry, phenol-formaldehyde (PF) resin is widely recognized. Despite the widespread usage of resole resin (phenol: formaldehyde = 1:2), due to its durability and strong water resistance, the toxicity of formaldehyde and carbon footprint of high cost petroleum-derived phenol presents a significant problem. As a result, the focus of our study has shifted to low-cost biomaterials that can substitute phenol and replace formaldehyde while maintaining the adhesive properties of the resin. Due to presence of phenolic unit and 15-30% of the lignocellulosic biomass is composed of lignin, researcher found lignin as a good substitute of phenol. We used solid-state NMR technique to identify the methylene linkages formed by the reaction of lignin and ^{13}C labeled formaldehyde, which is one of the major factors behind the adhesive property of resin. We synthesized three resins by two lignin monomer (H and G-lignin monomer) and crude lignin with labeled formaldehyde. Basic 1D cross polarization (CP) helps to find the formation of methylene linkages is higher in case of cured resin than the liquid resin. Further, a 2D ^{13}C - ^{13}C correlation experiment was performed to understand different linkages which are associated with methylene linkages after curing. This finding explained the formation of ethers and formate esters. Understanding of the fundamental reaction of lignin with formaldehyde paves attention to investigate linkages to exploit this cost-effective biobased lignin-formaldehyde resin.

Poster 14

Small multidrug transporter EmrE unaffected by lipid composition when bound to substrate

Ashley Hiatt, Graduate Student Research Assistant, University of Wisconsin-Madison

Biological membranes are complex heterogeneous and dynamic systems consisting of roughly half lipids and half membrane proteins by mass. Because of their complexity, many in vitro biophysical and biochemical studies of membrane proteins have been carried out in simpler, more easily manipulated membrane mimetic environments. The lipids used in integral membrane protein reconstitution may have variations in headgroup size, charge, acyl chain lengths, and degrees of saturation. These properties often affect integral membrane protein function. Here, we used ITC and ZZ-exchange NMR to investigate the effects of varying lipid compositions on the substrate affinity and alternating access rate of the *E. coli* small multidrug transporter EmrE. EmrE is a small integral membrane protein with a large protein-lipid interface relative to its intra-protein interactions. Given these qualities, we expected lipid compositions to greatly influence its structure, function and dynamics. Surprisingly, EmrE substrate binding affinity and rate of alternating access remain constant among lipid environments of various acyl chain lengths,

CANMRDG 2023 PROGRAM

degrees of saturation, and headgroup charges. We therefore conclude that substrate has a greater impact on EmrE than lipid, and any impact of lipid composition on EmrE activity must only be due to the influence of lipid on substrate-free EmrE.

Poster 15 **NAN & NMRbox: resources for NMR data collection, management, processing, and analysis**
Mark Maciejewski, Assistant Professor, UConn Health

NMR faces challenges like diverse software needs, limited public data, and hard-to-search datasets. NAN, a joint effort between UConn Health, UGA, and UW-Madison, aims to democratize NMR by providing open-access to NMR instruments including two 1,1 GHz instruments, four knowledgebases to aid users of different expertise in NMR data collection, a web-portal, a data transport system (NDTS) with rich meta-data capture, and an advanced data browser. NMRbox is a well-established cloud-based platform for NMR data processing and analysis, boasting 5,500 users, 260 software packages, and substantial compute resources totaling over 1.2 PFLOPs with 2 PB+ storage. This poster will describe NMRbox, NDTS, and the advanced data browser.

Poster 16 **Poloxamer NMR Method Development for Covid-19 Vaccine**
Mikhail Gerasimov, Scientist, Sanofi

Sanofi with GSK and BARDA has co-developed a recombinant protein-based adjuvanted vaccine to prevent severe COVID-19 disease. Sanofi Manufacturing Technologies and Analytical Sciences departments collaborated in developing and qualifying a Nuclear Magnetic Resonance (NMR) analytical method for detection and quantitation of residual Poloxamer, a non-ionic surfactant, in the COVID-19 vaccine intermediates. Method feasibility was demonstrated by measuring samples from various process steps with and without spiking of Poloxamer into the matrix. The use of ^1H NMR presaturation water suppression pulse sequence allowed quantitation of neat samples with minimal dilution (10% D₂O added). The method qualification showed that accuracy, precision, linearity, and specificity of the method are fit for the purpose. The quantitation limit of 2.5 ppm was established using an external standard. In addition, adoption of NMR allowed to confirm removal of Poloxamer by the downstream processing. This analytical work highlights a cross-functional approach to address an analytical challenge in a short time frame.

Poster 17 **Objective Approaches to Acquire and Assess Multidimensional NMR Spectra of Biological Solids**
Benjamin Harding, Graduate Student, UW-Madison

We aim to improve workflows for protein structure determination using solid-state NMR (SSNMR). Best practices for data collection include non-uniform sampling and SMILE reconstruction, enabling acquisition of well-resolved 3D and 4D spectra of complex biomacromolecules including membrane proteins, fibrous proteins, and protein assemblies. For these experiments, two of the ongoing challenges are optimizing parameters and monitoring the progress of data acquisition. For optimization, we leverage the Nelder-Mead simplex algorithm to identify best parameters for shimming and cross polarization (CP), including comparison of multiple CP conditions with varying tangent shapes. We then use principal component analysis (PCA) to assess spectrometer stability by identifying PC loading spectra corresponding to suboptimal shimming, CP and decoupling parameters. In ongoing studies, we anticipate that the simplex and PCA approaches can be combined with machine learning models in order to maximize signal-to-noise ratio (SNR) and resolution, especially for samples with inherently low signal (i.e. membrane proteins) and a small range of acceptable cross polarization (CP) conditions. These synergistic objective approaches toward multidimensional experimental optimization and analysis yield well-resolved spectra of the dynamic membrane protein EmrE reconstituted in lipid bilayers.

CANMRDG 2023 PROGRAM

Poster 18 Solid-state NMR structure of the A30P hereditary mutant alpha-synuclein fibril

Moses Milchberg, PhD student, University of Wisconsin - Madison

The protein alpha-synuclein (Asyn) misfolds and forms fibrils in Parkinson Disease (PD). The A30P point mutation is a rare missense mutation associated with autosomal dominant PD yet forms fibrils more slowly than wild-type Asyn. The secondary structure of A30P fibrils was determined to be highly similar to that of WT fibrils, but the higher order structural organization of the fibril core remains unclear. Here, we use high-field solid-state NMR spectroscopy to determine the structure of A30P Asyn fibrils. The structure reveals a Greek key fold, further providing evidence for a common structural motif driving neurodegeneration.

Poster 19 SSNMR Fibril Structure Determination Using PASD and Strict Symmetry

Owen Warmuth, PhD Student, University of Wisconsin-Madison

Manual analysis of NMR datasets presents a major bottleneck in the determination of protein structures. Crosspeaks with ambiguous assignment possibilities also pose an obstacle for spectroscopists, especially in datasets with high assignment degeneracy. In the case of fibrils, another challenge results from the computational cost of modeling large, fibrillar assemblies with both intra-molecular and inter-molecular interactions present in the data. Here we utilize the PASD algorithm implemented in XPLOR-NIH to automatically assign a series of spectra containing ambiguous data with high assignment degeneracy. We also show that by utilizing the strict symmetry module in XPLOR-NIH, we can significantly decrease the computational hours required.

Poster 20 Substrate-dependent transport behavior of a Small Multidrug Resistance transporter from *P. aeruginosa*

Juliet Cheng, Graduate Student, University of Wisconsin Madison

The Small Multidrug Resistance transporters were thought to confer only resistance to drugs and antiseptics via tightly-coupled antiport. Recent evidence shows that EmrE, an SMR from *E. coli* can perform multiple modes of transport based on a free-exchange model. This model suggests that SMRs may induce susceptibility to some compounds rather than resistance. PAsmr, an EmrE homolog from *P. aeruginosa*, has been shown to confer resistance to some known EmrE substrates in vivo and in vitro. Here, we hypothesized that the phenotype of PAsmr in vivo is substrate-dependent, potentially conferring susceptibility to some compounds. Growth assays in *P. aeruginosa* demonstrated that PAsmr contributes to resistance. Growth assays of *E. coli* expressing PAsmr displayed both resistance and susceptibility phenotypes, depending on substrate. SSME experiments demonstrated that susceptibility phenotypes in *E. coli* are due to substrate-gated proton leak. Therefore, differences in phenotype between *P. aeruginosa* and *E. coli* expressing PAsmr point to differential impacts of triggering proton leak on resistance behaviors in *P. aeruginosa*. This prompts further investigation into the role that SMRs play in antibiotic resistance in pathogens.

Poster 21 Structural analysis of a *Pseudomonas aeruginosa* second messenger binding protein

Christopher Ptak, Assistant Director, University of Iowa

In *Pseudomonas aeruginosa*, exploratory motility driven by interspecies interactions is regulated by a chemosensory pathway involving the intracellular second messenger, cyclic di-GMP (cdG). Within this signaling pathway, the PilZ domain protein, PA0012, is a cytoplasmic cdG receptor that interacts with other proteins in a cdG-dependent manner. Here, we assign backbone chemical shifts for both the apo

CANMRDG 2023 PROGRAM

and cdG-bound states of PA0012. Insights derived from NMR experiments are further examined in the context of the cdG-bound PA0012 crystal structure.

Poster 22

Customizable In-house 3D-printed Tool Kit for SSNMR

Martin Olson, Undergraduate Research Assistant, NMRFAM

Successful outcomes of biomolecular MAS solid-state NMR experiments depend strongly on user expertise in rotor packing and handling. Aiming to improve the reliability of rotor packing, tach-marking, sample changing, and storage techniques, we developed a 3D printable tool kit for SSNMR sample manipulation and management. The tool kit is designed for flexibility between rotor sizes (1.2 mm - 3.2 mm), including centrifugal rotor packing devices for solid-state samples and other rotor-related tasks. SLA 3D printing has enabled rapid re-design and fast, straightforward, cost-effective manufacturing. These tools provide consistent means of sample handling and increased accessibility for novice to experienced users.

Poster 23

Benchtop NMR Spectroscopy of an Aqueous Polysaccharide Product in Manufacturing

Christina Szabo, Associate Director, Research Baxter International

The Kidney Care segment of Baxter Healthcare produces and tests an aqueous polysaccharide drug product. Baxter procures the raw material and produces the active pharmaceutical ingredient (API) followed by the finished drug product in our manufacturing facilities. Currently, wet chemical methods are performed for two of the three relevant specifications: identification and number of dextrose equivalents (DE) determination. High resolution ^{13}C nuclear magnetic resonance (NMR) spectroscopy is used off-site to determine the branching ratio. With a well-developed NMR method, in one experiment, not only can the drug product be 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 water suppression. These improvements make benchtop NMR equipment a possible solution for applications at manufacturing facilities. Implementation of benchtop NMR spectroscopy on-site could provide Baxter the ability to characterize both the API and finished product without external testing support. A robust NMR method could dramatically shorten the testing cycle, saving time and resources while reducing environmental impact.