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Welcome to the 50th Southeastern Magnetic Resonance Conference!

We are honored to welcome you to the 50th edition of the Southeastern Magnetic Resonance Conference. In particular, we are grateful that we can have this meeting in person, as the COVID pandemic has severely limited our ability to gather, visit, and discuss great science over the past several years. Our program this year features a diverse array of research across solution NMR, solid-state NMR, DNP, EPR, and more, and we feel the program nicely captures contributions from universities and institutions across the Southeastern United States. This meeting provides many opportunities for the discussion of new ideas and scientific advancements, developing collaborations, and seeing old friends (and making new ones), all in an informal environment.

We welcome two keynote speakers who are doing exciting research in magnetic resonance, Joe Zadrozny from Colorado State University and Yongchao Su from Merck, as well as a third keynote speaker, Tim Cross, who was integral in getting NMR and MRI programs going at the National High Magnetic Field Laboratory, and will provide us with a career retrospective.

The 50th SERMC would not be possible without our sponsors, which include both private and public institutions. For their munificent contributions, we thank Bruker, PhoenixNMR, Doty Scientific, JEOL, Knowles, Tecmag, and Bridge12, as well as the MagLab, the FSU Office of Research, the FSU College of Arts and Sciences, the Departments of Physics, Chemistry and Biochemistry, Chemical and Biological Engineering at FSU, the FSU Institute of Molecular Biophysics, and the International EPR Society.

We are also extremely grateful to faculty and staff who participated in the preparation of this conference. In particular, we are indebted to Ms. Kim Mozolic and Ms. Heather Barnes for handling budgets, logistics, and communications, and for their diligence and patience throughout this process. We thank Nilubon Tabtimtong for her work on the SEMRC web site and Mary Desilets for assistance handling participant travel. We are also thankful for the help of our committee members, including Thierry Dubroca, Hans van Tol, Zhehong Gan, Sungsool Wi, and Fred Mentink.

We hope that you enjoy the meeting, learn new things, and make some new connections! Have a great weekend at the 50th SEMRC!

Sincerely,

Rob Schurko and Steve Hill, SEMRC co-Chairs

(on behalf of the entire organizing committee)

CONFERENCE ORGANIZING COMMITTEE

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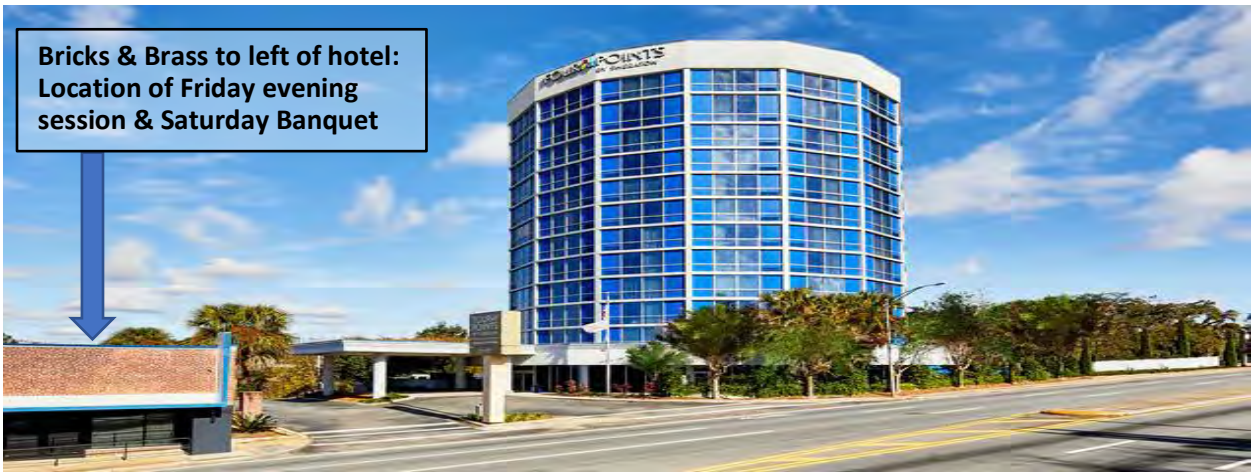
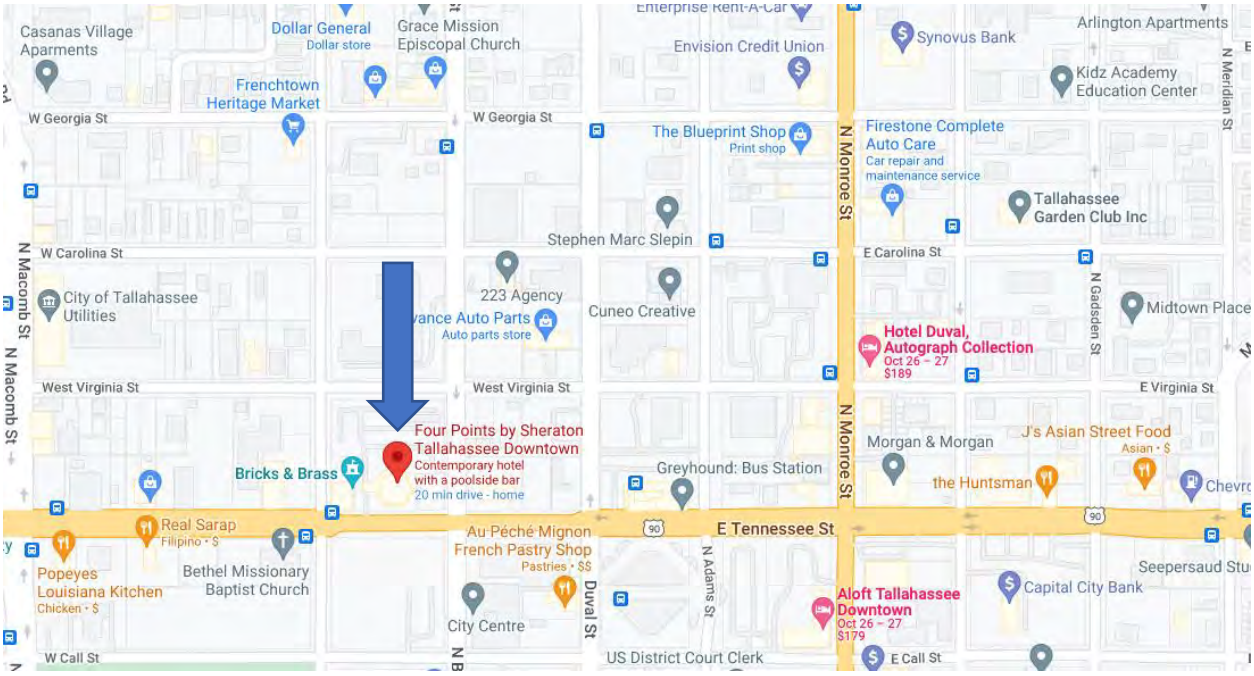
CONFERENCE LOCATION

All sessions will be held at the conference hotel, **Four Points by Sheraton Tallahassee Downtown**. This is the iconic round building located very close to the center of Tallahassee and the Florida State University Campus. You cannot miss it!



Four Points by Sheraton Tallahassee Downtown
316 W Tennessee St, Tallahassee, FL 32301
Tel.: **(850) 422-0071**



Free Parking is available directly behind the main hotel building

Free Wi-Fi is available to conference guests by just turning on their Wi-Fi

Complimentary airport shuttle for hotel guests

THANKS TO OUR SPONSORS!

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https://dotynmr.com/

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https://tecmag.com/

FSU – Departmnet of Physics
https://physics.fsu.edu/

FSU Department of Chemistry
https://www.chem.fsu.edu/

FSU Institute of Molecular Biophysics
http://biophysics.fsu.edu/

Dept. of Chemical & Biomedical Engineering
https://eng.famu.fsu.edu/cbe

International EPR Society
https://ieprs.org/

BRIDGE12
https://www.bridge12.com/

GENERAL INFORMATION

Friday events, including registration and check-in, will be held at **Bricks and Brass** (the building to the left from the hotel entrance – see information on conference location).

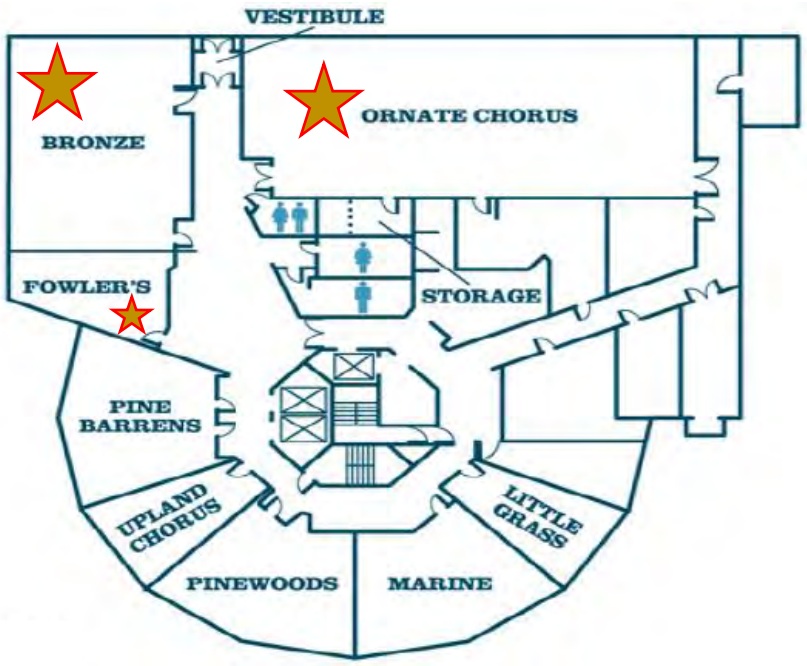
The conference **Banquet on Saturday** will also be held at **Bricks and Brass**.

Presentations and posters on Saturday and Sunday will be held in the main hotel.

Oral presentations: Ornate Chorus Ballroom.

Poster sessions: Bronze Ballroom.

Sponsors: please take time to visit several of our on-site sponsors in Fowler's.



Other Important Information

- Name tags must be worn at all times during the conference, including the oral sessions, poster sessions, all mixers, all breaks and all meals.
- Food and beverages will be provided throughout the conference, including:
  - Heavy hors d'oeuvres during the Friday Reception. In addition, 2 tickets will be provided for beer and wine; additional drinks may be purchased from the cash bar.
  - Lunch will be provided on Saturday along with morning/afternoon coffee at breaks.
  - The Banquet is included with the conference registration on Saturday evening, again with 2 tickets provided for beer and wine.
  - Morning coffee and a take-home box lunch will be provided on Sunday.
- If you would like to tour the MagLab on Sunday, please sign up at the registration table.
- Free parking is available behind the hotel.

INFORMATION FOR PRESENTERS

- For oral presentation, we ask that you use your own computer or bring a flash drive that can be connected to the main computer. We plan to make available various adapters to connect to the projector. However, the safest is to bring your own adapters if you plan on using your own computer. If, on the other hand, you plan on using the main computer, we ask that you upload your talk before your session.
- Speakers may upload/review their presentations during the breaks between sessions. Please look for one of the conference organizers (red dot on badge) or volunteers (green dot on badge) as early as possible, but no later than one session break prior to your scheduled talk. If you are the first speaker in the morning, please arrive early or review your presentation the prior evening.
- A laser pointer and microphone will be provided.
- Posters may be set up beginning Friday, November 4th, between 5:00 and 10:00 p.m. All posters need to be in place at their designated locations by Saturday, November 5th, at 3:30 p.m. Each Poster presentation has a code, which can be found in this abstract booklet. Poster boards will be numbered according to these codes, and we ask that you place your poster at the correct location in order to aid viewing. Posters must be removed no later than 10 a.m. on Sunday. Any posters remaining after this time will be discarded. The poster boards are 6ft wide and 4ft high; they are better suited to landscape formatting. Materials for attaching your posters to the boards will be provided.
- Student posters will be eligible for a poster awards. Therefore, students should stay near their presentation during the poster sessions so that judges can discuss with them.
- The conference organizers and several volunteers will be on hand throughout the conference to assist participants. These individuals will be identifiable via their name tags: a red dot denotes one of the organizers, and a green dot one of the volunteers.

CODE OF CONDUCT

- All participants will conduct themselves in a professional manner that is welcoming to all attendees and free from any form of discrimination, harassment, or retaliation. Participants will treat each other with respect and consideration to create a collegial, inclusive, and professional environment.
- Photography is not permitted at the oral or poster sessions.
- Cell phones must be turned off or set to vibrate during oral sessions.
- As a courtesy to the speakers, we ask that you take advantage of the many available spaces outside of the main presentation room to conduct conversations with collaborators or vendors during the oral sessions. In particular, the vendors will be located in a separate room adjacent to the presentations (Fowler's).

KEYNOTE SPEAKERS

Joseph M. Zadrozny

Assistant Professor of Chemistry, Colorado State University

From early in his career, Joe has been enchanted by magnetism and spin. Thus, he went to the University of California, Berkeley, and obtained a Ph.D. under the direction of Prof. Jeffrey R. Long, studying molecular magnetic properties in two-coordinate complexes, followed by a postdoc with Prof. Danna Freedman working on molecular quantum bits. At Colorado State University, Joe and his coworkers exploit this background to employ synthetic inorganic chemistry to command the static and dynamic magnetic properties of unpaired electrons and magnetic nuclei in metal complexes. In the long term, this knowledge will be vital for new applications in disparate fields spanning from reaction discovery, to quantum information processing, to biomedical imaging.

Yongchao Su

Principal Scientist, Analytical Research and Development, Merck & Co, Inc., United States

In addition to research at Merck, Dr. Su teaches as an Adjunct Associate Professor in the College of Pharmacy at the University of Texas at Austin and Purdue University, and as a Professor of Practice at the University of Connecticut. Dr. Su's research aims to investigate the fundamental mechanism of drug delivery, stability, and bioavailability by elucidating the molecular details. He has contributed over 110 peer-reviewed articles (H-Index=34) on pharmaceutical, biophysical and analytical topics, and several patents on drug developments of Islatravir™ and Keytruda™. He currently serves on the Editorial Advisory Board for Molecular Pharmaceuticals, Pharmaceutical Research, the Journal of Pharmaceutical Sciences, Magnetic Resonance in Chemistry, and as an Editor of Magnetic Resonance Letters. In 2022, he was selected as a Fellow of the Royal Society of Chemistry (FRSC).

Timothy A. Cross

Emeritus Faculty, Dept. of Chemistry and Biochemistry and National High Magnetic Field Laboratory, Florida State University

Tim grew up in Southeastern Massachusetts laboring on his parent's cranberry bogs before heading off to boarding school, Trinity College (Hartford, CT) and then to Univ. of Penn., Department of Chemistry, where he obtained his PhD with Prof. Stanley Opella and stayed for a short postdoctoral fellowship in the same lab. The subject of these studies was the structural characterization of the coat protein of the E. coli filamentous virus, fd. In addition to MAS spectroscopy, he obtained spectra of uniformly oriented samples of the virus. He did a second postdoctoral fellowship with Prof. Joachim Seelig in Basel, Switzerland, working on the development of in vivo spectroscopy and MRI imaging. He then spent 36 years on the faculty in the Department of Chemistry and Biochemistry at Florida State University, continuing structural biology research on membrane peptides and proteins and was part of the founding scientific team for the National High Magnetic Field Lab in Tallahassee.

CONFERENCE SCHEDULE

Friday, November 4

5:00 – 10:00 PM **Registration**
5:00 – 6:30 PM **Poster Set Up and Viewing**

Session A – Welcome
Chairs: Rob Schurko and Stephen Hill, MagLab – Florida State University

6:30 – 6:40 PM Welcome Remarks
Dr. Stacey Patterson, FSU Vice President for Research

6:40 – 7:20 PM **Keynote 1: Joseph Zadrozny, Colorado State University**
The Metal Complex as a Magnetic Vessel That Controls Coherence

7:20 – 8:00 PM **Keynote 2: Yongchao Su, Merck & Co, Inc., New Jersey**
Investigating Structural Basis for Pharmaceutical Drug Product Design of Small Molecule Solid Oral Dosages and Lyophilized Biologics

8:00 – 10:00 PM **Poster Set Up and Viewing**

8:00 – 10:00 PM **Welcome Reception with Heavy Hors D'oevres**

Saturday, November 5

Session B – Biosolids NMR
Chair: Riqiang Fu, MagLab – Florida State University

8:00 – 8:10 AM Opening Remarks and Announcements

8:10 – 8:50 AM **Keynote 3: Timothy A. Cross, MagLab – Florida State University**
A Career Perspective in Biological NMR Spectroscopy

8:50 – 9:10 AM **(C1)** Anant K. Paravastu, Georgia Institute of Technology
Solid-State NMR as a Tool for Rational Design of Peptide Nanoscale Assemblies

9:10 – 9:30 AM **(C2)** Azamat Galiakhmetov, North Carolina State University
Highly Aligned Peptoid-Based Macrodiscs for Structure-Function NMR Studies of Membrane Proteins

9:30 – 9:50 AM **Invited 1: Tuo Wang, Michigan State University**
Structure of Fungal Cell Walls and Remodeling by Antifungal Drugs Elucidated Using Solid-State NMR

9:50 – 10:10 AM **(C3)** Maria Luiza Caldas Nogueira, MagLab – University of Florida
Using DNP to Unravel Tyrosine Phenol-Lyase Enzymatic Mechanisms

10:10 – 10:30 AM **(C4)** Bo Chen, University of Central Florida
Structural Characterization of the Self-Assembly Formed by Largely Disordered Reflectin-Derived Polypeptide

10:30 – 10:50 AM **Coffee Break**

Session C – Dynamic Nuclear Polarization
Chair: Joanna Long, MagLab – University of Florida

10:50 – 11:10 AM **Invited 2: Alexander Nevzorov, North Carolina State University**
A Resonator-Based NMR Probe for DNP of Thin Film Samples and Overhauser DNP

11:10 – 11:30 AM **(C5)** Faith Scott, MagLab – University of Florida
600 MHz MAS-DNP Probe Designed with Microwaves in Mind

11:30 – 11:50 AM **(C6)** Yifan Quan, Massachusetts Institute of Technology
Pulsed DNP with Frequency Chirps

11:50 – 12:10 PM **(C7)** Frédéric Mentink-Vigier, MagLab – Florida State University
PyrroTriPol: A Semi-Rigid Trityl-Nitroxide for High Field Dynamic Nuclear Polarization

12:10 – 12:30 PM **Invited 3: Frédéric A. Perras, Ames Laboratory**
Methyl-Driven Overhauser MAS-DNP

12:30 – 1:30 PM **Lunch**

Session D – EPR in Chemistry and Biology
Chair: Gail Fanucci, MagLab – University of Florida

1:30 – 1:50 PM **Invited 4: Troy A. Stich, Wake Forest University**
Exploring Mechanism and Plasticity of Radical SAM Enzymes

1:50 – 2:10 PM **(C8)** Alexander Angerhofer, MagLab – University of Florida
5-Hydroxytryptophan as a Probe for Long Range Electron Transfer in Oxalate Decarboxylase

2:10 – 2:30 PM **(C9)** Afnan M. Jaufer, MagLab – University of Florida
Understanding Bacillus Subtilis Lipase-A From a Structure-Dynamics Perspective Through Site-Directed Spin Labeling and Molecular Dynamics Simulations

2:30 – 2:50 PM **(C10)** Tatyana I. Smirnova, North Carolina State University
Membrane Composition Drives Sidechain Ionization in Transmembrane Protein Domains

2:50 – 3:10 PM **(C11)** Alvaro Montoya, MagLab – University of Florida
Enzyme-Substrate Complex in B. Subtilis Oxalate Decarboxylase Revealed by Pulsed EPR Spectroscopy

3:10 – 3:30 PM **(C12)** Jurek Krzystek, MagLab – Florida State University
Terahertz EPR Spectroscopy Using a 36-Tesla High-Homogeneity Series-Connected Hybrid Magnet

3:30 – 3:50 PM **Coffee Break**

Session E – SSNMR: Materials and Methods
Chair: Faith Scott, MagLab – University of Florida

3:50 – 4:10 PM **Invited 5: Robbie Iuliucci, Washington and Jefferson College**
Potential of Second Order Møller–Plesset Perturbation Theory Towards NMR Crystallography

4:10 – 4:30 PM **(C13)** Cameron S. Vojvodin, MagLab – Florida State University
Synthesis, Characterization, and NMR Crystallography of Ionic Multi-Component Crystals

4:30 – 4:50 PM **(C14)** Riqiang Fu, MagLab – Florida State University
Cross Polarization using ^{1}H Adiabatic Demagnetization in Solid-State NMR of Aligned Samples

4:50 – 5:10 PM **(C15)** Clifford R. Bowers, MagLab – University of Florida
Adiabatic Passage Through Level Anti-Crossings in Systems of Chemically Inequivalent Protons Incorporating Parahydrogen: Theory, Experiment, and Prospective Applications

5:10 – 5:30 PM **(C16)** James J. Kimball, MagLab – Florida State University
Broadband Adiabatic Inversion Cross-Polarization under Magic-Angle Spinning Conditions

5:30 – 7:30 PM **Poster Session**

7:30 – 11:00 PM **Banquet Dinner**

Sunday, November 6

Session F – Solution NMR: Biomolecular & Environmental Applications
Chair: Sungsool Wi, MagLab – Florida State University

8:00 – 8:10 AM Opening Remarks and Announcements

8:10 – 8:30 AM **(C17)** James H. Prestergard, University of Georgia
Sparse Isotope Labeling for Structural Analysis of Glycoproteins

8:30 – 8:50 AM **(C18)** Emily Qingqing Peng, MagLab – University of Florida
Characterization of the C-Terminal Segment of the Streptococcus Mutans Adhesin P1 by Solution NMR Spectroscopy

8:50 – 9:10 AM **(C19)** Leah B. Casabianca, Clemson University
Interactions Between Small Molecules and Polystyrene Nanoparticles Examined by Multiphase NMR and Molecular Dynamics Simulations

9:10 – 9:30 AM **(C20)** Omid Sanati, University of Georgia
HTS ^{13}C-optimized NMR Probe at 21.1 T

9:30 –9:50 AM **Coffee Break**

Session G – EPR Technique Development
Chair: Hans van Tol, MagLab – Florida State University

9:50 – 10:10 AM **Invited 6: Mike Ozerov, MagLab – Florida State University**
Magnetic Resonance in Far Infrared Spectral Range

10:10 – 10:30 AM **(C21)** Elvin Salerno, MagLab – Florida State University
Pulsed 94 GHz EPR for Spin Population Transfer in a Gd(III) Molecular Crystal

10:30 – 10:50 AM **(C22)** Aulden K. Jones, Georgia Institute of Technology
Accessible Electron Spin Resonance Instrumentation within Cryostat Environments; a Step Towards Sub-Kelvin ESR

10:50 – 11:10 AM **(C23)** Timothy J. Keller, Bridge12
Open-Source Loop-Gap Resonator for X-Band EPR Spectroscopy

11:10 – 11:30 AM **(C24)** Alex I. Smirnov, North Carolina State University
High-Volume High-Q Resonators Drastically Improve Concentration Sensitivity of W-Band (95 GHz) Pulsed EPR

11:30 – 12:00 PM **Boxed Lunch and Adjournment**

11:40 – 12:30 PM **SEMRC Business Meeting**

12:45 – 2:00 PM Optional visit to the MagLab (sign up at registration table)

POSTERS

P1 Zain Becerra
PROGRESS ON EPR STUDIES TO PROBE THE ROLE OF DIOXYGEN AND LONG-RANGE ELECTRON TRANSFER IN OXALATE DECARBOXYLASE

P2 Wei-Hao Chou
GIANT MAGNETIC ANISOTROPY IN A TRIGONAL Ni(II) COMPLEX

P3 A. Ligia Focsan
CAROTENOID RADICALS: STRUCTURE AND PROPERTIES DETERMINED FROM HIGH FIELD EPR MEASUREMENTS AT NHMFL

P4 Dylan Graham
LOSS OF CHROMIUM(III) FROM MIXED METAL Cr(III), Fe(III)-TRANSFERRINS

P5 Brittany Grimm
INVESTIGATION OF THE SPIN-CROSSOVER TRANSITION IN A METALORGANIC Mn^{3+} COMPLEX WITH CONTINUOUS-WAVE HIGH-FIELD POWDER EPR SPECTROSCOPY

P6 Jakub Hrubý
TUNABLE CLOCK TRANSITIONS IN LANTHANIDE COMPLEXES FOR QUANTUM INFORMATION TECHNOLOGIES

P7 Tianyan Li
INVESTIGATING THE IMPACT OF CHARGE DISTRIBUTIONS ON FOLDED AND UNFOLDED CONFORMATIONS OF IA3

P8 Wei Li
GENERATION OF HYDROXYL RADICAL FROM REACTION OF HYDROGEN PEROXIDE WITH PROTEIN THIOLS

P9 Andrew Ozarowski
INTER-SPIN STATE TRANSITIONS IN HIGH-FIELD EPR SPECTRA OF COPPER CUBANE TETRAMERS

P10 Ganesh R. Rana
ELECTRON PARAMAGNETIC RESONANCE STUDIES OF TRANSITION METAL PHTHALOCYANINES

P11 Aorada Sripunya
SYNTHESIS OF GALLIC ACID-CONJUGATED PAMAM DENDRIMERS FOR AGE-RELATED MACULAR DEGENERATION - ANTIOXIDANT PROPERTIES DETECTED BY EPR

P12 Robert Stewart
54 GHz CLOCK TRANSITION IN A Ho(III) COMPLEX

P13 Manoj V. H. Subramanya
WIDEBAND FOURIER-TRANSFORM-DETECTED EPR AT W-BAND

P14 Johan van Tol
MULTIFREQUENCY PULSED EPR IN THE 120-400 GHz RANGE

P15 Kurt Warncke
CONFINEMENT DEPENDENCE OF PROTEIN-COUPLED SOLVENT DYNAMICS FOR DIFFERENT CLASSES OF PROTEINS

P16 Katie L. Whitcomb
PROTEIN AND COUPLED SOLVENT DYNAMICS OF OLIGOMERIC AND FIBRILLAR ALPHA-SYNUCLEIN

P17 Mingwei Zhou
COMPARATIVE STUDY OF CELL SURFACE α2,3- AND α2,6-SIALOGLYCANS BY ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY

P18 Adam R. Altenhof
TOWARDS THE DESIGN AND IMPLEMENTATION OF A DNP-NQR SPECTROMETER

P19 Swapna Bera
DNP NMR ASSISTED 'IN-CELL' STRUCTURAL CHARACTERIZATION OF MITOCHONDRIA LOCALIZED α-SYNUCLEIN TOXIC CONFORMERS

P20 Liyanage D. Fernando
STRUCTURE OF FUNGAL CELL WALLS AND REMODELING BY ANTIFUNGAL DRUGS ELUCIDATED USING SOLID-STATE NMR

P21 Sungsool Wi
PROTON-OBSERVED ^{13}C OVERHAUSER DNP AT 14 T

P22 Zachary T. Dowdell
MECHANOCHEMICAL SYNTHESES OF PHARMACEUTICAL COCRYSTALS AND THEIR STRUCTURAL CHARACTERIZATION USING ^{35}Cl SOLID-STATE NMR AND DISPERSIONCORRECTED DFT CALCULATIONS

P23 Isaac Eason
COUPLING SOLID-STATE NMR AND MOLECULAR DYNAMICS SIMULATIONS TO INVESTIGATE MEMBRANE PROTEINS

P24 Alexander Eletsky
NETWORK FOR ADVANCED NMR: COMMUNITY ACCESS TO HIGH-FIELD NMR

P25 Carl H. Fleischer III
NEW DIRECTIONS FOR QUADRUPOLAR NMR CRYSTALLOGRAPHY ENHANCED CRYSTAL STRUCTURE PREDICTION (QNMRX-CSP)

P26 Sean T. Holmes
DFT/ZORA CALCULATIONS OF ^{195}Pt MAGNETIC SHIELDING TENSORS

P27 Michelle P. Lapak
ADVANCED INSTRUMENTATION FOR PARAHYDROGEN BASED HYPERPOLARIZATION: CLOSED-LOOP, CONTINUOUS-FLOW HYPERPOLARIZATION FROM PARAHYDROGEN AND HETEROGENEOUS CATALYSIS

P28 Mira Menon
DEVELOPMENT OF IMPROVED BRAIN PHANTOMS TO IMPROVE UNDERSTANDING MOLECULAR DYNAMICS USING NMR

P29 Austin A. Peach
^{17}O SOLID-STATE NMR OF CARBOXYLIC ACIDS AND PHARMACEUTICAL COCRYSTALS ENRICHED USING MECHANOCHEMISTRY

P30 Arka Prabha Ray
ROLE OF CHOLESTEROL AS AN ALLOSTERIC MODULATOR FOR HUMAN A_{2A} ADENOSINE RECEPTOR CONFORMATIONAL DYNAMICS

P31 Jazmine E. Sanchez
MECHANOCHEMICAL SYNTHESIS AND MULTINUCLEAR SOLID-STATE NMR SPECTROSCOPY OF SILVER-CONTAINING COORDINATION POLYMERS

P32 Jasmin Schoenzart
^{103}Rh SOLID-STATE NMR SPECTROSCOPY: ULTRA-HIGH FIELDS, OPTIMIZED PULSE SEQUENCES, AND FIRST PRINCIPLES COMPUTATIONS

P33 Robert B. Smith
^{67}Zn, ^{27}Al, AND ^{71}Ga SOLID-STATE NMR SPECTROSCOPY OF ZINC OXIDE NANOCRYSTALS

P34 Naveen Thakur
ANIONIC PHOSPHOLIPIDS CONTROL MECHANISMS OF GPCR-G PROTEIN RECOGNITION

P35 Tyrone Thames
DISTINCT PORE-FORMING CONFORMATION OF AMYLOID BETA PEPTIDE Aβ1–42 IN MEMBRANE ENVIRONMENTS

P36 Yijue Xu
RHENIUM-185/187 SOLID-STATE NMR INVESTIGATION OF PERRHENATE COMPOUNDS

P37 Wancheng Zhao
REVEALING MOLECULAR ARCHITECTURE AND CARBOHYDRATE-AROMATIC INTERFACE OF WOODY PLANT CELL WALLS BY SOLID-STATE NMR

P38 Jamini Bhagu
PROBING ADSORPTION OF MONOCLONAL ANTIBODIES AT WATER-OIL INTERFACES VIA SPATIALLY RESOLVED MR SPECTROSCOPY

P39 Samuel W. Holder
^{23}Na MRI AT 21.1 T REVEALS SEX DIFFERENCES IN A PRECLINICAL MIGRAINE MODEL

P40 Jenna M. Radovich
FUNCTIONAL AND STRUCTURAL CONNECTIVITY IN THE TRANDGENIC RAT MODEL OF ALZHEIMER'S DISEASE USING RESTING-STATE fMRI & DIFFUSION TENSOR IMAGING

P41 Dayna L. Richter
BLOOD-CSF BARRIER PERFUSION IN A PRECLINICAL MIGRAINE MODEL

KEYNOTE ABSTRACTS

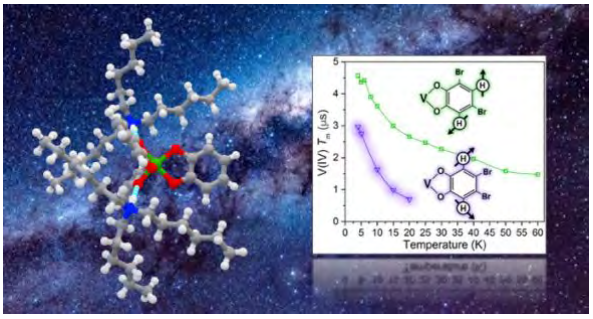
THE METAL COMPLEX AS A MAGNETIC VESSEL THAT CONTROLS COHERENCE

Joseph Zadrozny,[1] Cassidy Jackson,[1] Chun-Yi Lin,[1] Roxanna Martinez,[1] Spencer Johnson,[1] Johan van Tol,[2] Ökten Üngör,[1] Thacien Ngendahimana,[3] Sandra Eaton,[3] Gareth Eaton[3]

[1]*Colorado State University, Department of Chemistry*
[2]*National High Magnetic Field Laboratory*
[3]*University of Denver, Department of Chemistry*

Controlling spin coherence by molecular design is a grand challenge at the intersection of chemistry and physics. In particular, robust, long-lived spin coherence is important for long term applications for spins, either in quantum computing, quantum sensing, or future unforeseen applications. For unpaired electrons, spin coherence is largely seen to be detrimentally affected by environmental magnetic noise. The sources of this noise are generally attributed to the magnetization dynamics of nearby molecules with unpaired electrons or environmental magnetic nuclei (e.g. of the solvent). Understanding specifically how these species shorten spin coherence is a daunting challenge. In many cases, synthetic strategies are chosen to completely eliminate magnetic nuclei, or focus in dilute conditions toward robust coherence. While these proofs of concept show exciting results about what is possible with spin coherence, they nevertheless leave a persistent lack of knowledge on how *exactly* different environmental magnetic species affect spin coherence.

In this talk, I will present our work studying the exact role of specific types of magnetic nuclei on spin coherence in S = 1/2 V(IV) complexes. 1-5 The aggregate of our results underline one key point: that the effective “environment” governing the spin coherence of the V(IV) ion is far closer than the frozen solvent or matrix that the molecule is isolated in. Indeed, the environment is the nuclei on the periphery of the V(IV)-containing molecule itself! In this way, we can now think about metal complexes as “tiny magnetic vessels” that control spin coherence. The results thus provide an important underlining of the necessity of molecular tuning toward understanding this quantum phenomena.



References

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INVESTIGATING STRUCTURAL BASIS FOR PHARMACEUTICAL DRUG PRODUCT DESIGN OF SMALL MOLECULE SOLID ORAL DOSAGES AND LYOPHILIZED BIOLOGICS

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Solid dosages of small molecule and protein therapeutics represent a major component in modern medicines. However, the structural details of these pharmaceutical solids, elucidating fundamental mechanisms for designing effective drug substances and products to overcome physicochemical barriers to improve stability, are underexplored. For example, amorphous pharmaceuticals are designed to enhance the dissolution profile but often exhibit physiochemical instability via recrystallization. For this reason, polymer additives serve to elevate the energy barrier for the undesired amorphous-to-crystalline conversation of drug molecules. The molecular details of the drug-polymer complex are unknown. Moreover, despite their predominant role in the pharmaceutical market, biological therapeutics carry inherent limitations of structural instability. Formulating protein drugs in frozen solution or lyophilized form enhances shelf-life stability. Sugar additives are included in these solid-state protein formulations. The hypothesis which describes how sugar-protein interaction facilitates protein stability is not experimentally explored. In this presentation, we will demonstrate solid-state NMR as a high-resolution technique to probe drug-drug and drug-excipient interactions that are relevant to the stability of solid-state pharmaceutical formulations.

A CAREER PERSPECTIVE IN BIOLOGICAL NMR SPECTROSCOPY

Timothy A. Cross

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Having built ssNMR probes in Opella's lab for 8 mm diameter RF coils; I built an imaging probe for a 20 cm horizontal bore 1.9 T Bruker BNT-80 spectrometer. Having built the coil I was told my arm should be the first to experience the kilowatt RF pulses. The images were amazingly good and the next day the first hospital patient had their leg in the magnet – regulations in Switzerland were a little different than the US.

At Florida State I set out to characterize the atomic resolution structure of Gramicidin A, a transmembrane polypeptide antibiotic using primarily uniformly aligned samples of the peptide in lipid bilayers (Fig. 1). The lab then shifted to the structural characterization of the Influenza A M2 protein, a proton channel and then on to structural characterization of several membrane proteins from Mycobacterium tuberculosis.

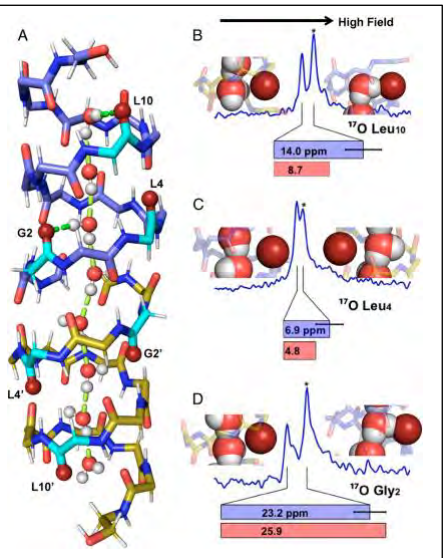

Fig. 1: ^{17}O Oriented Sample NMR spectroscopy in the 35.2 T SCH magnet showing carbonyl oxygen asymmetry between the monomers due to waters in the pore (Paulino et al., 2020).

Along the way there has been the development of the Magnet Lab led by three Physicists. As biochemists, a group of us wanted to adulterate the rarified air of the physicists with some biology and biophysics and to make sure there would be appropriate facilities for world class NMR and MRI. The development of the 21.1 Tesla superconducting magnet by Magnet Lab engineers was a great success and to this day represents the highest field MRI instrument. In addition, developing sophisticated instrumentation to stabilize a powered magnet has led to unique spectroscopy, such as the ^{17}O spectroscopy of the dimeric structure of gramicidin A that demonstrated for the first time that water molecules interacting with the gramicidin pore broke the structural dimeric symmetry.

INVITED ABSTRACTS

STRUCTURE OF FUNGAL CELL WALLS AND REMODELING BY ANTIFUNGAL DRUGS ELUCIDATED USING SOLID-STATE NMR

Malitha Dickwella Widanage[1], Liyanage Fernando[1], Isha Gautam[1], Frederic Mentink-Vigier[2], Tuo Wang[1]

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Life-threatening fungal infections have become a major threat to human health, with high mortality even after treatment. To counter the emerging drug resistance, efforts have been devoted to the development of novel antifungal agents targeting the components in the fungal cell walls. However, we still lack in-depth knowledge of the polymorphic structure and assembly of polysaccharides in native fungal cell walls. Here we will discuss three recent advances using solid-state NMR (ssNMR) and dynamic nuclear polarization (DNP) to understand the structural dynamics of the cell walls in a major fungal pathogen Aspergillus fumigatus. First, high-field ssNMR spectroscopy on living fungal cells has changed our view on the organization of cell wall polymers [1]. The cell wall of A. fumigatus was found to contain hydrophobic scaffolds of chitin and α-glucans, which are surrounded by a hydrated matrix of β-glucans and capped by a dynamic layer containing mannan and galactan polymers as well as glycoproteins. Second, ssNMR results of carbohydrate-deficient mutants revealed how the gene deletion induces significant changes in the composition and water accessibility of biopolymers [2]. Third, we employed DNP methods to characterize unlabeled fungal materials at different developmental stages and identified a conserved carbohydrate core [3]. Fourth, we identified the structural mechanism employed by fungal pathogens to remodel their cell walls in response to antifungal drugs and environmental stresses. These studies collectively provide the structural foundation for the design of better antifungal medication targeting the structure and biosynthesis of cell wall components.

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A RESONATOR-BASED NMR PROBE FOR DNP OF THIN FILM SAMPLES AND OVERHAUSER DNP

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The use of resonators in DNP makes it possible to take advantage of relatively inexpensive, frequency-agile solid-state microwave (MW) sources, which are currently limited to several 100 mW's of the output power. By employing high-Q resonators one can significantly – by factor of 50 or more – increase the MW power density at the sample *vs*. a non-resonant configuration. Thus, a 400 mW solid-state source can potentially yield MW powers equivalent to 20 W. Moreover, one-dimensional photonic band-gap resonators (PBGR) can accommodate relatively large sample volumes, up to several microliters vs. 10-100 nl as in single-mode mm-wave cavities. We have built a 198 GHz/300 MHz electron/^{1}H frequency probe by integrating PBGRs with a radiofrequency NMR double saddle coil, which can be tuned to ^{1}H, ^{13}C, and ^{31}P nuclei. DNP measurements on synthetic diamonds embedded in thin polyester 3M lapping films show that, depending on the dielectric properties of the alternating $\lambda/4$ layers forming the PBGR, the relative power enhancements afforded by the resonator varies from 30- to 50-fold as compared to the probe configuration without a resonator. Moreover, small liquid droplets (up to 2 µl volume) can be deposited within the resonator for liquid-state DNP measurements. By using a 100 mM BDPA radical solution in toluene with 1M of deoxygenating triphenylphosphine (Ph_3P), considerable Overhauser DNP enhancements are obtainable with just ca. 400 mW of output microwave powers. Supported by NIH 5R01GM130821 to AIS and AAN.

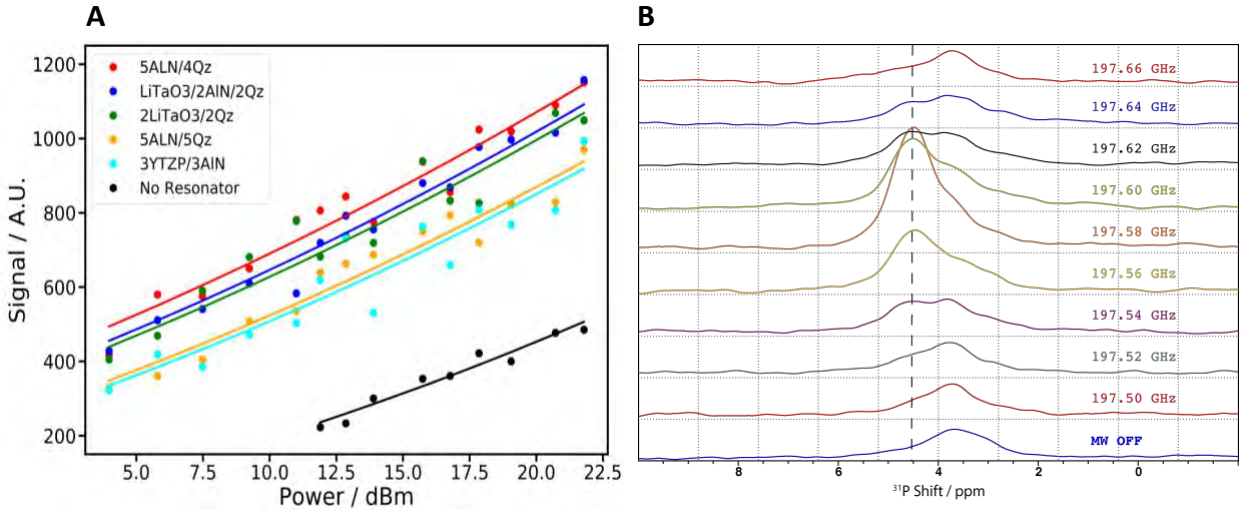

Figure. A. DNP-enhanced natural-abundance ^{13}C signal amplitudes of microdiamonds embedded into a 3 mil polyester film as a function of incident MW powers in dBm scale. The greatest horizontal displacement between the power profile for 5AlN/4Quartz PBGR (red) and no-resonator configuration (black curve) is ca. 17 dB, thereby indicating up to 50-fold increase in power afforded by the resonator. **B.** Overhauser DNP ^{31}P NMR spectra as a function of MW frequency recorded with the PBGR probe at 197 GHz/141 MHz electron/^{31}P frequencies. The enhancement was measured for ^{31}P NMR signal of Ph_3P in 100 mM BDPA solution in toluene (ca. 2 µl volume). The peak at *ca*. 3.5 ppm is not enhanced and is due to a part of the solution being outside the resonator and the B_{1e} field.

METHYL-DRIVEN OVERHAUSER MAS-DNP

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The Overhauser effect is unique among DNP mechanisms in that it requires the dynamic modulation of the electron-nuclear hyperfine interactions. While it dominates DNP in liquids and metals, where unpaired electrons are highly mobile, Overhauser DNP is possible in insulating solids if rapid structural modulations are linked to a modulation in hyperfine coupling. The first observation of Overhauser effects in insulating solids (in BDPA), by Griffin *et al.*,[1] showed a highly promising behavior: namely, that the DNP performance increased with increasing magnetic field strength, counter to all other known DNP mechanisms. As such there has been tremendous interest in designing high-field polarizing agents that make use of BDPA or designing new Overhauser MAS-DNP polarizing agents.

In this presentation, we will show that Overhauser effects can be triggered by the strategic addition of a methyl group, demonstrated in a Blatter's radical. The rotation of the methyl group leads to a modulation of the hyperfine coupling to its protons which in turn facilitates electron-nuclear cross-relaxation. Removal of the methyl protons, through deuteration, quenches the process, as does the reduction of the hyperfine coupling strength. Notably, the deuterated radical is active in ^{2}H Overhauser DNP but only solid effect ^{1}H DNP. Importantly, this result suggests the possibility for the design of tailormade Overhauser DNP polarizing agents for high-field MAS-DNP. We investigate the MAS rate, field, and temperature dependence of this new form of Overhauser MAS-DNP and note how it differs from existing Overhauser polarizing agents, which are all mixed-valence compounds.

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EXPLORING MECHANISM AND PLASTICITY OF RADICAL SAM ENZYMES

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[1]*Wake Forest University, Department of Chemistry*

Radical *S*-adenosyl-L-methionine (rSAM) enzymes employ a [4Fe–4S] cluster to reductively cleave SAM, yielding a 5'-deoxyadenosyl (5'dAdo•) primary carbon radical. This 5'dAdo• radical can then abstract an H-atom from substrate to initiate its transformation to product. And while only 100 different products are known, genetic clues suggest more than 150 000 different rSAM enzymes may exist. We explore the influence of substrate analogs and enzyme variants on these transformations to decode the roles of individual amino acids and then use mutagenesis to reprogram enzymes to manufacture non-native products. EPR spectroscopy is used to identify paramagnetic intermediates and report back on possible mechanisms.

The recent success in using cytochromes P450 for directed evolution applications in order to achieve new protein-catalyzed chemistries has posed the question: what other enzyme families might be ripe for similar exploitation? Given their unparalleled diversity, radical SAM enzymes are a natural candidate, save for the liability of the O_2-sensitive [FeS] cluster. To overcome this, we have engineered formerly "radical SAM" enzymes to no longer need the [FeS] and instead achieve deoxyadenosyl radical formation using the much more oxygen-tolerant adenosylcoablamin cofactor.

POTENTIAL OF SECOND ORDER MØLLER–PLESSET PERTURBATION THEORY TOWARDS NMR CRYSTALLOGRAPHY

Robbie Iuliucci[1]

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As computational resources and techniques continue to improve, the potential of expensive correlated quantum mechanical methods become more applicable for molecules of chemical interest. To address electron correlation deficiencies of the Hartree-Fock method, second order Møller–Plesset (MP2) theory includes an electron potential as a perturbation to the Fock operator. Being the most economical of the post-Hartree-Fock methods, the use of MP2 is on the rise. This presentation will summarize the results of MP2 calculations for two applications of NMR crystallography – predicting lattice energies and determining magnetic properties. Planewave density functional theory (DFT) is the go-to method for predicting lattice energies of organic crystals even though delocalization errors are well established. The DFT lattice energy can be improved by applying a MP2 monomer correction. In the study "Effect of Fluorination on Polymorphism and Photomechanical Properties of Cinnamalmalononitrile Crystals" (Crystal Growth & Design, manuscript in press), the spin-component scaled, dispersion-corrected MP2 method at the complete basis set limit was employed to improve the DFT crystal energy landscapes of cinnamalmalononitrile polymorphs. The MP2 corrections confirm that fluorination of cinnamalmalononitrile leads to unreactive polymorphs coexist within a few kJ/mol of the photoreactive crystal structure. A second study explores predicting $^{13}C/^{15}N$ chemical shifts and $^{14}N/^{17}O$ electric field gradient tensor components with MP2 and double hybrid DFT on a test set of organic crystals. Improvements to predicting magnetic properties at the general gradient approximation level has been demonstrated by employing hybrid DFT. Such improvements beg the question if further accuracy can be achieved by incorporating more sophisticated methods. Using both the 2-body fragment approach and monomer corrections to planewave DFT, we show no benefit is achieved by the more expensive methods when compared to experimental values.

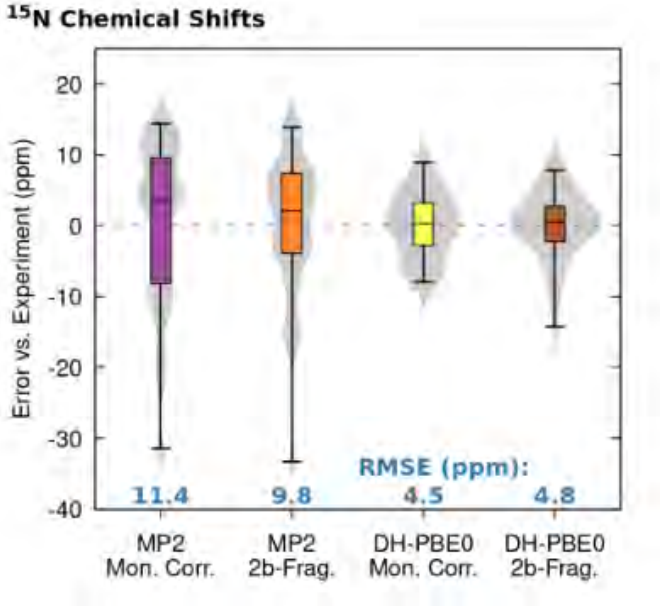

Figure 1. Comparison of the error distributions for 37 ^{15}N chemical shifts relative to experiment for MP2 and double hybrid DFT as computed with the monomer-corrected planewave DFT and the 2-body fragment approaches.

MAGNETIC RESONANCE IN FAR INFRARED SPECTRAL RANGE

Mike Ozerov[1]

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Magnetic resonance is a physical phenomenon that occurs across a wide spectral range. Far-infrared magneto spectroscopy (FIRMS) offers an experimental routine to probe the magnetic excitation spectrum between 0.4 and 22 THz (12- 720 cm-1). In this talk, I present the FIRMS technique that is used at the National High Magnetic Field Laboratory, with a specific emphasis on how it is successfully applied to the study of single-molecule magnets. A short introduction includes a description of the measurement protocol, the data analysis and a recent sensitivity improvement. The advantages and limitations compared to other spectroscopic techniques are discussed as well. Typical examples illustrate how the FIRMS technique allows direct measurements of zero-field splitting energy and enables the accurate determination of spin-Hamiltonian parameters, particularly when combined with high-frequency EPR data. Finally, spin-phonon coupling can be highlighted in the FIRMS spectra.

CONTRIBUTED ABSTRACTS

SOLID-STATE NMR AS A TOOL FOR RATIONAL DESIGN OF PEPTIDE NANOSCALE ASSEMBLIES

Paravastu, Anant K.[1], Robang, Alicia S.[1], Wong, Kong M.[1], Roberts, Evan. K.[1], Hudson, Benjamin C.[1]

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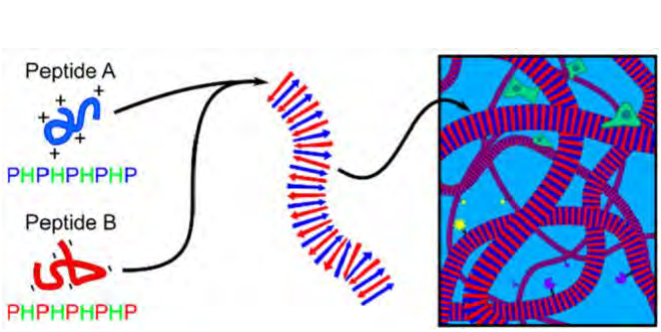
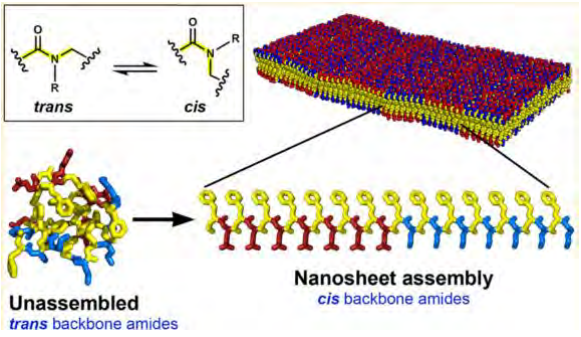
We have been applying solid-state NMR methodology to investigate designer peptide (and peptide analog) assemblies. These assemblies, which often resemble amyloid-like cross-β structures or α-helical coiled coils, have been used for antigen presentation, extracellular matrix mimicry, and drug delivery. Rational design has made it possible to incorporate bio-active peptide domains on nanofiber surfaces, modulate assembly kinetics as a function of salt concentration, and trigger assembly through molecular recognition. Our investigations have revealed several unexpected phenomena. Some of the phenomena we have discovered in designer assemblies are also relevant to naturally occurring assemblies. Lately, we have integrated computational methods to design and predict assembled structures with solid-state NMR methods to test computational predictions. We are producing better-controlled nanostructures and pursuing mechanism-inspired amino acid patterning.

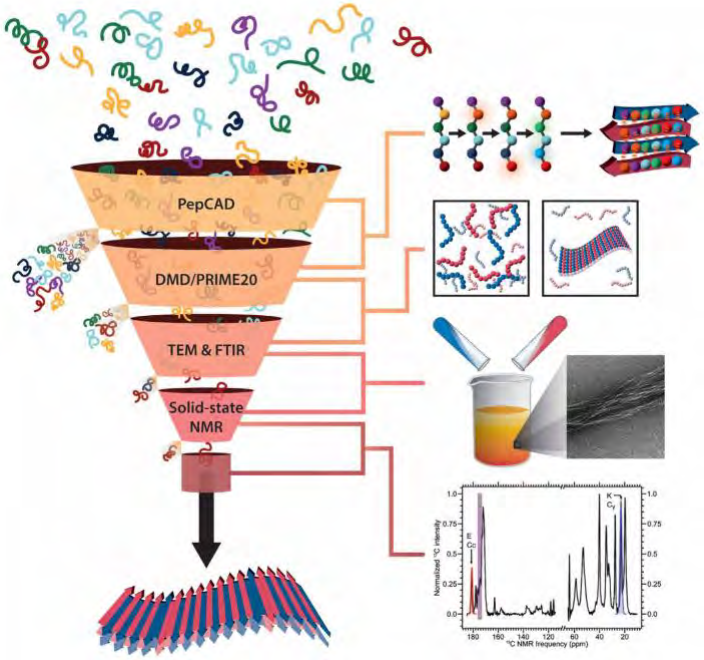
Co-assembly Concept:



Example Molecular Structural Model:



Design Workflow:



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HIGHLY ALIGNED PEPTOID-BASED MACRODISCS FOR STRUCTURE-FUNCTION NMR STUDIES OF MEMBRANE PROTEINS

Azamat Galiakhmetov[1], Carolynn Davern[1], Caroline Proulx[1], Alexander Nevzorov[1]

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We report on the recently developed detergent-free, magnetically alignable macrodiscs that exhibit the order parameters superior to the conventionally used bicelles, peptide-belt lipodiscs, or SMA particles. The peptoids were prepared by a solid-phase synthesis to yield a 15-mer polyglycine with its amide protons being replaced by alternating phenethyl/carboxyethyl side chains at the 2:1 molar ratio. The macrodiscs were prepared by thermally mixing DMPC with the 15-mers, and the optimized lipid/peptoid molar ratio was varied from 20:1 to 27:1. The peptoid macrodiscs exhibit an exceptionally high degree of alignment over a broad temperature range, between 25°C and 45°C. Moreover, the addition of a negatively charged lipid, DMPG, further improves the alignment as evidenced by an even more increased order parameter and sharper NMR line widths. The data have been analyzed by a combination of DLS, TEM, and ^{31}P and ^{15}N NMR measured to the sample of Pf1 coat protein reconstituted in the peptoid-based macrodiscs. The results expand the utility of solid-state NMR of oriented samples for structure-function studies of membrane proteins in their native-like planar lipid environment.

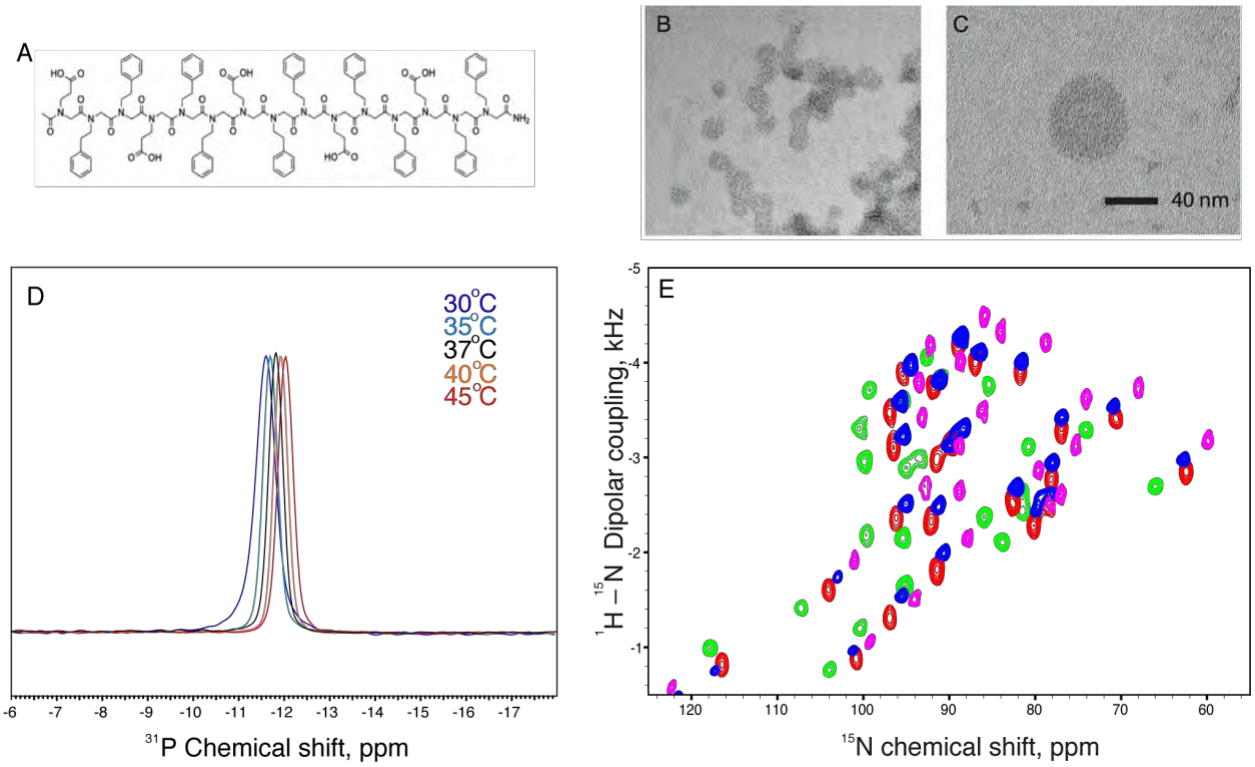

Figure. A. Structure of 15-mer peptoid belt. **B,C.** TEM micrograph of the formed macrodiscs. **D.** ^{31}P NMR spectra of peptoid macrodiscs at various temperatures. **E.** Superposition of ^{15}N 2D NMR spectra of Pf1 coat protein reconstituted in magnetically aligned bicelles (green), 14-mer peptide belt macrodiscs (red), 15-mer peptoid DMPC macrodiscs (blue), and DMPC/DMPG (85%/15%) peptoid macrodiscs (magenta). An increase in the order parameter is clearly evidenced by the higher dipolar couplings in the latter two cases.

USING DNP TO UNRAVEL TYROSINE PHENOL-LYASE ENZYMATIC MECHANISMS

Maria Luiza Caldas Nogueira[1], Rittik K. Ghosh[2], Leonard J. Mueller[2], Joanna R Long[1]

[1]*The University of Florida*
[2]*The University of California – Riverside*

The Tyrosine phenol-lyase (TPL) participates in the nitrogen and tyrosine metabolism by catalyzing reverse hydrolytic cleavage of the L-tyrosine into phenol, pyruvate, and ammonium ion (Raboni et al. 2010). Its cofactor is pyridoxal-5'-phosphate (PLP), also known as vitamin B6, is covalently bound as Schiff base to the active site lysine ε-amino group. PLP dependent enzymes carry out diverse array of chemical transformations such as β-elimination, decarboxylation, racemization, and transamination reactions. TPL carries out β-elimination and racemization reactions with diverse spectrum of amino acids. Therefore, it has great potential for industry biosynthesis of non-canonical amino acids (Zhu et al. 2022). However, its mechanism is not completely understood, how protonation state of the active site and substrate-cofactor complex modulate reaction specificity and diversity in TPL remains an open-ended question. Protonation/deprotonation states is critical in acid-base catalysis, and in understanding the enzyme efficiency, allosteric transitions, and substrate affinity (Huang et al. 2016) and is consequently important for understanding the enzymatic transformations. Here we used natural abundance TPL and ^{15}N or ^{15}N-^{13}C methionine to study the interaction between the substrate and the enzyme active site. We took advantage of the enhancement provided by MAS-DNP at 90K and 10.4 kHz MAS to trap and identify the intermediates species' ^{15}N chemical shift and 2D NCACX correlation spectroscopy to measure ^{13}C chemical shifts of the bound L-methionine. The NMR data was coupled with computational calculations to obtain information about the protonation state of the quinonoid intermediate. These results contribute to our understanding of canonical PLP catalysis at the molecular level.

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STRUCTURAL CHARACTERIZATION OF THE SELF-ASSEMBLY FORMED BY LARGELY DISORDERED REFLECTIN-DERIVED POLYPEPTIDE

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Cephalopods can shift their dazzling body color instantaneously owing to the self-assembly formed by a structural protein called reflectin.[1] However, its structural basis remains unclear at the molecular level. Circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy analyses showed that reflectin assumes largely disordered structure in its assembly, not suitable for high-resolution characterization techniques such as X-ray crystallography or cryoEM.

To elucidate the structure-function correlation, we characterized the self-assembly of $Ref(2C)_4$ by solid state NMR (ssNMR). $Ref(2C)_4$ is a polypeptide designed by linking four repeats of the conserved sequence DPRYYDYYGRFNDYDRYYGRSMF in *E. Scolopes* reflectin 1b.[2] Its assembly preserves the color shifting function with superb proton conductivity.[3] We found its NMR spectra display broad resonances (1.2 to 3 ppm), consistent with prior CD and FTIR analyses of the assembly formed by full length reflectin. To obtain accurate residue-specific assignments, spectra were recorded with ^{13}C, ^{15}N uniformly labeled and selective-residue labeled samples. The residue type assignments of different correlation spectra were further cross-checked by python scripts to ensure their chemical shifts correlation, further enhancing the accuracy. Due to the broad NMR lines and repetitive sequence compositions, the residue-specific assignments can still be sequentially connected in different orders. Statistical analyses of TALOS-N predictions were performed for 1000 sequential assignments, and a common secondary structural theme was revealed: most residues in $Ref(2C)_4$ assembly adopt random coiled structure with large dispersion of backbone torsion angles, while Tyr and Arg residue stretches consistently form short β-strand and α-helix segments with small variations of backbone torsion angles. Assembly interface was resolved by an assembly sample absorbing $MnCl_2$ to quench resonances of solvent exposed residues. Tertiary contacts were identified between Tyr and Arg with mixed labeled samples. Combined, our results indicate that the $Ref(2C)_4$ assembly is stabilized by scaffolds of short β-strands and α-helixes comprising Tyr and Arg residues linked by disordered loops.

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600 MHz MAS-DNP PROBE DESIGNED WITH MICROWAVES IN MIND

Faith Scott[1,2], Peter Gor'kov[1], Jason Kitchen[1], Thierry Dubroca[3], Wenping Mao[1], Stephen Hill[3], Fred Mentink-Vigier[1], Joanna Long[1,2]

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[3]National High Magnetic Field Laboratory, EMR

Dynamic Nuclear Polarization (DNP) increases solid-state NMR sensitivity via unpaired electron spins added to the NMR sample. Under high field Magic Angle Spinning (MAS) conditions, microwave (μw) sources must output a beam of a few watts and cold gas temperatures are required.

We have constructed a custom 3.2 mm (^{1}H or ^{19}F)-XY MAS-DNP probe with several key advantages over our current commercial MAS-DNP probe with (1) better cryogenic insulation, (2) better radio frequency (RF) channel circuits, and (3) better microwave coupling. First, the cold volume in the new probe is smaller due to extended insulation, cutting cooling time by about half. The RF circuit implements replaceable circuit chips that tune for a specified nuclear isotope combination on the X and Y channels thereby improving the flexibility of the RF circuit. This approach enables better RF isolation in between channels and improves decoupling efficiency. By a combination of efficient waveguide and sample coupling, the custom probe gives the same NMR enhancement on the same sample with 30% less microwave power than the previously used Bruker probe. This has been accomplished using knowledge of dielectric constants of probe and sample components previously unpublished in the literature(*1*, *2*).

Using a network analyzer and quasi-optical system, we characterized the dielectric constants for common DNP matrices such as frozen glycerol-water mixtures or 1,1,2,2-tetrachloroethane, and rotor materials in frequency ranges spanning 70 GHz to 960 GHz. We then correlated the dielectric constant measured with DNP enhancement performance. A stock solution was prepared and packed into a variety of rotors of different materials designed to maximize the μw penetration. Those rotors were then compared against a commercial sapphire 3.2 mm rotor in MAS-DNP experiments.

Microwave simulations were performed using the derived dielectric constants to support the experimental observations and provide an estimate of the μw nutation field in the rotor and sample space. The same simulations carried out at different frequencies provide new insights relative to the low performance of MAS-DNP at high field in 3.2 mm rotors. Finally, lens designs at the end of the waveguide were simulated and tested in the finished probe to maximize μw nutation field in the sample. Designing the MAS-DNP probe with microwave simulations to a new probe that yields the same enhancement with 30% less microwave power at the probe base. This has potential for applications with lower power sources such as solid-state diodes.

Acknowledgements

This work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Agreement No. DMR-1644779 and the State of Florida. This work was additionally funded by the National Institute of Health grant P41 GM122698.

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PULSED DNP WITH FREQUENCY CHIRPS

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One of the most promising time domain dynamic nuclear polarization (DNP) methods is the integrated solid effect (ISE), which utilizes either magnetic field modulation [1,2] or microwave frequency sweeps with an arbitrary waveform generator (AWG) [3, 4] to perform DNP. While exploring the ISE, we discovered the stretched solid effect (SSE), where the frequency center of the chirp pulse is off the EPR frequency [4]. Subsequently, when the chirped pulse sweep was near one of the solid effect (SE) conditions [5], we observed the adiabatic solid effect (ASE). Fig. 1 illustrates the frequency modulation schemes of the ISE, SSE and ASE. Furthermore, it was observed experimentally that the SSE and ASE can be more efficient than a full ISE, but the theoretical understanding of this observation and these two new DNP mechanisms is still lacking.

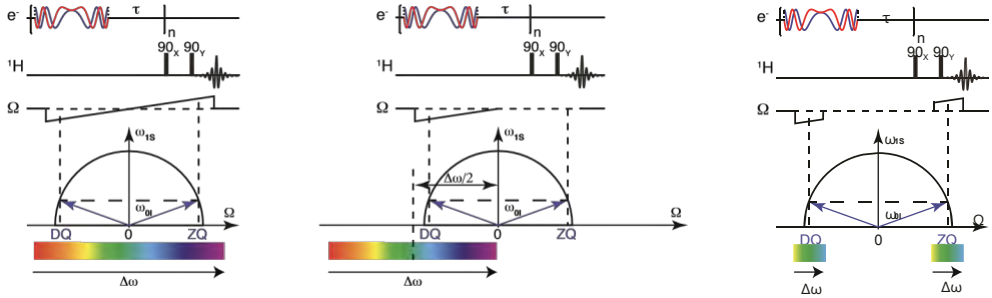

FIG. 1: Schemes of the (a) ISE, (b) SSE and (c) ASE in the rotating frame

Here we present a theoretical description of DNP induced by an arbitrary frequency-swept microwave pulse, based on the Landau-Zener theory. It shows that a strong microwave sweep can be highly efficient and transfer twice the electron polarization (much more than originally considered for ISE [2]) to the surrounding nuclei. The theory is used to explain the ISE, SSE and ASE and experimentally verified at 9.4 GHz (0.34 T), showing that SSE and ASE can be more effective than the ISE. At high magnetic fields, where the EPR line width is narrower than the nuclear Larmor frequency, the theory predicts that SSE can be optimally efficient. In addition, we show that the physics underlying the ISE, SSE and ASE is similar and we provide improved definitions to distinguish the three mechanisms from one another.

Finally we realize that the electron polarization after each field/frequency sweep may remain high and can be further utilized to perform DNP. This provides a new understanding of approaches to optimize frequency swept DNP and time domain DNP in general.

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PyrroTriPol: A SEMI-RIGID TRITYL-NITROXIDE FOR HIGH FIELD DYNAMIC NUCLEAR POLARIZATION

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Magic Angle Spinning (MAS) Dynamic Nuclear Polarization (DNP) has significantly broadened the scope of solid-state NMR to study biomolecular systems and materials. In recent years, the advent of very high field DNP combined with fast MAS has brought new challenges in the design of polarizing agents (PA) used to enhance nuclear spin polarization. Here, we present a trityl-nitroxide PA family based on a piperazine linker, named PyrroTriPol, for both aqueous and organic solutions. These new radicals have similar properties to that found in TEMTriPol-I and can be readily synthesized, and purified, at the gram scale thereby ensuring widespread application. The family relies on a rigid bridge connecting the trityl and the nitroxide offering a better control of the electron spin-spin interactions thus providing improved performance across a broad range of magnetic fields and MAS frequencies while requiring reduced microwave power compared to bis-nitroxides. We demonstrate the efficiency of the PyrroTriPol family at a high magnetic field of 18.8 T and fast MAS of 40 kHz, with respect to TEMTriPol-I. This was further demonstrated on γ-Al2O3 nanoparticles, enabling the acquisition of a high signal-to-noise CP MQMAS experiment at 18.8 T and 40 kHz.

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5-HYDROXYTRYPTOPHAN AS A PROBE FOR LONG RANGE ELECTRON TRANSFER IN OXALATE DECARBOXYLASE

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Oxalate decarboxylase (OxDC) from *Bacillus subtilis* is a Mn-dependent stress-response enzyme that breaks down the oxalate mono-anion into formate and carbon dioxide. The tryptophan pair W96/W274 at the interface of two monomer subunits of the protein serves as an electron hole transfer relay between the N-terminal and C-terminal Mn ions of the neighboring subunits. W96 was replaced by 5-Hydroxytryptophan (5HTP) using genetic code expansion protocols developed for *Escherichia coli* by the Chatterjee group at Boston College.[1] The standard one-electron reduction potential of the 5HTP/5-HTP$^{\bullet}$ couple is approximately 420 mV below the corresponding TRP/TRP$^{\bullet}$ couple. This makes 5HTP a 'hole sink,' blocking the long range electron transfer (LRET) between the two Mn ions and shutting down activity. Instead of Mn(III) which is observed in wild-type OxDC, a persistent carbon-based EPR signal is found in the W96(5-HTP) mutant enzyme. Using partially deuterated 5-HTP leads to additional splittings of the EPR signal. DFT calculations were used to identify the source of the signal as the neutral 5-HTP$^{\bullet}$ radical. Its linewidth of 70 G can be explained by dipolar coupling with two Mn(II) ions located at a distance between 10 amd 13 Å. The presentation will discuss the utility of genetic code expansion and specifically 5-HTP as a probe for LRET in redox proteins.

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UNDERSTANDING BACILLUS SUBTILS LIPASE A FROM A STRUCTURE-DYNAMICS PERSPECTIVE THROUGH SITE-DIRECTED SPIN LABELING AND MOLECULAR DYNAMICS SIMULATIONS.

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Lipases are enzymes whose natural function is to hydrolyze ester bonds. Lipases can also catalyze esterification, interesterification, and transesterification reactions, and this versatility makes lipases important to the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. Site directed spin labeling is an effective tool for studying the structure and local dynamics of proteins using electron spin resonance (EPR). Understanding the changes that occur when labeling different sites of a protein is important, as an effective reporter site is one that minimally perturbs the structure of the protein from its native state.

The structure, dynamics, and hydration environment of Bacillus subtilis Lipase A (BSLA) is hence investigated via EPR,Circular dichroism spectroscopy, and atomistic molecular dynamics simulations. 5 MTSL (1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)methanethiosulfonate) labeled spin constructs of BSLA generated through site-directed spin labeling yield insights on both the structure-activity effects of spin labeling. I12R1 and A20R1 were identified to be the most structurally similar to wild type, while other constructs show notable changes to secondary structure. Water dynamics analysis done on the atomistic simulation data shows water survival probability variations across different labeling sites. The largest variation was seen to be between the local hydration environment of S163R1 and I12R1. X-band CW EPR shows the mobility of each labeling site through line-shape analysis, and the I12R1 site was the most mobile.

MEMBRANE COMPOSITION DRIVES SIDECHAIN IONIZATION IN TRANSMEMBRANE PROTEIN DOMAINS

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Ionization states of individual amino acid residues play significant roles in membrane protein assembly and function; however, they are difficult to decipher or assign experimentally. The analysis is further complicated by the dearth of data on gradients in polarity, electric potentials, and hydration at the protein-membrane interface. In this work we examine how electrostatic interactions, suggested to be essential for the assembly of transmembrane domains of the T-cell receptor (TCR), could be manipulated by modifying the membrane composition. Specifically, we employed novel pH-sensitive ionizable EPR labels to profile a heterogeneous dielectric environment along the transmembrane protein-lipid interfaces. Model transmembrane α-helical WALP peptide integrated into bilayers was used for obtaining the profile of effective pK(a) as a function of membrane depth. We have shown that the effective pK(a) of membrane-buried sidechain can be significantly shifted by varying the membrane surface charge density. A peptide mimicking the transmembrane domain of TCR-α was labeled with pH-sensitive nitroxide and incorporated into liposomes. EPR of this label reported on the sidechain protonation state, membrane insertion, and association of the helices within the membrane. It was found that an increase in negative change density at the membrane surface alters the protonation state of membrane-buried model ionizable sidechain in the transmembrane domain of TCRα. Hyperfine Correlation Spectroscopy (HYSCORE) was used to assess effects of ionization state of model sidechain on water penetration at membrane-peptide interface. Turning the charge of the sidechain “off” also increases tendency of the transmembrane domain to agglomerate - a phenomenon that could be critical for both the TCR assembly and its degradation.

Overall, developed ionizable EPR probes can help to uncover mechanisms as to how the changes in local environment along the protein-lipid interface, can result in a “tunable” pKa of the ionizable sidechains and drive the structural changes in membrane proteins.

ENZYME-SUBSTRATE COMPLEX IN *B. SUBTILIS* OXALATE DECARBOXYLASE REVEALED BY PULSED EPR SPECTROSCOPY

Alvaro Montoya[1], Anthony Pastore[1], Justin Goodsell[1], Alex Angerhofer[1]

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Oxalate Decarboxylase (OxDC) is a Mn-containing enzyme from *B. subtilis* that catalyzes the dissociation of the mono-protonated oxalate anion into formate and CO_2 under acidic stress conditions. A high-spin Mn^{2+} site exists in both the N-terminal and C-terminal domains with similar coordination (three histidine residues and a glutamate).[1] The N-terminal domain is the site of catalysis. To date, the natural binding mode of the substrate to the active site is still uncertain. Here we present pulsed EPR studies probing the active site of site-directed mutant W96F OxDC. W96F possesses structural fidelity to the WT but an order of magnitude slower catalysis, making it an ideal candidate for pulsed EPR studies under active conditions.[2] ESEEM water counting experiments on W96F show the displacement of all water molecules bound to Mn upon addition of oxalate. Results are then corroborated by X-Band ^{13}C-ENDOR data on both WT OxDC at pH 8.5 (inactive pH range) and on W96F at pH 5.0 (active pH range) using ^{13}C-labeled oxalic acid. Density Functional Theory (DFT) calculations support that oxalate prefers a side-on, bidentate binding mode to Mn^{2+} in the active site as opposed to a monodentate arrangement. EPR-derived hyperfine coupling constants were validated by DFT and showed each ^{13}C in the labeled substrate at an approximately equal distance from the Mn^{2+} ion (~3 Å). The ENDOR spectra were simulated using the EasySpin toolbox for MATLAB. Good simulations of the experimental spectra were observed for the bidentate binding model providing the first glimpse of the enzyme-substrate complex for *B. subtilis* OxDC.

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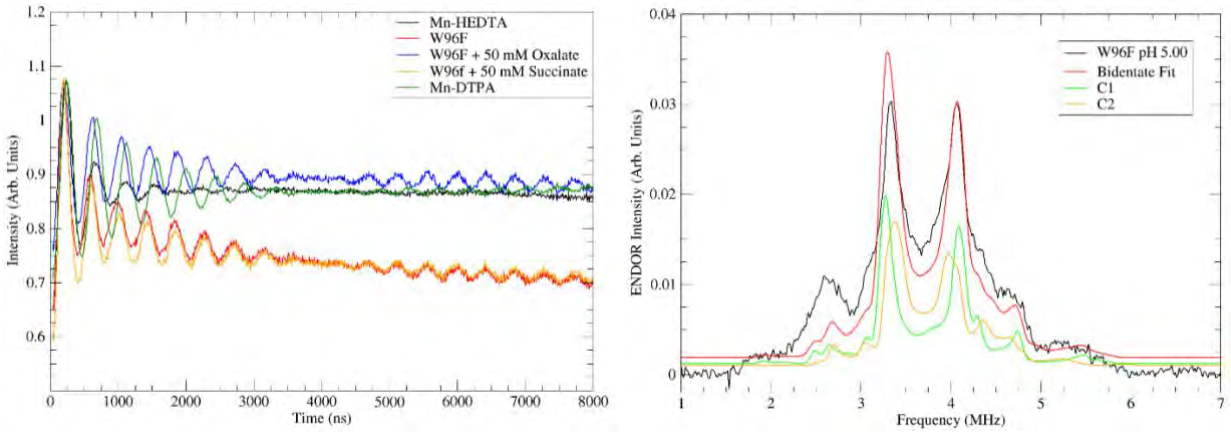

TERAHERTZ EPR SPECTROSCOPY USING A 36-TESLA HIGH-HOMOGENEITY SERIES-CONNECTED HYBRID MAGNET

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Electron Paramagnetic Resonance (EPR) is a very powerful technique to study materials and biological samples at the atomic scale. High-field EPR is able to resolve very small g-anisotropies in radicals and half-filled 3d and 4f metal ions, such as Mn(II) ($3d^5$) and Gd(III) ($4f^7$). In addition, it can differentiate unpaired spins with very close g-values, allowing extremely sensitive probing of the local atomic environment. Before the recent commissioning of the high homogeneity Series Connected Hybrid (superconducting outsert and resistive insert) magnet[1] at the NHMFL, the highest-field, high-resolution EPR available was limited to 25 T using a purely resistive "Keck" magnet.[2] Herein, we report the first EPR experiments performed using the SCH magnet capable of reaching the field of 36 T, corresponding to an EPR frequency of 1 THz for $g = 2$. The magnet's intrinsic homogeneity (25 ppm, that is 0.9 mT over 1 cm diameter, 1 cm length cylinder) was previously established by NMR.[1] We characterized the magnet's temporal stability (0.1 mT over 5 minutes) using the well-known radical DPPH, and fully resolved the very small (~2 x 10^{-4}) axial *g*-anisotropy of another model radical, BDPA, obtained from measurements at 932 GHz / 33.3 T. Furthermore, we recorded EPR spectra at multiple frequencies of Gd(III) complexes with potential applications as spin labels. The experiments were performed on 500 μM solutions in a 50/50 water/glycerol mixture at cryogenic temperatures. In conclusion, we demonstrated a significant reduction in second-order zero field splitting broadening and a resolution enhancement of *g*-tensor anisotropy for half-integer spins in an unprecedented high field/frequency regime. Funded by the National Science Foundation (DMR-1644779) and the State of Florida.

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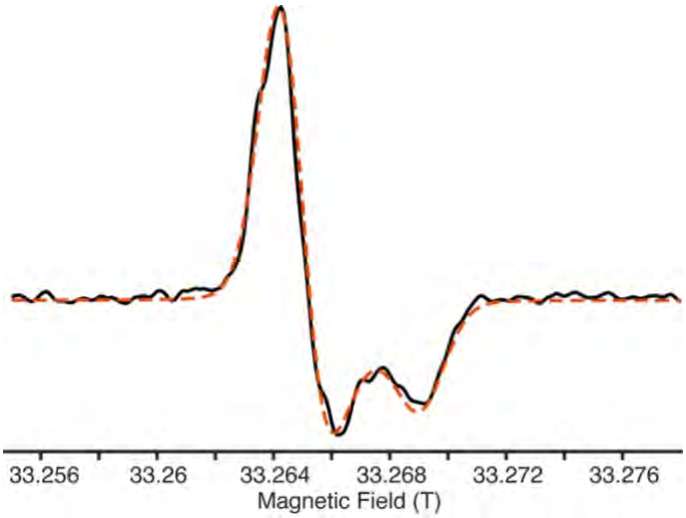

Figure 1. An EPR spectrum of BDPA in polystyrene measured at 932.4 GHz and 82 K (solid back line; 9-point average smoothing filter was applied to the collected data, ~0.8 mT smoothing). The red dashed line is the simulated spectrum using the following g-tensor principal values [g_{xx}, g_{yy}, g_{zz}] = [2.00265, 2.00263, 2.00240]. Note: the magnetic field was shifted arbitrarily by –0.1955 T from the current-calibrated values, to yield coherent *g* values with previous publications and DFT predictions.

SYNTHESIS, CHARACTERIZATION, AND NMR CRYSTALLOGRAPHY OF IONIC MULTI-COMPONENT CRYSTALS

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The rational design of multicomponent crystals (MCCs) is a flourishing area in crystal engineering and pharmaceutical sciences.[1,2] It is possible to rationally design MCCs with optimized physicochemical properties *via* careful consideration of coformer selection and mode(s) of preparation.[3,4] Of particular interest is preparation of MCCs by mechanochemical synthesis, which can rapidly produce novel MCCs with high yields and purities, is a form of green chemistry, and even provides avenues for the synthesis of novel MCCs that may be inaccessible through other synthetic pathways.[5,6]

The combination of SSNMR, PXRD, and density functional theory (DFT) calculations provides a route for NMR crystallography (NMRX) studies, which can be used to solve, refine, and validate crystal structures based on comparison of experimentally-measured and theoretically-derived NMR interaction tensors.[7,8] Quadrupolar nuclei ($I > ½$) are of great value for NMRX studies of MCCs, due to the great sensitivity of the quadrupolar interaction, which manifests in SSNMR spectra, to the local environments of ionic species that are involved in intricate hydrogen bonding networks.[9–12]

In this work, we present an investigation of MCCs of the form NR_4Cl:*x*Urea·*y*H_2O (R = H, Et, *n*-Pr; *x* = 1, 2, 3; *y* = 0, 2) made by novel ball milling methods, and their characterization by ^{35}Cl SSNMR, which provides insights into their molecular-level structures and their concomitant use for site assignment and NMRX.[13] We describe the inclusion of SSNMR data of other quadrupolar nuclei (*i.e.*, ^{7}Li, ^{23}Na, ^{87}Rb, and ^{133}Cs) for refining and validating the structures of a series of known MCCs of the form MCl:cyanuric acid. Lastly, we take the first steps in developing NMRX methods to investigate previously unreported MCCs of the form MCl:Urea:*x*H_2O (M = Li, Na, Cs; *x* = 0, 1), which are characterized by multinuclear (^{35}Cl, ^{23}Na, ^{7}Li, and ^{133}Cs) SSNMR spectroscopy and synchrotron PXRD. Plane-wave DFT calculations and Rietveld refinement of synchrotron XRD data aid in determining the crystal structures of these model systems and demonstrate promise for more general applications of NMRX methods utilizing quadrupolar nuclei.

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CROSS POLARIZATION USING ^{1}H ADIABATIC DEMAGNETIZATION IN SOLID-STATE NMR OF ALIGNED SAMPLES

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Cross polarization (CP) is a widely used solid-state nuclear magnetic resonance (NMR) technique for enhancing the polarization of dilute *S* spins from much larger polarization of abundant *I* spins such as ^{1}H. To achieve such a polarization transfer, the *I* spin should either be spin-locked or be converted to the dipolar ordered state through adiabatic demagnetization in the rotating frame. In this work, we analyze the spin dynamics of the Hartmann-Hahn CP (HHCP) utilizing the ^{1}H spin-locking, and the dipolar-order CP (DOCP) having the ^{1}H adiabatic demagnetization [1]. We further propose an adiabatic demagnetization CP (ADCP) where a constant radio-frequency pulse is applied on the *S* spin while ^{1}H is adiabatically demagnetized, as shown in Fig.1a. Our analyses indicate that ADCP utilizes the adiabatic passage to effectively achieve the polarization transfer from the ^{1}H to *S* spins. In addition, the dipolar ordered state generated during the ^{1}H demagnetization process could also be converted into the observable *S* polarization through DOCP, further enhancing the polarized signals. Fig. 1b indicates that ADCP has dramatically broadened the CP matching condition over the other CP schemes. While Fig. 1c illustrates that the polarized ^{15}N signals are rapidly increased when the ^{1}H transient RF amplitude coincides with the applied ^{15}N RF amplitude, as projected in the theory. As an example, it has been shown experimentally that, for the gA sample mechanically aligned in hydrated lipids, the ADCP scheme gives rise to 50% more ^{15}N signals over the traditional HHCP scheme.

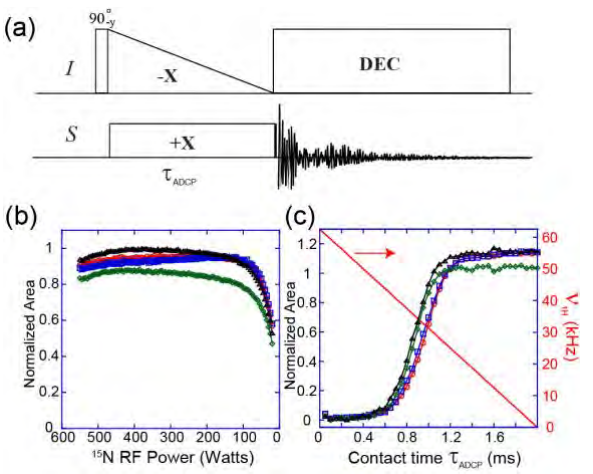

Fig. 1 (a) CP pulse sequence using ^{1}H adiabatic demagnetization. (b) The CP matching profile as a function of the applied ^{15}N RF power. (c) The buildup of the ^{15}N signals during the CP contact time τ_{ADCP}. The red line corresponds to the transient ^{1}H RF amplitude during τ_{ADCP}, while the ^{15}N RF power of 200 Watts ($\omega_{1N}/2\pi$ = 30.7 kHz) was applied. A ^{15}N-acetyl-valine (NAV) crystal sample was used in these experiments.

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ADIABATIC PASSAGE THROUGH LEVEL ANTI-CROSSINGS IN SYSTEMS OF CHEMICALLY INEQUIVALENT PROTONS INCORPORATING PARAHYDROGEN: THEORY, EXPERIMENT, AND PROSPECTIVE APPLICATIONS

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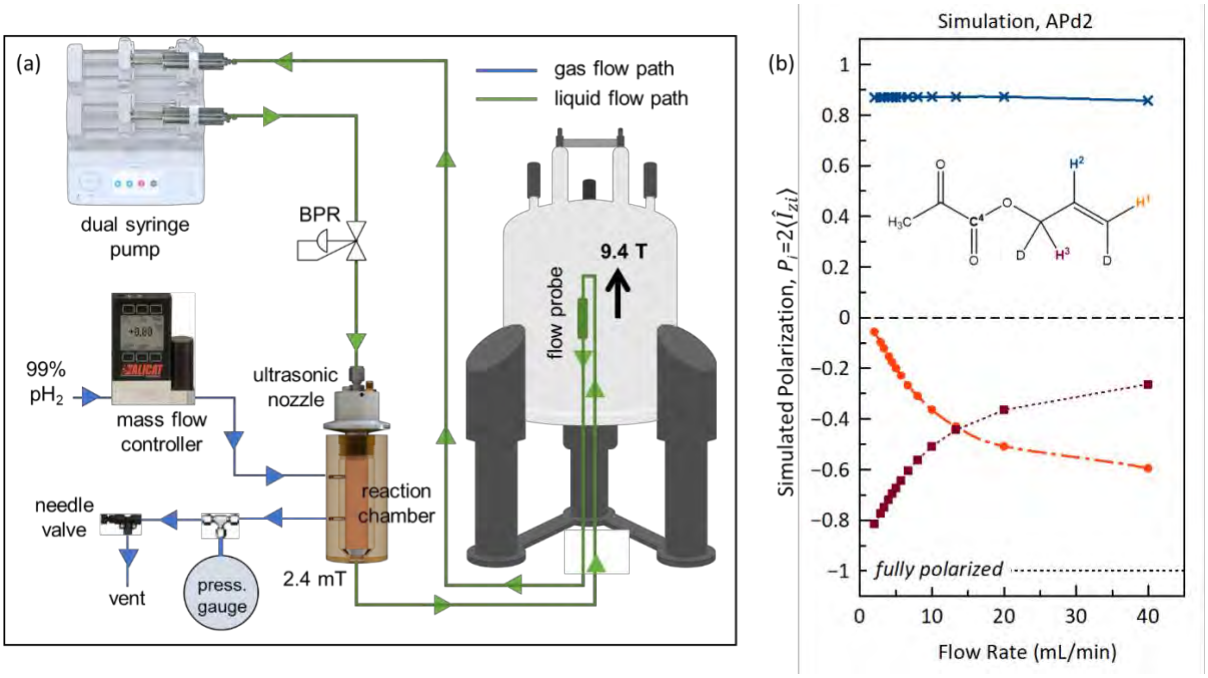
Department of Chemistry and National Maglab, University of Florida, Gainesville

Level anti-crossings (LACs) are ubiquitous in quantum systems and have been exploited for spin order transfer in hyperpolarized NMR. We have predicted and experimentally demonstrated that adiabatic passage through a specific type of LAC found in homonuclear systems of chemically inequivalent coupled protons incorporating parahydrogen (pH_2) could elicit translation of pH_2 spin order in the hydrogenation adduct.[1] After ultrasonic spray injection of a precursor solution containing propargyl pyruvate and a dissolved Rh catalyst into a chamber pressurized with 99% para-enriched H_2, the products were collected and transported to high magnetic field for NMR detection at precisely controlled transport rates. Proton spin polarizations of 19.8±2.6% on the methylene protons and 68.7±0.5% on the vinylic protons of selectively deuterated allyl pyruvate ester were demonstrated. Carbon-13 spin polarizations in pyruvate of up to 36.4% were demonstrated after selective INEPT transfer. Experiments also confirmed that selective deuteration of the side-arm increases polarization levels. LAC-mediated translation of parahydrogen spin order completes the first step toward a new and highly efficient route for ^{13}C NMR signal enhancement of pyruvate via side arm hydrogenation with parahydrogen.



(a) National MagLab's Ultrasonic spray-injection reactor system interfaced with the flow NMR spectrometer. The precursor solution is infused through an ultrasonically vibrated nozzle (3.5 W, 120 kHz) into the reaction chamber at a flow rate of 3 mL/min. Hydrogenation adducts accumulate and are drawn into the flow probe at precisely controlled flow rates. (b) Numerically simulated spin polarization of individual protons in allyl-pyruvate-d_2 as a function of the syringe pump withdrawal rate.

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BROADBAND ADIABATIC INVERSION CROSS-POLARIZATION UNDER MAGIC-ANGLE SPINNING CONDITIONS

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Large anisotropic NMR interactions can give rise to ultra-wideline (UW) NMR powder patterns ranging from 250 kHz upwards of 10 MHz in breadth, making it difficult to acquire uniform spectra with high signal-to-noise ratios.[1] The use of conventional methods for the acquisition of UWNMR powder patterns is limited due to the relatively narrow excitation and refocusing bandwidths of rectangular pulses. This problem is readily overcome by the use of frequency swept (FW) pulses, such as the wideband uniform-rate smooth truncation (WURST) pulse.[2,3] Signal enhancement via cross-polarization (CP) can be achieved using FS pulses in the broadband adiabatic-inversion cross polarization (BRAIN-CP) sequence.[4] Even further signal enhancement by means of magic-angle spinning (MAS) is often desired. While conditions do exist which aid in obtaining high resolution wideline NMR powder patterns under MAS,[5] CP bandwidths are still very much limited and are oftentimes inefficient in UWNMR applications. The WCPMG sequence has previously been implemented for optimal performance under MAS;[6] however, the BRAIN-CP sequence has yet to be fully explored in applications to UWNMR powder patterns of rotating solids.[7]

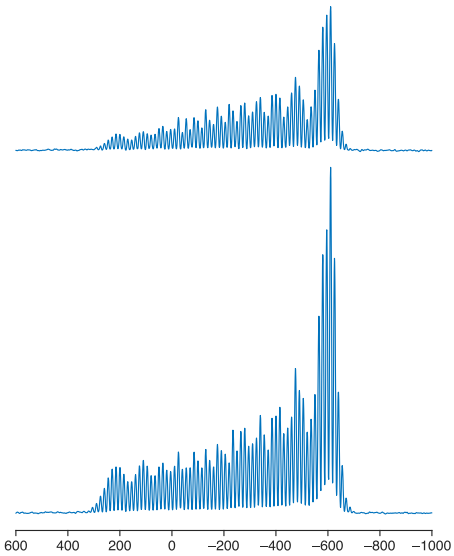

Figure 1: Experimental ^{195}Pt spectra of $Pt(NH_3)_4Cl_2{\cdot}H_2O$ acquired using WCPMG (top) and BRAIN-CP (bottom) at a spinning speed of ν_{rot} = 15 kHz. Both spectra consist of 256 transients and are acquired at one transmitter offset.

Herein we present theoretical and experimental considerations for the acquisition of UWNMR powder patterns under MAS using the BRAIN-CP pulse sequence. Numerical simulations are used to gain insight into the spin-dynamics of CP and to obtain an understanding of rotary resonance conditions upon which the CP bandwidth ultimately depends. The wideline ^{119}Sn powder pattern of dibutyl tin oxide is used for optimization of experimental conditions, and as a true test of the applicability of these methods to UWNMR powder patterns, ^{195}Pt powder patterns of $Pt(NH_3)_4Cl_2{\cdot}H_2O$ (*ca*. 1 MHz in breadth at 14.1 T) are acquired using both DE (WCPMG) and IE (BRAIN-CP) methods (**Figure 1**). These methods will enable the study of a wide range of unreceptive spin-1/2 nuclei, especially those with low gyromagnetic ratios, where the benefits of MAS and CP enhancement play crucial roles in efficient acquisition of high quality NMR spectra.

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SPARSE ISOTOPE LABELING FOR STRUCTURAL ANALYSIS OF GLYCOPROTEINS

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Glycoproteins constitute the majority of all human proteins, and structural characterization would facilitate efforts to combat disease in many cases. However, structural characterization presents special problems for NMR. Producing natively glycosylated glycoproteins with uniform isotopic labeling in mammalian cell cultures usually requires isotopically labeled versions of all amino acids, rather than the inexpensive substrates required by E. coli cultures. Moreover, mammalian cells do not typically tolerate perdeuteration. However, uniform labeling is primarily required for de novo structure determination. Structural validation of computational models and functional characterization of proteins may require only sparse labels. We have been exploring a simple way of producing glycoproteins labeled in all alanine methyl groups and all residues of attached glycans. Production is based on supplementation of media with 13C1-glucose. The resulting resonances are well dispersed and of high sensitivity, but they require an alternate assignment strategy. Combining easily collected data, such as 13C-edited NOESY and chemical shifts from 13C-detected HSQCs, with paramagnetic data from tagged proteins and a computational model, provides a path to these assignments. This path has been integrated into a software package, ASSIGN_SLP. We will illustrate application of this package to resonance assignment and structure validation of a cell-surface adhesion and signaling molecule, CEACAM1. We also, illustrate the use of paramagnetic data in conformation analysis of the glycans attached to CEACAM1.

CHARACTERIZATION OF THE C-TERMINAL SEGMENT OF THE *STREPTOCOCCUS MUTANS* ADHESIN P1 BY SOLUTION NMR SPECTROSCOPY

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Streptococcus mutans is a gram-positive bacterium and a major causative agent generating dental cavities, one of the most common human diseases in the world [1]. The cell surface-localized adhesin P1 (aka Antigen I/II, antigen B, or PAc) of the *S. mutans* mediates sucrose-independent adhesion of the bacteria, which further facilitates colonization and biofilm formation on the tooth surface [2]. Our previous studies indicate that the C-terminal region of adhesin P1 (C123, aka AgII) is also liberated as a separate polypeptide in saliva, which contributes to the pathogenesis of *S. mutans*. The released C123 segment interacts specifically with the intact P1 on the cell surface, and forms amyloid fibrils by self-assembling [3-4].

Identifying how C123 forms amyloid fibrils at the atomic level is essential for understanding the related virulence properties of *S. mutans*. However, the full-length C123, at 51 kDa, is not amenable to achieve high-resolution data for full structural analysis by solution NMR spectroscopy using uniform double isotopic labeling strategies. Although we used triple-labeling with ^{15}N, ^{13}C, ^{2}H for the backbone assignment on C123 (*Figure 1*), it's still very challenging during data processing and analysis. In addition, we would also like to locate the key functional sites between the three domains in C123.

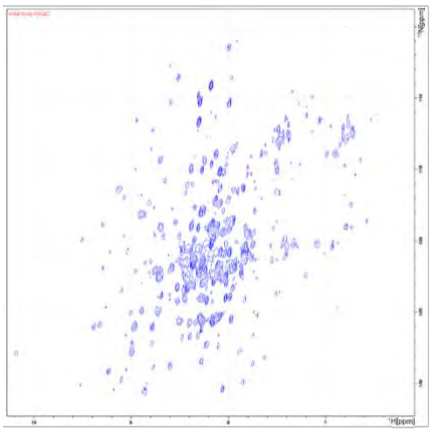

Figure 1. 2D ^{1}H-^{15}N TROSY spectra of *wt* U- [^{1}H, ^{13}C, ^{15}N]-C123

We are now working on the backbone assignment of the C3 domain of C123 NMR with triple labeling strategy. In our previous study, approximately 60% of the C3 backbone amide protons were assigned. By deuteration, we are expecting to have 20% more C3 backbone amide protons assigned.

The other strategy we have considered is segmental enrichment. We will produce isotopically labeled C3 domain and natural abundance C12 domain, then link them by intein assay to mimic the intact C123 polypeptide. Inteins are interpreted as extraneous polypeptide sequences into ordinary proteins. They do not contribute to the original protein function but perform an autocatalytic splicing reaction after protein translation. An issue is that protein splicing reaction will leave behind an extein scar, so we need to make sure the insertion of the leftover scar residues does not affect the C123 protein structure, which can be done by a quick 2D HSQC NMR experiment.

Via making segmental enriched C123, we will be able to assign the C3 domain within C123. In this case, even though the *wt* C123 NMR data is challenging, we should be able to tell which segments are involved in hetero-interaction study with A3VP1, AgA and Cnm. This will enable us to overcome current limitation of protein structural study by solution NMR in a certain level, and will provide insights into pathogenic mechanism of S.mutans, help us understand the sequence of events for the early stages of bacterial adhesion and subsequent biofilm development, including the formation of functional amyloids.

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INTERACTIONS BETWEEN SMALL MOLECULES AND POLYSTYRENE NANOPARTICLES EXAMINED BY MULTIPHASE NMR AND MOLECULAR DYNAMICS SIMULATIONS

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Plastic pollution in world waterways is a growing concern. Over time, large plastic particles break down into smaller pieces, eventually forming micro-and nano-scale plastic particles.[1] These particles pose a danger to wildlife as they can be mistaken for food, leading to choking hazards or malnutrition.[2] Another concern is that plastic particles can concentrate other small molecules that are present in polluted waterways, such as carcinogenic polycyclic aromatic hydrocarbons, pharmaceuticals, and pesticides.[3] As plastic particles can move up the food chain, these toxic adsorbed molecules may be released in higher organisms or even humans.[4] Plastic has also been suggested as a medium for removal of toxic small molecules in remediation efforts,[5] and understanding the interactions between small molecules and plastic surfaces is important for this application as well.

In this talk, we will present recent work we have done aimed at understanding the intermolecular interactions that are responsible for sorption of small molecules on the surface of nanoscale plastic particles. From a solution-state NMR perspective, saturation-transfer difference (STD) NMR has been a useful tool to identify small molecules that interact with a plastic nanoparticle surface.[6] STD-NMR has been used for epitope mapping, allowing us to determine the driving forces for binding between polystyrene nanoparticles and amino acids as model small molecules.[7] STD-NMR has also been used to examine binding between plastic nanoparticles and a selection of antibiotics, which are also expected to be present in polluted waterways.[8] However, solution-state NMR only gives half the picture, as it can only observe small molecules that are free in solution. Thus, we have used comprehensive multiphase NMR to examine the solid, liquid, and gel-like phases of the same sample containing polystyrene nanoparticles and amino acids.[9]

Molecular dynamics simulations complement the insight gained by the multiphase NMR, by predicting the binding propensity of various small molecules and illuminating the various binding geometries of these molecules. Results of this work could be useful to the design of biodegradable plastics that are resistant to sorption of small molecule toxins, regulatory policies regarding the concentrations of specific molecules that are allowed in fish intended for human consumption, and choosing plastic with optimal characteristics for environmental remediation.

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HTS ^{13}C-OPTIMIZED NMR PROBE AT 21.1 T

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We present a newly developed NMR probe (Fig. 1) that utilizes our previously published high-temperature superconductor (HTS) ^{13}C coils[1] for the innermost channel. This probe uses a normal metal coil double-tuned to ^{1}H and ^{2}H frequencies for decoupling, lock, and/or magnetization transfer from ^{1}H rather than direct detection because of its lower sensitivity.

We modified the shape of the center tube to accommodate a rectangular (6.2 x 3 mm) NMR sample tube in addition to cylindrical 3mm tubes. The ^{13}C ASTM S/N with the rectangular tube was ~5400 and with the 3 mm tube was ~2300. We verified that our HTS coil design provides sufficient current for 15 µs pulse width. Compared to commercial carbon-optimized probes, this probe gives better performance for 3 mm cylindrical mass limited samples as well as samples with low conductivity in the rectangular tube such as chloroform, pure water/DMSO and TRIS buffer.

Further, we will present initial findings about the salt dependence of the ^{13}C channel and show NMR spectra of a variety of biological samples to demonstrate the capabilities of this probe.

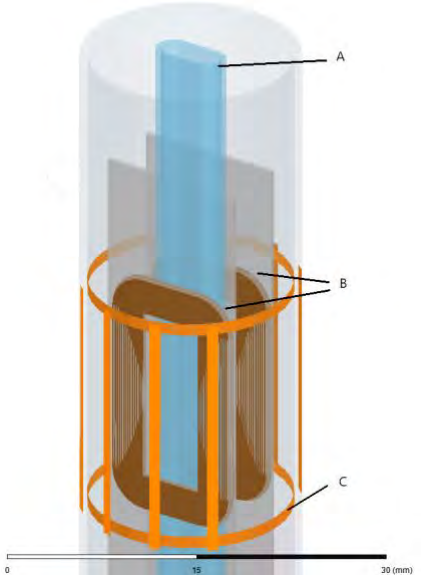

Figure 1: the schematic of the shaped sample tube (A), inner ^{13}C coils (B) and outer double tuned ^{1}H/^{2}H coil (C)

Acknowledgement: We thank J. A. Kitchen and J. N. Thomas at NHNMFL, Florida State University for their help in building up the probe as well as M. Lucci, C. Luchinat, L. Banci for testing the 3 mm version of the probe at University of Florence. This work was financially supported by NIH grant no. R01GM120151 and Bruker Switzerland AG. A portion of this work was performed at NHMFL, which is supported by NSF Cooperative Agreement No. DMR-1644779 and the State of Florida.

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PULSED 94 GHz EPR FOR SPIN POPULATION TRANSFER IN A Gd(III) MOLECULAR CRYSTAL

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Gd^{3+} is a spin $S = {}^{7}/_{2}$ ion that possesses a half-filled $4f^7$ electron occupancy, with no first order orbital angular momentum. Consequently, the $2S + 1 = 8$ spin levels are minimally mixed and can typically be considered quite pure, with very weak zero-field splitting expected. For these reasons, Gd^{3+} has been proposed as a $d = 8$ level qudit system approximating 3 coupled qubits (i.e., $d = 2^3 = 8$), with each $\Delta m_S = \pm 1$ transition being fully allowed via EPR, permitting spin state manipulation using resonant microwave pulses.[1,2] To access this proposed application, the pulsed EPR spectrometer must meet several requirements. These include: (1) sufficient power to achieve wide bandwidth excitation capable of accessing multiple spectrally separated spin transitions; (2) nanosecond time resolution with a high degree of control over the pulse waveform shaping; and (3) sufficient detection sensitivity for studying small samples with low spin concentrations. The 94 GHz quasioptical HiPER spectrometer at the National High Magnetic Field Laboratory meets all of these requirements and additionally allows for *in situ* single crystal rotation. Using this unique spectrometer, we have demonstrated dynamic population transfer within the ${}^{8}S_{7/2}$ ground manifold of a Gd^{3+} coordination compound, paving the way towards implementation of simple quantum logic operations within a $d = 8$ molecular spin qudit.

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ACCESSIBLE ELECTRON SPIN RESONANCE INSTRUMENTATION WITHIN CRYOSTAT ENVIRONMENTS; A STEP TOWARDS SUB-KELVIN ESR

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Electron spin resonance (ESR) spectroscopy is a versatile experimental technique to study and control the spin dynamics of various material systems through microwave-induced spin transitions (typically) under an applied magnetic field. The technique can be implemented in continuous wave (CW) and pulsed forms, providing direct determinations of a system's g-factors, relaxation times, and magnetic susceptibilities. ESR has been increasingly used within the field of quantum information for coherent manipulation and readout of qubit states. These advances have brought about the growing use of unconventional and homebuilt ESR instruments, allowing for a high degree of control and customization over experimental parameters. In addition, ESR has recently been used as a probe of exotic excitations in quantum magnets, mainly focusing on studying the existence and dynamics of spinons in quantum spin chain materials [1,2]. Investigating these types of systems requires sub-Kelvin temperatures, only reachable inside dilution refrigerators. Apart from a few innovative laboratories, most low-temperature ESR is limited to above few Kelvin. As a step towards full implementation at millikelvin temperatures, we utilize this modular approach to design and assemble a simple CW spectrometer to characterize the g-factors of materials of interest within our lab. Our probe is easily implemented into an existing measurement platform (Quantum Design Physical Property Measurement System), facilitating reproducibility in groups with similar equipment. The design and realization of this probe not only provide access to sample characterization but supply a platform to review the versatility of loop gap resonators in ESR experiments.

*Initial project funded by the Georgia Tech - Sandia Alliance. The work at the Center for Integrated Nanotechnologies supported under proposal # 2021ARA009. The work at Georgia Tech was supported by the NSF under NSF-DMR-1750186.

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OPEN-SOURCE LOOP-GAP RESONATOR FOR X-BAND EPR SPECTROSCOPY

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In recent years, the open-source hardware community has experienced tremendous growth. Open-source hardware designs ensure all necessary information needed to reproduce the design is made publicly available. In the EPR field, where instrumentation remains relatively expensive, the community would benefit from more open-source hardware designs.

Figure 1: Assembled Open-Source PCB LGR from stackable PCBs.

Loop-gap resonators (LGRs) have many advantages over cavity resonators, including a high microwave conversion factor B1, a low quality factor and high filling factor [1]. LGRs are particularly sensitive in the case of limited sample volume, where they outperform both cavity and dielectric resonators [2]. In most cases, LGRs are fabricated by metal plating a Macor ceramic material or electric discharge machining (EDM) of a metal blank. These fabrication techniques produce high quality LGRs, however the manufacturing process is expensive and requires expertise to get right. Modern printed circuit board (PCB) manufacturing capabilities can hold extremely tight tolerances and resonators can be constructed for much lower cost (prototype PCBs often cost less than $20). In this work, we demonstrate an open-source X-band LGR designed from PCBs. The design consists of stackable PCBs in a modular configuration to adjust the resonator height. One advantage of designing a resonator from PCBs is the skin depth of the copper layer exceeds X-band microwaves, but is less than 100 kHz, making field modulation penetrate the sample. We will discuss HFSS simulations, mechanical design, and EPR measurements.

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HIGH-VOLUME HIGH-Q RESONATORS DRASTICALLY IMPROVE CONCENTRATION SENSITIVITY OF W-BAND (95 GHz) PULSED EPR

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Magnetic resonance experiments at high magnetic fields offer the same advantages of increased spectral resolution and improved sensitivity for both NMR and EPR. However, the transition of EPR to resonant frequencies above conventional X- (9 GHz, resonance fields of *ca*. 0.3 T for g=2 species) and Q-band (35 GHz/1.2 T) has been significantly slower *vs*. NMR experiments, which are mainly conducted today at magnetic fields of 7 T (300 MHz ^{1}H frequency) and above. Clearly, this rather striking gap in the resonance fields between the two "sister" magnetic resonance methods cannot be attributed to the lack of magnet technology but rather a lower performance and/or higher cost of mm-wave components required for high field (HF) EPR. The gap is particularly apparent for time domain HF EPR as evidenced by, perhaps, only about a dozen spectrometers operating at frequencies of 95 GHz and above. The main reason for this is the remaining technical challenge to generate sufficiently high B_1 on the sample at these mm-wave frequencies. Currently, this challenge is approached by employing high power (up to *ca*. 1 kW) pulsed amplifiers based on EIK (extended interaction klystron) technology at W-band (95 GHz) but at the same time non-resonant sample holders to increase sample volume and signal-to-noise ratio. However, EIK devices are expensive and extensions of this technology to even higher frequencies (>200 GHz) is even more challenging. Here we describe one way to solve this problem by developing and characterizing a series of high-*Q* high-*F*inesse resonators based on one-dimensional photonic band gap (PBG) structures. Such structures allowed us to take advantage of all solid-state W-band components with a significantly lower output power (*ca*. 2 W amplifier was tested) to not only increase B_1 on the sample but also significantly increase the sample volume *vs*. single-mode cavities, thus, drastically increasing he signal-to-noise ratio for the same spin concentration. Specifically, by increasing the diameter of the resonator from 13 to 26 and then 32 mm and optimizing PBG configuration together with mirror geometry, we have improved unloaded *Q*-factor from the initial Q≈500 to up to Q=8,083. The conversion factors of these resonators were then evaluated in nutation experiments using 100 μm thick polystyrene film doped with 1 mM of BDPA. While the shortest 90° pulses for the best PBG were still longer than those measured for a cylindrical TE_{012}-type cavity with a comparable Q (34 ns vs. 23 ns, respectively), an increase in sample volume from ≈0.8 to ≈180 μl resulted in up to 60-fold signal gain for the same spin concentration. The PBG resonator design described here is readily scalable to higher resonance frequencies. Supported by NIH 5R01GM130821 to AIS and AAN.

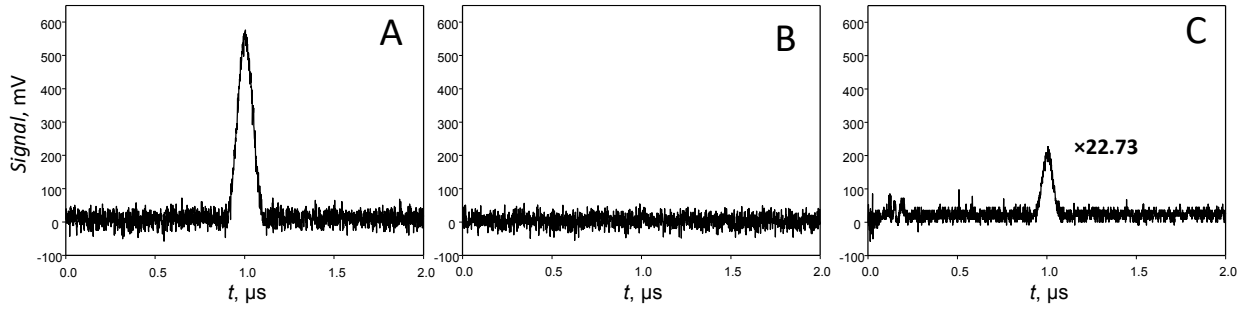

Figure 1. A. Single shot 2-pulse spin echo (1 μs delay) measured from 1 mM BDPA in polystyrene (100 μm thick film) using a PBG with Q_L=1,538. While no echo was observed after the single shot when using TE_{012}-type cavity (Q_L=1,241) by loading the powdered polymer into i.d.=0.7 mm (**B**), the signal appeared after 512 averages. All measurements were carried out at room temperature.

POSTER ABSTRACTS

PROGRESS ON EPR STUDIES TO PROBE THE ROLE OF DIOXYGEN AND LONG-RANGE ELECTRON TRANSFER IN OXALATE DECARBOXYLASE

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Oxalate Decarboxylase (OxDC) is an acid stress regulatory enzyme native to *Bacillus subtilis*. The enzyme catalyzes the disproportionation of oxalic acid to formate and carbon dioxide. For activity, OxDC requires Mn and dioxygen. A Mn(II) is coordinated in both the N-terminal and C-terminal domains of each subunit of OxDC, with the active site located near the N-terminus. In the current literature mechanism, dioxygen is bound to the N-terminal Mn site along with the substrate. However, there is no experimental evidence that supports dioxygen binding to this site. On the other hand, Mn(III) is needed for catalysis and needs to be initiated for the enzyme to be active.[1]

Here we present cryogenic CW Electron Paramagnetic Resonance (EPR) studies exploring the effect of dioxygen on Mn(II) and Mn(III) in OxDC. OxDC was prepared under aerobic and anaerobic conditions for EPR experiments. We observe a decrease of a parallel-mode EPR signal associated with Mn(III)[1] in the absence of dioxygen which is, however, present in aerobic OxDC samples. Interestingly, the signal does not recover when dioxygen is introduced to the anaerobic samples suggesting that dioxygen is not directly involved in producing Mn(III). However, the Mn(III) EPR signal immediately recovers upon the addition of hydrogen peroxide suggesting that it may not be dioxygen but hydrogen peroxide that leads to Mn(III) formation. Dioxygen may be only indirectly involved in catalysis through the generation of hydrogen peroxide.

In order to study the proposed long-range electron transfer (LRET) between the two Mn ions in OxDC[2] we are working to introduce photoactive Ru(II) probes as well as nitroxide spin labels on the protein surface through covalent linkage to cysteine residues. The installation of the Ru(II) probes in the vicinity of the C- and N-terminal Mn(II) binding sites should allow to photo-inject electron holes into the protein and facilitate the observation of LRET by time-resolved optical spectroscopy. The installation of nitroxide spin labels will allow us to monitor protein mobility and conformation by measuring distances to the paramagnetic Mn ions. Residues S109, E147, E239, and N250 were chosen as possible candidates. We report on initial EPR experiments on the double mutants E239C/C383A and N250C/C383A with 3-(2-Iodoacetamido)-proxyl (IAP) spin label attached to the surface cysteines. Our experiments demonstrate that these sites are accessible to site-directed spin labeling.

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GIANT MAGNETIC ANISOTROPY IN A TRIGONAL Ni(II) COMPLEX

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The trigonal bipyramidal Ni(II) complex, Ni[(Me_6tren)Cl]PF_6, has a ground state spin of S = 1 with a giant first-order axial magnetic anisotropy. By studying the angle-dependence of the EPR spectra of a single crystal sample, we can gain unique insights into the stability of the orbitally degenerate ground state of this molecule. In particular, such studies may provide signatures of Jahn-Teller-type structural distortions, leading to a lowering of the trigonal symmetry and a reduction in the overall magnetic anisotropy [1,2].

In Figure. 1, the angle-dependence of the EPR spectra associated with the transition within the isolated ground m_s = ±1 quasi-doublet is plotted, showing three angle-dependence EPR transitions. Here we follow the model in [1]. By considering the crystallographically molecular C_3 symmetry is broken due to a Jahn−Teller-type distortion, three different molecular orientations related by the threefold rotational symmetry in the unit cell of the crystal contribute three angle-dependence EPR transitions. With the field aligned in the magnetic easy-axis ($\theta = 0$), three EPR peak positions overlap each other, indicating the easy axes of three orientation molecules are parallel. Finally, we note that the parallel mode EPR transition within the ground m_s = ±1 quasi-doublet displays a giant 69 GHz clock transition, which could be of interest for quantum information applications.

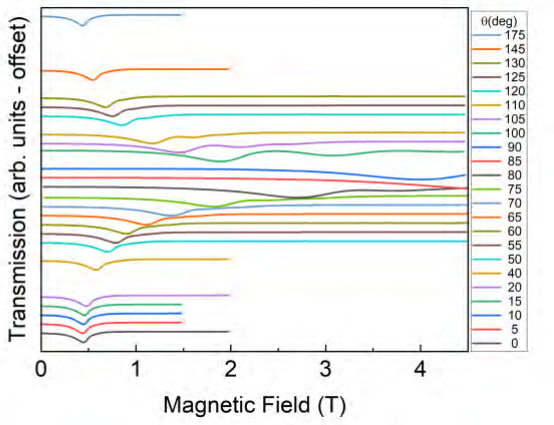

Figure 1. The angle-dependence of EPR spectra

This work was supported by the Center for Molecular Magnetic Quantum Materials (M^2QM), an Energy Frontier Research Center funded by the US Department of Energy, Office of Science, Basic Energy Sciences under Award DE-SC0019330. Experimental work performed at the National High Magnetic Field Laboratory is supported in part by the National Science Foundation (under DMR-1644779) and the State of Florida.

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CAROTENOID RADICALS: STRUCTURE AND PROPERTIES DETERMINED FROM HIGH FIELD EPR MEASUREMENTS AT NHMFL

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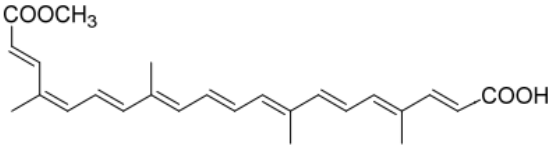
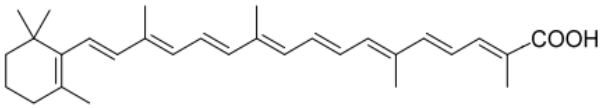
The continuous support from the U.S. Department of Energy from 1986 until May 2014 enabled the structures and properties of carotenoids to be established. A book describing these results, including the used EPR techniques in characterization of carotenoid radicals, is in progress.[1] Among the numerous EPR techniques that have been employed on carotenoid radicals, there are several high frequency (95-670 GHz) measurements done at NHMFL that will be described here.[2] Measurements at 327-670 GHz were used to demonstrate that the symmetrical unresolved EPR line at 9 GHz was due to a carotenoid π-radical cation. High field EPR measurements also demonstrated the incorporation of metal ions like Fe(III), Ni(II), or Al into the mesoporous MCM-41 molecular sieves and the photo-oxidation of carotenoids occurring in this framework. Replacement of Si(IV) in the mesoporous MCM-41 molecular sieves by metal ions allowed the study of electron transfer from the carotenoid to the metal electron acceptor sites.

Because of the increased awareness of the involvement of carotenoids in survival of photosynthetic plants, and their association with numerous health benefits, characterization of carotenoid radicals using EPR among other techniques, is of considerable importance. Scientists are continuously looking for answers and applications. For example, recently, two naturally occurring carotenoids with carboxyl groups, bixin and β-apo-8'-carotenoic acid (structures shown below), were found to exhibit remarkable anti-SARS-CoV-2 activity.[3] We studied these two carotenoids electrochemically and they both exhibit high oxidation potentials, bixin having the highest oxidation potential measured up to date.[4]

It is extremely important to note that only a very small fraction of the about 1200 of carotenoids known to date were studied and are still being studied. There is a great deal of knowledge to be gained from studying carotenoid radicals, their reactions in vitro and in vivo, and their biological mechanisms of action. Such studies are essential if the complex functions of the ubiquitous natural carotenoids are to be understood.



bixin



β-apo-8'-carotenoic acid

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LOSS OF CHROMIUM(III) FROM MIXED METAL Cr(III), Fe(III)-TRANSFERRINS

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Trivalent chromium has been shown to be transported *in vivo* from the bloodstream to the tissues via endocytosis by transferrin (Tf), the major iron transport protein in the blood. Recent *in vitro* studies using $Cr(III)_2$-Tf have shown that under physiologically relevant conditions, the binding of Cr(III) to Tf and the loss of Cr(III) from the $Cr(III)_2$-Tf/Tf receptor complex are rapid. However, the major form of transferrin in the bloodstream is mono-ferric Tf. Thus, given the low concentration of Cr(III) in the bloodstream, the form of Cr(III)-containing Tf in the bloodstream that is transported via endocytosis is monochromic, monoferric-Tf (Cr(III),Fe(III)-Tf). Given that Tf has two specific metal-binding sites, one in both the C-terminal and the N-terminal lobes of Tf, two forms of Cr(III),Fe(III)-Tf can form. The loss of Cr(III) from both forms of Cr(III),Fe(III)-Tf have been examined for the first time. The mixed-metal transferrins loose Cr(III) in similar fashions to Cr(III) losses from $Cr(III)_2$-Tf.

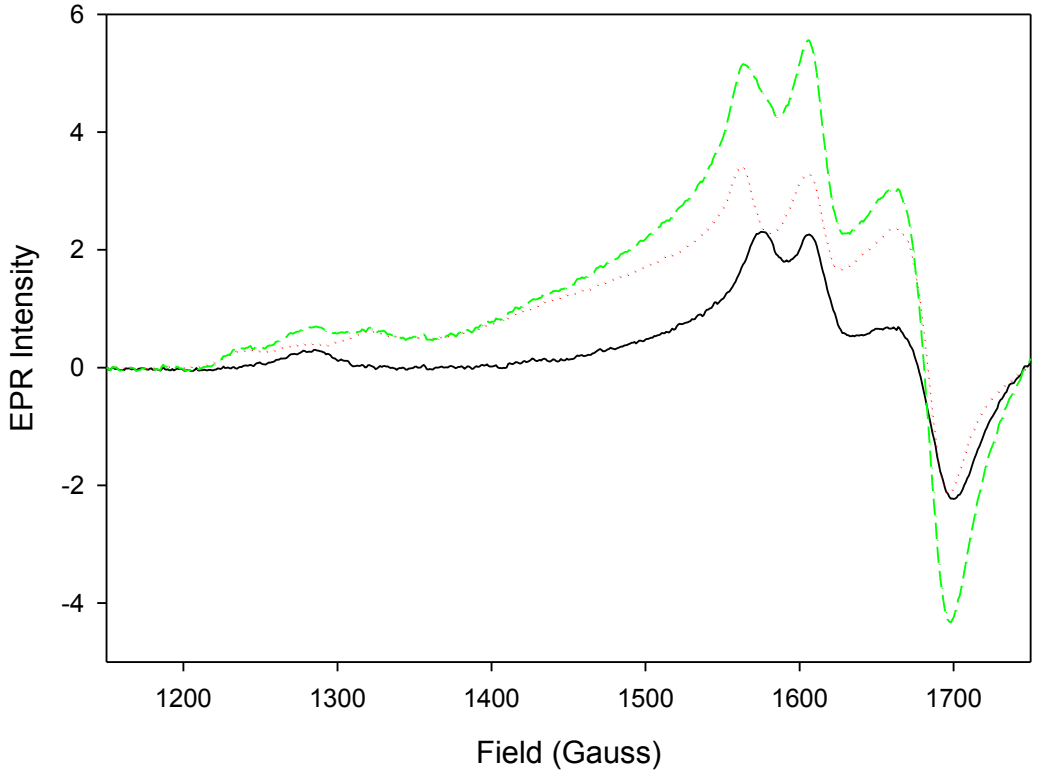

Figure 1. X-band EPR spectra of human serum Cr(III),Fe(III)-Tf's in 100 mM HEPES with 25 mM HCO_3^- at 37 ºC. Red, dotted line - Cr(III) in the N-lobe binding site. Solid black line - Cr(III) in the C-lobe binding site. Dashed green line - Sum of spectra of mixed Cr(III),Fe(III)-Tf's. Features at approximately 1240 and 1320 G (g = 5.1 and g = 5.6, respectively) correspond to Cr(III) in the N-lobe metal-binding site. The feature at approximately 1280 G (g = 5.4) corresponds to Cr(III) in the C-lobe metal-binding site. Features between 1,375 and 1,750 G arise from the Fe(III) centers.

INVESTIGATION OF THE SPIN-CROSSOVER TRANSITION IN A METALORGANIC Mn^{3+} COMPLEX WITH CONTINUOUS-WAVE HIGH-FIELD POWDER EPR SPECTROSCOPY

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Magnetoelectrics are a class of multiferroic material whereby coupling between the electric and magnetic properties are present. Spin crossover (SCO) metalorganics make for appealing magnetoelectric candidates due to the relative ease with which the electron occupancy of the atomic d-orbitals can be altered via external stimuli, such as temperature, pressure, electric field, magnetic field, and optical irradiation. In turn, changes in metal-ligand bond lengths are strongly correlated with the change in occupancy and overall spin state of the ion. Coupling between the molecular magnetic properties and the charge/elastic degrees of freedom of the lattice in these systems yield magnetic/charge bistability and, more importantly, a strong spin-lattice component that drives cooperativity via local lattice strains. Thus, spin-lattice coupling allows for rapid propagation of spin switching to adjacent sites when one site begins to undergo a spin state change, allowing for large changes in magnetic susceptibility with relatively small perturbations.

In this investigation, a metalorganic Mn^{3+} SCO complex that undergoes a complete transition from a high-spin (HS) $S = 2$ state to a low-spin (LS) $S = 1$ state below a sharp transition temperature ($T_{1/2} = 51$ K; with < 10 K hysteresis) was studied using continuous-wave high-field powder electron paramagnetic resonance (EPR) spectroscopy. Magnetic anisotropy in d-block transition metals is dominated by spin-orbit coupling, which admixes crystal field states, leading to anisotropic g and zero-field splitting, D, tensors in the effective spin Hamiltonian. In some SCO complexes, it can be too energetically costly to convert all of the sites in the lattice. This results in an inhomogeneous mixture of phases below the transition temperature, complicating the characterization of the EPR spectrum. Therefore, with the advantage of studying a complex exhibiting a complete and sharp SCO transition at temperatures amenable to EPR characterization, the ZFS parameters were obtained for both the LS ($D = +21.23$ cm^{-1}, $E = +2.275$ cm^{-1}) and HS states ($D = +5.66$ cm^{-1}, $E = +1.31$ cm^{-1}).

This work was supported by the US National Science Foundation (NSF DMR-2004732) and the Science Foundation of Ireland. Work performed at the National High Magnetic Field Laboratory is supported by the NSF (DMR-1644779) and the State of Florida.

TUNABLE CLOCK TRANSITIONS IN LANTHANIDE COMPLEXES FOR QUANTUM INFORMATION TECHNOLOGIES

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Bottom-up chemical synthesis of molecular spin qubit architectures represents a novel way for pursuing next-generation quantum technologies that could substantially influence all fields of human activity from complex structural biology to finance.[1,2] Our work focuses on fine-tuning resonant clock transitions (CTs) within $4f^n5d^1$ Ln(II) complexes, such that the associated transition frequencies, f, are insensitive to the local magnetic induction, B_0, with $\mathrm{d}f/\mathrm{d}B_0 \rightarrow 0$ at the CT minimum. This offers protection from magnetic noise and up to 10 times longer phase memory times, T_m, compared to conventional EPR transitions.[3] As an added bonus, hyperfine CTs associated with significant *s-d* mixing in $4f^n5d^1$ Ln(II) complexes minimizes spin-orbit coupling, leading also to enhanced spin-lattice relaxation times, T_1.[4]

This work was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences at LBNL under Contract No. DE-AC02-05CH11231.

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INVESTIGATING THE IMPACT OF CHARGE DISTRIBUTIONS ON FOLDED AND UNFOLDED CONFORMATIONS OF IA3

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The YPRA inhibitor, IA3 of Saccharomyces cerevisiae is an IDP consisting of 68 amino acid residues that form an alpha-helix when bound to YPRA. The helical structure traditionally obtained when bound to YPRA can be mimicked in the presence of the secondary structure stabilizer, TFE. Previous studies demonstrated that the alpha-helix of YPRA-bound IA3 remains in the N-terminus (residues 2-34) of the protein, leaving the C- terminus (residues 35-68) structurally unresolved and disordered. Site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR), as a robust method for characterizing the dynamics of biological macromolecules, showed site-specific variations in the helical character of the N-termini region with a constant degree of helicity of the C-terminus. Further comparison with CD spectroscopy reveals that the degree of helical transition in the C-terminus is not affected by mutations. X - Band Overhauser Dynamic Nuclear Polarization (ODNP) is a low-field method that combines CW - EPR and NMR techniques to amplify the 1H NMR signal of local water molecules within 5-10 Å of an electron spin probe. that upon a TFE-induced transition, the diffusive water dynamics surrounding the surface of IA3 remains minimally affected, but water is restricted near the surface of the protein. The changes to water environments from bulk-like properties in the absence of TFE, to buried properties in 30% (v/v) TFE was similarly reflected in both the N- and C- termini. Through ODNP, it was determined that differences between the N- and C- termini were strikingly apparent in the absence of TFE, where high-magnitude bulk-water properties were observed through the C-terminus. Taken together these results with computational simulation data suggest that the ordered and disordered conformations of the C-terminus can be likely impacted by the charge distribution along its amino acid sequence.

GENERATION OF HYDROXYL RADICAL FROM REACTION OF HYDROGEN PEROXIDE WITH PROTEIN THIOLS

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Hydrogen peroxide (H_2O_2) is innocuous to most biomolecules, that lack a transition metal center, in contrast to other reactive oxygen species (ROSs)[1]. Emerging evidence shows that H_2O_2 plays a crucial role in numerous cellular redox regulation and signal transduction events[2]. However, the cysteine thiol group (-SH) in proteins can be directly oxidized into sulfenic acid (-SOH), through a two-electron transfer process, and to further oxidized forms[1]. These site modifications are of great significance for structural proteomics and protein interaction characterization[3]. The hydroxyl radical (HO•), has been identified from the reaction of H_2O_2 with small molecules containing the thiol group, which may help to elucidate oxidation mechanisms involving H_2O_2[4]. It is not known whether HO• formation is general in the reaction of intrinsic protein thiols with H_2O_2. The challenge of detection and quantification of short-lived radicals, such as HO•, has been overcome by using the spin trap method, which effectively extends radical lifetime and affords line shape identification of the trapped species. Herein, time-resolved electron paramagnetic resonance (EPR) spectroscopy of the protein-external spin trap, DEPMPO, is employed to demonstrate HO• generation in the reaction between H_2O_2 and thiols in different proteins, and to characterize the mechanism, under controlled confinement conditions[5] in frozen H_2O_2 aqueous solution. Supported by NIH R01 GM142113.

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INTER-SPIN STATE TRANSITIONS IN HIGH-FIELD EPR SPECTRA OF COPPER CUBANE TETRAMERS

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Several tetrameric cubane Cu(II) complexes $[Cu_4L_4(bae)_4]\cdot nH_2O$ were prepared, where bae is the anion of 2-benzylethanolamine and L represents various carboxylic acids. The compounds are dimers of dimers, with stronger ferromagnetic interactions within the dimers and weaker interactions between them. The exchange interactions in Cu(II) tetrameric systems lead to the formation of a quintet state with spin S=2, three triplet states with S=1 and two singlet states with S=0 [1]

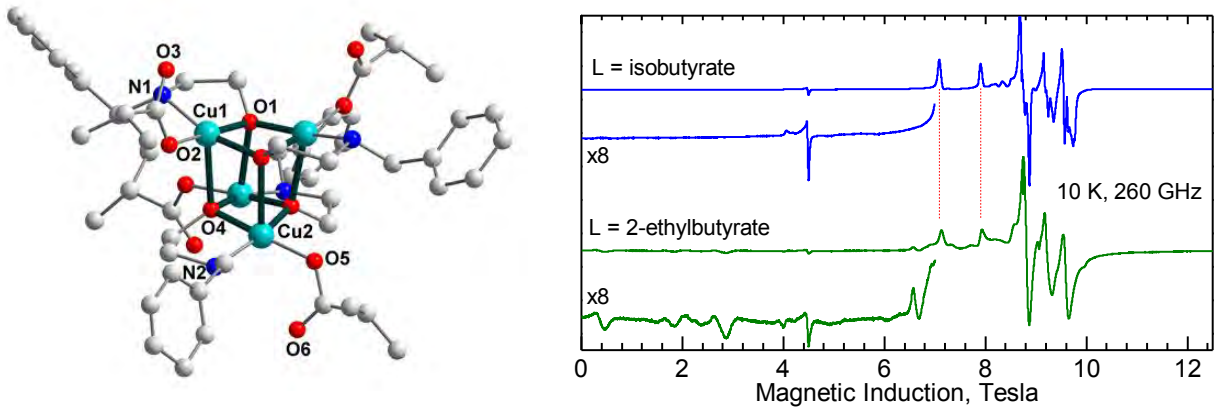

Figure 1. Left: The molecular structure of the complex with L=isobutyrate; Right: HFEPR spectra of two tetramers with different L ligands.

The ground state in present compounds is a spin quintet, as revealed by the magnetic susceptibility measurements and the high-field, high-frequency EPR (HFEPR) spectra (Figure 1). The L=isobutyrate complex exhibited the strongest ferromagnetism in this series and its HFEPR spectra could be simulated using a standard spin Hamiltonian for S=2. In other complexes, resonances at very low magnetic field were observed at very high frequencies, which cannot correspond to the transitions within either the S=1 or S=2 spin states and are therefore thought to occur between the ground S=2 state and other spin multiplets (see spectrum at the bottom of Figure 1). Considerable intensity of these nominally forbidden transitions indicates that the antisymmetric exchange (Dzialoshinskii-Moriya) interaction [2] may be operative in our non-centrosymmetric molecules.

Acknowledgements The National High Magnetic Field Laboratory is supported by National Science Foundation through NSF/DMR-1644779 and the State of Florida.

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ELECTRON PARAMAGNETIC RESONANCE STUDIES OF TRANSITION METAL PHTHALOCYANINES

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Transition metal phthalocyanines (MPCs) possess remarkable thermal and chemical stability, and are promising non-precious metallic catalysts, including for the oxidation reactions. EPR spectroscopy can give to insight into transition metal oxidation state and the electronic and molecular structure of catalytic states. For X-band EPR at a microwave frequency of ≈ 9.5 GHz, in liquid nitrogen the phthalocyanine is at the rigid limit. If the MPC is well dispersed in a glassy solvent, well resolved spectra can be obtained. Well resolved cw-EPR spectra are obtained using concentrated sulfuric acid, that dissolves most of MPCs and gives a good glass when frozen. However, the corrosive nature of this solvent makes alternatives solvents attractive. Here, we report a study of alternative solvent systems. Various mixtures of pyridine, quinoline, glycerol, ethylene glycol, DMF, and ethanol were studied that dissolve the MPCs and give a good glass. For an instance: Copper phthalocyanine disulfonate gave a well resolved spectra in pyridine/water and pyridine/ethylene glycol in 1:1 respectively. Similarly, cobalt phthalocyanine disulfonate in pyridine/EtOH mixtures in various ratios, gave well resolved spectra. Cobalt perfluorophthalocyanine in DMF/glycerol and DMF/ethylene glycol to some extent gave satisfactory spectra. The experimental spectra were then simulated using EasySpin in MATLAB as well as SpinCount.

SYNTHESIS OF GALLIC ACID-CONJUGATED PAMAM DENDRIMERS FOR AGE-RELATED MACULAR DEGENERATION - ANTIOXIDANT PROPERTIES DETECTED BY EPR

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PAMAM (Polyamidoamine) dendrimers are attracting significant interest in a variety of research fields, particularly medicinal compound delivery.[1,2] The versatile applications of PAMAM dendrimers are due to their ability to conjugate with various functional molecules on their surface. The purpose of our research is to synthesize antioxidant dendrimers with gallic acid conjugated on the PAMAM dendrimer surfaces. This may enhance the radical scavenging of intracellular reactive oxygen species (ROS), which will potentially retard age-related Macular Degeneration (AMD) progression.

TEMPOL and DPPH free radicals were used to investigate the radical quenching properties of PAMAM dendrimers using quantitative EPR (Electron Paramagnetic Resonance) spectroscopy. The radical quenching effect can be observed by the reduction of the EPR spectra and/or other properties of the free radicals in aqueous solution as a function of antioxidant concentration.[3] This poster will present our first results on the antioxidant properties of gallic acid-PAMAM conjugates.

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54 GHz CLOCK TRANSITION IN A Ho(III) COMPLEX

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Molecular lanthanide complexes are promising candidates for development of next-generation quantum technologies [1]. In particular, high-symmetry structures can give rise to well-isolated crystal-field quasi-doublet ground states, i.e., quantum two-level systems that may serve as a basis for spin qubits. More importantly, recent work has shown that the coordination environment around the lanthanide can be tailored to produce an avoided crossing, or clock transition within the ground doublet, where the first-order sensitivity to fluctuations in the local magnetic field is suppressed, leading to significantly enhanced coherence times [2]. Here, we employ single-crystal high-frequency electron paramagnetic resonance (EPR) spectroscopy to interrogate a new molecular Ho(III) complex. An axial coordination environment with distorted D_{4d} symmetry gives rise to a ground state $m_J = \pm 4$ crystal-field quasi-doublet with a 54 GHz clock transition, where m_J denotes the projection of the $J = 8$ spin-orbital moment associated with the Ho(III) ion. These states are further split into eight $(2I + 1)$ sub-levels due to the hyperfine interaction with the $I = 7/2$ nuclear spin (100% natural abundance).

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WIDEBAND FOURIER-TRANSFORM-DETECTED EPR AT W-BAND

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We report true wideband Fourier transform (FT) EPR detection capabilities for a quasioptical 94 GHz (W-Band) spectrometer with 1 kW peak power. It is based on the state-of-the-art pulsed HiPER spectrometer developed at the University of St. Andrews [1], into which we have integrated an arbitrary waveform generator (AWG) that is used to modulate the phase and amplitude of the microwaves prior amplification. A schematic of the AWG implementation in the HiPER spectrometer is shown in Figure 1. The spectrometer employs a non-resonant sample-holder providing an instantaneous bandwidth of 1 GHz. Benchmark experiments are presented for the standard TEMPOL Radical, which consists of a 500 MHz wide EPR spectrum at W-Band. We demonstrate an efficient inversion of this broad inhomogeneous spectrum using a single adiabatic chirp pulse, facilitating frequency dependent studies of longitudinal magnetization recovery in the time domain, via FT-detected EPR. An anisotropy in the spin-lattice relaxation time, T_1, of the TEMPOL radical is determined from these measurements. Additionally, the FT-detection scheme was implemented for multi-dimensional experiments such as CHEESY-detected NMR [2], demonstrating the full capabilities of the spectrometer.

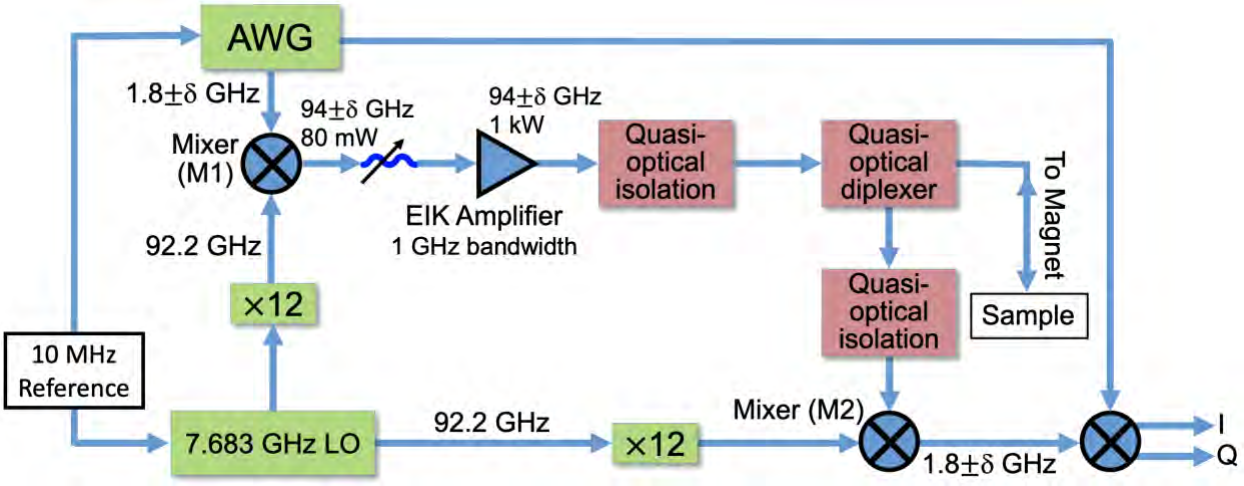

Figure 1. A schematic of the AWG implementation in the W-band HiPER spectrometer.

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MULTIFREQUENCY PULSED EPR IN THE 120-400 GHz RANGE

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At high fields and low temperatures both the electron spin lattice relaxation time (T_1) and spin-spin Relaxation time (T_2) can change dramatically with respect to those at lower fields. Relaxation in spin systems is of crucial interest with respect to various possible applications like quantum information processing, information storage, spintronics, and dynamic nuclear polarization (DNP). High frequencies and fields in combination with low temperatures polarize the electron spins, and allow for considerably longer spin memory times at high fields and frequencies as compared to X-band [1] for more concentrated spin systems. On the other hand, high frequencies lead to a significantly increased contribution from direct single phonon processes in the spin-lattice relaxation, and at low temperatures T_1 can be a few orders of magnitude shorter at fields of the order of 10T with respect to the typical 0.3 T at X-band frequencies.

At the NHMFL, users have the opportunity to explore spin-relaxation at a range of frequencies and fields. Here we provide an overview and description of the spectrometers available, and discuss results of frequency dependent spin-lattice relaxation measurements in a variety of spin systems.

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CONFINEMENT DEPENDENCE OF PROTEIN-COUPLED SOLVENT DYNAMICS FOR DIFFERENT CLASSES OF PROTEINS

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Solvent-protein dynamical coupling and the degree of confinement in the surrounding solvent modulate protein function.[1-3] The protein class, size and topology dependence of the solvation dynamics and the influence of confinement are characterized by using the rotational correlation time, t_c, of the electron paramagnetic resonance (EPR) spin probe, TEMPOL, which is restricted to regions vicinal to protein in frozen aqueous solution.[4,5] Weak (protein in fluid aqueous-dimethylsulfoxide cryosolvent mesodomain) and strong (no added crysolvent) conditions of ice boundary confinement are addressed over the wide temperature, *T*, range of 200 – 265 K. The panel of soluble proteins represents large oligomeric (ethanolamine ammonia-lyase, 488 kDa), small oligomeric (streptavidin, 52.8 kDa) and monomeric (myoglobin, 16.7 kDa) globular proteins, an intrinsically disordered protein (IDP, β-casein, 24.0 kDa), and two nominally unstructured peptides (protamine, 4.38 kDa; amyloid-β fragment, residues 1-16, 1.96 kDa). The spin probe method resolves expanded and condensate structures of β-casein and protamine under weak and strong confinement, respectively. Strong confinement induces emulation of globular protein behavior by the amyloid-β peptide. The soluble globular proteins display common *T*-dependences of rotational correlation times and normalized weights under each confinement condition, for two mobility components: protein-associated domain, PAD, and surrounding mesodomain. Thus, confinement uniformly influences the PAD dynamics of soluble globular proteins, and is therefore a generic control parameter for modulation of soluble globular protein function.[6] Supported by NIH GM142113.

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PROTEIN AND COUPLED SOLVENT DYNAMICS OF OLIGOMERIC AND FIBRILLAR ALPHA-SYNUCLEIN

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Alpha-synuclein (α-syn) is a protein (140 amino acids, 14.5 kDa) that has an, as yet, unspecified functional role in neurotransmitter release in brain neurons, and dysfunction, that is associated with Parkinson's disease (PD) pathology in humans and animal models.[1] α-Syn is an intrinsically-disordered protein (IDP), whose primary structure includes three domains: an N-terminal domain (NTD), “non-amyloid-β component” (NAC), and a dynamically disordered C-terminal domain (CTD). Toward understanding the role of protein and coupled solvent dynamics in α-syn function and dysfunction, our established electron paramagnetic resonance (EPR) spin probe (TEMPOL) methodology was applied.[2,3,4] Controlled-temperature ice-boundary confinement in frozen aqueous solutions of induced oligomeric and pre-formed fibrillar α-syn was used to localize TEMPOL to probe solvent phases specifically associated with α-syn. TEMPOL mobility in the presence of α-syn shows two distinct components at all temperatures from 220 – 265 K, as for soluble globular proteins,[5] but with dramatically higher fluidity. The temperature-dependence of the spin probe rotational correlation times and component weights, and hysteresis in these parameters for directional temperature change, are interpreted in terms of a high-fluidity, aqueous-CTD mesophase, and protein-associated domain (hydration layer). The results are relevant to the function of α-syn under conditions of high confinement in the neuron presynaptic terminal, and dysfunction, that involves oligomer and fibril permeation of phospholipid bilayer membranes. Supported by NIH GM142113.

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COMPARATIVE STUDY OF CELL SURFACE α2,3- AND α2,6-SIALOGLYCANS BY ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY

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In this study, α2,6- and α2,3-sialoglycans on HeLa cells were spin-labeled for the study of EPR spectroscopy with a click reaction between an azide-modified sialic acid (Neu5Ac9N3) installed through enzymatic glycoengineering (EGE) and our previously reported DBCO-SL. The α2,6-sialyltransferase (ST) Pd2,6ST and α2,3-ST CSTII were utilized for EGE to install α2,6- and α2,3-linked Neu5Ac9N3, respectively. Analysis of the EPR spectra showed both types of sialoglycans possess intermediate- and fast-motion components which indicated these glycans are surrounded by diverse packing environments in the cell glycocalyx or extracellular matrix. Results also demonstrated different distributions of the two motion components of these glycans, i.e., a higher average population of the slow-motion component for α2,6-sialoglycans (78%) than that for α2,3-sialoglycans (53%), further suggested Pd2,6ST and CSTII can target different glycans or different sialylation sites on the cell surface. These results are biologically important as they are useful for interpreting the different functions of α2,6- and α2,3-sialoglycans.

TOWARDS THE DESIGN AND IMPLEMENTATION OF A DNP-NQR SPECTROMETER

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Nuclear quadrupole resonance (NQR) is a spectroscopic technique that is similar to NMR, but can only be applied to quadrupolar (*i.e.,* spin > ½) nuclei. ^{14}N NQR has been previously implemented for the study and characterization of small organic compounds such as amino acids, pharmaceuticals, narcotics, explosives, *etc*. and is a promising tool for an inexpensive and high-throughput package scanner for illicit materials. However, NQR is an inherently insensitive spectroscopic technique with respect to the amount of detectable signal - far lower than NMR. To date, NQR is rarely utilized as a routine spectroscopic tool, despite the increasing interest in solid-state NMR (SSNMR) spectroscopy for studying small molecules. State of the art SSNMR techniques have successfully implemented dynamic nuclear polarization (DNP) for enhancing signal by several orders of magnitude in some cases. DNP relies on the transfer of spin polarization from electrons, e^-, in stable radicals to ^{1}H nuclei under the right conditions. If subsequent transfer to ^{14}N can be accomplished efficiently, then similar order-of-magnitude signal enhancements can be achieved in NQR spectra (*i.e.,* DNP-NQR).

Herein, I will describe and demonstrate the key aspects of the design of a DNP-NQR spectrometer including: (i) low-field S-band (*ca.* B_0 = 71 mT, $\nu_0(e^-)$ = 2 GHz, $\nu_0(^1H)$ = 3 MHz) Overhauser and solid-effect DNP-NMR; (ii) $^1H \rightarrow {}^{14}N$ spin polarization transfers and sensitivity enhancement in NQR spectra; and (iii) combining these processes to meditate full $e^- \rightarrow {}^1H \rightarrow {}^{14}N$ transfers and realize DNP-NQR. Crucial to the design of this spectrometer is the use of low electric-field (low E) inductors for DNP, such as loop-gap resonators (LGR) and Alderman Grant coils (AGC). These inductors cause minimal sample heating compared to conventional solenoids and enable stable and efficient DNP near ambient conditions. ^{1}H SSNMR spectra must be measured to monitor solid-effect DNP, but it is challenging at $\nu_0(^1H)$ = 3 MHz. This is accomplished by using a low dead-time transceiver circuit in conjunction with the steady-state free-precession (SSFP) pulse sequence to enable single-shot acquisitions at low field.

DNP-NQR methods can result in low-cost chemical characterization and screening of many organic and inorganic materials. Access to fast and routine measurement strategies will open up the field of NQR to a wide variety of researchers in chemistry, physics, and materials science.

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DNP NMR ASSISTED 'IN-CELL' STRUCTURAL CHARACTERIZATION OF MITOCHONDRIA LOCALIZED α-SYNUCLEIN TOXIC CONFORMERS

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α-Synuclein's (α-Syn) role in modulating mitochondrial function in both physiological and pathological conditions has long been a topic of intense debate.[1-3] This pre-synaptic protein, whose misfolding and neurotoxic aggregation into Lewy bodies represent the pathological trade of Parkinson's,[4,5] mainly localizes to mitochondria[6] and causes mitochondrial impairment.[7-9] Although many studies have already emphasized α-Syn's role in neurotransmission, the mechanism of α-Syn mediated mitochondrial toxicity and peripheral synaptic loss is still debated. One prevailing hypothesis states that neurotoxicity arises through α-Syn directly interacting with and disrupting mitochondrial membrane and complex-I activity, ultimately leading to ROS mediated oxidative stress.[10] Unfortunately, studying protein structures that are meta-stable and/or dynamically interchanging inside cells like α-Syn, is very challenging with most traditional structural methods.[11,12] Besides, the cell's interior atmosphere and complexity which control cellular functions is hard to replicate under in-vitro conditions. We accomplished a novel cellular platform with sensitivity-enhanced DNP-NMR that allows studying large proteins' structure, interaction, and chemical modification directly inside living mammalian cells.[13] Using this powerful structural tool, I aim to determine α-Syn's conformational ensemble with mitochondrial toxicity inside live cells and reveal how cellular environments such as membrane, chaperon depletion influences protein's amyloid core structure. We can determine protein's toxic/non-toxic conformers tightly coupled to genotypes, phenotypes, and environments with this 'In-cell' DNP experimental system. Collectively, this structural information in live cells will directly link cellular toxicity to protein conformation and will transform our mechanistic understanding of protein misfolding and mitochondrial impairment in Parkinson's etiology.

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STRUCTURE OF FUNGAL CELL WALLS AND REMODELING BY ANTIFUNGAL DRUGS ELUCIDATED USING SOLID-STATE NMR

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Invasive fungal infection has high occurrence and mortality among immunocompromised patients. Most of the currently available antifungal agents have limited efficacy, relatively high toxicity. The carbohydrate-rich cell wall is a promising target for antifungal drugs, but we lack in-depth knowledge of the structure, assembly, and dynamics of cell wall biomolecules. To overcome this barrier, we have explored the use of magic angle spinning (MAS) solid-state NMR (ssNMR), a nondestructive and atomic-resolution technique combined with 3D experiments and dynamic nuclear polarization, for characterizing the polysaccharides in intact and living fungal cells.

First, we investigated the structural heterogeneity of chitin and chitosan using a massive set of chemical shift data, with the interpretation assisted by principal component analysis (PCA) and linear discriminant analysis (LDA). The structure of chitin is found to be intrinsically heterogeneous, with unique fingerprints documented across six fungal species including *Aspergillus fumigatus*, *Aspergillus sydowii, Aspergillus nidulans*, *Rhizopus delemar*, *Candida albicans* and *C. auris.* Commercially available antifungal drugs and variation in salts concentrations did not significantly perturb the chemical shifts, revealing the structural resistance of chitin to stresses. Two and three-dimensional (2D and 3D) solid-state NMR have made major inroads in overcoming barriers to structural elucidation in isotopically (^{13}C) enriched plant and fungal cell walls comprising mainly of complex carbohydrates. Yet, these efforts are partially hampered by heavily congested regions even in the 3D spectra. Replacing the two indirect single quantum (1Q) dimensions with INADEQUATE 2Q-1Q dimensions, followed by a CORD mixing, eliminates the body diagonal of the 3D cube and relieves congestion, ushering in dramatic resolution enhancement in fungal cell wall spectra. Finally, we used an innovative approach combing the ssNMR with the dynamic nuclear polarization (DNP) technique to characterize the cell wall organization in different morphotypes varied along with the life cycle and growth conditions, revealing a highly conserved carbohydrate core in both conidia and mycelia [3].

These studies and the biophysical technique yield essential information about carbohydrate components and structures of the cell walls and their packing interfaces at atomic levels that can serve as potential targets for discovering novel antifungal compounds with broad spectrums and improved efficacy.

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PROTON-OBSERVED ^{13}C OVERHAUSER DNP AT 14 T

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DNP is a very efficient method to hyperpolarize solids at cryogenic temperatures to increase NMR's sensitivity. On the other hand, DNP in liquids is challenging, particularly at high magnetic fields, where resolution is needed to solve a wide variety of scientific questions. Liquid DNP via the Overhauser effect relies on either large scalar coupling between a free radical and the nuclei of interest, or strong dipolar coupling which requires fast correlation times. In this work, we have developed a large sample volume (≥100 µL) double resonance (^{1}H, ^{13}C) liquid NMR probe (Fig 1.) capable of irradiating a sample with a high-power microwave beam from a gyrotron operating at 395 GHz (14.1 T). A microwave B_1 field of 0.5 $G \cdot W^{1/2}$ was estimated using in-situ pulsed EPR with micro-diamonds. Using this newly developed probe we applied it to conduct scalar ODNP experiment, that polarize mostly the nuclei of heavier, electron-rich atoms. These leaves ^{1}H NMR outside the realm of direct application. This work also presents an experiment that can deliver ^{1}H-detected NMR experiments, such as INEPT and HSQC, while relying on scalar ODNP enhancements from ^{13}C. This is achievable thanks to the polarization transfer mechanisms relying on one-bond J_{CH}-couplings. Such $^{13}C \rightarrow {}^{1}H$ polarization transfer, which then enabled acquisitions at higher frequencies and with higher sensitivities than what a direct ^{13}C ODNP detection would provide while utilizing both the Overhauser effect as well as the ^{1}H-^{13}C nuclear Overhauser effect. For example, for a model solution of labeled $^{13}CHCl_3$ comixed with a nitroxide-based TEMPO derivative as polarizing agent (fig.2), an enhancement factor of 30 (^{13}C) could thus be imparted to the ^{1}H signal. In general, extensions of this approach might prove advantageous for enhancing even further the sensitivity provided by ODNP.

MECHANOCHEMICAL SYNTHESES OF PHARMACEUTICAL COCRYSTALS AND THEIR STRUCTURAL CHARACTERIZATION USING ^{35}Cl SOLID-STATE NMR AND DISPERSION-CORRECTED DFT CALCULATIONS

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The solid forms of active pharmaceutical ingredients (APIs), such as polymorphs, cocrystals, and hydrates, have distinct physicochemical properties (*e.g.,* stability, crystallinity, and solubility), which can affect their bioavailability, shelf lives, and general utility as useful drug products. These properties are dependent on their molecular-level structures; as such, it may be possible to select for desirable properties via modification of the solid forms with targeted synthetic procedures. HCl salts of APIs are the most common solid forms, many of which have with desirable properties; however, these properties can often be further improved by the production of *pharmaceutical cocrystals* (PCCs). For this reason, cocrystalline forms of APIs, which feature the API and a pharmaceutically acceptable coformer (PAC), are highly sought after.[1-4]

In 2019, Borodi *et al.* demonstrated syntheses of PCCs of promethazine HCl using mechanochemical and slow evaporation methods with four carboxylic acid coformers: fumaric acid, succinic acid, adipic acid, and oxalic acid.[5] These PCCs were characterized using a combination of DSC, IR, and XRD; however, SSNMR may offer new insights into understanding their mechanisms of formation and solid-state properties, which is key in the rational design of PCCs.

Herein, we describe the use of ^{35}Cl solid-state NMR (SSNMR) spectroscopy for probing the structures of known promethazine HCl PCCs,[6] and applying similar methods for the structural elucidation of novel PCCs of promethazine HCl and chlorpromazine HCl. First, we discuss mechanochemical syntheses of all the PCCs and demonstrate (i) the advantages of mechanochemical methods over previously reported synthetic procedures, (ii) the optimization of mechanochemical syntheses to maximize yield, and (iii) the application of these optimized protocols to the discovery of novel PCCs. Second, we use ^{35}Cl SSNMR to obtain spectral fingerprints of each API and their corresponding PCCs. Each solid form has Cl^- environment(s) with unique hydrogen bonding networks, and therefore, distinct sets of ^{35}Cl electric field gradient (EFG) tensor parameters, which in turn lead to clearly distinguishable central transition ($+1/2 \leftrightarrow -1/2$) powder patterns influenced by the second-order quadrupolar interaction. Lastly, experimentally measured ^{35}Cl EFG tensors are compared to those calculated using dispersion-corrected plane-wave density functional theory (DFT) methods[7] in order to (i) refine the crystal structures of known PCCs, and (ii) use this information to help elucidate the molecular level structures of new PCCs.

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COUPLING SOLID-STATE NMR AND MOLECULAR DYNAMICS SIMULATIONS TO INVESTIGATE MEMBRANE PROTEINS

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Human inward rectifier potassium (Kir) channels set and reestablish the resting membrane potential in excitable cells. Cholesterol is a known allosteric regulator of these channels. In some protein families, including G Protein Coupled Receptors (GPCRs), cholesterol has a binding affinity for sequential protein motifs including cholesterol recognition/interaction amino acid consensus (CRAC) motifs, an inversion of the CRAC motif named the CARC motif, and cholesterol consensus motifs (CCM). However, while similar binding motifs appear in Kir channels, it is not clear what variations might appear in cholesterol binding clefts between transmembrane helices and between subunits. Thus, determining how cholesterol binds to transmembrane channels is a complex endeavor. Because it is difficult to conserve long-range order within a lipid membrane, many structural techniques such as Cryo- EM and X-ray crystallography are unable to elucidate native behaviors under physiological conditions. KirBac1.1 is a bacterial Kir channel homologous to human Kir channels and can be expressed from E. coli in NMR quantities. KirBac 1.1 is activated by anionic lipids, however, cholesterol is known to attenuate this mechanism. Recently, we characterized a structural basis for this attenuation, in which a dimer of cholesterol binds sufficiently close to occlude the binding pocket responsible for binding activatory lipids. Using distances derived from Solid-State NMR and a modified version of XPLOE-NIH we were able to construct a dimer of cholesterol and were able to dock this dimer to KirBac1.1. Here, we use a similar method to investigate the cholesterol activation method of the gated inwardly rectifying potassium channel GIRK2. Through the clever use of solvent accessibility NMR data and XPLOR-NIH we were able to open the pore of KirBac1.1, thus offering a first look at these channels in an open state

NETWORK FOR ADVANCED NMR: COMMUNITY ACCESS TO HIGH-FIELD NMR

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Network for Advanced NMR (NAN) is a partnership between UW-Madison, UGA, and UConn. Our mission is to provide expanded access to state-of-the-art NMR instrumentation to the scientific community. This includes remote access to current NMR spectrometers at NMRFAM (UW-Madison) and UGA, as well as two new 1.1 GHz instruments, solid-state at UW-Madison and solution-state at UGA, to be installed in late 2023 and early 2024, respectively. NMR data will be automatically archived on a storage system integrated with NMRbox for further processing and analysis. A web portal will be used for resource discovery, instrument time allocation, and search of public NMR data. NAN partner centers are developing knowledgebases (KBs) in the areas of solution structural biology, biological solid-state NMR, materials solid-state NMR, and NMR metabolomics. KBs will include validated parameter sets, pulse sequences and example data for commonly used NMR experiments, as well as workflows and protocols for sample preparation, data acquisition setup and processing.

NAN
Network for Advanced NMR

NEW DIRECTIONS FOR QUADRUPOLAR NMR CRYSTALLOGRAPHY ENHANCED CRYSTAL STRUCTURE PREDICTION (QNMRX-CSP)

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NMR crystallography (NMRX) combines information from solid-state NMR (SSNMR) spectroscopy, X-ray diffraction, and quantum chemical calculations for the prediction, refinement, and validation of crystal structures. NMRX studies largely depend on the accurate measurement and calculations of key NMR parameters (*i.e.*, chemical shifts and dipolar couplings). Of these, chemical shifts are the most widely used for NMRX studies.[1] In contrast, electric field gradients (EFGs) of quadrupolar nuclides (*e.g.,* ^{14}N, ^{17}O, ^{23}Na, and ^{35}Cl) are seldom used for NMRX protocols.[2] EFGs are highly sensitive to their surrounding electronic environments, which include both the short- and long-range interactions and/or non-covalent interactions (*e.g.,* hydrogen bonding, π-stacking, *etc.*) that do not strongly impact chemical shifts. In addition, EFG tensors are easier to calculate than chemical shifts, as the former are dependent only on the ground-state electron density.[3] The use of EFG tensors in NMRX/CSP protocols could provide a new route for the *ab initio* predictions and refinements of crystal structures. Our group has constructed an NMRX crystal structure prediction protocol based on quadrupolar nuclei, the first iteration of which uses ^{35}Cl EFG tensor parameters for the prediction, refinement, and validation of simple organic HCl salts. This protocol, dubbed QNMRX-CSP, has been benchmarked with five model organic HCl salts and has been tested on one blind system.

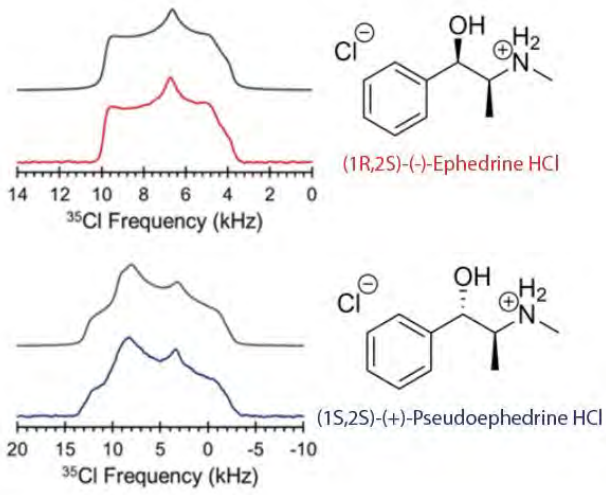

Figure 1. Experimental (bottom) and simulated (top) static ^{35}Cl SSNMR spectra of ephedrine and pseudoephedrine acquired at 21.1 T.

Herein, we discuss the QNMRX-CSP protocol, its applications to systems of increasing complexity, and new efforts in determining the uncertainties associated with atomic positions. Specifically, we describe the prediction of crystal structures of the diastereomers ephedrine HCl and pseudoephedrine HCl, which are clearly distinguishable by their ^{35}Cl spectra **(Figure 1)** and powder X-ray diffraction techniques. This represents a first step towards verifying that the protocol can differentiate between stereoisomers in the absence of single-crystal XRD data. Then, we report new methods aimed at generating ORTEP-like thermal ellipsoids that can be used to assess uncertainties in atomic positions generated by the protocol, thereby allowing direct comparison to structures determined from diffraction methods, along with their own measures of uncertainty. It is anticipated that this work will open new pathways to study a wide range of organic solids, with particular applications to HCl salts of active pharmaceutical ingredients.

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DFT/ZORA CALCULATIONS OF ^{195}Pt MAGNETIC SHIELDING TENSORS

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Quantum chemical calculations are invaluable for relating chemical shift tensors obtained from solid-state NMR measurements with molecular-level structure and dynamics. For heavy atoms like platinum, as well as for directly bonded light atoms, one must employ relativistic electronic structure approximations such as the zeroth-order regular approximation (ZORA) at the spin-orbit level to obtain agreement with experimental chemical shift tensors.[1] Additionally, when modeling NMR parameters for solids, calculations must consider long-range intermolecular effects to obtain the best agreement with experiment. Thus, the development of robust protocols for calculating the magnetic shielding tensors of heavy atoms in solids using relativistic density functional theory (DFT) is desirable.

One method of modelling extended structure in crystalline solids is to employ periodic boundary conditions, as is done in the gauge-invariant projector augmented wave (*i.e.*, GIPAW) method.[2] However, cluster-based methods can also be employed effectively to model long-range lattice effects; currently, two advantages that cluster-based approaches maintain over periodic calculations are (i) the ability to implement more advanced computational methods such as hybrid DFT and (ii) more rigorous relativistic treatments that include spin-orbit coupling.[3-7] Cluster-based calculations afford an opportunity to study the role of the ZORA spin-orbit Hamiltonian, as well as to explore the use of exchange-correlation functionals containing an admixture of Hartree-Fock exchange (*i.e.*, hybrid functionals). Furthermore, cluster-based models have been developed for both molecular solids and infinite network solids, allowing ^{195}Pt chemical shifts to be calculated for a wide array of materials.

In this work, I will discuss relativistic DFT calculations of ^{195}Pt magnetic shielding tensors, and consider the calculation of magnetic shielding tensors for light atoms bonded to platinum. We compare the results of GIPAW calculations with all-electron cluster-based calculations and evaluate the importance of relativistic effects by comparing calculations using the ZORA Hamiltonian at the scalar and spin-orbit levels. Finally, we contrast the results obtained through generalized gradient approximation functionals with those of hybrid functionals.

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ADVANCED INSTRUMENTATION FOR PARAHYDROGEN BASED HYPERPOLARIZATION: CLOSED-LOOP, CONTINUOUS-FLOW HYPERPOLARIZATION FROM PARAHYDROGEN AND HETEROGENEOUS CATALYSIS

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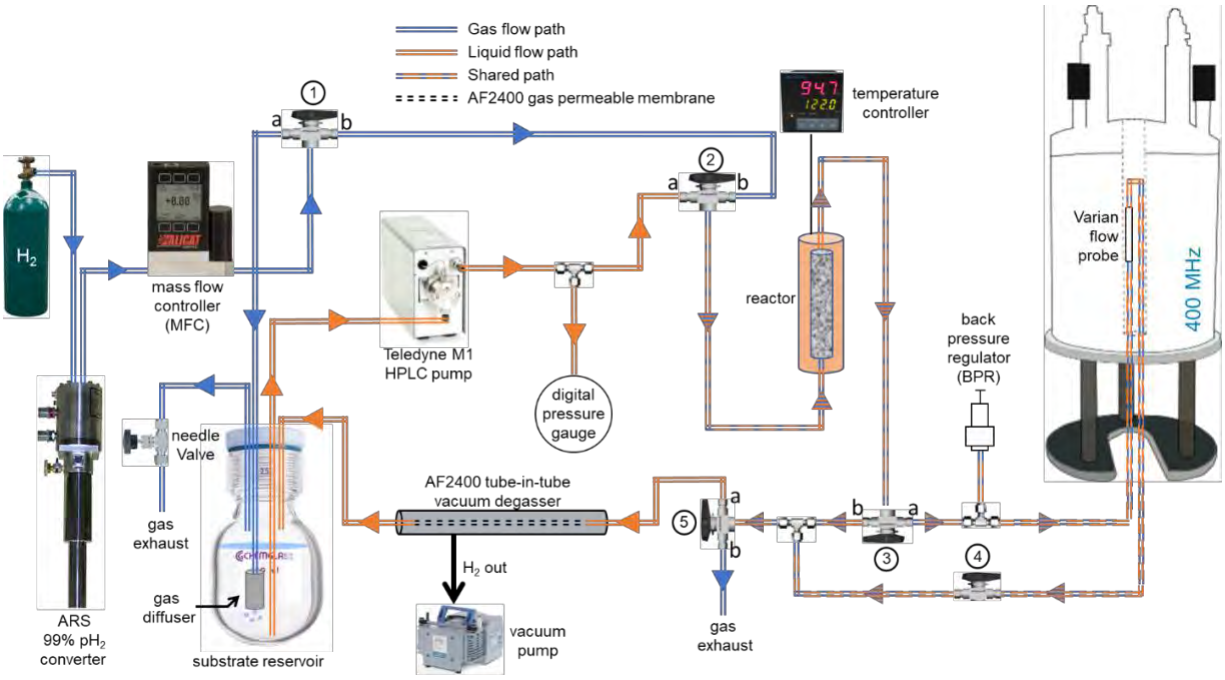
As part of the development of advanced facilities for parahydrogen-based hyperpolarization (UCGP funded) at the National Maglab, a novel closed loop, continuous flow (CF) reactor system for parahydrogen enhanced NMR of liquids via heterogeneous catalysis is introduced which enables extended operation with recycling of unreacted liquid precursor. This system incorporates an HPLC pump, a liquid substrate reservoir incorporating a gas diffuser, an all-metal packed bed catalytic reactor, and an AF-2400 tube-in-tube gas permeable membrane to remove normal H_2. Two types of supported metal nanoparticle catalysts were tested in this reactor system in the hydrogenation of propargyl acetate: mesoporous silica encapsulated Pt_3Sn intermetallic nanoparticles and Rh nanoparticles supported on anatase TiO_2. In the CF hydrogenation of propargyl acetate to allyl acetate, the hyperpolarized signals exhibited stability for over 20 minutes of recirculation with signal enhancements of up to 626 using 99% para enriched H_2. ICP-MS analysis confirmed negligible leaching of the catalyst into the flowing solutions. The results demonstrate the feasibility of performing systematic optimization of conditions for continuous flow heterogeneous catalysis and polarization transfer to heteronuclei with prospective applications to biomedical magnetic resonance imaging.



Closed loop continuous flow parahydrogen reactor system. Blue, orange, and alternating double lines represent the flow path of gas, liquid, or both. The grey double dashed line represents the AF-2400 gas permeable membrane in the tube-in-tube vacuum degasser. Arrows indicate the flow direction.

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DEVELOPMENT OF IMPROVED BRAIN PHANTOMS TO IMPROVE UNDERSTANDING MOLECULAR DYNAMICS USING NMR

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To determine the quantitative accuracy and reliability of applying existing NMR techniques *in vivo* for measuring metabolites concentrations as well as to assist with the development of new methods for their quantitation, high quality phantoms that mimic the *in vivo* composition of metabolites and their local environments, which impacts their molecular motions and relaxation properties, are required. In previous studies, 'Braino 2.0' was developed as a mimic of brain tissue and used as a model for *in vivo* spectroscopy.[1] This phantom used alginate (a long chain polysaccharide) to increase sample viscosity to demonstrate the changes in linewidths induced by biomacromolecules slowing down the translational and rotational motions of metabolites. To further improve this phantom, this study focuses on identifying alternative thickening agents that broaden metabolite linewidths and more accurately reflect the resolution and relaxation phenomena observed *in vivo*. Separate phantoms containing sodium alginate, gelatin (a long chain polypeptide), or multilamellar vesicles (MLVs) composed of lipids extracted from porcine brains were used to evaluate how each thickening agent affects small molecule metabolite linewidths and motion. To assess the various phantoms, T_1, T_2, T_2* and diffusion measurements were made using optimally shimmed sample preparations. We hypothesized that alginate, a long chain polysaccharide not present in brain tissue, uniformly slows down molecular motions by increasing viscosity. In contrast, metabolites that differentially partition into cell membranes within the brain might best be modeled using the MLV-based phantom. By comparing metabolite spectral properties across the samples, we can identify differences in metabolite interactions with the various thickening agents as well as identify the superior phantom design.

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^{17}O SOLID-STATE NMR OF CARBOXYLIC ACIDS AND PHARMACEUTICAL COCRYSTALS ENRICHED USING MECHANOCHEMISTRY

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^{17}O solid-state NMR (SSNMR) spectroscopy is a powerful tool for studying the structures, properties, and reactivities of a plethora of organic molecules, inorganic materials, and biological solids. ^{17}O is a quadrupolar nucleus (I = 5/2) with central transition (CT, +1/2 ↔ –1/2) powder patterns influenced by both the second-order quadrupolar interaction and chemical shielding anisotropy.[1] Although ^{17}O SSNMR is an attractive tool for materials characterization, it remains challenging, largely due to the low natural abundance of ^{17}O (0.037%). In most cases, ^{17}O enrichment is necessary, but involves impractical and/or hazardous labeling schemes, which can often be cost-prohibitive. However, ^{17}O labeling via *mechanochemical saponification* is emerging as a rapid, quantitative, and cost-effective means of isotopically enriching a wide range of chemicals and materials that adheres to the tenets of green chemistry.[2-4]

Herein, we demonstrate that mechanochemical saponification can be utilized to enrich a series of three carboxylic acids (*i.e.*, benzoic, fumaric, and succinic acids) with ^{17}O, which are known to form cocrystals with fluoxetine HCl and numerous other active pharmaceutical ingredients. We also demonstrate the great value of ^{17}O SSNMR for their structural characterization and quantification of isotopic enrichment. First, a detailed synthetic work-up involving unlabeled, ^{18}O-labeled, and ^{17}O-labeled water is described. IR spectroscopy, powder X-ray diffraction (pXRD), mass spectrometry (MS), and solution NMR are used to optimize this protocol, and quantify enrichment levels, synthetic and enrichment yields, and impurities. Second, we discuss the use of 1D, 2D, and variable-temperature ^{17}O SSNMR experiments to probe molecular-level structure and potential dynamics (*i.e.,* tautomerization) of the carboxylic acids. Third, the preparation of cocrystals of fluoxetine HCl with the ^{17}O-enriched carboxylic acids using mechanochemical methods is reported.[5] ^{17}O SSNMR of each cocrystal reveals distinct CT patterns arising from unique chemical shift and electric field gradient tensors, demonstrating its great utility for analyzing pharmaceutical cocrystals. Finally, it is demonstrated that plane-wave DFT calculations can be used to establish key relationships between NMR interaction tensor parameters, molecular-level structures, and assessment of dynamics. This work creates new avenues for the routine use of ^{17}O SSNMR analyses of ^{17}O-enriched materials in future crystal structure prediction studies guided by NMR crystallography.[6]

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ROLE OF CHOLESTEROL AS AN ALLOSTERIC MODULATOR FOR HUMAN A_{2A} ADENOSINE RECEPTOR CONFORMATIONAL DYNAMICS

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Cholesterol forms an extremely important part of eukaryotic systems and has major role in membrane organization, dynamics, and membrane protein trafficking and function[1]. Cholesterol has been well documented to be an allosteric modulator for G protein-coupled receptors (GPCRs)[2], sensory proteins that control numerous physiological processes and are targeted by 35% of all FDA approved drugs[3]. Computational modelling and structural analysis have predicted the presence of putative sites for cholesterol interaction with GPCRs, but their role in preferential cholesterol interactions is an evolving field. We used ^{19}F-NMR in aqueous solutions to decipher the structural basis of how cholesterol modulates the conformational dynamics of the human A_{2A} Adenosine Receptor (A_{2A}AR). Reconstitution of A_{2A}AR in lipid nanodiscs allow us to carefully regulate the amount of cholesterol and phospholipids in our samples over a wide range of compositions. We covalently attach a ^{19}F-NMR to a judiciously selected located on the intracellular end of helix VII in A_{2A}AR, which has previously been shown to respond to ligand efficacy[4] and more recently to its surrounding lipid environment in nanodiscs. We show that in the presence of zwitterionic lipids, A_{2A}AR complexes with activating drugs remained in its predominantly inactive state, while presence of cholesterol in the zwitterionic membrane environment led to activation of the receptor. We varied the amount of cholesterol in the nanodisc sample to show that this effect on the conformational dynamics of the receptor is due to specific interactions of cholesterol with A_{2A}AR, and not due to changes in the bulk membrane environment in the nanodiscs. We also show that temperature has an innate effect on the exchange rate between the different conformers of A_{2A}AR and increase in temperature leads to increased activation of A_{2A}AR in specific lipid environments. Furthermore, we explore the structural basis of this specific allosteric effect by NMR experiments with variant proteins containing amino acid replacements at key residues in putative cholesterol interaction sites, and by substituting cholesterol with its structural analogs in our nanodisc system. Overall, our data show that cholesterol allosterically modulates the conformational dynamics of A_{2A}AR and provides motivation to explore the structural basis of such allosteric control of membrane protein function.

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MECHANOCHEMICAL SYNTHESIS AND MULTINUCLEAR SOLID-STATE NMR SPECTROSCOPY OF SILVER-CONTAINING COORDINATION POLYMERS

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Over several decades, the overuse of antibiotics has created resistant pathogens, thereby negating the effectiveness of antibacterial agents.[1] Recently, a new class of silver-containing coordination polymers (CPs) has been shown to exhibit excellent antibacterial properties and tunable silver ion (Ag^+) release.[2,3] CPs involve the self-assembly of organic ligands and metal ions, leading to one-, two-, or three-dimensional networks. Mechanochemical preparation of CPs has garnered recent interest, since there is a need for environmentally-friendly syntheses that are rapid, high-yielding, and scalable.[4]

Structural characterization of the products of mechanochemical synthesis can be challenging since it is often difficult to obtain suitable crystals for single-crystal diffraction studies. However, the combination of solid-state NMR (SSNMR) spectroscopy, powder X-ray diffraction (PXRD), and quantum chemical calculations, can in principle be used to validate, refine, and/or solve crystal structures (*i.e.*, NMR crystallography or NMRX).[5] Commonly, ^{1}H, ^{13}C, and ^{15}N SSNMR measurements are employed for NMRX studies;[6,7] however, the use of quadrupolar nuclei (*i.e.*, nuclear spin, $I > 1/2$) and spin-1/2 nuclides with large chemical shift anisotropies (*i.e.*, CSAs of heavy metal nuclides) for NMRX studies have largely gone unexplored.[8–10] This is surprising, due to the rich information available on coordination environments, bonding, and oxidation states that is readily available from NMR spectra of such nuclides.

In this poster, we discuss the mechanochemical synthesis and characterization of three CPs of the form $AgNO_3$:*x*R (R = 4-aminosalicyclic acid (4ASA), 5-aminosalicyclic acid (5ASA), and pyrazinamide (PYZ)) using a combination of PXRD and multinuclear SSNMR (*i.e.*, ^{109}Ag, ^{14}N, and ^{13}C) to garner insight into their molecular-level structures. The known 4ASA and PYZ CPs[11,12] are used to design and benchmark an NMRX protocol using both ^{109}Ag CS tensors and ^{14}N electric field gradient tensors. This new protocol will be applied to characterize the novel 5ASA CP, as well as two CPs of the form MCl_2:4ASA (M = Sn, Zn), which will incorporate NMR interaction tensors obtained from ^{119}Sn and ^{67}Zn SSNMR spectra. These results could provide insight to unique structural motifs in CPs that could lead to useful physicochemical and/or pharmacological properties, which in turn may aid in the rational design of new antibacterial agents.

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^{103}Rh SOLID-STATE NMR SPECTROSCOPY: ULTRA-HIGH FIELDS, OPTIMIZED PULSE SEQUENCES, AND FIRST PRINCIPLES COMPUTATIONS

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^{103}Rh is a spin-1/2 nuclide that is very unreceptive to the NMR experiment due to its low gyromagnetic ratio, γ, which presents challenges for the signal detection and construction of probe circuits free of acoustic ringing. ^{103}Rh solid-state NMR (SSNMR) presents additional challenges, since ^{103}Rh powder patterns are likely to be dominated by chemical shift anisotropy (CSA) and T_1(^{103}Rh) values are likely to be very long. Accordingly, only a handful of applications of ^{103}Rh SSNMR to chemical compounds has been reported to date.[1,2,3] In this poster, I will discuss ^{103}Rh SSNMR spectra obtained for a series of compounds with optimized ^{1}H-^{103}Rh BRAIN-CP pulse sequences,[4] using the 21.1 T ultra-wide bore and 36 T series-connected hybrid (SCH) magnets at the National High Magnetic Field Laboratory, and featuring probes adapted for low-γ experimentation. ^{103}Rh SSNMR spectra acquired at 35.2 T feature reduced experimental times and uniform CSA patterns, allowing for high-precision measurement of rhodium chemical shift (CS) tensors. This experimental data is complemented, for the first time, by ^{103}Rh magnetic shielding tensors obtained from relativistic DFT calculations.[5] We explore computational protocols to obtain the best agreement between calculation and experiment; these include the use of (i) cluster-based methods, (ii) relativistic effects at the spin-orbit level, and (iii) hybrid exchange-correlation functionals.

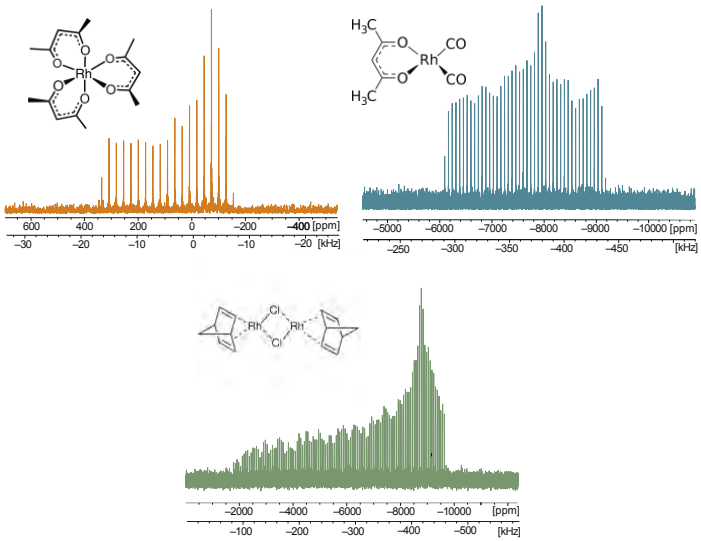

Fig. 1: ^{1}H-^{103}Rh BRAIN-CP/WURST-CPMG spectra acquired at 35.2 T for select Rh-containing compounds.

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^{67}Zn, ^{27}Al, AND ^{71}Ga SOLID-STATE NMR SPECTROSCOPY OF ZINC OXIDE NANOCRYSTALS

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Nanocrystals (NCs) are defined as particles that have a diameter between 1 and 100 nm.[1] They are of interest in a wide variety of fields because of their high surface area to volume ratios and tunability of their physicochemical properties via alteration of their sizes, dopants, and/or surface ligands.[2] Zinc oxide (ZnO) NCs have found widespread use in optoelectronics because they are intrinsic *n*-type semiconductors, made of earth abundant elements, and possess band gaps that can be manipulated by doping with various group III and IV elements.[3] Solid-state NMR (SSNMR) spectroscopy has emerged as a valuable tool for studying NCs, since it provides information on both ordered and disordered solid phases, allows for study of dopants and their incorporation (*i.e.*, concentrations, coordination environments, and net structural changes), and permits exploration of the interactions between the NCs and surface ligands.[2] SSNMR of quadrupolar nuclei (*i.e.*, nuclei with spin $I > ½$) is especially useful for studying atomic-level structure and dynamics in NCs, since the quadrupolar interaction, which manifests in most spectra of quadrupolar nuclei, is extremely sensitive to the local atomic environments and structural changes.

Herein, we demonstrate the utility of ^{67}Zn (I = 5/2), ^{27}Al (I = 5/2), and ^{71}Ga (I = 3/2) SSNMR (i) to make structural comparisons between the bulk and NCs ZnO phases; (ii) to probe the structure of ZnO NCs with varying types and concentrations of dopants; (iii) to understand the origin of Knight shift anisotropies (KSAs) that are observed in the SSNMR spectra; and (iv) to make correlations between the observed KSAs and generation of free carriers.[4] An understanding of dopant environments and their relationships with free carriers may enable the rational design of ZnO NCs with optimized electronic properties as their effects can be compared with that of bulk ZnO.[5]

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ANIONIC PHOSPHOLIPIDS CONTROL MECHANISMS OF GPCR-G PROTEIN RECOGNITION

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G protein-coupled receptors (GPCRs) are embedded in phospholipids that strongly influence drug-stimulated signaling[1]. Anionic lipids are particularly important for GPCR signaling complex formation, but a mechanism for this role is not understood. Using NMR spectroscopy, we visualized the impact of anionic lipids on the function-related conformational equilibria of the human A_{2A} adenosine receptor (A_{2A}AR) in bilayers containing defined mixtures of zwitterionic and anionic phospholipids. Anionic lipids primed the receptor to form complexes with G proteins through a conformational selection process. Without anionic lipids, signaling complex formation proceeded through a less favorable induced fit mechanism. In computational models, anionic lipids mimicked interactions between a G protein and positively charged residues in A_{2A}AR at the receptor intracellular surface, stabilizing a pre-activated receptor conformation. Replacing these residues strikingly altered the receptor response to anionic lipids in experiments. High sequence conservation of the same residues among all GPCRs supports a general role for lipid-receptor charge complementarity in signaling. Protein structure and function are known to be highly sensitive to temperature[2]. We observed that the increase in temperature had no effect on the conformational equilibria of A_{2A}AR reconstituted in detergent micelles whereas interestingly in nanodiscs containing anionic lipids, the increase in temperature shifted the conformational equilibria towards the active conformation.

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DISTINCT PORE-FORMING CONFORMATION OF AMYLOID BETA PEPTIDE Aβ1–42 IN MEMBRANE ENVIRONMENTS

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The extracellular accumulation of fibrillar assemblies of amyloid beta (Aβ) peptides in patients' brains is a hallmark of Alzheimer's disease. The interactions of lipid membrane and Aβ peptides are known to further modulate the assembly and cytotoxicity of Aβ peptides. However, there is no consensus regarding the effect of their interactions.

In this work, we reconstituted $A\beta_{1-42}$ peptides in lipid bilayers emulating various important components (POPC: POPG: Cholesterol = 6 : 3 : 1) in the cell membrane and applied solid state NMR (ssNMR) to characterize the resulting structure. Our ssNMR results found that $A\beta_{1-42}$ peptides adopt a distinct conformation from those observed previously, due to the presence of lipids. The charge-residue populated N-terminus forms two short pieces of β-strands, with considerable flexibility. The C-terminus comprises two long pieces of β-strands with a short one at the end of the peptide. The observed non-sequential contacts indicate that the peptide assumes an extended parallel β-sheet format. The relative position between the lipid acyl chain and $A\beta_{1-42}$ peptides was measured by heteronuclear correlation experiments, which suggests that the peptide is inserted deep into the lipid bilayer. Based on our NMR restraints, molecular dynamics simulations were performed to establish a high-resolution model. It indicates that $A\beta_{1-42}$ aggregate to form a pore-like structure in the bilayer lipids, which can disrupt the membrane integrity and may lead to ion leakage.

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RHENIUM-185/187 SOLID-STATE NMR INVESTIGATION OF PERRHENATE COMPOUNDS

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The nuclei of the two stable isotopes of rhenium ($^{185/187}$Re) are NMR-active and are present in high natural abundance (37.4% and 62.6% for ^{185}Re and ^{187}Re, respectively). They are quadrupolar nuclei with a spin of 5/2. Despite the high natural abundance and relatively high magnetogyric ratios that they possess ($\gamma(^{185}\text{Re}) = 6.1057 \times 107$ rad s^{-1} T^{-1}; $\gamma(^{187}\text{Re}) = 6.1682 \times 107$ rad s^{-1} T^{-1})[1], very few solid-state NMR studies exist. This is mainly because the very large nuclear electric quadrupole moment associated with each isotope (2180 mb and 2070 mb for ^{185}Re and ^{187}Re respectively)[2] results in a strong rhenium quadrupolar interaction that can broaden the NMR power pattern to the point that is undetectable. High magnetic fields can not only reduce the broadening effects of the second-order quadrupolar interaction but also improve the spectral resolution and signal sensitivity by enhancing the polarization. Therefore, the highest possible external applied magnetic fields are required for $^{185/187}$Re studies.

When powder patterns exhibit breadths wider than the excitation bandwidth of the probe, the variable offset cumulative spectrum (VOCS) approach is necessary to cover the full powder pattern breadth. For the ~36T series-connected-hybrid (SCH) magnet, the tuning access is restricted due to safety reasons when the magnet is above 18 T. Therefore, instead of frequency-stepping, the field-stepped NMR method[3] is the only option for acquiring ultra-wideline powder patterns.

In this study, we have analyzed five perrhenate compounds. Our aim is to determine whether the rhenium NMR response can be related to specific structural features present in these perrhenate compounds. We report here $^{185/187}$Re SSNMR spectra for all five samples acquired using VOCS on an 18.8 T superconductive magnet, and acquired using the field-stepped NMR method on the 35.2 T SCH magnet. Even with the reduced second-order quadrupolar broadening at 35.2 T, their central-transition spectra can still be over 8 MHz wide. The EFG and chemical shift tensors were extracted using the 'QUadrupolar Exact Software' (QUEST)[4] program to take high-order quadrupole interaction effects into account. Two of the compounds (pyridinium perrhenate and acetylcholine perrhenate) exhibit significantly faster T_2 relaxation compared to the other three compounds, implying the presence of dynamics. $^{185/187}$Re SSNMR spectra of acetylcholine perrhenate at variable temperatures were acquired at both 18.8 T and 35.2 T. Further molecular dynamics simulations are underway to provide insight into the potential nature of the chemical dynamics.

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REVEALING MOLECULAR ARCHITECTURE AND CARBOHYDRATE-AROMATIC INTERFACE OF WOODY PLANT CELL WALLS BY SOLID-STATE NMR

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Plant cell walls regulate cellular integrity, provide structural support, and serve as the majority of lignocellulosic biomass, an inexhaustible resource of biomaterials and biofuel. Rigid scaffolds of cellulose microfibrils are enclosed either in a soft biopolymer matrix (hemicellulose and pectin) in primary cell walls, or in a hemicellulose and aromatic polymer (lignin) mixture in secondary cell walls. Understanding these biopolymer networks will facilitate the development of more digestible crops and cost-effective conversion technology for biofuel production. However, most current methods, such as solution NMR and chromatography, need isolation, chemical modification, and solubilization procedures before characterization of cell wall materials, which processes can compromise the physical properties and chemical structures of biomacromolecules in native cells. The use of multidimensional solid-state NMR spectroscopy has become a powerful technique to investigate whole plant cells in their native environment, without pre-chemical treatments [1].

Here, we employ three wood samples, that is eucalyptus and poplar (hardwood), and spruce (softwood), to achieve in vivo investigation of biopolymer contacts of lignocellulosic components of plant secondary cell walls using solid-state NMR and dynamic nuclear polarization (DNP) approaches [2]. Our results show that the extent of glycan-aromatic association increases sequentially across grasses, hardwoods, and softwood. Lignin principally packs with the xylan in a non-flat conformation via electrostatic interactions and partially binds the junction of flat-ribbon xylan and cellulose surface as a secondary site. All molecules are homogeneously mixed in softwoods; this unique feature enables water retention even around the hydrophobic aromatics. Besides, we confirm that lyophilization and rehydration processing of the wood samples has few effects on the NMR result. This work will guide the rational design of more digestible plants and more efficient biomass-conversion pathways.

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PROBING ADSORPTION OF MONOCLONAL ANTIBODIES AT WATER-OIL INTERFACES VIA SPATIALLY RESOLVED MR SPECTROSCOPY

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Monoclonal antibodies (mAb) represent an important class of biologic therapeutics that can treat a variety of diseases including cancer. Despite many advantages, their processing, storage and/or administration remains challenging because of either the high flow environment in processing or presence of hydrophobic interfaces during administration and storage promote mAb aggregation. In this work, we used spectrally and spatially localized Point RESolved Spectroscopy (PRESS) NMR spectroscopy to investigate the adsorption of a model mAb to the oil-water interface.

Experiments were performed on a model mAb that consists of a maltose-based protein with a molecular weight of 155 kDa at different concentrations. Using a modified relaxation-enhanced PRESS (REPRESS), the structure of a model mAb was evaluated in the bulk (mAb in sodium phosphate buffer) as well as in incrementally closer voxels (375 μm) that approach a model water-oil interface, as well as voxels at the interface and within the oil. Both short echo time and diffusion-weighted REPRESS acquisitions were acquired from nine adjacent voxel locations in 375-μm steps, with six voxels in the bulk mAb above the interface, one voxel centered at the water-oil interface and two voxels below the interface in the oil. Localized MR spectroscopy results indicate that REPRESS spectra of mAb approaching the interface differ dramatically from the bulk mAb.

With further modification of the REPRESS sequence, diffusion- and T2-weighted experiments on the sample at and near the interface were conducted to determine if the changes are a result of aggregation or adsorption to the interface. Although T2 changes at the hydrophobic interface compared to the bulk were somewhat minimal, apparent diffusion coefficient changes at the interface were evident, indicating potential alteration of the size or diffusive environment of the mAb.

These results highlight the utility of localized MR spectroscopy in identifying potential alterations of the in situ higher order structure of monoclonal antibodies at hydrophobic interfaces, and point to the potential of MRS to elucidate mechanisms involved in aggregation and conformational changes of mAb that may impact immunogenicity and therapeutic utility.

^{23}Na MRI AT 21.1 T REVEALS SEX DIFFERENCES IN A PRECLINICAL MIGRAINE MODEL

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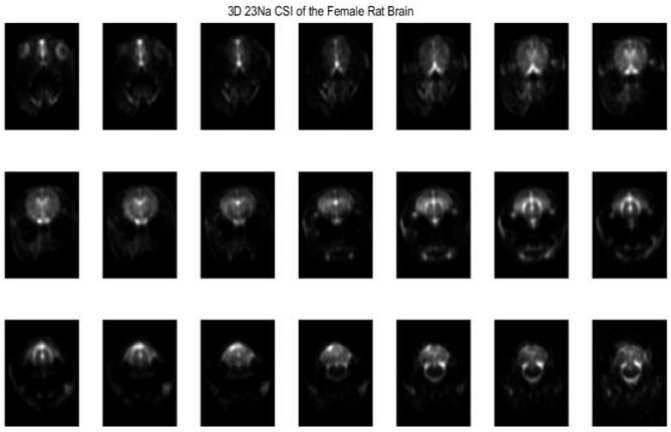
The vast majority of migraineurs are female. Despite this, preclinical examinations of migraine rely primarily on male animal models. Previously under the nitroglycerin (NTG) chemical model of migraine, male Sprague-Dawley (SD) rats showed widespread increases in neural sodium[1]. In this study, the female SD rat model response to NTG-triggered central sensitization is evaluated via 3D ^{23}Na chemical shift imaging (CSI) at 21.1 T to elucidate potential sex differences.

Methods:

Prior to scanning, animals were anesthetized with 5% isoflurane in oxygen to implant an intraperitoneal (IP) line for *in situ* NTG delivery in the vertical magnet. During scanning, rodents were maintained at 2-3% isoflurane. Post baseline acquisition, the NTG dose was administered IP. Groups examined were 2-mg/kg body weight NTG (N=14), 10-mg/kg body weight NTG (N=14) or an equivalent volume of saline (N=14).

For 3D ^{23}Na imaging, Hamming-weighted, 10-min FID-based 3D CSI scans were acquired every 20 min out to 2 h post-injection a 1x1x3-mm resolution. Reported times correspond to the scan end. Manual reconstruction and segmentation were performed in MATLAB 2020b. An exponential filter was applied to each FID; during Fourier transform, the image was zero-padded to a final resolution of 0.25x0.25x1 mm (Figure 1).



Segmentation was performed directly on sodium images, based on the Waxholm SD rat brain atlas. Regions segmented were the brainstem, thalamus, cisterna magna and ventricular system. The ventricles were sub-segmented to the aqueduct, lateral, 3rd and 4th ventricles. Statistical analyses were performed in JMP Pro 15. A mixed model with repeated measures with a Toeplitz covariance matrix was performed; post-hoc Tukey HSD tests were performed with $p < 0.05$ for all significances.

Results:

Male SD rats previously have demonstrated increases in neural sodium under 10-mg/kg NTG in the brainstem, neocortex, 3rd and 4th ventricles and cisterna magna. The female model, at both doses of NTG, is resilient to these increases. Transient increases are seen in the 3rd ventricle; otherwise female sodium remains at or below baseline levels. It is unlikely differences in baseline sodium play a role, as the brainstem sees similar slight increases under saline across both sexes.

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FUNCTIONAL AND STRUCTURAL CONNECTIVITY IN THE TRANDGENIC RAT MODEL OF ALZHEIMER'S DISEASE USING RESTING-STATE fMRI & DIFFUSION TENSOR IMAGING

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A hallmark of preclinical Alzheimer's Disease (AD) is spatial disorientation, such as getting lost in new locations. One potential cause is disrupted exchange between egocentric and allocentric reference frames. Both the parietal (PC) and restrosplenial cortex (RSC) have garnered attention for their roles in encoding and transforming information between these reference frames. The RSC and PC also are earlier sites of dysfunction in humans with AD and rodents modeling aspects of AD. This study aimed to examine resting-state functional MRI (rs-fMRI) and diffusion tensor imaging (DTI) in relationship with coordination between reference frames in an AD rat model. We hypothesized that pathology development in transgenic AD rats leads to brain network dysfunction, which causes impaired coordination between reference frames.

In double transgenic TgF344-AD rats and littermate controls, longitudinal MRI data acquired *in vivo* at 21.1 T to assess functional and structural connectivity alterations by rs-fMRI and structural DTI using the application of graph theory. Under light anesthesia (1-2% isoflurane), full brain rs-fMRI datasets were acquired at 250x250x1000 μm using 300 repetitions without stimulation followed by acquisition of an 18-direction DTI dataset at 200x200x100 μm at 2, 4, 6, 10, 12, 16 & 18 mos. MR data was compared to behavioral tasks in which reference frame coordination was assessed. Using an action-oriented spatial navigation task, rats were required to associate actions (left or right turn) with locations.

Data suggest that both age and genotype lead to declines in action-orientation performance, with differences in genotype emerging at 5 months of age and increasing at older timepoints. These alterations were reflected in the functional and structural networks in at least the PC, with more profound changes developing longitudinally. These findings highlight a new focus for understanding cognitive deficits in AD by using allocentric and egocentric coordination as a novel predictor of early declines in AD.

BLOOD-CSF BARRIER PERFUSION IN A PRECLINICAL MIGRAINE MODEL

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Migraine occurs from excess neuronal activation, likely from elevated sodium, leading to pain and other symptoms. Thus, waste products in the brain increase, and waste clearance is critical to achieving homeostasis. Because cerebrospinal fluid (CSF) is the primary mediator for clearance and a dysregulated choroid plexus (CP) is already thought to be involved in the propagation of migraine, the Blood-CSF-Barrier (BCSFB) function should be evaluated for determining brain clearance dynamics to restore brain homeostasis.

With the 900-MHz, 21.1-T vertical scanner at the National High Magnetic Field Laboratory, Tallahassee, Florida, this study utilizes ultra-long TE pulsed Arterial Spin Labeling (PASL) to measure perfusion across the BCSFB in the lateral ventricles during nitroglycerin (NTG) triggered preclinical migraine in female Sprague Dawley rats.

Three baseline scans were acquired prior to NTG administration, and then 7-min PASL scans were performed every 20 min for 3 h post-NTG administration using an EPI-based flow-sensitive alternating inversion recovery (FAIR) technique. BCSFB perfusion was measured using selective and nonselective inversions (TI = 100, 500, 1000, 1600, 2000, 3500, 5000 and 6500 ms) from a single slice placed over the choroid plexus of the lateral ventricles and having a long echo time (TE = 80 ms) to eliminate tissue contributions. Additionally, conventional BBB perfusion was assessed using a short echo time (TE = 16 ms) using the same inversion scheme but focusing analysis on a cortical region. Both dataset acquisitions were fitted to a two-compartment perfusion model to estimate across the BCSFB and BBB.

This presentation will discuss the ramifications of these results, comparing NTG injected female Sprague Dawley rats to similarly aged female rats receiving saline as a control.

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