Dear SEMRC Participants,

Welcome to the 48th Southeastern Magnetic Resonance Conference (SEMRC), held this year at the University of Florida! We are thrilled that you all could join us for an exciting program bringing together researchers across the Southeast and several other geographic areas. The magnetic resonance community benefits greatly from interactions among researchers in related, yet quite distinct, scientific areas, and we hope that this meeting provides opportunities for catalyzing stimulating collaborative research in years to come.

A major goal was to encourage attendance by students and early career scientists by keeping registration fees low. Thus, we are very grateful to acknowledge the financial support of the following companies and organizations that allowed us to do this. Specifically, we would like to thank Avanti Polar Lipids, Bridge 12, Bruker, Anatrace/Molecular Dimensions, Cortecnet, Doty Scientific, Cambridge Isotopes, Eppendorf, Fisher Scientific, Genesee Scientific, IKA, JEOL, Millipore Sigma, Isotec, New Era, Phoenix NMR, QOne Instruments, Shared EPR, USA Scientific, VWR and Wilmad-Lab Glass. In addition, we are also grateful to the National High Magnetic Field Laboratory (NHMF), the University of Florida (UF), and Visit Gainesville provided generous financial support, along with contributions from the University of Florida Department of Chemistry, Department of Biochemistry and Molecular Biology, Center for Chemical Physics, Center for Structural Biology, College of Medicine, Division of Sponsored Research, and McKnight Brain Institute. The conference would not be possible without this support.

We are also grateful for the input and advice of the executive steering committee, including Gail Fanucci, Joanna Long, Tom Mareci and Marcelo Febo who significantly helped shape the program. We also wish to thank Brianna Blassneck and her colleagues at UF Conferences Services and also acknowledge the significant contributions to the conference organization by Stacie Austin, Kelly Deuerling, and Amy Howe who really made all of this come together. Finally, we thank Nilubon Tabtimtong for maintaining the conference website. In closing, welcome to Gainesville and the 48th SEMRC!

Sincerely,

Anil Mehta and Matt Eddy
PLEASE SHOW YOUR GRATITUDE TO OUR GENEROUS SPONSORS

And to the following Centers and Departments at the University of Florida:

Center for Chemical Physics
College of Medicine - endowment
Division of Sponsored Research
McKnight Brain Institute
DIRECTIONS TO CAMPUS – University of Florida

From I-75: Take exit 384 (SR 24/Archer Road) and head east on Archer Road for 2.6 miles to Gale Lemerand Drive (formerly North-South Drive). Turn left onto Gale Lemerand Drive for 0.5 mile to turn right at Museum Road, or continue north to Stephen O’Connell Center lot at the intersection with West University Ave.

From I-95: Heading south toward Jacksonville, take exit 362B to merge onto I-295. Then take exit 21B and merge onto I-10 West. Next take exit 343 towards Starke and merge onto US 301 South. Follow US-301S to NE State Road 24, then take a right on West University Ave, Gainesville. Continue west to the O’Connell Center Lot at Gale Lemerand Drive or take a left on SW 13th Street (US 441) then turn right (west) at the light on Museum Road for 0.5 mile. The UF Bookstore and Welcome Center is on the right, just past Center Drive.

From Gainesville Regional Airport: Exit the airport drive and turn right on SR 222 (39th Avenue) for 4 miles to US 441 (13th Street). Turn left on US 441 to continue 2.9 miles until the right turn on University Ave, or 3.4 miles to the right turn at Museum Road.

SCHEDULE OVERVIEW – SESSION START TIMES

JHH = Joseph Hernandez Hall atrium, CLB = Chemistry Laboratory Building, C130 or lobby

Friday, October 25, 2019
4:00 PM  JHH  On-site registration and badge pickup open
5:30 PM  CLB  Welcome Address
5:40 PM  CLB  Keynote Speaker 1 and Session A
7:00 PM  JHH  Reception and Poster Session I

Saturday, October 26, 2019
7:30 AM  JHH  Breakfast
8:30 AM  CLB  Session B
10:00 AM  lobby  coffee break
10:20 AM  CLB  Keynote Speaker 2 and Session C
12:00 PM  JHH  Lunch
1:00 PM  CLB  Keynote Speaker 3 and Session D
3:10 PM  lobby  coffee break
3:30 PM  CLB  Session E
5:20 PM  lobby  break
5:40 PM  CLB  Lightning Talks
7:00 PM  JHH  Dinner
8:30 PM  JHH  Poster Session II

Sunday, October 27, 2019
7:30 AM  JHH  Breakfast
8:30 AM  CLB  Session F
10:00 AM  lobby  coffee break
10:20 AM  CLB  Session G
11:50 AM  CLB  Wrap-up and Business Meeting (Box Lunch in JHH – to go!)
CONFERENCE VENUE: University of Florida – Chemistry Dept.
Activities held in Joseph Hernandez Hall (JHH) atrium, 1645 W. University Ave, Gainesville, FL
Talks will be held in the in adjacent Chemistry Laboratory Building (CLB), room C130

Parking lot restrictions are in effect Monday – Friday, 7:30 am – 4:30 pm, and for select weekend lots as noted by signage.

After 4:30pm Friday, most lots and garages are available free of charge.

During all decal-restricted hours, visitors to campus can find pay parking for $6/day at the UF Visitor Welcome Center Parking Garage, 1900 Museum Road, at the corner of Museum Road and Reitz Union Drive. Pay stations are located at the stair towers and elevators in the garage. The distance from the Bookstore lot heading north via Buckman Drive to Hernandez Hall is ~0.6 miles (10-12 min walk).

Additional Free Parking – Stephen C. O’Connell Center

After 4:30pm Friday, visitors will be able to park without restrictions in the Stephen C. O’Connell Center Lot, 250 Gale Lemerand Drive. UF staff/students with the proper decal may be able to park in closer, restricted lots. The distance from the O’Connell lot traveling east via West University Ave to Hernandez Hall is ~0.5mi (8-10 min walk).
GUIDELINES FOR PRESENTERS

• Oral presentations – please allow 3 min for questions – i.e. for a 15min slot, please plan on 12 min for the presentation and 3 min for questions.

• Posters should be no wider than 36" horizontal and no taller than 48" vertical. Each poster board will thus accommodate 2 posters per side. All posters will be displayed during the entire meeting, with half of the attendees presenting Friday evening (odd numbered posters) and the second half presenting Saturday evening (even numbered posters). Push-pins will be provided; you may bring an alternative.

• Posters can be set up in Joseph Hernandez Hall Atrium (2nd floor) on Friday, October 25th starting at 4:00 pm. All posters should be in place by Friday at 7:00 pm. Posters should remain on display through 10:15 am Sunday, to allow viewer access during all meals and breaks. Posters must be removed no later than Sunday, October 29th at 12:30 pm. Posters remaining after that time will be discarded.

• Student posters will be eligible for poster awards. Judging will take place from 8-10pm Friday (odd numbered posters) and 8:30-10:30pm Saturday (even numbered posters). You should be at your poster during these times as a courtesy to all attendees and if you wish to be considered for an award.

GENERAL INFORMATION FOR ALL ATTENDEES

• We are attempting to go green by not printing a full program, but handouts of the schedule will be available onsite. The full program will be available online during and after the conference.

• Event badges must be worn at all times during the conference, including during the oral and poster sessions, breaks, receptions, and meals.

• Meals will be provided at the conference, including dinners on Friday and Saturday evenings, plus breakfast and lunch on both Saturday and Sunday (box lunch “to go” on Sunday). Various beverages will be provided at all times and additional snacks will be available during the coffee breaks.

• Conference organizers and volunteers will be on hand throughout the conference to assist participants. Their badges will be labeled so you can identify them easily.

• Photography of the presenters’ content is not permitted at the oral or poster sessions.

• Cell phone ringers and alerts must be turned off or set to vibrate during oral sessions.

• Several vendors will be on-site in the same location as the breaks and poster sessions. As a courtesy to our speakers, we ask that you take advantage of the vendor space to conduct your conversations.

• UF WiFi: When you join the ufguest network, an Acceptable Use Policy (AUP) screen will pop up. Upon accepting the AUP, you will have access to the Internet.

• We have not arranged any official shuttles to/from the conference venue. Many of the recommended conference hotels are less than 0.5 mile from campus. If you are concerned about your transportation or safety, please contact one of the conference organizers or volunteers for assistance with your travel to campus.

• UF is a non-smoking facility. Smoking is not permitted anywhere on the campus.
DETAILED CONFERENCE SCHEDULE – Abstracts to follow
JHH = Joseph Hernandez Hall atrium (two levels), CLB = Chemistry Laboratory Building room C130

**Friday October 25, 2019**

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>4:00 PM</td>
<td>JHH</td>
<td>On-site registration and Badge pickup open</td>
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<tr>
<td>5:30 PM</td>
<td>CLB</td>
<td>Welcome Address: Matt Eddy and Anil Mehta</td>
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### Session A – CLB

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<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
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<tr>
<td>5:40 PM</td>
<td><strong>Keynote 1:</strong> Dorothee Kern, Brandeis</td>
<td>-</td>
<td>NMR AT ITS BEST: EVOLUTION OF PROTEIN DYNAMICS - TIME TRAVEL TO THE PAST AND FUTURE</td>
</tr>
<tr>
<td>6:25 PM</td>
<td>Ben Wylie, Texas Tech</td>
<td>-</td>
<td>SOLID-STATE NMR STUDIES OF KIR CHANNEL-LIPID INTERACTIONS AND THE FIBRILLATION OF FUNCTIONAL AMYLOIDS IN THE EPIDIDYMIS</td>
</tr>
<tr>
<td>6:45 PM</td>
<td>Woonghee Lee, Wisconsin</td>
<td>-</td>
<td>INTEGRATIVE HIGH-THROUGHPUT NMR PLATFORM FOR BIOMOLECULAR RESEARCH</td>
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<th>Time</th>
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<tr>
<td>7:00 PM</td>
<td>JHH</td>
<td>Reception and Poster Session I (authors at odd numbered posters 8-10pm)</td>
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<tr>
<td>11:00 PM</td>
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**Saturday October 26, 2019**

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<th>Time</th>
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<tbody>
<tr>
<td>7:30 AM</td>
<td>JHH</td>
<td>Breakfast</td>
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### Session B – CLB – Manish Mehta, Session Chair

<table>
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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
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<tbody>
<tr>
<td>8:30 AM</td>
<td>Tim Cross, FSU</td>
<td>-</td>
<td>GRAMICIDIN A: THE PEPTIDE THAT REFUSES TO DIE, UNIQUE INSIGHTS FROM 17O SPECTROSCOPY</td>
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<tr>
<td>8:50 AM</td>
<td>Shella Keilholz, Emory</td>
<td>-</td>
<td>DYNAMIC PATTERNS OF SPONTANEOUS BRAIN ACTIVITY AND FUNCTIONAL CONNECTIVITY</td>
</tr>
<tr>
<td>9:10 AM</td>
<td>Katie Dunleavy, UF</td>
<td>-</td>
<td>HYDRATION ENVIRONMENT CHARACTERIZATIONS OF THE FOLDING OF Iα, AN INTRINSICALLY DISORDERED PROTEIN</td>
</tr>
<tr>
<td>9:25 AM</td>
<td>Thorsten Maly, Bridge12</td>
<td>-</td>
<td>HIGH-RESOLUTION, SOLUTION-STATE ODNP-ENHANCED NMR SPECTROSCOPY AT LOW MAGNETIC FIELDS</td>
</tr>
<tr>
<td>9:45 AM</td>
<td>Tuo Wang, LSU</td>
<td>-</td>
<td>REVEAL THE FUNCTIONAL STRUCTURE OF COMPLEX CARBOHYDRATES IN INTACT FUNGAL AND PLANT CELLS USING DNP SOLID-STATE NMR</td>
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<tbody>
<tr>
<td>10:00 AM</td>
<td>CLB lobby</td>
<td>coffee break</td>
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### Session C – CLB – Joanna Long, Session Chair

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
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<tbody>
<tr>
<td>10:20 AM</td>
<td>Keynote 2: Mei Hong, MIT</td>
<td>-</td>
<td>STRUCTURE &amp; MECHANISM OF MEMBRANE PROTEINS AND AMYLOID PROTEINS FROM SOLID-STATE NMR</td>
</tr>
<tr>
<td>11:05 AM</td>
<td>Leah Casabianca, Clemson</td>
<td>-</td>
<td>STD-INEPT NMR FOR EXAMINING SMALL MOLECULE INTERACTIONS WITH NANOPARTICLES</td>
</tr>
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</table>
Saturday October 26, 2019

Session C continued

11:25 AM  Joel Lapin, NCSU - A SIMULATED ANNEALING ALGORITHM FOR DE NOVO PULSE SEQUENCE DESIGN OF SEPARATED LOCAL FIELD NMR EXPERIMENTS
11:40 AM  Bo Chen, UCF - A SITE-SPECIFIC COMPARISON OF THE PENTAMERIC AND HEXAMERIC ASSEMBLY OF THE ROUS SARCOMA VIRUS CAPSID PROTEIN
12:00 PM  JHH  Lunch

Session D – CLB – Marcelo Febo, Session Chair

1:00 PM  Keynote 3:  Nanyin Zhang, Penn State - MANIPULATING AND MONITORING THE BRAIN NETWORK FUNCTION IN AWAKE RODENTS USING MULTI-MODAL APPROACHES
1:45 PM  Tom Mareci, UF - FLOW, AND ELECTRIC CURRENT INDUCED PHASE IN MR IMAGING
2:05 PM  Brad Wilkes, UF - VOLUMETRIC MAGNETIC RESONANCE AND DIFFUSION TENSOR IMAGING OF C58/J MICE: NEURAL CORRELATES OF REPETITIVE BEHAVIOR
2:20 PM  James Collins, UF - INVESTIGATION OF BRAIN TISSUE METABOLITE LOCAL ENVIRONMENT WITH HR-MAS
2:35 PM  Mukundan Ragavan, UF - BINOMIAL EXCITATION FOR DETECTION OF HYPERPOLARIZED DIHYDROXYACETONE METABOLISM IN PERFUSED MOUSE LIVER
2:50 PM  Tatyana Smirnova, NCSU - EFFECTIVE ACIDITY OF FROZEN SOLUTIONS AND AT LIPID BILAYER - WATER INTERFACE BY EPR OF IONIZABLE SPIN LABELS AND PROBES
3:10 PM  CLB lobby  coffee break

Session E – CLB – Anant Paravastu, Session Chair

3:30 PM  Steve Hill, FSU - ADDRESSING NUCLEAR SPINS USING PULSED BROADBAND 94 GHz EPR
3:50 PM  Veronika Szalai, NIST - EPR SPECTROSCOPY MEASUREMENT DEVELOPMENT FOR NANOBIO MATERIALS
4:10 PM  A. Ligia Focsan, Valdosta - ENHANCEMENT OF ANTIOXIDANT ACTIVITY OF CAROTENOIDS IN SUPRAMOLECULAR COMPLEXES AND THE ROLE OF THEIR REDOX POTENTIALS STUDIED BY EPR SPIN TRAPPING TECHNIQUE
4:25 PM  Alexander Nevzorov, NCSU - THIRTY-FOLD GAIN IN MM-WAVE POWERS OBTAINED BY PHOTONIC BAND-GAP RESONATOR PROBEHEADS FOR DNP OF THIN FILM SAMPLES AT 7T
4:45 PM  Evan Akeroyd, NCSU - HYPERPOLARIZATION OF COMMON PRESCRIPTION DRUGS AND METABOLITES
5:00 PM  Brad Pierce, UA - COORDINATION NUMBER AND SUBSTRATE-BINDING DENTICITY DETERMINED FOR NON-HEME THIOL DIOXYGENASES BY ANALYTICAL CW X-BAND EPR
5:20 PM  CLB lobby  break

5:40 PM  CLB  Lightning Talks
7:00 PM  JHH  Dinner
8:30 PM  JHH  Poster Session II (authors at even numbered posters 8:30-10:30pm)
11:00 PM  (end day)
Session F – CLB – Kurt Warncke, Session Chair

8:30 AM  Douglas Kojetin, Scripps  - LIGAND-INDUCED STRUCTURAL DYNAMICS AND FUNCTION OF PPARγ LEARNED THROUGH NMR
8:50 AM  Robert Silvers, FSU  – NEURODEGENERATIVE DISEASES AND THE MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE
9:05 AM  Megan A. Macnaughtan, LSU  - STRUCTURAL INDEPENDENCE OF SCC4’S DUAL FUNCTIONS IN CHLAMYDIA TRACHOMATIS
9:25 AM  Monique Rogals, UGA  - OPTIMIZING SOLUTION NMR FOR STRUCTURAL STUDIES OF GLYCOSYLATED MAMMALIAN PROTEINS
9:40 AM  Will J. Placzek, UAB  - CONNECTING APOPTOSIS TO THE CELL CYCLE USING A NEW PROTEIN-PROTEIN INTERACTION MOTIF

10:00 AM  CLB lobby coffee break

Session G – CLB – Alex Smirnov, Session Chair

10:20 AM  Rob Schurko, FSU  - FREQUENCY-SWEPT PULSES AND PULSES DESIGNED WITH OPTIMAL CONTROL THEORY FOR ULTRA-WIDELINE NMR SPECTROSCOPY
10:40 AM  Alyssa Rose, FSU  - SOLID-STATE NMR INVESTIGATIONS OF CHLORANILIC ACID AS ORGANIC ELECTRODE MATERIAL FOR LITHIUM-ION BATTERIES
10:55 AM  Goncalo Gouveia, UGA  - COMBINING THE GENETICS OF A MODEL ORGANISM WITH QUANTUM CHEMISTRY FOR UNKNOWN COMPOUND ID IN METABOLOMICS
11:15 AM  Nia Harmon, FSU  - OPTIMIZATION OF POLARIZING AGENTS FOR SCALAR OVERHAUSER DNP
11:30 AM  Faith Scott, UF  - DIELECTRIC CONSTANTS OF MATERIALS FOR OPTIMIZED DYNAMIC NUCLEAR POLARIZATION, IN THE RANGE OF 70 TO 960 GHZ
11:45 AM  Russ Bowers, UF  - HYPERPOLARIZATION FROM PARAHYDROGEN AND HETEROGENEOUS CATALYSIS

12:05 PM  Conference Wrap-up and Business Meeting

12:20 PM  JHH  Box lunch (to go) – Thank you for another successful SEMRC!
KEYNOTE SPEAKER ABSTRACTS

Dorothee Kern, Dept. of Biochemistry, HHMI/ Brandeis University -- NMR AT ITS BEST: EVOLUTION OF PROTEIN DYNAMICS - TIME TRAVEL TO THE PAST AND FUTURE

As a direct manifestation of molecular kinetic energy, temperature is a fundamental evolutionary driver for chemical reactions. However, it is currently not understood how the natural evolution of catalytic efficiency responds to dramatic changes in environmental temperatures. Using Ancestral Sequence Reconstruction (ASR) we resurrect and biophysically characterize the oldest common ancestral kinase and enzymes along the evolutionary path to modern kinases. Strikingly, enzymes coped with an inherent drop in catalytic speed caused as the earth cooled down over 3.5 billion years by accelerating protein dynamics and adapting thermostability by unexpected mechanisms, as characterized by NMR. Tracing the evolution of enzyme activity and stability from the hot-start towards modern hyperthermophilic, mesophilic and psychrophilic organisms illustrates active pressure versus passive drift in evolution on a molecular level. NMR combined with characterization of the evolution of kinase dynamics over 1 billion years reveals that the root for kinase inhibitor selectivity lies in target dynamics in the drug-bound state, a new concept that opens novel opportunities for rational drug design. I will shed light into a 20 year-old puzzle: why the wonder drug Gleevec is a highly selective inhibitor of Abl kinase. We will explore the molecular mechanism of improving enzyme function by forward evolution. Several rounds of directed evolution of Cyclophilin A accelerated catalysis by accelerating protein dynamics as measured by CEST and CPMG experiments. The presented data will demonstrate the power of NMR to characterize the essential role of protein dynamics for function and its evolution.

Mei Hong, Department of Chemistry, MIT -- STRUCTURE & MECHANISM OF MEMBRANE PROTEINS AND AMYLOID PROTEINS FROM SOLID-STATE NMR

In this talk I will present my group’s latest advances in determining membrane protein and amyloid protein structures. In the membrane protein area, we solved 1.5 Å resolution structures of the influenza B virus M2 (BM2) proton channel in the closed and open states. The structures give insight into why BM2 conducts protons bidirectionally, in contrast to the influenza A M2 protein (AM2), which conducts protons only from the N-terminus to the C-terminus. I will also show the determination of the cholesterol-binding site of AM2, from which we gained novel mechanistic insight into the virus budding function of this protein. Both the BM2 proton channel structures and the cholesterol-binding study crucially benefitted from 19F-NMR based distance measurements. I will present four high-field and fast-MAS 19F NMR techniques that we have developed and benchmarked to measure inter-atomic distances to about 2 nm. In the amyloid protein direction, I will describe the novel structure of the amyloid fibrils formed by the peptide hormone glucagon. Glucagon forms an antiparallel hydrogen-bonded cross-β fibril with two coexisting molecular conformations. The structure shines light on the extraordinary stability of this pharmaceutical peptide fibril and provides a rational basis for designing glucagon analogs that resist fibril formation in order to better treat diabetic hypoglycemia.

Nanyin Zhang, Biomedical Engineering and Electrical Engineering, The Pennsylvania State University -- MANIPULATING AND MONITORING THE BRAIN NETWORK FUNCTION IN AWAKE RODENTS USING MULTI-MODAL APPROACHES

A major challenge in research on the pathophysiology of brain disorders has been the difficulty to directly translate from human symptoms to animal models that have unique behavioral repertoire. The brain circuit/network function, which has become accessible through the broad application of fMRI in humans, might provide a link between animal models and human patients. However, this task has been largely unsuccessful, primarily due to the confounding effects of anesthesia in most animal fMRI experiments. Our lab has established an approach that allows animal’s brain circuit/network function to be examined at the awake state and investigation of the link between animal models and human pathophysiology for psychiatric disorders. This method can be combined with multiple cutting-edge neuroscience approaches including optogenetics, DREADDs and electrophysiology to manipulate and monitor brain-network function with multi-dimensional information.
OTHER SPEAKERS – Listed alphabetically by last name

Titles and Abstracts to follow

Evan Akeroyd, NCSU
Russ Bowers, UF
Leah Casabianca, Clemson
Bo Chen, UCF
James Collins, UF
Tim Cross, FSU
Katie Dunleavy, UF
A. Ligia Focsan, Valdosta
Goncalo Gouveia, UGA
Nia Harmon, FSU
Steve Hill, FSU
Joel Lapin, NCSU
Shella Keilholz, Emory
Douglas Kojetin, Scripps
Woonghee Lee, Wisconsin
Megan Macnaughtan, LSU
Thorsten Maly, Bridge12
Tom Mareci, UF

Alexander Nevzorov, NCSU
Brad Pierce, UA
Will Placzek, UAB
Mukundan Ragavan, UF
Monique Rogals, UGA
Alyssa Rose, FSU
Rob Schurko, FSU
Faith Scott, UF/FSU
Robert Silvers, FSU
Tatyana Smirnova, NCSU
Veronika Szalai, NIST
Tuo Wang, LSU
Brad Wilkes, UF
Ben Wylie, Texas Tech

TRAVEL AWARD
VSP AWARD
Evan Akeroyd, NCSU

HYPERPOLARIZATION OF COMMON PRESCRIPTION DRUGS AND METABOLITES

Evan Akeroyd¹, Patrick TomHon¹, Soeren Lehmkuhl¹, Thomas Theis¹ (Arial. Font size 11pt)

¹ North Carolina State University, Department of Chemistry

NMR and MRI have proven to be invaluable tools for chemical analysis, biomolecular structure elucidation and disease diagnosis. However, magnetic resonance suffers from inherent low sensitivity. Sensitivity can be increased using hyperpolarization techniques such as Dynamic Nuclear Polarization (DNP) which has a wide substrate scope but is limited by long polarization build-up times and expensive equipment. Other hyperpolarization techniques such as Signal Amplification by Reversible Exchange (SABRE) use parahydrogen (p-H₂) and a polarization transfer catalyst (PTC) to transfer spin order from the p-H₂ to the target molecule through the J-coupling network¹. Because the substrate and p-H₂ are in reversible exchange, and no chemical modification occurs, SABRE conveniently allows for hyperpolarization to be repeatedly generated in the same sample. Unfortunately, the substrate scope of SABRE is limited and to date few biomolecules have been hyperpolarized.

This work demonstrates SABRE can produce hyperpolarization on common prescription drugs and metabolites. A robust pneumatic shuttling setup, developed in our lab, and a large solenoid conveniently allow for polarization transfer to occur at a wide range of magnetic fields. The polarization transfer efficiency at various magnetic fields and the temperature dependence were evaluated for the anti-fungal drugs including fluconazole, clotrimazole, and voriconazole as well as letrozole, a common aromatase inhibitor used for the treatment of breast cancer. Hyperpolarization could enable real time monitoring of the action of drug or could find applications in MRI as a contrast agent.

In this work, we have demonstrated that SABRE can be used to generate high levels of polarization on common drugs and metabolites. Such molecular markers can be used as MRI contrast agents, enabling real time imaging of metabolism and fast, high resolution MRI imaging. The hyperpolarized signal generated by the SABRE process lends itself toward biomedical applications as a hyperpolarized agent injected into a patient could greatly enhance MRI resolution reduce acquisition time.

References:

Figure 1: Successfully hyperpolarized prescription drugs voriconazole, letrozole, fluconazole, and clotrimazole. Nuclei gain polarization during the SABRE process are highlighted.
HYPERPOLARIZATION FROM PARAHYDROGEN AND HETEROGENEOUS CATALYSIS

C. Russell Bowers,¹ Ranjan K. Behera,² Minda Chen,² Diana Choi,¹ Yong Du,¹ Maria-Jose Ferrer,¹ Wenyu Huang,² Qiuying Li,¹ Bochuan Song,³ Helena Hagelin-Weaver,³ Tommy Zhao¹

¹University of Florida Department of Chemistry; ²Iowa State University Department of Chemistry and Ames Lab; ³University of Florida Department of Chemical Engineering

Nuclear spin singlet-states are attracting a lot of attention nowadays because they can significantly extend the usable lifetime of hyperpolarization. Singlet order decays more slowly than ordinary magnetization because the former is protected from the intra-pair dipole-dipole relaxation mechanism. Singlet-triplet imbalance (STI), which refers to the population differential between the singlet and triplet manifolds for a pair of strongly coupled spin-1/2 nuclei, can be prepared directly from parahydrogen, the singlet spin isomer of dihydrogen that is easily generated by cooling the gas in the presence of an ortho-para conversion catalyst. With perfect magnetic equivalence and isolation, the parahydrogen singlet is the quintessential long-lived state with a lifetime of many weeks. The transformation of singlet order into observable Zeeman order can be achieved by symmetry breaking chemistry.¹ The key requirement is pairwise addition with retention of inter-pair spin-spin coupling. This talk will survey some highlights from our recent research utilizing heterogeneous catalysis to convert dihydrogen STI into magnetization on various target molecules in gases and solution. One of the main challenges that needs to be overcome is the fast diffusion of H ad-atoms that is typically observed on the catalytically active metal surfaces. We show that through rational design of heterogeneous catalysts, significant improvements can be made in the efficiency of the singlet-to-magnetization transformation.²³ Three examples are presented: ultra-low loadings of Pt on shaped cerium oxide nanocrystal supports, PtSn intermetallic nanoparticles for hyperpolarization of water and alcohols, and certain other bimetallic combinations that have delivered substantially higher performance than monometallic nanoparticle catalysts. Lastly, a new approach utilizing heterogeneous catalysis for the continuous-flow synthesis of symmetric and pseudo-symmetric parahydrogen adducts hosting long-lived states will be presented.

Acknowledgements: NSF grants CHE-1808239 (CRB and WH), CBET-1933723 (HH-W and CRB) and the NHMFL-UCGP which is supported by the National Science Foundation Cooperative Agreement No. DMR-1644779 and the State of Florida. Technical assistance from Greg Labbe, John Graham and Bill Malphurs for construction of the 99% parahydrogen converter is gratefully acknowledged.

2. EW Zhao, R Maligal-Ganesh, Y Du, TY Zhao, J Collins... - Chem, 2018
3. EW. Zhao, R. Maligal-Ganesh,...., -Angew. Chem. 2017
STD-INEPT NMR for Examining Small Molecule Interactions with Nanoparticles

Hui Xu, Danielle Lustig, and Leah B. Casabianca
Department of Chemistry, Clemson University, Clemson, SC, 29634 USA

Previously, we have shown that Saturation-Transfer Difference (STD)-NMR experiments can be used to examine binding between small molecules and the surface of polystyrene nanoparticles.¹

The most common STD-NMR technique is based on a simple one-dimensional proton NMR spectrum, although two-dimensional STD-NMR experiments are also standard. When examining larger molecules binding to nanoparticle surfaces, spectral overlap becomes a problem in the proton NMR spectrum. When two or more NMR peaks overlap, STD-NMR information cannot be obtained for the individual nuclear sites. Two-dimensional STD-NMR techniques can improve spectral resolution, but are time consuming. The long experimental times required for two-dimensional NMR experiments are especially problematic when several experiments at different saturation times are required to construct an STD buildup curve. However, due to the large chemical shift dispersion of the ¹³C nucleus, carbon NMR generally has less spectral overlap than ¹H NMR. Therefore, we explored STD experiments in which the protons in the nanoparticle are saturated, and proton polarization is transferred to nearby carbon nuclei using an INEPT pulse sequence. ¹H→¹³C INEPT and ¹H→¹⁹F INEPT STD experiments have been previously suggested.²,³ In this work, we examine using ¹H→¹³C INEPT to explore small molecules binding to nanoparticles, quantity the STD effect, and compare the buildup curves generated with this experiment to those generated with standard two-dimensional STD NMR experiments. Figure 1 (a-c) shows how the spectral overlap in the ¹H NMR spectrum of pentanol is resolved in the ¹³C NMR spectrum. STD buildup curves generated using ¹H STD-NMR, Heteronuclear Single Quantum Coherence (HSQC)-STD NMR, and ¹H→¹³C INEPT STD-NMR. The INEPT buildup curve is in agreement with that from the HSQC experiment, but each HSQC experiment took 3 hours, while each INEPT experiment only took 16 minutes.

Figure 1. Comparison of various STD-NMR techniques for examining pentanol binding to the surface of carboxylate-modified polystyrene nanoparticles. (a) Chemical structure of pentanol with peak assignments. (b) ¹H STD-NMR spectrum of pentanol (c) ¹H→¹³C INEPT STD-NMR spectrum of pentanol. In (b) and (c), blue is the reference spectrum and red is the difference spectrum obtained at a saturation time of 3 seconds. (d) Comparison of STD buildup curves obtained from ¹H STD-NMR, Heteronuclear Single Quantum Coherence (HSQC)-STD NMR, and ¹H→¹³C INEPT STD-NMR. The INEPT buildup curve is in agreement with that from the HSQC experiment, but each HSQC experiment took 3 hours, while each INEPT experiment only took 16 minutes.

A site-specific comparison of the pentameric and hexameric assembly of the Rous sarcoma virus capsid protein

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In the maturation process, the immature spherical retroviral capsids reorganize into polymorphic mature capsids to encapsulate the viral genome. The retroviral capsid is a promising antiviral drug target, as its shape and stability is vital to the viral infectivity. The polymorphism of mature retroviral capsids is caused by insertion of twelve pentameric capsid proteins (CAs) into the hexameric lattice. However, due to the strong polymorphism, structural information of retroviral capsids is limited at the molecular level, especially the pentameric assembly that underpins the shape and size of the mature capsid. Here we report our solid state NMR (ssNMR) studies of the assembly of the 237-residue Rous sarcoma virus (RSV) CA, a widely used retroviral template. Torsion angles and dynamics are derived from chemical shift assignments of 220 residues in the RSV CA spherical assembly, formed entirely by CA pentamers. By comparing with our prior work¹ of the same protein in its tubular assembly comprising exclusively CA hexamers, we reveal, for the first time, site-specific structural differences that dictate the assembly morphology in its native state. This information lays the foundation for further study of the mechanism of capsid assembly at the molecular level and may assist rational design of anti-viral drugs against deadly diseases caused by retroviruses.

Reference:

Investigation of Brain Tissue Metabolite Local Environment with HR-MAS

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High Resolution Magic Angle Spinning (HR-MAS) is commonly used to study a variety of excised tissues, including brain tissue. Spectra obtained using HR-MAS approaches the line-widths of those seen in solution state NMR experiments on tissue polar extract, and allows for over a dozen small molecule metabolites to be identified¹. HR-MAS is also more physiologically relevant than polar extraction NMR, but there are inevitable metabolic changes which occur during the animal sacrifice and tissue removal, the most obvious being the higher levels of Lactate often seen in deceased tissue. However, significant line broadening is observed for in-vivo spectra, which limits the number of identifiable metabolites. HR-MAS thus represents a compromise, allowing many of the metabolites present to be identified while maintaining much of the tissue structure. This provides the potential to use HR-MAS to not only study the metabolite concentrations, but potentially their local environments within the tissue, providing further information not available through other techniques. The local environment of the metabolite, such as their presence in cytosol, extracellular space, cell membranes, etc., lead to changes in their molecular dynamics. These changes in motion, can be measured by NMR in a number of ways, depending on the timescales and changes in dynamics involved. We have measured the T₁ and T₂ relaxation times, as well as diffusion measurements, of the metabolites present in brain tissue. T₂ is measured using a modified version of the PROJECT sequence² in order to remove unwanted effects from J-coupling. PFG diffusion measurements were made using the PROJECTED sequence³. By contrasting relaxometry and diffusion data of metabolites, with that obtained from improved gel and MLV tissue phantoms, further information about the local environment of the metabolites can be obtained.

Furthermore, new MATLAB code has been developed to allow better metabolite peak deconvolution from the HR-MAS data in datasets with imperfect shimming, which will help improve the quality of data from the diffusion and relaxation measurements, as well as basic measurements of metabolite concentration in tissue samples.

Gramicidin A (gA) is a dimeric structure that spans lipid bilayers and forms a monovalent cation selective channel. This was the first peptide to be structurally characterized by solid state NMR when it was published in 1993 based on the newly developed structural technique of Oriented Sample Solid state NMR (OS ssNMR). This all atom structure broke with early models that had the opposite helical sense for the $\beta$-strand that wraps into a helix with 6.3 residues per turn. The helical structure is made possible by an alternating sequence of D and L amino acid residues – in other words the side chains are all on one side of the strand permitting the formation of a helix. Over the following decade the dimeric structure was highly refined and cross-validated.

Throughout all of those studies the structure was shown to perfectly symmetric in the presence and in the absence of monovalent cations. Initial $^{17}$O OS ssNMR studies at 21T (900 MHz for $^1$H) and 19.5T (830 MHz for $^1$H) more than a decade ago continued to show no reason to believe that there was any break in the dimeric symmetry.

The much improved spectral sensitivity and resolution for $^{17}$O spectroscopy at 35.2 T obtained in the Magnet Lab’s DC powered Series Connected Hybrid Magnet clearly shows that the symmetry is broken and yet recent $^1$H-$^{15}$N PISEMA spectra continue to show no evidence for such a break in symmetry. The single file of water molecules that populates the gA pore is uniformly aligned from one side of the membrane to the other side forming an electric dipole that spans the membrane. These 7-8 water molecules are hydrogen bonded together leaving a proton per water available to hydrogen bond with 7 or 8 of the 26 carbonyl oxygens that line the pore of the dimeric gA structure. Indeed, it is these waters that break the dimeric symmetry, not the structural symmetry, but the hydrogen bonding symmetry to carbonyl oxygens as detected by $^{17}$O NMR. The strength of the electric dipole accounts for the difference in cation affinities at either end of the gA structure. Molecular Dynamics and DFT calculations have been used to confirm the $^{17}$O spectral shifts, but the instability of the water wire in most MD simulations appears to be inconsistent with the $^{17}$O spectral results that are dependent on millisecond timescale stability of the water wire and electric dipole orientation. It appears that the water models used for MD based on the properties of bulk water may not be appropriate for the single file waters in the gA pore.
HYDRATION ENVIRONMENT CHARACTERIZATIONS OF THE FOLDING OF IA3, AN INTRINSICALLY DISORDERED PROTEIN

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IA3 is an intrinsically disordered protein (IDP), found in Sacchoromyces cerevisiae, that has been previously shown to adopt α-helical secondary structure when bound to yeast proteinase A (YPRA). The α-helical structure of IA3 can be stabilized in the absence of YPRA, by using 2,2,2-trufluoroethanol (TFE). Site-directed spin-labeling (SDSL) along with electron paramagnetic resonance (EPR) and circular dichroism (CD) has been used to characterize the TFE induced folding of IA3. Here we report results from cysteine scanning through the N-termini, which reveals evidence for the degree of unfolded-ness of the region. Additionally, results demonstrate a sensitivity of the N-terminal region to amino acid substitution. Particularly, sites V8 and S9 are sensitive to SDSL, where alterations in amino acid structure are shown to limit or strengthen the TFE induced structural transition, respectively. We find that site S14C-SL behaves similarly to WT in TFE-induced folding, making S14 a useful spin-label reporter site to probe the impact of mutations at V8 and S9. Overhauser dynamic nuclear polarization (ODNP) was used to further understand hydration effects on IA3 protein folding. Hydration effects impact proper folding of proteins, and the study of IA3’s hydration when disordered or ordered in structure using mutants of IA3, with WT deviating behavior, can lead to a clearer representation of the secondary structure characteristics of this model IDP. Results from CD, EPR and ODNP will be presented. Through the use of these combinatorial techniques, a clearer representation of the disordered and ordered states of IA3 will be determined. This model system can further be used to hypothesize about other functional IDPs and their structural transition upon protein function.
A. Ligia Focsan, Valdosta

**ENHANCEMENT OF ANTIOXIDANT ACTIVITY OF CAROTENOIDs IN SUPRAMOLECULAR COMPLEXES AND THE ROLE OF THEIR REDOX POTENTIALS STUDIED BY EPR SPIN TRAPPING TECHNIQUE**

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Electron transfer plays an important role in many biological processes with participation of carotenoids, including their antioxidant activity. We have found using EPR spin trapping technique that the ability of carotenoids to scavenge the reactive oxygen species (ROS) is extremely sensitive to their redox potentials, and increases sharply with increasing oxidation potentials of carotenoids [1]. Carotenoid bixin exhibits the highest measured oxidation potential (0.94 V) to date and its scavenging ability towards ROS was estimated to be 17 times higher than that of astaxanthin [2]. Another advantageous property of bixin is that it does not form H-aggregate in aqueous solutions like zeaxanthin, lutein and astaxanthin do [3]. H-aggregation significantly reduces the scavenging ability of carotenoids towards ROS. We have demonstrated that incorporation of highly hydrophobic carotenoids into water soluble delivery systems significantly affects the properties of carotenoids including their water solubility, stability, optical properties, self-association ability, oxidation potentials and even stability of radical paramagnetic forms [4,5]. For example, inclusion of some carotenoids into glycyrrhizin complex affected their oxidation potentials resulting in increased antioxidant activity and prevented aggregation of xanthophyll carotenoids in aqueous solutions. This work was supported by The Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Sciences, U.S. Department of Energy, grant DEFG02-86ER-13465, and by the National Science Foundation for EPR instrument grants CHE-0342921 and CHE-0079498 (L.D.K.). This work was also supported by Faculty Research Seed Grants (FRSG) Program at Valdosta State University (A.L.F.) and the Russian Science Foundation (grant no. 18-13-00047) (N.E.P.).

COMBINING THE GENETICS OF A MODEL ORGANISM WITH QUANTUM CHEMISTRY FOR UNKNOWN COMPOUND ID IN METABOLOMICS

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University of Georgia

Unknown compound identification remains a major challenge in metabolomics. We are combining genetics and quantum chemical calculations as tools to aid traditional analytical chemistry. Our approach leverages the extensive genomic data available for the small nematode, *Caenorhabditis elegans*. This project is a Compound Identification Core as part of the NIH Metabolomics Common Fund. I will present the overall structure and rationale of the project, which is still in its early stages. We have developed several new components that are critical for its overall success, including an approach to making a stable and long-term reference material, a robust method to organize metadata across a large study, new methods for producing large numbers of worms sustainably, optimization of LC-MS measurements, and attempts to extract stage-specific information from mixed developmental stage cultures.
Dynamic nuclear polarization (DNP) is an increasingly popular approach used to enhance weak nuclear magnetic resonance (NMR) signals. DNP involves microwave irradiation of paramagnetic species at their electron frequency, which results in the transfer of a large electron spin polarization to surrounding nuclei. Thus far, many applications have been developed for solid state DNP including some structural analysis of membrane proteins and enzyme-inhibitor complexes. However, liquid state DNP applications are still in their infancy due to the challenge of applying the technique at higher magnetic fields (above 10 T). At higher magnetic fields, the efficiency of the transfer of polarization via the Overhauser effect is greatly reduced. This effect is driven by electron-nuclear interactions, namely scalar interactions and dipole-dipole interactions. Due to major improvements in instrumentation, large DNP enhancements are achievable via scalar interactions. In this work we focus our efforts on improving the DNP enhancement of $^{13}$C by optimizing sample preparation with new bi-radicals. Our group has developed new bi-radicals, which are more effective than traditional mono-radicals in liquid DNP. It is hypothesized that the two closely bound electrons in the bi-radicals interact via direct exchange, which reduces the linewidth of their electron paramagnetic resonance (EPR) spectrum, thereby making it easier to saturate and increase the DNP enhancement.
This talk will highlight recent developments in the area of pulsed broadband EPR at the National High Magnetic Field Laboratory in Tallahassee. These efforts have been facilitated by the acquisition of a high-power quasi-optical 94 GHz (W-band) spectrometer – HiPER [1] – that was developed at the University of St. Andrews. Microwave pulses are generated at 7.833 GHz and upconverted to 94 GHz via a $\times 12$ solid-state multiplier chain. The microwave power is then boosted via an extended interaction klystron (EIK) amplifier with 60 dB of gain and a 1 GHz instantaneous bandwidth. Transmission of microwaves to the magnet cryostat and isolation of the returning inductive mode EPR signal in the polarization orthogonal to the excitation pulse is achieved via a quasi-optical bridge. The ability of the EIK amplifier to deliver up to a kilowatt of instantaneous power does away with the need for a microwave resonator (i.e., Q ~ 1). This enables generation of broadband 94 GHz pulses via modulation of the 7.833 GHz base frequency, limited only by the 1 GHz bandwidth of the amplifier. After a brief overview of HiPER, the talk will focus on recent applications that take advantage of this instrument’s considerable bandwidth, which enables pulsed excitation of relatively broad electron- and electron-nuclear magnetic resonance spectra. The first example involves two-dimensional pulsed electron-electron double resonance (PELDOR) measurements in which a frequency-pure saturation pulse is used to burn narrow holes in the inhomogeneously broadened spectrum, followed by a spin-echo detection sequence. The full spectrum can be collected either by scanning the frequency of the detection sequence [2,3] or, using an arbitrary waveform generator (AWG), it is possible to image the complete spectrum using chirped pulses followed by Fourier-transform detection [4]. Examples involving both approaches will be presented, where holes are generated at both the electron- and weakly-allowed electron-nuclear transition frequencies, thereby enabling rapid acquisition of NMR signals over a wide frequency range and with the advantages of EPR sensitivity. The second example also exploits the near simultaneous excitation of electron-only (single-quantum) and weakly-allowed electron-nuclear (zero- and double-quantum) transitions using frequency-chirped pulses, giving rise to improvements in the efficiency of dynamic nuclear polarization in solids [5].

A SIMULATED ANNEALING ALGORITHM FOR DE NOVO PULSE SEQUENCE DESIGN OF SEPARATED LOCAL FIELD NMR EXPERIMENTS

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A typical NMR pulse sequence design involves finding the phases, amplitudes, and timings of rf pulses that would evolve the specific Hamiltonian terms of interest while averaging out the unwanted terms. For the evolution of heteronuclear dipolar couplings, also known as separated local-field (SLF) spectroscopy in solid-state NMR, the homonuclear interactions are canceled out through a series of alternating symmetric and antisymmetric pulses applied on the low and high spin channels. In conventional analytic treatments, the pulses usually have quadrature phases in order to keep the calculations tractable. Non-quadrature phases are not routinely employed due to the sharply rising complexity of an analytical treatment. By contrast, computer simulations of NMR experiments can greatly aid pulse sequence design with virtually any complexity of pulses. However, these simulations can become costly, with the dimensions of the density matrix rising exponentially with the number of spins considered. Nevertheless, when properly optimized, such simulations can be of great use in designing pulse sequences for selectively detecting the desired NMR observables, such as heteronuclear dipolar couplings used for structure determination of uniaxially oriented membrane proteins.

We have developed a fully automated computer program to design SLF pulse sequences that have optimal linewidths for $^1$H-$^{15}$N dipolar couplings. By utilizing highly efficient GPU computing, the program searches for the optimal experimental parameters for a pulse sequence, i.e. the pulse phases and timings, by simulating its spectra and finding the best parameters using the Monte Carlo Simulation Annealing algorithm. Given a pre-defined pulse sequence architecture, i.e. the number of subdwells and an rf-amplitude scheme on the different channels, this program can numerically optimize the parameter combinations to produce the sharpest linewidths for the heteronuclear dipolar doublets. Such pulse sequences termed “ROULETTE”, produce linewidths that are on average 20% sharper on an NAL crystal (fig. 1), and 11% sharper on a Leucine labeled Pf1 coat protein reconstituted in magnetically aligned bicelles as compared to the existing SAMPI4 pulse sequence (Nevzorov and Opella 2007). Moreover, the signal to noise ratio was improved from 17:1 in SAMPI4 to 20:1 in ROULETTE. The simulations allowed for including non-quadrature pulse phases that otherwise would have been too cumbersome to treat analytically, as well as pulse sequences with varying levels of symmetry between their dwells and subdwells. While exemplified for SLF experiments that evolve $^1$H-$^{15}$N dipolar couplings, this method of pulse sequence design is extendable to practically any experiment that can be simulated with reasonable accuracy.

**Fig. 1.** SAMPI4 pulse sequence (blue) vs. the computer designed ROULETTE pulse sequence (red) on an NAL crystal. Peak linewidths in the $^1$H-$^{15}$N dimension are given for each peak as indicated.

Spontaneous fluctuations in the blood oxygenation level dependent (BOLD) MRI signal are correlated in brain areas that are strongly interconnected (e.g., left and right motor cortex), a feature called functional connectivity. Despite the widespread research into functional connectivity differences related to cognitive performance, aging, and neurological/psychiatric disorders, the neurophysiological origins of functional connectivity are poorly understood. Using simultaneous microelectrode recordings and MRI in the rat, we show that the BOLD signal fluctuations reflect a coordinated spatiotemporal pattern of infraslow electrical activity that is repeated over time. This suggests that the functional connectivity networks are actually snapshots of particular phases of a brain-wide organization, possibly controlled by neuromodulatory centers.
Douglas Kojetin, Scripps

LIGAND-INDUCED STRUCTURAL DYNAMICS AND FUNCTION OF PPARγ LEARNED THROUGH NMR

Douglas J. Kojetin

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To ensure the correct formatting, please replace this text with your abstract text. Structural studies of nuclear receptor transcription factors have revealed a conserved mechanism for agonist-induced coactivator interaction and transcriptional activation. In contrast, limited structural data indicate that ligand-induced corepressor-dependent nuclear receptor repression occurs through structurally diverse mechanisms. Furthermore, the conformational ensemble of the apo/ligand-free nuclear receptor ligand-binding domain, which is hypothesized to exchange between transcriptionally active and repressive conformations, remains poorly characterized. I will discuss our efforts to characterize how small molecule pharmacological ligands—agonists, antagonists, and inverse agonists—influence the conformational ensemble and function of the nuclear receptor PPARγ.
Integrative High-Throughput NMR Platform for Biomolecular Research

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Three major bottlenecks in characterizing structures and functions of biomolecules by NMR are: a. biological limitations such as low yield and stability of the sample, b. instrumental limitations such as cost, time, spectral resolution and pulse sequences, and c. lack of expertise for handling and interpreting data. Among three bottlenecks, the biological limitations have been tackled by many groups testing a variety of plasmids and developing cell-based and cell-free technologies because the production of a reliable protein sample is fundamental and ubiquitous in the biochemical study. However, technologies towards two other aspects have been driven by only a few groups.

To address these issues, I have developed a computational platform called Integrative NMR, which provides a seamless and interactive environment for biomolecular research [1]. This system makes biomolecular NMR spectroscopy much more accessible by integrating software tools so that they interact efficiently in ways that support automation, validation, and visualization. These include non-uniform sampling (NUS), reduced dimensionality (RD) [2], automated signal detection and chemical shift assignment [3] and automated structure determination [4,5]. One of the main components, the NMRFAM-SPARKY software package has become the most popular analysis tool in biomolecular NMR and the 70+ YouTube video tutorials I recorded and uploaded have amassed over 56k views worldwide in just four years [6]. My group continues working on developing more advanced IHT-NMR (Integrative High-Throughput NMR) for both solution and solid-state NMR that can finish structural and functional analyses of proteins routinely within a week by connecting the Integrative NMR and fast data collecting technologies that reduce actual cost. IHT-NMR will provide an interactive bio-NMR platform for scientists to carry out NMR experiments, data analyses, and structure calculations.

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Megan Macnaughtan, LSU

**Structural independence of Scc4’s dual functions in *Chlamydia trachomatis***

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*Chlamydia trachomatis* is an oligate intracellular bacteria pathogen responsible for the most common sexually transmitted bacterial disease in the world. The bacterium has a unique bi-phasic developmental cycle with a type III secretion system to invade host cells. Scc4 is a protein that functions both as a type III secretion system chaperone and as an RNA polymerase binding protein to regulate σ⁶⁶-dependent transcription. Our research group has worked to determine the high-resolution structure of Scc4 and to understand its binding interactions with its protein partners. As a transcription factor, Scc4 binds the RNAP holoenzyme between σ⁶⁶ region 4 and the beta flap of the beta subunit. As a type III secretion system chaperone, Scc4 binds to its partner protein, Scc1, to chaperone the essential virulence factor, CopN. The structure of the Scc4:Scc1 complex has been a focus of intense study in our laboratory. A mutant of Scc4 was identified that disrupts the Scc4:Scc1 interaction, but maintains Scc4’s ability to bind RNA polymerase holoenzyme. The progress made to date on the structure of Scc4, the chaperone complex, and the mutant will be discussed.
Dynamic Nuclear Polarization (DNP) is a technique capable of boosting the sensitivity of an NMR experiment by two to three orders of magnitude. Currently, the method is mostly used in solid-state NMR experiments to enhance signal intensities at magnetic field strengths corresponding to NMR frequencies up to 900 MHz. As a result, DNP enables scientists to conduct experiments that were unthinkable even a decade ago.

DNP for solution-state NMR experiments is a much more challenging task, due to the high dielectric losses of the solvent, which leads to microwave induced sample heating. Solution-state DNP, based on the Overhauser effect (ODNP), is therefore typically performed at low magnetic field strengths. At 0.35 T, corresponding to a proton Larmor frequency of 14.5 MHz, many concepts from X-band EPR spectroscopy can be applied to build efficient ODNP resonators with minimal sample heating. However, at this low magnetic field the overall sensitivity is low, especially for low-gamma nuclei, and the resolution is often poor.

Figure 1: ODNP-enhanced proton NMR spectra of neat ethyl crotonate (left) and aspirin 144 mM in DCCl₃ (right) at 0.35 T.

Here, we demonstrate high-resolution, ODNP-enhanced NMR spectroscopy at 0.35 T on samples of small molecules such as ethyl crotonate and aspirin (see Figure 1). Even at a polarizing agent concentration of 10 mM TEMPO we are able to resolve the J-coupling and many features of the NMR spectrum. Until now, X-band ODNP spectroscopy has been typically used to study hydration dynamics of water molecules of bio-macromolecular surfaces. Here we demonstrate in-situ ODNP to enhance NMR signals at low magnetic fields. The application to reaction monitoring of small molecules and studies of crude oil samples will be discussed along with its application to enhance NMR signals of different nuclei such as ¹⁹F and ³¹P and two-dimensional NMR spectroscopy.
FLOW, AND ELECTRIC CURRENT INDUCED PHASE IN MR IMAGING

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The coherent precession of resonating magnetization induces a measurable voltage in the receiver, which is expressible as a complex-number value at each measurement point, using quadrature detection. In a complex–number plane, this value has a magnitude (peak of the voltage) and a phase (relative angle of magnetization precession). MR phase is routinely used to encode frequencies in spectroscopy and spatial information in imaging. But most MR images are viewed as magnitude intensity without considering the image phase. However, image phase is a unique tool to encode additional subtle features of the physical system. Two examples of physically induced changes in magnetic resonance image phase are discussed.

The first example uses a phase-sensitive imaging method to measure flows [1], which we modified to measure very slow, creeping flow in fluids [2]. This application required accommodation for thermal effects, and a characterization of the spatial-gradient system performance to compensate for eddy current induced artifacts. This method of measuring very slow creeping flow may allow the visualization of glymphatic flow in the perivascular spaces in the brain [3]. The second example is a phase-sensitive imaging method to indirectly measure electric current [4], which we modified to measure the magnetic field induced by electric current flow in the human brain resulting from transcranial electric stimulation [5,6]. Using the measured phase change, the magnetic field induced by the electric current is used to estimate the electric current density in the tissue. This application required a sequence modification to accommodate for the static magnetic field drift of a 3 T superconducting magnet, that caused a time-dependent shift in measured phase, which was larger that the phase shift caused by the induced electric current.

This examples illustrate how magnetic resonance phase can encode a phenomena into a magnetic resonance image to provide a map of a physical process not directly observable in magnitude intensity images.

A particularly promising avenue for advancing Dynamic Nuclear Polarization (DNP) in chemistry and biology is in developing frequency-agile high-power mm-wave sources. Currently, significantly less expensive solid-state devices are still trailing behind gyrotrons by more than two orders of magnitude in terms of the available power. We are aiming at closing this gap by concentrating mm-waves on the sample using μl-volume resonators. A next-generation 200 GHz mm-wave photonic band gap (PBG) resonator based on LiTaO$_3$/optical quartz has been constructed and integrated with the doubly tuned DNP NMR probehead operating at 75/300 MHz $^{13}$C/$^1$H frequencies. The use of the high-$\varepsilon$ ($\varepsilon=40$) perovskite LiTaO$_3$ improved the gain in mm-wave power by about 2.5-fold, i.e., from 12- to 30-fold as compared to the previously employed AlN/fused silica configuration [1].

A comparison of the signal amplitudes demonstrates ca. 30-fold gain in mm-wave power obtained with the resonator (green line) at just 3% of the input mm-wave power as compared to 100% input power for the empty configuration (purple line). 80 scans were acquired with 100 s DNP time.

The PBG resonator was constructed from two (2) disks of LiTaO$_3$ (diameter=6.7 mm, thickness=60 μm) and two (2) disks of optical quartz (thickness=190 μm). The sample consisted of 3M™ 666XW lapping film (3M Inc., Maplewood, MN) having 1 μm sized microdiamonds embedded into 3 mil thick polyester film with a pressure-sensitive adhesive (PSA) backing (ca. 30 μm thickness). The experimental mm-wave resonator tuning curve shown in Fig. 1A yielded the quality factor $Q=340$, which exceeds the experimental $Q=252$ we have demonstrated last year [1]. More importantly, the gain in power is largely due to the much improved resonator finesse estimated at $F=166$ vs. the previous $F=75$. Direct comparison of the NMR intensities in Fig. 1B demonstrates that the same DNP gain is obtained at just 3% input mm-wave power with the new resonator as compared to the empty (no resonator) configuration at full power. Thus, the gain in power at the sample with the LiTaO$_3$/quartz resonator is about 30-fold, which corresponds to effectively 4.5 W at the sample with just 150 mW input power. The obtained gains are also analyzed and supported by extensive mm-wave COMSOL simulations.

COORDINATION NUMBER AND SUBSTRATE-BINDING DENTICITY DETERMINED FOR NON-HEME THIOL DIOXYGENASES BY ANALYTICAL CW X-BAND EPR

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The X-ray crystal structure for the substrate-bound Rattus norvegicus cysteine dioxygenase (CDO) reveals a bidentate coordination of L-cysteine (cys) to the mononuclear non-heme iron active site via a thiolate and neutral amine. While the structure for the substrate-bound 3-mercaptopropionic acid (3mpa) dioxygenase (MDO) remains unsolved, we have argued on the basis of kinetic and spectroscopic studies (Mössbauer and EPR) that substrate-binding occurs via thiolate Fe-coordination only. As a consequence of this difference in the CDO and MDO Fe-coordination sphere, CW X-band EPR experiments, in which nitric oxide (NO) is used as a surrogate for molecular oxygen, reveal a spin-state change for the for the resulting substrate-bound iron-nitrosyl site that is correlated to substrate binding denticity [bidentate (S = 1/2) versus monodentate (S = 3/2)]. Complementary EPR studies in which the catalytically inert Fe(III) enzymes were titrated with cyanide (CN⁻) were performed in to analytically determine the Fe-coordination number for each enzyme. These studies verify that CDO which binds substrate bidentate is 6-coordinate whereas MDO which binds substrate monodentate is 5-coordinate. Crucially, the active site coordination number appears to be largely controlled by interactions with a single outer Fe-coordination sphere tyrosine (Y159) residue. While the physiological relevance of the iron-nitrosyl spin state is uncertain, it suggests that these enzymes may produce different transient iron-oxo intermediates in reactions with O₂ despite catalyzing the same oxidation of a free thiols (-SH) to produce the corresponding sulfinic acid (-SO₂⁻). Moreover, this shift in NO-reactivity appears to be gated by interactions with outer Fe-coordination sphere residues which influence the active site coordination number.
Will Placzek, UAB

CONNECTING APOPTOSIS TO THE CELL CYCLE USING A NEW PROTEIN-PROTEIN INTERACTION MOTIF

William J. Placzek and Robert H. Whitaker

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Commitment to cell cycle entry and cellular duplication is a tightly coordinated and regulated process. Once initiated, a series of multiple checkpoints ensure both accurate genomic replication and chromosomal separation. In the event of unsuccessful cell division, parallel pathways exist that induce the cell to undergo programmed cell death, or apoptosis. At the center of such stress-induced, intrinsic apoptotic regulation lies the BCL2 family of pro- and anti-apoptotic regulatory proteins (1-3). In a proliferative state the balance of pro- and anti-apoptotic signaling proteins would be expected to favor an excess population of anti-apoptotic members. While the anti-apoptotic BCL2 family member, MCL1, has been identified to oversee mitotic progression (4), direct communication between the BCL2 family and cell proliferation has not been observed. In this study we demonstrate a direct protein-protein interaction between MCL1 and the G1/S checkpoint protein, P18\(^{\text{INK4C}}\). This interaction is mediated by a reverse BH3 (rBH3) motif located in P18\(^{\text{INK4C}}\)’s C-terminal ankyrin repeat. MCL1 is further shown to decrease P18\(^{\text{INK4C}}\) expression and thereby regulate cell cycle entry in a retinoblastoma (RB1)-dependent manner. Our findings establish a mechanism for translation independent and direct communication between the BCL2 family regulation of apoptosis and early G1/S transition CDK4/6-RB regulation of cellular division/growth.
Binomial Excitation for Detection of Hyperpolarized Dihydroxyacetone Metabolism in Perfused Mouse Liver
Mukundan Ragavan¹, Anthony Giacalone¹, Alan Carter¹, Matthew Merritt¹

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Hyperpolarization has enabled measurement of ¹³C spectra reporting on metabolic turnover in biological systems that were previously not accessible due to signal-to-noise constraints¹,². In this work, we have employed a simple binomial excitation scheme to observe hyperpolarized dihydroxyacetone metabolism in liver perfused under challenging physiological conditions. C57/BLKS mice were used as models for all experiments. Animals were handled in compliance with the University of Florida IACUC regulations. Livers were perfused through the portal vein with oxygenated Krebs-Henseleit buffer containing 1.5%(w/v) bovine serum albumin, mixed fatty acids, lactate and pyruvate (lac:pyr is 10:1). After 30 min of perfusion, 4 mM HP [2-¹³C] dihydroxyacetone was injected directly into the liver and ¹³C NMR spectra were acquired in real time. All experiments were carried out in a NMR spectrometer (14.1 T magnet) equipped with a 18 mm (Doty Scientific, USA) or 20 mm broadband probe (QoneTec, Switzerland). ¹³C spectra were collected using a binomial excitation scheme (45° {x, -x} pulses) with ¹H decoupling (WALTZ-16) during acquisition. Hyperpolarized DHA metabolism in perfused liver observed using this scheme is shown in Figure 1. The parent DHA signal is much lower when using the binomial excitation thus enabling the use of higher receiver gain. Signal intensities of downstream metabolites are substantially higher when measured with binomial pulses as compared with small flip angle (in this case, 30°).

To summarize, we have used a simple binomial scheme to observe resonances within a large bandwidth of frequencies (>200 ppm) and enabling robust detection of metabolites in perfused livers.

Monique Rogals, UGA

Optimizing Solution NMR for Structural Studies of Glycosylated Mammalian Proteins

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As much as 80% of mammalian proteins are glycosylated, many requiring glycosylation for proper folding and function. Producing isotopically labeled samples of these proteins with native glycosylation is best done in mammalian cell culture. Today expression in mammalian cell culture often produces samples in amounts and on a time scale that is competitive with expression in bacterial cultures. However, it does come with some limitations; uniform isotopic labeling with 13C and 15N can be prohibitively expensive (a mix of labeled amino acids is needed), and perdeuteration is not well tolerated. For larger proteins these limitations virtually eliminate triple resonance approaches for structural studies. We describe a procedure for assigning glycosylated proteins that begins with an efficient sparse labeling strategy that uses a single or a few types of the less expensive isotopically labeled amino acids. For larger, fully protonated proteins, observation methods that directly detect 13C, such as HETCOR instead of HSQC experiments, prove advantageous. For assignments of proteins that have a crystal structure or good homology model, we use an approach that uses a genetic algorithm to search for a best match between experimental and predicted observables (NOEs, RDCs, PCSs and chemical shifts) for each crosspeak. Illustrations of the procedure will be drawn from work on glycosylated proteins such as Robo1, CEACAM1, and ST6Gal1, as well as a 150 kDa heterotetramer (SH1) produced by an archaeal hyperthermophile.
Organic materials are considered promising to be used as electrodes in lithium-ion batteries with high energy density and low cost due to their high theoretical capacities, high redox potentials and eco-friendliness. However, organic electrodes face critical challenges, such as poor electronic and ionic conductivities and high solubility in the electrolyte solution. In this study, we investigate the electrochemical properties and intermediates of chloranilic acid during cycling, correlating the cycling profile with the evolution of $^1$H and $^6,^7$Li NMR as well as UV-Vis. The results elucidate the causes of capacity degradation of chloranilic acid cathode: side reaction products and the solubility of chloranilic acid and its intermediates. Through NMR characterizations, this study reveals the mechanism of electrochemical cycling of chloranilic acid cathode and the origin of deterioration of cycling performance, providing insights for the improvement of organic cathodes.

FREQUENCY-SWEPT PULSES AND PULSES DESIGNED WITH OPTIMAL CONTROL THEORY FOR ULTRA-WIDELINE NMR SPECTROSCOPY

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Solid-state NMR (SSNMR) spectra often feature patterns that range from hundreds of kHz to several MHz in breadth. Ultra-wideline NMR (UWNMR) patterns are those that exceed ca. 250 kHz, since conventional rectangular pulses are unsuitable for producing uniformly excited patterns. Frequency-swept (FS) pulses are often utilized for broadband excitation and refocusing, and have found much use for the acquisition of UWNMR spectra.[1] Of the FS pulses, Wideband Uniform-Rate Smooth-Truncation (WURST) pulses are the most widely used in UWNMR experiments on both spin-1/2 and quadrupolar nuclei; however, they have limitations in terms of yielding uniformly excited, distortion-free spectra.[2]

In this lecture, I will present our recent explorations into two new classes of pulses for use in UWNMR experiments: (i) hyperbolic secant (HS) and tanh/tan (THT) pulses for broadband excitation and refocusing,[3] and (ii) new pulses designed with optimal control theory (OCT),[4] which feature uniquely optimized amplitude and phase modulations. In the former case, HS and THT pulses were tested via simulation and experiment on systems featuring spin-1/2 or quadrupolar nuclei, including $^{119}$Sn, $^{195}$Pt, $^2$H, and $^{71}$Ga, and resulting spectra were compared with those obtained from analogous WURST experiments. In the latter case, new OCT Optimized Broadband Excitation and Refocusing (OCTOBER) pulses were generated from WURST, HS, and THT pulses as starting points (the SIMPSON software package[4] was utilized for this purpose), and demonstrated to work under experimental conditions. It is hoped that OCTOBER pulses will lead to new insights into developing a generalized class of pulses for the acquisition of uniform, distortion-free, UWNMR patterns.

References:


Dynamic Nuclear Polarization (DNP) increases NMR sensitivity via unpaired electron spins added to the NMR sample. The resonance frequency for a radical is approximately 660 times that of \( ^3 \text{H} \) nuclei, and thus is in the GHz range for modern NMR spectrometers. The dielectric properties of materials used in NMR and DNP have been studied in the low GHz range but data is often missing in the range currently employed for DNP (200-600 GHz). Dielectric constant values are critical in designing DNP-NMR probes and rotors to minimize microwave loss, as well as new types of DNP and EPR resonators to maximize Q-factors\(^1\)-\(^3\).

Dielectric constants were extracted from spectra obtained with a network analyzer and quasi-optical system. The refractive index \((n)\) and damping constant \((k)\) are extracted from the interferograms by fitting an interference pattern model, as shown in Figure 1. Our experiments show that refractive index values are fairly constant with frequency, while changes in the damping constant over frequency are observed. When microwaves are reflected from a rotor surface or absorbed before reaching the sample center, DNP effectiveness is diminished. Common matrices for DNP such as frozen glycerol-water mixtures and rotor materials such as sapphire have been measured in a variety of frequency ranges from 70 GHz to 960 GHz. Glassy matrix measurements were taken in a bath of liquid nitrogen while sapphire and yttrium-stabilized zirconia\(^4\), and other common rotor materials were measured at \( \sim 300 \) K. These dielectric properties will be used in microwave simulations to inform MAS-DNP probe, rotor, and even sample mixture design.

**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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**Figure 2.** Reflection spectra of a glycerol-water mixture (60/40) at 80 K (green) and sapphire at room temperature (red). Each spectrum is fit to a mathematical model to find the refractive index \((n)\).
Robert Silvers, FSU

Neurodegenerative diseases and the mitochondrial unfolded protein response

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The mitochondrial matrix possesses a stringently regulated machinery to maintain proteostasis, the mitochondrial unfolded protein response (UPRMT). The UPRMT is highly conserved in eukaryotes and allows cells to manage stress that is imposed by unfolded proteins accumulating mitochondrial matrix. Because of that central position, the UPRMT is involved in the onset of a multitude of diseases such as neurodegenerative diseases including Creutzfeldt-Jakob disease, Alzheimer’s disease, Parkinson’s disease, and Huntington's disease, as well as cancer.

Unlike other cellular compartments, however, the mitochondrial matrix is particularly unique in the way that it integrates folding and assembly of proteins that are derived from nuclear as well as mitochondrial genomes. The UPRMT senses the accumulation of unfolded proteins in the mitochondrial matrix and induces changes in nuclear gene expression (e.g. mitochondrial chaperonins). Additionally, the UPRMT also affects the matrix localized protein synthesis through translational inhibition by downregulating the mitochondrial RNase P, a protein that is involved in tRNA maturation.

Human mitochondrial RNase P is the first of its kind in the sense that it does not require any RNA component unlike all previously discovered RNases P. In humans, it only works as a trimeric complex consisting of mitochondrial RNase P proteins 1, 2, and 3 (MRPP1 3). Whereas all three mitochondrial RNase P proteins appear to bind RNA to some extent, their exact stoichiometry and modes of interaction, their individual contribution to substrate recognition and specificity, and their precise role in hydrolysis/catalysis remain elusive.
Many biophysical experiments with biological systems including proteins, lipid bilayers, and cells are carried out with frozen aqueous samples with temperatures down to ca. 150-77 K and even to ca. 2 K in some cases. Cryopreservation at 77 K by freezing samples in liquid nitrogen is the most common means to preserve integrity of biological samples including whole cells and tissues as all the biological activity and biochemical reactions stop at such a low temperature. Cryogenic temperatures are also a necessity for DNP NMR to provide for penetration of mm-waves into aqueous samples and to increase electronic relaxation time of polarization agents. For similar reasons the optimal temperature for DEER experiments with nitroxides is usually 40-60 K. However, freezing is known to change the samples at least in some extend. The most notable is an exclusion of solute molecules by growing ice crystals. While formation of ice crystals could be suppressed by adding cryoprotectants or co-solvents, the dielectric properties of frozen glasses still will be different from the corresponding liquid phase. The latter could affect surface charges of proteins and membranes with possible consequences for their structural organization. Limited research literature on these effects indicates that freezing of aqueous samples would also shift $p$H up to 2-3 units in either directions depending on the buffer and the nature of the ions present.

Here we describe the use of EPR of ionizable nitroxides employed as soluble probes or labels attached to biomolecules to probe effective acidity of bulk glassy matrices and also micelle and lipid bilayer interfaces upon freezing. Specifically, for bulk water- $i$-PrOH glassy mixtures (40:60 vol%) acidity of the solution at 290 K should be increased by $\Delta p$H=3.7 in order to maintain IKMTSL-2-mercaptoethanol (IKMTSL-ME) in the same protonation state at 77 K as at 290 K. However, this difference decreased to $\Delta p$H=1.4 when fraction of $i$-PrOH decreased to 20%. While significant $\Delta p$H values for water-detergent micelle interfaces upon freezing, remarkably, the ionization of the same reporting nitroxide tethered to the polar heads of a lipid and incorporated into DMPG lipid bilayers did not change upon freezing when compared to lipid bilayers existing in a gel phase at 290 K (see Fig. 1, right). Thus, it appears that the properties of the biomolecule-water interface rather than bulk properties of the solvent such as dielectric constant govern the ionization of the surface-exposed residues upon sample freezing.

**Figure 1.** Left: An example of least squares decomposition of a rigid-limit (77 K) 9.5 GHz EPR spectrum of IKMTSL-ME in a buffer solution containing 20 %v/v isopropanol (A) into two components (B) and (C). Right: Fractions of the non-protonated form of IKMTSL-PTE (1 % mol) in multilamellar DMPG vesicles at 321 K (○, red solid line), 290 K (●, pink solid line), and rapidly cooled from 290 K to 77 K (▲, cyan dashed line), and from 321 K to 77 K (●, blue solid line). The pH scale corresponds to experimental values measured at 290 or 321 K using a conventional pH electrode.
Veronika Szalai, NIST

**EPR Spectroscopy Measurement Development for Nanobiomaterials**

Veronika A. Szalai,¹ Alan Band,¹† Matthew P. Donohue,¹,²,³ Boris Epel,¹,⁴ Nandita Abhyankar,¹,² Amit Agrawal,¹,² Robert D. McMichael,¹ Shraeya Madhu¹

¹National Institute of Standards & Technology, Gaithersburg, MD ²Institute for Research in Electronics & Applied Physics, University of Maryland, College Park, MD ³Current Address SalubrisBio, Inc., Gaithersburg, MD ⁴Center for EPR Imaging in Vivo Physiology, University of Chicago Medical Center, Chicago, IL †Deceased

Recent advances in nanofabrication and biotechnology rely on biomacromolecules, which are often used to marry bottom-to-top self-assembly with top-down lithographic methods. In biotechnology, biomacromolecules are either themselves therapies (monoclonal antibodies) or are used in combination with nanoscale drug delivery vectors. Structural measurements on biomacromolecules enable design and engineering of robust, reliable systems. Electron paramagnetic resonance (EPR) spectroscopy is a structural biology method that is particularly powerful for nanoscale systems lacking long-range (crystalline) order, a regime in which many biomacromolecules fall. We present an overview of our work at NIST to advance pulsed EPR measurements on biomacromolecules, ranging from synthetic model systems to biomacromolecular complexes. This presentation highlights our work to measure distances between cationic copper porphyrins bound to guanine quadruplex DNA structures of increasing length¹ as well as our newest efforts in instrumentation development.²

Financial support from the Cooperative Research Agreement between the University of Maryland and the National Institute of Standards and Technology Center for Nanoscale Science and Technology, Award 70NANB10H193 (MPD, NA, AA), the Summer High School Intern Program at NIST (SM), NIH P41 EB002034 (BE), and NIH R50 CA211408 (BE) are gratefully acknowledged.

**References**


REVEAL THE FUNCTIONAL STRUCTURE OF COMPLEX CARBOHYDRATES IN INTACT FUNGAL AND PLANT CELLS USING DNP SOLID-STATE NMR

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Complex carbohydrates play crucial roles in energy storage, cell recognition and structural building. Their functional structure is often elusive due to the technical difficulty in characterizing these molecules, which are typically polymorphic and disordered in structure. Here we present two solid-state NMR and DNP studies of carbohydrate-rich biosystems: the disease-relevant, pathogenic fungi and the energy-rich plant biomass. High-resolution of such complex biomaterials is accomplished by systematically investigating the composition, sub-nanometer packing, site-specific hydration and ns-μs motion of polysaccharides and other biomolecules in the near-native cells through a series of 2D $^{13}$C-$^{13}$C/$^{15}$N experiments. DNP are often needed to overcome sensitivity limitation as well as specifically probe the interaction interface between biomolecules. The fungal cell walls of a major pathogen *Aspergillus fumigatus* is found to contain a hydrophobic scaffold of chitin and α-1,3-glucan, which is surrounded by a hydrated matrix of diversely linked β-glucans and capped by a dynamic, outer layer rich in glycoproteins. This study provides the first high-resolution model of fungal cell walls and enables in-cell, high-resolution characterization of the drug effect to promote the development of wall-targeted antifungals. In the intact stems of multiple energy crops, such as maize and switchgrass, lignin is found to self-aggregate to form hydrophobic nanodomains, which are bridged to cellulose microfibrils by xylan via extensive interface. The flat conformers of xylan are coating the even surface of cellulose microfibrils and the non-flat conformers bind the intrinsically disordered aromatics of lignin through electrostatic interactions. This study has substantially revised our contemporary views of lignocellulose and has the great potential to facilitate the development of crops with higher digestibility for improving biomass deconstruction and conversion to biofuels. These studies provide invaluable insight into the functional structure of carbohydrates, their interaction with other polymers such as lignin and proteins, and the evolutionary structure of cell walls.

In addition, recent results collected on the 1.5 GHz (35 T) Series Connected Hybrid magnet at National High Magnetic Laboratory, the development of DNP methods for structural elucidation and statistical analysis of polysaccharide structure in unlabeled whole-cells, as well as the efforts for developing a carbohydrate solid-state NMR database, will also be briefly discussed.
Brady Wilkes, UF

VOLUMETRIC MAGNETIC RESONANCE AND DIFFUSION TENSOR IMAGING OF C58/J MICE: NEURAL CORRELATES OF REPETITIVE BEHAVIOR

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Restricted, repetitive behavior (RRB) involves sequences of responding with little variability and no obvious function. RRB is diagnostic for autism spectrum disorder (ASD) and a significant feature in several neurodevelopmental disorders. Despite its clinical importance, relatively little is known about how RRB is mediated by broader neural circuits. In this study, we employed ultra-high field (17.6 Tesla) magnetic resonance imaging (MRI) to study the C58/J mouse model of RRB. We determined alterations in brain morphology and connectivity of C58/J mice and their relationship to repetitive motor behavior using structural MRI and diffusion tensor imaging (DTI). Compared to the genetically similar C57BL/6 control mouse strain, C58/J mice showed evidence of structural alterations in basal ganglia and cerebellar networks. In particular, C58/J mice exhibited reduced volumes of key cortical and basal ganglia regions that have been implicated in repetitive behavior, including motor cortex, striatum, globus pallidus, and subthalamic nucleus, as well as volume differences in the cerebellum. Moreover, DTI revealed differences in fractional anisotropy and axial diffusivity in cerebellar white matter of C58/J mice. Importantly, we found that RRB exhibited by C58/J mice was correlated with volume of the striatum, subthalamic nucleus, and crus II of the cerebellum. These regions are key nodes in circuits connecting the basal ganglia and cerebellum and our findings implicate their role in RRB, particularly the indirect pathway.
Ben Wylie, Texas Tech

**SOLID-STATE NMR STUDIES OF KIR CHANNEL-LIPID INTERACTIONS AND THE FIBRILLATION OF FUNCTIONAL AMYLOIDS IN THE EPIDIDYMIS**

Benjamin J. Wylie¹, Collin G. Borcik¹, Reza Amani¹, Nazmul Khan¹, Maryam Yekefallah¹, and Derek B. Versteeg¹

¹Texas Tech University Department of Chemistry and Biochemistry

**KirBac1.1.** KirBac1.1 is an inward-rectifier K⁺ (Kir) channel. KirBac1.1 exists as a tetramer and its structural components include a selectivity filter (outer gate), which binds K⁺ ions and allows them to pass through the bilayer, a transmembrane region exhibiting an “inverted tepee” structure, and a cytoplasmic domain or “Kir domain”. This gating bundle is coupled to the activation gate (inner gate) and responds to anionic lipids. We have discovered how bilayer composition modulates the activity and conformational states of KirBac1.1 using Solid-state NMR (SSNMR) spectroscopy partnered with fluorescence assays and Forster Resonance Energy Transfer (FRET). We identified the activating anionic lipid binding pocket within KirBac1.1 and determined the changes in dynamic structure associated with channel gating. Through extensive chemical shift assignment, we further identified two states of the channel under gating conditions. One state is activated and conductive and the second state appears to be shallowly inactive. Further, using ¹³C-labeled biological lipids, we show that KirBac1.1 lowers the gel-liquid crystalline phase transition of the surrounding bilayer and increases the liquid-ordered (Lₒ)/liquid-disordered (Lᵈ) phase separation of these membranes.

**Figure 1.** SSNMR studies of KirBac1.1 protein. (a) Chemical shift perturbations measured using NUS sampled 3D MAS-SSNMR reveal allosteric communication between the activation gate and selectivity filter of the channel. (b) Using ¹³C-enriched biological lipids we compared cross polarization and refocused-INEPT (rINEPT) ¹³C-detected ¹D SSNMR as a function of temperature to discover that KirBac1.1 introduces liquid-ordered domains into biologically derived bilayers. (c) Fluorescence assays and FRET confirm channel activity and gating motions as a function of lipid environment.

**CRES.** The cystatin-related epididymal spermatogenic (CRES) protein is found in the male reproductive tract. CRES proteins form non-pathogenic functional amyloids within the epididymal lumen. We use liquid-state and solid-state NMR to reveal how CRES transitions from its monomeric to amyloid fold. In aggregate, these chemical shifts revealed that CRES transitions to a unique amyloid fold with multiple contact surfaces centered around a domain-swapped β-stacked amyloid core. This helps to explain the complex, non-fibrillar structures formed by this family of proteins in vivo.

**Figure 2.** NMR studies of CRES protein. (a) Liquid-state NMR structure of CRES with secondary structural elements labeled. This structure is colored by chemical shift perturbations between the chemical shifts measured in liquid-state and solid-state NMR. (b) A domain-swapped model for the amyloid core of the CRES protein. This model was generated using CS-ROSETTA fragment selection using the ROSETTA Fold-and-Dock algorithm. This analysis reveals CRES forms a domain-swapped amyloid core flanked by highly amyloid-prone regions comprising intermolecular contact surfaces. This model suggests a mechanism for the web-like structure these proteins form in vivo.
### POSTER PRESENTERS – Listed alphabetically by last name, with numbers for poster location and author present on designated night

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*TRAVEL AWARD*  
*VSP AWARD*  

*Not included in Student Poster Competition*
THE ACQUISITION OF ULTRA-WIDELINE NMR SPECTRA OF INTEGER-SPIN NUCLEI

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Of the non-radioactive NMR-active nuclei in the periodic table, 74% are classified as quadrupolar (i.e., having a nuclear spin > ½). The majority of quadrupolar nuclei are half-integer spins, with only seven possessing integer spins (i.e., $^2$H ($I = 1$), $^6$Li ($I = 1$), $^{11}$B ($I = 3$), $^{14}$N ($I = 1$), $^{50}$V ($I = 6$), $^{138}$La ($I = 5$), and $^{176}$Lu ($I = 7$)). Of these, only $^2$H is widely studied using solid-state NMR spectroscopy (SSNMR), owing to its value as an excellent probe of molecular-level dynamical motions and rates, and relative ease of spectral acquisition for most diamagnetic samples; isotopic enrichment is almost always required, but fortunately, it is relatively inexpensive. $^6$Li SSNMR has been applied in some studies of battery materials, with the only challenge to spectral acquisition being its relatively low natural abundance (n.a. = 7.59%), as $^6$Li powder patterns tend to be relatively narrow. $^{10}$B and $^{14}$N are often overlooked due to the availability of simpler NMR experimentation on $^{11}$B ($I = 3/2$, n.a. = 81.1%, small quadrupole moment) and $^{15}$N ($I = 1/2$, n.a. = 0.36%, enrichment is generally required). The three heavier elements of this list are rarely studied, due to very low n.a.’s, very large nuclear quadrupole moments, and/or the availability of more easily accessible isotopes from the perspective of SSNMR.

The emergence of methods and instrumentation for conducting ultra-wideline NMR (UWNMR) spectroscopic experiments (i.e., efficient acquisition of SSNMR powder patterns ranging from ca. 250 kHz to tens of MHz in breadth) has opened up large swaths of the periodic table to routine investigation by SSNMR, including both spin-1/2 and quadrupolar nuclei.1,2 As such, there is growing interest in the use of UWNMR methodologies to examine “neglected” integer-spin quadrupoles such as $^{10}$B and $^{14}$N (which have very broad patterns), as well as for use in obtaining $^2$H SSNMR spectra (i.e., in natural abundance studies and for much broader patterns arising from large quadrupolar interactions). For such experiments to be efficient and generally applicable to a wide range of systems, a better understanding of the spin dynamics surrounding the acquisition of SSNMR spectra of integer-spin nuclei is needed.

To this end, I will present some of our recent SSNMR investigations of integer-spin quadrupolar nuclei, including $^2$H and $^{10}$B. I will discuss (i) the use of frequency-swept (FS) pulses and pulses designed with optimal control theory (OCT) for broadband direct-excitation and cross-polarization of integer-spin powder patterns, (ii) a density-matrix model that allows us to understand how FS and OCT pulses produce significant enhancements in signal, (iii) the implementation of targeted acquisitions that avoids the need for acquisition of the entire powder pattern, and (iv) a series of interesting examples for exploring dynamics ($^2$H), ceramics, and glasses ($^{10}$B) in both simulations and experiments.

A NOVEL MULTISHELL ACQUISITION WITH INCREASED B-SHELLS AND SPARSE ORIENATIONS

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Introduction
Complex directional, multi-shell diffusion acquisitions are feasible with advanced MRI technology. To fit scan time constraints, diffusion acquisitions must optimize the number of b-shells and directions per shell with the goal of keeping scan time the same, while optimizing the distribution of acquisitions across shells to improve accuracy in microstructure analysis, as well as improve signal decay fitting in the high-b regime. To optimize acquisition, a multishell acquisition with increased b-shells and sparse orientations was developed and tested against common acquisition schemes.

Methods
To test the novel acquisition scheme, a large ground-truth diffusion weighted image dataset (Full Set 9 x 60) was acquired using the parameters listed in the Table 1. Data was pre-processed using the FSL pipeline. The b-values were selected to provide uniform spacing in q-space (1) and multi-shell diffusion directions were optimized for uniform angular coverage (2).

From the Full Set, two data subsets requiring equal time were formed (Subset 9 x 20 with 9 shells & 20 directions/shell, and Subset 3 x 60 with 3 shells & 60 directions/shell). Each of the subsets were created to have an optimal direction distribution per shell across shells. Diffusion datasets were processed using the DTI (3), NODDI (4), and CSD (5) models to analyze the microstructural differences relative to the Full Set (Figure 1).

References:

Table 1

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Figure 1

Spreading diffusion weighted directions over more b-shells gives more accurate microstructure.
NMR Studies of a Human G Protein in Aqueous Solutions

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G proteins are guanine nucleotide binding proteins that transmit signals from stimuli outside the cell to the interior of the cell through their interaction with the integral membrane proteins, G protein coupled receptors (GPCRs). Several loss and gain of function mutations can alter the ability of G proteins to signal effectively, resulting in several metabolic disorders and diseases, which makes these important potential drug targets.

Studies of the structure and dynamics of GPCR tertiary complexes with G proteins and drugs can reveal important information on the mechanisms of receptor signaling. However, these complexes can be difficult to isolate and study. An important set of biochemical tools that have recently emerged are so-called “engineered” G proteins¹, which are G proteins containing the GTPase domain and modified to improve their stability with minimal alteration of their functional activity. In this study, we use NMR spectroscopy in aqueous solution to study the dynamics and structure of one of these engineered G proteins, the “mini-GaS” protein and its complexes with GPCRs²,³.

References

1. Byron Carpenter, Christopher G. Tate, Engineering a minimal G protein to facilitate crystallization of G protein-coupled receptors in their active conformation, Protein Engineering, Design and Selection, (2016), 29, 583–594.


Structure determination of membrane proteins in planar native-like bilayers by oriented-sample (OS) solid-state NMR involves measurements of orientationally dependent dipolar couplings and chemical shifts, which provide angular restraints for structure calculations. So far, only $^1$H-$^15$N dipolar couplings together with $^{15}$N chemical shift anisotropy have been routinely assessed in uniformly $^{15}$N labeled protein samples by the separated local field (SLF) techniques. While highly efficient for determining helical tilts in short alpha-helical peptides, the $^1$H and $^{15}$N measurements alone are likely insufficient for determining protein structures of arbitrary topology. Complete de novo structure determination of membrane proteins by OS NMR, therefore, requires measurements of additional angular restraints, for which doubly ($^{15}$N, $^{13}$C) labeled protein samples need to be employed. However, the presence of the abundant $^{13}$C spins represents an essential complication arising from the $^{13}$C-$^{13}$C homonuclear couplings. Here we have developed a triple-resonance pulse sequence that enables detection of the $^1$H-$^{13}$Ca couplings at the $^{15}$N sites. The pulse sequence consists of three blocks: (i) the evolution of $^1$H-$^{13}$C couplings under both $^1$H-$^1$H and $^{13}$C-$^{13}$C homonuclear decoupling; (ii) proton-mediated magnetization transfer of the $^{13}$C dipolar evolution to the proximal $^{15}$N sites; (iii) detection at the $^{15}$N sites. The performance of the pulse sequence has been tested with Pf1 phage coat protein reconstituted in magnetically aligned bilayers. By overlaying the $^1$H-$^{15}$N and $^1$H-$^{13}$Ca SLF experiments, the latter dipolar couplings can be directly assigned. Moreover, all three measurements per peptide plane have been fitted simultaneously to a consensus $\alpha$-helical transmembrane structure for Pf1 coat protein using the recently developed structure fitting method [1]. The previously inaccessible $^1$H-$^{13}$Ca couplings represent a powerful chiral restraint for structure determination of membrane proteins in oriented lipid bilayers, thus greatly enhancing utility of the method as a tool for "NMR crystallography".

[1]. Lapin, J. and Nevzorov A.A., Validation of protein backbone structures calculated from NMR angular restraints using Rosetta, J. Biomol. NMR, 2019; 73(5):229-244.

Figures: A. A triple-resonance pulse sequence for measuring $^1$H-$^{13}$Ca dipolar couplings at the $^{15}$N sites. Correlation of the $^1$H-$^{13}$Ca couplings to $^{15}$N amide sites is achieved by proton-mediated magnetization transfer (MMHH). B. Overlay of 2D triple-resonance spectra of a uniformly ($^{13}$C, $^{15}$N) labeled Pf1 protein. Shown in blue: Correlation of $^1$H-$^{13}$Ca dipolar couplings and $^{15}$N chemical shifts. Shown in red: double-resonance correlation of $^1$H-$^{15}$N dipolar coupling and $^{15}$N chemical shifts. C. Consensus structure of Pf1 TM domain obtained from simultaneously fitting three NMR angular restraints.
ANOMALOUS RELATIONSHIP BETWEEN MOLECULAR SIZE AND DIFFUSIVITY OF ETHANE AND ETHYLENE IN ZIF-11 BY NMR, IRM, AND VOLUMETRIC TECHNIQUE

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Zeolitic imidazolate frameworks (ZIFs) represent a sub-group of metal organic frameworks (MOFs) that have found application in gas separation due to their chemical and thermal stability, tunable framework, and large surface area. Owing to ZIFs flexible framework, guest molecules with sizes larger than or comparable to ZIF nominal pore aperture size can diffuse through these porous systems.

Multinuclear pulsed field gradient (PFG) NMR was used to study self-diffusion of ethane and ethylene inside several ZIF-11 crystals batches with different mean crystal sizes. Diffusion measurements were performed in a broad range of sorbate concentrations and temperatures. It was observed that for ZIF-11 crystals loaded with a single sorbate at the same or similar concentration, ethane diffuses faster than ethylene despite its larger molecular size compared to ethylene. It was also observed that replacing a fraction of ethane molecules by ethylene molecules reduces self-diffusivity of ethane (Fig. 1A). Additionally, higher activation energy of diffusion was found for ethylene compared to ethane (Fig. 1B). For further investigation of observed anomalous relationship between molecular size and diffusivity of ethane and ethylene, additional measurements were performed using IR microscopy (IRM) and volumetric uptake technique for single ZIF-11 crystals and thin ZIF-11 crystal beds, respectively. The data obtained by these two techniques for the same molecules and samples showed consistency with the data obtained by PFG NMR which further confirms the absence of any measurement artifacts. This anomalous relationship can be related to reduced framework flexibility of ZIF-11 in the presence of ethylene molecules that reduces the effective pore aperture size in ZIF-11 [1].

Figure 1. A) self-diffusivities of ethane and ethylene measured by PFG NMR in ZIF-11 beds at 296 K as a function of the total sorbate concentration of ethane and ethylene. B) Temperature dependence of ethane and ethylene self-diffusivities measured by PFG NMR.

References
Zeolitic imidazolate frameworks (ZIFs) are novel porous molecular sieves, which are composed of metal centers interconnected by organic linkers. Substitution of a small fraction of native linkers by a different linker type, viz. linker doping, can potentially be used to fine-tune ZIF transport properties. It has been shown that bulkier 2-ethylimidazole (elm) linkers can be incorporated along with the native 2-methylimidazole (mlm) linkers in ZIF-8 without disturbing the topology of this ZIF.[1] In this work, we utilize $^1$H and $^{13}$C pulsed field gradient (PFG) NMR with high magnetic fields (up to 17.6 T) and large gradients (up to 25 T/m) to study the effect of elm linker doping on diffusion of gases in ZIF-8.

Our PFG NMR data show that linker-doping with elm does not have an effect on the self-diffusion of the C2 species studied, ethylene or ethane. However, the data obtained for larger C3 molecule propylene shows a strong increase in the self-diffusivity as elm doping is increased (Fig. 1). Furthermore, this increase in diffusivity with increasing doping is more pronounced at the limit of low propylene concentration. The observed influence of the doping on the propylene self-diffusivity is explained by an increase in the framework flexibility of ZIF-8 induced by the doping. Such flexibility increase can lead to distortions in the aperture size and/or shape in the presence of larger molecules, such as propylene, resulting in a faster diffusion of these molecules.

![Figure 1. Corrected self-diffusivities of propylene in elm doped ZIF-8 at 296 K. “High” and “low” loadings correspond to intra-ZIF concentrations of around 7.0 and 1.3 mmol/g, respectively.](image)

Dilute magnetic semiconductor quantum dots (DMSQDs) have emerged as potential materials for future spin-based technologies. A critical parameter for the development of this technology is a relatively long electron spin phase memory time, $\tau$, which could be gleaned from relaxation measurements via pulsed EPR (pEPR). $\tau$ must be long enough for the data storage and manipulation processes. Thus pEPR studies of lightly-doped Quantum Dots (QDs) have recently yielded important spin dynamical data on DMSQDs. The earlier studies have, however, been carried out mostly at 3.5 T and 9.5 GHz. Since relaxation dynamics depend sensitively on the Zeeman field and temperature, this work reports the use of higher microwave frequencies up to 224 GHz, in continuous wave (CW) and pEPR modes. The samples studied are ~2 nm 0.8, 1.6, 3.2 % Mn-doped ZnSe QDs. Unlike at 9.5 GHz, the use of higher frequencies enables us to resolve EPR peaks from surface and core sites. A detailed examination of the temperature and frequency dependence of the spin-lattice relaxation time $T_1$ and spin-spin relaxation time $T_2$ across a wide range of temperatures (1.8-20 K) and frequencies (9.7-240 GHz) revealed that the relaxation mechanism involves the Direct as well as the Orbach processes, with little contribution from the Raman process. The $T_1$ decay is found to be bimodal, in contrast to the earlier low-frequency data. Additionally, the observation of large $T_2$ times and Rabi oscillations indicate that Mn spins in the doped ZnSe QDs can be successfully manipulated, and thus promising components in quantum computation devices.

Figure 1. Schematic of a quantum dot showing surface and core Mn sites and cw/pEPR spectra (black and green) for 2 nm 3.2% Mn doped ZnSe at 240 GHz. 5 K.
NMR CHARACTERIZATION OF ANTIGEN A/C3 BINDING IN STREPTOCOCCUS MUTANS

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¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, USA
²Department of Oral Biology, University of Florida, Gainesville, USA

Streptococcus mutans has been studied extensively since it was first isolated from dental caries in 1924.¹ S. mutans is the primary etiologic agent in human dental caries, although it has also been implicated in other infections throughout the body, including infective endocarditis.² ³

Wall protein A (WapA) is produced by S. mutans in the early stages of biofilm establishment when sucrose is not present.⁴ This enables S. mutans to anchor to the dental surface and create a site for biofilm formation that can progress to becoming dental caries. WapA exists outside of the cell as several natural derivatives, including Antigen A, which are important to biofilm formation. In particular, Antigen A plays a role in amyloid fibril formation in S. mutans, which is vital to the structure and stability of a biofilm.

C3 is another protein that is expressed at a similar time as AgA and is also involved in biofilm formation. To better understand the roles that these two proteins play in amyloid fibril formation, we needed to investigate if they are able to interact with each other.

In this study we use NMR to characterize the AgA-C3 interaction. Earlier studies of Antigen A failed to optimize the production of a purified AgA sample, however we were able to produce a pure sample of Antigen A and are using ¹³C/¹⁵N-NMR to assign the amino acid residues of AgA and developed an NMR-based approach for future studies of the protein. A preliminary ¹⁵N-¹H-HSQC showed the possibility of interaction between AgA and C3. Since it is important to consider both the binding site on Antigen A and on C3, we ran experiments on ¹⁵N-AgA/unenriched C3 and unenriched AgA/¹⁵N-C3. This understanding is applicable to the use of Antigen A and C3 as targets for biofilm inhibition as a way of improving the treatment of dental caries.

THE PARA-CUBE: A SMALL, INEXPENSIVE, AND PORTABLE PARA HYDROGEN GENERATOR

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² Joint Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC, and North Carolina State University, Raleigh NC

NMR experiments provide key data for science ranging from chemical analysis to clinical imaging. The primary challenge facing NMR is absolute concentration sensitivity. Hyperpolarization overcomes the low, Boltzmann distributed, polarization and breaks current sensitivity limitations. There are several hyperpolarization techniques, among them is parahydrogen induced polarization (PHIP)¹,², which is simple and fast compared to other methods. Parahydrogen is one of the spin isomers of dihydrogen. At room temperature there is a 25/75 split of ortho to para. At lower temperatures para hydrogen is thermodynamically favored, thus ortho hydrogen can be converted to parahydrogen by thermalization at low temperature with a suitable catalyst, typically iron oxide or activated charcoal.

Here we present small and efficient parahydrogen generators, that can easily be adopted in other labs interested in entering the field of hyperpolarization / PHIP. We call them “Para-Cubes”. Currently in our lab, there are three different styles of generators. Figure 1A shows the stationary unit that sits beside a magnet. This generator produces around 50% para hydrogen using liquid nitrogen and has sample shuttling capabilities. The large frame and flat surface make it convenient to use.

Figure 1B shows our Para-Cube, a mobile version that can be brought to different labs or buildings, fits in a car and is easily shipped. It is a lightweight cube with dimensions of 14” by 14” by 14”. The para-cube uses liquid nitrogen (available in any research lab) to achieve 50% para hydrogen. Material costs are approx. $500.

Lastly, we show in Figure 1C a generator, which can produce >99% para hydrogen using a cryo-stat, however, it is a larger unit as it requires an external helium compressor. This unit can produce portable bottles of parahydrogen.

Using the para-cube and the portable bottles parahydrogen can be accessed anywhere without the huge initial cost. A promising next step may be replacing the current cooling methods with Peltier plates. This would reduce the size further and lead towards true miniaturization.


Figure 1. Para Hydrogen Generators
a) stationary unit using liquid nitrogen and used next to a magnet.
b) mobile unit using liquid nitrogen (“para cube”) c) unit using liquid helium that produces 99% para-H.
PROBING THE LOCAL ENVIRONMENT OF PH-RESPONSIVE POLYPEPTIDES WITH ELECTRON PARAMAGNETIC RESONANCE TO OBSERVE SECONDARY STRUCTURE TRANSITIONS

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The George and Josephine Butler Polymer Research Laboratory, Department of Chemistry and Center for Macromolecular Science and Engineering, University of Florida, Gainesville, FL 32611

Polypeptides are a promising alternative to other common synthetic polymers for \textit{in vivo} cargo delivery vehicles due to their bioinspired characteristics, but there is still a need to fundamentally understand what governs the intra- and intermolecular interactions between polypeptide chains. Herein, we use continuous wave electron paramagnetic resonance (CW EPR) spectroscopy to describe the inter- and intramolecular interactions between polypeptides that contribute to secondary structure formation. EPR is a powerful technique that probes the local environment of a spin probe on the nanometer scale. In this study, a nitroxide radical was installed onto poly(L-glutamic acid) (PE) to probe local mobility and polarity changes, via EPR spectroscopy, as a function of molecular weight and pH. We utilized 4-amino-TEMPO as an initiator for the ring-opening polymerization (ROP) of N-carboxyanhydrides (NCA), and as a spin-probe to observe conformational changes of the latter. By exploiting the pH-responsive nature of PE, we hypothesize that the helix-to-coil transition can be observed via mobility and polarity information obtained through the lineshape of the spin-labeled polypeptide CW EPR spectra.
NMR relaxometry of small molecules encapsulated in reverse micelles

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NMR spectroscopy of proteins encapsulated within reverse micelles (RM) prepared in low-viscosity fluids such as liquid propane was first applied as a strategy to overcome the size limit in protein NMR.¹ The TROSY approach is sometimes limited by the increased cost and decreased yields from protein perdeuteration and the loss of the richness of information provided from the abundance of 1H nuclei. RM-NMR affords many advantages in protein studies by NMR in addition to the tumbling advantage. Sensitivity gains from use of cryogenic NMR probes are partially attenuated in high-conductivity (high-salt) samples, thus limiting the allowable ionic strength of the sample.² RM samples effectively replace ~98% of the aqueous buffer with alkane, thus eliminating this dielectric penalty, allowing very high salt concentrations to be used in the aqueous RM core.³ In fact, for large proteins dissolved in low-viscosity solvents the increased tumbling can enhance the data obtained from NOESY experiments. The faster tumbling will require less (per)deuteration for optimization of $T_2$ relaxation, which allows for the detection of more intramolecular nuclear Overhauser effects (NOEs). However, no information regarding the dynamics of small molecules/proteins in RM have been provided in literature till date. We aim to investigate the relaxation parameters ($T_1$, $T_2$ and diffusion rates) in RM using sucrose as a target molecule and compare the data in similar systems like aqueous solution, surfactant micelles etc. This can provide us mechanistic insights of molecules in RM and can help us to better design experiments in various solubilizing systems for small molecules/proteins.

References:

A SECOND ACTIVE SITE IN ZEOLITE CATALYSTS REVEALED BY TWO-DIMENSIONAL CORRELATION NMR UP TO 35 TESLA

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Ultra-high field $^{1}\text{H}$-$^{27}\text{Al}$ 2D correlation NMR experiments demonstrate that a new active site can exist in acidic zeolite catalysts. In addition to the known framework bridging acid site (BAS), a new site created by a second tetrahedral Al atom and its hydroxyl group proton in zeolite HZSM-5 are clearly resolved at 35.2 T field strengths, enabled by recently developed series-connected hybrid (SCH) magnet technology. $^{27}\text{Al} \{^1\text{H}\}$ D-HMQC experiments at 3 different field strengths, i.e., 14.1, 19.6 and 35.2 T, indicate the second Al site (denoted Al(IV)-2) experiences unique quadrupolar parameters relative to the well-known bridging acid site (BAS) in both dehydrated and hydrated states, and more importantly, is tetrahedrally-coordinated. Detailed characterizations, including chemical treatments and $^{27}\text{Al}$ MQMAS experiments, suggest the second site arises from partially-bonded framework structures that can result either from framework crystallization defects or from incomplete post-synthetic hydrolysis of a framework Al. The results herein communicate the highest magnetic field strength data on active zeolite catalyst structures to date and enable for the first time the detection of Al and H association on a dry HZSM-5 catalyst, i.e., under conditions representative of typical end-use processes.

$^{1}\text{H}-^{27}\text{Al}$ correlation of dehydrated a zeolite catalyst via dipolar-based Heteronuclear Multiple-Quantum Correlation (HMQC) method, revealing a new active aluminum site: Al(IV)-2
ULTRA-LOW LOADINGS OF PLATINUM ON SHAPED CERIUM OXIDE NANOCRYSTAL SUPPORTS FOR PARAHYDROGEN ENHANCED POLARIZATION NMR

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Parahydrogen Enhanced Polarization (PEP) is a hyperpolarization technique used to achieve high-field signal enhancements of up to four times the magnitude.[1] Hydrogenative PEP NMR requires the pairwise addition of parahydrogen. Here, the synthesis of ultra-low loadings Pt catalyst on well-defined ceria nano shapes by a modified Atomic Layer Deposition (ALD) is proposed as an approach to increase the pairwise selectivity.

Previous research[2] revealed that smaller Pt nanoparticle sizes are more effective at increasing pairwise selectivity, which makes Pt single atom catalysts a promising candidate for parahydrogen enhanced NMR. In this study, ultra-low loadings of Pt were deposited onto CeO₂ nanocrystals with two well defined shapes – octahedra and cubes – and the resulting catalysts were tested in the hydrogenation of propene using normal hydrogen and 50% enriched parahydrogen (p-H₂). The ceria nanocrystal supports only expose (111) and (100) surface sites for the octahedra and cubes respectively, which affects the dispersion and stability of Pt atoms on the surface of the support. The (111) surface on octahedra was more effective at stabilizing the atomic dispersion of reduced Pt on CeO₂, which leads to higher pairwise selectivity. Additionally, we report that a CeO₂ octahedra supported Pt catalyst with only 8.2ppm loading exhibits a pairwise selectivity of 8.8%. Past results[3] for metal-oxide supported monometallic Pt nanoparticle on TiO₂ yielded lower pairwise selectivity for propene, 2.4% at 0.8% loading, respectively. Hence, the ceria-supported ultra-low loadings of Pt catalysts were tailored to offer higher performance for applications requiring the hyperpolarized propane.

Works Cited
INEXPENSIVE 99% PARAHYDROGEN ENRICHMENT
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Parahydrogen-based hyperpolarization techniques can enable orders of magnitude signal enhancements for nuclear magnetic spectroscopy and imaging.1 The parahydrogen-derived polarization is related to the parahydrogen (p-H2) mole fraction \( x_p \) by \( \left(\frac{1}{3}\right)\left(4x_p - 1\right) \). Hence, by using 99%-enriched p-H2, obtained by equilibrating hydrogen gas at desired temperature, the signal enhancement is approximately 3\( \times \) greater than that obtained using 50% enrichment at 77 K.2 Nevertheless, because conversion at liquid nitrogen temperatures is so much simpler, many labs opt to work with 50% enrichment for convenience. Turn-key commercial p-H2 generators, such as the Bruker BPHG 90, utilize a cryo-cooler to keep the conversion temperature below 36 K where 90%-enriched p-H2 is produced. While simple to operate, such systems are expensive (~$150k for BPHG 90) and thus not accessible to many labs. Furthermore, the cryo-coolers typically require 1-2 hours before obtaining stable para-enrichment, and due to the usage of a compressor, water cooling equipment is required.3 Here we present an inexpensive and easy-to-operate system for continuous-flow 99% para-enrichment. The ortho-para converter coil, packed with a high-surface area activated iron oxide (i.e. Fe(O)(OH)) ortho-para conversion catalyst, is mounted inside a home-built cryostat can containing a few hundred torr of helium exchange gas, and the cryostat is inserted into liquid helium in a standard (79 mm neck opening) liquid helium dewar. Continuous-flow 99% para-enriched hydrogen was confirmed at 30 K and 65 psi H2 pressure. Further details about the construction and performance data of this low-cost parahydrogen generator will be presented in this poster. The enrichment system provided here offers a low cost for construction and operation, only requiring a liquid helium storage dewar of the type that is commonly found in NMR labs for filling superconducting magnets.

This work was funded by NSF grant CHE-1808239 (C.R.B. and W.H.) and the NHMFL-UCGP which is supported by the National Science Foundation Cooperative Agreement No. DMR-1644779 and the State of Florida.

Figure 1. 300 MHz proton NMR stack spectra of hydrogen gas flowing through the 5 mm NMR tube continuously at 300 mL/min after conversion at different temperatures.

Reference
SURFACE WATERS ARE MAGNETIZED BY PARAHYDROGEN

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In the PASADENA (parahydrogen and synthesis allows dramatically enhanced nuclear alignment) effect, introduced in 1987, singlet proton spin order of parahydrogen is revealed by symmetry-breaking chemistry.¹ Scores of different substrates have since been hyperpolarized by this technique. However, hyperpolarization of liquid water from parahydrogen using heterogeneous catalysis was not reported until only last year.² In this SWAMP effect (Surface Water Are Magnetized by Parahydrogen), hyperpolarization of the solvent protons (liquid water, methanol and ethanol) is induced simply by the bubbling of parahydrogen through a suspension of insoluble Pt₃Sn intermetallic nanoparticles (iNPs). The conversion of parahydrogen singlet spin order into proton magnetization is mediated by symmetry-breaking interactions on the surface of the iNPs. The hydroxy protons exhibit stimulated emission NMR signals relative to those of Boltzmann polarized water. Moreover, in the alcohols, hyperpolarization of the nonexchangeable methyl or methylene protons is also observed, which is key to the interpretation of the mechanism and spin-dynamics of SWAMP effect. Details about the catalyst, systematic studies, and a model for the molecular mechanism for the interfacial process, including density operator model calculation, will be presented. A key advantage of the heterogeneous Pt₃Sn@mSiO₂ catalyst is its insolubility, which allows it to be quickly and completely separated from the hyperpolarized water without any leaching. SWAMP can generate NMR-observable hyperpolarization of liquids that are free of radicals, catalyst residues, or other additives at low magnetic field. This could enable low-field MRI without superconducting magnets, which would be transformative for MRI and signal enhancement of biomolecules with exchangeable protons.

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Figure 1. Liquid hyperpolarized water produced by the SWAMP effect.

Reference
The Bologna Stone is a barium sulfate (baryte) derived luminescent compound of historical interest named for the Italian city of Bologna. Baryte is a common mineral found in many locations. However, the persistent luminescence after calcination and UV irradiation was historically only observed with stones found at Monte Paderno in Bologna, Italy. Previously lost in time, the secret of making the Bologna Stone Recipe has been rediscovered in the modern era. Part of the secret was the realization that the luminescence is strongly dependent on the presence of trace amounts of transition metal ions in the stone. Copper in a narrow concentration window is necessary, and the luminescence is quenched entirely by the presence of iron. This is attributed to Cu(I) filling a vacancy in the mineral which is transformed into BaS after calcination. EPR spectroscopic evidence indicates there is also Mn(II) present within the baryte. Current efforts are focused on elucidating the mechanism that causes this luminescence.

References

ASSESSING FUNCTIONAL CONNECTOMIC MEASURES IN ANIMAL MODELS OF TBI USING HIGH-FIELD RESTING-STATE FMRI

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TBI-induced diffuse axonal injury (DAI) can produce brain-wide functional connectivity disruptions. Here we used high field resting-state fMRI (rsfMRI) and brain-wide functional connectivity analysis to measure intrinsic resting state neural activity between sensory, motor, affective and cognitive brain areas after controlled cortical injury (CCI) or fluid-percussive injury (FPI). Adult male rats were imaged on a 11.1-Tesla MRI scanner controlled by Bruker Paravision 6.0.1 under 1.5% isoflurane at Day 2 and at Day 30 post-injury.

Respiratory rate was monitored continuously, and body temperature was controlled. fMRI scans were collected using a single-shot spin echo planar imaging sequence [TE=15ms, TR=2sec, 300 repetitions, field of view=24x18mm, data matrix=64x64x25, slice thickness=0.9mm].

A high-resolution turbo rapid acquisition with relaxation enhancement was used for functional image overlays and alignment with a segmented atlas of rat brain. We employed both manual-drawing-assisted seed-based functional connectivity analysis, and atlas-based seed localization.

Manual seed analysis indicates a diffuse disruption of connectivity in ipsilateral hippocampus and somatosensory cortex in CCI/FPI animals relative to controls. This was consistent with the atlas-based analyses. We further conducted connectomic metric analysis and observed clear suppression of connectivity patterns, which was most robust in CCI relative to controls. Standard anatomical MRI in TBI is limited in terms of the characterization of functional network properties. High field functional connectivity can help fill this gap and provide markers reflecting functional deficits, progression and potential treatment responses.

Figure: Structural analysis using Advanced Normalization Tools (ANTS diffeomorphic registration). T2 anatomical scans are normalized to a reference image (Waxholm Space Atlas of the SD Rat Brain, University of Oslo). Mean Log Jacobian determinant map shows intensity-based information regarding the degree of warping used in subject-to-atlas transformation. Darker regions in the CCI and FPI lesion areas suggest smaller volume in the TBI subjects (relative to the reference atlas image). Plots of Log Jacobian values illustrate significant hippocampal structural differences between control, CCI and FPI rats on day 2, which are only partially recovered by day 30.
Molecular Effects of Antifungal Drugs on the Cell Wall Structure of Pathogenic Fungi from Solid-State NMR

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Inefficiency of the antifungal drugs demand the development of new agents with efficient drug responses and targets. The carbohydrate-rich fungal cell wall is a promising target of antifungal drugs, and new compounds have recently been developed to disrupt glucan-synthesis and perturb cell wall integrity with reduced toxicity. Due to highly polymorphic structure of biopolymers and the lack of high-resolution techniques, it is difficult to elucidate the structure of this complex biosystems. Here we present solid-state NMR and DNP studies of the cell wall of Aspergillus fumigatus, a major fungal pathogen, with and without antifungal treatment. A series of 2D ¹³C-¹³C/¹⁵N experiments were measured to determine the sub-nanometer packing, ns-μs motion and hydration of biopolymers in intact cell walls of native fungi. The fungal cell walls is found to contain a hydrophobic scaffold of chitin and α-1,3-glucan, which is surrounded by a hydrated matrix of diversely linked β-glucans and capped by a dynamic, outer layer rich in glycoproteins¹. Treatment by the Echinocandins drug substantially reduces the amount of β-glucan, and α-glucan but the fungi enhances the synthesis of chitin instead. Relaxation data further reveal lower mobility of carbohydrates in drug-treated cell walls. These studies provide invaluable insight into the functional structure of carbohydrates and the drug response of A. fumigatus, which has the potential to promote the development of better wall-targeted antifungals. In addition, we will also present the high-resolution spectra collected on the world-record 1.5 GHz magnet which shows substantial improvement on the ¹³C resolution for wild-type A. fumigatus, its genetic mutants and drug-treated cultures. The ¹³C FHMW linewidths of cell wall carbohydrates decrease from the 0.45-0.65 ppm range observed on an 800 MHz spectrometer to 0.25-0.55 ppm on the 1.5 GHz magnet, which allows us to better resolve the heavily overlapped signals of cell wall biomolecules.


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SYMMETRIC AND PSEUDOSYMMETRIC PARAHYDROGEN ADDUCTS HOSTING LONG-LIVED STATES

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Parahydrogen Enhanced Nuclear Magnetic Resonance (PE-NMR) is proving to be a facile and inexpensive alternative to DNP for the preparation of hyperpolarized metabolites for disease detection and monitoring. However, as in all hyperpolarization methods, the useful time-window is limited by the spin-lattice relaxation time, T₁. Singlet-states and their analogs, which can be directly formed by addition of parahydrogen to symmetric alkenes, can prolong the relaxation time of hyperpolarized spin order by several orders of magnitude. However, the presence of finite symmetry-breaking interactions is required to read-out these Long-Lived States (LLS). We are exploring a new approach based on the synthesis of alkene isotopomers that can be used for the generation of LLS by homogeneous catalysis. Preliminary results will be presented.
USE SOLID STATE NMR TO CHARACTERIZE THE STRUCTURE OF Aβ₁₋₄₂ 150-KDa OLIGOMERS: AN ANTI-PARALLEL β-SHEET AND A SPECIAL PARALLEL β-SHEET

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In the latest study of Alzheimer’s disease, the small oligomers formed by Amyloid-β (Aβ) peptide were supposed to be the main toxic species for the neurone. Our lab kept focusing on the structural study of a stable 150-kDa Aβ₁₋₄₂ oligomer. Previously, we found two β-strand regions on the Aβ₁₋₄₂ sequence, the C-strand (A₃₀ – A₄₂) and the N-strand (E₁₁ – V₂₄), and the C-strands were found to form an antiparallel β-sheet with the center at V₃₆. The most recent solid state NMR constraints indicate the N-strands would assemble into an out-of-register parallel β-sheet, which has uncommon registry shifts of alternating +3 and -3.

At the meantime, the preliminary Cryo-EM class averages of the oligomer sample showed it has 4-fold symmetry. We proposed one reasonable structure based on the symmetry and the two β-sheet configurations, which also matches the dimension of the Cryo-EM data. A dimer-addition mechanism was proposed to explain the stability and the size limitation of the oligomer.
EXPERIMENTS WITH SELECTIVE EXCITATION OF OVERLAPPING SIGNALS – SCOPE AND LIMITATIONS

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NOes display a complicated dependence on distance and other factors, however recent work demonstrated that in certain conditions inter-proton distances can be extracted from NOe spectra with the precision of X-ray crystallography. This revived our interest in 1D experiments in which just one of several overlapping signals is selectively excited.

The methods to achieve this are:
1. Chemical Shift Selective Filters (CSSF) – several spectra are added, with varying delay for chemical shifts evolution. The target signal is on-resonance and is reinforced upon addition, while the other signals are cancelled by destructive interference.
2. Selective TOCSY Edited Preparation Function (STEP) – magnetization is transferred to the target proton B from a source proton A in the same coupling network through a TOCSY step, then B is selectively excited.
3. In-Phase Editing of Coupled Spins (IPECS) – the same as STEP, but the magnetization is transferred between directly coupled spins in a COSY step.

CSSF requires that the chemical shift of the target signal does not overlap other chemical shift. The multiplet signals themselves can overlap. CSSF is the only choice when the target is a singlet. STEP and IPECS can be applied for overlapping chemical shifts, as long as the signals overlapping the target are not in the coupling network of a signal overlapping the source. STEP is more robust, but is the least sensitive of the three, since the magnetization of the source is distributed also to other protons than the target.

We implemented these experiments in vnmrj, and are going to present examples of applications of these experiments to chemical problems.

Example of 1D NOESY spectrum with IPECS selection:

a) proton spectrum.
b) spectrum of proton B free from overlap. Magnetization was transferred from A in an IPECS step.
c) NOESY 1D spectrum with selective inversion of B.

GENETIC DIVERSITY DIFFERENTIALLY IMPACTS DIFFUSION MRI MEASURES IN CORTEX AND HIPPOCAMPUS OF WILDTYPE AND 5XFAD MICE

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Genetic mutations in amyloid precursor protein (APP) and presenilin-1/2, each of which lead to an increase in toxic beta-amyloid, are linked to a high risk of Alzheimer’s disease (AD). However, the extent to which individual genetic variation affects neurobiological factors to regulate resilience/vulnerability to cognitive and non-cognitive symptoms of AD is not well understood. To begin to address this gap, the present study investigated brain-wide microstructural characteristics of genetically diverse mice expressing the 5xFAD transgene, each of which show differential susceptibility to AD-related symptoms. Specifically, we used high angular resolution diffusion MRI (HARDI) and quantified well-known diffusion tensor imaging (DTI) metrics such as the fraction anisotropy (FA) and mean diffusivity (MD), along with intracellular volume fraction (neurite density, NDI) and orientation dispersion (ODI) to investigate detailed morphological differences in hippocampal and cortical tissue. Young (6-8 m.o.) and aged (>12 m.o.) male and female 5xFAD mice on C57BL/6 (B6), F1-B6/DBA/2J (D2), or various BXD backgrounds, and their sex- and age-matched wildtype counterparts, were imaged at 11.1 Tesla. Two-way ANOVA indicated a significant strain x mutation effect in the primary motor cortex and dorsal hippocampal commissure of the D2 strain for FA, and in left and right entorhinal cortex and subiculum of BXD strains (Bonferroni p <0.05). These results suggest that strain-specific variation in cognitive outcomes previously demonstrated to exist in this panel (Neuner et al 2019) may perhaps be due to strain-specific variation in 5XFAD-induced microstructural alterations. Background strain was also seen to effect measures including MD, FA, NDI, and ODI in a broad range of brain regions implicated in learning and memory. We are currently processing functional magnetic resonance (fMRI) data sets in order to determine the effect of background strain on brain networks and testing a broad range of behaviorally-phenotyped and genetically-characterized F1-B6/BXD recombinant lines. Results that highlight brain regions involved in resilience to high-risk AD mutations may improve biomarkers for susceptibility and provide clues as to the nature of resilience to AD.
Quantum chemical calculations of solid-state NMR (SSNMR) parameters, including magnetic shielding tensors, are invaluable for establishing relationships between spectroscopic measurements and molecular-level structure and dynamics. Because of the insights they can afford, the calculation of magnetic shielding tensors has been an active field of research for several decades. Such calculations frequently employ density functional theory (DFT) because of the relatively efficient scaling of this class of methods with the number of basis functions, therefore making them suitable for systems that are large and/or have many electrons. For heavy atoms such as cadmium and platinum, one must employ relativistic approximations (such as the zeroth-order regular approximation, ZORA) to obtain reasonable agreement with experimentally-derived chemical shift tensors.1-2

When calculating NMR parameters in solids, one must consider long-range intermolecular effects; this requirement necessitates significant computational resources to make the calculations tractable. One method of modelling extended structure is to employ periodic boundary conditions, as is done in the gauge-including projector augmented wave (i.e., GIPAW) method. However, cluster-based protocols can also be employed effectively to model long-range 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 at a reduced computational cost.3 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).

In this presentation, relativistic calculations of the magnetic shielding tensors of the elements cadmium and platinum are discussed. To this end, we will compare the results of GIPAW calculations, using several protocols for generating pseudopotentials, with all-electron cluster-based calculations. We also evaluate the importance of relativistic effects by comparing calculations using the ZORA Hamiltonian at the scalar and spin-orbit levels. Finally, we compare results obtained through generalized gradient approximation functionals with those of hybrid functionals.

A COMPARISON STUDY OF DIFFERENT BRAIN EXTRACT METHODS

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The brain serves as the center of the nervous system in all vertebrate and most invertebrate animals and has many metabolites involved in energy metabolism and neurotransmission processes. In metabolomics, tissues are extracted by many different methods for different study purposes. Here we evaluate two common extraction methods of using either methanol:chloroform:water or methanol:water for extracting metabolites from brain tissue and evaluate metabolomics outcomes for these methods. We use several solution NMR and multivariate statistical analysis methods to compare metabolites detected using the two extraction methods. Our results indicate that methanol:water extraction enables more concentrated NMR samples but worse sample homogeneity as some lipids and macromolecules are co-extracted with the metabolites. The primary difference in metabolite stability for these two methods is that adenosine can be extracted by the methanol:chloroform:water method while adenosine was fully converted to inosine using the methanol:water method. Overall, we conclude that the methanol:chloroform:water method provides good quality data.
MECHANISM OF 2-AMINOPROPANOL SUBSTRATE RADICAL REARRANGEMENT CATALYSIS IN B_{12}-DEPENDENT ETHANOLAMINE AMMONIA-LYASE

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The coenzyme B_{12}-dependent ethanolamine ammonia-lyase (EAL) is the signature enzyme in ethanolamine metabolism in Salmonella and Escherichia coli under homeostatic and pathogenic conditions in the human gut. Toward elucidating the molecular mechanism of the radical-mediated reactions and the role of solvent-coupled protein dynamics in catalysis, time-resolved, full-spectrum electron paramagnetic resonance (EPR) spectroscopy has been used to measure the first-order reaction kinetics of the cryotrapped substrate radical intermediate in EAL from Salmonella enterica serovar Typhimurium at low-temperature (T) values, following T-step reaction initiation. Over 220-250 K, reaction of the non-native substrate, 2-aminopropanol, proceeds from two sequential, interconverting substrate radical intermediate states along two parallel channels: (1) S_1^- (radical pair capture configuration) reacts through a destructive pathway to form a dead-end radical species; (2) S_2^- (reaction enabling configuration) reacts through the native pathway to form diamagnetic products. Here, we report decay kinetics at 210 K, which show that interconversion of S_1^- and S_2^- is effectively quenched. This indicates that activation of detectable S_1^-; S_2^- exchange occurs over the relatively narrow T interval of 220-240 K. The possible coupling of the T-dependent exchange process to protein-associated domain (PAD) and concentric mesodomain solvent dynamics is addressed by using TEMPOL spin-probe EPR spectroscopy. The spin-probe results indicate an order/disorder transition in the PAD over the T-range of 230-235 K. Understanding of the solvent-protein-reaction coupling in EAL for 2-aminopropanol substrate is emerging from comprehensive kinetic analysis of the T-dependence of the S_1^- and S_2^- exchange interaction and reaction kinetics over 210-240 K, including extension to physiological T values, and correlations with the T-dependence of the solvent dynamics under pristine and dimethylsulfoxide cosolvent-tuned conditions. Supported by NIH DK054514.

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PROBING THE SELF-ASSEMBLY OF SQUID PROTEIN REFLECTIN AS A POTENTIAL BIO-PHOTONIC AND BIO-ELECTRONIC MATERIAL

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Cephalopods are very well-known for their remarkable ultrafast camouflage and proton conductivity, a potential platform for bio-inspired photonic and electronic materials. Understanding these processes requires molecular-level characterization of several attributes such as complex skin circuitry, self-assembly of the associated protein and signaling cascade in the nervous system. Although the protein responsible for this structural coloration, known as reflectin is widely expressed in cephalopods, the exact structural and self-assembly pathways are highly desirable but still elusive. In this work, we used solid and liquid phase characterization to probe the secondary structure and self-assembly of the second conserved domain of reflectin namely ref2cx4. Our data show that this protein assumes relatively fixed structure in the assembly. The residues Met, Phe and Ser were found in disordered conformations while Tyr, Thr, Pro, Asn, Gly, Asp and Arg were in ordered conformations. We also identified multiple conformations of Tyr, Thr, Asp and Arg with Asp and Arg being more flexible. The NMR chemical shifts identified the secondary structure of the residues Tyr, Thr, Pro, Asn, Gly, Arg, Phe, and Ser as alpha-helical type while Asp and Met are in beta-sheet conformation consistent in both solid and liquid phases. Using the sequential assignments with the help of 3D-NCACX and 3D-NCOCX spectra, we will reveal the complete assembly model of this protein.

References:

HIGH-FREQUENCY EPR STUDY OF THE UNUSUAL MULTIFERROIC NH₄CuCl₃

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We present orientation and temperature dependent high-frequency 240 GHz EPR experiments undertaken on NH₄CuCl₃ from 11--290 K. At 290 K, the system is axially symmetric, producing a single Lorentzian peak with \( g_\parallel = 2.2674 \) and \( g_\perp = 2.0520 \). The linewidth, \( \Delta H_{pp} \), varies according to an \( A + B(\cos \theta)^2 \) dependence, which is ascribed to the Dzyaloshinsky-Moriya antisymmetric interaction. Additionally, \( \Delta H_{pp} \propto (g - g_e) \), a consequence of spin-orbit coupling. Temperature measurements along each principal \( g \)-axis showed little temperature variation in \( g \) until around 50 K, at which point \( g_\parallel \) began to increase and \( g_\perp \) began to decrease with decreasing temperatures, with \( g \) remaining essentially constant. \( \Delta H_{pp} \) was found to significantly vary with respect to temperature for both principal \( g \) directions, with the line rapidly narrowing with decreasing temperatures from 290-45 K, while slowly beginning to broaden again until 11 K. We propose that an Orbach relaxation process is responsible for the linewidth behavior from 290-45 K, with \( \Delta \) on the order of 370 ± 15 cm⁻¹, and is related to the librational mode of the ammonium ion.
MEMBRANE INSERTION OF HUMAN β-DEFENSIN ANALOG DETERMINED BY INTEGRATING SOLID-STATE NMR AND MOLECULAR DYNAMICS

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The antimicrobial molecules of the immune system protect the human body from bacterial overgrowth. Human β-defensin (hBD) is one of the most prominent antimicrobial molecules. In humans, six α-defensins (~30 residues) and twenty-eight types of β-defensins have been identified. The solution or crystallographic structures of five β-defensins, including hBD-1, -2, -3, -4 and -6 have been determined. These peptides have a net charge density ranging from +4 to +11, and hBD-3, the focus of this study, is the most cationic peptide in this family, therefore, stronger interactions with the negatively charged bacterial membranes are expected. Using 2D solid-state NMR with molecular dynamics, we reveal functional-relevant structure and dynamics of hBDs in lipid bilayers and its effect on membrane morphology (Fig. 1a). hBD-3 analog exists in three major states with decreasing mobility: dissolved in solution, loosely associated with membrane surface, and deeply inserted into lipid bilayers. The structure of hBD-3 analog is highly polymorphic when bound to phospholipid membranes. The dynamic peptides in water exhibit relatively sharp linewidths of 1-1.5 ppm for aliphatic carbons because the conformational heterogeneity is averaged out by rapid motion. NMR-derived distance map revealed that the membrane bound topology is conformational dependent. The polymorphic L21 residue has its major conformer inserted in the hydrophobic core, whereas L24 has the trend reversed (Fig. 1b). The membrane-bound residues were found to be rigidified. By measuring 13C-1H dipolar coupling of the residue in POPC/POPG bilayer, most of the motionally averaged dipolar evolution curves exhibit asymmetric patterns indicative of intermediate time scale motion. We conducted 2D static 31P-31P exchange experiments to probe the rate of phospholipid reorientation along the membrane surface (Fig. 1c). Adding peptides increases off-diagonal intensity revealing higher diffusion coefficient and higher curvature and smaller vesicles indicating that hBD-3 analog has fragmented membranes into smaller, ellipsoidal vesicles that facilitates efficient reorientation of lipids (Fig. 1d).

In addition, we correlated the experimental results with MD simulation to better understand the peptide structure. The Root Mean Squared Fluctuation (RMSF) of the atomic positions in hBD-3 analog revealed that only a few hBD units form stable binding with the lipid membranes, with much smaller RMSF values than the other peptides. In general, hBD-3 analogs exhibit the lowest RMSF for the regions of residue 17-22 and 36-39, which generally match the MD-derived distance map and ssNMR-restrained structural topology. This study experimental, molecular-level evidence for understanding the functional structure and mechanism of β-defensin family.

Figure 1. Conformation, mobility and depth of insertion of hBD-3 analog. a 2D 13C-13C correlation spectra of VALIG with 100-ms DARR mixing. b Summary of distances from water and lipid acyl chains to the peptide. c 31P static spectra of control POPOC/G membranes without and with hhBD-3 analog 298 K. d RMSF of Hbd-3 analog binding POPC/G bilayers

Reference:

*Equal Contribution
The secondary cell wall, a complex composite of cellulose, hemicellulose and lignin, is the major constituent of plant biomass and is the central source for biofuel production. The degradation of carbohydrates is hindered by the deposition of lignin, which locks the energy in the cell wall and makes the lignocellulosic materials recalcitrant to chemical and enzymatical treatments during biofuel production. Using 2D $^{13}$C-$^{13}$C correlation spectra, we have investigated the structure of the cell wall and how lignin interacts with carbohydrates. The long range correlation spectra revealed that lignin has abundant electrostatic interactions with xylan (Fig 1. a) The irregular 3-fold helical screw conformation facilitates xylan’s binding to the aromatics of lignin. This xylan-lignin interface is linked to another domain of xylan in the flat-ribbon conformation that coats the even surface of cellulose microfibrils. The degree of lignin-carbohydrate interactions correlates with the content of methyl ether groups, revealing the central role of electrostatic interactions in maintaining these contacts. Relaxation and water-edited spectroscopy reveals that lignin, xylan and cellulose have distinct characteristics of hydration and motion (Fig. 1b). This study has revised our understanding of the plant secondary cell wall and provide the structural basis for designing and optimizing the biomass degradation process to facilitate the production of biorenewable energy.

We have further employed DNP to investigate the functional structure of natural abundance cotton and rice. The DNP enabled 2D $^{13}$C-$^{13}$C correlation spectra allows us to monitor the loss of $\alpha$ and $\beta$ allomorphs and the generation of a novel structure during ball-milling which reveals the importance of large crystallite size for maintaining $\alpha$ and $\beta$ model structures (Fig. 1c). After 2-hr ball milling, the remaining crystallites adopt a structure that could be viewed as B’ stacked sheet wrapped surface chains (Fig. 1d). In addition, we have studied the genetic rice mutants with altered degree acetylation in the hemicellulose arabinoxylan and revealed that deacytalation of xylan will decrease the flat conformer that intrinsically bind cellulose.

Figure 1. Structure of maize and cotton. a Lignin-polysaccharide cross peaks in maize in the 0.1 s spectrum. b water-edited intensities showing hydration level of molecules in maize. c 2D$^{13}$C-$^{13}$C spectra of unlabeled cotton. d $^{13}$C chemical shift RMSD map between cotton and other cellulose sources.

A full understanding of the dopant site occupation and dynamic processes that occur continues to be one of the remaining unresolved challenges in QD doping community. The synthesis of new, precisely doped QDs with different sizes and dopant concentrations provides a unique opportunity to initiate a systematic high-resolution NMR study on the nano-materials to correlate local structure and observed properties. This work focuses on the elucidating site of occupation of Al dopants in ZnSe Quantum Dot host lattice using MAS-NMR. Minimum of two types of dopant sites (Oh and Td) are anticipated to be formed in wurtzite type of host lattice. A series of samples with different concentrations of Al dopants and QD sizes is investigated. Preliminary structural studies were done to confirm QD size and lattice type using pXRD. Chemical shift measurements were performed using a standard pulse sequences, $^{27}$Al signals corresponding to Oh, Td sites and a third one corresponding spinel type phase are observed for doped wurtzite type Al:ZnSe QDs. With increase in Al$^{3+}$ concentration formation of a crystalline side-phase of spinel type ZnAl$_2$Se$_4$ is observed to form in 15% doped sample. Further multinuclear (C, Al, Zn, H, Se) spectroscopic investigation was done using number of 1D MAS NMR techniques.
SABRE Chemistry meets low field NMR

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SABRE1 (Signal Amplification By Reversible Exchange) is a parahydrogen based hyperpolarization technique, which allows for rapid, affordable and repeated hyperpolarization of molecules directly in room temperature solutions. SABRE has many applications, ranging from biomedical to high precision measurements. To achieve the full potential, we investigated key steps in spin physics, chemistry, and engineering:

(1) We build small, inexpensive and portable “paracubes” that generate 50% parahydrogen at liquid nitrogen temperature. This will enable parahydrogen experiments to be conducted easily in new locations that don’t have any hyperpolarization infrastructure yet.

(2) We used a modular immobilization system to generate a new class of heterogeneous catalysts from known homogeneous catalysts for hyperpolarization experiments. Heterogeneous catalysts allow for facile catalyst removal of the often toxic metal organic catalysts.

(3) High SABRE polarization was obtained under continuous flow using a membrane reactor for parahydrogen dissolution.2 This enables a continuous stream of hyperpolarized solution for injection or to feed a high precision Zeemann MASER.

(4) We hyperpolarized common prescription drugs (e.g. anti-tumor, fungicides and antibiotics) and amino acids. Furthermore, we hyperpolarize water3 and find an atypical dependence on polarization transfer field, which speaks to a novel One-H polarization transfer mechanism.

(5) We were able to acquire high resolution NMR spectra of 1H, 13C, 15N and 19F at very low magnetic fields between 1-10 mT. We used simulations to investigate the dynamics of the hyperpolarization systems and understand the surprisingly strong field dependence.

(6) Finally, our advances enabled the parahydrogen fueled NMR RASER (radiowave amplification by stimulated emission of radiation).4 The continuous hyperpolarization pumps the RASER and multimode operation allows for ultra-high precision measurements in the micro-Hz regime and beyond.5

All these aspects advance the SABRE method towards important applications in various areas of science including biomedical applications and high precision measurements.

32-Wei Li, Emory

**Tuning the Phase Transitions in Solvent Regions that Surround B12-Dependent Ethanolamine Ammonia-Lyase in Frozen Aqueous Solution by using Sucrose and Dimethylsulfoxide**

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The adenosylcobalamin (coenzyme B12) -dependent ethanolamine ammonia-lyase (EAL) is the signature enzyme in the ethanolamine utilization (EUT) metabolic sequence associated with microbiome homeostasis, and *Salmonella* - and *Escherichia coli* -induced disease conditions, in the human gut.\(^1\) Characterization of the molecular mechanism of EAL from *S. enterica* serovar Typhimurium advances toward therapeutic modulation of the EUT pathway. We have developed time-resolved, full-spectrum electron paramagnetic resonance (EPR) spectroscopy\(^2\) at low temperature (\(T\); 203 – 250 K) to reveal first-order kinetics of the substrate radical rearrangement and hydrogen transfer reactions in EAL.\(^3\)\(^-\)\(^5\) In order to correlate the substrate radical reactivity with solvent structure and dynamics, parallel methods of TEMPOL spin-probe EPR were developed.\(^6\)\(^,\)\(^7\) Here, the \(\sim10^{-11} – 10^{-7}\) s correlation timescale of the standard spin-probe measurement is extended to \(\sim10^{-3}\) s by using techniques of saturation transfer (ST) EPR spectroscopy\(^8\)\(^,\)\(^9\) applied to samples of EAL and 0.6% v/v aminoethanol substrate at varying concentrations of sucrose (0–5% w/v) or DMSO (0–5% v/v). The results confirm the order-disorder transition (at \(T_{ODT}\)) in the protein-associated domain (PAD; hydration layer) and detect the glass transition (at \(T_g\)) in the interstitial mesodomain that surrounds the PAD and EAL in the frozen aqueous samples. Values of \(T_g\) are consistent with the maximum freeze concentration condition for solutes in the mesodomain. The \(T_{ODT}\) and \(T_g\) values display opposite trends with added concentration of sucrose and DMSO, with higher sensitivity of the \(T_g\). Calibration of the \(T\)-dependent ST EPR line shapes by using a glycerol standard allows the viscosity dependence in the aqueous sucrose/DMSO and aminoethanol/sucrose/DMSO mesodomains to be addressed over the measurement range (180 – 260 K). The results provide fundamental information toward elucidation of the mechanisms of solvent-protein-reaction dynamical coupling in EAL catalysis.

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33-Christopher Lopez, UF

**31P-MRS BIOMARKERS OF MUSCLE PATHOLOGY RELATED TO INFLAMMATION IN YOUNG MDX MICE**

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**Background** Previous studies have reported altered energetic and acid-base status in dystrophic muscle using 31P phosphorus magnetic resonance spectroscopy (31P-MRS). However, the relationship between these alterations and inflammation is unclear. Young mdx mice undergo a well characterized phase of enhanced inflammation early in life. Furthermore, whether resting ATP turnover is elevated during this phase due to enhanced degeneration/regeneration has not been examined.

**Aims** The purposes of this study were to: 1) examine energetic status (Pi/PCr and ATP flux) at a young age in dystrophic mice and relate it to 1H2O T2, a marker of inflammation/muscle damage and 2) determine the effects of enhancing inflammation/muscle damage on energetic status and 31P markers of sarcolemma integrity by performing downhill running.

**Methods** In vivo 31P-MRS spectra were acquired from the gastrocnemius and soleus muscles in C57BL/10ScSn-DMDmdx (mdx, n=44) and wild-type mice (C57BL/10, n=21) between 4-10 weeks of age at 11.1T. ATP flux was measured by saturation transfer experiments. Downhill running was performed in a subset of wild-type and mdx (n=10/group). Relative concentrations of high-energy phosphates were measured and intracellular pH and magnesium (Mg2+) were calculated. 1H2O T2 was measured using single voxel 1H-MRS from the gastrocnemius and soleus at 4.7T.

**Results** At rest, T2 was elevated (p<0.05) in mdx in the gastrocnemius (mdx: 30.8±3.1ms; wild-type 28.2±1.2ms) and soleus (mdx: 31.6±3.9ms; wild-type 27.5±1.8ms). Intracellular pH was also elevated in the gastrocnemius in mdx (7.21±0.07) vs. wild-type (7.14±0.05). No differences were observed in Pi/PCr and ATP flux was, on average, slightly lower (p=0.10) in mdx (10.5±1.1mM/s) vs. wild-type (11.1±0.8mM/s). Downhill running further amplified the differences in T2 between wild-type and mdx. No changes were observed in wild-type pre to post running, while mdx showed a decrease (p<0.05) in Mg2+ and increase (p<0.05) in pH, with no changes in Pi/PCr and ATP flux.

**Conclusions** Despite clear differences in T2 between mdx and wild-type, Pi/PCr and ATP flux was not significantly altered in mdx, suggesting there is no direct relationship between inflammation and energetic status in young mdx mice. However, downhill running resulted in a reduced Mg2+ and increased pH in mdx mice, consistent with impaired sarcolemma integrity.
BRANCHED-CHAIN AMINO ACIDS ALTER THE HEPATIC DE NOVO LIPOGENESIS - A POTENTIAL IMPLICATIONS FOR NONALCHOLIC FATTY LIVER DISEASE (NAFLD)

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Nonalcoholic fatty liver disease (NAFLD) is a rapidly growing public health problem worldwide. The disease is closely associated with the presence of metabolic diseases, such as obesity, type 2 diabetes and insulin resistance (1). NAFLD describes a spectrum of liver disease characterized by progressive steatosis and inflammation, leading to the nonalcoholic steatohepatitis (NASH), and subsequent development to cirrhosis and liver cancer (2). NAFLD and T2D have been associated with increased hepatic lipid accumulation as well as production via re-esterification of the circulating free fatty acids (FFAs) and de novo lipogenesis (DNL) respectively.

Deuterated water is widely used for measuring de novo lipogenesis, based on quantifying lipid ²H-enrichment relative to that of body water enrichment (3). Hence, a ¹H/²H NMR approach has been utilized to determine the de novo lipogenesis (DNL) in the liver of mice (4). Three groups of mice were fed with low fat (LF), high fat diet (HFD) and high fat diet with branched-chain amino acids (HFD+BCAA) for 16 weeks and subsequent 4 days of D₂O exposure with total 4% body D₂O enrichment. NMR results showed that the HFD suppresses de novo lipogenesis (DNL) but triglycerides (TG) accumulation significantly increases because of the re-esterification of the circulating FFAs. Interestingly, HFD mice supplemented with BCAAs significantly decrease the DNL with less accumulation of the triglycerides (TG) compared to the HFD whereas low fat diet mice have highest percent of new lipids contrast to the lowest total hepatic triglycerides accumulation. In conclusion, BCAAs affects the DNL in the liver of the mice but a comprehensive investigation is needed to find out the pros and cons of BCCAs supplementation.

![Figure](a) (A) ¹H and (B) ²H NMR spectra of lipids: non ω-3 methyl (A); partial ω-6 methyl (B); ω-3 methyl (C); aliphatic chain (methylene) (D); α3 aliphatic (-CH₂-CH₂-COO⁻) (E); monounsaturated aliphatic (F); polyunsaturated aliphatic (G); All α2 aliphatic (-CH₂-CH₂-COO⁻) (H); DHA α2 and α3 (I); linoleic acid bis-allylic (J); other bis-allylic (K); sn-1, sn-3 of esterified glycerol (L); sn-2 of esterified glycerol (M); olefinic (N); chloroform (O); and pyrazine standard (P). (b) % DNL in the low fat diet (LF), high fat diet (HFD) and HFD supplemented with branched-chain amino acids (HFD+BCAA) mice.

MAGLAB HIGH B/T AND AMRIS CONVERGENCE LAB AT UF: NEW OPPORTUNITIES

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The mission of the High B/T Facility of the National High Magnetic Field Laboratory (MagLab), located on the University of Florida (UF) campus, is to provide users with experimental access to high magnetic fields (up to 16.5 T) and ultra-low temperatures (down to 0.5 mK) in an ultra-quiet electromagnetic environment [1]. The mission of the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) Facility of the MagLab, also located on the UF campus, is to enable research leveraging state-of-the-art magnet resonance technologies for MRI and NMR in high magnetic fields [2].

During the Fall 2019 semester, these two facilities have started to expand and relocate some of their operations to the new High Bay Convergence Lab, Figure 1, located in the New Physics Building operated by the UF Department of Physics. This exciting new development, made possible by resources provided by the MagLab and the University of Florida, will provide the first shared laboratory space for the High B/T and AMRIS Facilities. Together, this interdisciplinary group will explore new probe development and experimental platforms based on extensions of cryogenic applications and combined EPR/NMR (GHz/MHz) expertise. The resulting convergent atmosphere will also provide undergraduate and graduate students with opportunities to expand and diversify their horizons.

Figure 1. A sketch of the MagLab High Bay Convergence Lab at the University of Florida, a shared, common resource for the MagLab High B/T and AMRIS Facilities. The sketch shows the provisional site locations for several 89 mm, room-temperature bore superconducting magnets, one 800 MHz and two 400 MHz instruments. The arrangement will allow one group to be in high field while other groups refine their probes in the low field stations. The high bay space, with ceiling clearance of nominally 27 ft and equipped with a 10 ton ceiling crane, is located left-hand half of the lab. The East side of the interior space (mostly highlighted in light blue) opens to the external loading dock. The Crogenics Lab, which houses the helium gas recovery and liquefaction facilities, is located in the same building, and the North wall is shared with the Precision Instrument Shop.

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[1] https://nationalmaglab.org/user-facilities/high-b-t-facility
Oxalate Decarboxylase (OxDC) is a bicupin enzyme from Bacillus subtilis that catalyzes the redox-neutral decarboxylation of oxalate to formate and carbon dioxide. OxDC has two cupin domains each containing a Mn(II) ion. The N-terminal domain (domain I) hosts the active site of the enzyme. The C-terminal domain (domain II) has been proposed to be important in the folding of the enzyme into a hexameric quaternary structure. The mechanism proposed in the literature shows an oxidation of the N-terminal Mn(II) to Mn(III) under catalytic conditions and is a crucial step to the formation of the final product. In recent work, redox cycling of Mn(II) in WT OxDC with hexachloroiridate (IV) shows an apparent increase in Mn(III). A mutant was produced with structural homology to WT but is catalytically impaired due to a mutation at the critical W96 position. The data shows that it is more difficult to oxidize Mn(II) in the mutant than in WT suggesting that the mutation affects the reduction potential of the N-terminal Mn(II) ion.

¹Twahir et al., 2016, Biochemistry 2016, 55, 6505–6516
G proteins are guanine nucleotide binding proteins that transmit signals from stimuli outside the cell to the interior of the cell through their interaction with the integral membrane proteins, G protein coupled receptors (GPCRs). Several loss and gain of function mutations can alter the ability of G proteins to signal effectively, resulting in several metabolic disorders and diseases, which makes these important potential drug targets.

Studies of the structure and dynamics of GPCR tertiary complexes with G proteins and drugs can reveal important information on the mechanisms of receptor signaling. However, these complexes can be difficult to isolate and study. An important set of biochemical tools that have recently emerged are so-called “engineered” G proteins\textsuperscript{1}, which are G proteins containing the GTPase domain and modified to improve their stability with minimal alteration of their functional activity. In this study, we use NMR spectroscopy in aqueous solution to study the dynamics and structure of one of these engineered G proteins, the “mini-GaS” protein and its complexes with GPCRs\textsuperscript{2,3}.

References


ALPHA-KETOISOCAPROATE AS A PROMISING BIOMARKER FOR CANCER DETECTION

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Nuclear Magnetic Resonance (NMR) is relatively insensitive when applied to low-gyromagnetic ratio (γ) nuclei, such as ¹³C (γ₁³C = 10.705 MHz/T), due to the strong dependence on γ that is inherent in NMR. Electron Spin Resonance (ESR), on the other hand, is very sensitive due to the high thermal polarization of electrons (γₑ = 28,024.952 MHz/T). Dynamic Nuclear Polarization (DNP) is one method by which the NMR signal may be enhanced via transferring the incredibly high polarization of the electrons at cryogenic conditions (~1 K) and high field strengths (~3.35 T) to the nuclei. Now, DNP was at first usually restricted in application to solids due to its requirement that the sample to be hyperpolarized be placed in cryogenic conditions. However, in 2003, its applicability was extended to liquids through the invention of dissolution DNP by Ardenkjaer-Larsen et al.¹ This extended the high signal enhancement (at least 10,000-fold) of DNP to the biomedical realm. Significantly, dissolution DNP allows real-time tracking of in vivo metabolism via labeling the relevant substrate with ¹³C. This observation has led many groups in the hyperpolarization community to investigate the possibility of non-invasively locating and diagnosing malignant tumors. In order for such to be feasible, one must first find a promising biomarker (like ¹³C-labeled pyruvate²) that, upon injection into a subject, results in an overproduction of some labeled downstream metabolite if in the presence of cancer. With such in mind, this study followed real-time metabolism of [¹-¹³C] alpha-ketoisocaproate (α-KIC) and [¹-¹³C] leucine (Leu) in the prostate and glioblastoma cancer cell lines PC-3 and SfXL (see Figure 1). Results of in vitro cell extract experiments will be discussed in light of whether α-KIC or Leu represent a promising biomarker for the non-invasive detection of these two cancers when combined with dissolution DNP.


Anthony Pastore, UF

5-HYDROXYTRYPTOPHAN LABELED OXALATE DECARBOXYLASE SIPHONS AWAY OXIDATIVE POWER FROM THE ACTIVE SITE AT LOW pH

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Oxalate decarboxylase (OxDC) oxidizes a Mn²⁺ co-factor, to break apart monoprotonated oxalate into carbon dioxide and formate, through a redox neutral process. Dioxygen is a required co-factor for decarboxylase activity, however, the role and location for binding of dioxygen is unknown. Site-directed mutagenesis experiments combined with enzyme kinetics and X-ray crystallography have demonstrated the catalytic significance of a π-stacked tryptophan dimer (W96 and W274). W96 substitution with the unnatural amino acid, 5-hydroxytryptophan (5-HTP), was utilized to further study the role of the tryptophan pair upon catalysis. This report encompasses CW-EPR experiments, enzyme kinetics, and supporting LC-MS/MS data on 5-HTP labeled OxDC to evaluate Mn³⁺ formation in the active site. Our results correlate low activity with impaired Mn³⁺ formation in the active site. We also observed the appearance of a carbon based radical under turnover conditions without substrate. The EPR signal of the carbon based radical significantly increased over time when substrate is added. Our results suggest 5-HTP substitution alters the reduction potential for Mn³⁺ formation in the active site and dioxygen binding in OxDC.

Figure 1. Formation of the Carbon Based Radical in 5-HTP Labeled OxDC.
MECHANOCHEMICAL SYNTHESIS OF HCL API MULTI-COMPONENT CRYSTALS AND THEIR STRUCTURAL REFINEMENT AND ELUCIDATION USING $^{35}$CL SSNMR AND DISPERSION-CORRECTED DFT CALCULATIONS

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The solid forms of active pharmaceutical ingredients (APIs), including polymorphs, pseudopolymorphs, and amorphous solid dispersions, have unique properties such as stability and solubility that can affect their bioavailability and shelf lives. These properties are dependent on the molecular-level structure of the solid form of the API; as such, they can be modified with targeted synthetic or preparative procedures. HCl salts of APIs are the most common solid forms with improved solubility and stability; however, these can often be further improved by the production of multi-component crystals (MCCs). For this reason, MCC forms of APIs, which feature the API and a pharmaceutically acceptable coformer, have garnered much recent attention.

In 2004, Childs et al. showed that fluoxetine HCl could be cocrystallized via solvothermal methods with three different carboxylic acid coformers: benzoic acid, fumaric acid, and succinic acid.¹ In 2019, Borodi et al. also demonstrated MCC formation by MC methods of promethazine HCl with four carboxylic acid coformers: fumaric acid, succinic acid, adipic acid, and oxalic acid.² Structural characterization of these MCCs is crucial for understanding their mechanisms of formation, their solid-state properties, and the rational design of other novel MCCs.

Herein, we describe the use of $^{35}$Cl solid-state NMR (SSNMR) for probing the structures of known fluoxetine HCl and promethazine HCl MCCs, and applying similar methods for the structural elucidation of novel MCCs of fluoxetine HCl, promethazine HCl, chlorpromazine HCl, and promazine HCl. First, we discuss MC synthesis of all of the MCCs and demonstrate (i) the superiority of MC methods to previously reported synthetic procedures³ and (ii) improvements in comparison to previous MC syntheses. Second, we use $^{35}$Cl SSNMR to obtain spectral fingerprints of each API and corresponding MCCs. 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 second-order quadrupolar powder patterns. Lastly, experimentally measured parameters are compared with those calculated using dispersion-corrected DFT methods⁴ in order (i) to improve the structural refinement of MCCs with known crystal structures and (ii) to use this information to help elucidate the molecular level structures of new MCCs.


Streptococcus mutans is the virulent bacteria generating dental cavities, one of the most common human diseases in the world. Adhesin P1 (Antigen I/II) is a key functional protein on the surface of S. mutans which initiates adherence of the bacteria to form plaque. Our previous studies indicate that the C-terminal region of adhesin P1, known as the C123 domain, plays an important role in the formation of amyloid fibrils by self-assembling. It is also critical to the pathogenesis of S. mutans. We are now focusing on characterizing the C123 fibril amyloid formation by NMR spectroscopy. However, full-length C123, at 51 kDa, is not amenable to full structural analysis by solution NMR using uniform isotopic labeling strategies. We would also like to locate the key functional sites between the three domains in C123. Therefore, we have created constructs which enable us to specifically isotopically enrich the C12 or C3 domains in C123 protein, in order to simplify and to increase the resolution and sensitivity of DNP and solution NMR spectra.
Lack of Effect of Chronic Cannabidiol Treatment During Early Adolescence on Brain Neuroimaging Indices in Rat

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Several lines of evidence support the use of cannabidiol (CBD) as a broad acting medication to alleviate childhood disorders typically comorbid with autism spectrum conditions. CBD has been reported to alleviate psychosis and anxiety, facilitate REM sleep, and suppress seizure activity, which represent highly beneficial outcomes. Whether chronic treatment during a developmental epoch such as early adolescence exerts unwanted neurobiological and behavioral effects, however, remains to be determined. The present study was carried out to determine the in vivo neurobiological effects of CBD treatment during adolescence on various functional and diffusion neuroimaging markers. Pregnant rats arrived at gestational day 10 (GD10) and were left undisturbed, singly housed with food and water ad lib and nesting material. On postnatal day 1 (PD1), pups were assessed for body weights, sex ratios, anogenital distances and developmental defects. Pups were randomly assigned to either vehicle- or CBD-treated groups (CBD at 100 mg/kg in 1:1:18 ethanol:cremaphore:saline vehicle; volume at 1 ml/kg subcutaneous, SC). CBD or vehicle was administered from PD32 to PD47. We conducted the study using a cross sectional design, with imaging at 4.7 Tesla on PD48 and behavioral testing PD49-52. Rats were imaged at 4.7 Tesla under 0.2 mg/kg/h dexmedetomidine, SC and 0.5% isoflurane in 70%N2/30%O2. Data were processed using pipelines established and published by our group. We carried out network analyses on the multiple groups: Vehicle-Female, CBD-Female, Vehicle-Male, CBD-Male (n=5/group). We used a robust analysis approach involving network (connectomic) metrics from the field of graph theory. This allowed us to focus the analysis on correlation values above a significance threshold (in this case z>0.3), and to determine not only changes in connectivity strength but also the arrangement of neuronal interactions in the brain (network organization). In spite of this robust approach there was no effect CBD on any of these measures. In support of these negative findings, we also failed to observe behavioral changes (locomotor activity and ultrasonic calls) with CBD treatment. In conclusion, CBD treatment during adolescence did not produce significant effects on functional connectivity, locomotor activity, or ultrasonic vocalizations. At this time, we are processing diffusion MRI data sets, which we collected using a multi shell sequence in order to determine the effect of CBD (sex) on neurite orientation dispersion and density, and standard diffusion tensor imaging metrics.

Acknowledgements

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Through X-ray crystallography and kinetics assays, it has been previously found that the Mn-dependent enzyme Oxalate Decarboxylase (OxDC) binds small carboxylates such as acetate in its active site in the absence of the substrate oxalate. Using carbon-13 (C-13) labeling, Electron Nuclear Double Resonance (ENDOR) Spectroscopy can be used to determine binding conformation and bond distances between these small molecules and the manganese in the active site of wild-type (WT) and mutant OxDC. Resolving details of binding may illuminate factors critical to the enzymatic mechanism of the protein.

In collaboration with Dr. Robert Bittl’s lab at the Freie Universität Berlin, Germany, the ENDOR spectrum of WT OxDC and the double mutant E280QC383A was investigated in the W-band. The protein was poised at high pH and carbon-13 labeled bicarbonate, oxalate, formate, and acetate were used to probe binding. Despite a strong continuous wave EPR spectrum and a relatively high concentration of both protein and substrates, neither the WT nor the mutant displayed a carbon-13 ENDOR signal in the presence of any of the labeled small molecules. This is surprising because similar experiments in the X and Q-band have shown strong C-13 – Mn coupling in WT and the related mutant, E280Q. To test the approach, a model system was used containing carbon-13 labeled bicarbonate in aqueous MnCl$_2$ which yielded a strong C-13 peak, indicating that the instrument was sensitive enough for the experiment. We are currently exploring at lower fields how sample preparation affects the ENDOR signal and plan to move back to W-band once a reliable protocol has been established to test how the signals depend on the instrument’s frequency.
Spin-labeled Retinal for Studying Rhodopsin Oligomerization in a Synthetic Lipid Bilayer and Native Cellular Membranes by DEER
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Oligomerization of integral membrane proteins is a common biophysical phenomenon that is often the final step of protein folding into functional assemblies. Stability of protein oligomers and their organization varies and could be affected by a number of factors including physicochemical interactions with other membrane constituents such as lipids and proteins. Thus, the protein oligomers observed in different membrane mimetics including bilayers formed from synthetic or highly purified natural lipids could be different from those formed in native membranes. These considerations warrant further development of experimental methods suitable for quantitation and structure determination of protein oligomers in the native cellular membranes. One of such methods is DEER spectroscopy which provides long range (ca. 2-9 nm) constraints between specific protein sidechains labeled with paramagnetic tags. Previously, we employed Q-band (34 GHz) DEER to refine NMR-based structure of a ~81 kDa trimer formed by hepta-helical transmembrane photoreceptor Anabaena Sensory Rhodopsin (~27 kDa) from Anabaena sp. PCC7120 in membranes composed from synthetic lipids.

Here we describe the use of a spin-labeled derivative of retinal (4-[2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxyl]-retinal, Fig. 1A)) to assess the oligomeric state of bacterial rhodopsin in lipid vesicles composed from synthetic DMPC-DMPA and compare the results with the native cellular membrane preparation. The spin-labeled retinal analog exhibits a strong binding to apo-rhodopsin and, therefore, no site-directed mutations are required for DEER experiments. Furthermore, retinal binding is a useful probe for oligomerization of functional rhodopsins as the protein known to lose the retinal upon denaturing. Because rhodopsins would form both homo pentamers and hexamers in lipid bilayers and even higher order nanodomains, the spin-labeled retinal was magnetically diluted with native retinal for binding to apo-rhodopsin to avoid strong electronic spin-spin interactions. Experimental DEER signals from such spin-labeled rhodopsin revealed significant difference in protein oligomerization in synthetic vs. native membranes. Specifically, while oligomers with well-defined distances were found to form in DMPC-DMPA (Fig. 1B), some shorter distances were determined to be present in the native cellular membranes (Fig. 1C). The latter distances were attributed to smaller oligomers (dimers, trimers, etc.) that could be intermediates to larger oligomers or incomplete oligomers which further oligomerization was terminated by the membrane defects and/or other protein components. The work at NCSU was supported by U.S. DOE Contract DE-FG02-02ER15354.

Figure 1. (A) Structure of a spin-labeled derivative of retinal (4-[2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxyl]-retinal synthesized for this study. (B) Experimental Q-band (34 GHz) DEER signals and (C) corresponding distance distributions for spin-labeled retinal incorporated into bacterial rhodopsin reconstituted in DMPC-DMPA and native cellular membranes.
Protonation of Ionizable Probe at the Protein-Lipid Interface

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Interfacing biological and artificial systems at the nano-scale level is essential for developing novel living-nonliving biotechnology platforms for applications in biology and medicine as well as for designing of biosensors. Despite an impressive progress achieved in new bio-nano hybrid systems, the needs remain high to understand the influence of a nanostructured support and nanoconfinement on structure and properties of the membrane-protein interface.

In this work we report on spin-labeling EPR studies to assess effects of solid inorganic interface, specifically, silica support, on effective $pK_a$ of the membrane-burred peptide ionizable sidechains. A model transmembrane $\alpha$-helical WALP peptide was covalently modified with cysteine-specific pH-sensitive nitroxides and incorporated into bilayers of various compositions. We have determined the effective $pK_a$ of the probe positioned in a site-directed manner at the interface of the peptide and the membrane. We have shown that the profile of $pK_a$ across the bilayer is somewhat different for unilamellar and multilamellar vesicles. We report that the $pK_a$ of the peptide sidechain increases by more than 2 pH units upon replacing zwitterionic PC with anionic PG lipids. We have also investigated the effect of placing a phospholipid bilayer with the integrated transmembrane $\alpha$-helical WALP peptide on the surface of silica nanoparticles on the peptide dynamics and the effective $pK_a$ of the probe. The silica support caused shift in the $pK_a$ of the probe consistent with the negative charge on the silica surface but induced a peptide transition upon the probe protonation not observed in liposomes. Supported by NSF 1508607 to TIS.
EFFECT OF RADICAL CONCENTRATION ON $^{13}$C SCALAR ODNP at 14 T

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Dynamic Nuclear Polarization in the solution state, by the Overhauser Effect (ODNP), makes use of two competing cross-relaxation mechanisms: scalar and dipolar hyperfine coupling. While the ODNP coupling factor for the dipolar mechanism is mainly influenced by molecular size and molecular motions, it scales unfavorably with increasing magnetic field. The scalar ODNP coupling factor, on the other hand, while independent of magnetic field, depends strongly on the target nuclear site; large enhancements have been observed at high field at the $^{31}$P site in triphenyl phosphine (TPP) but not in TPP-oxide, and the $^{13}$C enhancement in chloroform has been observed to be about four times larger than in CCl$_4$.

Here, we present $^{13}$C scalar ODNP enhancements at 14 T ($\omega_n/2\pi = 150$ MHz) in a wide range of target molecules, including hexane and pentane, which are generally believed to be unsuitable for scalar ODNP. These enhancements were obtained at low radical concentrations (2-8mM), which eliminates any competing dipolar cross-relaxation. The low radical concentration causes long nuclear spin-lattice relaxation times, necessitating long microwave irradiation times and solvents with low dielectric loss at 395 GHz.
NMR Studies of Human GPCRs in Lipid Nanodisc Environment.

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G-protein coupled receptors (GPCRs) represent the largest family of membrane proteins in the eukaryotic genome and play key roles in signal transduction at the cellular surface. GPCRs drive numerous primary physiological responses and thus are important targets for the development of new therapeutics¹. GPCR function is inherently linked to simultaneous population of multiple conformational states, the relative populations of which are modulated by the efficacies of bound drugs². Additionally, endogenous allosteric modulators such as lipids can control the relative populations and life times of different functional states; however, little experimental data exist that determine structural mechanisms of lipid allosteric modulation. Motivated by this need, we study the effect of lipids on the human A2A adenosine receptor (A2AAR). Using lipid nanodisc preparations of A2AAR, we use solution NMR to study the mechanisms of receptor allosteric modulation by lipids, with the goal of providing fresh insights into how endogenous lipids alter the conformational equilibria of GPCRs in cells.

A site-specific comparison of the spherical and tubular capsid structure and assembly of the Rous Sarcoma Virus

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Retroviruses are a class of RNA viruses that replicate by inserting a copy of their genome into host cells before replicating. One of the most commonly known retroviruses is the human immunodeficiency virus (HIV), which infects immune T-cells in humans. A more studied retrovirus, however, is the Rous Sarcoma Virus (RSV), responsible for causing connective tissue cancer in chickens. RSV carries an oncogene, making it useful to study both retroviruses and cancer-causing genes. For retroviruses, the correct formation and disassembly of mature capsids is a crucial step in the effective infection of host cells. As part of full capsid formation in vivo, RSV capsid protein monomers assemble into irregular polyhedrons that arrange into larger structures consisting of twelve pentamers inserted into a larger hexagonal lattice. In vitro, they can be induced to form spherical and tubular assemblies, consisting entirely of pentamers and hexamers, respectively.

The primary goal of this research is to determine the structure of both capsid assembly types. In vitro, we obtained highly uniform samples of both the tubular and spherical assemblies of the RSV capsid protein, which produced sharp spectral lines in solid state NMR experiments. This allowed site-specific identification of chemical shifts, which were used to predict the conformation of the protein in each assembly via the TALOS-N protein backbone dihedral angle prediction program. Our results reveal site-specific differences of the capsid protein between assembly morphologies, including notably large torsion angle shifts in several flexible regions between alpha helices such as the cyclophilin loop regions. This information lays the foundation for further study of the mechanism of capsid assembly at the molecular level and may assist rational design of anti-viral drugs against deadly diseases caused by retroviruses.
PROBING THE MAGNETO-OPTICAL PROPERTIES OF GaMnAs: A $k.p$ STUDY

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At Mn concentrations above about 1%, (Ga,Mn)As is a dilute ferromagnetic semiconductor (DFS) supporting Curie temperatures as high as 190 K. Despite substantial literature demonstrating the ability to tune the magnetic and spin-related properties in this material, a fundamental understanding of the band structure and the origin of free holes is still debated. Because of the possibility of controlling the magnetic coupling among localized Mn spins through electric field gating, light pulses, or hydrostatic pressure, (Ga,Mn)As and other DFS materials have attracted attention for semiconductor spintronics. However, few studies have examined the nuclear spin interactions in DFS materials, and to our knowledge, light-induced nuclear spin dynamics in (Ga,Mn)As has not been previously reported.

We employed high-field inductively-detected nuclear magnetic resonance (NMR) spectroscopy to investigate the effects of continuous-wave near band-edge optical illumination on lattice nuclear spins in a Ga$_{1-x}$Mn$_x$As film sample, (with $x \approx 0.04$), grown on semi-insulating GaAs. Control experiments were performed on a piece of the bare GaAs substrate wafer. Samples were immersed in superfluid liquid helium at 1.5 K, well-below the Curie temperature of 47.5 K of the DFS sample. Our Ga-71 NMR experiments revealed light-activated nuclear spin relaxation. We consider the effect of the ferromagnetic exchange interactions and the role of Mn centers, as well as various other types of defects that are inevitably introduced by the low-temperature MBE growth. Theoretical $k.p$ calculations for absorption coefficients and electron spin polarization were performed to gain insight into the experiments. The evolution of the band structure, Fermi-level, and optical properties as a function of Mn concentration and magnetic field were calculated. The action spectra can be interpreted as due to the combined effect of band-gap renormalization, Burstein-Moss effect, and intervalence band transitions.
A Complete Study of Metronidazole Hyperpolarization with SABRE using Automated Pneumatic Shuttling

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Signal Amplification By Reversible Exchange (SABRE) is a parahydrogen based hyperpolarization modality that is particularly simple and fast or even continuous\(^1\). A more recent variant, SABRE-SHEATH, (SABRE in SHield Enables Alignment Transfer to Heteronuclei) enables targeting \(^{15}\text{N}\) and \(^{13}\text{C}\) nuclei in a wide range of substrates\(^2,3\). SABRE relies on reversible exchange of parahydrogen and substrate on a polarization transfer complex (PTC) (Fig. 1A).

The common antibiotic metronidazole (Fig. 2A) is a promising SABRE substrate due to its high polarization (~20%) and long relaxation times (~8 min)\(^4,5\). With these ideal hyperpolarization properties metronidazole has strong potential as a hyperpolarized imaging agent in MRI, for diagnosis such as hypoxia imaging in tumors. Here, we present a complete study providing novel insights into the metronidazole hyperpolarization mechanism with SABRE (See Fig. 2B-D).

To enable our study, we engineered a pneumatic shuttling apparatus synchronized with pulse programs to enable high throughput experiments and multidimensional NMR exploiting SABRE hyperpolarization (Fig. 1B). In this automated system, parahydrogen (\(p\)-\(\text{H}_2\)) is bubbled through a SABRE active solution at various magnetic fields, established above the NMR magnet. This includes mu-metal shielding for heteronuclear SABRE-SHEATH (e.g. \(^{15}\text{N}\)) experiments or a solenoid for \(^1\text{H}\) SABRE experiments. The study demonstrates the utility of the designed pneumatic shuttling for robust, reproducible, and convenient analysis of parahydrogen hyperpolarization that can be extended to any desired substrate. Using metronidazole, we demonstrate a variety of shuttling experiments to elucidate hyperpolarization mechanisms in a target substrate, including identification of previously unknown binding states in the SABRE complex using hyperpolarized multidimensional NMR.

DNP Assisted REDOR: Applications to Clinical Pulmonary Surfactant Therapeutic KL4

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Here we present dynamic nuclear polarization (DNP) results at 600 MHz/395 GHz for a lung surfactant peptide mimetic, KL4, utilizing a novel sterol lipid tethered biradical, cholesterol-AMUPol, and water soluble biradicals AsymPolPoK and AMUPol. Most notably, we identify conditions where the peptide/membrane system is insensitive to the presence of polarizing agents. This enables us to structurally characterize the KL4 peptide in its native membrane environment under conditions replicating clinical peptide/lipid formulations for respiratory distress syndromes. In this study, we compare DNP polarization and reproducibility between cholesterol-AMUPol, AMUPol, and AsymPolPoK containing samples for KL4 in hydrated liposomes. We address effects of sample preparation on NMR spin coherence times using MAS solid state NMR and evaluate biradical distribution and relaxation properties in liposomes with X-band EPR. In addition, we use dipolar recoupling, assisted with DNP polarization enhancement, to measure mid-range interatomic distances within KL4 (dipolar couplings <100 Hz) for comparison to measurements via conventional solid state NMR at ambient temperatures.

Our results show the superior DNP performance of AsymPolPoK, with enhancements $\epsilon \sim 45$ achieved for the peptide carbonyl. At 5 mM polarizing agent concentration, $T_2$ spin coherence times were comparable to those measured under ambient conditions. This allowed us to implement long dipolar evolution periods (up to 80 msec) in the REDOR pulse sequence for accurate measurement of peptide interatomic distances. Notably at 100 K, we observe two distinct distance populations for KL4 [$^{1,13}$C] L8, [$^{15}$N] L12, that are also present both at ambient temperatures and in molecular dynamics simulations. Interestingly, interatomic distances for both populations at 100 K are $\sim 10\%$ shorter compared to those measured at ambient conditions, suggesting an effect of cryogenic temperatures on the peptide structure and/or water/lipid membrane interface. We plan to support our experimental results with MD simulations at 100 K, and address effects of cryogenic temperatures on lipid membrane systems.
The glycocalyx composition is diverse between various cells. Changes in composition can result in disruption in certain cell functions and are associated with diseases. Studies of glycan organization and interactions can aid in cancer therapy and cancer immunotherapy development. This study will improve our understanding of the organization of glycocalyx using electron paramagnetic resonance (EPR). In this study, metabolic glycan engineering was utilized to incorporate unnatural sugars, azido mannosamine (ManNAz) and azido glucosamine (GlcNAz), in cells. A nitroxide spin label was then incorporated onto the modified sugars in the glycocalyx via copper-free click chemistry. Information on mobility at spin labeled glycan sites were obtained using EPR line shape analysis. ManNAz shows higher mobility compared to GlcNAz. Line shape analysis of Ls174T-GlcNAz spectrum reveals a high mobility component and a low mobility component. GlcNAz is also metabolized differently in different cell lines.
NMR Crystallography of Multi-Component Crystals of Urea
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The rational design of single-phase multi-component crystals (MCCs) is a flourishing area in crystal engineering; MCCs come in a variety of different solid forms, such as solvates, salts, and cocrystals. MCCs are of particular interest to the pharmaceutical industry, as they can be made from an active pharmaceutical ingredient (API) and one or more pharmaceutically acceptable coformers. By carefully selecting the appropriate constituents and mode of preparation, it is sometimes possible to tailor the physicochemical properties of the crystalline solid to have better solubility, stability, bioavailability, and/or shelf-life.[1,2] Two common methods of synthesizing MCCs are slow evaporation and mechanochemistry; of these, the latter has been less explored.

The mechanochemical method of liquid-assisted grinding (LAG) incorporates all modern tenets of “green chemistry” (i.e., it requires little solvent, has low energy input, and no harsh reagents or waste are involved).[3] There is great interest in the syntheses of MCCs containing APIs, predominantly for the production of stable dosage formulations; however, there are few reports describing their rational design or the reaction mechanisms underlying their formation.[4,5] Powder X-ray diffraction (pXRD) and solid-state NMR (SSNMR) spectroscopy are well suited for studying the formation of MCCs, both allowing for the identification and characterization of distinct crystalline phases and the detection of impurities. Most importantly, SSNMR is extremely sensitive to local structural differences that result from unique hydrogen bonding networks. Thus, careful characterization of these materials by SSNMR provides an avenue for structural solution, and aids in the development of NMR crystallography protocols that can be applied to a diversity of solids.[6-8]

Here, we present an NMR crystallographic investigation of MCCs of the form MCl:Urea:xH2O (M = Li, Na, Cs; x = 0, 1, 2) made by mechanochemical syntheses; this includes characterization by multinuclear (35Cl, 23Na, 7Li, and 133Cs) SSNMR spectroscopy, synchrotron XRD, and thermogravimetric analysis. The study of these model systems using plane-wave density functional theory calculations[9] and Rietveld refinement of synchrotron XRD data aid in determining the crystal structures of these solids; these methods could find general applicability in NMR crystallographic studies using quadrupolar nuclides.

Deuterium Kinetic Isotope Effects Resolve Low-Temperature Substrate Radical Reaction Pathways and Steps in B12-Dependent Ethanolamine Ammonia-Lyase

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The decay reaction kinetics of the cryotrapped $^2\text{H}_4$-aminoethanol substrate radical intermediate state in the adenosylcobalamin (B$_{12}$) -dependent ethanolamine ammonia-lyase (EAL) from *Salmonella enterica* serovar Typhimurium are measured over 203 – 225 K by using time-resolved, full-spectrum electron paramagnetic resonance (EPR) spectroscopy. The studies target fundamental understanding of protein configurational dynamics that control the function of EAL, the signature enzyme in the process of ethanolamine utilization (EAT) associated with microbiome homeostasis, and *Salmonella* - and *Escherichia coli*-induced disease conditions, in the human gut. The incorporation of $^2\text{H}$ in the hydrogen transfer step that follows the substrate radical rearrangement step in the substrate radical decay sequence leads to an observed $^1\text{H}/^2\text{H}$ isotope effect of approximately 2, that preserves, with high fidelity, the idiosyncratic piecewise pattern of rate constant versus temperature dependence over 203 – 225 K, that was previously reported for $^1\text{H}$-substrate, including monoexponential ($T\geq 220$ K) and two distinct biexponential ($T=203 – 219$ K) decay regimes. In the proposed global kinetic model for $^2\text{H}$- and $^1\text{H}$-substrate radical decays, reaction proceeds through two parallel channels of rearrangement and hydrogen transfer, from two substrate radical progenitor states, $S_1^*$ and $S_2^*$, that are distinguished by different protein configurations, representing nascent substrate radical capture and rearrangement-enabling functions, respectively. Decay from $S_1^*$, or $S_1^*$ and $S_2^*$, is rate-determined by radical rearrangement ($^1\text{H}$) or by contributions from both radical rearrangement and hydrogen transfer ($^2\text{H}$). Non-native direct decay of $S_1^*$ to products is a consequence of the abrupt rise of the free energy barrier to the native $S_1^* \rightarrow S_2^*$ protein configurational transition. In EAL at physiological temperatures, this is averted by the fast collective protein configurational dynamics that guide the $S_1^* \rightarrow S_2^*$ transition.

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INVESTIGATING THE OXIDATIVE POTENTIAL OF SECONDARY ORGANIC AEROSOLS ON POLYUNSATURATED FATTY ACIDS AND CELLULAR MEMBRANES

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Airborne particulate matter found in industrial byproducts can promote lung, cardiovascular, and even cerebrovascular diseases. Much attention has been given to PM₂.₅, a particulate matter found to penetrate the human lung barrier. A large fraction of PM₂.₅ consists of secondary organic aerosols (SOAs), a gas-to-particle conversion of volatile oxidized hydrocarbon vapors found in the atmosphere(1-2).

Recently, we have identified SOAs ability to oxidize polyunsaturated fatty acids (PUFAs) and cellular membranes of live mammalian carcinoma cells, as well as human small airway epithelial cells (SAEC). In our study, three developed strategies were used to probe and assess the oxidative potential of SOA on several different lipid membrane systems. (1) Electro-Paramagnetic Resonance (EPR) with the usage of 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) spin trapping identify the presence of carbon-based radicals in SOA-DMPO samples. We additionally show in vitro evidence for fatty-acid free radical formation using spin trap 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) with oxidized PUFAs in organic solvent. EPR spectra demonstrates an increase in radical adduct formation upon reaction of DMPO and SOA-oxidized PUFAs. (2) A Diphenyl-1-pyrenyphosphhine (DPPP) fluorescent probe employed in both PUFAs, and live-mammalian and human SAEC cells showed a significant increase in DPPP oxide fluorescence when exposed to SOA, compared to controls. Our data further supports the production of free radicals in secondary organic aerosols, and their oxidative effect on different cellular membranes. (3) A 2',7'- Dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe was assayed to measure intracellular reactive oxygenated species (ROS) formation via flow cytometry. Our results demonstrated a larger shift in fluorescent cell population upon incubation with SOA. Microscopy imaging further supports a significant increase in green fluorescence denoting an increase in ROS production upon incubation of SOA. Our results demonstrate the oxidative potential of secondary organic aerosols and its profound oxidative damage in both in vivo and in vitro experiments. Results from this study will be used assess oxidative damage as a result of multiple forms of SOA exposure, to both pulmonary and cardiovascular cell strains, as well as various compositions of PUFA-derived liposomal membranes.

AL FRESCO: A New, Efficient Scheme for Establishing $^{13}$C-$^{13}$C Correlations Under Ultrafast MAS Rates in Solid State 2D NMR

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We introduce an efficient chirp pulse mixing scheme for the two-dimensional (2D) $^{13}$C-$^{13}$C homonuclear dipolar correlation spectroscopy, Adiabatic Linearly FREquency Swept reCOupling (AL FRESCO), that can be used under an ultrahigh MAS spinning rate. This method is suitable for producing $^{13}$C-$^{13}$C cross-peaks efficiently between sites that exist within a broad offset frequency window, especially for sites with large chemical shift differences. The AL FRESCO mixing scheme is also very tolerant to the dipolar truncation effect and also has an advantage of minimizing the RF heating effect, even under a very long mixing time, because this method employs a very weak RF pulse strength for mixing. Key considerations required for optimizing this chirp pulse mixing scheme, such as dwell time ($\beta t$), sweep bandwidth (BW), and total pulse duration ($t_p$), will be presented. Our experiments demonstrated on a few different samples, including U-$^{13}$C-labeled Barstar protein, will be shown.
PROBING DRIVING FORCES BETWEEN NANOPARTICLES AND AMINO ACIDS BY STD-NMR

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Nowadays, lots of nanoparticles (NPs) are produced and applied as biomedicines with applications in diagnostics and therapeutics, such as drug delivery, biosensors and imaging contrast agents. However, the effects of nanoparticles on biological systems are not well known. Some studies show that once the nanoparticles enter the biological systems, they will be surrounded by proteins immediately\(^1\). These nanoparticles could change the conformation of proteins, resulting in new epitope expose on the protein surface\(^2\). This could further lead to unexpected reactions and toxicity. If there is a way to determine the structure change of proteins, we can better predict and prevent the unexpected side effects of NPs.

Due to its non-invasive and high-resolution properties, NMR techniques have been widely used to characterize dynamics and surface morphology of small ligands on NPs\(^3\). In the latest work, by using STD-NMR, amino acids binding to negatively charged polystyrene NPs surface were studied, without controlling pH. It was found that there are three driving forces, hydrophobic force, π-π stacking force, and electrostatic force\(^4\). However, there is still an open question about which driving force dominates the binding process and which interaction contributes less to the binding. What if the surrounding environment changes, like pH, will the binding of amino acids change or stay the same?

To address this problem, we studied the binding process under three different pH conditions. Also, we investigated zwitterionic polystyrene nanoparticles, whose surface charge would change sign based on different pH. Through this system, we look more deeply at how pH changes the binding process of amino acids (Figure 1) and further sheds light on the contribution of each binding force based on the pH result. Knowing the relative intensity of different driving forces can facilitate predicting amino acids interaction with polystyrene NPs, and even further, more accurately predict the structure of peptides and proteins on the surface of nanoparticles.

**Figure 1.** The maximum initial slope of the STD buildup curve of amino acids that show significant STD effects at high, neutral and low pH. The initial slope = \(S_{\text{max}} \times K\), which come from the fitting equation \(S(t) = S_{\text{max}} (1 - e^{-kt})\). The error bar on each column is the propagated error of the fitting error of \(S_{\text{max}}\) and \(K\).

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THE EFFECT OF pH, CONCENTRATION, AND SOLVENT ON 5-FLUOROURACIL NMR CHEMICAL SHIFTS

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5-Fluorouracil is an anticancer metabolite, used as a model compound due to its small size and presence of nuclei. In this work, we looked at the effects of concentration, pH, and solvent on ¹H, ¹³C, and ¹⁹F chemical shifts of 5-fluorouracil and how this data compares to theoretical calculations.

Samples were prepared by dissolving the correct amount of 5-Fluorouracil in either DMSO or 100mM sodium phosphate buffer solution to make a 40mM sample. The samples were diluted to the appropriate concentration and the pH of each sample was obtained. The pH and concentration, in each solvent type, were altered to observe chemical shift effects. We found that concentration had no chemical shift effect for buffered D₂O solvent regardless of the nucleus type. A small increase was observed, for ¹H and ¹⁹F nuclei, when the concentration was increased in DMSO solvent. The largest change in chemical shift occurred when the pH of the sample was changed. Increasing the pH caused an increase in chemical shift for ¹³C and ¹⁹F nuclei and a decrease for ¹H nuclei. All of the curves resembled a titration curve with the steepest region of the graph close to the pKa of 5-fluorouracil. The largest shift occurred in ¹³C nuclei as seen in Figure 1.

We were able to model the increase of ¹³C NMR chemical shifts using Density Functional Theory as implemented in the Gaussian Program. Theoretical NMR chemical shifts were calculated with and without deprotonation. The same increases in chemical shift was observed in the experimental and theoretical data as seen in Figure 2.

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**Figure 1.** The effects of pH on ¹³C Chemical shifts in buffered D₂O solvent.

**Figure 2.** Theoretical NMR shifts for ¹³C NMR. B3LYP density functional theory and 6-31G* basis set for geometry optimizations and 6-311G for NMR Calculations.

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**Coordination geometry of low spin Fe(III) in the active site of *Azotobacter vinellandi* 3-mercaptopropionate dioxygenase**

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Thiol Dioxygenases are non-heme iron enzymes that catalyze the $O_2$-depentent oxidation of thiol-bearing amino acid derivatives to their corresponding sulfinic acid. The active site of this enzyme class is comprised of a mononuclear Fe(II) ion coordinated by 3 protein derived histidine residues along a single octahedral face (3-His facial triad). Considerable work has been focused on this class of enzymes recently as sulfur metabolite imbalances have been correlated with neurodegenerative disorders such as Autism, Alzheimer’s, and Parkinson’s disease. Unlike the mammalian cysteine dioxygenase (CDO), which is highly specific for its L-cysteine substrate, bacterial 3-mercaptopropionate dioxygenase (MDO) is promiscuous in that it oxidizes a variety of thiol substrates. In this work, we use CW X-band EPR spectroscopy to probe the nature of substrate coordination among this class of enzymes. In these studies, enzymes, and selected active site variants, were oxidized to their catalytically inert Fe(III) state. Cyanide (CN) was used as a spectroscopic probe to analytically titrate the available coordination sites within the active site. The strong $\beta$-accepting character of cyanide was exploited to produce a low-spin ($S = 1/2$) (cyano/substrate)-bound enzyme. In principle, the resulting ternary complex would have a similar formal charge and size to the proposed FeIII-superoxide intermediate in the CDO catalytic cycle. Moreover, the Fe-bound cyano-ligand serves as a spectroscopic probe to observe hydrogen-bonding interactions as well as geometric perturbations arising from the Ser-His-Tyr amino acids adjacent to the outer Fe-coordination sphere (~3 Å). Collectively, these residues are referred to as the ‘catalytic triad’. These studies demonstrate that the specificity of these enzymes is largely controlled by substrate coordination denticity (mono- versus bidentate) at the Fe-site. It was also observed that the coordination number at the active site is regulated through hydrogen bonding with the conserved sequence of outer sphere Ser-His-Tyr residues.
59-Kong Wong, GT

STRUCTURAL HETEROGENEITY IN CO-ASSEMBLED β-SHEET PEPTIDE NANOFIBERS

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Co-assembling peptides are a growing frontier in the design of peptide-based supramolecular materials for biotechnological applications. Compared to traditional functional biomaterials, co-assembled peptides produce scaffolds with a well-defined site for functionalization, control over concentration and density of biomolecules, and facile co-immobilization. These properties make peptide co-assembly well-suited for biocatalysis, tissue engineering, and immunotherapy applications.1 Existing co-assembling β-sheet designs utilize charge-complementary peptides that individually resist self-assembly but upon mixing, produce co-assembled peptide nanofibers. It is hypothesized that these charge-complementary systems form monomorphic assemblies with ideal alternation along the β-sheets. However, solid-state NMR measurements combined with discontinuous molecular dynamics (DMD) simulations reveal significant structural heterogeneity in two established co-assembling systems, King-Webb and CATCH peptides.2,3 In agreement with previous Fourier transformed infrared (FTIR) spectroscopy measurements, antiparallel β-sheet structures are observed by REDOR measurements and computational simulations.2,3 Surprisingly, however, a significant proportion of β-strands are aligned with parallel nearest neighbors as assessed by PITHIRDS-CT results. DMD simulations also identify β-sheets with registry shifts, wherein the alignment of β-strands do not maximize hydrogen bonds. Ideal alternation of the two complementary peptide components within β-sheets would produce weak dipolar couplings in samples where only one peptide component is isotopically enriched. In contrast, attenuated signal decays in PITHIRDS-CT measurements suggest a minor population of strongly coupled spins consistent with peptide self-association (AA or BB pairings). Finally, these different β-sheet structures are seen in computational simulations to coexist within the same nanofiber indicating a lack of preference. Structural heterogeneity in current co-assembling β-sheet peptide pairs may be inherent to charge-complementary designs and highlight the complexity of interactions in these systems.

Tuberculosis (TB) is a leading infectious disease killer worldwide caused by Mycobacterium tuberculosis (Mtb). CwsA and CrgA are both small integral membrane proteins that belong to Mtb cell divisome. Previous studies by Plocinski et al. showed that the interaction between CwsA and CrgA resulted in the elongation at the poles and division at midcell. However, the structural details on the atomic level of this interaction in a lipid bilayer environment are not clear. Here, we strive to understand this interaction through both oriented (OS) and magic angle spinning (MAS) solid-state NMR (ssNMR) approaches. We first determined the tilt of a carefully designed CwsA fragment in a POPC/POPG lipid bilayer to be ~20° with glass slides supported OS ssNMR. His-tag pull-down assay was utilized to confirm the interaction happens within transmembrane helices. The DARR pulse sequence was then applied to probe the interacting interface with isotope specific labeled CwsA and CrgA. Given the determined tilt and rotation angle of CwsA in a lipid bilayer, along with published transmembrane helices structure of CrgA, an interacting model was proposed. The structural characterization of CwsA and CrgA interaction will help to understand the mechanism of Mtb cell division machinery.
Carbohydrates, which include polysaccharides, monosaccharide, and their derivatives, play key roles in many life activities such as structural building, energy storage, and cell recognition. Solid-state NMR (ssNMR) spectroscopy, as a new emerging method and with readily improving resolution, has the distinctive capability to reveal structural polymorphism of insoluble complex carbohydrates, involving identity and configuration of the subunits, the eventual branching pattern on the main chain and substitution by methyl or acetyl groups, the position of the glycosidic linkages and their anomeric configuration, and more subtly, variations in the torsional conformations. Here, we developed Complex Carbohydrates Magnetic Resonance Database (CCMRD), aiming to promote future ssNMR database and benefit the carbohydrate-research community. The beta version of CCMRD is released on March 27, 2019, which web address is: www.ccmrd.org. Currently, the CCMRD holds around four hundred compounds from fungi, plants to algae, and it supports searching by atom, chemical shifts and residue names. The current version supports user deposition, expecting rapid expansion. New portals and functions are being coded and implemented, and accessory tools that facilitate structural analysis of carbohydrates and NMR spectra are also under development as follow-up projects.

Figure 1. Database structure and example of a search result.

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BIMETALLIC NANOPARTICLES TAILORED FOR PARAHYDROGEN ENHANCED NMR BY HETEROGENEOUS CATALYSIS

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Parahydrogen enhanced NMR, or PHIP, can achieve nuclear spin hyperpolarization of order unity, thus significantly boosting the sensitivity of NMR and MRI. The overpopulated singlet spin state in para-enriched hydrogen (p-H₂) is converted into NMR-observable Zeeman order via symmetry-breaking pairwise hydrogenation. PHIP by heterogeneous catalysis over supported metal nanoparticles has drawn great interest in the past decade owing to the easy separation of the solid catalyst from the hydrogenation adduct, enabling to the fast and continuous generation of contaminant-free hyperpolarized metabolites that is essential for disease detection and monitoring. However, due to rapid diffusion of H atoms and stepwise addition to the alkene on the metal surface, the pairwise selectivity and the resulting signal enhancements achieved by het-PHIP over monometallic nanoparticle catalysts have been modest. Recent work in our group has demonstrated that the pairwise selectivity of addition is significantly increased in certain binary intermetallics composed of an active and an inert metal. The inert metal can increase the energy barriers for both H₂ dissociation and H ad-atom diffusion on metal surfaces, thereby prolonging the lifetime of the singlet. PtSn@mSiO₂ bimetallic catalysts achieved 10.9 % pairwise selectivity, a 5000-fold increase relative to the 0.002% pairwise selectivity achieved by monometallic Pt@mSiO₂, as shown in Figure 1.¹ To further increase the pairwise selectivity, while retaining high yield, we are pursuing the tailored design of new bimetallic nanoparticles with higher ratios of inert metal and we are also exploring a wide range of bimetallic compositions. Some remarkable new results will be presented.

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SEMRC History – Can you fill in the blanks in our history from 1981-2001? Prizes for filling in the blanks!

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- 1982
- 1983
- 1984: Duke University (22)
- 1992: North Carolina State University (23)
- 1993:
- 1995: Florida State University and the National High Magnetic Field Laboratory (26)
- 1996:
- 1997: University of Florida (28)
- 1998:
- 1999: Florida State University and the National High Magnetic Field Laboratory (30)
- 2000:
- 2001: University of Florida (32)
- 2002: North Carolina State University (33)
- 2003: Florida State University and the National High Magnetic Field Laboratory (34)
- 2005: Emory University (35)
- 2006: University of Florida (36)
- 2007: University of Alabama (37)
- 2008: Florida State University and the National High Magnetic Field Laboratory (38)
- 2009: Vanderbilt University (39)
- 2010: University of Florida (40)
- 2011: Georgia State University (40)
- 2012: North Carolina State University (41)
- 2013: Florida State University and the National High Magnetic Field Laboratory (42)
- 2014: University of Alabama (43)
- 2015: University of Florida (44)
- 2016: Emory University (45)
- 2017: Florida State University and the National High Magnetic Field Laboratory (46)
- 2018: Clemson University (47)
- 2019: University of Florida (48)

We look forward to seeing you next year at a place to be determined (it could be yours!). Then we’ll see you in Tallahassee in two years, and back in Gainesville in four!