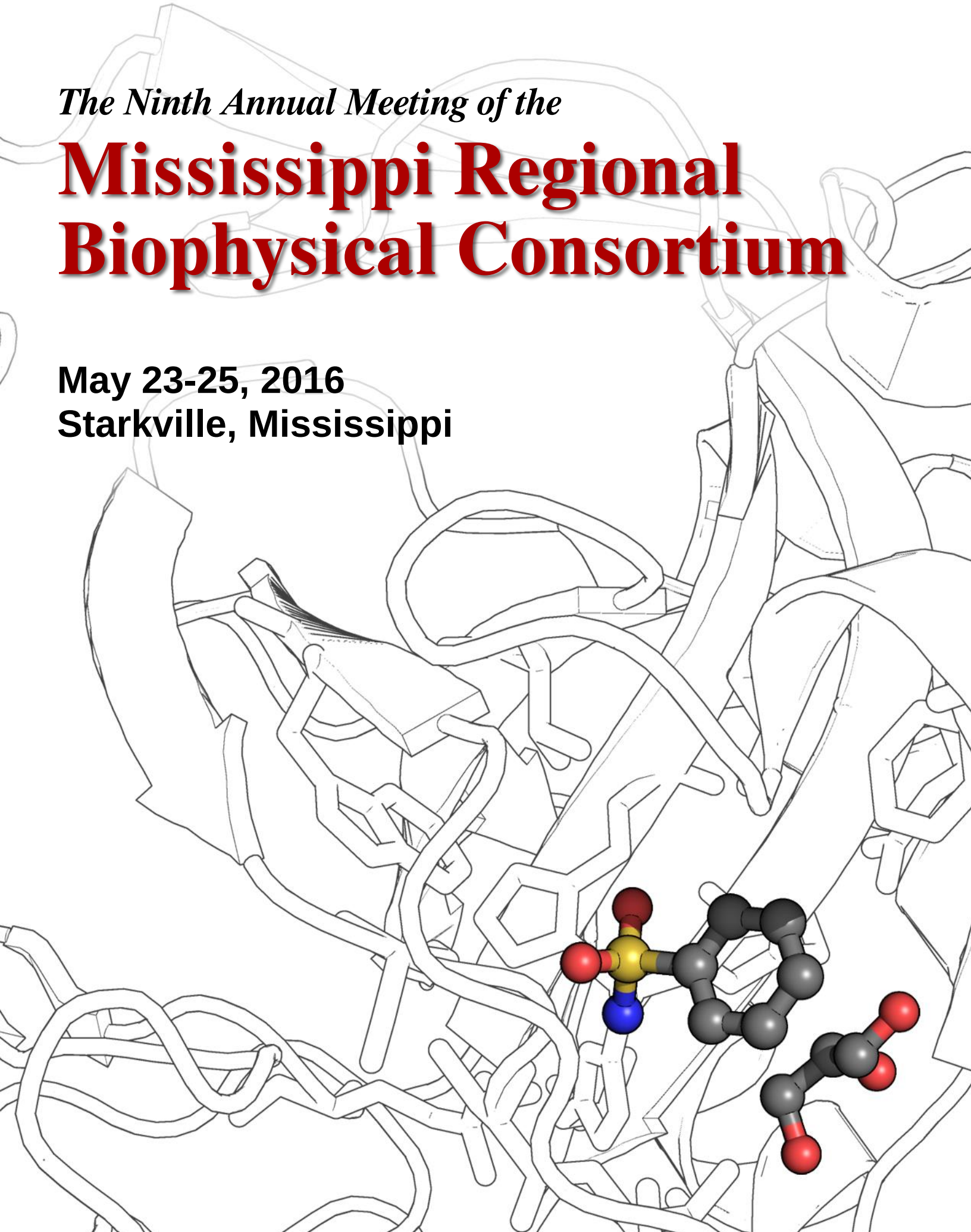
The Ninth Annual Meeting of the

Mississippi Regional Biophysical Consortium

May 23-25, 2016
Starkville, Mississippi



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Mississippi State University
Starkville, Mississippi

Abstract Book and Program

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Mission of the Mississippi Regional Biophysical Consortium

The mission of the Mississippi Regional Biophysical Consortium is to promote biophysics and biophysical research in Mississippi and the surrounding states. Founded in 2008, our goal has been to pool resources and promote collaborations in our region. Our activities include: advertisement and sharing of biophysical instrumentation, promoting topical seminars, organizing workshops and meetings, and facilitating collaborations and funding applications.

Sponsor Listing

The following organizations and individuals have generously donated toward supporting this year's meeting. Their donations have been used to support student housing and registration, and we encourage you to support them in return.

We also thank the MSU Department of Chemistry and the MSU College of Arts and Sciences for their generous administrative assistance. This conference could not have happened without their expertise and support.

General Information

Conference Location and Directions

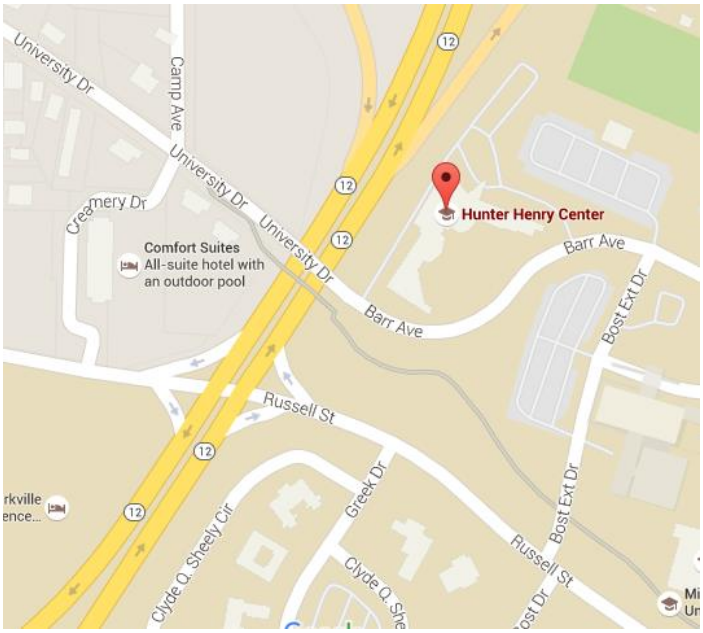
This year's conference will be held at the Hunter Henry Center on the campus of Mississippi State University. The center boasts over 40,000 square feet of offices and meeting spaces, along with dedicated parking convenient to Highway 12. Conference registration will be held in the lobby, with a dedicated meeting room for the Biophysical Consortium talks. The concurrent Lester Andrews Graduate Research Symposium will be held in the same building, allowing participants to choose talks that are most suited to their interests. Several sessions will be shared between the meetings.

Addresses to the conference center and conference hotels are given below.

Hunter Henry Center
100 Hunter Henry Blvd.
Mississippi State, Mississippi 39762
(662) 325-7000

Comfort Suites
801 Russell St
Starkville, MS 39759
(662) 324-9595

Hilton Garden Inn
975 Highway 12 East
Starkville, MS 39759
(662) 615-9664



Conference Hotels

Two conference hotels are available for the MRBC meeting. The Comfort Suites is located within a 10 minute walk from the conference venue (see map above) and is convenient to several Starkville restaurants and the downtown area. The Hilton Garden Inn is a short (5 minute) drive away from the conference venue, and is conveniently located along Highway 12 just outside of town. Both hotels include free wifi and other amenities. The Comfort Suites offers a complimentary breakfast.

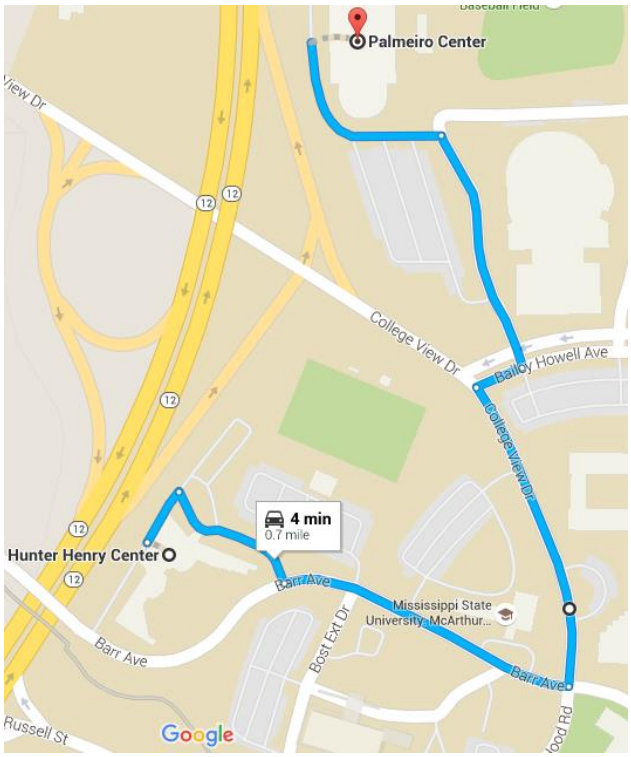
Parking Information

Parking at all conference hotels is free, as is parking for guests at the Hunter Henry Center. To ensure that you do not get a ticket, you should park in the lot adjacent to Hunter Henry center. Other campus lots do not permit visitor parking without a permit, and you will be ticketed if you park there.

Conference Internet Access

Guests wishing to use the Internet during the conference are invited to join the "msuguest" wireless network. You will need to obtain the daily wireless guest password, which will be available at the registration table.

Monday's Banquet and Poster Session



Monday night's combined banquet and poster session will be held on the MSU campus at the Palmeiro Center. Parking is available at the Palmeiro Center, which is located off of Bailey Howell Ave. From the Hunter Henry Center, turn left on to Barr Avenue, and then take the next left on to College View Dr. Bailey Howell Ave is your next right, and the driveway for the Palmeiro center is the first immediate left. Poster boards will be provided at the Palmeiro center.

Monday Night Social

A joint social will be held on Monday night at Rosey Baby, a local bar and eatery. All participants are encouraged to attend. The address is *300 S. Jackson St., Starkville, MS 39759.* Directions will vary depending on where you are parked, although there is plenty of parking and there may be carpools from the Palmeiro center as well.

Tuesday's Banquet

Tuesday's banquet will be held off campus at the Hilton Garden Inn. To get there, get on to Highway 12 heading north. In the map above, note that Barr Avenue does not allow direct access to Highway 12; use Russell Street or College View Drive instead. After merging on to Highway 12, the Hilton Garden Inn will be on your left after approximately 1.5 miles.

Recording and Photography of Sessions and Posters

Collaboration and collegial discussion of unpublished data is encouraged at the MRBC meeting, but photography and recording of conference sessions is strictly prohibited. Attendees found to be recording sessions or photographing posters without the presenter's consent will be asked to stop, and violators may be asked to leave at the discretion of the MRBC board.

Conference Web Page and Additional Information

Additional information can be found at the conference web page:

http://folding.chemistry.msstate.edu/mrbc9/

For other questions, please contact Dr. Nicholas Fitzkee, the conference organizer at nfitzkee@REMOVE-MEchemistry.msstate.edu.

Program At A Glance

	Monday, May 23
10:00 AM	Registration Opens (Hunter Henry Center)
	Lunch (On Your Own)
12:45 PM	**Welcome and Opening Remarks** *Dr. Nicholas Fitzkee, MSU Department of Chemistry* *Dr. Giselle Thibaudeau Munn, Associate Dean for Research, College of Arts and Sciences, MSU*
1:00 PM	**Lester Andrews Keynote Address: "Beyond Li-Ion: The Lithium/Sulfur Cell"** *Dr. Elton Carnes, UC Berkeley and Lawrence Berkeley National Laboratory*
2:00 PM	Break
2:15 PM	**Session 1: Covalent Modification of Biomolecules**
3:35 PM	Break
3:45 PM	**Session 2: Energetics**
5:00 PM	Break (Depart for Posters and Dinner at Palmeiro Center)
5:15 PM	Poster Set-Up at Palmeiro Center
5:30 PM	**Poster Session**
7:00 PM	Combined Lester Andrews and MRBC Dinner at Palmeiro Center
8:30 PM	Joint Social at Rosey Baby Bar *300 S. Jackson St., Starkville, MS 39759*
	Tuesday, May 24 (Morning)
7:30 AM	Breakfast in your Hotel
9:00 AM	**Joint Lester Andrews and MRBC Session**
10:15 AM	Break
10:30 AM	**Session 4: Disordered Systems**
11:50 AM	Break
	Lunch (On Your Own)

Tuesday, May 24 (Afternoon)	
1:15 PM	**Session 5: NMR Structure and Dynamics**
2:25 PM	Break
2:45 PM	**Session 6: Nano-Scale Biophysics**
4:00 PM	Break
4:15 PM	**MRBC Keynote: "The coordinated action of RPA and DNA primase at the replication fork"** *Dr. Walter Chazin, Vanderbilt University*
5:15 PM	Break
5:45 PM	Dinner at Hilton Garden Inn *975 Highway 12 East, Starkville, MS 39759*

Wednesday, May 25	
7:30 AM	Breakfast in your Hotel
9:00 AM	**Session 7: Biophysics of Catalysis & Function I**
10:15 AM	Break
10:30 AM	**Session 8: Biophysics of Catalysis & Function II**
11:50 AM	Break
12:00 PM	**Lunch Presentation with NanoTemper Technologies**
1:00 PM	MRBC 10 Planning Meeting
1:30 PM	Depart

Detailed Program

Monday, May 23

10:00 AM Registration Opens (Hunter Henry Center)

Lunch (On Your Own)

12:45 PM **Welcome and Opening Remarks**
Dr. Nicholas Fitzkee, MSU Department of Chemistry
Dr. Giselle Thibaudeau Munn, Associate Dean for Research, College of Arts and Sciences, MSU

2016 Lester Andrews Graduate Research Symposium Keynote Address

1:00 PM **Beyond Li-Ion: The Lithium/Sulfur Cell**
Dr. Elton Carnes, UC Berkeley and Lawrence Berkeley National Laboratory

2:00 PM Break

Session 1: Covalent Modification of Biomolecules

Moderator: Kayla McConnell, Mississippi State University

2:15 PM Glycan Structural Changes to Notch1 Epidermal Growth Factor-like Repeats
Megan Macnaughtan, Louisiana State University

2:45 PM Global Profiling of Lysine Acetylome in Developing Rice (*Oryza sativa*) Seeds
Xiaoxi Meng (Peng Lab), Mississippi State University

3:05 PM Preliminary characterization of model erythromycin resistance methyltransferases
Jack Dunkle, University of Alabama

3:35 PM Break

Session 2: Energetics

Moderator: Valeria Zai-Rose, University of Mississippi Medical Center

3:45 PM Quantification and Interpretation of Weak Preferential Interactions between Folate and Betaine
Purva P. Bhojane (Howell Lab), University of Tennessee Knoxville

4:05 PM Mechanisms of Enzyme Catalyzed Protein Unfolding and Translocation by Class 1 AAA+ Motors
Aaron L. Lucius, University of Alabama at Birmingham

4:35 PM Characterization Studies of Isolated Domain 1 of Neural Cadherin
Samantha Davila (Pedigo Lab), University of Mississippi

5:00 PM Break (Depart for Posters and Dinner at Palmeiro Center)

5:15 PM Poster Set-Up at Palmeiro Center

5:30 PM **Poster Session**

7:00 PM Combined Lester Andrews and MRBC Dinner at Palmeiro Center

8:30 PM Joint Social at Rosey Baby Bar
300 S. Jackson St., Starkville, MS 39759

Tuesday, May 24 (Morning)

7:30 AM Breakfast in your Hotel

Joint Lester Andrews and MRBC Session

9:00 AM Near Infrared Spectroscopy and Chemometrics can be used to Determine Physiological Status in the Endangered Snow Leopard (*Panthera uncia*)
Kristen Counsell (Vance Lab), Mississippi State University

9:25 AM The thermodynamics of metal and substrate binding with an taurine/α-ketoglutarate-dependent oxygenase (TauD)
Mingjie Li (Emerson Lab), Mississippi State University

9:50 AM Typical and Atypical Prion-like Propagation of Neurotoxic Amyloid-β Oligomers
Dexter N. Dean (Rangachari Lab), University of Southern Mississippi

10:15 AM Break

Session 4: Disordered Systems

Moderator: Michael Molnar, University of Mississippi

10:30 AM Disorder within Cysteine-Rich Proteins: Curious Case of Granulins
Vijay Rangachari, University of Southern Mississippi

11:00 AM Optimizing doxorubicin derivative delivery using temperature sensitive biopolymers in multidrug resistant breast cancer cells
Sonja Dragojevic (Raucher Lab), University of Mississippi Medical Center

11:20 AM Developing the Elastin-like Polypeptide Biopolymer for Delivery of Growth Factor Therapeutics
Gene L. Bidwell, III, University of Mississippi Medical Center

11:50 AM Break for Lunch (On Your Own)

Session 5: NMR Structure and Dynamics

Moderator: Becca Hill, Mississippi State University

1:15 PM Computational and experimental studies of ADP-ribose binding proteins
Margaret A. Johnson, University of Alabama at Birmingham

1:45 PM Inexpensive production of Site Specific ^{1}H-^{13}C Phenylalanine and Tyrosine
Bhargavi Ramaraju (McFeeters Lab), University of Alabama at Huntsville

2:05 PM Internal motions prime cIAP1 for rapid activation
Allyn J. Schoeffler, Spring Hill College

2:25 PM Break

Session 6: Nano-Scale Biophysics

Moderator: Melody Williams, University of Memphis

2:45 PM Modulating Protein-Nanoparticle Binding Capacity Using Site-Directed Mutagenesis
Y. Randika Perera (Fitzkee Lab), Mississippi State University

3:05 PM Magnetic-Plasmonic Core-Shell Nanoparticles: Shape- Controlled Synthesis, Properties and Applications for Capture and Detection of Circulating Tumor Cells
Xiaohua Huang, University of Memphis

3:35 PM Can Surface-Enhanced Raman Scattering Identify the Drug Mechanism of Platinum-Based Anticancer Drugs?
Sidrah Khan (Mirsaleh-Kohan Lab), Texas Women's University

4:00 PM Break

2016 Mississippi Regional Biophysics Consortium Keynote Address

4:15 PM The coordinated action of RPA and DNA primase at the replication fork
Dr. Walter Chazin, Vanderbilt University

5:15 PM Break

5:45 PM Dinner at Hilton Garden Inn
975 Highway 12 East, Starkville, MS 39759

Wednesday, May 25

7:30 AM Breakfast in your Hotel

Session 7: Biophysics of Catalysis & Function I

Moderator: Clint Mikek, Mississippi State University

9:00 AM Building nonheme metal sites in biological systems
Joseph P. Emerson, Mississippi State University

9:20 AM Non-Specific Interactions of Dihydrofolate Reductase Ligands in Crowded Environments
Michael Duff (Howell Lab), University of Tennessee Knoxville

9:50 AM An Investigation into the Potential Dual Role of SirC in the Alternative Heme Biosynthetic Pathway of *Methanosarcina acetivorans* C2A
Victoria L. Owens (Mansoorabadi Lab), Auburn University

10:15 AM Break

Session 8: Biophysics of Catalysis & Function II

Moderator: Claudette Fraire, Texas Women's University

10:30 AM Using Molecular Modeling to Decipher the Molecular Targets of Bioactive Molecules.
Ifedayo Victor Ogungbe, Jackson State University

11:00 AM Biophysical Characterization of i-Motif Capped with Flanking Duplex Ends: A Model for c-MYC NHE-III$_1$ Complementary C-rich Strand
Amanda M. Metz (Lewis Lab), Mississippi State University

11:20 AM Elucidation of the Biosynthetic Pathway for the Key Coenzyme of Methanogenesis and Anaerobic Methane Oxidation
Steven O. Mansoorabadi, Auburn University

11:50 AM Break

12:00 PM **Lunch Presentation with NanoTemper Technologies**
Hunter Henry Center

1:00 PM **MRBC 10 Planning Meeting**
Anyone interested in the future of MRBC is welcome and encouraged to attend this meeting.

1:30 PM Depart

Keynote Speakers

Dr. Elton Cairns
Department of Chemical & Biomolecular Engineering, UC Berkeley
Energy Storage Group, Lawrence Berkeley National Laboratory
Lester Andrews Graduate Research Symposium Keynote Speaker

Dr. Elton Cairns is a Professor of Chemical and Biomolecular Engineering at the University of California, Berkeley, and a Faculty senior Scientist at the Lawrence Berkeley National Laboratory. After completing his Ph.D in Chemical Engineering at UC Berkeley, Dr. Cairns moved on to work for General Electric, Argonne National Laboratory, General Motors, and finally back to UC Berkeley and the Lawrence Berkeley National Laboratory. Dr. Cairns' research includes electrochemical energy conversion, electrocatalysis, electrodes, X-ray absorption spectroscopies (XAS), synchrotron radiation, nuclear magnetic resonance spectroscopy (NMR), fuel cells, batteries, and chemical engineering electrochemistry. Dr. Cairns is also active in many outside professional activities such as editing scientific journals, organizing scientific symposia, and serving on professional committees. Has served as both the President of the Electrochemical Society and President of the International Society of Electrochemistry.

Dr. Cairns' selection as the Lester Andrews Keynote was determined by the graduate students in the MSU Department of Chemistry.

Dr. Walter Chazin
Departments of Biochemistry and Chemistry, and Center for Structural Biology
Vanderbilt University
Mississippi Regional Biophysical Consortium Keynote Speaker

Dr. Walter Chazin is the Chancellor's Professor of Biochemistry and Chemistry and the Ingram Professor of Cancer Research and Vanderbilt University. He also directs the Center for Integrated Structural Biology and the Molecular Biophysics Training program at Vanderbilt. After completing his Ph.D in Chemistry at Concordia University, he performed postdoctoral research with Dr. Kurt Wütrich at E.T.H. in Switzerland and Dr. Peter E. Wright at the Scripps Research Institute. Dr. Chazin has been on faculty at Vanderbilt since 1999. Dr. Chazin has made significant contributions to the field of biomolecular NMR, where he has focused on proteins involved in calcium signaling, including calmodulin. More recently, he has been investigating the mechanism of DNA replication and single strand DNA priming. Dr. Chazin is a fellow of the American Association for the Advancement of Science, and in 2016 he was elected as a fellow of the Biophysical Society.

Keynote Session Abstracts

Lester Andrews Graduate Research Symposium Keynote

Beyond Li-Ion: The Lithium/Sulfur Cell

Elton J. Cairns
University of California, Berkeley, and Lawrence Berkeley National Laboratory

The lithium/sulfur cell is widely regarded as the next-generation high specific energy rechargeable cell. This is true because of its very high theoretical specific energy (~2600 Wh of energy stored per kg of reactants) compared to that of the lithium ion cell (~600 Wh/kg). In spite of the very attractive specific energy, the development of the Li/S cell has been slow and difficult due to the facts that sulfur and its reaction product Li_2S are both electronically non-conductive, sulfur is dissolved as polysulfides into most organic solvent based electrolytes (resulting in capacity loss), and the soluble polysulfides can react chemically at the lithium electrode. During the last few years, we have been successful in ameliorating the above problems, resulting in laboratory coin cells with very high sulfur utilization (~1400 mAh/gS) and very long cycle lives (1500 deep cycles) for two different versions of sulfur electrode formulations. We are now in the process of developing electrode structures and formulations that will be useful in commercial Li/S cells. Our recent advances will be reviewed and discussed. If the commercialization of our technology is successful, it will be feasible to have electric automobiles with a range of at least 300 miles per charge.

Mississippi Regional Biophysical Consortium Keynote

The coordinated action of RPA and DNA primase at the replication fork

Walter J. Chazin
Departments of Biochemistry and Chemistry, and Center for Structural Biology, Vanderbilt University, Nashville, TN

Faithful copying of our genome requires the coordinated action of multi-domain proteins operating within dynamic multi-protein machines that operate at the replication fork. Replication Protein A (RPA) is the primary ssDNA-binding protein in eukaryotes. Beyond protecting and organizing the ssDNA, it serves as an essential scaffold in virtually all DNA transactions. During replication, RPA and DNA primase together play a central role in the transition from unwinding the duplex to initiating synthesis on both the leading and lagging strand templates. In this presentation, I will introduce the unique characteristics of multi-domain proteins and multi-protein machinery and new perspectives on structural biology that are required for such complex dynamic systems. The concept of remodeling of protein architecture will be defined. I will then describe how X-ray crystallography, NMR, X-ray scattering and computation modeling have been used to build a picture of how RPA and primase function in the transition from duplex unwinding to template priming. I will also present provocative new data suggesting that DNA charge transport driven by the 4Fe-S cluster of primase can be used as a molecular switching mechanism to control primer length counting and hand off to DNA polymerase α for primer extension.

Oral Presentation Abstracts

Session 1: Covalent Modification of Biomolecules

Glycan Structural Changes to Notch1 Epidermal Growth Factor-like Repeats

Huimin Zhong[1], Thomas Weldeghighoris[2], Megan A. Macnaughtan[1]
[1]Department of Chemistry, Louisiana State University
[2]NMR Facility, Louisiana State University

Epidermal growth factor-like (EGF) repeats are small protein domains (~ 40 amino acids) held together by three disulfide bonds. The repeats are often found in tandem and in long chains. The Notch1 protein is a notable example: its extracellular domain includes 36 EGF repeats, which mediate cell signaling through protein-protein interactions with other EGF repeat-containing proteins. EGF repeats can have a variety of characteristics that affect structure and function, including calcium-binding sites, hydrophobic packing, and O-glycosylation sites. The affinity for calcium, presence of hydrophobic residues at key amino acid positions, and the presence of glycans are variable among EGF repeats, but the pattern of these characteristics is highly conserved, suggestive of a code. While calcium-binding and hydrophobic residues at the interface of tandem EGF repeats have been shown to produce a rigid rod-like structure, less is known about the structural effects of O-glycosylation and elongated glycans. For Notch1 EGF27, the loss of the highly conserved O-fucose site causes defects in Notch1 trafficking and processing. Using NMR spectroscopy, we have observed a large change in the backbone structure of EGF27 upon glycosylation with the disaccharide, GlcNAc-β1,4-Fuc-O. We are currently performing NMR peak assignment and computational method to investigate the mechanism of this structural change and predict similar effects for other EGF repeats.

Global Profiling of Lysine Acetylome in Developing Rice (*Oryza sativa*) Seeds

Xiaoxi Meng[1], Yuanda Lv[1, 2], Mariola J. Edelmann[3], Han Zhao[2], Zhaohua Peng[1*]
[1]*Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, USA*
[2]*Provincial Key Laboratory of Agrobiology, Institute of Biotechnology, Jiangsu Academy of Agricultural Sciences, China*
[3]*Department of Microbiology and Cell Science, University of Florida, USA*

Protein Lysine acetylation is a highly conserved post-translational modification with various biological functions. However, only limited acetylation sites have been reported in plants, especially in cereals, and the function of non-histone protein acetylation is still largely unknown. In this report, we found that developing rice seed is an organ with intensive protein lysine acetylation. We identified 1003 lysine acetylation sites in 692 proteins of developing rice seeds, which greatly extended the number of known acetylation sites in plant. Eight distinguished acetylation motifs were found in the identified acetylation sites after motif analysis. While five of the motifs are shared between eukaryotes and prokaryotes, at least one is first reported in this study. Biological process analysis showed that 437 of the 692 proteins are related to metabolism. Further pathway-based enrichment analysis showed that carbon metabolism, starch and sucrose metabolism, citrate cycle, glycolysis/gluconeogenesis, biosynthesis of amino acids, ribosome components, oxidative phosphorylation, proteasome components, metabolic pathways, and protein processing in ER are significantly enriched with lysine acetylation. Interestingly, the seed storage proteins were heavily acetylated as well. Our results suggest that lysine acetylation is involved in seed storage nutrient production processes via modifying the key metabolic enzymes and the storage proteins themselves.

Preliminary characterization of model erythromycin resistance methyltransferases

Joshua Halliday, Alyshah Lakhani, Andrew Beckman, Malcolm Luttrull, Sawyer Foyle and Jack A. Dunkle
Department of Chemistry, University of Alabama, Tuscaloosa, Alabama 35487

Modification of bacterial ribosomes by the erythromycin resistance methyltransferase (Erm) family of enzymes is a widespread mechanism used by bacteria to achieve multi-drug resistance to antibiotics, such as erythromycin and clindamycin, that exert their effect by binding to the bacterial ribosome and interfering with protein synthesis. Methylation of a specific adenosine residue adjacent to the ribosome's peptidyl transferase center sterically occludes antibiotic binding. Erm induced antibiotic resistance is found in both in soil dwelling bacteria that biosynthesize antibiotics (antibiotic producers) that are not a threat to humans and pathogenic bacteria that do pose a risk to humans. We are mechanistically characterizing two evolutionarily distant Erm family members, ErmC present in 'pathogens' and ErmE present in 'producers'. We are using biophysical approaches to understand how Erm enzymes accomplish substrate recognition and positioning of the target nucleotide within the enzyme active site.

Session 2: Energetics

Quantification and Interpretation of Weak Preferential Interactions between Folate and Betaine

Purva P. Bhojane, Michael R. Duff, Jr., Gabriella Rimmer, and Elizabeth E. Howell
Department of Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville

In vitro studies with two different dihydrofolate reductases (EcDHFR, *E.coli* chromosomal and R67 DHFR, plasmid encoded) have shown that weak interactions between osmolytes and the substrate, dihydrofolate (DHF), decreases the affinity of DHF towards these enzymes. The unique changes in binding affinity with water activity for each osmolyte indicate preferential interactions between osmolyte and folate and its derivatives. Characterization of these interactions is essential for better understanding of *in vivo* effects of folate and its various redox states with available functional groups inside the cell. Quantitation of weak interactions using a vapor pressure osmometry method yields a preferential interaction coefficient, or μ_{23}/RT value. This provides a scale for measuring the preference of folate for betaine relative to water. Experimental measurements found a folate concentration dependence of the μ_{23}/RT values, consistent with dimerization of folate. Our results also indicate neutral folate preferentially interacts with betaine whereas the anionic form excludes betaine. Studies with other model compounds suggest aromatic rings prefer to interact with betaine as compared to water. The preferential interaction coefficients or μ_{23}/RT values obtained for additional nitrogen containing aromatic compounds were dissected into additive contributions from chemically distinct functional groups. The atomistic interaction potentials for each of the surface types (alpha values) were calculated. The calculated set of values coupled with the water-accessible surface areas (ASA) can be used to predict the μ_{23}/RT of any compound with betaine. Additionally, solubility assays were done to quantify the free energy of transfer of folate from water to 1 M betaine solution. Data indicate favorable interactions between betaine and folate at a lower pH with a negative free energy of transfer whereas at higher pH, the free energy of transfer is positive. These results are consistent with our μ_{23}/RT dependence of pH.

Can μ_{23}/RT values be used to predict osmotic stress effects on ligand binding? In some cases, yes. However, the caveat is whether all the ligand atoms are used in binding. As glutamate excludes betaine, calculation of the μ_{23}/RT value for polyglutamylated folates (pteroyltetra-γ-glutamate (PG4)) predicts an overall exclusion of betaine. This should translate into tighter binding of PG4 to DHFR. Our studies found betaine addition weakens binding of both folate and PG4 to R67 DHFR to similar extents. This result indicates the polyglutamylated tail does not contact the enzyme.

Mechanisms of Enzyme Catalyzed Protein Unfolding and Translocation by Class 1 AAA+ Motors

Aaron L. Lucius
Department of Chemistry, The University of Alabama at Birmingham

AAA+ molecular motors involved in protein quality control are at the heart of many biological functions. Here I will discuss our efforts on two model AAA+ driven motors, prokaryotic ClpA and ClpB. ClpA is a hexameric ring motor that uses the energy from ATP binding/hydrolysis to processively translocate a polypeptide substrate. The enzyme does this for protein remodeling functions and ATP dependent proteolysis. ClpA associates with the tetradecameric serine protease, ClpP, to form the ATP dependent protease ClpAP, which is architecturally identical to the 26 S proteasome in humans. ClpB, on the other hand, has the unique ability to disrupt protein aggregates in vivo. Due to the structural similarity between ClpA and ClpB it has long been proposed that ClpB processively translocates a polypeptide through the axial channel of its hexameric ring structure; as does ClpA. However, this has been difficult to show because ClpB does not covalently modify the substrate on which it translocates. We have developed a single-turnover stopped-flow polypeptide translocation strategy that has allowed us to examine processive translocation catalyzed by ClpA in the presence or absence of proteolytic degradation catalyzed by the protease ClpP. Using this approach, we have now shown that ClpB takes at most two translocation steps before rapid dissociation from the polypeptide substrate. Thus, we have shown that ClpB is a non-processive translocase and is not likely to fully translocate a polypeptide through its axial channel during protein disaggregation. The implications of these findings will be discussed.

Characterization Studies of Isolated Domain 1 of Neural Cadherin

Samantha Davila and Susan Pedigo
Department of Chemistry and Biochemistry, University of Mississippi

The dimerization of the transmembrane protein, cadherin, leads to cell-cell adhesion in multicellular organisms and is essential for critical processes including synaptogenesis and synapse maintenance. Classical cadherins have a common arrangement of five extracellular domains, each connected by a 7-residue linker region. Cadherins mediate adhesion via Adherens Junctions by forming a strand-swapped structure between identical protomers from apposing cells. Upon calcium binding to the linker region, strand-crossover dimer forms between the first N-terminal extracellular domains (EC1) of the adhesive partners by swapping their βA-sheets and docking tryptophan-2 in the opposing hydrophobic pockets. The adhesive interface has been characterized structurally, and biophysical studies have attempted to explain the forces that stabilize the strand-swapped dimer. Fundamental questions remain regarding the striking difference in the calcium-dependent kinetics of dimerization between Neural-cadherin (NCAD) and Epithelial-cadherin (ECAD). We hypothesize that differences in the kinetics of dimerization may be due to a difference in the intrinsic stability of the first domains. The scope of this work addresses the characterization studies of EC1 stability. Notably, the binding pocket for calcium is not complete in the isolated domain constructs, therefore dimerization was not expected. However, we observed a kinetically trapped dimeric form of NCAD1. This NCAD1 dimer was reversibly converted to a monomeric form upon heating the protein to 50°C. Thermal denaturation studies of NCAD1 indicated that the isolated domain is very stable with a Tm of 75°C, and a ΔH_m of 130 kcal/mol. Additionally, significant hysteresis in the refolding transition for NCAD1 that persisted upon reheating, and a difference in susceptibility to proteolysis indicates that the formation of the stable NCAD1 structure is pathway dependent. Estimates of global stability for both thermal and chemical induced denaturation studies varied considerably between the isolated domain 1 construct. Overall, ECAD1 studies have indicated it has significantly lower stability compared to NCAD1, and ongoing studies aim to elucidate impact of findings.

Lester Andrews and Biophysical Joint Session

Near Infrared Spectroscopy and Chemometrics can be used to Determine Physiological Status in the Endangered Snow Leopard (*Panthera uncia*)

Kristen Counsell[1], Beth Roberts[2], Brandie Balkenbusch[2], Andrew Kouba[3], Scott Willard[1], Carrie Vance[1]

[1]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University

[2]Research and Conservation, Memphis Zoo, Memphis, TN.

[3]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University

Aquaphotomics is the study of changes in patterns of water H-bonding structure with near infrared spectroscopy (NIRS). Water band coordinates in the first overtone of the O-H stretching band (1300-1600nm) act as solute biomarkers; specific wavelength shifts correspond to the solute's physical properties and concentration. We used aquaphotomics to evaluate steroid and non-steroid reproductive hormones in urine of snow leopards to detect pregnancy and parturition. This study measures NIR efficacy in distinguishing and quantifying the hormones estradiol (E_1G), progesterone (P_4) and prostaglandin (PGFM) as a rapid and non-destructive assay for estrus, pregnancy and parturition, respectively. Triplicate sampling of standards and snow leopard urine (37^{o}C) had NIR spectra (1nm resolution) collected using a quartz ℓ=0.1 cm cuvette and ADS-FieldSpec®3 fiber-optic system. Chemometric analysis (Grams AI/IQ 9.1) using mean centering and 2nd derivative GAP spectral pre-processing revealed unique water spectral patterns corresponding to each hormone. NIR water coordinates were determined for E_1G (1380nm, 1414nm, 1448nm, 1492nm), P_4 (1418nm, 1440nm, 1478nm) and PGFM (1442nm, 1450nm, 1510nm) standards. Quantitative calibration of NIR water signatures reflecting E_1G (0-4000 pg/ml), P_4 (0-3 pg/ml) and PGFM (100-1600pg/ml) levels were R^2=0.92, 0.99 and 0.97, respectively. In the samples of urine from a pregnant snow leopard, spectral shifts of the water O-H stretch occurred over the course of three days as P_4 values dropped to baseline, and concurrently, PGFM levels increased from 708 pg/ml to 1625 pg/ml, signaling parturition. These spectral shifts demonstrate NIR has the ability to detect steroid and non-steroid hormones in urine for reproductive analysis.

The thermodynamics of metal and substrate binding with an taurine/α-ketoglutarate-dependent oxygenase (TauD)

Mingjie Li[1], Kate L Henderson[1,2] Salette Martinez[3] Robert P Hausinger,[3] and Joseph P Emerson[1*]

[1]Department of Chemistry, Mississippi State University
[2]Department of Biochemistry, University of Wisconsin
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The biochemistry of iron has been studied for the last 80+ years, however, little has been done to characterize how iron(II) is mediated throughout living systems, and more importantly how iron(II) coordination impacts biomolecule stability. Here we have focused our efforts on a nonheme iron(II) oxygenase, TauD. TauD is a taurine and α-ketoglutarate (αKG) dependent metalloenzyme, which catalyzes the hydroxylation of taurine leading to its decomposition into aminoacetaldehyde and sulfite; where sulfite is a key metabolite in *E. coli*.. Here, we explore the thermodynamic properties and global stability of TauD and a variety of TauD complexes using a combination of circular dichroism spectroscopy and calorimetry techniques. The results show a general increase of the melting temperature as substrate and cofactors are added to TauD. However there are interesting discrepancies in the spectroscopically measured change in enthalpy (ΔH_{VF}) versus the calorimetric enthalpy (ΔH_{DSC}), which could suggest that denaturing these metalloenzymes costs more than just the energy needed to unfold the protein. Additionally, the thermodynamic associated with of bioavailable, divalent metal ions (including Fe, Co and Mn) binding with TauD are elucidated.

Typical and Atypical Prion-like Propagation of Neurotoxic Amyloid-β Oligomers

Dexter N. Dean[1], Kayla M. Pate[2], Pradipta K. Das[3], Sarah E. Morgan[3], Melissa A. Moss[2,4], and Vijayaraghavan Rangachari[1]
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Soluble oligomers of the amyloid-β (Aβ) peptide have emerged as the primary neurotoxic agents in Alzheimer disease (AD). Recent evidence from animal models also implicates aggregates of Aβ to undergo prion-like propagation towards seed-specific fibril deposition. However, dearth in a molecular understanding of Aβ oligomers has confounded the insights into propagation and dissemination of toxic amyloid aggregates. Our recent reports on a distinct 12-24mer oligomer of Aβ, called large fatty acid-derived oligomers (LFAOs), have opened doors in investigating this elusive mechanism. We have previously established that LFAOs undergo replication upon interacting with monomers to form quantitative amounts of identical oligomers. Here, we sought to investigate the concentration-dependent dynamics of LFAOs to reveal how such transitions manifest in their ability to replicate and induce neuronal apoptosis. We have also investigated the ability of LFAOs to undergo prototypical prion-like propagation, where LFAOs grow as distinct repeating units leading to morphologically unique fibrils. We discovered that LFAOs undergo a concentration-dependent transition between 12mers and disperse 12-24mers, which correlates with their ability to replicate and induce apoptosis. At low concentrations (sub-μM), LFAOs exist as 12mers and undergo atypical prion-like propagation (replication) upon interaction with Aβ monomers. While at high concentrations (> 10 μM), LFAOs exist as disperse 12-24mers and propagate towards morphologically unique fibrils in typical prion-like fashion. The observations reported here may have profound significance in deciphering the emerging roles of Aβ oligomer phenotypes in prion-like propagation and dissemination of toxicity in AD.

Session 4: Disordered Systems

Disorder within Cysteine-Rich Proteins: Curious Case of Granulins

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Granulins (Grns) are a family of small, cysteine-rich, pro-inflammatory proteins that are generated upon proteolytic cleavage of their precursor, progranulin. All seven Grns (A-G) contain twelve conserved cysteines (~20% per molecule) that is believed to form six intramolecular disulfide bonds, rendering this family of proteins unique. Grns are involved in multi-functional roles, including wound healing, embryonic growth, and inflammation. Recently, they are also implicated in neurodegenerative diseases such as frontotemporal dementia (FTD) and Alzheimer disease. Despite their manifold functions, there exists a dearth of information regarding their structure-function relationship. We sought to establish the role of disulfide bonds in structure and function by studying the native, oxidized (intramolecularly disulfide bonded) form of GrnB and the completely reduced GrnB (rGrnB). We establish that rGrnB is intrinsically disordered (IDP) at low concentrations and at elevated concentrations forms a fuzzy homodimer without a net gain in the structure– a characteristic increasingly believed to be a hallmark of some IDPs. Interestingly, rGrnB also activates inflammatory receptor, NF-κB in human neuroblastoma cells in a concentration-dependent manner, which correlates with the observed monomer-dimer dynamics. With native GrnB, we observed that only les thank 10% of recombinantly expressed protein is present in the form intramolecularly disulfide bonded monomers. A majority of the expressed protein are multimeric with intermolecular disulfide bonds. Furthermore, surprisingly monomeric GrnB also largely lacks structure suggesting disulfide bonds do not facilitate folding, and resemble kringle-like domains. Our data brings forth the question whether intact disulfide bonds are required for functional GrnB. Efforts are underway to ascertain this by investigating the behavior of various forms of GrnB on inflammatory responses in glial cells.

Optimizing doxorubicin derivative delivery using temperature sensitive biopolymers in multidrug resistant breast cancer cells

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The anticancer agent doxorubicin is an anthracycline compound that shows high potency in treating cancer, and it is one of the most widely used chemotherapeutics. However, efficiency of doxorubicin treatment is limited by low blood plasma solubility, poor blood pharmacokinetics, and non-selective cell killing that results in serious toxicity to healthy tissues. Additionally, cancer cells often develop drug resistance, which is a significant limiting factor to the drug's effectiveness. Motivated by these problems, we have designed a drug delivery system that can specifically deliver drug to the tumor site while overcoming doxorubicin resistance in breast cancer cell lines. This drug delivery system consists of: ELP – Elastin like polypeptide, CPP – Cell penetrating peptide, a cleavable linker -- to enable doxorubicin release in the targeted low pH environment, and a derivative of the anticancer agent Doxorubicin (modified by a 6-maleimidocaproyl moiety for conjugation to a terminal cysteine residue on ELP). ELP is thermally responsive and improves the complex's pharmacokinetics by prolonging its clearance rate while the CPP mediates cellular uptake of large macromolecules. The linker is an acid sensitive amino acid sequence (Gly-Phe-Leu-Gly) that serves as a substrate for lysosomal enzymes. In this study, we compared cytotoxicity of a cleavable (cDox) and non-cleavable (ncDox) doxorubicin derivative delivered by ELP biopolymer in the breast cancer cell lines MCF-7 and MCF7/ADR (doxorubicin resistant cell line). We showed that cDox had two fold higher cytotoxicity than ncDox in both cell lines. When ncDox, however, was conjugated to the ELP biopolymer containing a lysosomally degradable GFLG spacer, the drug delivery construct was equally cytotoxic to both sensitive and resistant cell lines, indicating that the construct delivers doxorubicin into cells by a mechanism that bypasses doxorubicin resistance. Confocal fluorescence microscopy showed that after two hours of exposure to the doxorubicin derivatives, cDox was predominantly localized in the nucleus in both cell lines. However, ncDox was localized in the plasma membrane and cytosol. Intracellular doxorubicin accumulation examined by flow cytometry indicated 3 fold higher uptake of ELP-cDox in sensitive MCF 7 cells compared to resistant MCF7 ADR cells. Cellular uptake of ELP-cDox was further enhanced two fold when conjugated with CPP. In conclusion, our current results indicate that ELP-doxorubicin conjugates may successfully overcome drug resistance in breast cancer cells, providing a promising approach for the use of chemotherapeutic agents such as doxorubicin in patients with drug resistant breast cancers.

Developing the Elastin-like Polypeptide Biopolymer for Delivery of Growth Factor Therapeutics

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Elastin-like Polypeptide (ELP) is a biopolymer often used for drug delivery. The biopolymer is composed of repeated pentapeptide blocks of the sequence VPGxG, where x may be any amino acid except proline. Its use for drug delivery takes advantage of its desirable physical properties. The protein undergoes a reversible phases transition in response to hyperthermia, forming insoluble aggregates at temperatures above a distinct transition temperature (Tt) and re-dissolving when cooled below the Tt. This phase transition allows for easy purification of ELP and ELP-fused proteins by selective precipitation and centrifugation above the Tt. In addition to its use as a purification tag, ELP fusion to therapeutics provides drug delivery advantages when used as a direct fusion to therapeutic peptides, proteins, or small molecules. ELP fusion to a peptide or small protein therapeutic slows plasma clearance relative to the unconjugated therapeutic, protects the agent from degradation, and reduces its immunogenicity. Also, because ELP is genetically encoded, modulation of its sequence to modify its molecular weight (via altering the number of VPGxG repeats) and to add targeting peptides or reactive sites is a matter of straightforward molecular biology manipulation of the coding sequence. Recently, we have used ELP for delivery of growth factors for several therapeutic applications. Free growth factors tend to have very short plasma half-lives when administered exogenously. Fusion of growth factors to ELP allows for extended plasma half lives and *in vivo* bioavailability, the opportunity to add tissue targeting sequences, and the added protein stability makes alternative delivery routes such as subcutaneous administration feasible.

Session 5: NMR Structure and Dynamics

Computational and experimental studies of ADP-ribose binding proteins

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Mono- and poly(ADP-ribosyl)ation are widespread post-translational modifications that affect a number of biochemical pathways. Molecular dynamics studies were employed to investigate the structural effects of mono(ADP-ribosyl)ation (mARylation) of peptides and proteins. Force field parameters were developed to model mono-ADP-ribosylated aspartate and glutamate residues in the Amber 12 program and the automodification region of the poly (ADP-ribose) polymerase enzyme was modeled. The results showed that mARylation led to changes in secondary structure content.

We also investigated a family of mono- and poly(ADP-ribose) binding proteins, the macrodomains. Divergent macrodomains and associated domains from coronaviruses were cloned and characterized using biophysical methods. Five proteins, from the bat coronavirus strains HKU4 and HKU9, were shown to be stable and independently folded domains. Two proteins exhibited unexpected nucleic acid binding affinity. NMR studies, computational predictions, and biochemical experiments for studying the binding of nucleic acids and NAD^+ metabolites will be presented.

Inexpensive production of Site Specific ^{1}H-^{13}C Phenylalanine and Tyrosine

Bhargavi Ramaraju, Hana McFeeters, Robert McFeeters
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Innovations in site specific isotope labeling have increased the size and number of systems studied by NMR spectroscopy. More options are needed to complement methyl labeling to extend utility. Aromatic amino acids are excellent probes since they are often found at important interaction interfaces and play significant roles in terms of structure and macromolecular interactions. Aromatics amino acids also have distinct side chain ^{13}C chemical shifts, multiple magnetically equivalent ^{1}H positions, and reduced effective correlation times due to ring flipping that make them well suited for studying large systems and membrane proteins. Presented here is the inexpensive production and purification of phenylalanine and tyrosine with isolated ^{1}H-$^{13}C^{\alpha,\varepsilon}$ spin systems. Yields on the laboratory scale are in excess of 300 mg per liter of media utilizing 1 gram of ^{13}C carbon source. With cost less than and compatibility with methyl labeling, these reagents hold considerable promise to improve understanding of large macromolecular systems.

Internal motions prime cIAP1 for rapid activation

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Cellular inhibitor of apoptosis protein 1 (cIAP1) is a RING-type ubiquitin ligase that constitutes one of the last brakes on programmed cell death. X-ray crystallography and small-angle X-ray scattering (SAXS) have elucidated the dramatic structural changes that accompany dimerization and activation of this auto-inhibited protein, but several lines of evidence suggest that the static picture afforded by these techniques obscures complex dynamics critical to protein function. Using NMR relaxation dispersion measurements and resonance broadening, we detected micro- and millisecond motions in specific interdomain regions of the auto-inhibited, monomeric state. In addition, we conducted time-resolved SAXS measurements of cIAP1's peptide-induced monomer-to-dimer transition. We find that dimerization occurs over hundreds of milliseconds, and opening is the rate-limiting step of the transition. Together, these data form a picture of a potent anti-apoptotic brake in which selectively dynamic interfaces balance rapid activation against stringent control.

Session 6: Nano-Scale Biophysics

Modulating Protein-Nanoparticle Binding Capacity Using Site-Directed Mutagenesis

Y. Randika Perera, Ailin Wang, Alex Hughes and Nicholas C. Fitzkee
Department of Chemistry, Mississippi State University

Understanding the interactions of gold nanoparticles (AuNPs) with biological macromolecules is becoming increasingly important. This is in part due to the potential applications in drug delivery, bio sensing, diagnostics, and imaging. Our long-term goal is to use protein-functionalized AuNPs as a general tool for molecular sensing and drug delivery. We hypothesize that electrostatic interactions play an important role in the protein-AuNP interaction, since citrate-stabilized AuNPs carry a net negative charge. Using an NMR-based approach developed by our group, we have monitored apparent binding capacity of two similarly structured, but differently charged proteins. Previous work on the GB3 protein hypothesized that positively-charged lysine residues are involved in protein-AuNP binding, and a potential binding site was proposed involving three specific lysine residues, K4A, K19A, and K50A. To test this hypothesis, we mutated the lysine residues to alanine one at a time using site directed mutagenesis. NMR was then used to observe how the binding capacity of these variants changed relative to the wild-type protein. In particular, the K19A and K50A variants significantly reduced binding, while other variants did not. This result supports our original hypothesis, and suggests that GB3 adopts a specific orientation on the AuNP surface. In the future, we believe that experiments like these will result in a better understanding of protein-AuNP interactions.

Magnetic-Plasmonic Core-Shell Nanoparticles: Shape- Controlled Synthesis, Properties and Applications for Capture and Detection of Circulating Tumor Cells

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Due to their high integrity, facile surface chemistry, excellent stability, and combined properties from the core and shell, magnetic-plasmonic core-shell nanoparticles are promising for applications in many areas including catalysis, energy conversion, biological separation, medical imaging, cancer detection and treatment. Here we report a facile method to prepare magnetic-plasmonic iron oxide-gold core-shell nanostructures in different sizes and shapes including sphere, popcorn and star. The nanostars exhibit greatest optical tunability and strength, followed by popcorns and spheres. Using spiked cancer cells in human whole blood, we demonstrate that the anisotropic iron oxide-gold hybrid nanoparticles allow on-line magnetic separation and surface enhanced Raman scattering detection of circulating tumor cells, with the detection sensitivity down to 1-2 cells per milliliter of blood. We further demonstrate the capability of the hybrid nanoparticles for multiplexed multicolor detection of surface protein markers on cancer cells at the single cell level using the highly sensitive and specific surface enhanced Raman scattering technology.

Can Surface-Enhanced Raman Scattering Identify the Drug Mechanism of Platinum-Based Anticancer Drugs?

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Department of Chemistry and Biochemistry, Texas Woman's University

This study focuses on the application of Surface-Enhanced Raman Scattering (SERS) to explore modifications to DNA when bound to carboplatin or cisplatin. SERS enhances Normal Raman (NR) by adsorbing the sample on metal nanoparticles such as silver and gold. Previous studies have shown interaction of cisplatin with DNA leads to a number of changes in DNA such as unwinding, formation of cross-links, and bending. In general, SERS is an ideal method for probing biological systems including drug-oligonucleotide complexes discussed here, therefore we are employing SERS technique to examine the changes to DNA in the presence of the drugs. SERS spectra of a variety of DNA oligonucleotides (2-18 mers) and single DNA bases were collected. Similar sample conditions were repeated when combining oligonucleotide with drugs (carboplatin and cisplatin). SERS samples were prepared by adjusting incubation time and testing a variety of salts. The SERS spectra of the combination drug and DNA were compared to baselines. Comparisons between spectra show distinct changes in the spectrum which is indicative of unique distortions to DNA. Pending computational analysis will provide information regarding the precise molecular distortions.

Session 7: Biophysics of Catalysis & Function I

Building nonheme metal sites in biological systems

Joseph P Emerson
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Nearly half of all enzymes contain a metal ion cofactor. Divalent metal ions can be found in each of the six enzymes classes, and more often than not these metal ions play roles in directing their chemistry. Nature uses a number of strategies to match metal ions with specific proteins, including sequestration and compartmentalization. However, understanding the driving forces behind metal ion selectivity and how these metal ions influence the fidelity of biocatalytic processes remains ambiguous. Here we report our most recent efforts using isothermal titration and differential scanning calorimetry to studying proteins, peptides, and small molecule coordination chemistry in aqueous media, which is new insight into these complex, complex-ion equilibria. Specifically, the thermodynamics of metal binding to the 3-histidine site of carbonic anhydrase II is explored with biologically relevant transition and main group metals, and our more recent efforts to study metal ion coordination chemistry in TauD and homoprotocatechuate 2,3-dioxygenase, which use a 2-His 1-carboxylate facial binding site are discussed.

Non-Specific Interactions of Dihydrofolate Reductase Ligands in Crowded Environments

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The reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR), using NADPH as a cofactor, is an essential part of the folate cycle. The product, THF, is necessary for the synthesis of methionine, purine nucleotides, thymidylate, and other compounds. Inhibition of DHFR leads to interruption of DNA synthesis, making this enzyme a crucial target in the treatment of cancer and other diseases. Previous studies examined the effects of small molecule osmolytes on the substrate interactions with two non-homologous DHFRs, *E. coli* chromosomal DHFR (EcDHFR) and R67 DHFR, with vastly different active site structures. The results indicated that DHF weakly interacts with the osmolytes in solution, shifting the binding equilibrium to unbound DHF. It is hypothesized that similar interactions may also occur between cellular proteins and DHF, where the *in vivo* concentration of the cellular milieu is approximately 300 g/L. Under the crowded conditions in the cell, there is a higher propensity for intermolecular interaction.

Effects of crowding macromolecules in concentrations similar to those *in vivo* were examined. ITC and enzyme kinetic assays were used to detect effects of molecular crowders by monitoring the binding of the enzyme-ligand complexes in the presence of molecular weight crowding agents. Analysis of the K_d's and K_m's indicated a correlation between increased molecular crowding in the solution and weakened binding of the ligands to two structurally unrelated DHFRs. These findings indicate an importance of weak, transient interactions between molecular crowding and DHFR ligands.

An Investigation into the Potential Dual Role of SirC in the Alternative Heme Biosynthetic Pathway of *Methanosarcina acetivorans* C2A

Victoria L. Owens, Kaiyuan Zheng, Steven O. Mansoorabadi
Department of Chemistry and Biochemistry, Auburn University

The methanogenic archaeon, *Methanosarcina acetivorans* C2A, utilizes tetrapyrroles such as heme, factor III, and coenzyme F430 for energy production. Each of these tetrapyrroles contain different metal ions (iron, cobalt, and nickel, respectively), which are generally thought to be inserted into sirohydrochlorin, a common biosynthetic intermediate of each pathway, by a unique chelatase. Interestingly, the genome of *M. acetivorans* C2A encodes only two class II chelatase (CbiX) homologs, and one precorrin 2 dehydrogenase (SirC). SirC is likely responsible for the production of sirohydrochlorin for each of the aforementioned tetrapyrrole biosynthetic pathways, and is homologous to the N-terminal dehydrogenase/ferrochelatase domain of the trifunctional enzyme siroheme synthase (CysG), which converts uroporphyrinogen III to siroheme. Since SirC is found within the alternative heme biosynthetic (*ahb*) gene cluster, which does not contain a chelatase homolog, it is possible that SirC is responsible for both the formation and ferrochelation of sirohydrochlorin in *M. acetivorans* C2A. Here, the ability of SirC to carry our both of these reactions is examined and the implications on the biosynthesis of tetrapyrroles in *M. acetivorans* C2A is discussed.

Session 8: Biophysics of Catalysis & Function II

Using Molecular Modeling to Decipher the Molecular Targets of Bioactive Molecules

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Department of Chemistry and Biochemistry, Jackson State University

Exposure to the non-caloric constituents of the human diet is one of the major ways by which the environment influences the expression of genes as well as alters homeostatic regulations. One of the major groups of these non-caloric food components are phenolic and polyphenolic compounds. These compounds have been associated with several biological processes and pathways; however, there is very limited understanding of their mode(s) of action and/or their molecular target(s). Using cheminfomatics and molecular modeling tools, we have made some predictions on the molecular targets of some dietary polyphenols. These predictions have been tested experimentally, and the results of this work will be presented.

Biophysical Characterization of i-Motif Capped with Flanking Duplex Ends: A Model for c-MYC NHE-III_1 Complementary C-rich Strand

Amanda M. Metz, Savannah J. West, and Edwin A. Lewis
Department of Chemistry, Mississippi State University

Complementary guanine rich and cytosine rich DNA oligonucleotides have the potential to fold into G-Quadruplex and i-Motif structures respectively. These sequences can be found within promotor regions of several genes related to cancer and are possible regulatory elements for gene expression and transcription. Currently, there is an abundance of research on single stranded G-Quadruplex and i-Motif structures making it necessary to develop a model of c-MYC NHE-III_1 that caps the G-Quad and i-Motif forming cores with flanking duplex ends. The wild-type sequence of both G-Quad and i-Motif cores has the potential to fold into 1-2-1 or 1-6-1 loop isomers. Both mutants are studied alongside the wild-type to determine the folding pattern. This new model is needed for a better understanding of the stability and binding of small molecules to the structural cores flanked with duplex DNA. A capped i-Motif was formed by annealing an 18-mer oligonucleotide with a midsection of 6G's and tail ends of 3'-AAATTT and TTTAAA-5' to a 32-mer or 29-mer oligonucleotide having a c-MYC i-Motif forming core with 3'-TTTAAA and AAATTT-5' complementary sticky ends. In this presentation, the structural stability of the single stranded and capped i-Motif models will be discussed using molecular dynamics, CD, and DSC. Also, small molecule binding with TMPyP4, a common G-Quadruplex binding drug, will be reviewed by CD, DSC, and ITC experiments. Furthermore, there is an ongoing development of covalently cross-linking the flanking duplex ends using Psoralen, a known duplex DNA intercalator, to ensure that the duplex ends do not come apart.

Elucidation of the Biosynthetic Pathway for the Key Coenzyme of Methanogenesis and Anaerobic Methane Oxidation

Steven O. Mansoorabadi
Department of Chemistry and Biochemistry, Auburn University

Methyl-coenzyme M reductase (MCR) is the terminal enzyme in the pathway of biological methane formation in methanogenic archaea. Recently, a homolog of MCR has been found in anaerobic methanotrophic archaea (ANME) and is thought to catalyze the first step in the anaerobic oxidation of methane (AOM). In each case, MCR requires a unique nickel-containing tetrapyrrole, coenzyme F430, for activity. However, the genes and corresponding enzymes responsible for the biosynthesis of coenzyme F430 are unknown. A comparative genomics strategy was used to identify putative coenzyme F430 biosynthetic genes. These genes were expressed in a heterologous host and the recombinant enzymes were shown to be capable of synthesizing coenzyme F430 from a common intermediate in the biosynthesis of C2 and C7 methylated tetrapyrroles. In addition, a role for a gene of unknown function from the *mcr* cluster in the production of *holo* MCR was identified. This study significantly advances our understanding of coenzyme F430 biosynthesis and MCR maturation, identifies new biological targets for inhibitors of natural greenhouse gas emissions, and sets the stage for the production of engineered methanogenic and methanotrophic microorganisms utilizing MCR.

Poster Presentation Titles

Posters should be set up in the Palmeiro center following the keynote presentation on Monday. Please arrange to be at or near your poster for the duration of the session. There is no assigned poster board space, so you may hang your poster on any board. Posters should be removed following dinner on Monday night.

Note that several student speakers have elected to present posters as well. To save space, those abstracts/titles are not reproduced here.

1. **Pinpointing the Molecular Basis for Metal Ion Effects on Plasminogen Activator Inhibitor-1 (PAI-1)**
   Joel C. Bucci, Morten Beck Trelle, Carlee S. McClintock, Yuzhuo Chu, Tihami Qureshi, Gregory Ware, Thomas J.D. Jørgensen, Cynthia B. Peterson

2. **Characterization of Putative Peptidyl-tRNA Hydrolase PTRHD1**
   Geordan Burks, Hana McFeeters, Robert McFeeters

3. **Determination of Thermodynamic Parameters Associated with Alkyne-substituted Diminazenes Binding to G-quadruplexes**
   Zachary G. Cuny, Changhao Wang, Brandon Carter-Cooper, Yixuan Du, Jie Zhou, Musabbir. A. Saeed, Jinbing Liu, Benjamin T. Roembke, Clinton G. Mikek, Edwin Lewis, Rena G. Lapidus and Herman O. Sintim

4. **Modeling Intrinsically Disordered Proteins with Chemically Realistic Monte-Carlo Simulations**
   Nic Ezzell, Yue Zhang, and Nicholas C. Fitzkee

5. ***In vivo* effects of macromolecular crowding on the binding affinity of dihydrofolate to dihydrofolate reductase**
   Gabriel Fuente, Michael Duff and Elizabeth Howell

6. **Testing a Model for Rapid Dimerization Kinetics in Epithelial Cadherin**
   Xiaoyun Howard, Martha Kaitlyn Coghlan, Nagamani Vunnam, and Susan Pedigo

7. **An NMR Study of the Binding of Pin1 to Histones**
   Dinusha Jinasena, Hawa Gyamfi and Nicholas C. Fitzkee

8. **Heteroaromatic Salts as Precursors of Reactive Species**
   Wolfgang H. Kramer*, Courtney B. Mullins, Melinda K. Solomon, Lauren M. Hoth, Anna K. Allred, Irene S. Corrao, James P. McVaugh, Emily H. Stewart, B. Woods Curry, Ian R. Gould

9. **Isothermal Titration Calorimetric and Circular Dichroism Spectrophotometric Studies of $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ Derivative Binding with B-DNA**
   Logan R. Martin, Clinton G. Mikek, Jesse I. DuPont, Nagham Alatrash, Frederick M. MacDonnell, and Edwin A. Lewis

10. **Small Molecule Docking Indicates Narrow Spectrum Potential for the Novel Antibiotic Target Bacterial Pth1**
    Paul P. Ferguson, W. Blake Holloway, William N. Setzer, Hana McFeeters, and Robert L. McFeeters

11. **Effect of osmotic stress on enzymes of the folate pathway**
    Deepika Nambiar, Robert Shew, Bryan Schwarz, Michael Duff, Timkhite Kulu-Berhane and Elizabeth Howell

12. **Effects of 5-Hydroxymethylcytosine Epigenetic Modifications within the VEGF Promoter Region on G-Quadraplex and I-Motif DNA Structure and Stability**
    Michael M. Molnar, R.K. Morgan, B. Summerford, Tracy A. Brooks, Randy M. Wadkins

13. **An investigation of $[Ru(II)(L)(phen)_2]^{2+}$ analog binding properties to understand the binding thermodynamics of Ruthenium complexes with B-DNA**
    Clinton G. Mikek, Jesse I. DuPont, Nagham Alatrash, Frederick M. MacDonnell, and Edwin A. Lewis

14. **A Computational Investigation of Dinoflagellate Bioluminescence: Evidence for an Unprecedented Biological Twisted Intra-molecular Charge Transfer Reaction**
    Phong Ngo and Steven Mansoorabadi

15. **Photosensitizer-loaded gold nanorods for combined photodynamic and photothermal cancer therapy**
    Ryan T. O'Connor, Saheel Bhana, and Xiaohua Huang

16. **Synthesis of MTSL Labeled Elastin-Like Proteins for Paramagnetic NMR & EPR**
    Cody Price, Yue Zhang, Nicholas Fitzkee

17. **Long-lived Radical Anions in Aqueous Solution**
    Donya Razinoubakht, Gurjit Kaur, Sabrina Molitor, Axel G. Griesbeck, Wolfgang H. Kramer*

18. **Inhibition of breast cancer cells by Hedgehog-inhibitory peptide conjugated with Elastin-like biopolymers**
    Jung Su Ryu, Drazen Raucher

19. **Linking pH, Temperature, Ionic Strength and Conformation for the DNA i-Motif**
    Thomas Sutorius, Tra Nguyen, Claudette Fraire and Richard D. Sheardy

20. **Near Infrared Spectroscopy and Chemometrics Analysis of Complex Traits in Animal Physiology**
    Carrie Vance, Xiaoguang Ouyang, Andrew Kouba and Scott Willard

21. **c-MYC NHE-III1 C-rich Complementary Strand i-Motif Structural Stability is Influenced by Flanking Duplex Regions and Drug Interactions**
    Savannah J. West, Amanda M. Metz, and Edwin A. Lewis

22. **Examining the Effects of Various Salts on the SERS Spectra of Short DNA Oligonucleotides**
    Aimee S. Williams, Paige D. Hall, Dr. Nasrin Mirsaleh-Kohan

23. **Studying ELP Aggregation Using Paramagnetic Methods**
    Yue Zhang, Cody J. Price, Gene L. Bidwell III, John J. Correia and Nicholas C. Fitzkee

Poster Presentation Abstracts

Pinpointing the Molecular Basis for Metal Ion Effects on Plasminogen Activator Inhibitor-1 (PAI-1)

Joel C. Bucci,[1,3] Morten Beck Trelle,[2] Carlee S. McClintock,[1] Yuzhuo Chu,[3] Tihami Qureshi,[1] Gregory Ware,[3] Thomas J.D. Jørgensen[2], Cynthia B. Peterson,[1,3]
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[2]Department of Biochemistry and Molecular Biology, University of Southern Denmark,
[3]Department of Biological Sciences, Louisiana State University

Plasminogen activator inhibitor type-1 (PAI-1) specifically inhibits the proteases tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) to control the activation of fibrinolysis. Vitronectin (VN) interacts with PAI-1 primarily through the somatomedin B (SMB) domain to stabilize and localize PAI-1 to sites of injury. Our laboratory observed that transition metals ions such as copper^{2+} have VN dependent, reciprocal effects on how long PAI-1 remains active. We aim to determine the molecular basis for effects of copper^{2+} on PAI-1 activity. We employed a computational algorithm (MUG) to predict metal binding clusters, and introduced amino acid substitutions hypothesized to create metal binding deficiency. We compared variants to wild-type by: measurement of stability kinetics, thermodynamic parameters using isothermal titration calorimetry, and protein dynamics using hydrogen deuterium exchange. Active PAI-1 binds copper^{2+} in the low nanomolar range. In a variant lacking the N-terminal histidines of PAI-1, we observed reduced copper^{2+} binding, but this does not abolish accelerated transition to the latent form. PAI-1 variants lacking the carboxylate containing resides in the gate region require more copper^{2+} than wild-type to promote accelerated latency formation, making these residues candidates for further metal binding characterization. SMB-PAI-1 complex binds copper^{2+} with comparable affinity and stoichiometry as PAI-1 alone. Finally, the SMB domain stabilizes PAI-1 by localized effects on dynamics in the same regions that are affected by copper^{2+}. Thus, binding of SMB does not sterically interfere with copper binding to PAI-1, but rather negates copper^{2+} effects directly through changes in dynamics.

Characterization of Putative Peptidyl-tRNA Hydrolase PTRHD1

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Peptidyl-tRNA hydrolases (Pths) are essential enzymes in all domains of life. They are responsible for recycling peptidyl-tRNA to prevent tRNA starvation. Based on sequence and structural prediction, PTRHD1 was hypothesized to be a human Pth, belonging to the Pth2 superfamily. Herein we present the recombinant expression and purification of PTRHD1. We also present inability of PTRHD1 to cleave peptidyl-tRNA and or complement $Pth1^{TS}$. Thus, PTRHD1 is not a peptidyl-tRNA hydrolase, but carries a different functionality.

Determination of Thermodynamic Parameters Associated with Alkyne-substituted Diminazenes Binding to G-quadruplexes

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G-quadruplex ligands have been touted as potential anticancer agents but despite years of active development of small molecules that bind to G-quadruplexes, none of the reported G-quadruplex-interactive small molecules have gone past phase II clinical trials. Recently, it was revealed that diminazene (Berenil, DMZ), a drug that is used to treat animal trypanosomiasis and which was previously thought to be a selective minor groove binder, actually binds to G-quadruplexes 3 orders of magnitude better than DNA duplexes with dissociation constants approaching 1 nM. However, DMZ does not have strong anticancer activities due to its “promiscuous” nucleic acid binding nature. Here we examined several DMZ analogues bearing alkyne substitutes for their selective binding to G-quadruplexes using calorimetric and spectroscopic techniques (Isothermal Titration Calorimetry and Circular Dichroism).

Modeling Intrinsically Disordered Proteins with Chemically Realistic Monte-Carlo Simulations

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Though it was once believed that protein function was inextricably linked to a definite three-dimensional structure, this paradigm has come under scrutiny as our understanding of intrinsically disordered proteins (IDPs) has improved. Given the importance that IDPs play, wherein molecular binding to globular protein domains can induce significant alterations in IDP structure, relevant and accurate simulations must be produced to understand the role of IDPs in biological mechanisms. Previously, we implemented a Monte Carlo simulation for generating chemically realistic, disordered protein conformations, but this model lacked rigorous conformational sampling of protein side chains and *cis-trans* proline isomers; instead, only phi and psi torsion angles were adjusted in simulations. Here, we have implemented side chain chi angle randomization, which provides improved chemical realism in structure generation. Torsion angles are selected using a library of frequently used side chain rotamers found in the Protein Data Bank (PDB). To ensure that structural snapshots are sufficiently independent, we have investigated the degree of torsion angle randomization with an autocorrelation analysis. We find that introducing side chains reduces the efficiency of generating structures as measured by the acceptance rate, but that average chain dimensions are not affected by the change. Future work will focus on introducing prolyl isomerization into the simulation. A long-term goal is to accurately model the protein conformations of the N-terminus of the p53 protein, an intrinsically disordered protein region involved in cell cycle control.

In vivo effects of macromolecular crowding on the binding affinity of dihydrofolate to dihydrofolate reductase

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Escherichia coli is capable of growing in low and high osmolality environments, causing a rapid change in the movement of water across its cell membrane. To deal with the loss of water, many osmotically stressed cells either uptake or synthesize small molecules, called osmoprotectants. Examples of osmoprotectants include proline, betaine, trehalose, and glutamate. As a result, the water activity in the cells is decreased, leading to higher macromolecular crowding. We are interested in understanding how folate metabolism enzymes work under crowded conditions.

Dihydrofolate reductase is an enzyme that catalyzes the NADPH-dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF). DHFR is an important enzyme in folate metabolism as generation of THF is required for the synthesis of thymidylate, methionine and other metabolic intermediates. Two types of DHFR exist, the chromosomally encoded DHFR and the plasmid encoded R67 DHFR. Previous *in vitro* osmotic stress studies showed that upon addition of various osmolytes a tighter binding of the NADPH cofactor and weaker binding of DHF was observed in both enzymes. However, *in vitro* studies are far from representative of the heterogeneous and crowded intracellular environment. A crucial difference between *in vivo* and *in vitro* conditions is the high concentration of macromolecules, which can range in cytoplasm from 200 mg/mL in eukaryotes to >400 mg/mL in prokaryotes. In contrast, most biochemical studies are conducted under dilute (<10 mg/mL) macromolecular conditions. These arguments lead to the longstanding question as to what extent do the experiments observed *in vitro* reflect the behavior *in vivo*[1]? In this work we will address this question by determining the binding affinity of DHF to DHFR in the interior of *E. coli* cell by NMR. Here, an osmotic stress approach will be used to determine the *in vivo* effects of the ligand binding on the ^{19}F-labeled tryptophans of chromosomal DHFR and R67 DHFR. NMR will used to monitor the K_d for the DHF binding to DHFR as a function of folate concentration. Thus far, we have standardized the purification of R67 DHFR and we are in the process of labeling our protein.

Testing a Model for Rapid Dimerization Kinetics in Epithelial Cadherin

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Cadherins are Ca^{2+}-dependent adhesion molecules that are crucial for cell-cell junction formation and tissue remodeling. They form adhesive dimers through exchange of the βA-strand from EC1 domains of interacting protomers. Our current interest is understanding the molecular components responsible for fast dimer assembly and disassembly. Recent crystallographic and cell adhesion studies of epithelial cadherin proposed an intermediate, low-affinity dimeric structure between protomers termed the X-dimer, which is necessary for rapid disassembly of the adhesive dimer (1, 2). Of particular interest is the residue K14, which appears to have an interaction with D138 on the other protomer that stabilizes the X-dimer interface. The wild-type protein displays behavior characteristic of a rapidly exchanging monomer-dimer equilibrium. The X-dimer mutant, K14E, exhibits slow exchange kinetics presumably due to electrostatic repulsion between E in position 14 and D138, two residues in close proximity at the X-dimer interface (1).

In this study, we investigated the link between Ca^{2+}-dependent disassembly kinetics of an X-interface mutant. Previously, we created three mutants of X-dimer at position 14, K14A, K14E, and K14S. Analytical size exclusion chromatography (SEC) was performed to assay for the effect of the mutation on the rate of dimer disassembly. The results of SEC experiments exhibited disproportional formation of dimer from these mutants due to disulfide linkage between protomers. To avoid this problem, the C9A mutant was created, and three double mutants, K14A/C9A, K14E/C9A, K14S/ C9A. Mutant C9A showed rapid monomer-dimer exchange like wild type in the presence or absence of Ca^{2+}. Mutant K14E/C9A exhibited slow monomer-dimer exchange in the presence or absence of Ca^{2+}. Mutant K14A/C9A had intermediate behavior; in the apo-state it showed slow monomer-dimer exchange and in Ca^{2+} added-state, it displayed contrast result ----- a rapidly exchanging monomer-dimer kinetics as seen for neural cadherin. Current studies are underway to assess the effect of K14S/C9A mutations on dimerization kinetics. In conclusion, it is clear that the dimerization kinetics for epithelial cadherin are more complex than structural studies would indicate.

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An NMR Study of the Binding of Pin1 to Histones

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Pin1 is an essential Peptidyl-prolyl isomerase (PPIase) that catalyzes cis-trans prolyl isomerization in peptides containing pSer/Thr-Pro motifs. It has an N-terminal WW domain and a C-terminal PPIase domain. Pin1 targets pSer/Thr-Pro motifs by its WW binding domain and catalyzes isomerization through the PPIase domain. The Pin 1/histone H1 interaction plays a key role in pathogen response in bacterial infected host cells. This makes anti-Pin1 therapeutics an important target for treating infections as well as cancer. The H1 histones (H1.0-H1.5) each contain several potential Pin1 recognition (pT/pS)-P motifs. For example H1.1 has two (pT/pPS)-P sites and H1.5 has five sites. It is presently unknown whether Pin1 interacts with a subset or all of these possible sites and influence of histone peptides on two domain interactions of full length Pin1. To understand this interaction fully, it is important to determine how both the WW and PPIase domains bind to these H1 histone substrates. NMR studies can be used to measure the thermodynamics of biopolymer binding. This technique probes the dissociation constant (K_d) and monitors the residues involved in substrate binding. Here, we investigate the binding affinities of several peptides corresponding to putative binding sites from the H1 histone proteins. We observe different K_d values depending on the binding site, suggesting that energetics plays a role in guiding the Pin1-histone interaction. Estimated overall correlation times derived from ^{15}N relaxation data were analyzed to characterize the effect of Histone binding on the domain flexibility of full length Pin1. For all peptides studied to date, binding does not appear to induce any significant inter-domain interactions. In the future, this information may be useful in determining how Pin1 may be involved in regulating histone and therefore chromatin structure.

Heteroaromatic Salts as Precursors of Reactive Species

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N-alkoxy substituted heteroaromatic compounds based on pyridine, quinoline, isoquinoline and phenanthridine allow the photochemical generation of transient species that can be used to damage biomolecules and induce controlled cell death. The transient species, heteroaromatic radical cations and a methoxy radical are produced with a quantum yield of about 0.55 as determined by trapping experiments.

The N-substituted heteroaromatic salts were combined with 1,8-naphthalimide to enhance ground state association with biomolecules such as DNA. The increased binding was detected with UV/Vis, fluorescence and CD titrations. The DNA-cleaving efficiency and mode was determined by irradiation experiments and gel electrophoresis. Laser flash photolysis was used to analyze the photophysical properties of the bifunctional compounds.

The N-methoxy substituted heterocycles produce a radical cation and a methoxy radical, each of which can initiate DNA cleavage. By comparison with restriction endonuclease, cleaving assays indicates that both transient species might be involved in the cleaving process.

DNA double strand cleavage is desired for efficient cleavage. The bifunctional compounds presented in this project have the ability to induce DNA damage by two different mechanisms, thus showing potential for double strand cleavage.

Isothermal Titration Calorimetric and Circular Dichroism Spectrophotometric Studies of $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ Derivative Binding with B-DNA

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Ruthenium based complexes have been long sought as a potential solution to inhibit development and growth of cancerous tumors within the human body. The number of oncogenetic publications and *in vivo* studies have skyrocketed in recent years with experimental data indicative of Ruthenium complexes possessing the ability to interfere with hypoxic tumor cell function and to obstruct tumor growth. Through previous publications by our group, we found that $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ **(P^{4+})** bound to B-DNA by taking advantage of the "breathing" motion of DNA. In our recent studies we derivatized **P^{4+}** by truncating the bridging ligand from tetraazatepyridopentacene (tatpp) stepwise to phenanthroline (phen) and examined the thermodynamics of binding these derivatives to B-DNA. The $[Ru(phen)_2(tatpp)]^{2+}$ **(MP)** derivative was found to bind tighter than the parent compound **P^{4+}** to B-DNA.

Small Molecule Docking Indicates Narrow Spectrum Potential for the Novel Antibiotic Target Bacterial Pth1

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Peptidyl-tRNA hydrolase (Pth) enzymes are essential an ubiquitous in all living organisms. They return tRNA to participate in protein biosynthesis by removing the peptide moiety from peptidyl-tRNA. In *E. coli*, inhibition of bacterial Pth1 leads to accumulation of peptidyl-tRNA, depletion of aminoacyl-tRNA, and cell death. Eukaryotes have multiple Pths and Pth1 knock out was shown to have no effect on viability in yeast. Thus, bacterial Pth1 is a promising target for novel antibiotic development. From the abundance of Pth1 structural data, molecular docking was used for virtual screening of existing antibiotics to map potential interactions with Pth enzymes. Overall, compounds were docked to 8 different bacterial Pth1 and 3 different Pth2 structures. Multiple compounds demonstrated favorable docking with Pths. Whereas some compounds interacted favorably with all Pths (potential broad spectrum inhibition), more interactions were observed with selectivity for Pth1 or Pth2 and even specificity for individual Pth1s. While the correlation between computation and experiment still remains unknown, these findings support broad spectrum inhibition and point to the possibility of narrow spectrum Pth1 inhibition. Also suggested is that Pth1 can be distinguished from Pth2 by small molecule inhibitors. The findings support continued development of Pth1 as an antibiotic target.

Effect of osmotic stress on enzymes of the folate pathway

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Folate (Vitamin B9) is involved in one carbon transfer reactions required for the synthesis of important macromolecules such as DNA and amino acids. Our current understanding of the folate pathway is mostly based on in vitro studies, which are very different from the crowded environment that exists in the cell. E. coli is known to produce osmoprotectants during times of osmotic stress. This leads to perturbation of water activity inside the cell, and an increase in macromolecular crowding. We have shown earlier that, in vitro, osmolytes weaken the binding of dihydrofolate to dihydrofolate reductase of the folate pathway. We hypothesize that an increased osmolyte concentration in the cell will also prevent the functioning of other folate pathway enzymes by interaction of osmolytes with the various folate redox states. Here we studied the effect of osmolytes on the enzymes methylenetetrahydrofolate reductase (metF) and serine hydroxymethyltransferase (glyA) required for methionine and glycine synthesis respectively in E. coli and also dihydropteroate synthase (folP) responsible for biosynthesis of dihydrofolate. Studies were done with knockout and rescued strains for the metF, glyA and folP genes. The knockout mutants were restored to prototrophy by addition of folate end products while the rescued strains contain a pKTS plasmid containing the knocked out gene under tetracycline control. Osmotic stress studies for metF and glyA indicated that the rescued strain was unable to grow in higher osmolality conditions when compared to knockout strains. We predict this is due to an increase in osmolyte concentration in vivo which leads to interaction of osmolytes with folate intermediates in the pathway. This is turn decreases the efficiency of the folate pathway enzyme.

An investigation of $[Ru(II)(L)(phen)_2]^{2+}$ analog binding properties to understand the binding thermodynamics of Ruthenium complexes with B-DNA

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Ruthenium anticancer drugs have attracted an increasing interest in the last 20 years including examination in clinical trials and compared to platinum drugs, the complexes based on ruthenium are often identified as less toxic and capable of overcoming the resistance induced by platinum drugs in cancer cells. Ruthenium complexes have shown selective carbon radical formation in solid tumors versus healthy tissues due to the reducing environment present in tumors as opposed to the normoxic environments of healthy tissues. Complexes $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (**P^{4+}**) and $[Ru(phen)_2(tatpp)]^{2+}$ (**MP**) induce DNA cleavage in tumor cells through the abstraction of a deoxyribose hydrogen atom by a carbon radical located on the tatpp ligand, which occurs selectively in hypoxic environments. We previously reported on (**P^{4+}**) binding to B-DNA with an affinity of 4×10^5 M^{-1} through an entropically driven intercalative mechanism. A study of increasingly substituted heterocycle-containing $[Ru(II)(L)(phen)_2]^{2+}$ analogs was undertaken to understand the structure-function relationship of these ligands with dsDNA to guide ligand design to increase binding affinity of Ru(II) complexes. Calorimetric and spectroscopic titration experiments were performed in which **MP**, $[Ru(phen)_2(dppz)]^{2+}$ (**Rp2d**), and $[Ru(phen)_3]^{2+}$ (**Rp3**) were independently bound to B-DNA. We determined that **MP** binds to a model dsDNA with higher affinity than **P^{4+}**. **MP** has a larger entropic contribution to Gibbs free energy and a smaller enthalpic penalty resulting in an order of magnitude increase in affinity ($K_a \approx 2.0 \times 10^6$ M^{-1}, $\Delta G \approx -8.60$ kcal/mol).

Effects of 5-Hydroxymethylcytosine Epigenetic Modifications within the VEGF Promoter Region on G-Quadraplex and I-Motif DNA Structure and Stability

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Epigenetic modifications to DNA base sequences may regulate gene expression. CpG islands can contain methylated (5mC) or hydroxymethylated (5hmC) cytosine. Most CpG islands are found primarily in promoter regions that may also contain a high number of repeated cytosines and/or guanines. G-quadraplexes (G4) and i-motifs (iM) are two unique DNA secondary structures that can form in repeating sequences of either guanine or cytosine, respectively. Both G4 and iM sequences may contain CpG sequences that can be methylated or hydroxymethylated. The effects of CpG islands on DNA secondary structures were determined by incorporating a single 5hmC at varying positions in the Vascular Endothelial Growth Factor (VEGF) G4 and iM sequences. An Olis DSM-20 spectropolarimeter and a Cary 100 UV-visible spectrometer were used to monitor the effect of 5hmC on G4 and iM thermal stability. Two of the three 5hmC-containing loops showed a notable decrease in stability for G4's and increased intermolecular structure formation. Contrastingly, the iM stability increased when 5hmC was incorporated into its sequence. Additionally, there was little change in the iM pka. In summary, our results suggest the 5hmC has little effect on iM structures, but can destabilize the G4's.

A Computational Investigation of Dinoflagellate Bioluminescence: Evidence for an Unprecedented Biological Twisted Intra-molecular Charge Transfer Reaction

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Ubiquitous in the world's oceans, dinoflagellates are capable of fantastic displays of bright blue bioluminescence. This luminosity is a consequence of the oxidation of an open-chain tetrapyrrole, dinoflagellate luciferin, by the enzyme dinoflagellate luciferase. While many other bioluminescence systems have been thoroughly investigated (i.e., firefly, jellyfish, and bacterial), the mechanism of dinoflagellate bioluminescence remains enigmatic. A comprehensive time-dependent long-range corrected density functional theory (TDLCDFT) investigation was used to evaluate several competing reaction mechanisms of dinoflagellate luciferase catalysis employing distinct excited state luminophores. The results provide strong evidence in favor of a mechanism of dinoflagellate bioluminescence involving a biologically unprecedented twisted intramolecular charge transfer (TICT) reaction.

Photosensitizer-loaded gold nanorods for combined photodynamic and photothermal cancer therapy

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Photodynamic therapy (PDT) is a non-invasive cancer treatment that uses localized, non-invasive light for the treatment of certain cancers. Conventional PDT is limited by poor water solubility, non-specificity, and photobleaching. Delivery and efficacy can be improved using nanoscale deliveries, among which includes gold nanorods (Au NRs). Au NRs possess several advantages, including including small size with tunable features, easy preparation, excellent stability, and immense functionalization potential. We report a Au NR-based nanocomplex as a photosensitizer-carrying photothermal agent. The silicon 2,3-naphthalocyanine dihydroxide(SiNC) photosensitizer is densely trappedin a hydrophobic pocket on the surface of the Au NRs via PEG covalently linked with an alkyl-thiol segment with a specific chain length. Highly efficient SiNC release and cellular uptake was achieved through partition between the nanocomplex and cell membrane. Additionally, we demonstrate through in vitro studies that the nanocomplex provides a synergistic effect upon exposure to NIR light, generating cancer cell eradication superior to photothermal therapy (PTT) or photodynamic therapy alone. With the ability to deliver high concentrations of photosensitizer and be utilized in PTT, the nanocomplex has the potential ability to completely ablate tumors, thus preventing tumor reoccurrence.

Synthesis of MTSL Labeled Elastin-Like Proteins for Paramagnetic NMR & EPR

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Elastin-Like Proteins (ELPs) have been proposed as a novel drug delivery vector for treating cancer. These proteins aggregate reversibly above a specific temperature, allowing ELPs to be thermally targeted to cancerous tumors. Though proven successful in mouse models, without a molecular understanding of how ELPS aggregate, it remains extremely difficult to optimize these molecules for drug delivery in humans. Our long-term goal is to test the hypothesis that ELP aggregation is non-specific and does not originate at a specific nucleation point. In this project, we have completed the initial characterization of these proteins and begun paramagnetic labeling in preparation for critical EPR and NMR experiments. We successfully purified and expressed seven ELPs according to established methods with purity of ELP40 higher than 99% at a yield of 150mg/L. Using dynamic light scattering and one-dimensional proton NMR we have begun characterization of the temperature-dependent transition in our ELPs. In addition, we have labeled our protein using MTSL, a paramagnetic NMR probe that will help us to monitor protein association. Here, a series of chromatography methods are applied to achieve the MTSL-labeled ELP. Future work will utilize this MTSL-labeled ELP to monitor the extent to which protein interactions occur below the transition temperature.

Long-lived Radical Anions in Aqueous Solution

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Pyromellitdiimides are used as electron acceptors in photochemical applications due to their reduction potential and prominent radical anion absorption. Their high stability has led to the use of pyromellitic diimides in polymeric films such as Kapton. The similarity to phthalimide might make it a suitable chromophore for the decarboxylative photocyclization, a preparative photochemical method for the synthesis of small to medium rings. The strong one electron acceptor properties can be used to oxidation and thus selective cleavage of biomolecules.

The pyromellitic diimides were synthesized substituted with N-alkyl carboxylic acids. Irradiations in aqueous solution at pH 7, followed by extraction and column chromatography yielded the cyclization products. UV/Vis spectroscopy and EPR/NMR were used to detect the long lived radical anion.

Pyromellitic diimide undergoes decarboxylative photocyclization to yield a large number of regio- and stereoisomers. Interestingly, the radical anion formed after the first PET is extremely stable and has a lifetime of several days in deoxygenated solution. The radical anion was confirmed by EPR and NMR. An interesting spacer dependency was observed.

The long-lived radical anion of pyromellitic diimide makes it an ideal electron trap after oxidation of a donor. The dianion is a species with strong reducing power and can thus be used to selectively reduce acceptors.

Inhibition of breast cancer cells by Hedgehog-inhibitory peptide conjugated with Elastin-like biopolymers

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The Hedgehog(Hh) signaling pathway has been reported to be aberrantly activated in cancer. Studies have shown that suppression of this aberrantly activated Hh pathway may be effective in controlling the development and proliferation of many cancers. However, to date, there is no drug in clinical application, which can be used to inhibit the Hh signaling pathway and suppress cancer growth.

In attempt to address this problem we developed a thermally responsive polypeptide inhibitor of the Hh pathway. This polypeptide is derived from a mammalian tropo-elastin protein which is thermally responsive and it will aggregate and accumulate at the tumor site where local hyperthermia is applied. For this study, ELP was fused to a peptide which inhibits Hh signaling by blocking interaction of Sonic Hh ligand (Shh) and Patch-1 docking site. To increase cellular uptake ELP was further modified by cell penetrating peptide Tat.

The anti-proliferative activity of Tat-Hhi-ELP was examined in three breast cancer cell lines, MCF7, MDA-MB-231, and SKBR-3. Treatment of the cells with 20 uM of peptide for 2 days resulted in maximally 40% inhibition of cell proliferation. Observed cytotoxicity was further increased two fold by application of hyperthermia.

To validate that Tat-Hhi-ELP is targeting Hh pathway we determined the levels of GLI-1, which is a downstream target in Hh patway. Treatment of the breast cancer cells with Tat-Hhi-ELP resulted in the reduction of GLI-1 level indicating that the cytotoxicity is based on hedgehog pathway inhibition. Furthermore, the formation of mammospheres was significantly reduced by the Tat-Hhi-ELP's treatment in SKBR-3. These results suggest that thermal targeting of ELP-based Hedgehog inhibitory peptides to the breast cancer cells may be an effective and promising treatment strategy for breast cancer stem cells.

Linking pH, Temperature, Ionic Strength and Conformation for the DNA i-Motif

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DNA is highly polymorphic and the conformation a particular DNA segment assumes depends upon its sequence context and the environment under which it is prepared. To complement our findings with G-rich sequences related to the human telomere (i.e., $(XXXGGG)_4$, where X = T and/or A) we have been investigating the pH induced transition from single strand to i-motif for sequences related to the human telomere C-rich strand. We have investigated the effects of pH on the transition of $(CCCXXX)_4$, where X = A and/or T, from the unfolded single stranded structure to the folded i-motif at 25 ^oC. We have also investigated the thermally induced unfolding of the i-motif back to single strand at pH 5.0. We have also carried titrations of $(CCCTAA)_4$ from pH 7.0 to pH 5.0 at seven different temperatures (15 ^oC, 20 ^oC, 25 ^oC, 30 ^oC, 35 ^oC, 40 ^oC and 45 ^oC) at 115 mM K^+ and at five different K^+ concentrations (15 mM, 75 mM, 115 mM, 215mM and 325 mM) at 25 ^oC. DNA samples were prepared in phosphate buffer with least 12 different pH values from 7.0 to 5.0 and desired $[K^+]$. Circular Dichroism (CD) spectra were determined to monitor the transition. Preliminary analysis of the data indicates that the transitions are influenced by sequence context effects, are less favorable by higher temperatures but more favorable at higher K^+ concentrations. These results will be discussed from a thermodynamic point of view.

Near Infrared Spectroscopy and Chemometrics Analysis of Complex Traits in Animal Physiology

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Near infrared reflectance (NIR) applications have been expanding from the traditional framework of small molecule chemical purity and composition (as defined by spectral libraries) to complex system analysis and holistic exploratory approaches to questions in biochemistry, biophysics and environmental ecology. The increased complexity of compound biochemical systems depend on multivariate modeling in which the specific underlying chemistry is revealed in terms of matrix loadings and scores, which themselves reflect coupled groups of biomolecules that wax and wane correlatively. As such, we have employed NIR to ask basic questions about animal physiology and health across several taxa and species, and implement this biophotonic methodology for non-invasive, non-destructive real-time analysis of otherwise ill defined traits. Here we exemplify how NIR spectroscopy can be used to determine sex and reproductive status in wild mammalian species to map population demographics in conservation biology.

c-MYC NHE-III1 C-rich Complementary Strand i-Motif Structural Stability is Influenced by Flanking Duplex Regions and Drug Interactions

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Cytosine-rich sequences in slightly acidic pH solutions have the potential to form a non-canonical structure called an i-Motif, which is complementary to a G-Quadruplex forming sequence. These complementary guanine and cytosine rich sequences are known to be present within the promoter regions of oncogenes but additional studies are required to elucidate their biological function and stability. Here, we used molecular modeling to predict the influence of flanking duplex ends and other mutations on i-Motif structural stability. The c-MYC wild type (WT) C-rich sequence folds into numerous configurations *in vitro* necessitating the use of mutant structures to "lock in" a chosen configuration for study. Mutants were manually docked with a ligand, TMPyP4, on the top and bottom of the i-Motifs to predict the structural stability. Conformational energy of each structure was calculated and tabulated.

Examining the Effects of Various Salts on the SERS Spectra of Short DNA Oligonucleotides

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The Raman technique offers significant information regarding structures of a molecule and also the changes in the structure under certain conditions. Surface−Enhanced Raman Scattering (SERS) utilizes the properties of nanoparticles to enhance Raman signals especially for samples with low Raman cross sections. The SERS technique is used to detect a very low concentration of molecules in many fields such as biochemical, environmental and crime scene forensic analysis. In this study, SERS of short oligonucleotides of DNA are mixed with silver nanoparticles (50-100 nm) and are incubated up to 48 hours before obtaining the SERS data. Different conditions such as molar ratios, various salts and concentrations, and immobilization time will be examined to study structural characterization of DNA. We are specifically focusing on the effects of salts such as potassium chloride and potassium sulfate. In this work, we will present our preliminary results and will discuss our future directions.

Studying ELP Aggregation Using Paramagnetic Methods

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Elastin-like proteins (ELPs) are known to desolvate and aggregate reversibly above a defined transition temperature; therefore, ELPs can be a useful tool to study protein aggregation and solubility. Previously, a temperature-dependence of the secondary structure elements (type II beta-turn) has been observed in short elastin-like peptides. Here, we investigate a hypothesis for ELPs aggregation: ELP aggregation is non-specific and does not originate at a specific nucleation point. To investigate the intermolecular interactions during the early-stage of ELP aggregation, a small ELP construct containing 40 repeats (ELP40, 16 kDa) is specially designed for NMR experiments. To monitor the physical properties of different regions in ELP40, a series of cysteine variants has also been created, where, a paramagnetic spinning label, MTSL, was attached. A series of temperature experiments were carried out using both NMR and EPR. NMR experiments focus on the domain-domain interactions; EPR experiments provide molecular motions during the aggregation. Combining the complementary DLS experiments, we will be able to study ELP aggregation from the molecular to residue level.