Symposium Schedule

1PM: Posters, Group 1

1:45PM: Move into Auditorium for Talks

2PM: Welcoming Address: **Dr. Jon Mogford, Vice Chancellor for Research**

2:20PM: Student Talks
- 15 minutes with 5 minutes questions
- 2:20PM: **Ana Chang-Gonzalez**
- 2:40PM: **James Gonzales**
- 3:00PM: **Michael Hebert**

Break

3:30PM: Posters, Group 2

4:15PM: Move into Auditorium for Talks

4:30PM: Talks
- 15 minutes with 5 minutes questions
- 4:30PM: **Lauren Kustigian**
- 4:50PM: **Isita Jhulki**
- 5:10PM: **Dat Truong**

Break

5:45PM: Keynote: **Dr. Alex Zabula, ExxonMobil**

6:30PM: Awards and unofficial event at Blackwater Draw Brewery

<u>Oral Presentation Abstracts</u>

Sequential Tuning of Adaptive Immunity During T-Cell Receptor Development

Ana C. Chang-Gonzalez

Department of Biomedical Engineering

In mammalian cell-mediated immunity, T lymphocytes play a key role to detect foreign peptides, such as viruses. The mature αβT cell antigen receptor (αβTCR) recognizes a foreign structure and induces a signaling response via its affinity to the Major Histocompatibility Complex (MHC) of the foreign structure. In order for a mammalian cell to appropriately identify and respond to a foreign threat, the αβTCR must exhibit robust specificity and ability to recognize peptides from the MHC. However, in solution, TCR ligands have low affinity to the MHC peptides.

While there are several models for how TCR adequately responds to the MHC, via optical tweezer experiments it has been found that αβTCR acts as a mechanosensor. This mechanosensing activity is triggered by the tangential force exerted when TCR binds to MHC ligands. The pre-T-cell antigen receptor (pre-TCR), which is the structure prior to the mature αβTCR expression, is missing one of the two variable domains required for αβTCR-MHC binding specificity. Regardless of its incomplete form, the pre-TCR has been found to induce T-cell signaling through its early intervention determining which MHC-bound peptides are recognized by mature T-cell receptors. This function is pivotal in adaptive immunity, ensuring that mature T-cells are able to discriminate between self- and non-self structures.

Although a crucial step in adaptive immunity, how pre-TCR directs peptide recognition before the mature αβTCR is fully developed and tweaks the ligand specificity with MHC peptides is not well understood. NMR methods have examined pre-TCR ligand folding and regions of the protein which could interact with MHC peptides, strengthening pre-TCR binding affinity based on the force generated through bonds. Molecular dynamics simulations is a tool that can help researchers detect the mechanosensing activities of the pre-TCR at an atomistic level. In our work, by integrating measurements found via mechanistic studies using optical tweezers and molecular dynamics simulations, we can elucidate on this ligand-dependent model of pre-TCR and MHC peptide binding. Through molecular dynamics simulations, we measure the relative motion of the pre-TCR domains in terms of hinge, rotation, and extension angles in order to classify the conformational transitions that may lead to the catch bond behavior responsible for immune response triggering. Atomistic level information about pre-TCR motion will be necessary for understanding the selectivity process for the MHC-recognizing variable domains in the mature αβTCR that ensure our cells respond appropriately to foreign objects.

Computer Simulation of Dynamic Behaviors of Microtubule Protofilaments

James E. Gonzales

Department of Biomedical Engineering

The purpose of this study was to explore and understand the behavior of microtubule protofilaments (MT PFs) in a dynamic environment through molecular dynamics simulations. MTs play a vital role in various cellular processes, such as: transport of materials within cells, cell structure and stability, and cell division. During normal cellular processes, MTs continuously polymerize and depolymerize depending on the nucleotidepresent in the end tubulin, either guanosine triphosphate (GTP) or guanosine diphosphate (GDP). While previous research has found that the nucleotide at the end of the filament is responsible, it is unclear why.

To understand the effects of the nucleotide state, molecular dynamics simulations were performed on four MT PF systems. One system contained tubulin bound only to GTP and the other system contained tubulin bound to both GTP and GDP. Both of these systems were simulated in two different sizes, as a 2-dimer and 4-dimer MT PF. The 2-Dimer system was simulated for 400 nanoseconds (ns), and the 4-Dimer system was simulated for 210 ns. The simulations were performed on Texas A&M's High Performance Research

Computing machines. The trajectories of these simulations were analyzed, using a state-of-the-art method developed in our lab, to quantify the mechanical properties of MT PFs as an elastic rod. Additionally, an analysis of the non-polar and polar contacts found between tubulin was performed to see the chemical effect of the nucleotide state on the mechanical properties of MT PFs.

Hydration of Guanidinium Ions: An Experimental Search for Like-Charged Ion Pairs

Michael J. Hebert, David H. Russell

Department of Chemistry

Guanidinium ions (GdmH+) are reported to form stable complexes (GdmH+/GdmH+) in aqueous solution despite strong repulsive interactions between the like-charged centers. These complexes are thought to play important roles in protein folding, membrane penetration, and formation of protein dimers. Although GdmH+ ions are weakly hydrated, semi-empirical calculations provide evidence that these like-charged complexes are stabilized by water molecules, which serve important structural and energetic roles. Specifically, water molecules bridge between the GdmH+ ions of GdmH+/GdmH+ complexes as well as complexes involving the guanidinium side chains of arginine. Potential biological significances of like-charged complexes have been largely confirmed by ab initio molecular dynamics simulations and indirect experimental evidence. We report cryo-ion mobility-mass spectrometry results for the GdmH+/GdmH+ ion pair confined in a nanodroplet---the first direct experimental observation of this like-charged complex. A second like-charged complex, described as a water-mediated complex involving GdmH+ and H3O+, was also observed.

The Mechanism of Membrane fission at the Recycling Endosome of *C. elegans*

Lauren Kustigian, Hays S. Rye

Department of Biochemistry and Biophysics

Endocytic recycling is the process by which endocytosed cargoes, such as membrane bound proteins and lipids, are returned to the plasma membrane. At the tubular recycling endosome, material is sorted from non-recycled material prior to its return to the plasma membrane in transport carriers. The release of these transport carriers requires a membrane separation process known as membrane fission. The mechanism of membrane fission at the tubular recycling endosome is poorly understood. At the basolateral tubular recycling endosome of *C. elegans*, two key proteins are required for membrane fission: receptor mediated endocytosis 1 (RME-1) and amphiphysin 1 (AMPH-1). Here we use Burst Analysis Spectroscopy (BAS), a free-solution, single particle fluorescence-based technique, to identify the minimal fission machinery of the recycling endosome. We identify AMPH-1, an N-BAR protein, as the key mediator of membrane fission. Surprisingly, the fission activity of AMPH-1 is stimulated by GTP. An unexpected result as AMPH-1 does not have a canonical nucleotide binding motif. Furthermore we show that the GTP-stimulated membrane fission activity may be a general feature of amphiphysin family of N-BAR proteins, as the S. cerevisiae amphiphysin homolog RVS161/167p also possesses GTP stimulated membrane fission activity. Finally we identify RME-1 as a negative regulator of the AMPH-1 mediated membrane fission activity. The data presented here supports a model in which the worm and yeast N-BAR proteins function as a core membrane fission machine, using the insertion of their amphipathic helices into the bilayer to induce membrane fission.

RosB Catalyzed Substitution of Aromatic Methyl Group with Amino: Novel Flavoenzyme Converts Vitamin into Antibiotic

Isita Jhulki

Department of Chemistry

Roseoflavin is a bright red-colored anti-vitamin biosynthesized by Streptomyces davawensis and Streptomyces cinnabarinus. It is the only naturally occurring riboflavin analog that exhibits antibiotic properties. Earlier studies show that roseoflavin is biosynthesized from riboflavin itself by substitution of the C8 methyl with a dimethylamino group. Recently, the methyltransferase catalyzing the final step has been characterized and a sub-genomic fragment containing genes necessary for the biosynthesis has also been cloned. However, the biosynthesis of the key intermediate 8-amino-flavin remains largely unexplored. Herein, we report the identification and characterization of a single flavin-dependent enzyme, RosB that replaces the C8 methyl group of Flavin mononucleotide (FMN) and uses the amino group of glutamate to convert FMN to 8-amino-FMN via the intermediacy of 8-formyl-FMN. The enzyme was cloned, overexpressed and purified, and subsequently tested for the predicted activity. A mechanism for the RosB-catalyzed reaction is proposed based on the identification of reaction products and intermediates. In the first step of this reaction, the enzyme oxidizes the C8 methyl group of FMN to a formyl group using molecular oxygen. Next, the intermediate, 8-formyl-FMN catalyzes the transfer of the amino group from the glutamate to itself in a fashion, similar to that observed for a PLP-dependent aminotransferase. The resulting reactive FMN intermediate then rearranges to yield 8-amino-FMN and the C8 methyl group is lost as formate. To gain further insights into this unprecedented amine transfer reaction various substrate analogs were designed and studied. Additionally, the crystal structure of the enzyme containing a bound inhibitor complemented the results of the biochemical analysis. Overall, this work reveals the existence of yet another incredible new flavin transformation involved in natural product biosynthesis.

The Important Role of a Second-Shell Amino Acid in Determining *N*-Succinylamino Acid Racemase Reaction Specificity

Dat P Truong, Simon Rousseau, Jamison Huddleston, Frank M. Raushel, James Sacchettini, & **Margaret E. Glasner**

Department of Biochemistry and Biophysics

Several lines of evidence show that catalytic promiscuity, which refers to the ability of an enzyme to catalyze more than one reaction in the same active site, plays a role in the evolution of new enzyme functions. Studying catalytic promiscuity can help identify structural features that predispose an enzyme to evolve new functions. Our research will address this problem using the catalytically promiscuous *N*-succinylamino acid racemase/*o*-succinylbenzoate synthase (NSAR/OSBS) subfamily, which is a branch of the OSBS family. We found that the residue R266 is conserved in the NSAR/OSBS subfamily, in which most members catalyze both NSAR and OSBS reactions. However, the homologous position is usually hydrophobic in other nonpromiscuous OSBS subfamilies, which lack NSAR activity. R266 is a second-shell amino acid that is close to the catalytic K263, but it does not contact the substrates, suggesting that R266 affects the catalytic mechanism, rather than substrate binding. Mutating R266 to glutamine in *Amycolatopsis* NSAR/OSBS reduces both NSAR and OSBS activities, but it is more deleterious for NSAR activity. Specifically, the R266Q mutant decreases the rate of proton exchange between the alpha proton of the NSAR substrate and the general acid/base K263 without affecting that of the other general acid/base K163. Preliminary analysis of the crystal structure of *Amycolatopsis* NSAR/OSBS R266Q shows that K263 forms a salt bridge with the conserved metal ligand D239, which normally forms a salt bridge with R266 in the wild type protein. This interaction reduces the acid/base reactivity of K263 in the NSAR reaction. However, this interaction is less deleterious for the OSBS reaction because K263 forms a cation-π interaction with the OSBS substrate and/or the intermediate, rather than acting as a general acid/base. Together, the data explain the switch in the reaction specificity by the R266Q mutant and show that R266 is important for determining NSAR reaction specificity.

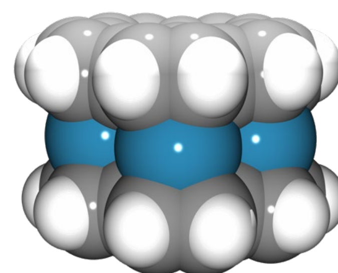
Keynote

Organic Materials for Superior Electron Acquisition and Corrosion Prevention

Alexander V. Zabula

[a] P. Roy and Diana T. Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, 231 S. 34th Street, Philadelphia, PA 19104, USA. Current position: Senior Research Chemist, ExxonMobil Chemical Company.

Polycyclic aromatic hydrocarbons or polyarenes represent one of the most important and interesting classes of organic compounds showing unique electronic structures and intriguing chemical and physical properties. In the present research, non-planar polyarenes have been used as models to study the reactivity and properties of carbon allotropes with curved π-surfaces including their ability to reversibly acquire additional electrons. Structural elucidations for supramolecular sandwich-type assemblies between highly charged non-planar polyarenes and alkali metal cations allowed the elucidation of energy storage mechanisms in prospective anode materials fabricated from fullerenes or nanotubes.



In the second part of my talk I will discuss the development of high throughput screening techniques for fast and convenient elucidation of anti-corrosion activity of molecular corrosion inhibitors, including environmentally friendly materials. The developed methods enabled the discovery of a new family of cheap coordination compounds that act as corrosion inhibitors and demonstrate superior metal protection compared to standard chromate solutions. The structure-property relationship for prospective lanthanide coordination compounds was elucidated based on structural studies both in solution and in the solid state.