

**The 10th Annual Frontiers in
Chemistry and Biology Interface
Symposium**

University of Delaware

Saturday, May 6, 2017

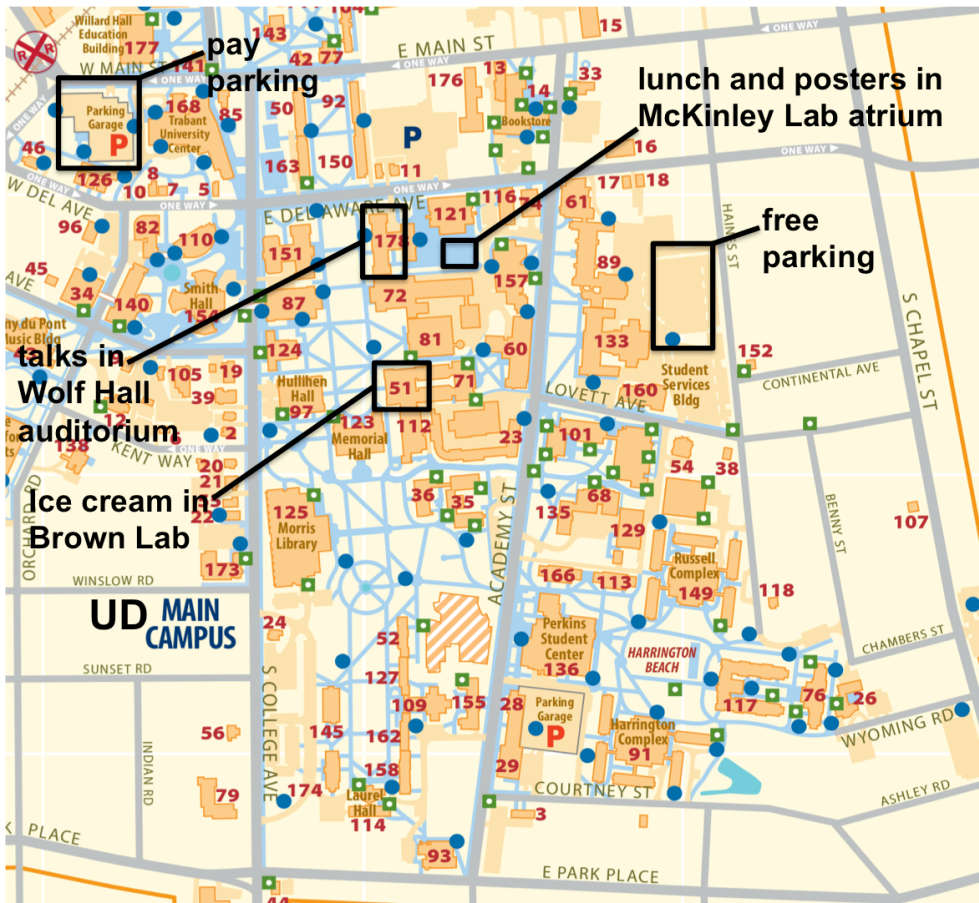


The 10th annual FCBIS will take place on the University of Delaware main campus on May 6, 2017.

Talks will be in Wolf Hall auditorium and lunch/posters sessions will be in the McKinley Lab Atrium. Post symposium ice cream social will be housed in Brown Lab.

For driving directions from your current location to the Pearson parking lot, which will have free parking click the link below:

<https://www.google.com/maps/dir/39.6792998,-75.7477284/@39.680237,-75.7489059,18z>



Driving Directions to UD's Newark campus

From the north - Take I-95 South to Delaware Exit 1-B — Route 896 North, which becomes South College Avenue at the intersection of Route 4. Continue straight on South College Avenue for about two miles, past such landmarks as the Bob Carpenter/Sports Convocation Center, Delaware Stadium, and the Delaware Field House. Continue going straight through the intersection of South College Avenue and Park Place, then take a right on Delaware Ave. Take a right at next light onto Academy St, then a left at stop sign onto Lovett Ave. The Pearson parking lot will be on your left.

From the south - Take I-95 North to Delaware Exit 1 — Route 896 North, which becomes South College Avenue at the intersection of Route 4. Continue straight on South College Avenue for about two miles, and follow directions listed above.

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Johns Hopkins University

Steve Rokita, Dept. of Chemistry

Caren Freel Meyers, School of Medicine, Dept. of Pharmacology & Molecular Sciences

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Greg Gatto, Sr. Scientific Investigator

National Cancer Institute-Frederick

Jordan Meier, Chemical Biology Laboratory

UD Local Organizing Committee – more info at <http://sites.udel.edu/fcbis2017>

Brian Bahnson, Dept. of Chemistry & Biochemistry

Catherine Leimkuhler Grimes, Dept. of Chemistry & Biochemistry

April Kloxin, Dept. of Chemical & Biomolecular Eng.

Ramona Neunuebel, Dept. of Biological Sciences

FCBIS 2017 Student Organizers:

Mackenzie Lauro, Dept. of Chemistry & Biochemistry

Yiben Wang, Dept. of Chemistry & Biochemistry

Nikodimos Gebreselassie, Dept. of Chemical & Biomolecular Eng.

Rebecca Noll, Dept. of Biological Sciences

Univ. of Pennsylvania will host FCBIS in 2018 led by:

David Chenoweth, Assistant Professor of Chemistry

Rahul Kohli MD/PhD, Assistant Professor of Medicine and Biochemistry & Biophysics

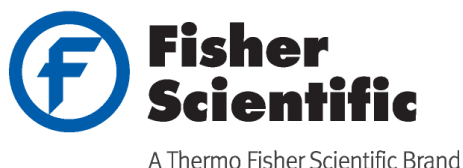
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Saturday, May 6, 2017
Program

- 8:30-9:00 Registration, Breakfast, Poster setup
- 9:00-9:05 Introductory Remarks – Wolf Hall Auditorium
- 9:05-10:00 **Session I** –Chair: April Kloxin Wolf Hall Auditorium
- 9:05-9:25 **Christian Kaiser** (Johns Hopkins University)
“Studying protein biogenesis at the single-molecule level”
- 9:25-9:45 **Rodrigo Maillard** (Georgetown University)
“Investigating the Conformational Landscape of Protein Kinase A with Optical Tweezers”
- 9:45-10:00 Founders Reflection on the FCBIS
- 10:00-10:20 Coffee Break
- 10:20-10:55 **Session II** – Chair: Christine Ott Wolf Hall Auditorium
- 10:20-10:40 **Lisa Jones** (University of Maryland School of Pharmacy)
“Extension of Hydroxyl Radical-Based Footprinting Coupled with Mass Spectrometry for In Cell and In Vivo Protein Analysis”
- 10:40-10:55 **Emily Shutsky** (University of Pennsylvania) “Defining and Exploiting APOBEC3A’s Activity on the Extended Epigenome “ poster speaker (poster #79, selected from abstracts)
- Morning Keynote**
- 10:55-11:55 **Carmen Drahl** – Keynote (Freelance Chemistry Journalist)
“I Communicate Chemistry (And So Can You!)”
- 11:55-12:00 Career Round Table Introductions – Wolf Hall Auditorium
- Carmen Drahl** (Freelance Chemistry Journalist)
Nikki Goodwin (Director of Medicinal Chemistry, GSK)
Benjamin Israel (Sr. Technical Team Lead, Siemens Healthcare Diagnostics)
Pamela Marino (Chief , Biochemistry & Biorelated Chemistry Branch, NIGMS)
Wilson Francisco (Program Director, NSF)
Joseph Rucker (VP Research and Development, Integral Molecular)

The Career Round Table will be in McKinly Atrium and run concurrent with poster sessions from 12:30-2:00

- 12:00-12:30 Lunch
- 12:30-1:15 Poster Session I (**Odd Numbered Posters**) – McKinly Lab Atrium
- 1:15-2:00 Poster Session II (**Even Numbered Posters**) – McKinly Lab Atrium
- 2:00-2:35 **Session III** – Chair: Myles Poulin, Wolf Hall Auditorium
- 2:00-2:20 **Mark Allen** (University of Maryland Baltimore County)
“A Biological Toolbox: Making a Connection”

- 2:20-2:35 **Alicia DeColli** (John's Hopkins University) "Investigating the mechanism of LThDP decarboxylation by DXP synthase" poster speaker (poster # 18, selected from abstracts)
- 2:35-2:45 Refreshment Break
- 2:45-3:20 **Session IV** – Chair: Mary Watson, Wolf Hall Auditorium
- 2:45-3:05 **Martin Schnermann** (National Cancer Institute)
"Near-IR Uncaging Chemistry: Discovery and Applications"
- 3:05-3:20 **Andrew Urmev** (University of Delaware) "Detecting Protein Tyrosine Nitration with Designed Peptides"
poster speaker (poster #89, selected from abstracts)

AFTERNOON KEYNOTE:

- 3:20-4:20 **Joel Schneider** – Keynote (National Cancer Institute)
"Racemic hydrogels from self-assembling mirror image peptides: Predictions from Pauling and Corey"
- 4:20-4:30 Poster Awards and Final Remarks – Wolf Hall Auditorium
- 4:30-5:30 Networking Ice Cream Mixer – Brown Lab

Speaker Abstracts

Session I Speakers:

Christian Kaiser (Johns Hopkins University)

Title: “Studying protein biogenesis at the single-molecule level”

Abstract: Multi-domain proteins often require help from molecular chaperones to fold productively, even before the ribosome has finished their synthesis. The mechanisms underlying chaperone function remain poorly understood. Using optical tweezers to study the folding of elongation factor G (EF-G), a model multi-domain protein, we find that the N-terminal G-domain in nascent EF-G polypeptides folds robustly. The following domain II, in contrast, fails to fold efficiently. Strikingly, interactions with the unfolded domain II convert the natively folded G domain to a non-native state. This non-native state readily unfolds, and the two unfolded domains subsequently form misfolded states, preventing productive folding. Both the conversion of natively folded domains and non-productive interactions among unfolded domains are efficiently prevented by the nascent chain-binding chaperone trigger factor. Thus, our single-molecule measurements of multi-domain protein folding reveal an unexpected role for the chaperone: It protects already folded domains against denaturation resulting from interactions with parts of the nascent polypeptide that are not folded yet. Previous studies had implicated trigger factor in guiding the folding of individual domains, and interactions among domains had been neglected. Avoiding these early folding defects is crucial, since they can propagate and result in misfolding of the entire protein.

Rodrigo Maillard (Georgetown University)

Title: “Investigating the Conformational Landscape of Protein Kinase A with Optical Tweezers”

Abstract: Yuxin Hao¹, Jeneffer England¹, Susan S. Taylor², Rodrigo Maillard¹

1. Department of Chemistry, Georgetown University, Washington, DC,

2. Department of Pharmacology, University of California, San Diego, La Jolla, CA

Signaling proteins are dynamic macromolecular complexes that sample multiple conformational states. Such conformational plasticity allows these proteins to adapt and respond to different biological signals. Protein Kinase A (PKA) is a signaling protein that oscillates between inactive and active conformations depending on cAMP concentration. In this study, we use single molecule optical tweezers to dissect the pathways of communication between PKA domains that enable the progression from inactive to active conformations. By using this novel approach, we show that cAMP triggers networks of communication between the two cAMP binding domains of the PKA regulatory subunit that involve direct, interfacial domain contacts as well as long-range interactions between non-contiguous structural motifs. In contrast, without ligand the two cAMP binding domains behave as independent, non-interacting structures, illustrating how cAMP turns on and off domain communication networks. The selective manipulation of the regulatory subunit bound to the catalytic subunit reveal that the inactive PKA holoenzyme is in a dynamic equilibrium between two conformational states, wherein the two cAMP binding domains establish different sets of interactions with the catalytic subunit. By changing the pulling axis in the regulatory subunit, we identify an energetic hub in the regulatory subunit whose mechanical perturbation triggers the highly cooperative and coordinated dissociation of the PKA holoenzyme. Altogether, our results show how this signaling complex propagate ligand binding signals throughout the protein structure to regulate protein function. Our experimental approach based on optical tweezers should be readily applicable to dissect domain communication networks in other signaling proteins.

Session II Speaker

Lisa M. Jones, Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD 21201

Title: “Extension of Hydroxyl Radical-Based Footprinting Coupled with Mass Spectrometry for In Cell and *In Vivo* Protein Analysis”

Abstract: In recent years, protein footprinting coupled with mass spectrometry has been used to identify protein-protein interaction sites and regions of conformational change through modification of solvent accessible sites in proteins. Hydroxyl radical-based footprinting (HRBF) approaches utilize hydroxyl radicals to oxidatively modify the side chains of solvent accessible amino acids. There are several approaches to generate radicals for oxidation including synchrotron radiation and

electrochemistry. One HRBF method, fast photochemical oxidation of proteins (FPOP), utilizes an excimer laser for photolysis of hydrogen peroxide to generate hydroxyl radicals. To date, HRBF methods have been used *in vitro* on relatively pure protein systems. We have further extended the FPOP method for in cell analysis of proteins. This will allow for study of proteins in their native cellular environment and be especially useful for the study of membrane proteins which can be difficult to purify for *in vitro* studies. We have designed and built a single cell flow system to enable uniform access of cells to the laser. Results demonstrate that in cell FPOP (IC-FPOP) can oxidatively modify over 1300 proteins in various cellular compartments. Further, the method successfully probes solvent accessibility similarly to *in vitro* FPOP. We have further extended the method for *in vivo* analysis using *C. elegans*, members of the nematode family. *C. elegans* are widely used as model systems for human diseases including cancer, Parkinson's disease, and diabetes. Preliminary results indicate a number of proteins can be oxidatively modified in *C. elegans* by *in vivo* FPOP (IV-FPOP) leading to the possibility of studying protein structure in human diseases directly in animal model systems. However, further optimization of the method is required to increase the number of oxidatively modified proteins.

Morning Keynote Speaker

Carmen Drahl, Freelance Chemistry Journalist

Title: "I Communicate Chemistry (And So Can You!)"

Abstract: Most researchers don't become science journalists. But to thrive in this day and age, every researcher must be an effective science communicator. This talk explains who science journalists are, and what skills help someone thrive in this career. It also explains how researchers can improve their chances of getting work noticed by the press. Finally, this talk will address strategies for communicating more effectively to non-scientists and to policymakers.

Session III Speaker:

Mark A Allen, University of Maryland, Baltimore County, Baltimore, MD 21250

Title: A Biological Toolbox: Making a Connection

Abstract: Throughout the past it seems that nature has been confronted with countless challenges that eventually were surmounted through the application of natural selection. These challenges have involved thriving in toxic environments and developing ways of finding water in the driest of places; successful organisms have adapted by exploiting a diverse material toolbox. The tools in the toolbox include nucleic acids, lipids, polysaccharides, and proteins, each of which have been used for direct applications far different from their defined purposes to make sensors or other electronic devices. Proteins in particular represent nature's most diverse polymer with a range of functionality determined by 20 naturally encoded amino acids. In this presentation I will discuss the application of proteins or polypeptides for the purpose of addressing challenges that biological systems have never previously had to hurdle with an emphasis on bio-templated lithium ion batteries.

While natural selection ends at modifications to the environment that allow generations of organisms to continue, our lab uses a form of artificial selection called phage display in order to address technological problems that are not commonly thought of as relevant to nature. The toolbox remains the same however the application of the tools is to identify solid binding polypeptides that have very strong and specific interactions with electroactive materials and to identify and exploit these interactions in order to improve devices with an emphasis on the improvement of lithium ion batteries.

This talk will present findings that describe how solid binding polypeptides interact with functional inorganic materials. The application that is always on the horizon is the improvement in lithium ion batteries; however, fundamental organic/inorganic interactions will be explored and data will be presented that focus on how the polypeptides interact with the surface of the particles.

Session IV Speaker

Martin Schnermann, NIH

Title: Near-IR Uncaging Chemistry: Discovery and Applications

Abstract: The combination of caged small molecules and the spatially controlled application of light provides a conceptually powerful approach to modulate biological systems. Long wavelength photocaging groups are needed to translate these techniques out of cellular settings and into more complex organismal applications. Our research program centers on the design, development, and application of broadly useful uncaging reactions initiated by light in the near-IR range. The guiding hypothesis is that we can discover such reactions by defining and then using the innate reactivity patterns of frequently used fluorophores scaffolds. We have developed an uncaging reaction using C4'-dialkylamine-substituted heptamethine cyanines initiated by light of up to 800 nm. Release occurs through a sequence initiated by regioselective photooxidative C-C cleavage (a reaction previously only associated with cyanine photodegradation). We are developing a strategy to use these molecules as the linker domain for a near-IR light activated antibody-drug conjugate (ADC) strategy. Using rational design, compounds with properties suitable for *in vivo* use have been identified. The conjugates can be readily imaged using the fluorescence of the cyanine scaffold prior to uncaging with an external laser source. The optimal structures, which release a derivative of the natural product duocarmycin, exhibit significant anti-tumor efficacy. Details regarding the development of the uncaging reaction, ongoing mechanistic and optimization studies, and long-term goals will be described.

Afternoon Keynote Speaker

Joel Schneider, National Cancer Institute

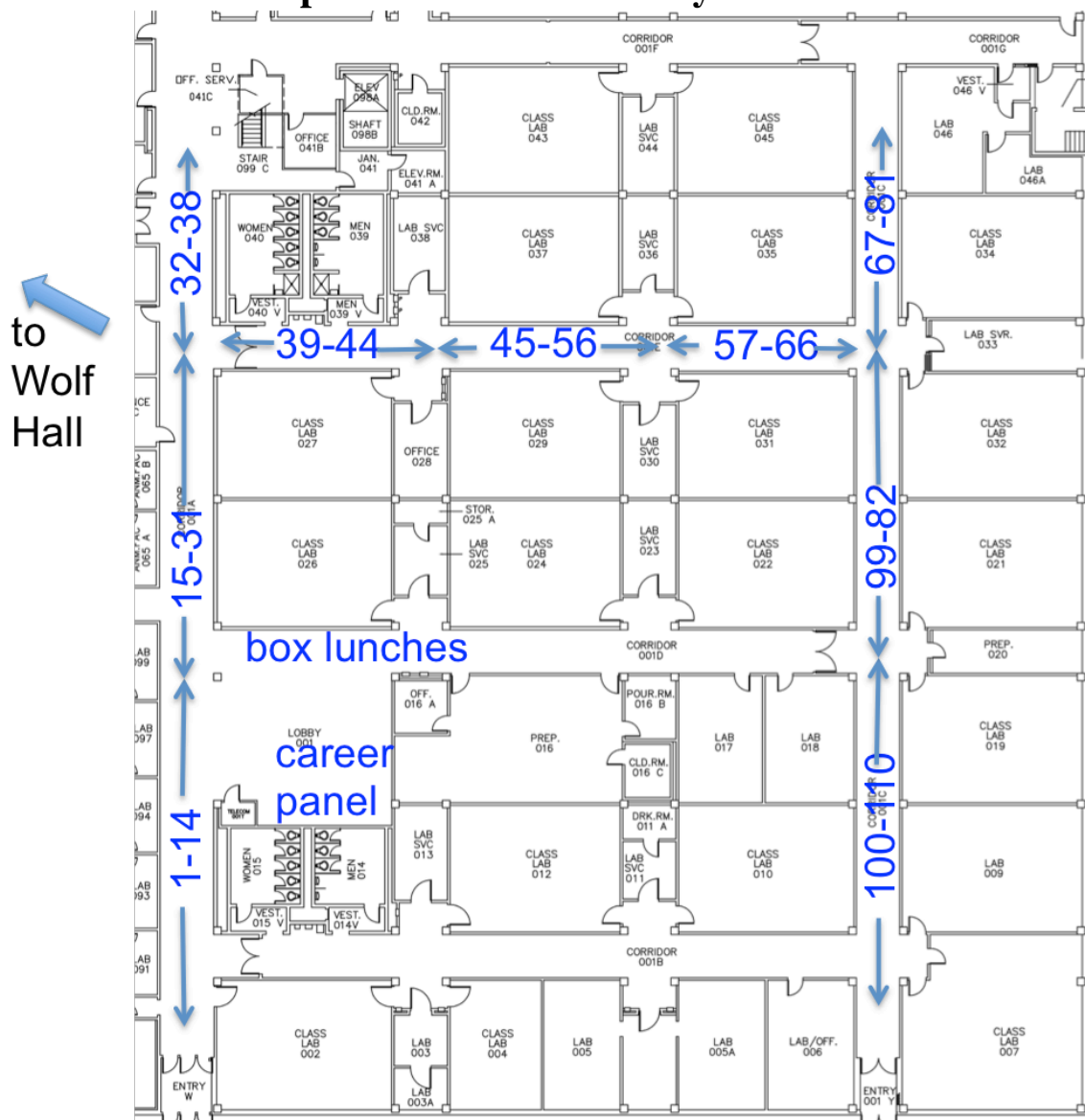
Title: "Racemic hydrogels from self-assembling mirror image peptides: Predictions from Pauling and Corey"

Abstract: Hydrogels prepared from self-assembling peptides are promising materials for medical applications, and using both L- and D-peptide isomers in a gel's formulation provides an intuitive way to control the proteolytic degradation of an implanted material. In the course of developing gels for delivery applications, we discovered that a racemic mixture of the mirror-image b-hairpin peptides, named MAX1 and DMAX1, provides a fibrillar hydrogel that is four-times more rigid than gels formed by either peptide alone – a puzzling observation. Transmission electron microscopy (TEM), small angle neutron scattering (SANS), solid state NMR, diffusing wave, infra-red, and fluorescence spectroscopies, and modeling was used to determine that enantiomeric peptides assembled into a structure predicted by Pauling and Corey in 1953, which provides the molecular basis for the increased mechanical rigidity of the racemic gel. Molecular level understanding of the peptide hydrogel network allows the rational design of materials for specific applications, for example, multiphase transitioning gels that facilitate the suturing of ultrasmall blood vessels.

12:30-1:15: Poster Session I (odd #s) – *McKinly Atrium*

1:15-2:00: Poster Session II (even #s) – *McKinly Atrium*

Map of Posters in McKinly Atrium



American Chemical Society Publications is sponsoring our poster awards. First, second and third prizes will be awarded from both ACS Chemical Biology and from Biochemistry. (1st - \$200, 2nd - \$125, 3rd - \$75)

The Career Panel will run concurrent with the poster sessions.

Speaker Abstracts

- Poster # 1 **Adeniji-Adele, Adetoun**, Donohue, Michael; Van Horn, Cassandra; Tomsho, John
University of the Sciences in Philadelphia
Title: Biosynthesis of anti-tuberculosis peptide, lariatin A
Abstract: Lasso peptides are ribosomally assembled and post-translationally processed natural products with a unique threaded lariat structure. This architecture makes them highly stable and is important for their activity as antibacterials, antivirals and antimycobacterials. The tuberculosis (TB) bacterium are worryingly resistant to drugs that target translational or transcriptional regulation. The lasso peptide Lariatin A has been chosen for study since it exhibits high specificity and activity against TB and may act via a novel mechanism of action. Because lasso peptides are inaccessible to organic chemical synthesis, we have successfully constructed a heterologous expression system for lariatin A in *E. coli*. Utilizing this system, we have shown that the wildtype and several alanine mutants have activity against *Mycobacterium smegmatis*. We are working to create a more robust production system by RBS optimization of the vectors in the system and differential induction of enzymes in the biosynthetic pathway. We believe that this process will produce a regulable and robust system for lariatin A production. This increased production will generate material that can be used to investigate the mechanism of action of lariatin A against mycobacteria.
- Poster # 2 **Ageeli, Abeer**, Baird, Nathan University of the Sciences in Philadelphia
Title: Mechanisms of RNA Triple Helix Stability: Biophysical Insights into the Functional Persistence of the Oncogenic MALAT1 Long Non-Coding RNA
Abstract: Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is a highly abundant nuclear long non-coding RNA involved in many cancer-related processes such as cell proliferation and migration leading to metastasis. A small, 94 nt triple helix found at the 3' end protects the transcript from degradation, resulting in persistently high levels in several cancer types. Therefore, the MALAT1 triple helix (TH) has been identified as a target for therapeutic intervention. We examine the dynamic conformations of the TH and their respective stabilities using UV melt and FRET assays. We designed five different MALAT1 TH constructs to probe the role of various structural elements in triple helix formation. Our results show that the stability of the W-C paired strands of the TH is significantly reduced in the absence of the TH Hoogsteen strand. Removal of a non-structured loop destabilizes the TH in a manner similar to the destabilization resulting from removal of an apical hairpin. We also employ a multidimensional, high throughput FRET assay to simultaneously monitor the TH conformation and stability in response to environmental conditions such as monovalent concentration, divalent concentration, and temperature. For these and other studies, we have developed a novel 3' end labeling protocol for template addition of modified nucleotides using the Klenow fragment. Our FRET results demonstrate that addition of monovalent ions destabilizes the MALAT1 TH over a broad range of Mg²⁺ concentrations. Future work will include mutational analyses and high-throughput chemical screening to identify binding sites and small molecules for therapeutic intervention.
- Poster # 3 **Al-Amin, Mohammad**, Moon, Daniel J.; Lewis, Robert S.; Arnold, Kimberly M.; Yap, Glenn P. A.; Mourtada, Jennifer Sims; Chain, William J. University of Delaware
Title: A General Approach to Icetexane Natural Products
Abstract: Icetexane diterpenoids are richly complex polycyclic natural products that have been described with a variety of medicinally significant biological activities. They are generally isolated as 6-7-6 tricyclic system. The decorated 6-7-6 tricyclic diterpenoids have been shown with a variety of important medicinal interests and particularly

Premanalitifolin A is an interesting member of this family. Premnalatifolin A, a novel dimeric diterpene was reported to have cytotoxic activity against seven human cancer cell lines in vitro. The most potent activity was exhibited against breast cancer (MCF-7) with an IC50 of 1.77 microMolar, which compared favorably with doxorubicin (Adriamycin), one of the current standards of care (IC50 = 3.68 microMolar). We are interested in the synthesis of monomeric and dimeric icetexane diterpenoid natural products. We will talk and discuss a general synthetic approach toward the important 6-7-6 tricyclic core structure of these interesting synthetic targets based on a two-step enolate alkylation and ring-closing metathesis reaction sequence.

Poster # 4 **Anderson, Erin**, Gorka, Alexander; Schnermann, Martin National Cancer Institute
Title: Photoredox-mediated Near-IR Uncaging from Silicon Phthalocyanines
Abstract: Established near-IR light-initiated approaches to treat cancer (photodynamic therapy (PDT), singlet oxygen-mediated near-IR uncaging) require oxygen. These approaches are not effective in solid tumors with prevalent hypoxic microenvironments. Alternative methods of near-IR uncaging that do not depend on oxygen are needed, but the identification of a physiologically stable chemical bond that can be directly cleaved with low-energy long-wavelength light is a challenge. We have optimized a photoredox-mediated silicon oxygen bond cleavage reaction that uncages a small molecule upon irradiation with 690 nm light under hypoxic conditions. Extensive mechanistic studies confirm that small molecule release is initiated by photoinduced electron transfer. Furthermore, we have established that glutathione, an intracellular reducing agent, is effective as the electron donor to initiate the uncaging reaction. Finally, we have applied this system in a biological context through the design of a silicon phthalocyanine that undergoes near-IR uncaging in hypoxia while remaining active as a PDT agent under normoxic conditions. Through cellular studies, we have confirmed an oxygen-dependent switch in mechanism of action: near-IR uncaging of a chemotherapeutic under hypoxia and reactive oxygen species mediated PDT under normoxia. The discovery of a near-IR uncaging method that is not dependent on oxygen allows near-IR light to target both hypoxic and normoxic tumor cells with a single molecule.

Poster # 5 **Arunendra Saha Ray**, Pak, Yewon; Joanna; Meares, Adam; Swaan, Peter; Ptaszek, Marcin; Daniel, Marie Christine University of Maryland
Title: Analysis of Toxicity and Cellular Localization of PPI Dendron-TEG Gold Nanoparticles For Bioimaging
Abstract: One of the promising avenues for delivery of poorly water soluble chemotherapeutics in increased effective concentration is through the use of nanoparticles and presently various novel nanoparticle-based drug delivery systems (liposomes, dendrimers, micelles, gold nanoparticles) are being investigated. The use of nanovectors has been a promising avenue due to their inherent versatility, increased multivalency and ability to passively target tumors through the enhanced permeability and retention (EPR) effect. Within the diverse field of nanovectors, dendrons and dendrimers (synthetic macromolecular polymers with tightly controlled geometry and structure) have shown impressive potential for targeted delivery of therapeutics.

This project is aimed towards the preparation of dendron carrying gold nanovectors (hydrodynamic radius 30 nm) with various terminal functional groups (amine, carboxylate, and hydroxyl) for their use in drug delivery and in vivo imaging. The aforementioned nanovectors have been designed with four complimentary components. (i) A 20 nm gold core (ii) Dendrons carrying imaging agents such as: (DOTA chelated Gd) for MRI and water-soluble Cy 7 dye for fluorescence imaging. (iii) Dendron carrying tumor recognition

moieties (e.g. carrying monoclonal antibodies) for active targeting (iv) Dendrons carrying chemotherapeutics with specific release profile (e.g. pH sensitive release of doxorubicin, docetaxel). In the present research, gold nanoparticles have been utilized as a non-classical dendrimer core due to its well-established biocompatibility. The dimension of the gold nanoparticles has been carefully selected to minimize rapid clearance via renal filtration or elimination via macrophages due to opsonization. For this project, poly(propyleneimine) dendrons were prepared with various terminal functionalities. Additionally, a TEG (Tetra Ethylene Glycol) spacer was incorporated at the focal point of the dendron to minimize steric repulsion between the branches as well as to increase biocompatibility of the nano construct. The cytotoxicity and cellular fate of the thus prepared dendronized gold nanoparticles was studied in MCF-7 breast cancer cell lines using fluorescence microscopy.

For the purpose of drug delivery, it is important that the drug delivery scaffold maintains the integrity of cellular membrane without exhibiting inherent cytotoxicity. To elucidate the cellular fate of the nanocarrier, chlorin-carrying dendrons were utilized to visualize the nanoparticle compartmentalization within cellular organelles. Furthermore, the integrity of the cellular membrane was studied as a function of the surface charge of nanoparticle coatings. From the studies, an optimum scaffold was identified for the creation of theranostics which will be tested for efficacy of drug delivery against PC3 cancer cell lines as well as MCF-7 breast cancer cell lines.

Poster # 6

Barannikova, Evgenia, Allen, Mark University of Maryland Baltimore County

Title: Dual affinity solid-binding peptides as templates for nanoscale organization of Li-ion battery electrodes

Abstract: Biotemplating presents a unique approach for the synthesis and organization of materials at the nanoscale. Among other biotemplates, solid-binding peptides (SBP) isolated for the material of interest provide high binding affinity and selectivity due to distinctive combination of functional groups found in amino acids. Applications of nanomaterials assembled and synthesized with SBP range from drug delivery to catalysis and energy storage, to list just a few. Specifically, my project is focused on developing a peptide-templated approach for assembly of heterofunctional cathode materials for Li-ion batteries using dual affinity solid-binding peptides. The cathode is composed of the two major materials, electroactive and conductive materials. When the battery undergoes multiple charge and discharge cycles, the two materials lose contact between each other leading to decrease in battery performance. Thus, my goal is to design a dual affinity nanoscale bridges that would bind to both entities and precisely control the assembly of electroactive nanoparticles in close contact with conductive material, which will result in better conductivity, structural support and electrochemical stability upon cycling. In particular, using phage display procedure I have isolated SBP that have an affinity for one of the promising high-voltage cathode materials $\text{LiNi}_{0.5}\text{Mn}_{1.5}\text{O}_4$ (LMNO). The binding affinity of the isolated peptides is determined via relative phage binding studies. In order to design a dual affinity polypeptide, SBP for electroactive material is combined with SBP for multiwall carbon nanotubes (MWCNTs), which are known to improve conductivity of Li-ion battery electrodes. Our preliminary data indicates that the cathode materials assembled with dual-affinity polypeptide exhibited a decrease in charge transfer resistance, which corresponds to improved movement of electrons in the system. We also observed improvement in Li-ion diffusion, as well as an increase in specific capacity during the initial cycles. The future research will be focused on exploring other binding peptides, modifying the composition of dual affinity polypeptide, and optimization of system parameters for cathode assembly. Ultimately, our goal is to show that fabrication of nanostructured electrodes through biotemplating with bifunctional solid-binding peptides

will result in higher conductivity, as well as improved morphological stability, and cyclability for Li-ion battery.

Poster # 7

Bartee, David, Sanders, Sara; Monts, Josh; Freel Meyers, Caren Johns Hopkins University

Title: Inhibition of 1-Deoxy-D-xylulose 5-phosphate (DXP) synthase towards the development of new antimicrobial agents targeting bacterial central metabolism

Abstract: Antibiotic-resistant infections are a growing problem in the US and globally. To fight this ever-increasing public health threat, new antibiotic agents are needed. 1-Deoxy-D-xylulose 5-phosphate (DXP) synthase is an emerging antibacterial target due to its importance in bacterial central metabolism and isoprenoid biosynthesis. DXP, a vital branch point metabolite, is processed to become vitamins B1 and B6 as well as the essential 5-carbon isoprenoid precursors dimethylallyl diphosphate and isopentenyl diphosphate; thus, its inhibition is an attractive approach to halting several essential metabolic pathways simultaneously. Towards this end, our lab has developed inhibitors of DXP synthase that exploit both its large active site and unique mechanism through the use of the acetyl phosphonate moiety as a pyruvate mimic. Here, we show the development of homopropargyl acetyl phosphonate as a versatile precursor for the synthesis of a library of chemically diverse acetyl phosphonates through CuAAC chemistry and their evaluation both in vitro and in vivo. Using this approach, we have identified several nanomolar inhibitors of DXP synthase with growth inhibitory activity against E. coli. These compounds show promise as probes to increase understanding of the unique mechanism of DXP synthase and leveraging that knowledge to develop new antimicrobial agents.

Poster # 8

Bawazir, Nada, Beshay, Mariam; Christopher, Janetopoulos University of the Sciences in Philadelphia

Title: Plasma membrane PI(4,5)P₂ levels regulate chemotactic signaling pathways and actin networks in Dictyostelium

Abstract: Phosphatidylinositol 4,5-bisphosphate (PIP₂) has been shown to be involved in the chemotactic signaling pathways in Dictyostelium. However, the role of PIP₂ in these signaling networks is not fully understood. In polarized cells, PIP₂ levels at the leading edge turn over by the activity of PI3'Kinase (PI3K) and phospholipase C (PLC). The tumor suppressor, PTEN, distributes to the back and contains a PIP₂ binding motif which may help maintain higher PIP₂ levels at the back in a positive feedback loop. We have found that the activity of the GTPase Ras is reciprocally regulated with local PTEN localization during polarity. This suggests that high PIP₂ levels inhibit Ras activity, regulating a negative feedback loop at the rear of the cell. We hypothesize that PIP₂ negatively regulate chemotactic signaling events leading to cell protrusions. To test this, we used the mutant strain lacking the PI5 kinase pik1 (pik1⁻). These cells have highly reduced PIP₂ levels and higher Ras activity compared to wild-type cells. We found that Pik1⁻ resemble other phenotypes we see when we deplete PIP₂. We observed that the PIP₃ and Ras biosensors diffuse to the cytosol when the pik1⁻ round-up and translocate to the plasma membrane when pik1⁻ spread indicating that the PIP₂ levels may further go down as cells spread, and rebound as cells contract. The localized changes of Rac GTPase, a small g-protein involved in actin remodeling, suggests that branching F-actin polymerization takes place when cell spreads which is confirmed by the spial and temporal activity of actin biosensor LimE. Remarkably, these signaling events are not affected when actin polymerization was greatly inhibited, as PIP₃ and Ras biosensors showed wide crescents in LatA-treated cells. Data suggest that chemotactic signaling network is regulated upstream of actin reorganization. This work shows that PIP₂ serves as an inhibitory role on the upstream chemotactic signaling molecule Ras. This might explain its high activity in pik1⁻. Production of PIP₂ in

pikI- by synthetically recruiting a PI5K using a rapamycin-inducible system should inhibit signaling events and lower basal levels of Ras and PI3K activity.

Poster # 9

Borowski, Joe, Carpenter, Megan; Kalburge, Sai; Boyd, E. Fidelma University of Delaware

Title: Diversity of CRISPR-Cas systems carried on pathogenicity islands in *Vibrio cholerae* and related species

Abstract: Pathogenicity islands (PAIs) are mobile integrated genetic elements that contain a diverse range of virulence factors. PAIs integrate into the host chromosome at a tRNA locus that contains a specific bacterial attachment site, known as attB, via integrase mediated site-specific recombination and generates attL and attR sites on either side of the newly integrated PAI. Previously, conserved recombination modules (integrases and att sites) in PAIs that contained novel cargo genes were identified. One of these PAIs was found to have a clustered regularly interspaced short palindromic repeat (CRISPR)-associated proteins (Cas proteins) that integrated at the *Vibrio* Pathogenicity Island-1 (VPI-1) insertion site in nineteen *V. cholerae* strains and contained the same recombination module as VPI-1. Two divergent CRISPR-Cas systems were identified among these strains, which differed in Cas protein homology and Cas protein content. In silico analysis suggests that the CRISPR-Cas systems are active against phages and plasmids. These data demonstrate a novel method of acquisition for functional CRISPR-Cas systems in bacteria. Further analysis identified the *V. cholerae* CRISPR-Cas system in several other *Vibrio* species. In addition, we identified a diversity of additional novel CRISPR types including type I-C, type II-B, and type III-B systems in 45 different species within the Vibrionaceae family.

Poster # 10

Brandis, Joel, Neu, Heather; Williams, Anne; Aaron, Nicole; Polli, James; Michel, Sarah University of Maryland School of Pharmacy

Title: Development of Physicochemical Quality Control, and Bioanalytical Mass Spectrometric Approaches to Measure Iron Release in Plasma of Patients Treated with IV Iron Preparations

Abstract: Patients with chronic kidney disease (CKD) associated iron anemia are most commonly treated with Intravenous (IV) iron preparations composed of iron colloidal nanoparticles suspended in sucrose. Currently, there is only one product for which both an FDA approved innovator (ferrlecit) and generic (sodium ferric gluconate) exist. Under normal conditions, these IV iron preparations function to deliver iron to transferrin, a plasma iron binding protein, to be used in later cellular processes. However, under iron overload conditions, transferrin becomes oversaturated and the remaining iron (labile iron) is non-specifically delivered to cells leading to damage by reactive oxygen species (ROS). Recently, there has been some concern, based on several toxicity reports, that generic sodium ferric gluconate may release iron in blood plasma differently than ferrlecit leading to the reported toxicity. To begin to determine if these toxicity concerns are warranted, we have developed and modified a series of bioanalytical assays to determine iron distribution in plasma. Additionally, several quality control experiments were conducted to compare the physicochemical properties of both brand and generic. Herein, we found plasma total iron (TI) concentrations to be quantifiable by an inductively coupled plasma mass spectrometer (ICP-MS). To validate the ICP-MS ability to quantify TI, a modified established colorimetric ferrozine assay was utilized. Further experiments with benzenesulfonic acid (BCX) silica gel resin have shown a potential to separate labile iron from other plasma iron binding species.

Poster # 11 **Burch, Jason**, Wykoff, Dennis; Grimes, Catherine University of Delaware
Title: Understanding the Molecular Recognition of Carbohydrates by the *C. albicans* Adenylyl Cyclase, CYR1p

Abstract: *C. albicans* is the most common human pathogenic fungus. Despite its prevalence as a member of the microbiota in the human gastrointestinal tract, most individuals do not experience a problem; however, in immunocompromised individuals the fungus changes into an opportunistic pathogen. To become pathogenic, the fungus undergoes a phenotypic switch from budding yeast to filamentous hyphae, which allows *albicans* to invade the epithelium and enter the blood stream. To develop methods to fight *Candida* infections, it is important to understand what is causing the cell to change phenotype. Expression of many hyphae related genes is induced by increased levels of cyclic AMP (cAMP). The *albicans* genome contains only one protein that exhibits adenylyl cyclase activity, CYR1p. Besides the adenylyl cyclase domain, CYR1p contains a G α , Ras association domain, and Leucine Rich Repeat (LRR) domain, which act as signal integrators to regulate the production of cAMP. Interestingly, the LRR domain, like those found in human innate immune receptors, recognizes fragments of the bacterial peptidoglycan, and these fragments have been shown to induce hyphae growth. We have expressed and purified the LRR domain of CYR1p, and using SPR and FP assays will determine the binding affinity of different peptidoglycan fragments. Additionally we are testing a variety of carbohydrates and peptidoglycan fragments to determine the minimal peptidoglycan motif necessary to induce hyphae growth in *C. albicans*.

Poster # 12 **Butchbach, Matthew E. R.**, Kirk, Ryan W.; Harris, Ashlee W.; Hinkle, Kyle M.; Connell, Andrew J.; Pesyan, Amir; Butchbach, Matthew E. R. Nemours/Alfred I. duPont Hospital for Children

Title: Identification of Novel CNS-Active Inducers of SMN2 Expression

Abstract: Spinal muscular atrophy (SMA), a leading genetic cause of infant death worldwide, is an autosomal recessive motor neuron disease caused by the loss of SMN1 but retention of SMN2. The number of copies of SMN2 inversely correlates with disease severity in SMA patients making SMN2 a target for therapeutics development. AurimMed Pharma, Inc. has developed a focused library of potent CNS active compounds. In this study, we screened this library for modulators of SMN2 expression using reporter cell lines as well as fibroblasts derived from SMA patients. The compound library was screened using NSC34 motor neuron-like cell lines expressing either a reporter gene under the control of the 3.4 kb SMN2 promoter or a mini-gene construct containing exons 6 through 8 of SMN2 as well as their intervening introns. The effects of hit compounds on SMN2 expression were measured in type II SMA fibroblasts (GM03813) by quantitative real-time PCR, immunoblot and immunofluorescence. Of the 64 compounds in this library, 26 showed enhanced SMN2 promoter activity relative to vehicle (DMSO)-treated cells and 5 showed increased inclusion of exon 7. Some of these hits were validated in GM03813 type II SMA fibroblasts. 5 compounds—AMP-X-0079, AMP-X-0080, AMP-X-0026, AMP-X-0009 and AMP-X-0024—increased SMN2 mRNA and protein levels in these cells. We have identified new compounds that increase SMN2 expression in NSC34 cells and in SMA fibroblasts. Future work will determine the mechanisms by which these compounds are increasing SMN2 expression. Furthermore, these compounds will be moved forward into preclinical studies using mouse models of SMA.

Poster # 13 **Byrne, Shane**, Hutchinson, Mark; Rokita, Steven Johns Hopkins University

Title: Reversible Alkylation of Nucleosomes by Quinone Methides

Abstract: Quinone methides (QMs) are a class of highly electrophilic compounds that are formed in vivo from the metabolism of various drugs and toxins, such as mitomycin C, butylated hydroxytoluene, and tamoxifen. QMs alkylate and cross-link DNA via the formation of both irreversible and reversible DNA adducts. The reversible covalent chemistry permits a dynamic alkylation and crosslinking of DNA. BisQMs, which contain two electrophilic methylene groups on the same aromatic ring, were developed to examine the migration of DNA cross-linking via sequential QM generation. A BisQM conjugated to acridine (BisQMPAc) was observed to migrate along a duplex DNA scaffold. The mobility of QMs along DNA may allow them to evade repair in vivo. Nevertheless, the initial design migrated too slowly and now new DiQMs conjugated to ammonium linkers have been synthesized in an attempt to facilitate QM migration. DiQMs contain the electrophiles on two distinct aromatic rings, which facilitates independent QM generation. However, in cells, DNA does not exist free in solution, but rather packaged around an octamer of four histone proteins to form the nucleosome core particle (NCP). This packaging serves to compact the DNA so that it fits within the volume of a cell. This raises numerous questions concerning the preference for QM reaction with the NCP. In particular, QMs react with both DNA and protein in the NCP, but their DNA reaction is significantly suppressed relative to free DNA. Here, we demonstrate that the compaction of DNA by the NCP provides protection against reaction with QMs. The histone proteins serve as a target for QM alkylation, both directly and as a result of transfer from DNA to protein. In addition to the role of the NCP in DNA compaction, the NCP also serves as a regulator of epigenetic information. The histone tails are subject to numerous electrophilic modifications in vivo that influence gene expression. Future studies will seek to determine how QMs affect epigenetic regulation.

Poster # 14 **Cawrse, Brian**, Lapidus, Rena; Seley-Radtke, Katherine University of Maryland, Baltimore County

Title: Antiproliferative activity of N-substituted pyrrolo[3,2-d]pyrimidines and their potential use as therapeutic agents.

Abstract: Halogenated thieno- and pyrrolopyrimidines have been developed by the Seley-Radtke lab and show potent antiproliferative activity against multiple cancer cell lines. The pyrrolo[3,2-d]pyrimidines, commonly called 9-deazapurines, are a medically important group of compounds that have shown activity as bactericides and protozoicides, and act as anti-tumor agents through inhibition of a variety of enzymes. A structure-activity relationship (SAR) study showed that KSR4 is a potent inhibitor of the TNBC MDA-MB-231 cell line, and caused an accumulation of cells in the G2/M stage with little apoptosis. This was consistent with earlier reports of 9-deazapurines used as antiproliferative agents. The introduction of an iodine at C7 (KSR6) resulted in increased activity, with the IC50 against MOLM14 cells decreasing from $1.0 \pm 0.8 \mu\text{M}$ for KSR4 to $0.055 \pm 0.07 \mu\text{M}$ for KSR6. Surprisingly, the halogen also altered the apparent mechanism of action, with the majority of the cells now undergoing apoptosis (data not shown). These findings led us to further investigate the effect of substituents on the pyrrole moiety of these compounds with the aim to retain or increase activity while decreasing toxicity.

Poster # 15 **Cheng-Chieh, Tsai**, Christopher Ellis; Robert Harris; Jack Henderson University of Maryland Baltimore

Title: Addressing Challenges in Drug Design through Novel Computer Simulations

Abstract: Targeting BACE1 with small-molecule inhibitors is a potential treatment for Alzheimer's disease. Despite enormous efforts made in the past decade, no inhibitors have progressed to the market. Designing BACE1 inhibitors is challenging because subtle pKa differences between inhibitors greatly affect their efficacy. Further, BACE1 inhibitors often have high affinity for other structurally similar proteases leading to side effects. Here we use continuous constant-pH molecular dynamics (CpHMD) to examine the pH-dependent binding of inhibitors to BACE1 and off-targets. The work demonstrates the potential of CpHMD as a useful tool in structure-based drug design.

Poster # 16 **Childers, Kenneth**, Amason, Joshua; Giannakoulis, Sam; Garcin, Elsa D. University of Maryland, Baltimore County

Title: Design of a medium-throughput assay to identify novel activating mutations in soluble guanylate cyclase

Abstract: Cardiovascular diseases contribute to the highest mortality rate in the United States. Soluble guanylate cyclase (sGC) catalyzes the conversion of guanosine triphosphate (GTP) into cyclic GMP (cGMP), which acts on downstream kinases to inhibit platelet aggregation and stimulate vasodilation, maintaining cardiovascular health. Dysfunction in this pathway including, but not limited to, low NO bioavailability or an oxidized heme group, both due to reactive oxygen species, can contribute to lowered cGMP output and decreased cardiovascular health. The sGC heterodimer uses an N-terminal reduced heme cofactor to bind nitric oxide (NO) and increase activity 100-200-fold. How NO binding at the N-terminus leads to increased catalytic activity at the C-terminus remains largely uncharacterized.

Our lab has solved the high-resolution structure of the catalytic heterodimeric wild-type $\alpha\beta$ GC domain in its inactive conformation. Activity assay results showed that this construct displays ~0.01% of full-length sGC basal activity, suggesting that additional sGC domains are required to align active site residues into a catalytically-competent position.

Our current efforts are focused on (1) obtaining a high-resolution structure of the active catalytic domain, and (2) identify networks of residues that promote formation of the catalytically competent active site. One strategy to accomplish this is to identify residues that increase basal activity. So far, only two such mutations have been identified in the catalytic domain and outside the active site. We have designed a medium-throughput luciferase assay in bacterial cells to identify novel activating mutations in various sGC constructs. We have obtained proof-of-concept data showing the feasibility of the assay with known activating and deactivating sGC mutations. Structure-based and random mutations are currently being tested to identify potential networks of residues tuning sGC activity.

Identifying residues that modulate sGC activity will provide crucial information to understand the mechanisms underlying sGC activation by NO and may suggest alternate routes for the design of novel small molecules that can modulate of sGC activity.

Poster # 17 **Clupper, Michael,** Tanis, Jessica University of Delaware

Title: Investigation of the localization of *C. elegans* ion channel CLHM-1 and its role in dye-filling sensory neurons

Abstract: Calcium (Ca^{2+}) level in neurons is tightly regulated and dysregulation of Ca^{2+} homeostasis can have cytotoxic effects and disrupt neuronal function, which can manifest in neurodegenerative disorders. Calcium homeostasis modulator 1 (CALHM1) is a Ca^{2+} -permeable ion channel that is expressed in the brain. Mutations in CALHM1 have been shown to accelerate the onset of late-onset Alzheimer's disease, yet the precise physiological function of CALHM1 in neurons is unknown. The versatile model organism *Caenorhabditis elegans* possesses a single CALHM1 homolog, CLHM-1, which is functionally conserved. This affords us a powerful tool to elucidate the precise localization and functions of CLHM-1 in *C. elegans* neurons. Using this model, we find that CLHM-1 is localized to the ciliary tips of the IL2 sensory neurons in the head. The IL2s take up the dye DiI when animals are exposed to low ionic extracellular solution. We have determined that loss of CLHM-1 does not prevent the uptake of DiI into the IL2s or other chemosensory neurons regardless of extracellular salt concentration. Thus, we will continue to perform additional assays to determine the role of CLHM-1 in the cilia of the IL2 neurons.

Poster # 18 **DeColli, Alicia,** Majumdar, Ananya; Nemeria, Natasha; Jordan, Frank; Freel Meyers, Caren Johns Hopkins University

Title: Investigating the mechanism of LThDP decarboxylation by DXPS synthase

Abstract: The rapid development of antimicrobial resistance underscores the need for novel antibiotics. The unexploited drug target 1-deoxy-D-xylulose synthase (DXPS) catalyzes the thiamine diphosphate (ThDP)-dependent formation of DXP from pyruvate and D-glyceraldehyde 3-phosphate (GAP). DXP is an intermediate in the biosynthesis of ThDP, pyridoxal phosphate, and isoprenoids in bacteria but not in humans. Thus, selective inhibition of this branch point enzyme is a potential antibacterial strategy. Previous studies show that DXPS possesses relaxed specificity for its acceptor substrate and proceeds through a unique random sequential, preferred order mechanism. GAP serves two roles, 1) as the trigger for decarboxylation of the first enzyme-bound intermediate lactylThDP (LThDP), stabilized by DXPS in the absence of a trigger, and 2) as acceptor in the carbonylation step. This unprecedented mechanism in ThDP enzymology implies distinct DXPS conformations exist along the reaction coordinate which could offer opportunities for selective inhibitor design and hints at the possibility that DXPS could possess other cellular functions. Studies aimed at understanding factors underlying DXPS stabilization of LThDP and GAP-induced decarboxylation have revealed oxygenase activity of DXPS, an unexpected finding given the observation of LThDP accumulation on DXPS and lack of evidence for post-decarboxylation intermediates in the absence of GAP. Here we present evidence for oxygenase activity of DXPS under conditions where LThDP was previously observed and new insights into triggers of LThDP decarboxylation. Our results suggest that LThDP decarboxylation takes place in an O_2 -dependent manner in the absence of GAP and peracetate is detected as an intermediate in acetate formation. The implications of these findings for DXPS mechanism will be discussed. The identification of distinct triggers of LThDP on DXPS decarboxylation combined with a remarkable flexibility for the acceptor substrate lends further intrigue and support to the notion that this novel mechanism may impart useful, yet uncharacterized cellular functions to DXPS.

Poster # 19 **DeMeester, Kristen E.**, Liang, Hai; Jones, Zachary S.; Grimes, Catherine L. University of Delaware

Title: Synthesis of bioorthogonal uridine diphosphate N-acetyl muramic acids for metabolic incorporation into bacterial peptidoglycan

Abstract: The bacterial cell wall is composed of a thick, web-like polymer known as peptidoglycan (PG). This structure is critical for bacterial survival, as it protects the cell from changes in osmotic pressure and environmental insults. PG structures are essential for complex chemical and biological processes related to human health, as antibiotics are designed to target its destruction while the innate immune system responds to small molecules teased out of the PG network. Building blocks of peptidoglycan that contain the carbohydrate unit N-acetyl muramic acid (NAM) are critical intermediates for innate immune recognition. We desired a method to selectively install bioorthogonal modifications at the NAM residue in order to fluorescently track and ultimately capture glycan immunostimulatory molecules. Uridine diphosphate (UDP) NAMs are the early stage PG biosynthetic intermediates in which we chose to install the bioorthogonal handle. While chemical synthesis and purification of UDP-glycan derivatives proves challenging throughout the carbohydrate community, we developed a robust chemoenzymatic synthesis and preparation of a variety of 2-N functionalized UDP NAMs utilizing relaxed substrate specificity of PG cell wall recycling enzymes MurNAc/GlcNAc anomeric kinase (AmgK) and uridyl transferase (MurU). We further explored the utility of the bioorthogonally tagged UDP NAMs as a tool to metabolically incorporate the chemical handle into the peptidoglycan of *Lactobacillus acidophilus* using click chemistry. This method will allow for rapid and scalable access of N-functionalized UDP NAMs and when metabolically incorporated into whole cell PG, can be used as a tool to track and isolate NAM containing PG fragments.

Poster # 20 **Devannah, Vijayarajan**, Amber A. S. Gietter-Burch University of Delaware

Title: Trifluoromethylation of Secondary Nitroalkanes

Abstract: We developed general method for trifluoromethylation of secondary Nitroalkanes using commercially available trifluoromethylating reagent. Variety of complex trifluoromethylnitroalkanes can be obtained with high yielding and good diastereoselectivity. These compounds can be rapidly converted into Biologically relevant trifluoromethylamine.

Poster # 21 **Diaz, Camil**, Hamaker, Nathaniel; Antoniewicz, Maciek University of Delaware

Title: Quid pro quo: Engineering nitrogen self-sufficient cocultures

Abstract: Diazotrophs, or organisms capable of converting atmospheric nitrogen into ammonia, are attractive coculture partners, offering a sustainable alternative to the Haber-Bosch process as a source of fixed nitrogen. However, efforts to engineer synthetic consortia are currently impeded by difficulties in predicting metabolic compatibility and achieving long-term stability.

In this contribution, we investigated the performance of five, fully nitrogen self-sufficient cocultures involving an ammonium-secreting strain of the aerobic diazotroph, *Azotobacter vinelandii*. Coculture pairings were systematically designed to range from pure commensalism (e.g. with wild-type *E. coli*) to fully nitrogen and carbon self-sufficient, mutualist partnerships with engineered cyanobacteria.

¹³C labeling studies and metabolic flux analysis (MFA) unexpectedly revealed that the diazotroph shared the majority of its fixed nitrogen, even in commensalist cocultures. For example, wild-type *E. coli* constituted more than 60-75% of a coculture population with a

total OD of ~5, and was maintained as 30-40% of the population in later stages, up to a total OD of ~40. Furthermore, negative interactions could be overcome by introducing metabolic interdependence through genetic manipulation, e.g. by engineering the partner to secrete a carbon source suitable for the diazotroph. Preliminary adaptive evolution studies highlighted the surprising stability of such cocultures, where synthetic cross-feeding persisted over weeks of passaging. Ongoing work dually harnesses deep-sequencing and ¹³C-MFA of evolved cocultures to identify additional genetic and metabolic traits that enable enhanced community stability and overall growth.

These results shed insight on how bioprocesses can benefit from the modularity, improved sustainability and potential cost reduction offered by diazotrophic cocultures.

Poster # 22 **Drake, Walter**, Bahnson, Brian; Grimes, Catherine University of Delaware
Title: Purification and Functional Characterization of Nod1, an Innate Immune Receptor
Abstract: The innate immune system serves as the body's first line of defense against invading pathogens. A key component of the innate immune system on the molecular level is a variety of receptors, both membrane bound and cytosolic, that are critical for specifically identifying different pathogens. One class of cytosolic receptors is the Nod-like receptors, which includes the proteins Nod1 and Nod2. Both Nod1 and Nod2 are activated by different components of peptidoglycan (PGN), the repeating sugar-peptide moiety that comprises the bacterial cell wall. Nod1 has been shown to be activated by molecules including GM-TriDAP, M-TriDAP, and iE-DAP, all of which are found in the PGN of Gram negative bacteria. After being activated by these PGN-derived ligands, the NF- κ B signaling cascade is triggered, resulting in the expression of pro-inflammatory cytokines and chemokines. Nod1 is expressed in various body tissues, including the stomach, lungs, and intestines, and is thus associated with various diseases, including stomach cancer, lung cancer, asthma, and irritable bowel syndrome. The structure of Nod1 has yet to be solved, so a homology model was created based off of the structure of rabbit Nod2. Different Nod1 constructs, including full length Nod1 and the ligand binding LRR-domain, have been expressed and purified in *E. coli* in order to assess their ability to bind ligands. Additionally, stable human cell lines have been constructed to express Nod1 in both inducible and over-expressed fashions. These cell lines have been used to detect *in vivo* Nod1 interactors, including heat-shock proteins, as well as post-translational modifications. Future work will determine the affect of these chaperones and modifications on Nod1 stability and activity.

Poster # 23 **Dutt, Reetika**, Thorpe, Colin; Galileo, Deni S. University of Delaware
Title: Emerging role of a sulfhydryl oxidase in glioma cell behavior
Abstract: Quiescin Sulfhydryl Oxidase 1 (QSOX1) is a flavo-enzyme that catalyzes the oxidation of free thiols to generate disulfide bonds with the reduction of molecular oxygen to hydrogen peroxide. QSOX1 has been reported to be upregulated in a number of cancers including breast, prostate, pancreatic and certain brain cancers. Overexpression of QSOX1 has been correlated with aggressive cancers and poor patient prognosis. Gliomas are primary brain tumors that occur mostly in adults. Glioblastoma Multiforme (GBM) is a stage IV glioma that is very aggressive and has been practically impossible to treat successfully. GBM cells invade normal brain tissue very quickly and, thus, escape surgery to lead to an often more aggressive recurrence. There is a continuing need to understand the mechanism of GBM cell migration and invasion. Here we investigate the function of QSOX1 in human gliomas. We lentivirally introduced shRNA to knockdown QSOX1 in a human GBM cell line, T98G. These QSOX1 knockdown cells showed decreased cell migration *in vitro* as demonstrated by a quantitative Super Scratch assay. We also used the embryonic chick brain xenograft model to demonstrate glioma cell invasion *in vivo*. Therefore,

decreased QSOX1 expression leads to decreased glioma cell migration and invasion both in vitro and in vivo, respectively.

Poster # 24 **Eisen, Margaret,** DL; Glotfelty, EJ; Ondeck, CA; Mangkhalakhili, MC; Nelson, MR; Lyman, ME; Schulz, SM; Hamilton, TA; McNutt, PMUS Army Medical Research Institute of Chemical Defense

Title: Acute and chronic pathologies in the rabbit corneal endothelium following in vivo ocular sulfur mustard exposure: towards a new understanding of corneal injury progression

Abstract: Sulfur mustard (SM) reactivity within biological tissues is rapid and irreversible, and consequently current treatment strategies are focused on mitigating pathological host responses and promoting healing. However, therapies to preserve the corneal epithelium and reduce inflammation following ocular exposures have been ineffective in preventing chronic symptoms of mustard gas keratopathy (MGK). Hypothesizing that SM toxicity to the non-regenerative corneal endothelium may present a novel injury modality that could explicate ocular SM injury progression, we characterized long-term effects of corneal exposure to SM vapor on the corneal endothelium in rabbits. Convergent methods revealed that ocular SM exposure causes acute endothelial toxicity and disruption of endothelial barrier function. Furthermore, MGK eyes exhibit long-term endothelial pathologies consistent with the endothelial-to-mesenchymal transition that is associated with endothelial failure. None of these features were observed in SM-exposed eyes that fully recovered. Importantly, the extent of endothelial toxicity was determinative of MGK development. The association of endothelial pathologies with MKG suggests that SM damage to corneal endothelial cells provides a novel mechanistic basis for ocular injury progression. Based on these findings, we hypothesize that (a) the severity of acute SM injury is determined by the degree of endothelial damage and (b) the efficiency of endothelial repair influences whether corneas resolve or develop MGK. These hypotheses explain the dose-dependence of corneal SM symptoms and predict that treatments to reduce endothelial toxicity or promote endothelial recovery will reduce or eliminate acute and chronic manifestations of corneal SM exposure.

Poster # 25 **Ekanayake Oshini,** Liu, Jun; Santoleri, Dominic; Rozovsky, Sharon; University of Delaware

Title: Creating Peptide Hydrazides via Intein Splicing for Expressed Protein Ligation

Abstract: Protein or peptide hydrazides are powerful thioester surrogates for chemical ligation. Hydrazides can also serve as an effective tool in introducing site-selective modifications. Currently, peptide hydrazides are generated mainly via solid phase peptide synthesis (SPPS), which comes with size or solubility limitations. Here we have introduced a recombinant approach to generate C-terminal hydrazides by protein trans splicing mediated by split inteins from *Pyrococcus horikoshii* (PhoRadA). The versatility of this recombinant hydrazide has been shown by its ability to undergo an aldehyde coupling reaction to produce a hydrazone. These hydrazides can further be utilized to generate thioesters for expressed protein ligation.

Poster # 26 **Fang, Bing,** Munsell, Erik; Sullivan, Millicent University of Delaware

Title: Towards Bone Repair: Histone Modified Gold Nanocarriers

Abstract: Large segmental defects are challenging in bone repair. Local substrate based gene delivery provides a capacity for sustained, spatiotemporally controlled release. Researches show that gene delivery of growth factor bone morphogenic protein-2 (BMP-2) is promising in bone healing. However viral gene delivery approaches always involve

concerns like immunogenicity and mutagenesis. Our lab has a strong interest in developing non-viral strategies with improved efficacy and safety. Recent findings have shown that the histone tails play important roles in native gene regulatory control and subcellular trafficking. Previous work in our lab has proved that polyplexes displaying modified histone 3 (H3) tails promote nuclear accumulation, DNA release, transcription, and enhanced transfection. Herein, we developed optimized multifunctional gold nanoparticle (AuNP) carriers decorated by histone motifs, which are supposed to induce efficient gene transfer. We hypothesize that polycationic AuNPs coupled to histone motifs will mimic the native presentation of these sequences on the histone octamer and create structures with the capacity to stably bind as well as controllably deliver plasmid DNA (pDNA).

AuNPs bearing ~2nm cores are prepared based on the well-established Brust-Schiffrin two-phase method involving tetrachloroaurate reduction in the presence of 1-pentanethiol. Through Fmoc solid-phase synthesis procedures, short peptides of poly-Lysine (cationic) in 5 repeating units and histone peptides comprised of 1-25 residues of H3 are successfully fabricated. These peptides were thiolated by conjugation with 11-mercaptoundecanoic acid. Subsequently, Murray place-exchange was employed to install various combinations of thiolated peptides onto the AuNPs. Electron microscopy (EM) was used to analyze the core dimensions of the modified AuNPs. Thermogravimetric analysis (TGA) coupled with C/H/N/S elemental analysis was used to determine the average composition of multi-component ligands on the AuNP surface, and showed that H3-containing ligands were successfully installed at a variety of densities. To test the gene transfer potential of the histone-inspired AuNPs, stable nanoplexes were obtained through self-assembly of AuNPs and pDNA. The dimension and toxicity of the nanoplexes were easily controlled by fine-tuning the ligand composition of the AuNPs and the N/P ratio. Initial analysis of bioactivity showed that the H3-targeted AuNPs were capable of activating histone effector (HBO1), encouraging subsequent studies on AuNP engagement of histone pathways within cells for gene delivery applications.

Poster # 27 **Ferrer, Alexander**, Jones, Aubrey; Wamser, Nicole; Talotta Leanna; Thévenin, Anastasia
Moravian College

Title: In vitro binding studies between Gap Junction Protein, Connexin 43 (Cx43) and its master-regulator, Zona Occludens-1 (ZO-1)

Abstract: Cell-cell communication is vital to maintaining cellular homeostasis and is accomplished through Gap Junctions (GJs). GJs are made up of transmembrane proteins called connexins (Cxs) that interact to form channels, allowing for passage of small molecules and ions. Previous research has shown that Connexin 43 (Cx43) GJ function is regulated by phosphorylation at ~15 serine residues on Cx43 C-Terminus. Zona Occludens-1 (ZO-1) is a known binding partner of Cx43, and work in mammalian cells demonstrated that when ZO-1 is bound. The S373 site is the closest to the last 4 amino acids that specifically bind to ZO-1. To understand how phosphorylation of Cx43 regulates its interaction with Zo-1 in a more quantitative fashion, Cx43 C-Terminus, along with Cx43 S373 A, and ZO-1 were expressed and purified from BL21 E. coli cells. Preliminary binding studies were conducted using affinity chromatography, taking advantage of different affinity tags present on purified Cx43 alanine and ZO-1. These binding experiments have demonstrated that this interaction is stable and easily detectable by SDS-PAGE, and Coomassie staining. Current work is focused on testing purified S373A along with other Cx43 mutants for future binding experiments with ZO-1, and for quantitative Kd value using isothermal titration calorimetry (ITC).

Poster # 28 **Foster, Celia,** Thorpe, Colin University of Delaware

Title: Inhibition in the face of thiols: complexities of protein disulfide isomerase inhibitor evaluation

Abstract: The enzyme protein disulfide isomerase (PDI), a member of the thioredoxin super-family, acts as both an isomerase and a reductase of disulfide bonds. It approaches mM concentrations in the endoplasmic reticulum and is found at lower levels in the mitochondrial outer membrane, the cytosol, and the cell surface. PDI has recently grown in medicinal and therapeutic importance as it holds key roles in thrombosis formation, cell adhesion and invasion. Here we describe a sensitive PDI reductase activity assay utilizing a commercially available self-quenched fluorescent disulfide reagent (BD-SS). PDI-mediated reduction can be followed using a plate-reader and can detect 5-10 nM isomerase in the presence of a range of small molecule and peptide thiol substrates. We have utilized BD-SS to address the difficulties in assessing the in vitro efficacy of proposed inhibitors of PDI that are themselves thiol-reactive. One assay involves a 15-minute preincubation of reduced PDI with the inhibitors and then follows the rate of fluorescence development after the addition of BD-SS. Alternatively, to approximate intracellular thiol concentrations, PDI is mixed with inhibitor in the presence of 5 mM reduced glutathione (GSH) and the assay is started by the addition of a small volume of BD-SS. Four mechanistically diverse inhibitors were tested: 3,4-methylenedioxy- β -nitrostyrene (MNS), 4-amino-phenylarsine oxide (APAO), 16F16, and quercetin-3-O-rutinoside. Reversible thiol-directed compounds MNS and APAO were found to inhibit PDI in the absence of competing thiols, but inhibition was completely lost when pre-incubated and assayed in the presence of 5 mM GSH. The irreversible chloroacetamide reagent, 16F16, showed inhibition of PDI that was only moderately attenuated with 5 mM GSH. Quercetin-3-O-rutinoside, a reversible non-thiol directed compound, showed similar inhibitory effects in both assay conditions. The results demonstrate the complexities of evaluating thiol-directed inhibitors in classical in vitro assays of PDI and provide a framework to assess the intricacies of inhibitor specificity in vivo.

Poster # 29 **Fritz, Matthew,** Caitlin M. Quinn, Mingzhang Wang, Guangjin Hou, Xingyu Lu, Leo Koharudin, Angela M. Gronenborn, and Tatyana Polenova University of Delaware

Title: Accurate Measurement and Prediction of ^{15}N and ^{13}C Chemical Shift Tensors in Proteins

Abstract: Magic angle spinning (MAS) NMR is uniquely positioned to obtain structural and dynamics information on systems not accessible to other structural biology techniques. The chemical shift tensor (CST) is a rich probe of structure and dynamics, dependent on electronic environment, local geometry, H-bonding, and dynamics. Isotropic chemical shifts and chemical shift anisotropies can be employed as structural restraints, in conjunction with distance restraints. To establish robust protocols for using chemical shifts in structure determination, accurate experimental measurements as well as computations of CST parameters need to be established first in proteins that are extensively structurally characterized. Herein, we present an analysis of experimental and computed ^{13}C and ^{15}N chemical shifts using a 132-residue lectin from *Oscillatoria agardhii* (OAA), a potent anti-HIV protein. OAA is an ideal benchmark system because it crystallizes readily and reproducibly (thus X-ray crystallography and MAS NMR studies are done on the same crystal form), its crystal structure has been determined with high (1.2 Å) resolution, and it has been extensively characterized by solution NMR.

We explored a hybrid quantum mechanics/molecular mechanics (QM/MM) approach for calculation of chemical shift tensors in OAA. We observed excellent agreement between MAS and solution NMR isotropic shifts. We recorded residue-specific chemical shift

anisotropy (CSA) lineshapes using RN symmetry sequences and correlated those to the computed tensors. We examined the influence of crystal contacts, H-bonding, ligand molecules, and dynamics on the accuracy of chemical shift tensor calculations. We will present the results of this study and discuss the potential of this approach for structure refinement.

- Poster # 30 **Gamrat, James M.** omsho, John W. University of the Sciences in Philadelphia
Title: Synthesis and Evaluation of Boron-Containing Inhibitors of the Non-mevalonate Isoprenoid Biosynthesis Pathway
Abstract: With the increase in resistance of pathogens to common anti-infective agents, there is an urgent need to develop new classes of therapeutic molecules and discover drug with new mechanisms of action to overcome this resistance. The non-mevalonate isoprenoid synthesis pathway (MEP pathway) is essential to the survival of several pathogens including *Plasmodium falciparum* and *Mycobacterium tuberculosis*. This pathway has emerged as an attractive therapeutic target for drug design since it is not present in the human body. A phosphonate-containing natural product, fosmidomycin, is the most potent inhibitor of the MEP pathway to date. The molecular target of fosmidomycin is 1-deoxyxylulose-5-phosphate reductoisomerase (IspC), an enzyme which catalyzes the first committed step in this biosynthetic pathway. Historically, the phosphonate moiety has been used to mimic the phosphate fragment of the natural substrates. The highly charged nature of this moiety hinders absorption and leads to poor pharmacokinetic properties thus fosmidomycin has found limited utility as a therapeutic agent. In an effort to overcome these issues, our work investigates the synthesis and evaluation of boron-containing compounds that may act as neutral phosphate/phosphonate isosteres. Here, we report the synthesis of a small library of aryl boronic acid and benzoxaborole analogs of fosmidomycin and their evaluation as inhibitors of IspC. Current work involves completing the compound library, assessing their inhibitory activity against IspC, and evaluating their anti-microbial activity via Kirby-Bauer disc diffusion assays.
- Poster # 31 **Ganguly, Himadri**, Pandey, Anil; Elbaum, Michael; Zondlo, Neal University of Delaware
Title: Molecular Effects of the R406W Mutation and Phosphorylation on the Dynamics of Tau
Abstract: Hyperphosphorylation of tau promotes its aggregation. Aggregated tau forms neurofibrillary tangles (NFTs) that are associated with neurodegeneration. Other than phosphorylation, certain mutations (e.g. R406W) and structural dynamics (like cis-trans isomerization) are also associated with tauopathy. The R406W mutation occurs close to phospho-epitopes pSer396 and pSer404. In order to understand the molecular basis for aggregation, we investigated the structural and phosphorylation dynamics in native and R406W-mutated peptide sequence derived from this region of tau. The R406W mutation increases the Ser-cisPro conformation via a C-H/ π interaction between the tryptophan side-chain and serine H α . The phosphorylation of Ser in the pSer404- τ -Pro405 sequence enhances the cis conformation, and the enhancement of cis conformation in presence of R406W mutation is cumulative. The R406W mutation is also capable of altering the phosphorylation/dephosphorylation kinetics in Ser409 and Ser404. R406W mutated sequence was phosphorylated at Ser404 by cdk5. Surprisingly, the R406W mutant was also dephosphorylated faster by PP2A with respect to the native sequence at Ser404 as well as Ser409. Both mutant and native peptides were phosphorylated at Ser404 at a comparable rate by GSK-3 β . Cis-trans isomerization in tau protein is involved in its aggregation via unknown mechanisms. Both the R406W mutation and Ser404 phosphorylation increase cis

conformation, indicating roles in tau aggregation and the onset of Alzheimer's disease. The R406W mutation can also alter the kinase/phosphatase activity around the mutation site, which can potentially control the cis-trans isomerization and global structure of tau.

- Poster # 32 **Gong, Ping**, Li, Guorui; Gui, Weijun; Zhuang, Zhihao University of Delaware
title: Activity-Based Ubiquitinated Protein Probes Reveal Target Protein Specificity of Deubiquitinases
Abstract: Ubiquitination is one of the post-translational modifications that regulate various cellular processes. The removal of ubiquitin from its target protein is catalyzed by deubiquitinating enzymes (DUBs). However, whether DUBs specifically interact and recognize ubiquitinated protein is not clear. Here we reported a new class of ubiquitinated protein probes mimicking native isopeptide linkage between ubiquitin and substrate protein. DUBs specificity was profiled in cell lysate using pull-down and mass spectrometry. Our results suggest a subset of DUBs recognize a given substrate protein and are also able to distinguish ubiquitination at different position on the target protein.
- Poster # 33 **Gregory, Gwendolyn**, Kalburge, Sai S.; Carpenter, Megan R.; Rozovsky, Sharon; Boyd, E. Fidelma University of Delaware
Title: Quorum sensing regulators control compatible solute biosynthesis gene expression in the halophile *Vibrio parahaemolyticus*
Abstract: *Vibrio parahaemolyticus* is a moderately halophilic Gram-negative bacterium that inhabits marine and estuarine environments. As such, *V. parahaemolyticus* encounters a range of abiotic and biotic stress factors, including fluctuations in osmolarity. In order to maintain the turgor pressure of the cell, bacteria have developed a strategy that involves accumulation of compatible solutes (CS) either through uptake from the environment or biosynthesis from available precursors. Compatible solutes are low molecular weight organic compounds such as glycine betaine, ectoine, trehalose, glutamate and proline. *Vibrio parahaemolyticus* has six transporters for CS and two biosynthesis pathways for ectoine and glycine betaine (GB), triple the number present in non-halophiles. Ectoine is synthesized from aspartate and glycine betaine is synthesized from choline taken up from the environment. Ectoine is essential for growth under high salinity conditions. Previous studies have shown that the ectoine and betaine biosynthesis genes are upregulated during osmotic upshock. Although the biosynthetic pathways have been well-characterized, transcriptional regulation is still poorly understood. Quorum sensing (QS) is a form of bacterial communication that involves synthesis of autoinducers (AI) which trigger a phosphorelay pathway. LuxO is a QS response regulator that controls expression of the output master quorum sensing regulators (MQSRs) OpaR (LuxR homologue) and AphA. Preliminary data suggest and previous studies have shown that the MQSRs play a direct role in the regulation of compatible solute biosynthesis. We hypothesize that expression of ectoine biosynthesis genes is controlled by the MQSRs in *Vibrio parahaemolyticus*.
- Poster # 34 **Hamaker, Nathaniel**, Antoniewicz, Maciek University of Delaware
Title: Harnessing cyanobacterial metabolism towards engineering carbon self-sufficient co-cultures
Abstract: *Synechocystis* sp. ATCC 27184 (Syn. 6803) is a freshwater, unicellular cyanobacterium capable of photoautotrophic metabolism, meaning it is able to fix atmospheric carbon dioxide given a suitable light source. Over recent decades, this strain has been well-characterized due to its use as a solar-powered cellular factory; in fact, its genome was the first of any photosynthetic organism to be fully sequenced, providing valuable insight into the evolution of modern chloroplasts. It was hypothesized that the

addition of an ammonium-secreting strain of the diazotroph *Azotobacter vinelandii* could lead to the development of a co-culture (CC) that would cooperatively fix both carbon and nitrogen without any other major inputs. For the purpose of utilizing the powerful mass spectrometry-based methods developed by the Antoniewicz group for the analysis of monoculture and CC metabolism, the mixotrophic growth mode of Syn. 6803 was exploited following glucose adaptation. A cyanobacteria-specific metabolic network model was developed using the most up-to-date findings regarding the unique metabolisms exhibited by this family of bacteria. Amino acid and sugar labeling data generated from six parallel singly labeled glucose tracer experiments facilitated monoculture metabolic flux analysis (MFA). Furthermore, preliminary CC trials showed evidence of bacterial cooperation in the absence of nitrogen supplementation, laying the groundwork for future experiments to perform CC MFA.

Poster # 35 **Hedrich, William,** Li, Linhao; Li, Daochuan; Lu, Yuanfu; Hassan, Hazem; Wang, Hongbing
University of Maryland School of Pharmacy

Title: Characterization of CITCO and Implications for Lymphoma Treatment

Abstract: Purpose: The constitutive androstane receptor (CAR, NR1h3) is recognized as the key transcription factor governing the inductive expression of CYP2B6. Cyclophosphamide (CPA), an alkylating prodrug, is metabolized to its active form, 4-hydroxycyclophosphamide (4-OH-CPA), in the liver primarily by CYP2B6. CPA has been prescribed for many years to combat cancers and autoimmune disorders and it still utilized clinically as the backbone of the CHOP (CPA, doxorubicin, vincristine, and prednisone) regimen which remains the front-line therapy for non-Hodgkin lymphoma (NHL). Despite the success of this regimen and several immunotherapies, prognosis for patients diagnosed with late-stage NHL remains poor and the need for improved therapeutics is evident. In the present study we aim to characterize CITCO, a selective CAR activator, as a small molecule with clinical utility for lymphoma treatment.

Methods: We have utilized a co-culture system containing human primary hepatocytes for drug metabolism, lymphoma cells as a model therapeutic target, and cardiomyocytes as an indicator of off-target toxicity, to demonstrate that the inclusion of CITCO improves the therapeutic index of CHOP in lymphoma treatment *in vitro*. We further explored the potential benefits of this combination *in vivo* in hCAR-transgenic mice through tumor inhibition in lymphoma xenograft studies. In this experiment, hCAR-transgenic mice (n=7/gp) received *i.p.* vehicle (saline and corn oil), CITCO alone (20 mg/kg in corn oil), vehicle (corn oil) and CHOP (40 mg/kg in saline), or CITCO and CHOP (20 mg/kg in corn oil, 40 mg/kg in saline). Pharmacokinetic studies examining the drug-drug interactions between CITCO and CHOP are ongoing.

Results: Results from our *in vitro* studies indicate that inclusion of CITCO with the CHOP regimen could achieve comparable anti-cancer activity at significantly lower concentration of CHOP, which attenuates the off-target cardiotoxicity. In our lymphoma xenograft studies in hCAR-transgenic mice, the CITCO/CHOP combination was able to inhibit the growth of the tumors to a greater extent than the current standard CHOP alone. Encouragingly, at the conclusion of the study, two mice from the CITCO/CHOP cohort did not have any remaining detectable tumor tissue.

Conclusions: Taken together, results from these studies offer compelling evidence that selective activation of CAR reduces the overall chemotherapeutic load required for CPA-based treatment and represents a promising approach for improving existing clinical treatments for lymphomas.

- Poster # 36 **Henager, Samuel**, Chu, Nam; Chen, Zan; Bolduc, David; Dempsey, Daniel; Hwang, Yousang; Wells, James; Cole, Philip Johns Hopkins University
Title: Enzyme Catalyzed Expressed Protein Ligation
Abstract: Here we show the utility of subtiligase, a previously described engineered form of the bacterial protease subtilisin that has been converted to a peptide ligase, for creating semisynthetic proteins from intein-generated protein thioesters and synthetic peptides. Expressed protein ligation (EPL) is an established method that exploits a chemoselective ligation between a recombinant protein thioester generated with an intein and an N-Cys-containing synthetic peptide. While a powerful method, EPL is limited by its standard requirement for a Cys residue at the ligation junction. In this study we investigate both ubiquitin and glutathione-S-transferase as model protein thioesters to assess the efficiency of subtiligase-mediated protein ligation with respect to the amino acid sequence proximal to the ligation junction. Many amino acid sequences at the junction were tolerated and led to ligation conversions as high as 50-70% in 10 minutes or less. We also show that subtiligase could be used to produce semisynthetic tetraphosphorylated PTEN, a lipid phosphatase and tumor suppressor gene, in its native form. This native form more accurately captures the effects of tail phosphorylation than did previous, non-native, semisynthetic PTEN constructs. By using this semisynthetic tetraphosphorylated PTEN as a protein standard, we could also more accurately quantify the level of tail phosphorylation of cell-derived PTEN, allowing insights into its regulation. The application of subtiligase described here therefore offers a rapid and efficient alternative to standard EPL for protein semisynthesis.
- Poster # 37 **Huang, Rong**, Li, Linjie; Mackie, Brianna Virginia Commonwealth University
Title: Development of novel photoaffinity probes for protein α -N-terminal methylation writers
Abstract: Protein α -N-terminal methylation is emerging as an important post-translational modification in regulating protein-chromatin interactions, mitotic division, and DNA damage repair. Therefore, elucidation of recognition specificity for the regulating enzymes is critical to advance our understanding of their biological functions. Herein, we report the design, synthesis, characterization, and application of the first photoaffinity probes for the protein α -N-terminal methyltransferases (NTMTs). We demonstrate that our probes exhibit high specificity for NTMTs in complex protein mixtures. Furthermore, our probes serve as powerful tools to discover unanticipated substrate preferences between two closely related homologs in the NTMT family, which will shed light on the development of selective inhibitors for individual NTMTs. This study also provides a paradigm to develop chemical probes for the identification of novel writers, readers, or erasers for N-terminal modifications.
- Poster # 38 **Hudson, Devin**, Caplan, Jeffery; Thorpe, Colin University of Delaware
Title: Designing flavoprotein-GFP fusion probes for analyte-specific ratiometric fluorescence imaging
Abstract: The development of genetically encoded fluorescent probes for analyte-specific imaging has revolutionized our understanding of intracellular processes. Current classes of intracellular probes depend on the selection of binding domains that either undergo conformational changes on analyte binding or can be linked to thiol redox chemistry. Here we have designed novel probes by fusing a flavoenzyme, whose fluorescence is quenched on reduction by the analyte of interest, with a GFP domain to allow for rapid and specific ratiometric sensing. Two flavoproteins, E. coli thioredoxin reductase (EcTrxR) and S. cerevisiae lipoamide hydrogenase (ScLpDH), were successfully

developed into E. coli thioredoxin and NAD⁺/NADH specific probes respectively and their performance was evaluated in vitro and in vivo. The steady-state cytosolic ratio of NAD⁺/NADH induced by pyruvate, lactate, and glucose was successfully determined in mammalian cell culture. These genetically encoded fluorescent constructs represent a new approach to intracellular probe design and will extend the range of metabolites accessible to in vivo interrogation.

Poster # 39 **Imamoto, Jason**, Bruist, Michael Academic

Title: A Molecular Dynamics Investigation of the Stability of Sarcin/Ricin Domains: Towards using Adaptively Biased MD to Find the Full Dynamic Range of RNA

Abstract: Over the past decade our understanding of RNA's biological role has greatly expanded. As much as 80% of the DNA in the human genome is transcribed, but only 2% of this is translated. The conservation of numerous nontranslated sequences across mammals, and even distantly related eukaryotes, indicates that these structures have specific functions. Understanding these RNAs will require efficient deciphering of their two- and three-dimensional structures as well as the dynamics of these structures, a daunting task. Luckily, RNA tertiary structure is guided by motifs. These are specific structures that occur frequently in RNA, often with the same or related functions. Therefore, understanding the dynamics of various RNA motifs will be pivotal to decoding the vast number of complex RNAs.

Our work focuses on the sarcin/ricin domain (SRD), an RNA motif that ranges from multiple occurrences in the ribosome, where it has structural and protein binding roles, to a replication control region in the potato spindle tuber viroid (PSTVd), where it may direct RNA ligation. The SRD is stable under normal MD and consists of non-canonical base pairs, one above and two below, that surround a base triplet. We use adaptively biased molecular dynamics (ABMD) to build a negative of the free energy surface (FES) based on two collective variables (CVs) that each measures a structural feature. ABMD increases the sampling speed of the surface by using multiple walkers that start at different places on the FES and a repulsive bias to prevent a walker from returning to the same CV value allowing us to observe structures that rarely occur in standard MD.

Three models of the SRD were made: one model contains only the SRD, while the other two contain GC and AU rich regions in the format of GC/SRD/AU or AU/SRD/GC. Numerous sequence variations of the simple SRD model are being studied. We are looking for changes that range from subtle alterations in the quality of base stacking to structural rearrangements in the modeled motifs. We have found that the SRD region is as stable as an AU-rich region, and the SRD tends to denature from the bottom of the domain. Insert a description of motif AG paired via Hoogsteen and sugar edges on top of a base triplet. These have at least two more noncanonical base pairs below them

Poster # 40 **Jeon, Miji**, Kohnhorst, Casey L.; Kyoung, Minjoung; Schmitt, Danielle L. ; Kennedy, Erin L.; Ramirez, Julio; Bracey, Syrena M.; Luu, Bao Tran; Russell, Sarah J. and An, Songon University of Maryland, Baltimore County

Title: A regulatory metabolic complex for glucose metabolism in living cells

Abstract: Glycolytic enzymes have been suggested to form multienzyme complexes in a variety of organisms. However, there is a lack of understanding of fundamental mechanisms that regulate the direction of glucose flux at single cell levels. Quantitative live cell imaging with fluorescently tagged human liver type phosphofructokinase 1 (PFKL) has allowed us to identify a variety of sizes of cytosolic enzyme clusters in human cells. Importantly, cell-based metabolic flux assays unveil the cluster size-dependent metabolic functions of the enzyme clusters at single cell levels. Collectively, we propose that the

varying sizes of this enzyme assembly represent various metabolic functions to guide the direction of glucose-mediated carbon flux in the cell.

- Poster # 41 **Jiang, Lingxi**, Thorpe, Colin University of Delaware
Title: Interrogating the thiol-disulfide redox status of the mammalian cell surface by ratiometric fluorescence imaging
Abstract: The exofacial/extracellular thiol/disulfide (SH/SS) redox status has been shown to play critical roles in a range of mammalian cell behaviors, including adhesion, migration, invasion and viral fusion. Two proteins, whose activities potentially oppose each other, the disulfide-generating Quiescin-sulfhydryl oxidase (QSOX), and the disulfide reductase activity of protein disulfide isomerase (PDI), are believed to contribute to extracellular SH/SS status. However the range of their substrates, and the possible synergy between these oxidoreductases remain cryptic. To investigate exofacial SH/SS status in a range of mammalian cells, we have developed conventional confocal and super-resolution ratiometric fluorescent imaging methods that can quantitatively assess the status of cell surface proteins using impermeant fluorescent maleimide reagents and selective reduction of exofacial disulfides. Using these approaches, the general surface redox status on different cell types are observed and quantitated. We also present the effects of extracellular QSOX and PDI on surface SH/SS as well as the impact of components of the extracellular matrix, and their in vitro mimics, on cell exofacial SH/SS status of a range of mammalian cell types.
- Poster # 42 **Johnson, Chad**, Coop, Andrew; Winger, Gail; Saccone, Phillip; Woods, James; Blough, Bruce University of Maryland, Baltimore
Title: Reinforcing Properties of Meta-Nicotine
Abstract: Metanicotine is much less potent than nicotine; it nevertheless substitutes completely for its discriminative effect at both low (0.03 mg/kg) and high (1.8 mg/kg) nicotine doses. Metanicotine's discriminative and response rate suppressing effects are blocked by dihydrobetaerythroidine suggesting that these effects are $\alpha 4\beta 2$ -nicotinic receptor mediated in the rat. Unlike nicotine, metanicotine (0.1 -3.2 mg/kg, i.v.) does not reinforce self-injection responding in animals that self-inject nicotine. These same rats also self-inject bupropion at a series of comparable doses. At these doses, metanicotine punishes responding that delivers small pellets of sucrose. Very preliminary data suggest that the profile of activity described for metanicotine is similar to the very potent agonist, epibatidine.
- Poster # 43 **Johnston, Melanie**, DeColli, Alicia; Meyers, Caren Johns Hopkins School of Medicine
Title: Understanding the Alternative Activities of DXP Synthase
Abstract: The essential metabolic enzyme 1-deoxy-D-xylulose 5-phosphate (DXP) synthase catalyzes the formation of DXP from pyruvate and D-glyceraldehyde 3-phosphate (GAP). DXP is an essential branch point metabolite in bacteria, as it is a precursor to isoprenoids, as well as the important cofactors thiamine diphosphate (ThDP) and pyridoxal phosphate. DXP synthase (DXPS) is not present in humans, highlighting it as a potential antibacterial drug target. The enzyme possesses unique structural and mechanistic features that could be exploited for selective inhibition of DXP synthase over mammalian ThDP-dependent enzymes. The large active site and unique domain arrangement of DXP synthase in addition to its novel random sequential mechanism are particular features of DXP synthase that can guide selective targeting strategies. In addition, we have shown that DXPS is promiscuous in terms of its acceptor substrate, as it can utilize several aldehydes and nitroso analogues, but previous reports have suggested a strict requirement for pyruvate as the donor substrate in the presence of the natural acceptor substrate, D-GAP. In this study, we show that DXPS can indeed utilize alternative donor substrates, and

propose that donor substrate usage may be dictated by the nature and availability of acceptor substrate. We demonstrate that although hydroxypyruvic acid is not efficiently coupled with D-GAP as an acceptor, it is turned over in the presence of O₂ to produce α -hydroxyperacetate. This alternative substrate pair on DXP synthase sets a precedent for unique donor/acceptor combinations which could have important implications for DXP synthase function in bacterial cells. These results highlight a unique toolset for studies of donor substrate specificity and advance our knowledge of alternative chemistries of DXP synthase which are valuable to guide inhibitor design and understand function.

Poster # 44 **Kalburge, Sai Siddarth**; Boyd, E Fidelma University of Delaware

Title: The Role of Quorum Sensing regulators in colonization and metabolic fitness of *Vibrio parahaemolyticus*

Abstract: Quorum sensing (QS) is a process that bacteria use to regulate gene expression patterns based on cell density. In *Vibrio* species, at low cell density, the sigma 54-dependent response regulator LuxO is active and regulates the two QS master regulators AphA, which is induced, and OpaR, which is repressed. At high cell density the opposite occurs: LuxO is inactive, and therefore OpaR is induced while AphA is repressed. *Vibrio parahaemolyticus* is a Gram negative bacterium that is ubiquitously found in marine environments. It is the leading bacterial cause for seafood-related gastroenteritis worldwide. The roles of the QS regulators in *V. parahaemolyticus* pathogenesis are less known. We examined deletion mutants of luxO, opaR, and aphA for in vivo fitness using an adult mouse model. We found that the luxO and aphA mutants were defective in colonization compared to levels in the wild type. The opaR mutant did not show any defect in vivo. Colonization was restored to wild-type levels in a luxO opaR double mutant and was also increased in an opaR aphA double mutant. These data suggest that AphA is important and that overexpression of opaR is detrimental to in vivo fitness. Transcriptome sequencing (RNA-Seq) analysis of the wild type and luxO mutant grown in mouse intestinal mucus showed that 60% of the genes that were downregulated in the luxO mutant were involved in amino acid and sugar transport and metabolism. These data suggest that the luxO mutant has a metabolic disadvantage, which was confirmed by growth pattern analysis using phenotype microarrays. Bioinformatics analysis revealed OpaR binding sites in the regulatory region of 55 carbon transporter and metabolism genes. Biochemical analysis of five representatives of these regulatory regions demonstrated direct binding of OpaR in all five tested. These data demonstrate the role of OpaR in carbon utilization and metabolic fitness, an overlooked role in the QS regulon.

Poster # 45 **Kljaic, Teodora**, Toonstra, Christian; Wang, Lai-Xi University of Maryland - College Park

Title: Highly efficient chemoenzymatic synthesis of prostate cancer associated glycopeptide antigens for diagnosis and therapeutic vaccine design

Abstract: Prostate cancer is the second leading cause of cancer death in American men, behind only lung cancer. These most recent statistics from American Cancer Society dictate an urgent need for a more sensitive and reliable method for diagnosis as well as a better vaccine candidate for therapeutics of prostate cancer. It is known that prostate specific membrane antigen (PSMA), a membrane bound glycoprotein, and the secreted prostate specific antigen (PSA) both are heavily overexpressed in prostate cancer. Recent studies have also demonstrated that highly branched and sialylated glycoforms are associated with progression of prostate cancer, making the glycoprotein antigen and related glycopeptides unique targets for diagnosis and therapeutics. However, pure chemical synthesis of the complex PSA- and PSMA-associated glycopeptides is still a challenging task. Here, we describe a highly convergent chemoenzymatic synthesis of homogeneous, PSA-associated

glycopeptides carrying sialylated bi- and tri-antennary N-glycans. The synthesis starts with the preparation of the GlcNAc-containing polypeptide chain selected from the PSA sequence and the N-glycans from bovine fetuin, followed by glycosynthase-catalyzed ligation of the GlcNAc-peptide and the glycans to give the target glycopeptides. We have synthesized a fucosylated bi-antennary complex type N-glycan-carrying glycopeptide on a semi-preparative scale, while tri-antennary complex type N-glycan-carrying glycopeptide is still in preparation. Conjugation of the synthetic glycopeptide to a carrier protein is in progress and the details of synthesis and application of the glycopeptides and their protein conjugates will be discussed further in my poster presentation.

Poster # 46 **Krause, Sarah**, Mcatee, Jesse; Watson, Donald University of Delaware

Title: An Improved Precatalyst for Silyl-Heck Reactions

Abstract: Previous work from our group has shown that allyl and vinyl silanes can be prepared from terminal alkenes via the silyl-Heck reaction. Mechanistic studies aimed at identifying the active palladium complexes in this reaction have revealed a new complex by ³¹P NMR and mass spectrometry. This complex has been isolated and a crystal structure has been solved, confirming the identity of an iodide dimer of the palladium complex, (LPdI₂)₂. This complex has been found to be a competent single component precatalyst for the silyl-Heck reaction, giving comparable yields to previous precatalysts. However, this precatalyst offers several advantages over previous systems. The complex can be easily prepared from PdI₂ and is temperature, moisture, and air stable. Additionally, this catalyst gives more reproducible results when compared to the Pd₂(dba)₃ precatalyst.

Poster # 47 **Ku, Therese**, Seley-Radtke, Katherine University of Maryland, Baltimore County

Title: Synthesis of Flexible, Purine Analogue Inhibitors of NCp7

Abstract: Anti-HIV-1 drug design has been notably challenging due to the virus' ability to mutate and develop immunity against commercially available drugs. This project aims to discover a new series of nucleobase analogues that not only possess inherent flexibility that could withstand active site mutations, but also target a non-canonical, more conserved target, NCp7. Interestingly, these compounds are not predicted to work by zinc ejection, which would endow them with significant advantages over currently reported zinc-ejectors, which are toxic. We have synthesized several series of these fleximer base analogues using palladium-catalyzed coupling techniques and tested them against NCp7 specifically, and HIV-1 in general. One analogue has shown interesting activity, displaying potential binding patterns with NC in a proton NMR experiment and HIV-1 inhibition. The results are shown herein.

Poster # 48 **Kulkarni, Rhushikesh**, Zengeya, Thomas; Shrimp, Jonathan; Garlick, Julie; Roberts, Allison; Montgomery, David; Meier, Jordan. National Cancer Institute

Title: Discovering Targets of Non-enzymatic Acylation by Thioester Reactivity Profiling

Abstract: Non-enzymatic protein modification driven by thioester reactivity is thought to play a major role in the establishment of cellular lysine acylation. However, the specific protein targets of this process are largely unknown. Here we report an experimental strategy to investigate non-enzymatic acylation in cells. Specifically, we develop a chemoproteomic method that separates thioester reactivity from enzymatic utilization, allowing selective enrichment of non-enzymatic acylation targets. Applying this method to cancer cell lines identifies numerous candidate targets of non-enzymatic acylation, including several enzymes in lower glycolysis. Functional studies highlight malonyl-CoA as a reactive thioester metabolite that can modify and inhibit glycolytic enzyme activity. Finally, we show that synthetic thioesters can be used as novel reagents to probe non-enzymatic acylation in living cells. Our studies provide new insights into the targets and

drivers of non-enzymatic acylation, and demonstrate the utility of reactivity-based methods to experimentally investigate this phenomenon in biology and disease.

Poster # 49 **Kumar, Sujeet**, Candelora, Christine; Kumar, Sujeet; Cope, Nicholas; Nan, Haihan; Wong, Kenneth & Wang, Zhihong University of the Sciences, Philadelphia

Title: Molecular Mechanisms of BRAF regulation: Looking beyond the kinase domain

Abstract: BRAF belongs to the RAF serine/threonine kinase family and is an established driver of oncogenesis. The spectrum of human cancers carrying BRAF mutations includes melanoma (40-60 %), thyroid (60%), colorectal (20%) and lung cancer (10%), which provides a rationale for targeting this kinase in cancer therapy. Development of ATP-competitive inhibitors based on the isolated kinase domain provides only short-term clinical benefits and stimulates secondary malignancy growth by paradoxically activating the same MAPK signaling pathway where BRAF lies in. In order to provide a better understanding of the extremely complex regulation mechanisms of full-length BRAF (FL-BRAF), we have purified and characterized the full-length enzyme expressed from mammalian cells. The FL-BRAF enzyme recapitulates the paradoxical activation of the enzyme in vitro, for the FDA approved drug Dabrafenib. In addition, the FL-BRAF is about 20-fold more active than the catalytic domain alone, highlighting the advantage of studying full-length instead of fragments of BRAF. We further enumerate how FL-BRAF is regulated by dimerization, phosphorylation and interactions among different domains. Our findings provide a platform to devise novel strategies to effectively treat the large patient population who are diagnosed with malignancies involving activated BRAF in melanoma and beyond, that currently remains poorly managed with current BRAF kinase inhibitors.

Poster # 50 **Labonte, Jason W.**, Gray, Jeffrey J. Johns Hopkins University

Title: RosettaCarbohydrate: Expanding the computational tools available to glycoscientists

Abstract: Modeling the structures of carbohydrates and glycoconjugates is an infamously challenging undertaking, and only a limited number of such tools are currently available. We have built a framework within the Rosetta structure-prediction and design suite for modeling saccharide ligands and glycoconjugates, including glycoproteins and -lipids. We have nearly completed full integration of carbohydrate functionality into the full range of Rosetta modules, including its de novo folding, docking, loop modeling, crystal refinement, and design algorithms. Our updates to the Rosetta code base have allowed access to all torsion angles (φ , ψ , ω , ν , and χ) for sampling all conformations and capture the high degree of flexibility, stereochemistry, and branching inherent in carbohydrate structures. We have added a glycosidic bond scoring method and low-energy ring conformer libraries to the Rosetta database.

We will present an assortment of real-world applications, highlighting a diversity of functions enabled by our modeling framework. We will highlight improvements of ligand docking to antibodies with carbohydrate antigens. We will demonstrate the use of glycan loop-modeling as a means of constructing reasonable structures for analysis into the activity of glycosylated carboxylesterases. We will show how the fitting of glycans into the electron density of poor-resolution crystal structures of HIV-1 envelope protein trimer allows for refinement to a quality model. Lastly, we will present accessibility studies for antibody-antibody receptor pairs.

Our work will expand the computational toolbox available to glycoscientists, furthering the understanding of the biomolecular mechanisms of glycobiology and advancing the efforts of glycoengineering. Financial support: National Research Service Award, NIH National Cancer Institute (1F32-CA189246-01); NIH R01-GM078221; and NIH R01-GM73151.

- Poster # 51 **Li, Chao**, Giddens, John; Zhu, Shi-Lei; Wang, Lai-Xi University of Maryland
Title: Facile chemoenzymatic synthesis of core fucosylated glycopeptides using an engineered bacterial α -fucosidase
Abstract: Structurally well-defined core-fucosylated glycopeptides are ideal compounds for further characterization of the key roles of core fucosylation and also for development of carbohydrate-based therapeutics. Here we demonstrate that a novel engineered α -(1,6)-fucosidase, which has very broad substrate specificity, is capable of core fucosylating various GlcNAc-peptides with high efficiency. The *E. coli* overexpressed enzyme mutant was applied into the transglycosylation from fucosyl fluoride to various sugar acceptors, including GlcNAc and GlcNAc-peptides such as the CD52 glycopeptide antigen. Compared with the wild-type enzyme, the mutant exhibits significantly higher transfer activity to produce core fucosylated GlcNAc-CD52 with up to 90% yield. Since the α -(1,6)-fucosylated GlcNAc-peptides thus obtained are the excellent substrates for the glycosynthase mutant we recently reported (EndoF3-D165A), full-size complex type glycopeptides can be efficiently synthesized through the enzymatic sugar chain extension. It is expected that the α -(1,6)-fucosidase mutant could be widely applied in the convergent synthesis of biologically interesting core-fucosylated complex type N-glycopeptides.
- Poster # 52 **Li, Tiezheng**, DiLillo, David; Bournazos, Stylianos; Giddens, John; Ravetch, Jeffrey; Wang, Lai-Xi University of Maryland, College Park
Title: Modulating IgG effector function by Fc glycan engineering
Abstract: IgG antibodies contain a conserved N-glycosylation site on the Fc domain to which a complex, biantennary glycan is attached. The fine structures of this glycan modulate antibody effector functions by affecting the binding affinity of the Fc to diverse Fc receptor family members. For example, core fucosylation significantly decreases antibody-dependent cellular cytotoxicity (ADCC), whereas terminal α 2,6-sialylation plays a critical role in the anti-inflammatory activity of human i.v. immunoglobulin therapy. The effect of specific combinations of sugars in the glycan on ADCC remains to be further addressed, however. Therefore, we synthesized structurally well-defined homogeneous glycoforms of antibodies with different combinations of fucosylation and sialylation and performed side-by-side in vitro Fc γ R-binding analyses, cell-based ADCC assays, and in vivo IgG-mediated cellular depletion studies. We found that core fucosylation exerted a significant adverse effect on Fc γ RIIIA binding, in vitro ADCC, and in vivo IgG-mediated cellular depletion, regardless of sialylation status. In contrast, the effect of sialylation on ADCC was dependent on the status of core fucosylation. Sialylation in the context of core fucosylation significantly decreased ADCC in a cell-based assay and suppressed antibody-mediated cell killing in vivo. In contrast, in the absence of fucosylation, sialylation did not adversely impact ADCC.
- Poster # 53 **Long, Christopher**, Antoniewicz, Maciek University of Delaware
Title: Pathway discovery and model development in *E. coli* central carbon metabolism via ^{13}C metabolic flux analysis of knockout strains
Abstract: Metabolic and physiological responses to genetic perturbations are of fundamental interest to systems biology and metabolic engineering. They reveal underlying information about network structure including kinetics, regulation, and the presence of otherwise "hidden" reactions. Quantitative prediction of these responses is essential for rational strain design, but has been a persistent challenge in the field. There has been much work done to develop in silico predictive models of metabolism, most notably the constraint-based models. Progress in this area has been limited, however, by a lack of high-quality, reproducible experimental metabolic flux (fluxomic) data of genetically perturbed strains.

In this study, state-of-the-art ^{13}C metabolic flux analysis (^{13}C -MFA) approaches were applied to ~50 *Escherichia coli* knockout strains spanning the major pathways of central carbon metabolism. A full physiological characterization of each strain during aerobic exponential growth was performed, including uptake and secretion rates as well as biomass composition. Fluxes are estimated based on an optimized parallel experimental design, coupled with mass spectrometry measurement of metabolite isotopic labeling and ^{13}C -MFA. Novel targeted tracer experiments were designed and executed to elucidate previously unknown reactions and identify the responsible enzymes.

The flux and physiological responses of these strains collectively provide valuable insights into the robustness of central carbon metabolic pathways, as well as areas of likely kinetic limitation. Severely growth-impaired knockout strains identify the most important enzymes in these pathways and the adaptability of *E. coli* to extreme perturbations. Particularly surprising responses, including the identification and quantification of significant reverse flux carried by Enzyme I of the sugar phosphotransferase system (PTS), will be emphasized. These data are being applied to the assessment of constraint-based metabolic models and the development of kinetic models.

Poster # 54 **Marangoz, Alize**, Pupillo, Rachel. C.; Marangoz, Alize; Wu, Wenbo; Burch, Jason M.; Grimes, Catherine Leimkuhler; Rosenthal, Joel University of Delaware

Title: The Development of Electrochemiluminescent Platforms for Interrogation of Biomolecule Binding Phenomena and Identification of New Therapeutics

Abstract: Many biological processes depend on complex non-covalent interactions between proteins and other biomolecules. Identification of small molecule antagonists for such interactions has largely been accomplished using fluorescence based high throughput screening assays. While such screening methods have been widely successful, they are incompatible with naturally fluorescent molecules and require fluorophore tagging. To address these limitations, we have worked to develop new platforms to probe biomolecule binding and screen for chemotherapeutics that are based on electrogenerated chemiluminescence (ECL). Our initial ECL platform is based on the biomolecular interaction of the transcription factor binding domain DNA sequence GGGACTTTC and a luminophore conjugated NF- κ B mutant. In developing this platform, we have immobilized the DNA and transcription-factor binding proteins onto ITO surfaces by first electrochemically modifying the conducting glass surface with COOH groups. EDC/NHS coupling allowed for DNA sequences to be coupled to the ITO, and these bioelectrodes were characterized XPS, EIS and TOF-SIMS. In parallel, we have developed a strategy to synthesize maleimide and $\text{N}\alpha,\text{N}\alpha$ -Bis(carboxymethyl)-L-lysine hydrate (NTA) appended derivatives of tris(bipyridine)ruthenium(II) ($\text{Ru}(\text{bpy})_3$) and Boron-dipyromethane (BODIPY) ECL emitters that can be conjugated to cysteine and histidine terminated NF- κ B protein mutants. To set up the foundation of detecting ECL signals from modified surfaces, we succeeded in attaching a $\text{Ru}(\text{bpy})_3$ -alkyne luminophore onto the azide functionalized magnetic particles using 1,3-Huisgen dipolar cycloaddition (also known as Cu-Catalyzed Azide Alkyne Cycloaddition [CuAAC]). The cyclic voltammogram (CV) of the modified particles together with tripropylamine (TPrA) co-reactant was collected. During the CV, the ECL emission as a function of potential was recorded. The ECL spectrum of the modified particles was also obtained, which was in accordance with the fluorescence spectrum of modified magnetic particles. Integration of these new ECL platforms into high-throughput screening assays will be discussed.

Poster # 55 **Mariam Beshay**, Bawazir Nada and Janetopoulos Christopher University of the Sciences in Philadelphia

Title: Corresponding Changes in Plasma Membrane PI(4,5)P₂ Levels with Cell Migration Rates

Abstract: During gradient sensing, many signaling and cytoskeletal markers become asymmetrically distributed, and cells establish a polarity circuit. A similar signaling pattern was shown in cells during cytokinesis. Phosphoinositide-linked signals at poles of dividing cells were equivalent to those found at the front of migrating cells. Signals at the furrow between the two daughter cells were similar in nature to those found at the rear of migrating cells. To better understand the regulation of the plasma membrane (PM) PI(4,5)P₂ in maintaining cell morphology and breaking cell symmetry during polarized morphologies, we have used the inducible rapamycin (iRAP) system. iRAP was used to recruit various enzymes to the PM that regulate PM PI(4,5)P₂ levels. Cell migration and the localization of various signaling and cytoskeletal biosensors were then monitored and recorded when PI(4,5)P₂ levels were depleted or elevated. We find that lowering PM PI(4,5)P₂ leads to cell spreading and increased migration, while elevating PM PI(4,5)P₂ leads to cell rounding, and reduced rates of cell migration. Interestingly, depletion of PM PI(4,5)P₂ leads to a dramatic increase in Ras and PI3 Kinase activity. Cells show a variety of phenotypes when PI(4,5)P₂ levels are depleted, including oscillatory behavior and dramatic changes in cell morphology. Similar effects were observed in Dictyostelium cells lacking the PI5 Kinase (pik1), whose loss leads to dramatic decreases in PM PI(4,5)P₂ levels. We also investigated the localization of ForminA, which has a putative PI(4,5)P₂ motif, and this protein localizes in a reciprocal manner with that of markers for PI(3,4,5)P₃ during cell oscillations, with ForminA moving to the cytosol during cell spreading. Interestingly, this Formin also localizes to the rear of cells undergoing chemotaxis and is found at the furrow of dividing cells. Our data suggests that the PM levels of PI(4,5)P₂ are critical in altering cell morphology, with lower levels contributing to cell protrusions. PM PI(4,5)P₂ levels above a threshold support regulators that contribute to actomyosin contraction, while levels below a threshold activate branching actin networks. Lowering PI(4,5)P₂ levels too much causes deleterious effects and cell death.

Poster # 56 **Martinez III, Ramon**, Huang, Weiliang; Centola, Garrick; Scheenstra, Jacob; Samadani, Ramin; Chen, Lijia; Mackerell, Alexander D.; Fletcher, Steven; Kane, Maureen; and Shapiro, Paul. University of Maryland-School of Pharmacy

Title: Mechanistic studies of a novel small molecule that selectively inhibits melanoma cells containing constitutively active ERK1/2

Abstract: The uncontrolled proliferation and growth of cancer cells is often a result of constitutive activation of the extracellular signal-regulated kinase (ERK 1/2) signaling pathway. In an attempt to identify ERK specific inhibitors for anti-cancer therapy, a novel thienyl benzenesulfonate compound was identified that selectively inhibits ERK at substrate binding sites. Additionally, this compound has been shown to selectively inhibit the growth of melanoma cells that have constitutively active ERK1/2 via mutations in upstream activators BRAF or NRAS. Proteomic and transcriptomic analysis of drug-treated lysates shows expression changes of important downstream substrates of ERK 1/2 (IEGs) such as c-Fos, Fra-1, and c-Myc. In addition, increased expression of the protein Nrf2, which is a potential regulator of pathways generating reactive oxygen species (ROS), suggests that this compound may be involved with impeding cell growth in melanoma cells via upregulation of oxidative stress pathways. In this work, we attempt to further characterize and validate the regulatory mechanisms and apoptotic effect this novel compound is having on cancer cells, and how it can serve as a promising alternate line of therapy to traditional ATP-competitive kinase inhibitors.

Poster # 57 **McDonald, Nathan,** Wang, Yiben; Grimes, Catherine; Boyd, Fidelma University of Delaware

Title: Structural characterization of nine carbon amino sugars involved in glycosylation of the bacterial outer membrane

Abstract: Nonulosonic acids (NulOs) are a diverse family of nine carbon α -amino sugars that are present across all branches of life. Bacteria have been shown to biosynthesize the common eukaryotic NulO, N-acetylneuraminic acid (Neu5Ac), as well as prokaryotic specific NulOs: legionaminic, pseudaminic, or acinetaminic acid. These bacterial specific NulOs, are incorporated into different cell surface structures and have been implicated in a variety of host-pathogen interactions.

All strains of *Vibrio vulnificus*, a marine bacterium and an opportunistic pathogen, are capable of producing NulO. Previous work showed that NulO is essential for biofilm, motility, antimicrobial resistance, and pathogenesis. Here we examine the diversity of NulO biosynthesis within *V. vulnificus* as well as other species. We characterize the structure of the NulO biosynthesized in *V. vulnificus* CMCP6 and provide evidence that a novel NulO is produced with an N-acetyl-D-alanyl functional group.

Poster # 58 **Mishra, Subrata,** Kancharla, Ashwini; Sgourakis, Nikolaus; Dowling, Daniel, Frueh, Dominique Johns Hopkins University, School of Medicine

Title: Insight into non-ribosomal peptide synthetase (NRPS) functioning from the solution structure and dynamics of a 52 kDa NRPS cyclization domain.

Abstract: Non-ribosomal peptides (NRPs) are a diverse array of secondary metabolites encompassing broad biological activity, from etiological agents in microbial infections to various pharmaceutical applications. NRPs are synthesized in bacteria and fungi by NRP synthetases (NRPSs); modular multi-domain enzymatic systems functioning in an assembly line-like manner. Despite their diversity NRPs are synthesized in a similar iterative manner, where each module of the synthetase adds a single substrate to the growing NRP chain. Chain elongation proceeds via peptide bond formation catalyzed by condensation domains (C) between substrates covalently tethered to thiolation domains (T) in sequential modules. Condensation domains are sometimes replaced by cyclization domains (Cy) that carry out both condensation and heterocyclization (e.g. cysteines to thiazolines). NRP chain elongation by C (or Cy) domains and product hand-off between sequential T domains is poorly understood, as structural investigations have generally been hampered by the transient nature of inter-domain interactions. We have used solution Nuclear Magnetic Resonance (NMR) techniques to probe excised NRPS domains and here we present the NMR solution structure of the 52 kDa cyclization domain from the NRPS Yersiniabactin synthetase. New methods were developed to overcome challenges in resonance assignment and improving distance constraint accuracy in the structure determination of this large protein and are briefly discussed. Relaxation dispersion revealed a network of dynamic residues that provide a rationale for allosteric effects necessary for directionality in sequential peptide bond formations. Lastly, these dynamic residues chart a pathway that connects the putative active site to sets of surface residues that have been previously implicated in C-T inter-domain interactions.

Poster # 59 **Montgomery, David**, Garlick, Julie; Sinclair, Wilson; Kulkarni, Rhushikesh; Meier, Jordan
National Cancer Institute

Title: Chemoproteomic Profiling of Epigenetic Signaling in Cancer

Abstract: Acetylation is a dynamic posttranslational modification involved in the epigenetic regulation of gene expression and other critical cellular processes. Lysine acetyltransferase enzymes (KATs) are the cellular writers of acetylation, whose aberrant activity has been strongly associated with cancer. However, the specific mechanisms by which KAT activity is regulated in the context of cancer are not fully understood. Here we have developed a chemoproteomic strategy capable of profiling of KAT enzymes within their endogenous contexts for this first time, allowing the quantitative analysis of KAT enzyme activity. This approach circumvents many of the challenges associated with traditional biochemical assays to study KAT enzymes, providing a powerful general platform for identification and target validation of KAT inhibitors, and allowing global analysis of KAT-ligand interactions. We have utilized this strategy to determine the binding profiles of specific metabolites with a range of KATs, implicating several enzymes that may be constitutively activated in the altered metabolic state of cancer cells. By identifying the previously uncharacterized molecular links between altered metabolism and epigenetic signaling, these studies have illuminated potential new therapeutic targets and strategies for the treatment of cancer.

Poster # 60 **Nani, Roger**, Gorka, Alexander; Nagaya, Tadanobu; Yamamoto, Tsuyoshi; Kobayashi, Hisataka; Schnermann, Martin National Cancer Institute

Title: Near-IR Light-Mediated Cleavage of Duocarmycin-Antibody Conjugates Using Cyanine Photocages

Abstract: The development of photocaging groups activated by near-IR light would enable new approaches for basic research and allow for spatial and temporal control of drug delivery. Research in our group has led to the discovery of a novel near-IR uncaging reaction, which utilizes heptamethine cyanine photooxidation chemistry to release bioactive phenols in response to low intensity 690 nm irradiation. Here we report the first in vivo application of our uncaging method, which incorporates a cyanine photocage into an antibody-drug conjugate (ADC), to provide a targeted drug-delivery system wherein drug release is controlled by application of tissue-penetrant near-IR light. Guided by rational design, including computational analysis, we conducted an SAR study to characterize the impact of structural alterations on the cyanine uncaging reaction. A modest change to the ethylenediamine linker (N,N'-dimethyl to N,N'-diethyl) leads to a bathochromic shift in the absorbance maxima, while decreasing background hydrolysis. Building on these structure-function relationship studies, we prepare antibody conjugates that uncage a derivative of duocarmycin, a potent cytotoxic natural product. The optimal conjugate, CyEt-Pan-Duo, undergoes small molecule release with 780 nm light, exhibits activity in the picomolar range, and demonstrates excellent light-to-dark selectivity. Mouse xenograft studies illustrate that the construct can be imaged in vivo prior to uncaging with an external laser source. Significant reduction in tumor burden is observed following a single dose of conjugate and near-IR light. These studies define key chemical principles that enable the identification of cyanine-based photocages with enhanced properties for in vivo drug delivery.

Poster # 61 **Neu, Heather**, Brandis, Joel; Williams, Anne; Alexishin, Serge; Sun, Dajun; Zheng, Nan; Jiang, Wenlei; Polli, James; Kane, Maureen; Michel, Sarah University of Maryland School of Pharmacy and U.S. Food and Drug Administration
Title: Iron, Where Does It Go? Bioanalytical Techniques to Measure Iron Speciation in Plasma

Abstract: Nearly 1 billion people worldwide suffer from chronic anemia, 70% of which are unable to take oral iron supplements due to adverse effects or limited therapeutic efficacy. In such cases, intravenous (IV) iron is the preferred method of treatment. There are currently 6 brand and 1 generic FDA approved intravenous iron treatments on the market used to treat anemia. Under normal conditions, iron is chelated by transferrin which transports it to the cell, thereby providing iron to proteins. However, under iron overload conditions, transferrin becomes saturated and the remaining iron, which is collectively called 'labile iron', is taken up by the cell via non-iron specific pathways. Once labile iron enters the cell it can promote the production of reactive oxygen species which can damage proteins, DNA, and lipids. Therefore, it is critical that intravenous iron products deliver the optimal level of iron at the optimal infusion rate to reduce the production of labile iron. High throughput bioanalytical mass spectrometric assays have been developed to measure total iron, transferrin bound iron, drug bound iron, and labile iron in plasma samples. These assays will be applied to a clinical trial in which the brand and generic sodium ferric gluconate will be compared.

Poster # 62 **Nguyen, John**, Akkiraju, Hemanth, Nohe, Anja University of Delaware

Title: Regulation of Osteoclastogenesis by Novel CK2-Interacting Peptide, CK2.3

Abstract: Osteoporosis is a bone disease characterized by the loss of bone mass in patients. Available treatments for osteoporosis, including bisphosphonates and parathyroid hormones, often limit their target to either inhibiting osteoclastogenesis or enhancing osteoblastogenesis. There has never been a treatment that can target both. Bone morphogenetic protein 2 (BMP2) was demonstrated to have effect on enhancing both osteoclastogenesis and osteoblastogenesis. In addition, our laboratory previously showed casein kinase 2 (CK2) to have a regulatory function on BMP2-induced osteoblastogenesis and osteoclastogenesis. We designed a synthetic peptide, namely CK2.3, to inhibit the interaction of CK2 to BMP receptor 1a (BMPRIa) at amino acid 475-479. CK2.3-injected mice showed increase of osteoblastogenesis and decrease of osteoclastogenesis, however, the mechanism was unknown.

The focus of this project was on the suppressing effect of CK2.3 on osteoclastogenesis. Akt, Erk, and p38 were shown to act downstream of BMP2 and played critical roles in osteoclastogenesis. Interestingly, we previously showed CK2.3-injected mice had an increase in p-Erk expression. These mice also had an increase in bone mineral density. To elucidate the mechanism of CK2.3, we used monocyte/macrophage RAW264.7 cells as a model to study its effect on osteoclastogenesis. Using inhibitors to inhibit pathways that were known to involve in osteoclastogenesis, we showed that U0126, a Erk inhibitor, increased osteoclastogenesis at 10M in the presence of 100nM CK2.3. Osteoclastogenesis was decreased in the presence of 100nM CK2.3 alone. Together, the result suggested CK2.3 suppressed osteoclastogenesis of RAW264.7 cells via activation of Erk. For the first time, we were able to show CK2.3 promoted osteoblastogenesis and inhibited osteoclastogenesis via activation Erk.

- Poster # 63 **Noll, Rebecca**, Yu, Xiaobo; Romero, Barbara; Allgood, Sam; Barker, Kristi; Caplan, Jeffrey; Machner, Matthias; LaBaer, Joshua; Qiu, Ji; Neunuebel, Ramona University of Delaware
Title: Identification of a novel function and host target of the Legionella pneumophila effector AnkX
Abstract: Legionella pneumophila is an intracellular bacterial pathogen that infects humans, causing the severe pneumonia known as Legionnaires' disease. Through the inhalation of contaminated aerosols, the bacteria gain access to alveolar macrophages in which they replicate inside a specialized compartment. Within this compartment, L. pneumophila evades phagosome maturation by translocating over 300 effector proteins into the host cell. One effector protein, AnkX, was shown to prevent lysosome fusion with the Legionella-containing vacuole as well as post-translationally modify host Rab GTPases. However, further characterization is required to fully understand this protein's role during infection. To gain insight into its cellular function, we sought to identify novel host proteins in AnkX's interaction network using the nucleic acid programmable protein array (NAPPA). The NAPPA detected PLEKHN1 as a top interaction candidate. Through in vitro pull-downs, co-immunoprecipitation, and cell-based assays, the interaction between AnkX and PLEKHN1 was confirmed and determined to be direct. Ectopically expressed HaloTag-AnkX and endogenous PLEKHN1 were found to co-localize on vesicular compartments in mammalian cells. Additionally, we found that PLEKHN1 had interaction candidates involved in the inflammatory response and AnkX was partially responsible for the activation of NF- κ B during infection. Overall, the interaction between AnkX and PLEKHN1 could reveal novel functions of this complex effector.
- Poster # 64 **Oates, Eleanor**, Antoniewicz, Maciek University of Delaware
Title: Applying ¹³C Metabolic Flux Analysis to Investigate Metabolic Shifts in 3T3-L1 Fat Cells Under Normoxic and Hypoxic Conditions
Abstract: Metabolic disorders, such as diabetes and obesity, are rapidly reaching epidemic proportions. Given the current and forecasted pervasiveness of these life-threatening conditions, it is imperative to identify the origins of these metabolic dysfunctions. Complicating such mechanistic studies, however, most metabolic disorders are multifactorial, caused by the interactions of numerous environmental, lifestyle, and genetic variables. This is why fluxomics, the study of metabolic fluxes, has received increased interest. Fluxomic techniques, such as ¹³C-metabolic flux analysis (¹³C-MFA), can capture the dynamic nature of system-wide metabolic fluxes, providing a high-level characterization of disease phenotypes and thus insights into disease mechanisms and origins. Here, ¹³C-MFA was applied to normoxically and hypoxically cultured mouse adipocyte cells (3T3-L1) to investigate how oxygen supply can perturb adipocyte central carbon metabolism. Adipocyte hypoxia is a symptom of obesity and has been suggested to play a role in the development of adipocyte dysfunction. Understanding adipocyte metabolic shifts under oxygen abundant and limited environments could therefore illuminate potential therapeutic treatments for this metabolic disorder.
- Poster # 65 **Ok, Kiwon**, Shimberg, Geoffrey; Li, Wenjing; Liang, Dong-dong; Neu, Heather; Michel, Sarah University of Maryland School of Pharmacy
Title: Gold Fingers: Investigation of the interactions of Gold Drugs with the Key Anti-inflammatory Protein, Tristetraprolin (TTP)
Abstract: Gold (I) complexes are used in the clinic to treat arthritis, which is a chronic autoimmune disease that causes severe inflammatory response in joints. Gold (I) and gold (III) complexes exhibit inhibitory potency towards cancer cells due to their high cytotoxicity, which is similar to the platinum (II) based anti-cancer drug, Cisplatin. For both diseases, the mechanism by which gold drugs modulate inflammation and cancer is not

known. One potential target for gold drugs is the cytoplasmic protein tristetraprolin (TTP), which is a thiol rich zinc finger protein that regulates inflammation and modulates the proliferation of cancer cells. TTP achieves this by targeting cytokine mRNAs, including TNF-alpha mRNA. To test our hypothesis that Au complexes target TTP, we prepared the gold (III) complex [Au(III)[terpy]Cl]Cl₂ (Auterpy) and investigated its interactions with a protein construct of TTP that contains just the two zinc finger domains, called TTP-2D. Using a combination of UV-visible and circular dichroism spectroscopy along with native electrospray ionization mass spectrometry, we determined that Au(III) exchanges with Zn(II)-TTP-2D forming a series of complexes, and that the resultant Au-TTP-2D complex is unstructured. The effect of Au on function was measured using fluorescence anisotropy. Zn(II)-TTP-2D bound to target RNA with nanomolar scale affinity, while Au-TTP-2D showed loss of RNA binding affinity. In contrast, the gold complex had no effect on the Zn(II)-TTP/RNA complex, suggesting a protective effect of the gold complex on TTP function. Efforts to further define this mechanism as well as to translate these findings to a cell model are in progress.

- Poster # 66 **Ortega-Escalante, Jose**, Jasper, Robyn; Miller, Stephen University of Maryland Baltimore County
Title: Developing a CRISPR/Cas9 genome editing system for *Volvox carteri*
Abstract: The success of any model organism depends on the availability of molecular techniques to manipulate or characterize that system. Recently, the Cas9/CRISPR system has become a powerful tool to edit genes due to its simplicity, versatility, and precision. The high precision is due to the CRISPR associated (Cas) endonuclease's ability to bind specific sequences of DNA via associated guide RNAs, permitting targeted INDEL mutations, reporter knock-ins, and other manipulations. To date, no genome editing system has been developed for the green alga *Volvox carteri*, and in fact no CRISPR/Cas9 system has been reported for any green algal species other than *Chlamydomonas reinhardtii*. Genome editing would greatly facilitate genetic analysis of *V. carteri*, which is an important model for investigating the evolution of fundamental developmental processes. In this study we adapted a Cas9-sgRNA vector that was designed for *C. reinhardtii* genome editing for use in *V. carteri* by inserting species-specific regulatory sequences and guide RNA sequence targeting test genes with known mutant phenotypes. Biolistic transformation of these vectors resulted in viable transformants, which were tested for guide RNA expression and Cas9 protein expression via RT-PCR and Western blots, respectively. RT-PCR and western analysis confirmed that guide RNA, and Cas9 transcripts, and Cas9 protein accumulate in the transformants. Transformants expressing two of the guide RNAs we tested, one for each of two different genes, gave rise to mutants with the expected phenotype, and sequencing of the targeted test-gene regions in those mutants showed that each contains an INDEL mutation that causes a frameshift and premature termination of the protein, demonstrating that Cas9-based genome editing works in *V. carteri*. We will use this system to mutate candidate cell differentiation and multicellularity genes in *Volvox*, and adapt it for use in related algae.
- Poster # 67 **Ott, Christine**, Bolormaa Baljinnyam², Alexey V. Zakharov², Anton Simeonov², Ajit Jadhav², Zhihao Zhuang¹ ¹Department of Chemistry and Biochemistry, 214A Drake Hall, University of Delaware, Newark, DE, 19716, USA; ²National Center for Advancing Translational Sciences, NIH, Bethesda, MD, 20892, USA
Title: A Cell Lysate-Based AlphaLISA Deubiquitinase Assay Platform for the Identification of Small Molecule Inhibitors
Abstract: Ubiquitination is an important and reversible post-translational modification that controls a large number of cellular signaling pathways in humans. Protein

ubiquitination is modulated by a class of enzymes known as the deubiquitinases (DUBs). Close to 100 DUBs have been identified in humans and are implicated in a number of human diseases including different types of cancer, neurodegenerative diseases, viral infections, and inflammatory diseases, thus making DUBs promising new targets for drug development. Currently deubiquitinase assays, particularly those amenable for high-throughput screening, have been largely limited to the minimal substrates (such as Ub-AMC) and DUBs that can be purified recombinantly. This has hindered the development of DUB inhibitors because many DUBs are large multi-domain proteins and function in complexes associated with accessory proteins, which make them difficult to obtain recombinantly. Additionally, assays run in a cellular environment may lead to more potent and specific inhibitors against the native form of the enzymes and/or complexes. Here we report a whole-cell lysate DUB assay that is based on AlphaLISA (amplified luminescent proximity homogenous assay linked immunosorbent assay) technology for high throughput screening. This assay platform uses a biotin-tagged ubiquitin probe and a HA-tagged DUB expressed in human cells. The assay was validated and adapted to a 1536-well format, which enabled a screening against UCHL1 as proof of principle using a library of fifteen thousand compounds. We expect that the new platform can be readily adapted to other DUBs to allow the identification of more potent and selective small molecule inhibitors and chemical probes.

Poster # 68 **Patel, Bhavyaben**, Janetopoulos, Christopher; Myers, Kenneth University of Sciences Philadelphia

Title: PI(4,5)P2 regulates cytoskeletal reorganization and extracellular matrix adhesion

Abstract: Angiogenesis is the process through which new blood vessels are formed from the existing blood vessels. During angiogenesis, endothelial cells (ECs) extend branched protrusions to generate a polarized leading edge through a process that involves the coordinated reorganization of the actin and microtubule cytoskeleton. Cytoskeletal reorganization and EC branching are necessary to generate productive physical contacts between the cell and the extracellular matrix (ECM), used to drive directional migration resulting in the formation of new blood vessels. The phosphatidylinositol lipids have been identified as mediators for directional migration. Phosphatidylinositol 3,4,5-triphosphate (PI(3,4,5)P3) is essential for actin polymerization at the leading edge of migrating cells. PI(3,4,5)P3 is produced by Phosphoinositide-3-kinase (PI3K) from Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). PI(4,5)P2 is known as the major phosphoinositide that interacts with actin binding proteins and is associated with various cellular signaling events. While studies suggests that PI(4,5)P2 restricts cortical actin polymerization, it is not known how PI(4,5)P2 is organized in the cell or how it regulates cell polarity and migration. We hypothesized that the regulation of PI(4,5)P2 levels functions to locally modulate cytoskeletal organization, EC-ECM adhesion, and directional migration. To test this hypothesis, we used an inositol polyphosphate 5-phosphatase (Inp54p)-fused rapamycin-triggered heterodimerization strategy to deplete plasma membrane PI(4,5)P2 levels in Human Umbilical Vein Endothelial Cells (HUVECS). High resolution live cell imaging of cells co-expressing GFP-F-tractin/GFP-Paxillin, and i-RAP constructs were collected and analyzed. Experimental data reveal that treatment of HUVECs with 100nM rapamycin, triggered disruption of the acto-myosin bundles, inducing cell spreading and inhibited the rapid turnover of the nascent focal adhesions at the cell protrusions. The results suggests that experimentally lowering PI(4,5)P2 induces actin reorganization that is coupled with cell-ECM adhesion that may contribute to HUVEC morphology and polarity. In conclusion, spatial and temporal dynamics of PI(4,5)P2 levels might have an important role in tuning different cellular functions. Future investigations will focus on the role of PI(4,5)P2 in controlling EC polarization and directional migration.

- Poster # 69 **Pogostin, Brett**, Casey Londergan and Karin Åkerfeldt Haverford College
Title: The pKa Determination of a His Residue in a Self-assembling Peptide Using Raman Spectroscopy
Abstract: Semonogelin I (SgI) is a protein found in human semen that is known to form a hydrogel. Previous studies found that a 13 amino acid residue segment of this larger protein self-assembles into an extended beta-sheet based hydrogel at pH 7-9. It is hypothesized that a histidine residue near the C-terminal end of the peptide sequence may explain this pH dependence due to the proximity of the pKa of histidine to the pH of the hydrogelation observed. The current project aims to determine the pKa of this histidine using Raman spectroscopy. Previous studies have found that this method could be used to determine the pKa of deuterated L-carnosine by observing a shift in a C-D stretch from 2360 to 2390 cm⁻¹ between the deprotonated and protonated forms of histidine. Thus, it was hypothesized that the same method could be used to determine the pKa of histidine within a peptide sequence, and potentially one that self assembles into a hydrogel. Initially, we investigated shorter peptide derivatives of the original sequence. We are currently increasing the length of the peptide in a stepwise manner in order to determine how far we can take this method. Ultimately, we are interested in evaluating the feasibility of using this method to determine the pKa of a histidine residue within the framework of a hydrogel with the aim of determining to what extent the protonation state of histidine is responsible for triggering hydrogelation.
- Poster # 70 **Potocny, Andrea**, Martin, Maxwell; Riley, Rachel; Day, Emily; Rosenthal, Joel*
University of Delaware
Title: Synthetically Accessible Tetrapyrrole Metal Complexes as Efficient Photochemotherapeutic Agents with Remarkably High Phototoxicity Index
Abstract: Photodynamic therapy (PDT), which involves the photoinduced sensitization of singlet oxygen is an attractive treatment for certain types of cancer. The development of photochemotherapeutic agents remains an active area of research because PDT is less invasive than surgical options, lacks the side effects associated with conventional chemotherapy or radiation treatments, and allows cancerous tissues to be selectively targeted via directed irradiation of the affected area. Macrocyclic tetrapyrrole compounds such as derivatives of porphyrins, phthalocyanines, chlorins, and bacteriochlorins have been pursued as sensitizers of singlet oxygen for PDT applications but historically are difficult to prepare/purify and can also suffer from high dark toxicity, poor solubility in biological media and/or slow clearance from biological tissues. Given these shortcomings, the widespread success of PDT treatment strategies awaits the development of new photochemotherapeutic agents. Toward this end, we have developed a series of novel tetrapyrrole architectures as potential PDT agents. More specifically, we have established a facile synthetic approach for the synthesis of linear tetrapyrrole complexes of late transition metals. We find that these dimethylbiladiene (DMBil1) tetrapyrrole complexes efficiently sensitize generation of singlet oxygen upon irradiation with light of wavelengths longer than 550 nm with quantum yields that are close to unity. Derivatization of the periphery of the tetrapyrrole scaffold allowed for installation of polyethylene glycol (PEG) functionalities, making these systems water soluble and biocompatible. Photophysical studies demonstrate that the DMBil1-PEG conjugates maintain their ability to sensitize singlet oxygen production and are highly stable under aqueous conditions. Preliminary cell studies with the DMBil1-PEG derivatives indicate that this new class of tetrapyrrole is an extremely promising candidate for photochemotherapeutics; millimolar concentrations of the DMBil1 complexes are well tolerated by cells in the dark, while incubation with nanomolar concentrations of these complexes are highly toxic upon illumination with light of $\lambda_{exc} > 550$ nm. As such, the DMBil1 conjugates show a remarkably high phototoxicity

index, approaching 10,000, which is significantly larger than that observed for traditional tetrapyrrole PDT agents. Additional efforts to elaborate the DMbil1 construct for development of photochemotherapeutics will also be discussed.

- Poster # 71 **Qingqing Chen**, Xingyu Lu, Yuliya Pepelyayeva, Mike Boeri University of Delaware
Title: ^{77}Se -selenomethionine as a probe of its environment in proteins
Abstract: ^{77}Se is a highly sensitive, NMR active nucleus whose chemical shift spans over 2000 ppm – a range that is an order of magnitude wider than ^{13}C and three times more than ^{15}N . The NMR signal of ^{77}Se readily responds to changes in the environment such as temperature, mobility, conformation geometry, and ionization state. While there are reports of selenium NMR in small molecules, currently there is no systematic investigation of selenium chemistry by NMR in biological macromolecules. This lack of peer-reviewed studies in turn hinders data analysis thus forming a feedback loop. To bridge the knowledge gap and fully take advantage of ^{77}Se sensitivity, we engineered a series of methionine mutants in the well-established GB1 protein model platform, and performed methionine to selenomethionine substitution to introduce selenium into the polypeptide. We demonstrate the effects of temperature, solvent exposure, and site mobility on ^{77}Se T1 relaxation and chemical shift. This work is part of our continuing efforts in constructing a database for the collection and interpretation of biological selenium NMR data.
- Poster # 72 **Redij Tejashree**, Chaudhari Rajan, Li Zhiyu, Li Zhijun University of the Sciences in Philadelphia
Title: Rational design of anti-diabetic agents.
Abstract: According to National Diabetes Fact Sheet 2014, 9.3% of US population is affected by diabetes and 27.7% of the US population is obese. Despite the advances in the medications to treat type II diabetes, the uncontrolled weight loss and glucose level still remains a matter of concern. A major hurdle in drug discovery process for targeting incretin receptors is the lack of their three-dimensional (3D) structures. Due to difficulty in G-Protein Coupled Receptor (GPCR) isolation and solubility, GPCRs are tremendously hard to crystallize. Currently, there are no complete crystal structures available for Glucagon Like Peptide 1 Receptor (GLP-1R), a member of class B GPCRs and a major target for diabetes treatment. Therefore, in our lab, a novel homology modeling method to predict the structure of class B GPCRs was proposed. This formed the basis to predict the GLP-1R structure and ligand-binding site in GLP-1R using the low sequence identity templates. This structure was subsequently used to screen small orally active incretin modulators using computer aided drug design techniques. The top-ranked compounds have been tested in vitro using luciferase assay. This assay was standardized to test both the agonist as well as antagonist effect of the incretin modulators on GLP-1R. Two compounds showed the agonist activity to GLP-1R and a few showed antagonist activity. The agonist and antagonist activities were further confirmed based on the synergistic effect in the presence of GLP-1 peptide. The activity of the incretin modulators towards GLP-1R confirms the reliability of our GLP-1R model and provides hit compounds for further drug development. In future, these active compounds will be tested to check the induction of insulin production in the presence or absence of glucose. Furthermore, we will perform rational design to improve the binding affinity and absorption, distribution, metabolism and excretion (ADME) properties of the hit compounds.

Poster # 73 **Regmi Abish**, Kalburge, Sai Siddharth; Whitaker, W. Brian; Boyd, Fidelma Ethna
University of Delaware

Title: Diverse and competitive nutrient utilization: bacterial strategy for host colonization

Abstract: *Vibrio parahaemolyticus* colonizes the human gastrointestinal tract to cause inflammatory diarrhea after consumption of contaminated shellfish. We developed a streptomycin pretreated adult mouse model of colonization to determine the bacterial factors required. Antibiotic pretreatment reduces the diversity of commensal species, reducing competition for resources such as nutrients allowing *V. parahaemolyticus* to colonize. UCM-V493, an environmental strain, lacks the known virulence factors present in the clinical isolate RIMD2210633. However, our data show that UCM-V493 outcompetes RIMD2210633 in the streptomycin pretreated mouse model.

To determine the mechanism of this superior colonization, we examined metabolic fitness between the strains. We determine growth in 190 different carbon sources using phenotypic microarray plates. UCM-V493 had enhanced metabolic diversity and activities in 27 of 77 carbon sources utilized, for example, galactose, mannose and a number of amino acids. We focused our attention on galactose and determined in UCM-V493 the presence of a transporter with homology to SGLT transporters from eukaryotes. Characterization of this transporter suggests that it is a high affinity galactose transporter that could explain UCM-V493's in vivo fitness. Overall the data suggests in vivo fitness correlates with in vitro metabolic fitness of UCM-V493.

Poster # 74 **Rezazadeh, Sina**, Devannah, Vijayarajan; Watson, Donald A. University of Delaware

Title: Nickel-catalyzed C-Alkylation of Nitroalkanes with Unactivated Alkyl Iodides

Abstract: Nitro group is a versatile functional group in organic synthesis. Historically, the C-alkylation of nitroalkanes using alkyl halides has been highly challenging due to the propensity of alkylation at oxygen by nitronate anions. Recently, our group has developed a variety of copper-catalyzed nitroalkane alkylations using alkyl bromides bearing radical stabilizing function groups alpha to the halide, such as benzyl bromides. However, to date non-activated alkyl halides have not been substrates for this transformation. We have now found an efficient and simple catalytic system that allows the reaction of unactivated primary, secondary, and tertiary alkyl iodides with nitroalkanes. With this transformation, a wide variety of complex nitroalkanes can be easily prepared in good yields.

Poster # 75 **Rujin Cheng**, Zhengqi Zhang, Sharon Rozovsky University of Delaware

Title: Chemical ligation of membrane selenoprotein S and its redox regulated function in ERAD

Abstract: Selenoprotein S is an intrinsically disordered membrane enzyme that is a member of the ER-associated protein degradation (ERAD) pathway. The ERAD governs the extraction of misfolded proteins or misassembled protein complexes from the ER's membrane and lumen to the cytoplasm where they are degraded by the proteasome. Selenocysteine (Sec) is a genetically encoded amino acid but its incorporation into proteins requires a dedicated suite of elements to reprogram the opal codon UGA to encode for Sec. Sec incorporation efficiency is low due to the opal codon being decoded as a termination signal. Hence it remains challenging to prepare selenoproteins. In order to characterize SELENOS's role in the ERAD membrane complex, we have utilized native chemical ligation to generate SELENOS from the protein fragments. We describe the preparation, characterization and redox regulated binding to its protein partner.

Poster # 76 **Samer Daher**, Lee, Miseon; Glassford, Ian; Jin, Xiao; Teijaro, Christiana; Andrade, Rodrigo
Temple University

Title: Alternative Approaches to Combat Antibiotic Resistance Utilizing Click Chemistry to Develop Next Generation Analogs

Abstract: The field of antibiotics and drug development is susceptible to onset of antibiotic resistance, and increasingly common problem, especially with the widespread use of antibiotics in healthcare. The demand for new classes of drugs that can overcome bacterial resistance threats is high and can be accomplished by synthesizing novel targets via structure-activity relationship (SAR), or developing enormous methodologies capable of screening larger scope of antibiotics, amongst others. Our lab is interested in exploring macrolides such as Solithromycin, developed in 2005 by Optimer Pharmaceuticals, via employing Cu (I) catalyzed combinatorial click chemistry. Currently, Solithromycin is in phase 3 clinical trials and has proven to be most efficacious amongst Ketolides developed to date. Macrolides' mechanism of action is by binding to the 50S subunit and inhibiting protein synthesis, which motivated us to investigate non-covalent interactions comprised of hydrogen bonding and Pi- stacking interactions. Accordingly, a series of analogs fulfilling the latter characteristics have been designed based on Cu click chemistry. Alternatively, inspired by target-guided in situ click chemistry, a library consisting of 5-15-membered alkynes has been developed in which E.coli 50S/70S ribosomes substituted the Cu in templating the macrolide azide with various alkyne fragments. An advantage of this powerful drug discovery platform is that it eliminates the arduous task of independent synthesis. All analogs have been evaluated by minimum inhibitory concentration (MIC) assays. Our initial data showed promising MIC levels compared to Solithromycin. In conclusion, the promising MICs values dedicate a positive impact for discovery of potential drug candidates that would have an enormous benefit on healthcare given the ever-expanding use of antibiotics.

Poster # 77 **Schaefer, Amy**, Melnyk, James E.; Wastyk, Hannah C.; Grimes, Catherine L. University of Delaware

Title: Requirements for the recognition and response to peptidoglycan fragments by the Innate immune receptor Nod2, a protein important in Crohn's disease

Abstract: Our innate immune system is an intricate collection of receptors responsible for recognizing pathogens and generating proper immune responses. Nod2 is one essential innate immune receptor that responds to peptidoglycan (PG) fragments by initiating the NF- κ B pathway. Mutations to this innate immune receptor are implicated in an increased susceptibility for Crohn's disease, a chronic inflammatory bowel disease caused by misregulation of the immune system. These Nod2 mutations (R702W, G908R, and 1007fsInsC) prevent it from recognizing or responding to its proposed ligand, muramyl dipeptide (MDP), a PG fragment. The objective of this study is to determine the requirements for Nod2 binding and activity. Using two independent analytical binding assays (surface plasmon resonance and backscattering interferometry) and a NF- κ B driven luciferase reporter assay we characterize the molecular requirements for recognizing and responding to PG fragments for both WT and mutant Nod2. Our data suggests that the receptor can bind a variety of peptidoglycan fragments other than MDP. Additionally we demonstrate that the membrane environment plays a crucial role in governing ligand specificity for activation of the NF- κ B pathway. Finally, our data suggest that the Crohn's disease-associated Nod2 mutants suffer from poor stability, but upon stabilization the mutants are able to properly bind to MDP and signal the NF- κ B pathway.

Poster # 78 **Schmitt, Danielle**, Dranchak, Patricia, Inglese, Jim, An, Songon University of Maryland Baltimore County

Title: Spatial Regulation of Glycolytic and Gluconeogenic Enzyme Compartmentalization by Small Molecules in Human Cells

Abstract: Glycolysis is catalyzed by ten enzymes, producing two molecules of pyruvate and two ATP molecules for every one molecule of glucose consumed. While much is understood about the process, the spatial compartmentalization of enzymes involved in glucose metabolism in human cells has remained elusive. Recently, using quantitative high-content imaging we have shown the association of glycolytic and gluconeogenic enzymes into cytoplasmic multienzyme complexes in human cells. However, the assembly mechanism of these enzymes into complexes is largely unknown. To understand the mechanism of enzyme compartmentalization in cells, we have developed a high-throughput screening assay which uses human phosphofructokinase I liver-type (PFKL) tagged with monomeric enhanced green fluorescent protein (mEGFP; PFKL-mEGFP) stably expressed in HeLa Tet-On cells as a marker for the promotion of the glycolytic and gluconeogenic multienzyme complex. A pilot screen using the Library of Pharmacologically Active Compounds (LOPAC) identified a small molecule previously implicated in cell cycle control regulates PFKL-mEGFP clustering in cells. Accordingly, we hypothesize the compartmentalization of PFKL-mEGFP is spatiotemporally regulated by the cell cycle in human cells. We envision that our study will advance our understanding of how metabolic multienzyme complexes are functionally orchestrated throughout the cell cycle.

Poster # 79 **Schutsky, Emily**, DeNizio, Jamie; Nabel, Christopher; Davis, Amy; Wu, Hao; Kohli, Rahul. University of Pennsylvania

Title: Defining and Exploiting APOBEC3A's Activity on the Extended Epigenome

Abstract: AID/APOBEC family cytosine deaminases canonically play crucial roles in immunity by converting cytosine to uracil in single-stranded DNA (ssDNA) in specific sequence contexts. Outside of this established physiological role, AID/APOBEC enzymes have also been implicated in the poorly-understood process of DNA demethylation through their proposed deamination of epigenetically-modified cytosine bases like 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). However, there has been no thorough biochemical characterization of AID/APOBEC activity on these substrates, or on the recently discovered 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) to inform this proposed role. Here, we provide the first steady-state kinetic measurements of the most active family member—APOBEC3A (A3A)—against various natural and unnatural modified substrates using a novel, restriction enzyme-based deamination assay. In this analysis, we determined that A3A has poor activity against larger oxidized cytosines, like 5hmC, 5fC, and 5caC, and therefore likely does not contribute substantially to active DNA demethylation via deamination of these bases. By contrast, 5mC is efficiently deaminated in a manner not fully explained by the steric discrimination observed with other AID/APOBEC family members. We also showed via sequencing of long single-stranded substrates with Cs or mCs that A3A's sequence preference is further exaggerated when targeting mC bases. Finally, due to A3A's proficiency on C and mC and potent discrimination against ox-mCs, we hypothesized that A3A could be developed as a biotechnological tool to localize ox-mCs in the genome. Through both locus-specific and whole genome validation on differentially modified phage genomes, we demonstrate that A3A's biochemistry permits its use as part of APOBEC Coupled Epigenetic Sequencing (ACE-Seq) – a nondestructive alternative to bisulfite-based methods for the single base resolution localization of genomic hmC. As this method requires as little as picograms of starting material, we propose that ACE-Seq can be used to characterize samples that have remained refractory to bisulfite-based methods, especially samples from small or transient cell populations.

Poster # 80 **Scinto, Samuel**, Ekanayake, Oshini S.; Boyd, Samantha; Liu, Jun; Rozovsky, Sharon; Fox, Joseph M. University of Delaware

Title: Development of trans-Cyclooctene as a highly reactive cell-permeable sulfenic acid probe

Abstract: Reactive oxygen species (ROS) are mediators for signal transductions pathways through their capability to activate and inactivate enzymes, generate post-translational modifications, and induce conformational changes in proteins. The increased hyperactivity of cancer cells and elevated levels of ROS leads to a unique and up-regulated redox environment. One of the primary mechanisms used by the cell to report and regulate oxidative stress is through the oxidation of cysteine to its reversible form of sulfenic acid. Sulfenic acids are highly reactive electrophiles and therefore often short-lived in a cellular environment; owing in part to their low intracellular concentration, this reactive intermediate has been difficult to detect. Current small molecule probes for the intracellular detection of sulfenic acids are primarily derived from the 5,5-dimethyl-1,3-cyclohexanedione (dimedone), which suffers from modest kinetics and decreased activity in low pH environments. We have developed a small molecule probe, derived from trans-cyclooctene, with fast and selective reactivity with sulfenic acid. Mutant fRMs_r, capable of generating a stable sulfenic acid, was used to determine an in vitro second order rate constant of 746 M⁻¹sec⁻¹. A biotinylated derivative was used for live-cell assays in order to identify intracellular sulfenic acids in LNCaP and T98G. Furthermore, we developed a cell permeable fluorophore capable of capturing sulfenic acids under induced oxidative stress. Our findings demonstrate the utility of a highly reactive, cell-permeable, trans-cyclooctene derived probe for detecting intracellular sulfenic acid.

Poster # 81 **Shihan, Mahbulul**, Pathania, Mallika; Wang, Yan; Duncan, Melinda University of Delaware

Title: Regulation of TGF- β bioavailability in lens

Abstract: Posterior capsular opacification (PCO) is the major side effect seen after cataract surgery. Although PCO is treatable by laser, this can lead to further complications such as macular edema and, retinal detachment. Thus, PCO prevention would improve the outcome of cataract surgery. Although activated transforming growth factor beta (TGF- β) signaling is known to mediate PCO, little is known about what controls the bioavailability of active TGF- β following cataract surgery. Fibronectin, an extracellular matrix (ECM) protein, has been shown to locally concentrate latent TGF- β into the ECM. However the mechanism of action of fibronectin in PCO pathogenesis is not well understood. Notably, in a mouse cataract surgery model, fibronectin mRNA levels upregulate by 24 hours post cataract surgery (PCS), and fibronectin protein is deposited around fibrotic lens epithelial cells (LECs), by 48 hours PCS while deposition continues as the fibrosis progresses at later times after surgery. So to understand the function of fibronectin in PCO pathogenesis, we created mice that lack the fibronectin gene (FN conditional knock out -FNcKO) from the lens, and I have shown that, while these animals normally undergo the initial fibrotic response, the long term scarring response is not maintained. As I have found that these animals also exhibit attenuation of canonical TGF- β signaling, this proposal seeks to test the hypothesis that fibronectin is essential for TGF- β bioavailability during PCO.

Poster # 82 **Shimberg, Geoffrey**, Michalek, Jamie; Ok, Kiwon; Neu, Heather; Zucconi, Beth; Rodrigues, Andria; Wilson, Gerald; Stemmler, Timothy; Splan, Kathryn; Michel, Sarah University of Maryland Baltimore School of Pharmacy

Title: A Tale of Two Zinc Fingers: CPSF30, a 'CCCH' Zinc Finger with an Unexpected 2Fe-2S Cluster that Regulates RNA Processing and TTP, a 'CCCH' Zinc Finger that is modulated by Cu(I)

Abstract: CPSF30 and TTP are non-classical zinc finger proteins (ZFS) that contain domains with a CCCH motif. CPSF30 has 5 CCCH domains and TTP has 2 CCCH domains. Both proteins are involved in RNA regulation; CPSF30 regulates pre-mRNA and TTP regulates mRNA; however, only TTP has been shown to directly bind to RNA (via its CCCH domain, targeting AU-rich sites). Given the sequence similarity between TTP and CPSF30, we hypothesized that CPSF30 directly binds AU-rich RNA sequences via its CCCH domains. To test this hypothesis, a construct of CPSF30 containing the five CCCH domains, was over-expressed and purified. Unexpectedly, CPSF30 was reddish in color, suggesting iron coordination. UV-visible, ICP-MS analysis and XAS spectroscopy revealed that the protein contains a 2Fe-2S cluster in addition to four zinc domains. The 2Fe-2S cluster utilizes a CCCH ligand set, and is the second example of this site in biology! RNA binding studies, using EMSA and fluorescence anisotropy (FA), with α -synuclein AU-rich pre-mRNA as a target, were then performed. From these studies, we determined that (1) CPSF30 binds directly to AU-rich targets on pre-mRNA via a cooperative binding mechanism and (2) CPSF30 requires both iron and zinc coordination for RNA binding (1) For further research, we are looking into how NS1A, an influenza A protein, inhibits CPSF30. Preliminary work shows that NS1A binds the CPSF30-RNA complex and inhibits CPSF30 binding to RNA when NS1A is present. Studies focused on Cu(I) binding to TTP will also be presented. Cu(I) is toxic in excess and there is emerging evidence that ZF sites may be target of Cu(I) toxicity. Using UV-visible and circular dichroism spectroscopies, we have determined that 3 Cu(I) ions bind to TTP and that Cu(I) binding inhibits the structure of the protein. In addition, RNA binding studies, using FA with the TNF- α AU-rich mRNA revealed that Cu(I) inhibits the TTP-RNA interaction. We propose that inhibition of TTP function by Cu(I) contributes to its mechanism of toxicity. (1) Shimberg G. M. et al. PNAS, 2016, 113, 4700-5

Poster # 83 **Shrimp, Jonathan**, Grose, Carissa; Esposito, Dominic; Meier, Jordan Government Institution

Title: Chemical Control of a CRISPR-Cas9 Acetyltransferase

Abstract: Lysine acetyltransferases (KATs) play a critical role in the regulation of gene expression and other genomic functions. A major challenge in studying KATs is determining their causal effects on transcription at specific genomic elements, particularly with spatial and temporal control. To address this challenge, we have developed a CRISPR-Cas9 assay to target and study the cellular activity of the lysine acetyltransferase p300. We find the acetylation activity of p300 can be targeted to specific genomic loci using either dCas9 or MS2 fusion proteins via engineering of short guide RNA architecture. Applying this reporter in a focused small molecule screen revealed that KAT-activated transcription requires acetyltransferase activity as well as a functional p300 bromodomain. This result provides insight into p300 activity, and implies that, in some cases, genomic-targeting of a KAT may be insufficient on its own to stimulate gene expression, and instead requires a coupled bromodomain to tune catalysis, localization, or processivity. Finally, we demonstrate that the activity of our CRISPR-Cas9 acetyltransferase can be acutely inhibited using a small molecule KAT inhibitor identified in the patent literature, providing spatial and temporal control over the system. Overall, our studies provide a powerful platform for studying the loci-specific consequences of acetyltransferase activity, and provide the

technological basis to better understand how acetylation of specific genomic elements can drive oncogenic signaling and heritable epigenetic phenotypes.

Poster # 84 **Shuler, Scott**, Yin, Guoyin; Krause, Sarah; Vesper, Caroline; Watson, Donald University of Delaware

Title: Synthesis of Secondary Unsaturated Lactams via an Aza-Heck Reaction

Abstract: Unsaturated lactams are highly valued synthons due to their prevalence in biologically-active molecules and natural products. In addition, they are also rich synthetic intermediates that can be transformed into a diverse array of complex structures. To this end, we have developed a general, catalytic method to synthesize unsaturated lactams. Using O-phenyl hydroxamates as an electrophilic nitrogen source, palladium catalyst, and fluorinated phosphite ligand, we were able to achieve an intramolecular cyclization in excellent yields, and demonstrate a great tolerance of varying substrates and functional groups. Mechanistic evidence supports a Heck-type mechanism, with the catalyst proceeding through a palladium(0)/palladium(II) catalytic cycle.

Poster # 85 **Spangler, Leah**, Chu, Roxanne; Lu, Li; Kiely, Christopher; Berger, Bryan; McIntosh, Steven Lehigh University

Title: Single enzyme biomineralization of CuInS₂, (CuInZn)S₂, and CuInS₂/ZnS core/shell quantum dots for bioimaging applications

Abstract: Biomineralization, the process by which living organisms generate minerals, has recently been utilized to synthesize crystalline materials under ambient conditions for catalytic and energy based applications. Semiconductor quantum dots, which are desirable for their size-tunable optical and electronic properties, have seen limited commercial use due to costly high temperature, inorganic synthesis routes. Quantum dots are also ideal for bioimaging applications, but commonly studied materials, such as CdS or CdSe, are toxic to cells and must be phase transferred to the aqueous phase before use. In this work, we discuss the direct, single enzyme, aqueous biomineralization of nontoxic CuInS₂, (CuInZn)S₂, and CuInS₂/ZnS core/shell quantum dots with fluorescence in the visible range for cell labeling applications. The biomineralized CuInS₂ nanocrystals are synthesized using an aqueous two-step approach at ambient temperature and pressure, reducing production cost and removing organic solvents from the synthesis process.

Previous enzymatic routes of nanocrystal synthesis exploit biomolecules for templating, but often lack size control and require the addition of a reactive sulfur precursor, such as Na₂S. In contrast, the cystathionine gamma-lyase (CSE) enzyme utilized by our group both templates nanocrystal growth and catalyzes mineralization by generating the reactive sulfur species H₂S from the amino acid L-cysteine. The as synthesized CuInS₂/ZnS nanocrystals can be easily transferred into biologically relevant buffers for bio-imaging applications, or into an organic phase for use in energy applications. Size tunable optical properties are demonstrated by UV-vis and photoluminescence spectroscopy. HRTEM and HAADF-STEM imaging are used to confirm the size and crystal phase of the nanocrystals. The CuInS₂/ZnS nanocrystals are conjugated to antibodies and used as fluorescent labels in THP-1 leukemia cells, demonstrating their potential for bioimaging applications.

- Poster # 86 **Sun, Zuodong,** Rokita, Steven E. Johns Hopkins University
Title: Towards A Halophenol Dehalogenase by Computational Design
Abstract: Enzymatic dehalogenation is a key step for bioremediation of environmentally hazardous halophenols. The mammalian flavoenzyme iodotyrosine deiodinase (IYD) provides a reductive strategy to cleave the carbon-halogen bonds for such compounds under aerobic conditions. However, native IYD is highly specific for its zwitterionic substrates: mono- and di-halogenated tyrosines. This substrate preference is created by the closure of an active site lid upon coordination of the zwitterion of halotyrosines. Herein we report the use of computational design to improve the dehalogenation efficiency of IYD towards a model non-zwitterionic halophenol 2-iodophenol (2IP). Iterative rounds of RosettaDesign simulations on the active site lid directed extensive mutation of 11 variants. The resulting enzymes ranged from a nearly inactive variant D1 to a variant D10 that was 450% more efficient with 2IP than the wildtype parent. The individual effects on deiodination activity of the 15 mutations of D10 were also evaluated. The local fitness landscape of the lid appears to be very rugged as the additive effect of each of the 15 mutations is highly deleterious, while only 2 surface mutations near the active site are beneficial. Stabilization of the dynamic active site lid for D1 and D10 relative to the wildtype enzyme was revealed by their resistance to limited trypsin proteolysis. D10 was even more resistant to proteolysis than D1 in the presence of 2IP. These findings represent the first step for altering the substrate specificity of IYD and highlight the importance of stabilizing a productive substrate-enzyme complex for designing an efficient enzyme.
- Poster # 87 **Szantai-Kis, D. Miklos,** Walters, Christopher R.; Wang, Yanxin J.; Barrett, Taylor M.; Hoang, Eileen M.; Petersson, E. James University of Pennsylvania
Title: Thioamides: Improved Incorporation Methods and Effects on Protein Stability
Abstract: In recent years, our laboratory has developed the thioamide, a single O to S substitution in canonical amide bonds, as a versatile probe for fluorescence and protease studies. Thus far, thioamides have been incorporated into the backbone of proteins via native chemical ligation (NCL) with a Cys or homocysteine (Hcs) at the ligation site. We utilized two different strategies to facilitate the site-specific incorporation of thioamides into proteins. First, we investigated selective thiol-desulfurization in the presence of thioamides. This allows us to perform traceless ligation reactions in the presence of thioamides and avoids have a Cys ligation scar. In another approach, showed that using alternative deprotection reagents in solid phase peptide synthesis, reduced epimerization and improved yield of thioamide containing peptides. With these strategies in hand, we synthesized different thioamide containing proteins and assessed their effect on protein stability using Circular Dichroism and thermal denaturation.
- Poster # 88 **Tong, Xin,** Li, Tiezheng; Wang Lai-Xi University of Maryland, College Park
Title: Site saturation mutagenesis of Streptococcus pyogenes endoglycosidase S and S2 leads to discovery of novel glycosynthases for antibody Fc glycan remodeling
Abstract: Endo- β -N-acetylglucosaminidases from pathogen Streptococcus pyogenes cleave at the beta-1, 4-linkage of the diacetylchitobiose core in the N-linked glycans of antibody IgG. Two endoglycosidases from two family 18 glycoside hydrolases (GH18), Endo-S and Endo-S2, possess intrinsically not only hydrolytic activity but also the ability to transfer synthetic glycan oxazolines to an alternative acceptor to form a new glycosidic linkage. The specific transglycosylation activity of endoglycosidases has proven to be indispensable in the chemoenzymatic synthesis and the site-specific N-glycosylation remodeling of intact antibody IgG. However, the inherent hydrolytic activity towards the product and the low transglycosylation efficiency of wild type enzymes has greatly limited

their broad applications. This study describes saturated site-directed mutagenesis on residues in the putative active site to create systematic libraries of glycosynthase mutants of both enzymes with superior transglycosylation efficiency. Interesting mutants were identified for both Endo-S and Endo-S2. Several interesting mutants were found to possess dramatically enhanced transglycosylation activity with much diminished product hydrolysis in comparison with the wild-type enzymes. Kinetic analyses, combined with structural information and molecular dynamics, revealed important properties of the mutants that help dissect the functional role of the aspartic acid residue in facilitating the formation of an oxazolinium ion intermediate in a unique substrate-assisted mechanism. This study also presents the first glycosynthases derived from Endo-S2 that modify IgG Fc N-glycans with distinct substrate specificity. Our discoveries provide further insights into the molecular basis for the hydrolysis-to-transglycosylation transition of endoglycosidases and the general substrate-assisted mechanism of GH18 family. The catalytic activity of Endo-S2 mutants with relaxed substrate specificity was exemplified by the efficient synthesis of different glycoforms of a therapeutic antibody, Rituximab, with distinct binding affinities to their endogenous IgG Fc receptors.

Poster # 89 **Urmey, Andrew, Zondlo, Neal** University of Delaware

Title: Detecting Protein Tyrosine Nitration with Designed Peptides

Abstract: Tyrosine nitration is an important biomarker indicative of oxidative stress and associated with disease states such as Alzheimer's disease, cancers, heart disease, and inflammation. Though it has historically been considered a type of oxidative damage, recent evidence indicates that this non-enzymatic post-translational modification (PTM) is involved in cellular signaling pathways. Because nitrotyrosine is not dependent on the activity of a specific enzyme, and because its specificity is based on 3-D structure rather than protein sequence, it is difficult to study using standard molecular biology techniques. In order to effectively visualize nitrotyrosine, and to elucidate its roles in cell signaling and disease, new tools are required which can apprehend nitrotyrosine at a molecular level. Toward this goal, we have designed peptides which contain nitration-susceptible tyrosine and respond with structural and luminescent changes upon nitration. Previous work in the Zondlo lab has shown that peptides based on the native calcium-binding EF hand can, when undergoing post-translational modifications, show significant changes in lanthanide binding. Incorporating a tryptophan in these peptides allows resonance energy transfer to a bound terbium ion, allowing for PTM-dependent fluorescence. Nitrotyrosine is known to bind lanthanides; in an EF-hand peptide, nitration of tyrosine was used to increase binding by 300-fold ($\Delta\Delta G = -3.3$ kcal/mol). A peptide designed to bind Tb³⁺ before nitration shows significant luminescence which can be quenched upon nitration of tyrosine. The sensor peptide was expressed as a tag on maltose binding protein (MBP) and used to detect nitration in vitro.

Poster # 90 **Vrathasha Vrathasha, Deibert, Jenna; and Nohe, Anja** University of Delaware

Title: Elucidating the mechanism behind peptide CK2.3 induced osteogenesis in C2C12 cells

Abstract: Osteoporosis is a debilitating bone disorder where patients are characterized with low bone density. Per the National Osteoporosis Foundation, one in two women and one in four men age 50 and older will break a bone due to osteoporosis. Osteoporosis is caused by: a) excessive bone resorption by osteoclasts and/or b) failure of osteoblasts to produce and replace the resorbed bone leading to weak and porous bone. Majority of the existing treatments target osteoclast activity, but osteoclasts are essential for maintaining body homeostasis. Thus, there is a need for a novel treatment that specifically drives osteoblastogenesis.

One of the well understood BMP2 induced signaling occurs via BMPR-IA and BMPRII. Our lab has reported an interaction between BMPR-IA and Casein Kinase 2(CK2). CK2 is a ubiquitously expressed enzyme that plays a key role in various cellular activities. Using the interaction site(2.3), a peptide was synthesized that would bind to CK2, preventing it from interacting with BMPR-IA at the respective site. Treatment of primary osteoblast cultures with CK2.3 enhanced bone formation without the increase in osteoclast activity; and increased overall BMD and mineral apposition rate in 8-week- old mice. We have established that CK2.3 mediates MSCs to take on osteoblastic lineage.

To determine the effects of peptide CK2.3 within the cell and in-vivo, quantum dots(QDots) will be used as a probe. QDots are semiconductor nanoparticles that are fluorescent under UV light and ideal for live-cell imaging. Peptide will be conjugated to QDots by forming an amide bond. FTIR spectroscopy will be used to confirm the conjugation, while confocal imaging will show its uptake into C2C12 cells(murine myoblasts) and its interaction with CK2. This technique will provide insight into the workings of the peptide within a cell and eventually in-vivo.

Poster # 91 **Walia Manish**, Christiana N. Teijaro, Manish Walia, Senzhi Zhao, and Rodrigo B. Andrade
Temple University

Title: Progress Toward the Syntheses of (-)-Conophylline a Bis-Aspidosperma

Abstract: In 1992 and 1993, Kam and coworkers isolated (-)-conophylline (0.065%) and (-)-conophyllidine (0.0043%) from the leaves of *Tabernaemontana divaricata*. In 2010 (-)-melodinine K, a deoxygenated version of (-)-conophylline, and (-)-conophyllidine, was isolated by Luo and coworkers from *Melodinus tenuicaudatus* in less than 0.006%. These dimeric alkaloids have a wide range of biological activities. (-)-Melodinine K was shown to have a low μM IC₅₀ against several human cancer cell lines compared to cisplatin and vinorelbine. (-)-Conophylline has been shown to be a potent inhibitor of the ras function, is capable of inducing Beta-cell differentiation in fetal and neonatal pancreatic tissue and pancreatic acinar carcinoma cells cultured in rats as well as in porcine neonatal pancreatic endocrine cells. These biological activities, low isolation yields and the structural complexity of these natural products have prompted their syntheses. These dimeric alkaloids can be disconnected via the dihydrofuran ring to afford a northern and southern fragment that can be arrived at from the syntheses of 16-methoxytabersonine and oxygenated analogs of tabersonine, respectively. Key steps in the synthesis include a Glorius indole synthesis to build the oxygenated indole core, a domino Michael/Mannich/N-allylation to form the E-ring and set the stereochemistry for the C and D rings, and a late stage electrophilic aromatic substitution (EAS) coupling.

Poster # 92 **Walters, Christopher R.**, Szantai-Kis, D. Miklos; Barrett, Taylor; Petersson, E. James
University of Pennsylvania

Title: The Impact and Utility of Thioamide Backbone Modifications in Diverse Protein Systems

Abstract: Backbone alterations of proteins can enable structure-function studies, bias conformational selectivity, and increase therapeutic potency and stability. In particular, we have investigated thioamides as surrogates for peptide linkages to enable photophysical studies of proteins. However, the impact of thioamides on the stability of proteins rich in secondary and tertiary structure has yet to be discussed in detail. We have recently incorporated thioamides in two structurally rich benchmark proteins through Native Chemical Ligation (NCL) to assess the local and global influence of thioamide substitutions on protein structure. We found that thioamides in the C-terminal loop and α -helix of the

calcium signaling protein calmodulin (CaM) can have either stabilizing, neutral, or destabilizing effects depending on their placement. Additionally, we examined thioamides in the β -sheet rich protein G-B1 domain (GB1) and observed that the severity of destabilizing effects is dependent on the position specific accommodation of the larger sulfur atom into the β -strand. The stabilizing and destabilizing effects described in these model systems exemplify the thioamide's potential for use as a modulator of structure-activity relationships in engineered proteins. Moreover, the advances in thioprotein synthesis and ligation made in these studies enables us to install one or more thioamides with increased efficiency at positions of our choosing in future protein and peptide targets for therapeutic applications and biophysical studies.

Poster # 93 **Wang, Jie-Liang**, Chen, Xing; Barrett, Taylor; Liu, Chunxiao; Szantai-Kis, Miklos; Lan, Matthews; Bazilevsky, Gleb; Marmorstein, Ronen; Kalb, Robert; Petersson, E.
JamesUniversity of Pennsylvania

Title: Peptide semi-synthesis as a versatile chemical biology tool to construct peptide probes

Abstract: Peptide semi-synthesis is a versatile chemical biology strategy to construct peptide probes. Different functionalities can be added to the sequence while the perturbation to the native structure can be controlled at the minimal level. We used a combination of methods to achieve this goal, which includes solid phase peptide synthesis (SPPS), native chemical ligation (NCL), protein expression, and copper-catalyzed click chemistry. We applied this strategy to three different studies. (1) We compared the difference in protein-protein interactions of N-terminal acetylated histone H4 versus non-N-terminal acetylated histone H4 using our probe. (2) We constructed probes to study the Proline/Arginine dipeptide (PR)_x from hexanucleotide repeat expanded C9ORF72, a gene shows strong association with amyotrophic lateral sclerosis (ALS). We found (PR)_x peptide inhibit the proteasome, which results in a reduction of flux through both autophagic and proteasomal degradation pathways. (3) We used thioamide-based fluorescent protease sensors to study the protease inhibition effect of backbone thioamide modification on pepsin, cathepsin-B, and thermolysin.

Poster # 94 **Wang, Xu**, Edwards, Rachel L.; Haymond, Amanda; Brothers, R. Carl; Boshoff, Helena I.; Couch, Robin D.; Odom, Audrey R.; Dowd, Cynthia S. The George Washington University

Title: Evaluating fosmidomycin analogs as antimicrobial agents through 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) inhibition

Abstract: Tuberculosis (TB) and malaria are severe, life-threatening infectious diseases that torture millions of people every year. Both diseases are caused by microorganisms: Mycobacterium tuberculosis (Mtb) causes TB and Plasmodium falciparum causes malaria. Due to the unavailability of new antibiotics and the increasing emergence of drug-resistant strains of these organisms, there is an urgent demand for novel drug therapies. We try to find new drug candidates that would effectively and efficiently kill Mtb and Plasmodium falciparum. The isoprene unit, made of 5-carbons, is used and made by all living cells. Halting isoprene production leads to cell death in Mtb and P. falciparum. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) is a crucial enzyme in the nonmevalonate pathway to make isoprenes. This pathway is found in many pathogenic organisms including Mtb and P. falciparum, but not humans. Thus, Dxr inhibitors may be promising therapeutic candidates with low human toxicity. It has long been known that fosmidomycin is a potent inhibitor of Dxr. Unfortunately, fosmidomycin is not effective against Mtb and has failed in clinical trials against malaria. We synthesized and evaluated fosmidomycin

analogs as improved Dxr inhibitors that act as potent antimicrobial agents, which shed light on its SAR and the potential of Dxr inhibitors becoming antimicrobial drug candidates.

Poster # 95 **Weidner, Hilary**, Eskander, Mark; Dibert, Debbie; Nohe, Anja University of Delaware
Title: A novel peptide increases bone mineralization in isolated human primary cells
Abstract: Osteoporosis is a bone disease that is characterized by low bone density. This leads to deterioration of the bones, which ultimately increases the occurrences of bone fractures or breaks. The treatments are very expensive (both emotionally and fiscally), and the costs will only steadily rise as the population of elderly increases, which is why it is necessary to find novel treatments. On a cellular level, normal bone has a balance between bone forming cells (osteoblasts) and bone remodeling cells (osteoclasts). Primary osteoporosis is characterized by having more osteoclast activity, less osteoblast activity, and an increased amount of adipocytes (fat cells). However the majority of treatments on the market focus on decreasing osteoclast activity and not increasing osteoblast activity or decreasing adipocyte population. Our lab has designed a novel peptide known as CK2.3 that binds to an interacting protein called CK2 thus inhibiting it from binding to a specific portion of its receptor, BMPRIa. Primary cells from patients undergoing osteoporosis or osteoarthritis were extracted and grown. The cells were from human femoral heads, which were removed via hip arthroplasty surgery from Christiana Care Hospital in Newark, Delaware. The cells were treated with our novel peptide, CK2.3 and a mineralization (osteoblast activity) and lipid droplet assay (fat cell formation) were conducted in order to measure cellular activity. Our lab has shown that treatment of CK2.3 increases the mineralization (osteoblast activity) and reduces lipid droplet formation (fat cells) in extracted primary cells from human femoral heads. The novel peptide CK2.3 could be utilized as a potential treatment for primary osteoporosis as it focuses on increasing osteoblast activity.

Poster # 96 **Welch, Matthew**, Ali, Izna; Lu, Yang; Brouwer, Kim; Swaan, Peter University of Maryland, Baltimore
Title: Development and In Vitro Validation of a Computational Model to Predict MRP3 Inhibitors
Abstract: Purpose: Multidrug resistance-associated protein 3 (MRP3), an efflux transporter on the hepatic basolateral membrane, may function as a compensatory mechanism to prevent the accumulation of anionic substrates (e.g., bile acids) in hepatocytes. Inhibition of MRP3 may lead to high intracellular bile acid concentrations and is one hypothesized risk factor for the development of drug-induced liver injury (DILI). MRP3 inhibition may further exacerbate the development of DILI during cholestasis. Therefore, identifying potential MRP3 inhibitors could help mitigate the occurrence of DILI.

Methods: Bayesian models were developed and validated using MRP3 transporter inhibition data for 86 structurally diverse drugs. Molecular descriptors were generated for these compounds based on physicochemical properties and substructure fingerprints. The compounds were split into training and test sets of 57 and 29 compounds, respectively, and four models were generated based on different inhibition thresholds and molecular fingerprint methods. The four Bayesian models were validated against the test set and the model with the highest sensitivity and specificity was utilized for a virtual screen of 1,488 FDA-approved drugs from DrugBank. Compounds that were predicted to be inhibitors were selected for in vitro validation. The predicted inhibitors that were tested included lovastatin, regorafenib, atazanavir, pravastatin, fidaxomicin, darunavir, amprenavir, suramin, dronedarone, amiodarone, and bortezomib. The ability of these compounds to inhibit MRP3 transport at a concentration of 100 μ M was measured in membrane vesicles

derived from stably transfected MRP3-over-expressing HEK-293 cells with [3H]-estradiol-17 β -D-glucuronide (E217G; 10 μ M; 5 min uptake) as the probe substrate. MK-571 (50 μ M) was used as a positive control and all experiments were conducted in triplicate.

Results: The most predictive of the Bayesian models had a sensitivity of 73.3% and specificity of 71.4% against the external test set used to rank the four models. The area under the Receiver Operating Characteristic (ROC) curve was 0.710 against the test set. The model was based on compounds that inhibited substrate transport at least 50% compared to the negative control, and functional-class fingerprints (FCFP) with a circular diameter of 6 atoms, in addition to one-dimensional physiochemical properties. All the predicted inhibitors tested at a concentration of 100 μ M inhibited MRP3-mediated E217G transport except darunavir, amprenavir, and bortezomib. The strongest inhibitors of MRP3-mediated E217G transport were fidaxomicin, suramin, and dronedarone. These drugs, which had not been identified as MRP3 inhibitors previously, inhibited E217G transport by 76%, 99% and 94%, respectively.

Conclusion: Bayesian models are a useful screening approach to identify potential MRP3 inhibitors. Novel MRP3 inhibitors were identified by virtual screening using the selected Bayesian model; MRP3 inhibition was confirmed by in vitro transporter inhibition assays. Information generated using this modeling approach may be valuable in predicting the potential for DILI and/or MRP3-mediated drug-drug interactions.

Poster # 97 **Wenjing Li**, Jianshi Yu, Maureen Kane University of Maryland, Baltimore

Title: Targeted Proteomics Analysis of CrbpI Reveals Diagnostic Value for Cancer

Abstract: Purpose: Cellular retinol-binding protein, type 1 (CrbpI) is a member of the intracellular lipid-binding protein family that bind hydrophobic ligands retinol, and delivers it to enzymes that synthesize active metabolite, all-trans-retinoic acid. RbpI, that encodes CrbpI, has been identified as an oncogene with aberrant expression in 10 of the 12 most common cancers including (in order of frequency): prostate, breast, lung, colon & rectal, melanoma, bladder, non-Hodgkin lymphoma, leukemia, endometrial, and pancreatic cancer. A lack of specific antibodies and conflicting reports on agreement between mRNA and protein level hampers a full understanding or investigation of its role in carcinogenesis. Here, we developed a bottom-up target proteomics approach to quantify protein levels of CrbpI in cancer cell models, to investigate its potential as a marker for cancer subtype diagnosis. Moreover, we profiled endogenous metabolites in the same models using mass spectrometry, to understand the role of CrbpI in retinoid signaling pathway in disease setting.

Methods: Cancer cell lines with various CrbpI gene expression levels measured through qPCR were selected for profiling. The lysed samples for each cell line were subjected to 1) in-solution digestion with heavy labeled CrbpI spiked in; 2) two-step liquid-liquid extraction for metabolite profiling, respectively. Recombinant heavy labeled CrbpI expressed in BL 21 E. coli was applied to optimize digestion condition, instrumental parameters and isotope labeled peptide selection. Proteome samples were then analyzed through a nanoACQUITY UPLC 1.7 μ m BEH130 C18 100 μ m \times 100 mm column, coupled to an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) with a nanoelectrospray ion source. Mascot was used for targeted qualitative and quantitative data extraction after data acquisition. Metabolites including retinol, retinyl ester, and retinoic acid isomers were quantified by HPLC and fast liquid chromatography-multistage tandem mass spectrometry using a Shimadzu Prominence UFLC XR coupled to an AB Sciex 5500 QTRAP hybrid tandem quadrupole-linear ion trap mass spectrometer. **Results:** The label-free workflow that developed for CrbpI quantitation enabled low-abundant protein to be identified and

quantified in complex biological samples. First, an In-solution digestion protocol for recombinant CrbpI were optimized to ensure the efficiency and coverage of the target. Two peptides were selected (ALDVNVALR; MLSNENFEEYLR) as putative stable isotope-labeled internal standards. A wide selected-ion monitoring, data-independent acquisition (WiSIM-DIA) workflow were optimized for CrbpI in the mass range of m/z 600-800 and 800-1000, with extremely high resolution and mass accuracy. This step enriched the target analyte ion abundance in the selected SIM mass range for low-abundant protein. In parallel, MS/MS spectra were acquired to detect fragment ions across each mass range for sequence confirmation. Selected cancer cell lines were grouped into CrbpI++(high), CrbpI+(medium), and CrbpI-(low) categories with a positive correlation between CrbpI mRNA expression and protein levels. We further found that the production of all-trans-retinoic acid, the major metabolite, was impaired in cell lines that had reduced CrbpI expression, was augmented in cell lines that had greater CrbpI expression, and was similar in cells that expressed similar amounts of CrbpI as compared to normal cells. Conclusion: Our bottom-up approach provided a robust assay for quantifying low-abundant protein, CrbpI, in selected cancer cell models. This enabled direct and accurate observation of subtle change in protein levels during carcinogenesis. Further investigation on major metabolite productions showed a positive correlation with CrbpI levels, indicating its critical role in maintaining retinoid homeostasis that was compromised in cancer development.

Poster # 98 **Wisthoff, Michael**, Lewis, Robert S.; Zheng, Weifeng; Yap, Glenn P. A.; Chain, William J.
University of Delaware

Title: Efforts Toward a Total Synthesis of Psiguadial A

Abstract: ortho-Quinone methides, or o-methylene cyclohexadienones, are highly reactive species that participate in a variety of organic reactions. Due to their highly reactive nature, it is advantageous to generate OQMs in situ at a rate comparable to their consumption. We have developed a coupling reaction between enolates and OQMs generated in situ simultaneously in a single flask under mild conditions that enables expedient synthesis of a natural product with exciting biological activity against human cancer cell lines, the meroterpenoid psiguadial A (Hep G2 IC50 = 61 nM). A simplified model system of the natural product is described in 5 steps in order to evaluate key late stage bond forming transformations – the enolate-OQM and oxa-Michael addition.

Poster # 99 **wong, kenneth**, Candelora, Chrissy; Cope, Nicholas; Gunderwala, Amber; Wang, Zhihong
University of the Sciences in Philadelphia

Title: Illuminating the intricate activating mechanism of CRAF in vitro

Abstract: Resistance to drug therapy in BRAF associated melanoma is a critical issue that can arise through activating other pathways or a plethora of secondary mutations. One notorious mechanism of resistance is transactivation of CRAF, a homolog of BRAF, through heterodimerization of BRAF and CRAF. Although the basal activity of CRAF is extremely low, when dimerized with BRAF, the activity overcomes that of BRAF alone. Here, we characterize the heterodimerization mechanism from a biochemical perspective in vitro via purified full-length RAF in a cell free system. The rare variant CRAFR391W is a recently discovered driver oncogene with enhanced expression level and kinase activity. Similarly, to oncogenic BRAF, CRAFR391W harbors the capacity to signal independent of upstream facilitation. These attribute highlights the variant as a potential culprit in BRAF inhibitor resistance. We verify the oncogenic traits of CRAFR391W and delve into the mechanism that augment kinase activity in vitro. By deciphering the activating mechanism of CRAF, we can develop insight into the aberrant regulation of CRAF in cancer cells and its role in tumorigenesis.

Poster # 100 **Xu, Feiyang**, Shuler, Scott A.*; Watson, Donald A. University of Delaware

Title: Synthesis of Diverse Imidazolidinones through a Unified Approach

Abstract: Imidazolidinones are widely found in biologically-active molecules and natural products, and are well recognized as important synthetic intermediates that can be transformed into a diverse array of complex structures. Motivated by their value, we have developed a palladium-catalyzed intramolecular cyclization to synthesize imidazolidinones. This method offers a rapid and efficient approach to prepare unprotected, unsaturated imidazolidinones with good functional group tolerance. It also provides the first example of metal-catalyzed cyclization for the synthesis of doubly-protected imidazolidinones.

Poster # 101 **Yang, Kun**, Greenberg, Marc Johns Hopkins University

Title: Reactivity of N7-methyl-2'-deoxyguanosine in nucleosome core particles – Identification of a new source of monofunctional alkylating agent cytotoxicity

Abstract: Alkylating agents are commonly used as anticancer drugs. DNA methylation predominantly occurs at the N7 position of guanine (N7mdG). N7mdG hydrolyzes to yield an abasic site (AP) with a half life of ~102-190 h in naked DNA. However, N7mdG reactivity in nucleosome core particles (NCPs) has not been reported. We reconstituted NCPs containing N7mdG at specific positions. N7mdG depurination in NCPs is ~1.3-5.7 fold slower than in naked DNA. Larger decreases in depurination rates were observed when N7mdG is proximal to the histone tails. Experiments using mutated proteins indicate that the histone tails are responsible for the majority of the decreased depurination rates. Importantly, we discovered that the histone protein tails form DNA-protein crosslinks (DPCs) with N7mdG, and the yields of DPCs are comparable to the amounts of AP. Similar results were also observed when a NCP was treated with methyl methanesulfonate (MMS). The overall depurination rate of MMS treated DNA is ~2 fold slower in NCP than in naked DNA. DPCs were detected in MMS treated NCP in comparable yields to AP sites. To our knowledge, DPCs have never been reported from monoalkylation agents in chromatin. The formation of DPCs from N7mdG suggests an alternative mechanism for the cytotoxicity of monofunctional DNA alkylating agents.

Poster # 102 **Yates, Mary**, Raje, Mithun; Peters, Hannah; Cau, Ylenia; Gharaibeh, Dima; Kenny, Tara; Retterer, Cary; Zamani, Rouzbeh; Bavari, Sina; Botta, Maurizio; Soloveva, Veronica; Seley-Radtke, Katherine UMBC

Title: Design, Synthesis, and Biological Evaluation of Flexible Acyclic Nucleoside Analogues Against Human Coronaviruses and Filoviruses

Abstract: To date, there are no FDA approved treatments or vaccines for diseases caused by coronaviruses (CoVs) or filoviruses. Over the past decade, two deadly human coronaviruses, Severe Acute Respiratory Syndrome CoV (SARS) and Middle East Respiratory Syndrome CoV (MERS), have emerged as lethal pathogens with high mortality rates. Filoviruses, such as the Ebola (EBOV), Sudan (SUDV), and Marburg (MARV) viruses, also represent a severe health threat with mortality rates reaching 90%. With the potential of global reemergence of SARS, as well as the recent outbreaks of MERS, EBOV, and SUDV, it is imperative that a viable and efficient treatment is developed in order to increase survival rates of these lethal diseases. Nucleoside analogues have long served as the cornerstone for antiviral therapeutics due to their ability to inhibit viral DNA or RNA replication; however, one major issue is the moderately high genetic mutation rate associated with these viruses, which alters the enzymatic binding site and renders the antiviral agents ineffective. One way to potentially overcome drug resistance is to create a more flexible nucleobase scaffold in order to increase adaptability of the drug once bound within the target enzyme. The Seley-Radtke lab has developed various types of flexible nucleoside analogues, called

“fleximers”, that have demonstrated the ability to overcome point mutations within the binding site of biologically significant enzymes, as well as to increase interactions in the binding pocket that were unattainable by the parent nucleoside. Preliminary results have shown that several acyclic Flex-analogues of the FDA-approved drug Acyclovir have shown activity against both SARS and MERS in vitro. These findings are groundbreaking since these compounds represent the first nucleosides to exhibit potent activity against SARS and MERS. More recently, studies have uncovered activity against various filoviruses including EBOV, SUDV, and MARV. The results of these studies are reported herein.

Poster # 103 **Yu, Tiantian**, Colin Thorpe University of Delaware

Title: Gaussia princeps luciferase: a bioluminescent substrate for oxidative protein folding

Abstract: The pathways for the generation and isomerization of disulfide bonds during the oxidative folding of proteins destined for secretion are still poorly understood. Reduced RNase and its fully oxidized, but mispaired counterpart, scrambled RNase, remain widely-used model substrates for these studies. However, there is a continuing need for additional convenient substrates that can be used to assess oxidative folding pathways. Here we introduce a bioluminescent assay for oxidative protein folding which utilizes reduced Gaussia princeps luciferase (GLuc). Native GLuc, a 19.9 kD monomeric protein containing 5 disulfides and no free cysteine residues, catalyzes the oxidation of coelenterazine independent of ATP or other cofactors with a burst of intense bioluminescence (at ~470 nm). Procedures have been developed for the quantitative generation of reduced GLuc (rGLuc) that exhibit less than 0.01% of the bioluminescence of the native protein. We show that up to ~80% activity can be recovered in a range of oxidizing environments including glutathione redox buffers, other small molecular oxidants, or nanomolar levels of avian and human Quiescin sulfhydryl oxidase (QSOX1). Scrambled GLuc has also been prepared and examined as a substrate for protein disulfide isomerase. Finally, we show that the oxidation of rGLuc to generate native-paired GLuc provides a very sensitive method for assessment of the potency of arsenical and heavy metal ion inhibitors of oxidative protein folding.

Poster # 104 **Zengeya, Thomas**, Garlick, Julie; Kulkarni, Rhushikesh; Miley, Mikayla; Roberts, Allison; Crooks, Daniel; Sourer, Carole; Linehan, Lineman; Meier, Jordan National Cancer Institute

Title: Co-opting a Bioorthogonal Reaction for Oncometabolite Detection

Abstract: Dysregulated metabolism is a hallmark of many diseases, including cancer. Methods to fluorescently detect metabolites have the potential to enable new approaches to cancer detection and imaging. However, fluorescent sensing methods for naturally occurring cellular metabolites are relatively unexplored. Here we report the development of a chemical approach to detect the oncometabolite fumarate. Our strategy exploits the reactivity of nitrileimines, which undergo a 1,3-dipolar cycloaddition with the electron-poor alkene of fumarate to form fluorescent pyrazoline cycloadducts. We demonstrate that hydrazone chlorides serve as readily accessible nitrileimine precursors, whose reactivity and spectral properties can be tuned to enable sensitive detection of fumarate and other dipolarophile metabolites. Finally, we show this approach can be used to detect enzyme activity changes caused by mutations in fumarate hydratase (FH), which underlie the familial cancer predisposition syndrome HLRCC. Our studies define a novel aspect of intrinsic metabolite reactivity that may be harnessed to facilitate biological profiling, imaging, and diagnostic applications.

Poster # 105 **Zhang, Zhengqi**, Liu, Jun; Cheng, Rujin; Hoffmann, W FuKun; Hoffmann, R Peter and Rozovsky, Sharon University of Delaware

Title: Autoproteolysis of Selenoprotein K requires selenocysteine

Abstract: Selenoprotein K (SELENOK) is membrane protein that assists the palmitoyl acyl transferase DHHC6 to conjugate fatty acids to protein substrates. In addition, SELENOK is a member of the Endoplasmic Reticulum Associated Protein Degradation (ERAD) pathway, which is responsible for translocation of misfolded proteins from the ER to the cytoplasm for subsequent degradation. Here, we report on SELENOK's ability in vitro to cleave its own peptide bond, releasing the cytoplasmic segment. Autoproteolysis is critically dependent on the presence of a nucleophilic serine and selenocysteine. The released segment contains the selenocysteine and an SH3 binding element and thus remains bound to DHHC6 following cleavage and potentially active. The cleavage is inhibited when SELENOK associates with protein partners. We propose that SELENOK's autoproteolysis could serve a regulatory role to control its association with protein complexes. The cellular fate of the 38-mer peptide containing Sec92, including its potential interactions with cellular targets, is not yet known.

Poster # 106 **Zhao, Xue Zhi**, Hymel, David; Burke, Jr. Terrence R. Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21702

Title: Application of oxime-diversification to optimize ligand interactions within a cryptic pocket of the polo-like kinase 1 polo-box domain

Abstract: Members of the polo-like kinase (Plk) family of serine/threonine protein kinases play crucial roles in cell cycle regulation and proliferation. Of five Plks (Plk1 – 5), Plk1 is recognized as an anticancer drug target. Plk1 contains multiple structural components that are important for its proper biological function. These include an N-terminal catalytic domain and a C-terminal non-catalytic polo-box domain (PBD). The PBD binds to phosphothreonine (pThr) and phosphoserine (pSer)-containing sequences. Blocking PBD-dependent interactions offers a potential means of down-regulating Plk1 function that is distinct from targeting its ATP-binding site. Oxime-based post-solid phase diversification is a form of directed fragment screening, which can be highly effective in optimizing protein-ligand interactions. As one example, starting from the known PBD-binding peptide "PLHSpT," we have previously used this approach to identify a hydrophobic cryptic binding pocket on the surface of the PBD, whose access can enhance peptide-binding affinity by approximately 1000-fold. As reported herein, we have employed this technology to further extend and optimize PBD-ligand interactions. By a process involving initial screening of a set of 87 aldehydes using an oxime ligation-based strategy, we were able to achieve a several-fold affinity enhancement over one of the most potent previously known Plk1 PBD-binding inhibitors. This improved binding may result from accessing a newly identified auxiliary region proximal to a key hydrophobic cryptic pocket on the surface of the protein. We have also shown that selectivity for the Plk1 PBD relative to the PBDs of Plk2 and Plk3 can be significantly enhanced by modulating interactions within this region. Our findings could have general applicability to the design of Plk1 PBD-binding antagonists.