8:20-9:00  Registration & Coffee
poster presenters (odd and even numbered) should set up posters.

9:00-9:05  Welcome and opening remarks.

Session I.
Session Chair: Catherine Grimes, U. Delaware

9:05-9:25  James Petersson, Assistant Professor, University of Pennsylvania, Dept. of Chemistry.
Thioamides as Fluorescence Quenching Probes: Minimalist Chromophores for Monitoring Protein Dynamics

9:25-9:45  Pumtiwitt McCarthy, CBER, U.S. Food and Drug Administration
Preparation of a meningococcal group C polysialic acid- tetanus Hc fragment glycoconjugate vaccine candidate by chemoenzymatic synthesis

9:45-10:05  Elsa D. Garcin, Assistant Professor, Dept. Chemistry & Biochemistry, UMBC
Down the nitric oxide signaling pathway: structural studies of soluble guanylate cyclase

10:05-10:30  Coffee break

Session II.
Session Chair: Caren Meyers, Asst. Prof. JHU

10:30-10:50  Yaming Hou, Professor, Thomas Jefferson University.
Differentiating analogous tRNA methyltransferases by Analogs of the Methyl Donor S-adenosyl Methionine.

10:50-11:10  Kory Blocker, Department of Chemical Engineering, University of Delaware,
Tunability and Tailorability of Cell-Triggered DNA Release from a Substrate-Mediated Delivery System

11:10-11:35  Shuwei Li, Assistant Professor, Dept. Chemistry and Biochemistry, Univ. of Maryland.
Reviving Deuterium-Labeled Tags for Quantitative Proteomics
11:35 1:10  
**Poster Session I & Lunch**
(even numbered posters)

**Session III.**
Session Chair: Elsa Garcin, Asst. Prof. UMBC

1:10-1:40  **Lynn Hyde**, Principal Scientist, Merck Research Labs.  
*Discovery of a Novel Class of Iminoheterocycle BACE1 (b-secretase) Inhibitors With Central Nervous System (CNS) Activity for the Treatment of Alzheimer's Disease*

1:40-2:00  **Jun Wang**, Department of Chemistry, University of Pennsylvania  
*Structure-Based Design Of Inhibitors Targeting Influenza A Virus M2 Proton Channel (A/M2)*

2:00-2:30  **Pam Green**, Professor, University of Delaware, Dept. of Plant and Soil Sciences.  
*The regulatory world of RNA: Genome-wide analysis of microRNAs and their targets*

2:30-3:45  
**Poster Session II /refreshments**
(odd numbered posters)

**Session IV.**
Session Chair: Ed. Lymann, Asst. Prof. UD

3:45-4:15  **James Stivers**, Professor, Pharmacology and Molecular Science, Johns Hopkins Univ.  
*A Look on the Dark Side of Fragment-Based Drug Design*

4:15-4:45  **Thomas Stevensson**, DuPont Crop Protection,  
*Chemistry at the Interface of Agriculture and Medicine: Pyrazinones as Tubulin Modulators*

4:45- 4:50  Closing remarks.

**We thank the following for their support:**

National Institutes of Health (NIGMS)  
The Delaware Biotechnology Institute  
The College of Arts and Sciences, University of Delaware  
The Johns Hopkins Pharmacology Department  
Johns Hopkins Department of Chemistry  
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University of Maryland Baltimore County, Dept. Chemistry & Biochemistry  
The Department of Chemical Engineering, University of Delaware  
The Department of Materials Science and Engineering, University of Delaware  
The Department of Chemistry and Biochemistry, University of Delaware

Organizing Committee: John Koh, Jennifer Codding and Millicent Sullivan (University of Delaware)  
Craig Townsend and Jin Zhang (Johns Hopkins University), Jim Fishbein (University of Maryland, Baltimore County), Steve Rokita (University of Maryland)
1. Linqing Li, University of Delaware: A Resilin-based Bio-elastomer for Engineering Mechanically Active Tissues. Elastomeric proteins are well known for their large extensibility before rupture, reversible deformation without loss of energy, and high resilience upon stretching. Motivated by these unique mechanical properties, we are particularly interested in resilin, the highly elastomeric protein found in specialized compartments of most arthropods; resilin possesses superior resilience and excellent high-frequency responsiveness. Enabled by biosynthetic strategies, we have designed and expressed recombinant resilin-like polypeptides (RLPs) bearing both multiple repetitive motifs and biological cascades. These approaches should allow for the engineering of microenvironments for the regeneration of mechanically active tissues such as blood vessels, cardiovascular tissues and vocal folds. The cross-linked bio-elastomeric hydrogels display changeable mechanical stability, energy dissipation capacity, and robust mechanical strength.

2. Matthew Weitzman, University of Delaware: Exploring the Ligand-Based Virtual Screening Study for the Hepatitis C Virus NS5B Polymerase by Docking and QSAR Analysis. The NS5B gene product is the RNA dependent RNA polymerase of HCV, which is essential for viral RNA replication. We study this typical right hand structure, and enzymatic studies will further be discussed herein.

3. Franziska Seeger, University of Maryland: A Biochemical and crystallographic characterization of the regulation and catalysis of soluble Guanylate Cyclase. Soluble guanylate cyclase (sGC) is the key enzyme in the NO-sGC-cGMP signaling cascade crucial in regulating the cardiovascular system. NO binding to sGC's regulatory domain enhances its basal catalytic activity to convert GTP to cGMP, which in turn modulates downstream targets leading to vasodilation. Low output of this system causes hypertension and acute heart failure, which are the leading causes of death globally. sGC is a heterodimer. Each of the two homologous subunits (α and β) contains three domains: an N-terminal regulatory domain (HNOX: Heme Nitric oxide OXygen), a central dimerization HNOX associated (HNOXA) and coiled-coil (CC) domain, and a C-terminal catalytic domain (GC). The enzyme is basally active, but NO binding to the heme group in the β subunit's regulatory domain enhances sGC's catalytic output several hundred fold. The exact mechanism by which the regulatory domain relays information to the catalytic domain remains unclear.

4. Andrew Napper, Nemours Center for Childhood Cancer Research: A homogeneous high-throughput screening assay for inhibitors of the interaction of MLL-AF4 and AF9 in pediatric leukemia. Translocations of the mixed lineage leukemia (MLL) gene occur in a subset of aggressive pediatric leukemias. The MLL protein fuses to one of a number of transcription factors, resulting in potent induction of leukemia through activation of transcription of the oncogene HOXA9, which blocks normal blood cell differentiation. The transcription factor AF4 is the most common MLL fusion partner, and MLL-AF4 is frequently associated with a particularly poor prognosis. Direct interaction of MLL-AF4 with the transcription factor AF9 appears to be critical to leukemic transformation. We have developed a homogeneous high-throughput screening (HTS) assay for inhibitors of the binding of MLL-AF4 to AF9 and validated this assay in pilot HTS. Others have shown that peptides derived from AF4 inhibit this interaction with low nanomolar potency and selectively kill MLL-AF4-containing leukemia cell lines. Our assay may offer a homogeneous screening strategy for the development of new therapeutic agents.

5. Iwona Weichard, UMBC: Exploring the Ligand-Based Virus Screening Study for the Hepatitis C Virus Gene CCR5. Hepatitis C Virus (HCV) affects about 170 million people worldwide. Due to the large number of people infected, HCV is an important public health problem. The non structural NS5B gene product is the RNA dependent RNA polymerase of HCV, which is essential for viral RNA replication. We study this typical right hand structure, with three distinct thumb, fingers and palm domains. 464 protein-ligand complexes of HCV as well as all related ligands have been downloaded from RCSB Protein Data Bank and Ligand Expo. The best hits were used as a query for pharmacophore development. The pharmacophore features of known HCV inhibitors were used as a filter to screen the ChemNavigator iResearch Library of about 59.3 million purchasable compounds. Docking of the known inhibitors and hits obtained from virtual screening was performed using Chem3D. Phenomena observed in this virtual screening were further discussed herein.

6. Qin Liang, University of Delaware: Small-molecule inhibitor of a human deubiquitylating enzyme complex. Abstract: Ubiquitin specific proteases (USPs) have been the subject of increased attention lately as a possible new therapeutic target class. Despite the identification of over sixty USPs in humans and the implication of many USPs in various human diseases, few small-molecule USP inhibitors have been reported. In this work we identified small-molecule inhibitors against the human USP1/UAF1, a heterodimeric deubiquitinating enzyme complex, through quantitative high throughput screening (qHTS) of a collection of bioactive molecules. And we also provided the first successful case of targeting the USP-WD40 repeat protein complexes for inhibitor discovery.

7. Sarah Zimmermann, UMBC: A Mutant of Platelet Activating Factor Acetylhydrolase Hydrolyzes Organophosphorus Nerve Agents. Stephen Kirby, Joseph Norris, Douglas Cerasoli and Brian Bahnsen. U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010. University of Delaware, Department of Chemistry & Biochemistry, Newark, DE 19716. Organophosphorus compounds (OPs) such as sarin (GB) and soman (GD) are the most toxic chemicals created. They exert toxic effects by inactivating acetylcholinesterase and binding to secondary targets. OP inhibitors are hemi-substrates for enzymes of the serine hydrolase superfamily; these enzymes have the potential to be engineered by point mutations into OP-hydrolyzing platforms that can be used as catalytic bioscavengers. Enzymes associated with lipoproteins, such as human plasma platelet activating factor acetylhydrolase (PAF-AH), have largely been ignored for engineering purposes due to complex interfacial kinetics and lack of structural data. We have expressed active PAF-AH in bacteria and crystallized the enzyme-OP adducts. Based on these structures, we engineered histidine mutations (L153H, W298H, F322H) near the permissive active site to enhance removal of the OP from the phosphorylated serine. Wild-type PAF-AH, L153H, and F322H have essentially no activity against GD, yielding nearly identical rates from negative control transformations. Surprisingly, W298H displayed novel somanase activity with a kcat of 5 min-1 and a KM of 593μM at pH 7.5. There was no selective preference for hydrolysis for any of the four GD stereoisomers. Not since the G117H variant of human butyrylcholinesterase has novel OP-hydrolase activity been engineered into an enzyme via a single amino acid substitution.
Mr. Jacob Neal  
UMBC  
Structural characterization of the glyceraldehyde-3-phosphate dehydrogenase-mediated regulation of endothelin-1 expression. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plays a key role in the regulatory pathways of endothelin-1 (ET-1), a vasoconstrictor critical for vascular homeostasis. Elevated ET-1 levels are associated with certain vascular diseases, thus production and secretion of ET-1 must be tightly regulated. This regulation occurs primarily at the level of RNA metabolism and transcription, and GAPDH binds particular adenine-uridine rich elements (AREs) in the 3’-untranslated region (3’-UTR) of the ET-1 gene. However, the structural details underlying the GAPDH-mediated control of ET-1 expression are lacking. With the techniques of molecular biology, electrophoretic mobility shift assay (EMSA), and x-ray crystallography, we seek to probe the structural interaction between GAPDH and the 3’-UTR of ET-1. To this end, we cloned, expressed, and purified human GAPDH. To identify the specific GAPDH binding sequences, we constructed small RNA transcripts of the implicated AREs using T7 RNA polymerase. We will next utilize EMSA to identify the smallest RNA fragments that bind to GAPDH. Ultimately, we will exploit these RNA fragments with GAPDH and solve the structure of the complexes. These studies will provide the first molecular basis for structure-based drug design, leading to the development of novel molecules that specifically target ET-1 mRNA destabilization.

Ms. Jasmina Cheung-Lau  
UPenn  
Self-assembly of thermophilic ferritin towards the encapsulation of gold nanoparticles. Abstract: Previously, the incorporation of 10 nm gold nanoparticles (AuNPs) into the hollow interior of ferritin, taken from the archaean, Archaeoglobus fulgidus, proved successful, as characterized by Joe Swift et al. (Langmuir 2009). At the conclusion of this study, we determined that the 10 nm AuNPs expand the native framework of the protein. Our current work involves further characterization of the ferritin-like NP system, synthesized with 5 nm AuNPs using methods that promote ferritin native assembly. The goals of this project include both the understanding of the bio-inorganic interface, and the development of a stable protein-metal NP with potential application as an in vivo biosensor. We expand upon earlier work by testing the enzymatic activity of ferritin when annealed to AuNPs, employing electrophoretic assays to study the stability of ferritin-like particle (FLP) systems, and functionalizing the FLP systems for organization into higher order structures.

10 Dr. Michael Massiah  
George Washington University  
Insights into the intrinsically unstructured C-terminus of alpha4, regulator of MID1 and PP2Ac.  

11 Mr. Vincent Pagnotti  
University of the Sciences  

12 Mr. Yubin Bai  
Chemistry Dept., UPenn  
Hyperpolarized 129Xe chemical exchange saturation transfer (129Xe Hyper-CEST) NMR for ultra high sensitivity detection of xenon host molecules in live human tissues.

13 Ms. Jasmina Cheung-Lau  
UPenn  
Self-assembly of thermophilic ferritin towards the encapsulation of gold nanoparticles. Abstract: Previously, the incorporation of 10 nm gold nanoparticles (AuNPs) into the hollow interior of ferritin, taken from the archaean, Archaeoglobus fulgidus, proved successful, as characterized by Joe Swift et al. (Langmuir 2009). At the conclusion of this study, we determined that the 10 nm AuNPs expand the native framework of the protein. Our current work involves further characterization of the ferritin-like NP system, synthesized with 5 nm AuNPs using methods that promote ferritin native assembly. The goals of this project include both the understanding of the bio-inorganic interface, and the development of a stable protein-metal NP with potential application as an in vivo biosensor. We expand upon earlier work by testing the enzymatic activity of ferritin when annealed to AuNPs, employing electrophoretic assays to study the stability of ferritin-like particle (FLP) systems, and functionalizing the FLP systems for organization into higher order structures.

14 Mr. Jung Choi  
Department of Chemical Engineering, University of Delaware  
APPLICATION OF TANDEM MASS SPECTROMETRY FOR QUANTIFYING LABELING DISTRIBUTIONS AND ESTIMATING METABOLIC FLUXES. We have developed and introduced novel methodologies for MFA based on tandem MS and stable-isotope labeling experiments. We demonstrate that tandem MS provides more labeling information than can be obtained from traditional full scan MS analysis and allows estimation of fluxes with much higher precision. We present a general modeling framework that takes full advantage of the additional labeling information obtained from tandem MS for MFA. In this framework, labeling information is obtained from daughter ion scanning and is represented in a matrix form, termed the tandem mass isotopomer matrix. We show that tandem MS can provide a more complete and a more robust estimation of fluxes using linear mapping of isotopomer fractions. The inherent advantages of tandem MS compared to full scan MS were demonstrated by comparing the number of independent measurements. For molecules with more than three atoms tandem MS always provided greater number of independent measurements. To investigate the relationship between the type of isotopomer measurement and flux identifiability, we generated a series of simulated data sets for a TCA cycle model. The results showed that tandem MS improved not only the sensitivity of measurements, but also the observability of estimated fluxes. In addition, we developed optimized tandem MS protocols for accurate and precise measurement of tandem mass isotopomers for TBDMS derivatized amino acids and organic acids. We provide a library of acceptable fragments that can be used for MFA.

15 Mr. William Bozza  
University of Maryland  
A Peptide-based Multivalent Antagonist of Human Proliferating Cell Nuclear Antigens. Proliferating cell nuclear antigen (PCNA) is a master regulator of cell proliferation via its interactions with numerous proteins, including replicative and specialized DNA polymerases. The natural tumor suppressor, p21, functions by binding to PCNA and thereby preventing other cellular proteins from accessing PCNA. Targeting this essential interaction, we have designed tandem repeats of the p21 protein. Our initial hypothesis was a multivalent p21 interaction would demonstrate improved affinity for PCNA than a single p21 peptide. Using isothermal titration calorimetry (ITC) we have confirmed the ability of our divalent p21 in binding PCNA. We have also developed a fluorescence polarization based competitive peptide binding assay to assess PCNA’s affinity for different p21 peptides. Our results showed that the tandem p21 repeat has a much improved affinity for PCNA than wild type p21. Our study validated the approach of designing multivalent antagonists of PCNA for controlling cell proliferation and will facilitate the development of highly potent disruptors of the essential protein interaction with PCNA.

16 Dr. Christopher Baker  
University of Maryland Baltimore  
From small molecules to macromolecules: progress towards a CHARMM Drude polarizable force field for the nucleic acids. Christopher M. Baker, Alexander D. MacKerell Jr. Electrostatic interactions play a crucial role in determining the structure and function of biomolecules, and an important aspect of the electrostatic interaction is polarizability, the response of the molecular dipoles to an external electric field. Towards development of a comprehensive force field for biomolecules, initial work on the CHARMM Drude polarizable force field for nucleic acids focused on optimizing the theoretical model and developing parameters for small molecule analogs of the sugar, phosphate and base moieties. With parameters for the small molecule analogs now in place, current work is focused on construction of the full nucleic acids, and details of this work will be presented here. Initial simulations of the full nucleic acids have demonstrated that the polarizable model is both robust and stable, and work is now underway to assemble the small molecule building blocks into the full nucleic acid chains. This procedure is associated with covalent connections between the constituent moieties. Following initial optimization of the intramolecular connections, multiple simulations will be performed to provide a detailed assessment of macromolecular properties in comparison to experimental data. These results will be used to identify and correct any weaknesses in the force field, and are also an essential tool for validation of the model in condensed phase environments; they will give new insights into the importance of polarizability in nucleic acid simulations. Protein-nucleic acid interactions are an essential process. Disruption of protein degradation interferes with numerous cellular activities. Analogous multistep pathways to degrade proteins are common amongst all living cells. The proteasome, a catalytic enzyme complex, is a major component of proteolysis. Crystal structures of eukaryotic and an increasing number of prokaryotic proteasomes provide information on their composition and enzymatic activities. Proteasome inhibitors, already approved for use against cancers, might be significant additions to antibacterial therapies. Our work uses the crystal structure of the Mycobacterium tuberculosis (Mt) proteasome as the basis for inhibitor design. Initial rounds of modeling and synthesis are based on analogues of bortezomib, a boronic acid dipeptide. Current synthetic efforts replace the leucine moiety of bortezomib with substituents that should provide a better fit into the protein active site, increasing inhibition of the Mt enzyme. Modeling results, synthesis and biological activity of these new compounds are presented.

17 Ms. Gail Clements  
The George Washington University  
From small molecules to macromolecules: progress towards a CHARMM Drude polarizable force field for the nucleic acids. Christopher M. Baker, Alexander D. MacKerell Jr. Electrostatic interactions play a crucial role in determining the structure and function of biomolecules, and an important aspect of the electrostatic interaction is polarizability, the response of the molecular dipoles to an external electric field. Towards development of a comprehensive force field for biomolecules, initial work on the CHARMM Drude polarizable force field for nucleic acids focused on optimizing the theoretical model and developing parameters for small molecule analogs of the sugar, phosphate and base moieties. With parameters for the small molecule analogs now in place, current work is focused on construction of the full nucleic acids, and details of this work will be presented here. Initial simulations of the full nucleic acids have demonstrated that the polarizable model is both robust and stable, and work is now underway to assemble the small molecule building blocks into the full nucleic acid chains. This procedure is associated with covalent connections between the constituent moieties. Following initial optimization of the intramolecular connections, multiple simulations will be performed to provide a detailed assessment of macromolecular properties in comparison to experimental data. These results will be used to identify and correct any weaknesses in the force field, and are also an essential tool for validation of the model in condensed phase environments; they will give new insights into the importance of polarizability in nucleic acid simulations. Protein-nucleic acid interactions are an essential process. Disruption of protein degradation interferes with numerous cellular activities. Analogous multistep pathways to degrade proteins are common amongst all living cells. The proteasome, a catalytic enzyme complex, is a major component of proteolysis. Crystal structures of eukaryotic and an increasing number of prokaryotic proteasomes provide information on their composition and enzymatic activities. Proteasome inhibitors, already approved for use against cancers, might be significant additions to antibacterial therapies. Our work uses the crystal structure of the Mycobacterium tuberculosis (Mt) proteasome as the basis for inhibitor design. Initial rounds of modeling and synthesis are based on analogues of bortezomib, a boronic acid dipeptide. Current synthetic efforts replace the leucine moiety of bortezomib with substituents that should provide a better fit into the protein active site, increasing inhibition of the Mt enzyme. Modeling results, synthesis and biological activity of these new compounds are presented.
Using the epigenetic code to promote unpackaging from a non-viral, gene delivery vector. Abstract: Non-viral gene delivery biomaterials must be able to release DNA inside the nucleus of a cell. Unfortunately, the majority of non-viral vehicles unpackaged prior to nuclear entry or did not unpackaged at all, promoting either premature degradation or inefficient transcription. Thus, we have created DNA polyplexes that are designed to release DNA upon arrival in the nucleus. These vehicles were designed utilizing homologous DNA activation, and consist of H3 histone tail peptides that are trimethylated at the fourth position (H3K4Me3) and grafted onto a PEG backbone. The H3K4Me3 modification has been associated with actively transcribing DNA in a variety of eukaryotes. When the H3K4Me3 peptide is mixed with pDNA, genetically tagged polyplexes are formed with a hydrodynamic diameter of approximately 100 nm. We have explored the effects of the N:P packaging ratio on DNA protection and transfection efficiency. Furthermore, upon insertion of the H3K4Me3 polyplexes into the nucleus, we observe protein production approximately 50% faster than with polyplexes containing nontrimethylated H3 peptides or with conventional gene delivery materials.

Pyrimidine Nucleotide Imbalances and Innate Immunity to Retroviral Infection. Amy F Guminski1, Robert F Siliciano2, James T Stivers1 1Johns Hopkins School of Medicine, Pharmacology and Molecular Sciences and 2Department of Medicine, Baltimore, MD, 21205 Uracil can be found in DNA after either deamination of cytosine or misincorporation of the deoxynucleotide dUTP during DNA synthesis. Recent work suggests that host organisms may utilize the detrimental effects of uracil incorporation. It has been suggested that dUTP misincorporation may act as one particular defense mechanism, as evidenced by the fact that several genera of retroviruses encode a dUTPase to hydrolyze cellular dUTP and reduce dUTP misincorporation. In these viruses, the encoded dUTPase is essential to permit infection of non-dividing cells, which have a naturally high dUTP:TTP ratio. Primate lentiviruses such as HIV-1, however, do not encode a dUTPase, and its potential increase their susceptibility to uracil misincorporation and its associated detrimental effects. We show that retroviruses that express a dUTPase can act as an innate barrier to retroviral infection. Using the drug Raltitrexed (RTX), which inhibits a key step in the de novo pyrimidine biosynthesis pathway, we have been able to greatly increase the dUTP:TTP ratio in HT29 cells. After RTX treatment, the HIV-1 cDNA of infected cells is significantly uracilated. Although the levels of HIV-1 cDNA are relatively unperturbed by RTX treatment, the level of integrated provirus is greatly reduced and viral protein production is virtually eliminated. This restriction seems to require UNG, a cellular Base Excision Repair (BER) enzyme that excises uracil lesions from DNA. We hypothesize that the excision of uracil by UNG from these over-uracilated cDNAs prevents stable integration of an infection competent viral genome.

Criteria for Increasing Efficiency of Excess Electron Transfer in DNA. Abstract: Charge transfer processes in DNA are actively studied in various fields ranging from biological chemistry to electronics. While parameters affecting the efficiency of hole transfer have been extensively studied, equivalent investigations on excess electron transfer (EET) in DNA are not yet well resolved. The roles of charge recombination, as a competing process with electron migration, and the diffusion freedom of electrons during EET in DNA are reported. Charge recombination is reduced using selective electron donation to pyrimidines by 1-aminooanthracene (AA) as electron donor, in contrast to 1,5-diaminonaphthalene (DAN) that can donate to both purines and pyrimidines. Results show that AA is about 3-fold more efficient than DAN in inducing EET in identical dsDNA. Studies on a series of dsDNA with a terminal or internal electron donor reveal that the terminal donor with only one diffusion direction exhibits a 2-fold higher efficiency in transferring electrons in DNA. These findings may be used in the future to design more efficient EET systems.

Hematopoietic Stem Cell Delivery of Therapeutics for the Treatment of Prion Diseases. Kyle M. Doolan and David W. Colby Prion diseases are a group of rapidly progressing, lethal neurodegenerative diseases for which there currently is no treatment available. Prion diseases are propagated by the conversion of the native form of the prion protein (PrPC) to an alternatively folded, disease causing form (PrPSc) catalyzed by the interaction of PrPSc with PrPC. Previous researchers have developed antibodies that bind PrPC and slow the conversion to PrPSc in cell culture and animal models, though few attempts have been made to engineer existing antibodies for greater therapeutic effect. Beyond protein engineering techniques, delivery of therapeutics using cell based delivery strategies has the potential to improve therapeutic distribution. Here we propose the development of anti-PrPC single chain antibody fragments (scFv) with improved affinity, stability, and endosomal recycling to be delivered by genetically engineered hematopoietic stem cells. Quantitative evaluation of scFv and cellular parameters will enable the development of a quantitative mathematical model, which will guide future improvements. The model and methods developed here will likely apply more generally to passive immunity therapies for prion diseases and for cell based antibiotic therapies for other neurological diseases.

We identified a family with a previously undescribed lethal X-linked disorder of infancy comprising a unique combination of craniofacial anomalies, hypotonia, global developmental delays, cryptorchidism, cardiac arrhythmia and eventual cardiomyopathy, in which four boys died by approximately one year of age. We used X-chromosome exome sequencing on an affected newborn individual and his relatives, followed by analysis with a powerful new software program, VAAST, developed by Mark Yandell at the University of Utah, to identify the causative mutation as c.109T>C; p.S37P in NAA10 (OMIM 300013), a gene that encodes the catalytic subunit of the major human N-terminal acetyltransferase (NAT). N-terminal acetylation is one of the most common protein modifications in humans, occurring on approximately 80% of all human proteins, but surprisingly mutations in the NAT genes have never before been linked to human genetic disease. Functional analysis of hNaa10p S37P compared to wild-type hNaa10p protein demonstrated significantly impaired biochemical activity, confirming that this mutation causes this disease. More broadly, we have demonstrated that exon sequencing and analysis with VAAST of just one affected individual, when coupled with pedigree genotype information and follow-up functional assays, can be a powerful technique to identify the mutations that cause extremely rare Mendelian disorders.

HEDGEHOG GENE REGULATION TO INHIBIT THE NEUROPATHOLOGY OF ALZHEIMER’S DISEASE. IN A TRANSGENIC AD DROSOPHILA MODEL. Alzheimer’s Disease (AD) is the world’s leading cause of dementia with an annual cost of 172 billion dollars. AD is characterized by two hallmark factors: neurofibrillary tangles (NFT) and amyloid-β plaques (Aβ-plaques). Aβ-plaques arise from the aggregation of the Amyloid-Beta (Aβ) peptide. Aβ is generated by the sequential cleavage of Amyloid Precursor Protein (APP) by β and γ-secretase enzymes. There is evidence that multiple genes may play a role in the metabolism of APP and neurodegeneration. Previously, our lab identified Sonic Hedgehog (SHH) as a putative regulator of APP metabolism. SHH plays a key role in the control of neuronal differentiation, growth, and proliferation. We have established transgenic AD Drosophila melanogaster that express the same neuropathology as demonstrated by human AD patients. Using this transgenic model we will determine if modulation of the Hedgehog pathway alters the phenotypes observed in this fly AD model. Determining if SHH plays a role in APP metabolism will further enhance our understanding of AD neuropathology and may also provide us with possible pharmaceutical interventions.
The unique methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis is essential in most human pathogens, including Mycobacterium tuberculosis and the malaria parasite Plasmodium falciparum. The first enzyme in this pathway, 1-deoxy-D-xylulose 5-phosphate (DXP) synthase, catalyzes the thiamin diphosphate (ThDP)-dependent formation of DXP from D-glyceraldehyde 3-phosphate (GAP) and pyruvate. This enzyme is mechanistically and structurally distinct from other ThDP-dependent enzymes and is a rational target for the development of new anti-infective agents. Toward our long-term goal to target non-mammalian isoprenoid biosynthesis, we have conducted mechanistic studies of DXP synthase. Here, we present the results of detailed kinetic analysis and substrate binding studies. Our results support a novel, random sequential mechanism of catalysis for DXP synthase. These results have important implications for the design of selective inhibitors of isoprenoid biosynthesis.

Electrophilic ortho-quinone methides (o-QM) are generated during xenobiotic metabolism and can adduct DNA, proteins, and lipids. They are also potent inhibitors of cellular enzymes and have been proposed as potential therapeutic agents. In this study, we have investigated the chemistry of o-QMs and their adducts with DNA, proteins, and lipids. Our results suggest that o-QMs are highly reactive and can undergo a variety of reactions, including electrophilic substitution, nucleophilic addition, and irreversible oxidation. The reactivity of o-QMs is influenced by the structure of the adduct and the nature of the adducting agent. The results of this study will help to better understand the chemistry of o-QMs and will have implications for the design of new therapeutic agents.

Trapping Reversible ortho-Quinone Methide – Nucleoside Adducts

Electrophilic ortho-quinone methides (o-QM) are generated during xenobiotic metabolism and can adduct DNA, proteins, and lipids. They are also potent inhibitors of cellular enzymes and have been proposed as potential therapeutic agents. In this study, we have investigated the chemistry of o-QMs and their adducts with DNA, proteins, and lipids. Our results suggest that o-QMs are highly reactive and can undergo a variety of reactions, including electrophilic substitution, nucleophilic addition, and irreversible oxidation. The reactivity of o-QMs is influenced by the structure of the adduct and the nature of the adducting agent. The results of this study will help to better understand the chemistry of o-QMs and will have implications for the design of new therapeutic agents.
**31. Mr. Meghan Reilly  
University of Delaware**

**Histone-Mimetic Polyplexes as Self-Activating & Tailorable Non-Viral Gene Delivery Vehicles**

Recent developments with non-viral gene carriers have focused on the effectiveness of gene therapies and improving understanding of the cell milieu and endocytotic machinery. A novel solution to address inefficient non-viral DNA delivery is the development of biomimetic materials capable of regulating DNA accessibility and transcriptional activation. For example, the recognition of the histone H3 tail peptides has been implicated in chromatin activation. This work examines H3 tail peptides with and without a trimethylated K4 for their potential as gene delivery materials. We have found that the H3 tail peptides, in combination with the helper cationic polymer poly(ethyleneimine) (PEI), bind, protect, and release DNA. Dynamic light scattering and agarose gel electrophoresis were used to demonstrate that both H3 peptides condense DNA into nanoscale particles that were stable in the presence of serum nucleases. The H3-PEI hybrid polyplexes were found to transfect a substantially higher number of CHO-K1 cells in vitro compared to both polyplexes that were formed with only PEI and the same total charge ratio. In addition, the PEI polyplexes exhibited a cytotoxic effect, whereas the H3-PEI hybrid polyplexes did not compromise cell viability. We have explored the observed synergies between the H3 peptides and PEI through the use of various chemical endocytic uptake and trafficking inhibitors, and have determined that the endocytotic pathways play a vital role in the fate of the polyplexes.

**32. Mr. Muhammad Noon  
School of Pharmacy, University of Maryland, Baltimore**

**MD simulation of the effect of oxidized methionine on the binding of DNA and CBFβ to the Runt domain of CBFβ**

Runt domain (RD) is DNA-binding domain of p53 type transcription factors known as core binding factor α (CBFα) subunits. Three related genes Runx1, Runx2 and Runx3 are involved in the expression of CBFαs. Runx genes are important for several developmental processes; Runx1 plays role in haematopoiesis, Runx2 is important for skeletal development (osteogenesis) and Runx3 is essential in the development of gastric epithelium. Mutation of Runx1 is linked with human leukemias, whereas mutation of Runx3 leads to development and progression of gastric cancers and testicular yolk sac tumor. Runx2 gene mutation is associated with inherited human skeletal disorder known as cleidocranial dysplasia. Biological activity of some proteins is known to be sensitive to oxidative modifications caused by variety of oxidants. Methionine, and Cysteine, residues of proteins are particularly sensitive to oxidation by reactive oxygen species (ROS). Methionine residues may be oxidized to sulfone form (reversible) and to sulfoxide form (permanent change). Here, we carry out molecular dynamic (MD) simulation studies of RD, which is the DNA-binding region of Runx genes, to see the effect of oxidized (sulfoxide form) methionine-106 of RD on its interactions with its binding partners. Methionine-106 is located at the interface with a related protein, known as core binding factor β (CBFβ), which binds to the RD to enhance RD-DNA interaction. The simulation studies indicate that oxidation of methionine-106 leads to improved binding of RD with CBFβ and DNA as dimers and in the DNA-RD-CBFβ ternary complex.

**33. Mr. Anil Pandey  
University of Delaware**

**Proline Editing: A Novel Tool for Controlling Peptide Structure and To Synthesize Chemically Diverse Peptides**

Abstract: Protein function is dependent upon protein conformation. Dramatic changes in peptide or protein structure may be achieved through utilization of stereoelectronic effects to control protein main chain conformation. We have developed a novel chemistry termed Proline Editing, which provides means of rapid modification and functionalization of a peptide or protein. A model peptide TYXN (X = 4-Hydroxyproline) was used to demonstrate the versatility of this approach by post-synthetic chemical modification at 4 position of proline (on resin) leading to conformational and broad chemical diversity. This easy to do chemistry utilizes a few washes of the derivatized resin, cleavage and subsequent HPLC purification, which significantly reduce time and material resources. These derivatives can be utilized as precursors for bio-orthogonal chemistry, as linkers, and sterol or electronic based switches. This stereoelectronic approach of tuning the local structure of peptide and protein via controlling ring pucker of proline and cis-trans isomerization of peptide backbone can be utilized globally to stabilize protein secondary structures. We have also demonstrated the bioorthogonal application of these post synthetically modified peptides by doing three parallel reactions together in one vial with excellent selectivity and yields within minutes of the reaction.

**34. Dr. Xue Zhi Zhao  
NIH**

**Hydroxy-pyrollopyridinidine-trione Based HIV-1 Integrase Inhibitors**

Abstract: After more than two decades of intensive research, approximately 30 drugs have been approved by the FDA for the treatment of HIV/AIDS. Merck’s Isentress™ (MK-0518 or Raltegravir), the first FDA-approved HIV-1 integrase (IN) inhibitor, shares key structural features with other IN inhibitors. These features include a planar arrangement of heteroatoms that chelate magnesium ions and halogen-substituted aromatic functionality linked to the chelating portion of the molecule that interacts with a region formed between a viral DNA base and the protein in the IN-DNA complex. The emergence of Raltegravir-resistant IN mutants demonstrates a need to develop new IN inhibitors that can overcome this resistance. A co-crystal of Raltegravir bound to the Prototype Foamy virus (PFV) IN complexed with DNA [Hare, S. et al. Nature, 2010, 464, 232-7] provides insights into the basis for the resistance caused by mutations in PFV. We have previously reported 4,5-dihydroxy-1H-isoido-1,3(2H)-diones as structurally simple IN inhibitors that exhibit good potency and strand transfer selectivity in vitro in the presence of Mg2+ cofactor. The co-crystal structure of one of these inhibitors bound to PFV-DNA complex has recently been solved. This allows comparison to be made of the binding interactions of our synthetic inhibitors with Raltegravir. As reported herein, a series of hydroxy-pyrollopyridinidine-trione-containing analogues were prepared by insertion of a nitrogen into the ring system of the original 4,5-dihydroxy-1H-isoido-1,3(2H)-diones. This simultaneously combines structural features of our original inhibitors with Merck’s pyrimidinone IN inhibitors. The efficient synthesis of these compounds relies on the application of a “Pummerer cyclization deprotonation cycloaddition” cascade of imidosulfoxides as well as [3+2] cycloaddition of isomünchnones. Introducing a nitrogen substituent onto the catechol ring to give the 2(1H)-pyridone moi

**35. Ms. Bernice Bediako  
University of Maryland Baltimore County**

**Regulation of the GOSPEL-GAPDH-Siah 1 apoptotic pathway by S-nitrosation**

The GOSPEL-GAPDH-Siah 1 pathway is a recently discovered pathway implicated in neurodegenerative diseases. The S-nitrosation of both GOSPEL (GAPDH’s competitor Of Siah Protein Enhances Life) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) in the presence of an apoptotic stimulus prevents Siah-1-mediated nuclear translocation of GAPDH, thus preventing cell death. The main aims of this research are to investigate 1) the effect of nitrosation on the association between GOSPEL and GAPDH and 2) the effect of nitrosation on GAPDH and GOSPEL 3D structure by combining molecular biology, enzymatic assay, x-ray crystallography and small-angle x-ray scattering. Our preliminary studies indicate that unmodified GAPDH follows Michaelis-Menten kinetics with Vmax, Km and Kcat values of 0.65 μM s⁻¹, 0.05 mM and 0.013 s⁻¹, respectively. By comparison, CysNO modified GAPDH shows decreased enzymatic catalytic activity (Vmax, Km and Kcat values of 0.16 μM s⁻¹, 0.08 mM and 0.002 s⁻¹, respectively). Understanding the effects of nitrosation on the apoptotic GOSPEL-GAPDH-Siah 1 pathway may help in the design of novel therapeutics for the treatment of neurodegenerative diseases.
36 Ms. Francine Morris  
Johns Hopkins University  
DXP synthase-catalyzed C-N bond formation: Implications for inhibitor design  
Authors Francine Morris and Caren Freel Meyers  
Abstract: The rising occurrence of microbial resistance to all currently available therapies has created a growing demand for new anti-infectives based on novel modes of action. Several opportunistic pathogens, including Pseudomonas aeruginosa, Mycobacterium tuberculosis and the malaria parasite Plasmodium falciparum utilize the mevalonate pathway. In mammals, the production of isoprenoids is essential and the MEP pathway is absent in mammals, the enzymes in this pathway are attractive targets for the development of new anti-infective agents. Currently, only a few inhibitors of non-mammalian isoprenoid biosynthesis are known. DXP synthase is the first step in this pathway and is known to catalyze the formation of a C-C bond in the production of deoxy-D-xylulose 5-phosphate (DXP) from pyruvate and D-glyceraldehyde 3-phosphate. In addition, DXP synthase catalyzes the early steps in thiamin diphosphate (THDP) and vitamin B6 biosynthesis. This similarity of this enzyme to the mammalian transketolase superfamily suggests that selective inhibition of DXP synthase may be beneficial. However, recent work has demonstrated that DXP synthase possesses a unique catalytic mechanism and structure, suggesting that development of selective inhibitors is possible. Here, we present a substrate specificity study that highlights DXP synthase-catalyzed C-N bond formation and reveals a remarkable affinity of the enzyme for naphthol-containing alternative substrates. Current efforts are focused on studying structure-activity relationships for alternative substrate turnover and identifying important binding elements as a starting point for inhibitor design.

37 Ms. Chengyun Huang  
University of Maryland, College Park  
Synthesis of an electron rich quinone methide precursor to expedite sequence-directed alkylation of DNA  
Chengyun Huang, Steven E. Rokita*  
Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20740  
A quinone methide (QM) is a very reactive electrophilic intermediate that can alkylate the most nucleophilic nitrogen in DNA reversibly. This reversibility has been used to construct sequence-directed alkylation reagents, but their reaction is slow. We have now synthesized an electron rich quinone methide precursor (QM') to accelerate QM (re)generation for selective alkylation. The DNA-QM conjugate was completely converted to DNA-QM self-adduct within 0.5 hour when the electron donating group was present compared to a period of 24 hours needed for a parent QM lacking the electron donating group. Alkylation study using the DNA-QM self-adduct with a single strand DNA target also showed that the electron rich self-adduct needed only half (2 days) as long as the unsubstituted self-adduct (4 days) to reach maximal alkylation of the target. This enhanced efficiency should shorten the time for a QM to take effect in a biological system.

38 Mr. Scott Crown  
University of Delaware  
Novel Insights into Odd-chain Fatty Acid Synthesis in Adipocytes  
Obesity and type II diabetes are increasing health epidemics in Western society and are characterized by dysregulation of lipid metabolism. A more complete understanding of de novo lipid synthesis at the metabolic level is required to develop novel therapeutics for the treatment of these disorders. Towards this end, we are implementing a systems biology approach using metabolic flux analysis and stable isotope 13C-tracers to determine the active reaction pathways in an adipocyte cell line 3T3-L1. In the work presented here, we investigated the synthesis of odd-chain length fatty acids in differentiated white adipocytes. The white adipocytes were incubated with [U-13C]-glutamine and incorporation of 13C into fatty acids was analyzed with gas chromatography mass spectrometry (GC-MS). Isotopomer spectral analysis (ISA), conducted on the mass isotopomer distributions of the fatty acids, suggested that odd-chain fatty acid synthesis proceeded from elongation of a three-carbon precursor rather than by alpha-oxidation as was previously assumed. To investigate this further, differentiated adipocytes were incubated with [U-13C,15N]-valine, and [U-13C,15N]-isoleucine. From these tracer experiments, valine and isoleucine were determined to be major contributors to the three-carbon precursor pool. Furthermore, novel mass spectrometry techniques validated that the three-carbon moiety acts as the primer on fatty acid synthase and is subsequently elongated by acetyl-CoA subunits. Lastly, it was verified that the incorporation of three-carbon units is highly specific to odd-chain fatty acids. The research demonstrated here provides an important link between amino acid and fatty acid metabolism.
A universal switch platform based on designed ankyrin-repeat proteins

Abstract: A universal protein switch platform that can be easily adapted to bind a variety of biological targets would greatly facilitate the development of biomolecular sensors for a variety of applications. Designed ankyrin-repeat proteins (DARPs) are currently recognized as potential antibody alternatives. DARPs have been engineered by others to specifically bind a variety of protein targets including G-protein coupled receptors (GPCRs), enzymes, and other protein-protein interactions. Here, we describe an excellent platform as a recognition motif for the development of a universal switch platform. We have used a combinatorial approach to create a library of hybrid proteins that are comprised of the MBP-specific DARPin off7 as the input domain and the enzyme TEM1 β-lactamase (BLA) as an output domain of the switch. Specifically, the gene encoding a circularly permuted variant of BLA was randomly recombined within the off7 gene to create a hybrid DNA library with a theoretical diversity of order of 10^5. An in vivo selection scheme based on a previously developed bacterial band-pass genetic selection system was implemented to identify fusion proteins in which MBP activated BLA enzyme activity. The identified switches would be used for the subsequent development of biosensors against a variety of targets by engineering new binding properties into the input domain.

High Rates of Epimerization During the Synthesis of Glycopeptides

Yalong Zhang, Saddam M. Muthana, David Farnsworth, Olaf Ludek, Jeffrey C. Gildersleeve

Glycoproteins play an important role in many biological processes including cell-cell recognition, inflammation, and metastasis. Homogenous glycopeptides or glycoproteins from natural sources are difficult to obtain due to the template-independent glycosylation with microheterogeneity. Glycopeptides can be obtained via solid phase synthesis using protected amino acids that contain the glycan (glyco-amino acids) as building blocks. Compared with standard peptides via automated methods, the synthesis of glycopeptides/glycoproteins can be significantly more challenging. Because of expensive or synthesized building blocks, use of large excess of glyco-amino acid to increase the rate and drive the reaction to completion is unappealing. Scientists typically use an automated synthesizer and then carry out the coupling of the glyco-amino acid manually. This process is especially disadvantageous when preparing large numbers of glycopeptides, such as in the construction of a glycopeptide library. In addition, epimerization of glyco-amino acid during coupling is not evaluated, which is critical for developing glycopeptides vaccines. In this study, we carried out a systematic comparison of published coupling conditions using four different Fmoc-protected glyco-amino acids as well as the non-glycosylated counterpart. Both efficiency and epimerization were evaluated using HPLC assays. Interestingly, epimerization at the alpha position of the amino acid backbone was found to be a substantial problem in a number of reactions with glyco-amino acids. In fact, epimerization resulted in as much as 80% of the non-natural epimer, indicating that the non-natural epimer can be the favored product for some glyco-amino acids. To better understand the origin of this problem, we also measured rates of...

The two rings in a GroEL molecule operate ~180° out of phase with one another to fold substrate proteins (SP). The release of ADP from the trans ring largely determines duration of the chaperonin cycle. A network of salt bridges break and form during the large, rigid body conformational changes in a ring. Sub-meta, inter-domain salt bridge D83-K237 and inter-subunit, inter-domain salt bridge R197-E386 break during the T to R transition. Elimination of these salt bridges by site directed mutagenesis destabilizes the T state, thus favoring the R state of GroEL rings. At high concentration of ATP and K+, this double mutant GroEL (D83A/R197A) undergoes only TR transition with a significantly decreased ATPase activity. However, at lower concentration of ATP and/or K+, transition is accelerated. This in accordance with our previous conclusion that the off-rate of ADP is the limiting step in a chaperonin cycle, and that K+ enhances nucleotide affinity greater to the R state than to the T state. The deficiency of SP-stimulated ATPase activity at physiological concentration of ATP and K+ can cause the ring to cycle through the R state, the SP-acceptor state, promoted by the salt bridges network, to efficiently assist protein folding.

The most prevalent problem for antiviral agents today is the rapid emergence of resistance to currently used therapeutics. As a result, there is a critical demand for drugs that can overcome mutations in order to stay active against resistant viral strains. Recent reports that two new drugs, tenofovir and etravirine, appear to be able to do just that are highly encouraging. They use their inherent flexibility to alter their conformation and position, allowing them to overcome mutations in HIV's reverse transcriptase binding site and retain their potency. Based upon these findings our lab has begun work on the design and synthesis of a series of nucleoside analogs in which flexibility has been introduced by splitting the purine nucleobase. We have applied this approach to several known nucleoside inhibitors such as tenofovir and the viral targets include the polymerases of HIV, HCV, HBV, HCMV and HSV. It is hypothesized that by introducing flexibility into already active nucleoside inhibitors they will be able to retain potency against mutant polymerases in which steric or electronic mutations have been introduced in the nucleoside binding site. The preliminary synthesis is presented herein.

Error-prone nucleotide incorporation by DinB, a Y-family polymerase from drug-resistant Pseudomonas aeruginosa

Abstract: Pseudomonas aeruginosa colonizes the distal tracts of lungs of patients with cystic fibrosis. Through the progressive disease course, the isolates of this opportunistic pathogen can become increasingly drug resistant, often greatly limiting therapeutic options. DinB, a member of the Y-family of DNA polymerases, is part of the SOS stress response pathway of the pathogen Pseudomonas aeruginosa. DinB promotes error-prone replication of the bacterial genome and as such has been implicated in hypermutation and consequent drug resistance. Targeting the activity of this polymerase, therefore, could slow the development of resistance and prolong the useful lifetimes of current antibiotics. Our studies have focused on the kinetics of nucleotide incorporation--and misincorporation--by the polymerase. We are also exploring the hypothesis that the more open active site of Y-family polymerases will make them amenable to inhibition by nucleoside analogs such as 3TC, commonly used to target the error-prone reverse transcriptase of HIV.

MicroRNAs Associated with Environmental Stress in Arabidopsis Thaliana

Environmental stress currently contributes to approximately 50% of crop loss world-wide, with climate change expected to exacerbate this problem. Genomics studies have indicated that differences in stress tolerance arise not only from the presence of stress-associated genes but also from their regulation. In plants, miRNAs are key gene regulators that have been shown to control various developmental processes and also to be involved in response to environmental stresses. In order to further assess the role of miRNAs in environmental stress, a series of stress treatments was carried out in Arabidopsis thaliana. Comparisons between small RNA populations of different tissues, mutants and stresses were made via the construction, sequencing and analysis of over 25 small RNA libraries, resulting in over 150 million sequences. Through this method, several new miRNAs, several from previously annotated miRNA precursors were also found. Additionally, some miRNAs and their target genes were shown to be regulated in a novel manner under stress conditions. Despite the prevalence of multiple simultaneous stresses in agriculture, this area remains largely unexplored. To address this, small RNA libraries were also generated from plants subjected to dual stresses. Several miRNAs were found to be regulated in a unique manner under these conditions, highlighting the importance of further study in this area.
Mr. Nicholas Corsepius  
University of Maryland  

Using Rhodamine Dimers to Explore the Role of Substrate Protein Binding on the GroEL Catalytic Cycle  

Despite being the most extensively studied chaperonin protein, the effect of substrate protein (SP) binding to the GroEL catalytic cycle is not well understood because it contains the various levels of heterogeneity: a given SP will have a variety of misfolded states, each of which can contain any number of GroEL-bound conformations. Our lab has employed the use of tetramethylrhodamine (TMR) dimers to address this problem. Using fluorescence or absorbance measurements, the TMR dimers can be used to monitor the allosteric state of GroEL. Simultaneously, the dimers impose a mechanical constraint on the enzyme, much like that of bound SP, that shifts the equilibrium between allosteric states, which stimulates ATPase activity. The uniformity of the non-covalent intermolecular interaction within a dimer removes many of the levels of heterogeneity that accompany SP binding. This allows us to quantify the additional energy the enzyme must exert in an allosteric state transition to overcome the mechanical constraint imposed by the TMR dimers.

Ms. Meagan Small  
University of Maryland, School of Pharmacy  

CONFORMATIONAL SAMPLING OF TELITHROMYCIN/CETHEROMYCIN AND DESMETHYL ANALOGS  
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Overcoming microbial resistance presents a major challenge in the development of new antibiotics. Approximately 50% of all antibiotics target the peptidyl transfer center (PTC) of the bacterial ribosome, inhibiting protein synthesis and thereby disrupting cellular growth. A recent class of 3rd generation macrolides, the ketolides, has been demonstrated to be effective against bacterial strains with macrolide-lincosamide-streptogramin B (MLSB) resistance. This activity is due in part to the replacement of a C-3 cladinose sugar with a carbonyl, which bypasses a gene-encoded macrolide efflux mechanism (mef) and addition of a alkyl-aryl chain to a C-11,12 cyclic carbamate, which increases ligand affinity via interaction with a second site. However, ketolides are still susceptible to resistance via mutation mechanisms, the most common of which is A2098G (E.coli numbering). Crystallographic studies show that this is due to the presence of a C-4 methyl group that sterically clashes with G2098. To probe the role of the C-4 methyl group as well as those at C-8 and C-10 for structural simplification purposes, 20 nanosecond molecular dynamics simulations in explicit solvent were performed on telithromycin and cethromycin and their desmethyl analogs. Analysis of selected distances distributions revealed that the conformations sampled by the parent compounds and their desmethyl derivatives are significantly different. The probability maxima of the distances for both telithromycin and cethromycin overlap well with crystal structure distances. Surprisingly, the largest difference is observed for the least chemically modified analog in both ketolides studied, C-4 desmethyl(ceth/tei)thyromycin. In addition, for b...
Insertion of selenocysteine into the redox-active cysteine-x-x-cysteine motif of the flavoprotein augmenter of liver regeneration. Stephanie A. Schaefer, Renee Rubenstein, Collin Thorpe, Sharon Rozovsky Augmenter of liver regeneration (ALR) is a mitochondrial enzyme that aids disulfide bond introduction into reduced unfolded proteins. ALR employs two active site cysteines to facilitate the formation of disulfide bonds. The reactive cysteines are part of a cysteine-proline-cysteine redox motif for activity. In order to study the catalytic mechanism of ALR we have modified the redox potential of ALR by introducing a selenocysteine in place of a cysteine within the cysteine-x-x-cysteine motif. Selenocysteine is a naturally occurring amino acid whose pKa and redox potential are lower than that of cysteine. Hence, while this mutation introduces no structural changes, the redox potential of the modified ALR is expected to be lower than that of the wild type protein. Selenocysteine was incorporated into ALR by using the Escherichia coli selenium incorporation machinery. As expected, the UV-VIS absorbance spectrum of the cysteine-x-x-selenocysteine containing protein is shifted from 456 to 461 nm. The enzyme containing enzyme forms a charge-transfer band in addition to a semiquinone complex when reacted with a small dithiol substrate. These spectral effects are attributed to the interaction of the selenocysteine with the cofactor FAD while the enzyme was turning over.

Chemical acetylation of hormone receptors with biomimetic protein acetyltransferases. Acetylation has increasingly been shown to be an important post translational modification for proteins. Acetylation is involved in the regulation of a number of steroid hormone receptors. Acetylation of lysines on the surface of steroid hormone receptors have been known to modulate hormone responsive behavior and is associated with a number of drug resistant cancers. As natural protein acetyltransferases function in these targeted reactions, we have designed reactive ligand conjugates that when bound position a thiocysteine in close proximity to reactive lysines on target proteins. Proximity directed acetylation represents a new paradigm in hormone receptor modulation by small molecules.

Diverse association paradigms of the AUF1 isoforms induce divergent mRNA structures. Christina Rossi, Jun Huang, Eric Toth, Gerald Wilson. AUF1 is a nucleic acid-binding protein that regulates the stability and translational potential of many mRNAs. AUF1 binds to AU-rich elements (AREs) that are common in mRNAs encoding many proteins involved in cardiovascular, inflammatory, and neoplastic diseases. There are four isoforms of AUF1 resulting from alternative splicing of exon 2 and/or 7 from a common pre-mRNA. Preliminary evidence suggests that the different isoforms have varied functional characteristics, but no detailed, quantitative analysis of the properties of each has been reported. Here we use differential expression and gel filtration assays to show that each isoform exists as a high affinity homodimer in solution. We then defined the association mechanisms of each isoform for an ARE-containing RNA substrate and quantified binding affinities using fluorescence anisotropy and electrophoretic mobility shift assays which varied dependent upon the alternative exons. Finally, fluorescence resonance energy transfer (FRET)-based assays showed that the different isoforms remodel bound RNA substrates into divergent structures as a function of protein-RNA stoichiometry. We predict that alterations in the local structure of AUF1-substrate mRNAs will impact accessibility for ancillary trans-acting RNA-binding proteins and microRNAs proximal to AUF1 binding sites, which implies a potential role for AUF1-bound mRNA structures in priming post-transcriptional regulatory events. Together, these data describe isoform-specific characteristics among AUF1 ribonucleoprotein complexes, which likely constitute a mechanistic basis for differential isoform function and regulation.

Probing a tRNA Target Base for Methylation by Atomic Substitution. The N1 methylation of guanosine 37 (m1G37) in tRNA is essential for maintaining the fidelity of translation and is required for cell growth in bacteria and in eukaryotes. The enzyme responsible for the synthesis of m1G37 is Trm37 in bacteria and Trm5 in eukaryotes and archaea. While TrmD and Trm5 catalyze the same reaction using S-adenosyl-methionine as the methyl donor, they have different active site structures. However, the molecular basis of how their distinct active sites recognize the target base is unknown. Understanding this difference will offer an important foundation to develop novel antibiotics that selectively target TrmD. We have developed an approach to probe the recognition of atomic groups in G37 by B. colli TrmD and M. jannaschii Trm5 as a model pair. In this approach, tRNA substrates with a specific atomic group substitution were created by a combination of chemical synthesis and enzymatic recoding. The activation of this modified substrate was monitored by the single-turnover rate constant of methyl transfer (kchem) and by the kinetic equilibrium binding constant (Kd) of the enzyme-tRNA complex. The kinetic parameter kchem/Kd of the normal over modified tRNA substrate is used to determine how TrmD and Trm5 discriminate the normal from the modified substrates. This approach has given new insights into the molecular mechanism of substrate recognition by TrmD and Trm5.

Reversibility of HNO-Induced Sulfinamide Formation. Gizem KEceli† and John P. Toscano†,* †Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218. Cysteine residues are susceptible to several post-translational modifications under oxidative conditions. HNO, a potential heart failure therapeutic, is known to be very thiolphilic. The reaction of HNO with thiols results in the formation of disulfide or sulfinamide, depending on the concentration of thiol. Traditionally, sulfinamide modification is considered to be irreversible in peptides and proteins. We have investigated the reversibility of HNO-induced sulfinamide formation in small organic molecules, peptides and proteins under physiological conditions. Proteins are known to undergo succinimide-mediated Asn deamidation at physiological pH and temperature. We hypothesized that the reversibility of sulfinamide modification might be facilitated by the presence of reducing agents, sulfinamide formation is reversible. Moreover, the sulfinamide reduction is increased by at least a factor of three in the peptide structure vs. in small organic molecules. Although peptide sulfinamides are prone to hydrolysis, it is slower than the reaction with thiols. Activity studies with a cysteine protease, papain, demonstrates the feasibility of sulfinamide reduction in a protein environment. We conclude that there is a significant contribution from a low energy, cyclic intermediate (analogous to that in Asn deamidation) in the reversibility of peptide sulfinamides.

Chemical acetylation of hormone receptors with biomimetic protein acetyltransferases. Acetylation has increasingly been shown to be an important post translational modification for proteins. Acetylation is involved in the regulation of a number of steroid hormone receptors. Acetylation of lysines on the surface of steroid hormone receptors have been known to modulate hormone responsive behavior and is associated with a number of drug resistant cancers. As natural protein acetyltransferases function in these targeted reactions, we have designed reactive ligand conjugates that when bound position a thiocysteine in close proximity to reactive lysines on target proteins. Proximity directed acetylation represents a new paradigm in hormone receptor modulation by small molecules.
Fluorescence recovery after photobleaching (FRAP) is a powerful microscopy technique to examine diffusion and flow processes in lipid membranes. The experiment proceeds as follows: several pre-bleach scans are collected, a small area is bleached with a high intensity laser, and the recovery of the fluorescence signal is monitored with respect to time. Fluorescence intensity over time corresponds to local exchange of fluorescent molecules attributed to diffusion or directed flow. The extent to which cellular changes affect some perturbation to the lipid environment tells us a great deal about the cross-linking, non-specific binding, and or protein aggregation. We have applied this method to membranes containing biotinylated lipids that we cross-linked with avidin.


Chemical Branch, Analytical Toxicology Division *Department of Chemistry, Ohio State University ABSTRACT To generate a novel defense against poisoning by organophosphorus (OP) nerve agents, a chimeric recombinant paraoxonase 1 (PON1) variant (MG-2-IA4) was generated by gene shuffling and multiple rounds of directed evolution to be expressible in bacteria and to have enhanced catalytic efficiency for the hydrolysis of G-type OPs. We have assessed the stereospecific activity of MG-2-IA4 and PON1, respectively, of the OPs GA, GB, GD and GF using a chiral gas chromatography / mass spectrometry approach. For racemic GD and GF, MG-2-IA4 displayed a modest preference for hydrolyzing the more toxic P(-) isomer (s). No stereopreference was detected with either GA or GB; both stereoisomers of each OP were hydrolyzed with almost equal relative catalytic efficiency. In vivo efficacy studies conducted using guinea pigs administered different doses of MG-2-IA4 via a carotid catheter prior to subcutaneous exposure to 2 x LDso of GA, GB, GD, or GF showed that pretreatment with an adequate dose of this enzyme afforded protection without any signs or symptoms of intoxication in exposed animals. At lower doses of enzyme, protection was observed against GA and GF but not GB or GD. Interestingly, the relative catalytic efficiencies do not correlate precisely with in vivo protective efficacy. Additional work to define both in vitro and in vivo parameters for MG-2-IA4 is ongoing.

Elastin mimic, cell adhesive multiblock hybrid copolymers. Abstract: Biocompatible elastomers that not only capture the elasticity of native elastin but also provide flexibility and tunability in a range of morphological, biological, and mechanical properties are needed for use as scaffolds for tissue repair and regeneration. Hybrid polymer-peptide materials offer greater tunability in mechanical and other material properties from the presence of synthetic polymer domains, while maintaining the structural and biological properties of the peptide domains. Elastin mimetic hybrid polymers (EMHPs) were synthesized via condensation polymerization of azide-terminated poly(ethylene glycol) with alkyne-functionalized peptides by orthogonal click chemistry. The peptide was composed of multiple alanine residues regularly spaced by lysine and terminally tagged with the RGDS sequence derived from fibronectin in order to promote cell attachment. The EMHPs were then crosslinked through the peptide lysine residues to form elastic hydrogels with mechanical properties comparable to natural elastin. Neonatal human dermal fibroblasts (NHDFs) adhered to the EMHPs within one hour in serum-free media, and F-actin staining showed the development and spreading of F-actin filaments after 24 hours of cell adhesion. Vinculin staining revealed that the cells attached to the gels via focal adhesions. Cell proliferation was significantly greater on the RGDS EMHP hydrogels in comparison with non-RGDS EMHP hydrogels, and competitive binding experiments with peptides containing cell adhesive sequences showed that NHDFs with soluble RGDS peptide or RGDS EMHP, indicating the involvement of the RGDS domains in integrin binding and cell attachment. These novel elastomeric, biocompatible EMHPs are promising candidates as conducing scaffolds for vocal fold tissue engineering.

Electronic properties of peptide scaffolds containing PPV based chromophores as side chains. Previous studies show that the alignment of the chromophores and the distance between them are crucial parameters which govern the structure-property relationships in electronically active molecules which are being studied extensively to enhance the performance of organic and polymeric semiconductor materials. However it is difficult to achieve control for this purpose at angstrom length scales. In this work, we have employed helical peptides as scaffolds to organize oxadiazole-containing phenyleninevinylen (PPV) oligomers at desired spacing to study the interaction between these functional groups. The oxadiazole-containing PPV oligomers will be coupled at specific positions on alanine-rich helical peptides. This system is designed to incorporate up to three chromophores strategically placed on the peptide backbone in the range of 6-17Å from each other. Circular dichroism spectroscopy is used to confirm the helical nature of the peptide. Other spectroscopic techniques such as exciton-coupled CD, photoluminescence, and absorption will elucidate how the chromophores interact with each other when placed on the same side of the peptide chain or on opposite side of the peptide chain.

Porphyrin-Peptide Self-Assembling Nanowires. We have designed and synthesized two porphyrin-peptide systems that self-assemble. Our goal is to create a nanowires that efficiently transfers energy using exciton coupling in a manner analogous to energy transfer between chlorophylls in the photosynthetic antenna complex of bacteria and plants. We have synthesized a 10-mer peptide, NAEEASAY, and coupled two monoaminotetraphenylporphyrins to the glutamic acid residues. This system shows ordered aggregation and increased a-helical character as observed with UV-Vis Spectroscopy, CD Spectroscopy, and Atomic Force Microscopy (AFM). We are also working with a system that couples a 7-mer peptide, GNNQQNY, with Deuteroporphyrin IX 2,4-bis ethylene glycol at the carboxylic group of the peptide. The 7-mer peptide is known to form P(-) helixes and based on observation and UV-Vis we see extensive aggregation. At pH 4 we observe a characteristic b-sheet peak in IR as well as potential frib formation according to AFM studies. We are now studying a new peptide, GSFSIQYTYHV, which is known to form a hydrogel. AFM indicates that this peptide forms large fibrils which seem promising as a structural framework for porphyrin. We are also exploring a new synthetic approach in this system where the porphyrin is coupled to the peptide before cleavage from the resin.

Structure and Function of Selenoprotein K. Jun Liu, Prabhavathi Srivinasa, and Sharon Rozovsky University of Delaware, Department of Chemistry and Biochemistry Newkirk BE 1976 SelK is critical to longevity. Selenoproteins contain the genetically encoded amino acid selenocysteine that is predominantly found in enzyme active sites where it provides high chemical reactivity and specificity. The function of SelK is unknown but it was shown to participate in anti-oxidant defense in vivo, possibly in the Endoplasmic Reticulum Associated Protein Degradation (ERAD) pathway. To facilitate SelK structural and biophysical characterization, we have developed bacterial overexpression strategy using a fusion partner to stabilize and solubilize SelK. The fusion can be cleaved from SelK in the presence of a variety of different detergents compatible with structural characterization by nuclear magnetic resonance spectroscopy. Furthermore, we demonstrate the incorporation of selenium in SelK forming the active selenoprotein.
Characterization of a Semenogelin I Peptide Hydrogel

Jamie DeNizio(1), Birgitta Frohm(2), Sara Line(2) and Karin Åkerfeldt(1) (1) Department of Chemistry, Haverford College, Haverford, PA 19041 (2) Department of Biochemistry, University of Lund, Sweden

The aim of this project is to investigate the hydrogel forming properties of a sequence derived from Semenogelin I, a protein found in seminal plasma. Hydrogel formation tests were performed varying the peptide concentration, pH, temperature, and time. Circular dichroism (CD) and FT-IR spectroscopy were used to determine the secondary structure, and atomic force microscopy (AFM) was used to look at the tertiary morphologies of the peptides in solution. It was found that the 11 residue sequence forms a hydrogel dissolved in water slightly above neutral pH. The CD spectra confirm the hypothesis that the peptide self-assembles to form b-sheets, both initially at acidic pH and after gel formation at basic pH. In addition, the IR spectra indicate strong b-sheet characteristics at basic pH, exhibiting extended sheeted extended sheets after the sample had gelled. The AFM images display different morphologies depending on substrate, incubation time and temperature. Current work includes the synthesis and purification of related sequences with an eye to increase peptide cross-linking in order to form more durable hydrogels.

Insights of the intrinsically unstructured C-terminal domain of alpha4, regulator of MID1 and PP2A

Yang Liu

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The structural properties of the C-terminal residues (Glu236-Leu280, alpha45) of Alpha4 are probed by NMR spectroscopy. We observed that even though there were no strong NH-NH(i,i+1) NOEs indicative of helical elements, chemical shift indices suggested the presence of an intrinsically unstructured helical C-terminus. Analysis with TALOS+ confirmed this observation. Interestingly, we observed that alpha45 adopted two alpha-helices in the solution with 1 % SDS. The residues that formed the alpha-helices were also predicted to be helical and identified by CSI and TALOS+ to be helical. HSQC experiment revealed that alpha45 binds tightly to the Bbox1 domain from MID1 in aqueous solution. Together, these results suggest that the C-terminus has intrinsically unstructured properties.

Preparation of a meningococcal group C polysaccharide-tetanus Hc fragment glycoconjugate vaccine candidate for chemoenzymatic synthesis Global pharmacodynamic and pathophysiologic characterization of lysine at position 2. Multiple minimal protein kinase-inducible domains were developed using combinations of these design elements and optimized as phosphorylation sensors for multiple targets.

Insights of the intrinsically unstructured C-terminal domain of alpha4, regulator of MID1 and PP2A

Haijuan Du

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Characterization of a Semenogelin I Peptide Hydrogel

Jamie DeNizio(1), Birgitta Frohm(2), Sara Line(2) and Karin Åkerfeldt(1) (1) Department of Chemistry, Haverford College, Haverford, PA 19041 (2) Department of Biochemistry, University of Lund, Sweden

The aim of this project is to investigate the hydrogel forming properties of a sequence derived from Semenogelin I, a protein found in seminal plasma. Hydrogel formation tests were performed varying the peptide concentration, pH, temperature, and time. Circular dichroism (CD) and FT-IR spectroscopy were used to determine the secondary structure, and atomic force microscopy (AFM) was used to look at the tertiary morphologies of the peptides in solution. It was found that the 11 residue sequence forms a hydrogel dissolved in water slightly above neutral pH. The CD spectra confirm the hypothesis that the peptide self-assembles to form b-sheets, both initially at acidic pH and after gel formation at basic pH. In addition, the IR spectra indicate strong b-sheet characteristics at basic pH, exhibiting extended sheeted extended sheets after the sample had gelled. The AFM images display different morphologies depending on substrate, incubation time and temperature. Current work includes the synthesis and purification of related sequences with an eye to increase peptide cross-linking in order to form more durable hydrogels.

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Synthesis of peptides containing 4-thiophenylalanine

Although Nature has evolved a plethora of protein functions from only 20 common amino acids, incorporation of non-natural amino acids into proteins can provide tools for reporting and tuning protein structure and function. We describe a copper-catalyzed reaction, which is compatible with all of the natural amino acids, that generates 4-thiophenylalanine on solid-phase without side products. By itself, 4-thiophenylalanine can function as a simple structural “switch” in proteins, where aromatic n–π-interactions can be strengthened or weakened an adjustment in pH. Once we also used simple thiophenol derivatives, to generate a wide variety of aryl/thiol derivatives (including thioethers, disulfides, sulfoxides and sulfones) and introduce greater functionalization on an aromatic amino acid. These derivatives can also be used to tune local structure within a peptide, and we report these electronic effects with a number of novel derivatives in the context of the aromatic-prolyl interaction, which is known to influence the Ktrns/cis of the amide bond by substituted aromatic residues adjacent to proline.