

Save the Date...

2nd Annual Chemistry-Biology Interface Scientific Picnic

WHAT: Graduate Student
and Post-Doc
Presentations and
Poster Session,
Breakfast, Lunch,
Dinner, and Fun!

WHO: UPenn Chemistry,
Biochemistry and Molecular Biophysics,
and Wistar

WHEN: Friday July 25th, 2008

WHERE: Swarthmore College

Sponsored by the NIH Chemistry-Biology Interface Fellowship
ALL DEPARTMENTS WELCOME (Biological, Organic, Inorganic, Physical)



Interested in presenting a talk or poster??

Speak to your PI or contact:

Julie Aaron julieaa@sas.upenn.edu

Chemistry-Biology Interface Scientific Picnic

Departments of Chemistry and
Biochemistry & Biophysics
University of Pennsylvania
& The Wistar Institute

Friday July 25th, 2008
Swarthmore College
Swarthmore, PA



Schedule of Events

- 9:20 – 10:00 Check-in and Breakfast, Science Center 101
- 10:00 – 12:00 **Morning Speakers**, Science Center 101
Peng Xie, Department of Chemistry/Wistar Institute
Christopher Butts, Department of Chemistry
Julie Aaron, Department of Chemistry
Julie Glasscock, BMB
- 12:00 – 1:00 Picnic Lunch
Box Lunch pickup at Upper Tarble, Clothier Hall
- 1:00 – 2:00 **Poster Session**
Upper Tarble, Clothier Hall
- 2:00 – 4:00 **Afternoon Speakers**, Science Center 101

Andre Isaacs, Department of Chemistry
Yao Zhang, Department of Chemistry
Claude Warzecha, Cell and Molecular Biology
Venkata Krishnan, Department of Chemistry
- 4:00 – 5:00 Reception, Outdoor games
Parish Lawn
- 5:00 – 6:00 Buffet Dinner
Upper Tarble, Clothier Hall

The Chemistry-Biology Interface Scientific Picnic Organization Committee:

Julie Aaron, Department of Chemistry (julieaa@sas.upenn.edu)
Sarah Chobot, BMB (chobot@mail.med.upenn.edu)
Diana Cabral, Department of Chemistry (dcabral@sas.upenn.edu)
Daniela Fera, Department of Chemistry (dfera@sas.upenn.edu)
Andre Isaacs, Department of Chemistry (isaacsa@sas.upenn.edu)

We would like to thank all of the speakers, poster presenters and attendees; this event would not have been possible without your enthusiastic participation.

We would also like to thank the following people for their support and guidance.

Ronen Marmorstein, Faculty Advisor
Mary Leonard, Webpage Designer
Ruth Keris, BMB Academic Administrator
Cheryl McFadden, Wistar Administrative Coordinator
Rita Manfre, Wistar Associate Director of Grants & Contracts
Matthew Lane, Executive Director, Department of Chemistry

Finally, we thank the Chemistry-Biology Interface (CBI) training grant from the National Institutes of Health (NIH) and Department of Chemistry for generous funding.

Poster Session

Crystal Structure of a p53 Core Tetramer bound to DNA

Kim Maleka, Marmorstein Lab, Department of Chemistry/Wistar

Photoactivatable Oligonucleotides for Controlling Gene Expression

Julia Richards & Julie Gripenburg, Dmochowski Lab, Department of Chemistry

Structure/Function Studies on the H3K56-acetylating HAT Complex Rtt109-Vps75

Yong Tang, Marmorstein Lab, Department of Chemistry/Wistar

Functionalization of colloidal gold using self-assembled ferritin-like systems

Joe Swift & Jasmina C. Cheung-Lau, Dmochowski Lab, Department of Chemistry

Calculating Binding Affinities of Zinc Proteins using QM/MM Methods

Seth Hayik,

Folding Kinetics of Naturally Occurring Helical Peptides

Smita Mukherjee, Gai Lab, Department of Chemistry

Probing the Role of Hydration in the Unfolding Transitions of Carbonmonoxy Myoglobin and Apomyoglobin

Lin Guo, Gai Lab, Department of Chemistry

Computational Design of Single-Chain Four-Helix Bundle Proteins That Bind Non-Biological Cofactors

Jose Manuel Perez Aguilar, Saven Lab, Department of Chemistry

Bundle Builder: An efficient method for generating large ensembles of helical bundle structures for directed protein design.

Christopher MacDermaid, Saven Lab, Department of Chemistry

Towards the Development of Xenon-129 Magnetic Resonance Biosensors

Aru Hill & Gary Seward, Dmochowski Lab, Department of Chemistry

Human AKRs display quinone reductase activity with PAH o-quinones

Carol Schultz, Penning Lab, BMB

Development of Bestatin-based Activity-based Probes

Michael Harbut, Greenbaum Lab, Department of Pharmacology

Structure-Based Design of A Potent and Specific Organoruthenium PI3K Inhibitor

Peng Xie, Marmorstein Lab

Mutations that constitutively activate the PI3K signaling pathway, including alterations in PI3K, PTEN and AKT are found in a variety of human cancers, implicating the PI3K lipid kinase as an attractive target for the development of therapeutic agents to treat cancer and other related diseases. In this study, we report on the combination of a novel organometallic kinase inhibitor scaffold with structure-based design to develop a PI3K inhibitor, called E5E2, with an IC₅₀ potency in the mid-low-nanomolar range and selectivity against a panel of protein kinases. We also show that E5E2 inhibits phospho-AKT in human melanoma cells and leads to growth inhibition. Consistent with a role for the PI3K pathway in tumor cell invasion, E5E2 treatment also inhibits the migration of melanoma cells in a 3D spheroid assay. The structure of the PI3K γ /E5E2 complex reveals the molecular features that give rise to this potency and selectivity towards lipid kinases with implications for the design of a subsequent generation of PI3K-isoform specific organometallic inhibitors. "

Fluorescence screening assay for apoferritin binders: A tool for gabaergic anesthetic discovery?

Christopher A. Butts, Jin Xi, Grace Brannigan, Roderic G. Eckenhoff, and Ivan J. Dmochowski

Horse spleen apoferritin (HSAF) assembles from 24 four-helix bundles to form a 480 kDa sphere with an 8-nm internal cavity. The secondary and tertiary structures of HSAF greatly resemble those observed in various membrane receptor proteins including the γ -aminobutyric acid type A (GABA_A) receptor. HSAF has the highest reported binding affinity for the inhalational anesthetics isoflurane and halothane of any biomolecule. We have developed a fluorescence assay employing HSAF and 1-aminoanthracene (1-AMA) to screen small organic compounds for their potential to induce anesthesia. Additionally, 1-AMA represents the first fluorescent anesthetic molecule capable of being imaged *in vivo* to probe anesthetic localization in albino *Xenopus laevis* tadpoles.

Structure of a ¹²⁹Xe-Cryptophane Biosensor Complexed with Human Carbonic Anhydrase II

Julie A. Aaron, Jennifer M. Chambers, Kevin M. Jude, Luigi Di Costanzo, Ivan J. Dmochowski and David W. Christianson

Cryptophanes represent an exciting class of xenon-encapsulating molecules that can be exploited as probes for nuclear magnetic resonance imaging. The 1.70 Å-resolution crystal structure of a cryptophane-derivatized benzenesulfonamide complexed with human carbonic anhydrase II shows how an encapsulated xenon atom can be directed to a specific biological target. The crystal structure confirms binding measurements indicating that the cryptophane cage does not strongly interact with the surface of the protein, which may enhance the sensitivity of ¹²⁹Xe NMR spectroscopic measurements in solution.

Using an Amino-Acid FRET Pair to Probe Protein Unfolding

Julie M. Glasscock, Yongjin Zhu, Pramit Chowdhury, Jia Tang and Feng Gai

Previously we have shown that p-cyano-phenylalanine (Phe_{CN}) and tryptophan (Trp) constitute an efficient fluorescence resonance energy transfer (FRET) pair that has several advantages over commonly used dye pairs. In this study, we aim to examine the general applicability of this FRET pair in protein folding/unfolding studies by applying it to the urea-induced unfolding transitions of two small proteins, the villin headpiece subdomain (HP35) and the lysin motif (LysM) domain. Depending on whether the Phe_{CN} is exposed to solvent, we are able to extract either qualitative information about the folding pathway, as demonstrated by HP35, or quantitative thermodynamic and structural information, as demonstrated by LysM. Our results show that the unfolding transition of HP35 reported by FRET occurs at a lower denaturant concentration than that measured by circular dichroism (CD) and that the loop linking helices 2 and 3 remains compact in the denatured state, which are consistent with the notion that HP35 unfolds in discrete steps and that its unfolded state contains residual structures. On the other hand, our FRET results on the LysM domain allow us to develop a model to extract structural and thermodynamic parameters about its unfolding, and we find that our results are in agreement with those obtained by other methods. Given the fact that Phe_{CN} is a non-natural amino acid and, thus, amenable to incorporation into peptides and proteins via existing peptide synthesis and protein expression methods, we believe that this FRET method is widely applicable to protein conformational studies, especially to the study of relatively small proteins.

Synthesis of Novel Steroidal Analogs of Cyclopamine

Andre Isaacs, Winkler Lab

The Sonic Hedgehog signaling pathway (SHH) plays an important role in the development of the cerebellum. However, its deregulation can lead to medulloblastoma. Cyclopamine, a plant-derived alkaloid, is a potent inhibitor of the SHH pathway. The difficulty in purification and synthesis of cyclopamine greatly hampers its clinical utility. We describe herein the synthesis of novel cyclopamine analogs prepared from commercially available steroidal precursors. Preliminary studies have showed that these analogs are highly potent inhibitors of SHH signaling.

Molecular design of TM helical bundle as a tool for probing the feature that drives folding in membrane

Yao Zhang, DeGrado Lab

The interaction of transmembrane helices is a fundamental step in the folding of membrane proteins. However, the energetics of interaction of transmembrane segments is not completely understood. We are interested in investigating the effect of the amino acid composition at the association interface, using a model peptide (MS1) derived from the soluble coiled coil GCN4-P1. Previous research in water soluble coiled coils showed that the stability of association increases with the hydrophobicity of the amino acid in the

a position. Here we have introduced Gly, Ala, Val and Ile at all the putative *a* positions in MS1 and measured the dimerization equilibria of all variants using a disulfide crosslinking equilibrium method. The results show that the association strength ranks in a reversed order as in the water soluble peptides, consistent with both the fact that hydrophobic interactions are not dominant in the membrane environment and that small residues can be favorable at protein-protein interfaces in membrane proteins. Computational modeling of the MS1 variants suggest smaller amino acids allow for closer and more favorable backbone interactions than the larger amino acids.

Epithelial Splicing Regulatory Proteins 1 & 2 are novel cell type-specific splicing regulatory proteins identified through a genome-scale functional screen in mammalian cells.

Claude Warzecha, Behnam Nabet, Trey Sato, John Hogenesch, and Russ Carstens

Cell type-specific expression of epithelial and mesenchymal isoforms of the Fibroblast Growth Factor Receptor2 (FGFR2) is achieved through tight regulation of cell type-specific splicing of mutually exclusive exons IIIb and IIIc, respectively. Partitioning of these splicing variants in their respective tissue compartments establishes intercellular communication pathways that are essential for development. Using a luciferase-based splicing reporter construct, we carried out a high-throughput cDNA overexpression screen and identified two functionally redundant, paralogous RNA binding proteins. The expression of either protein is restricted to epithelial cell types that express FGFR2-IIIb. Knockdown of both proteins by RNAi resulted in a complete splicing switch from exon IIIb to exon IIIc in the endogenous FGFR2 transcript in an epithelial cell line. Ectopic expression of either protein in mesenchymal cells that produce FGFR2-IIIc causes a switch to FGFR2 IIIb expression. These studies identify epithelial splicing regulator proteins 1 and 2 (ESRP1 and 2) as the essential regulatory factors that are sufficient and necessary to achieve epithelial cell type-specific splicing of FGFR2.

Functional biomolecular materials incorporating non-biological cofactors into amphiphilic 4-helix bundle peptides

Venkata Krishnan, Andrey Tronin, Joeseeph Strzalka, H.Chris Fry, Micheal J. Therien and J.Kent Blasie (Department of Chemistry, UPenn)
Ivan Kuzmenko and Thomas Gog (Advanced Photon Source, Argonne National Labs)

Biomolecular materials comprising of *de novo* designed amphiphilic 4-helix bundle peptides incorporating novel non-biological functional cofactors have been developed for charge separation and non-linear optical applications. Hierarchical organization of these nanoscale peptide-cofactor complexes into ordered 2-D to 3-D assemblies ensures the translation of their microscopic molecular properties into macroscopic material properties of the ensemble. Structural studies performed on monolayer ensembles of these complexes, using optical spectroscopy and x-ray scattering, indicate that these amphiphilic peptides specifically bind and orient the extended cofactors. Binding specificity is achieved via axial histidyl ligation of a metallo-porphyrin moiety in the cofactor while vectorial orientation of the complexes derives from the strong amphiphilicity of the peptide bundle. X-ray reflectivity studies of monolayer ensembles of the peptide with and without the cofactor reveal the location of the cofactor along the length of the bundle consistent with the design.