Welcome to the 6th Annual Chemical Biology Interface Summer Retreat!
July 23, 2013 – Bryn Mawr College

Schedule of Events:

9:30 a.m. - 10:00 a.m.
Welcome Breakfast (Coffee, Tea, Muffins)

10:00 a.m. – 10:30 a.m.
Joe Jordan, Radhakrishnan Lab
"Mining Cancer Databases"

10:30 a.m. - 11:00 a.m.
Chris Bialas, Dutton Lab
"The Engineering of Non-Natural Flavoproteins"

11:00 a.m. - 11:30 a.m.
Lee Speight, Petersson Lab
Acridonylalanine: Photophysics, in vivo incorporation, and derivative syntheses"

11:30 a.m. - 12:00 noon
Laura Castellano, Shorter Lab
"Using Molecular Tweezers to Counteract Amyloid-Mediated Enhancement of HIV Infectivity"

12:00 noon - 1:00 p.m. **Lunch**

1:00 p.m. - 2:30 p.m.
Poster Session – Prize Sponsored by Fisher Scientific

2:30 p.m. - 3:00 p.m.
Charlie Mo, Kohli Lab
"Characterizing and Targeting LexA, a regulator of bacterial mutation and resistance"

3:00 p.m. - 3:30 p.m.
Nataline Meinhardt, Greenbaum Lab
"Development of Inhibitors of Cysteine Proteases"

3:30 p.m. - 4:30 p.m.
KEYNOTE SPEAKER: Tarun Kapoor, Rockefeller University
"Examining cell division mechanisms using chemical approaches"

4:30 p.m. - 6:30 p.m.
Barbeque and Happy Hour

Co-Sponsored by:

Penn Chemistry Arts & Sciences

College Biochemistry Program

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The Department of Biochemistry and Biophysics

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Chemical Biology Interface Summer Retreat 2013 - Student Presenters

Joe Jordan, Radhakrishnan Lab

"Mining Cancer Databases"

The transformation from normal cell to cancerous cell is often marked by a gradual accumulation of mutations over time that eventually increase the ability of the cancer cell to sustain itself and reproduce. Cancer cells acquire mutations at a faster rate than normal somatic cells but not all of these mutations drive cancer progression. Mutations that confer selective advantage on the cancer cell line are known as driver mutations while passenger mutations are neutral in terms of selective advantage. Recent large scale sequencing projects have generated copious data on somatic mutations in cancer, allowing a look into which proteins are most frequently mutated in cancer, and therefore likely to be driver mutations. I have analyzed the catalog of somatic mutations in cancer (COSMIC), and found which proteins are frequently mutated. Interestingly, many of these proteins fall into a few protein families, namely kinases, g-proteins, and GTPases, all of which are involved in cell proliferation and differentiation. Further analysis has shown that in each protein class, the mutations segregate into subdomains in similar ways. This mutational clustering yields insight into why these mutations are driver mutations.

Chris Bialas, Dutton Lab

"The Engineering of Non-Natural Flavoproteins"

Flavoproteins are a ubiquitous class of redox enzymes that participate in a host of biological processes such as O2 activation, DNA repair, aromatic hydroxylation, phototaxis and magnetoception. Their diverse utility stems from the flavin cofactor's ability to accept one or two electrons and/or facilitate hydride transfer over redox potentials spanning ~600mV. These properties are modulated by specific interactions between the flavin and its protein matrix. In this work, we aim to understand the biophysical basis of this control by working in a robust and highly controlled model system: tetra-helical protein maquettes. In this system we covalently couple various flavin analogues (via a cysteine linkage) to a host of different maquettes. Our results demonstrate successful coupling of two flavin analogues to four different maquette topologies with midpoint potentials from -272mV to -70mV. We have utilized these designs to construct an enzyme capable of the oxidation of nicotinamide analogues. We have also constructed a photochemical triad that shows light activated electron transfer between a tryptophan, flavin and a bis-his ligated heme. These results not only help us understand the natural oxidoreductases but also bring us closer to realizing fully synthetic flavin enzymes for novel catalysis.

Lee Speight, Petersson Lab

Acridonylalanine: Photophysics, in vivo incorporation, and derivative syntheses"

Abstract Acridonyl-2-alanine(Acd) is a ribosomally permissible fluorescent amino acid that posses a long lifetime, resilience to photobleaching, a near unity quantum yield ($\varphi = 0.95$), and visible wavelength emissions. Recently, we have shown that it can be incorporated into proteins in vivo and can be a valuable probe of protein conformational change due to its red-shifted excitation wavelength, unique solvatochromic properties, and ability to participate in energy transfer with endogenous amino acids (Trp and Tyr) as well as exogenous fluorophores such as methoxycoumarin (Mcm). While many of the photophysical properties of Acd are prime for in vivo fluorescence experiments, we believe it is possible to improve the brightness of the fluorophore by increasing the excitation extinction coefficient (ε). Several literature reports detail the enhancement of fluorescent properties of a variety of conjugated systems by installing electron-donating substituents at the opposite end of a π system from electron-withdrawing substituents. Our previous report detailed a concise, high yielding synthesis of Acd in 5 steps from Tyr and preliminary data shows that this route is amendable to the inclusion of Tyr derivatives with substituents at the 3 position. Since the cornerstone of this synthesis is the Buchwald-Hartwig cross coupling between a protected Tyr triflate and methyl anthranilate, we are further expanding our substrate scope to include derivatized methyl anthranilate compounds in conjunction with 3-substituted Tyr derivatives. Presently we are synthesizing a library of Acd derivatives with diverse ring electronics, quantifying changes in photophysical properties, and assaying for in vivo translation permissivity

Laura Castellano, Shorter Lab

"Using Molecular Tweezers to Counteract Amyloid-Mediated Enhancement of HIV Infectivity"

Despite its discovery over 30 years ago, human immunodeficiency virus (HIV) continues to threaten public health worldwide. Semen is the principal vector for the transmission of this retrovirus and several endogenous peptides in semen, including fragments of prostatic acid phosphatase (PAP248-286 and PAP85-120) and semenogelins (SEM1 and SEM2), assemble into amyloid fibrils that promote HIV infection. For example, PAP248-286 fibrils, termed SEVI (Semen Derived Enhancer of Viral Infection), potentiate HIV transmission by up to 105-fold. Fibrils enhance infectivity by capturing HIV virions and facilitating their attachment and fusion to target cells. Despite the extremely stable structure of amyloid fibrils, certain agents can disrupt and reverse amyloid formation. CLR01 is a small molecule “molecular tweezer” that binds to lysine residues with high affinity. Through interactions with lysine residues, CLR01 has been found to inhibit the fibrillization of numerous amyloidogenic proteins. Here, we find that CLR01 inhibits the assembly of PAP248-286, PAP85-120, and SEM1 into mature amyloid fibrils. Furthermore, CLR01 disaggregates pre-formed PAP248-286 and PAP85-120 fibrils, yet is unable to disassemble SEM1 fibrils. HIV infectivity analysis indicated that CLR01 completely abrogated the infectivity-enhancing properties of all three peptides, and remarkably, it also diminished the infectivity-enhancing property of semen. Interestingly, CLR01 also has widespread antiviral effects in the absence of amyloid. In the future, it will be important to determine the inflammatory potential of CLR01 in an animal model to assess its use as a potential vaginal microbicide component.

Charlie Mo, Kohli Lab

"Characterizing and Targeting LexA, a regulator of bacterial mutation and resistance"

The rise of drug resistance in bacteria is a growing problem, and poses a formidable medical and scientific challenge. Mutations – the driving force behind evolution and drug-resistance – have long been thought to arise spontaneously in bacteria, However, the acquisition of mutations is in fact a regulated process, and is in part tied to the bacterial stress response or SOS pathway. Here we employ saturation mutagenesis, and kinetics to systematically elucidate substrate preference of the LexA, the bi-functional repressor-protease of the SOS pathway. Our studies elucidate the key determinants for substrate recognition and demonstrate that overall, LexA is a relatively tolerant protease. Furthermore, this study reveals positions in the LexA protein that are amenable for labeling with fluorescent probes, which can be used for conformational studies and inhibitor screens. Our studies can lead to insights about bacterial evolution and potentially open up new avenues for combating drug resistance.

Natalie Meinhardt, Greenbaum Lab

"Development of Inhibitors of Cysteine Proteases"

The human genome expresses 11 cysteine cathepsins (B, H, L, S, C, K, O, F, V, X, and W) that belong to the papain-like thiol protease family, (clan CA, family C1). These cysteine cathepsins are lysosomal proteases involved in endosomal/lysosomal protein turnover as well as other cellular processes including bone remodeling, immunity, apoptosis, and prohormone processing (Lecaille 2007). In addition to normal cell processes, cathepsins have been implicated in promoting several disease states. Due to the involvement of cathepsins in various diseases, cathepsins have become attractive target for drug development. Previous efforts to develop specific inhibitors of cathepsins have been focused on inhibitors, both covalent-irreversible and covalent-reversible, that bind to the unprimed side of the active site. A problem with these inhibitors is lack of specificity among different cysteine cathepsins. This is mainly due to the fact that cysteine proteases in the papain superfamily have very conserved active sites. Additionally, most of the inhibitors covalently modify the cysteine residue via an electrophilic warhead group, which in turn also reduces the specificity. This warrants development of non-covalent cysteine cathepsin inhibitors with novel mode of action. In this paper we present the development of non-covalent, α-helix-based inhibitors of the cathepsin L subfamily (cathepsin L, S and K). We arere especially interested in the prodomain (also called as the propeptide) as it acts as potent reversible inhibitor of its cognate enzyme. Each pro-domain is between 36 to 250 amino acid residues long and contains three α-helices: one α-helix occupies a unique binding site within the prime side of the substrate binding site and along with a beta strand occludes substrate entry into the active site (pdb: 1CS8, 2C0Y, 1BY8). The entire prodomain including this α-helix binds to the active site cleft in the reverse direction of a canonical peptide substrate so as to eliminate the chance of proteolytic cleavage. For each enzyme, this α-helix appears to have a unique sequence that creates specific interactions with its respective enzyme active site, suggesting that these helices could be used to create specific inhibitors. We have developed non-covalent helical inhibitors for the cysteine cathepsins L, S, and K using the respective prodomain active site helix sequence and the proenzyme crystal structures as our models by determining the best location for stabilization and establishing key mutations that enhance both specificity and potency through structure-based design.

Chemical Biology Interface Summer Retreat 2013 – Poster Presentations

Number	Presenter	Title
1	**Adrienne M. Pesce**	Triptycene Core Molecules as Structure Specific Nucleic Acid Binders
2	**Alec Riccuiti**	Hsp104 Quaternary Structure and M-domain Dynamics Elucidated via HX-MS
3	**Andrew Maguire**	Structural Characterization of an Eco1 Family Acetyltransferase
4	**Atrish Bagchi**	Understanding aberrant activation of the Epidermal Growth Factor receptor (EGFR) by extracellular oncogenic mutations
5	**Brittany Riggle**	Poster
6	**Bryan Marques**	Site-Resolved Measurements of Hydration Dynamics in Hen Egg-White Lysozyme
7	**David Schultz**	The Wistar Molecular Screening Facility
8	**Joy Li**	Enzymatic characterization of 2'-O-Me UTP incorporation into transcripts with a T7-RNA polymerase variant
9	**Lyndsay Wood**	Steroid-Derived Cyclopamine Analogs: Design, Synthesis and Biological Evaluation
10	**Rebecca Wissner**	with Solongo Batjargal
11	**Robert Rarig**	Synthesis and Conformational Dynamics of the Reported Structure of Xylopyridine A
12	**Sam Levi**	Novel Bisaminoquinoline Autophagy Inhibitors for Cancer Therapy
13	**Sara Goldstein**	Design of Riminophenazine Antibiotics as Mechanistic Probes
14	**Scott Ugras**	Elucidating the relationship between alpha synuclein aggregation and phosphorylation signaling in the brain
15	**Solongo Batjargal**	Development of Semi-Synthesis Methods to Incorporate Thioamide/Fluorophore Pairs into Proteins
16	**Steve Brown**	Exploring the Conformational Dynamics of Peptides with Tetrazine Phototriggers
17	**Charlie Mo**	Characterizing and targeting LexA, a regulator of bacterial mutation and resistance
18	**Chris Bialas**	The Engineering of Non-Natural Flavoproteins
19	**Anna Arnaudo**	Development of a Click-Chemistry Based Method to Quantitatively Study Propagation of Histone Post-Translational Modifications on Newly Synthesized Nucleosomes
20	**Anne Wagner**	N-TerminalProtein Modification by Aminoacyl Transferase: Enzylmology and Applications
21	**Chris Nabel**	Nucleic acid determinants for selective deamination of DNA over RNA by activation-induced deaminase
22	**Julianne Griepenburg**	Regulating gene expression with light-activated oligonucleotides
23	**Huixi Violet Zhang**	Molecular Structure and Flexibility of Designed Beta Fibrils as Studied with Molecular Simulations

Chemical Biology Interface Summer Retreat 2013 – Attendees and Lunch Selections

Name	Boxed Lunch Selection
Adrienne M. Pesce	chicken caesar salad
Alec Riciutti	turkey and cheese wrap
Allison L. Haigney	turkey and cheese wrap
Anatoly Kiyatkin	turkey and cheese wrap
Anatoly Zaytsev	ham and swiss cheese
Andrew Maguire	turkey and cheese wrap
Anna Arnaudo	ham and swiss cheese
Anne Wagner	turkey and cheese wrap
Atrish Bagchi	chicken salad with grapes and almonds
Benjamin Roose	turkey and cheese wrap
Brent Powell	chicken salad with grapes and almonds
Brittany Riggle	vegetables with hummus on greens
Bryan Marques	ham and swiss cheese
Chanat Aonbangkhen	chicken caesar salad
Charlie Mo	chicken salad with grapes and almonds
Cheryl McCullough	turkey and cheese wrap
Chris Bialas	ham and swiss cheese
Chris Nabel	roasted vegetables with feta
Claire Gober	chicken salad with grapes and almonds
Cory Rice	chicken salad with grapes and almonds
David Chenoweth	chicken caesar salad
David Schultz	chicken caesar salad
E. James Petersson	ham and swiss cheese
Eli Pollock	chicken salad with grapes and almonds
Emily Schutsky	chicken salad with grapes and almonds
Erik Nordgren	chicken salad with grapes and almonds
Greg Van Duyne	chicken caesar salad
Hannah Bucklin	vegetables with hummus on greens
Huixi Zhang	roasted vegetables with feta
Ivan Dmochowski	chicken salad with grapes and almonds
Jacob Nagy	chicken caesar salad
James Shorter	
Jarrett Remsberg	chicken salad with grapes and almonds
Jasmine Hwang	chicken caesar salad
Jasna Maksimoska	chicken salad with grapes and almonds
Jeff Winkler	chicken caesar salad
Joe Jordan	roasted vegetables with feta
Joel Courter	roasted vegetables with feta
John Philbin	turkey and cheese wrap
John Philbin	chicken salad with grapes and almonds
Jose Villegas	chicken salad with grapes and almonds

Josh Wand	roasted vegetables with feta
Joy Li	chicken caesar salad
Julianne Griepenburg	chicken salad with grapes and almonds
Julie Barber-Rotenberg	roasted vegetables with feta
Katie Pulsipher	chicken caesar salad
Kiran Gajula	turkey and cheese wrap
Laura Castellano	chicken caesar salad
Lee Speight	chicken caesar salad
Lyndsay Wood	roasted vegetables with feta
Mai Tran	chicken salad with grapes and almonds
Mark Nilson	turkey and cheese wrap
Michael Grasso	chicken caesar salad
Michael Soo	chicken salad with grapes and almonds
Moses Adenaike	turkey and cheese wrap
Nataline Meinhardt	turkey and cheese wrap
Rahul Kohli	ham and swiss cheese
Rahul Kohli	chicken caesar salad
Rebecca Wissner	ham and swiss cheese
Robert Rarig	ham and swiss cheese
Ronen Marmorstein	ham and swiss cheese
Rosie Molden	chicken salad with grapes and almonds
Roy Malamakal	turkey and cheese wrap
Sam Levi	turkey and cheese wrap
Sara Goldstein	vegetables with hummus on greens
Sara Manning	vegetables with hummus on greens
Scott Ugras	turkey and cheese wrap
Sean Yeldell	chicken salad with grapes and almonds
Shaina Carroll	turkey and cheese wrap
Solongo Batjargal	chicken salad with grapes and almonds
Stephanie Barros	turkey and cheese wrap
Steve Brown	ham and swiss cheese
Sung-Eun Suh	vegetables with hummus on greens
Tarun Kapoor	chicken salad with grapes and almonds
Teresa Rapp	chicken salad with grapes and almonds
Thu Duong	chicken salad with grapes and almonds
Uday Ghanty	roasted vegetables with feta
Yanfei Wang	ham and swiss cheese
Yanfei Wang	chicken salad with grapes and almonds
Yitao Zhang	chicken salad with grapes and almonds
Young Hoon Koh	chicken salad with grapes and almonds
Yubin Bai	chicken salad with grapes and almonds
Yuxiang Wang	chicken salad with grapes and almonds
Zach Hostetler	ham and swiss cheese
Priyanka Kothari	vegetables and hummus on greens