The 8th Annual Chemical Biology Interface Summer Retreat

June 13th, 2016

Bryn Mawr College (Thomas Great Hall)

Schedule of Events:

9:30 a.m. - 10:00 a.m.	**Welcome Breakfast** (Coffee, Tea, Muffins)
10:00 a.m. – 10:30 a.m.	**Claire Gober**, Joullié Lab
	Joining Forces: Studies of the Roquefortine C Biosynthetic Pathway
10:30 a.m. - 11:00 a.m.	**Emily Schutsky,** Kohli Lab
	Defining and Exploiting the Deaminase Activity of APOBEC3A Against the Extended Epigenome
11:00 a.m. - 11:30 a.m.	**Katie Pulsipher**, Dmochowski Lab
	Ferritin Mutants for Nanoparticle Encapsulation
11:30 a.m. - 12:00 noon	**Monica Liu**, Kohli Lab
	Deciphering the TETrad of Epigenetic Cytosine Modifications
12:00 noon - 1:00 p.m.	**Lunch**
1:00 p.m. - 2:00 p.m.	**Poster Session**
2:00 p.m. - 2:30 p.m.	**Jose Caro**, Wand lab
	Role of Conformational Entropy in Extremely High Affinity Protein
2:30 p.m. - 3:00 p.m.	**Meredith Jackrel,** Shorter Lab
	Engineering a Prion Disaggregase, Hsp104, to Counter Neurodegenerative
3:00 p.m. - 4:00 p.m.	**Keynote address - Lawrence Shapiro (Columbia)**
	Patterning the Nervous System with Clustered Protocadherins
4:00 p.m. - 6:00 p.m.	**Happy Hour**, the Cloisters

Co-Sponsored by
Department of Chemistry
Department of Biochemistry and Molecular Biophysics
Wistar Institute

CBI Summer Retreat 2016 - Student Presenters

Claire Gober, Joullié Lab

"Joining forces: Studies of the Roquefortine C Biosynthetic Pathway"

Indole alkaloids make up one of the largest families of secondary metabolites, and over the years, they have established a presence in the fields of pharmaceuticals and agrochemicals. Roquefortine C, an indole alkaloid produced by a number of species of Penicillium fungi, has been a compound of interest for several years due to its unique structure and its role as a biosynthetic precursor to related metabolites glandicoline B, meleagrin, and oxaline. These three compounds, each bearing a unique triazaspirocyclic core, have demonstrated noteworthy bioactivity, establishing that these skeletal features are significant in biological activity. Nature's transformations to form the unusual triazaspirocyclic scaffolds of glandicoline B, meleagrin, and oxaline have served as inspiration for my research in the lab. In this talk, I will discuss how I have gained insight into this unique transformation using a number of techniques and experiments, including biocatalysis, reaction progress analysis, and molecular modeling.

Emily Schutsky, Kohli Lab

"Defining and Exploiting the Deaminase Activity of APOBEC3A Against the Extended Epigenome"

AID/APOBEC family cytosine deaminases canonically play crucial roles in immunity by converting cytosine to uracil in single-stranded DNA (ssDNA) in specific sequence contexts. Outside of this established physiological role, AID/APOBEC enzymes have also been implicated in the poorly-understood process of DNA demethylation through their proposed deamination of epigenetically-modified cytosine bases like 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). However, there has been no thorough biochemical characterization of AID/APOBEC activity on these substrates, or on the recently-discovered 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) to inform this proposed role. Here, we provide the first steady-state kinetic measurements of the most active family member–APOBEC3A (A3A)–against various natural and unnatural modified substrates using a novel, restriction enzyme-based deamination assay. In this analysis, we found that A3A has an enhanced activity against 5mC that distinguishes it from the other AID/APOBEC family members, which discriminate against substrates via a purely steric mechanism. We also concluded that A3A has poor activity against larger oxidized cytosines, like 5hmC, 5fC, and 5caC, and therefore likely does not contribute substantially to active DNA demethylation via deamination of these bases. Importantly, given our discovery of A3A's vast preference for unmodified C and 5mC over larger bases like 5hmC, 5fC, and 5caC, we hypothesized that A3A could be utilized in an enzymatic, nondestructive alternative to bisulfite sequencing-based methods (APOBEC-coupled Epigenetic Sequencing, or ACE-Seq) to provide single-base resolution localization of genomic 5mC or 5hmC with up to 1000-fold less input DNA. Though much optimization remains, initial validation of ACE-Seq on genomic DNA from mutant T4 phage has fueled the plausibility of this method. By localizing epigenetic bases in important yet unexplored cell populations, this research will help refine the currently poorly-understood process of active DNA demethylation and our broader paradigms of genetic regulation.

Katie Pulsipher, Dmochowski Lab

"Ferritin Mutants for Nanoparticle Encapsulation"

Thermophilic ferritin is a unique nano-sized container whose multi-subunit cage structure can be disassembled and reassembled by changing solution ionic strength. Its native function is the oxidation and storage of iron as a hydrated ferric hydroxide nanoparticle. The iron can be removed and replaced

with useful cargo, including other nanoparticles and proteins, granting the cargo enhanced biocompatibility, targeted delivery potential, and greater ease of functionalization. The encapsulation of gold nanoparticles by tF is particularly noteworthy, as tF maintains its native overall diameter, secondary structure, subunit stoichiometry, enzymatic activity, and thermal stability after encapsulation is complete. The nanoparticle gains enhanced salt stability against aggregation compared to bare particles, demonstrating the synergy of this encapsulation product. Using biophysical and materials characterization techniques, we have explored how nanoparticle size and surface ligand, as well as mutations to the protein, affect encapsulation. We are currently exploring the use of these non-native cargo–ferritin conjugates in nanoparticle templating, catalysis, and medicine.

Monica Liu, Kohli Lab

“Deciphering the TETrad of Epigenetic Cytosine Modifications”

Ten-eleven translocation (TET) enzymes generate epigenetic DNA modifications by oxidizing 5-methylcytosine (mC) step by step to 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), and 5-carboxylcytosine (caC). A prevailing question is how these oxidized mC (ox-mC) bases form and function in epigenetic regulation, but so far, the individual roles of hmC, fC, and caC have been inseparably linked by stepwise oxidation. By targeting a single, conserved active site residue in TET2, we uncovered remarkable enzyme variants that allow proficient oxidation to hmC but largely abolish fC/caC formation. Combined biochemical and computational analyses further elucidated enzyme-DNA interactions that are required for efficient oxidation. Our results suggest that TET2 evolved in favor of generating highly oxidized cytosines and, for the first time, provide the field with TET variants to explore directly the biological importance of hmC separately from fC and caC.

Jose Caro, Wand lab

“Role of Conformational Entropy in Extremely High Affinity Protein Interactions”

Interactions of extreme affinity (Kd < 10-12 M) underlie many biochemical processes necessary to life. The physical determinants of such large binding energies are not well understood. Specific interactions at the interface (ΔHbinding) and the release of solvating water molecules (TΔSsolvation) are usually assumed to dominate the binding energetics. The role of conformational entropy (TΔSconf) in determining binding affinity has remained elusive, in part due to the difficulties in measuring such changes in entropy experimentally. Recent developments in the Wand laboratory have bridged this gap by using solution NMR measurements of dynamics to empirically calibrate a “conformational entropy meter.” It has enabled quantitative measurements of the change in conformational entropy in protein-ligand binding. The toxin-antitoxin system studied here, barnase-barstar, forms a complex with fM affinity (Kd ~ 10-14 M, ΔGbinding ~ 19 kcal/mol) without undergoing any major structural changes. To explore the role of conformational entropy, the fast (ps-ns time-scale) motions of backbone and side chains of these two proteins were measured in both the free (unbound) and the complexed (bound) states using NMR spectroscopy. The dynamic response is strikingly different in each protein, leading to changes in TΔSconf of opposite sign. Interestingly, the interface of both proteins, involving several salt bridges and buried waters, shows the largest increase in dynamics upon binding. This comprehensive view of the dynamic changes in both protein and “ligand” (in this case, another protein) reveals how the corresponding change in conformational entropy enables fM affinities.

Meredith Jackrel, Shorter Lab

“Engineering a Prion Disaggregase, Hsp104, to Counter Neurodegenerative Disease”

There are no therapies that reverse the proteotoxic misfolding events that underpin fatal neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and Parkinson’s disease

(PD). Hsp104, a conserved hexameric AAA+ protein from yeast, solubilizes disordered aggregates and amyloid but has no metazoan homolog and only limited activity against human neurodegenerative disease proteins. Here, we reprogram Hsp104 to rescue TDP-43, FUS, and a-synuclein proteotoxicity by mutating single residues in the middle domain or nucleotide-binding domain 1 of Hsp104. Strikingly, we have uncovered numerous missense mutations throughout these regions that potentiate Hsp104. Hsp104 potentiation stems from loss of amino acid identity rather than introduction of specific interactions. Potentiated Hsp104 variants enhance aggregate dissolution, restore proper protein localization, suppress proteotoxicity, and in a C. elegans PD model attenuate dopaminergic neurodegeneration. Potentiating mutations reconfigure how Hsp104 subunits collaborate, desensitize Hsp104 to inhibition, obviate any requirement for Hsp70, and enhance ATPase, translocation, and unfoldase activity. Our work establishes that disease-associated aggregates and amyloid are tractable targets and that enhanced disaggregases can restore proteostasis and mitigate neurodegeneration.

CBI Summer Retreat 2016 - Poster Presentations

Number	Presenter	Title
1	Nicholas Porter	Structure-Function Relationships of Conserved Residues in Histone Deacetylase 8
2	Korrie Mack	Engineering Potentiated Hsp104 Variants with Enhanced Substrate-Specificity to Counter Neurodegeneration
3	Miklos Szantai-Kis	Broadening the Utility Scope of Thioamides
4	Jose Caro	Role of Conformational Entropy in Extremely High Affinity Protein Interactions
5	Jiabei Lin	Examination of the Potential Posttranslational Modification of Hsp104
6	Jamie DeNizio	Role of TET Processivity in Shaping the Extended Epigenome
7	Charlie Mo	Modulating Bacterial Viability and Mutation with the LexA Cleavage Rate
8	Ben Roose	The Origin of Xenon-129 Hyper-CEST Signal in TEM-1 β-Lactamase
9	Stella Chen	Applications of Thioamides in Protease Studies
10	John Ferrie	Investigating the Trimethylamine-N-Oxide (TMAO) Induced Structure of Alpha-Synuclein
11	Conor M. Haney	Fluorescence Studies of a-Synuclein Fibril Formation and Disaggregation
12	Alice Ford	Mechanisms and Modifiers of IBMPFD/ALS-Linked hnRNPA2 Toxicity
13	Zachary March	Defining the Molecular Basis of Substrate Selection by Hsp104 Homologs
14	Lin Guo	Defining Kapβ2 as a Protein Disaggregase for ALS Disease Protein: FUS