Online Proceedings

2R

Exploring Protein Function at Scales from Whole Tissues to Single Atoms

Session Moderator: Celeste Berg, Genome Sciences
JHN 111
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Characterizing Control of a Metabolic Network Model
Yoshi Goto, Senior, Bioengineering
Mentor: Herbert Sauro, Bioengineering
Mentor: Kiri Choi, Department of Bioengineering

Cells, making up every living organism on earth, are extremely complicated biological machines. Much research has been done on how cells function, including how proteins made by cells work together to create biochemical pathways necessary for growth. Recently, computational simulations of biological processes have become possible, such as in the field of systems biology, which takes a holistic view of an organism to elucidate function. Previous research has constructed an accurate "whole-cell" model of *E. Coli* metabolic function. But, understanding metabolic behavior is still a challenge, especially from external perturbations in the environment or an internal alteration in the form of an insertion of an extra protein. This research uses an *E. Coli* whole-cell model—containing carbon-dependent metabolic pathways in *E. coli*— to study how perturbations will affect the pathway. External changes in the resources available to the cell or an internal change to the cell's components are simulated. In response, specific changes to the metabolic behavior of proteins will be made. These changes are made using optimization algorithms, which minimize the output of a growth "cost" function, which maximizes the growth rate of the cell. This represents the effect of evolution over time for the cell to reach an optimal level of growth. The "Tellurium" software package, using the Python programming language and created by the Sauro lab, was used for this experiment, and a collection of scripts that can be used in Tellurium were made to generalize the reproduction of this process to any model, and to visualize the results. This research can give greater insight to how cellular metabolic processes as a whole behave, and can give scientists working with *in vivo* experiments better predictions of the consequences of perturbations, externally or internally, on a cell.

Exploring the Role of Imaginal Disc Growth Factors in *Drosophila* Wound Healing
Bernice Lin, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Celeste Berg, Genome Sciences
Mentor: Anne Sustar, Genome Sciences

Wound healing is an essential physiological process that repairs damaged tissues through a collaboration of molecular and cellular events. For a wound to heal properly, hemostasis (blood clotting), inflammatory, proliferative, and maturation phases must occur sequentially at specific times and at optimal levels. I've been using the fruit fly *Drosophila melanogaster* as a model organism to study wound healing and the potential role of a family of six growth factors called Imaginal Disc Growth Factors (IDGFs). IDGFs are related to human chitinase-like proteins, which are upregulated in cancer and other diseases associated with inflammation; their function, however, is not yet understood. We hope to gain some insight by studying their role in wound healing in the *Drosophila* wing imaginal disc, a larval tissue that will become the adult wing. Using a method called *in situ* hybridization, which reveals the patterns of RNA localization in fixed tissue, I found that while IDGFs are normally expressed in wing imaginal discs, their expression is immediately turned off upon wounding. However, using transgenic fly strains that have IDGF2 and IDGF6 proteins tagged with a green fluorescent protein and an extended culture assay that allowed me to track protein expression over time, I found that IDGFs are upregulated at the wound sites after several hours. Combining these observations, I hypothesize that IDGFs are first turned off in the hemostasis phase and later are upregulated in the inflammatory or proliferative phase, when new tissue begins to form. To determine the cell types, cellular dynamics, and precise timing of IDGF upregulation, I will use confocal live imaging and label cell types that are known to be involved in wound healing, such as hemocytes. These studies will con-

tribute to our understanding of the genes that regulate tissue healing after mechanical injury.

Selective Inhibition of PARP4

Ashley Person, Junior, Biochemistry, Biophysics, and Molecular Biology, Whitman College
Mentor: Mike Cohen

Poly-ADP-ribose polymerases (PARPs) are enzymes that catalyze the post-translational transfer of adenosine diphosphate (ADP) from NAD+ to target proteins. Of the 17 PARPs, some have been studied extensively and are known to have important cellular functions while little is currenty understood about others. A cellular imbalance of one of these PARP enzymes, PARP4, has been correlated with several disease states, including cancers, though its cellular roles are largely unknown. Inhibitors of PARP4 are useful as tools to investigate the enzyme's functions and evaluate the inhibitors' therapuetic potential. In this research, several compounds were tested as inhibitors of PARP4 using a PARP inhibition screening assay. Initial results identified a potent, somewhat selective compound, and revealed which parts of the compound were important for PARP4 inhibition. This was used to direct the synthesis and testing of potentially more selective inhibitors of PARP4. This presentation discusses the inhibition results and their significance towards learning more about PARP4 biology, which can help inform researchers of this enzyme's role in disease states and give insight into developing treatments for such diseases.

Functional Interaction of Cyclic Adenosine Monophosphate (cAMP) and Hippo Signaling in Tumor Suppression and Tissue Homeostasis

Annie Shoemaker, Junior, Microbiology
Mentor: Martin Golkowski, Pharmacology
Mentor: Shao-En Ong, Pharmacology

The evolutionarily conserved Hippo cell signaling pathway is important in tissue and organ development through its regulation of cell death, proliferation, and differentiation; as such, the Hippo pathway is often dysregulated in cancer. The phosphotransferases serine/threonine-protein kinase 3 and 4 (STK3 and STK4), are core components of the Hippo pathway. When active, these kinases negatively regulate the transcription factors yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). The activities of STK3 and 4 are regulated by phosphorylation on multiple serine, threonine and tyrosine residues. Recently, we discovered that the central cellular signaling regulator cAMP-dependent protein kinase (PKA) can phosphorylate STK3 on serine 15 (S15). Since it has been shown before that PKA can suppress the transcriptional activity of YAP/TAZ, we hypothesized that this phosphorylation event activates STK3. To investigate the functional consequences of STK3-S15 phosphorylation, I used molecular cloning to construct active and kinase-dead (KD) FLAG-tagged STK3 mutants in which S15 was replaced by alanine (S15A, non-phosphorylatable) or aspartate (S15D, phosphomimic), respectively. I transfected plasmid DNA encoding these mutants into human embryonic kidney cells (HEK293T) to investigate if overexpression of each STK3 variant differentially affects activation of the Hippo pathway. Western blot analyses of transfected cells showed that active STK3 S15A and S15D but not the kinase-dead versions were able to phosphorylate known STK3 substrates. Further, I used anti-FLAG antibodies to enrich FLAG-tagged STK3 variants and co-precipitating protein complexes for mass spectrometric (MS) analysis to examine the effect of STK3-S15 phosphorylation on protein-protein interactions. MS analysis revealed that the STK3 S15D mutant preferentially bound to STK4, suggesting pathway activation. Insights into the mechanism of Hippo pathway regulation by PKA will have important implications for cancer research since targeted therapeutics modulating the cAMP - PKA signaling axis may be directly applicable in tumors with aberrant YAP/TAZ activity.

Computational Design of Symmetric Fc-Binding Homooligomers

Vanessa Thuy Anh Nguyen, Senior, Bioen: Nanoscience & Molecular Engr
Mary Gates Scholar
Mentor: Franziska Seeger

Protein homooligomers, multibodied assemblies built from identical polypeptide chains, comprise a large fraction of known cellular proteins. Homooligomers prove to be particularly amenable for many biological applications; they hold the potential as oligomerization domains, often have enzymatic functionality as a byproduct of their oligomeric configuration, and can serve as structural scaffolds for bionanomaterials. While there exists a multitude of protein homooligomers in the Protein Data Bank, the finite number of existing homooligomers limits the potential for custom applications. Our current work involves designing novel cyclic protein homooligomers from a set of de novo designed repeat proteins that bind the Fc region of antibodies. Using the Rosetta software suite, we generated a set of de novo homooligomer models by designing the oligomeric interface to direct self-assembly into a target configuration with three to six identical chains. After a round of refinement, we expressed the designs in *Escherichia coli* and purified them by immobilized metal affinity chromatography. Their oligomerization state was validated by measuring the molecular weight in solution by size exclusion chromatography paired with multi-angled light scattering and comparing it to the predicted molecular weight of the design. Designs that exhibited the desired molecular weight were submitted to collaborators for small angle X-ray scattering data and X-ray crystallography. The exclusive use of de novo proteins in homooligomer de-

sign granted a greater control over the shape and stability by nature of the repeats, thus making one successful interface design useful for a multitude of shapes and sizes. This variability opens up a wide scope of scaffolds for using these homooligomers for near atomic scale structural characterization by cryo-EM.

Regulating Disorder with Disorder: Molecular Insights to the Ubiquitin Conjugating Enzyme, Ube2W

Donovan Y Phua, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program,
Undergraduate Research Conference Travel Awardee
Mentor: Rachel Klevit, Biochemistry
Mentor: Tobias Ritterhoff, Department of Biochemistry

Posttranslational modification with the small protein ubiquitin (Ub) is an essential process in various regulatory pathways of eukaryotes, such as protein degradation, DNA repair, and the cell cycle. Misregulation of ubiquitylation has been associated with diseases such as cancer and neurodegenerative disorders. Ubiquitylation is accomplished by an enzymatic cascade that involves a family of ˜40 ubiquitin conjugating enzymes (E2), which form thioester intermediates with Ub, called E2conjugates. In most ubiquitylation reactions, it is the E2 that covalently attaches Ub to substrate proteins. While the overwhelming majority of E2s ubiquitylate the ϵ-amino group of substrates' lysine residues, important exceptions have recently been discovered. For example, Ube2W has been identified as the only human E2 that exclusively ubiquitylates the α-amino group of substrates' unstructured N-termini. Knockout of Ube2W in mice leads to severe developmental abnormalities, suggesting its importance for early postnatal survival. Little is known about the molecular mechanism of Ube2W's unusual specificity and structural insights to the catalytically-relevant species, the Ube2Wconjugate, are lacking. Here, I report the formation and purification of the first stable Ube2Wconjugate mimic. Using a combination of biochemical techniques and nuclear magnetic resonance (NMR), I characterized atomic-level changes within the Ube2Wconjugate during substrate recognition and catalysis. This investigation will expand our mechanistic understanding of E2s and establish insights to a biochemically fascinating and biologically relevant enzyme.

Computational Design of Artificial Metallo-Enzymes with Tunable Redox Properties

Tanu Priya, Senior, Materials Science & Engineering
Mentor: David Baker, Biochemistry
Mentor: Anindya Roy, Biochemistry

Metalloproteins account for at least one-third of all known proteins and take part in a myriad of redox chemistry. In spite of the long-standing interest, designing metalloproteins from scratch with structural precision and tunable redox properties remains an elusive goal. The underlying hypothesis for this project is to design redox active, iron- sulfur [Fe-S] proteins using protein designing software called Rosetta. Iron-sulfur proteins are nature's modular unit for electron transfer and redox catalysis. We used Rosetta to design classes of iron sulfur proteins including [4Fe-4S] and [2Fe-2S] clusters. We have expressed these designed proteins in E.coli and tested their binding properties to their respective inorganic clusters using biophysical techniques such as ultraviolet–visible spectroscopy (UV-Vis), circular dichroism (CD), and electron paramagnetic resonance spectroscopy (EPR). Initial results show that designed proteins can be expressed in E.coli and bind to [4Fe-4S] clusters as designed. The Cluster incorporation was verified by UV-Vis spectroscopy, which shows absorption centered around 410nm, characteristics of a [4Fe-4S] cluster. We also verified that the holo protein is folded and thermostable using CD. We are currently focusing on crystallizing these holoproteins to verify the accuracy of the design process and investigate their redox properties. These designed proteins will help in explaining the fundamental factors controlling the redox properties of metalloproteins and lay the foundation for designing a new class of metalloenzyme relevant to metabolic engineering.