Fall 2012

JHU Department of Biophysics
Undergraduate Newsletter

New this Spring: Biophysics Poster Session

Biophysics undergraduates will have their first Poster Session this spring. Mark your calendars for Tuesday, April 30, 2013 when current majors will share their research experiences with the new majors, our Faculty and our Research Sponsors. We hope this will develop into one of our most cherished departmental traditions, where undergraduate, graduate, postdoctoral researchers and faculty together celebrate science and in particular, biophysics.

How can you be involved? Starting Fall 2012, sudents involved in their second semester of research (or more) will now be required to prepare a poster describing their research, and we will invite all students to present their posters at this spring party where we will also celebrate the successes of our graduating seniors and welcome the new majors. More details will be available in the spring.

Research Highlights

Each fall we highlight the research experiences of some of our majors. In this issue, we learn about nucleosome repositioning, membrane protein folding, pulmonary hypertension, and multi-drug resistance.

How can you get involved in research?

Check out the guide for how to get involved in research written for Biophysics undergraduates at:

http://biophysics.jhu.edu/research_in_biophysics.html

Find a couple of Hopkins labs that interest you and meet with your Jenkins advisor to obtain futher guidance on the selection of your research home.

Brian Kohrs, Biophysics '14

Multi-Drug Resistance

Bryan is conducting his research with Dr. Herschel Wade in the JHMI Department of Biophysics & Biophysical Chemistry on the topic of multi-drug resistance.

Cells have developed numerous ways to keep themselves safe from toxins. The majority of these systems target a single drug or a set of structurally similar drugs.

However, cells also have developed characteristics of multi-drug resistance (MDR) to streamline this process, a characteristic that poses problems for those designing medically relevant drugs and biosensors. Multi-Drug Resistant (MDR) systems are able to recognize multiple, structurally variant drugs and then work to rid the cell of them. These systems pop up in both prokaryotic and eukaryotic cells throughout nature so Bryan works to elucidate the basic mechanisms and concepts that govern MDR systems, knowledge that could help to improve pharmacological design.

These systems typically consist of two proteins, which work together to confer drug resistance. First, a transcription factor is able to recognize an increase in cellular drug concentration through a multi-specific binding mechanism. This binding event allosterically induces overexpression of a MDR efflux pump. Overproduction of this pump leads to the continual extrusion of drugs and creates this MDR characteristic of cells.

Bryan studies the MDR system in the bacteria *Bacillus subtilis*, specifically the transcription factor BmrR. At this point in the research he is focused on figuring out how the binding event of BmrR leads to the up-regulation of the efflux pump. He is currently performing in-vitro transcription-based assays, which replicate BmrR cellular response and allows for direct study the BmrR response as a function of drug concentration. By conducting this experiment with a wide range of structurally unrelated drugs, his lab will be able to better understand what features of ligands drive transcription activation by BmrR.

The T. C. Jenkins Department of Biophysics Undergraduate newsletter is published twice yearly. The articles are predominantly written by current Biophysics majors and alumni. Announcements about the major are included, too. The Newlsetter is coordinated by Prof. Karen Fleming, Biophysics Director of Undergraduate Studies. Contact her at Karen.Fleming@jhu.edu to contribute articles. Previous issues can be found at http://biophysics.jhu.edu/undergraduate_newsletter.html

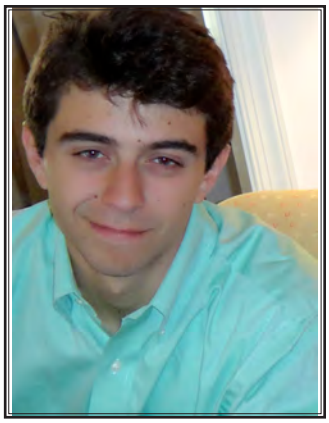
This is Bryan's first time working in a laboratory and he believes that he's learned more doing research than he ever could in a lecture, "Being able to see the things that you learn about in the classroom happen before your eyes is a very rewarding experience." He would like to thank Dr. Herschel Wade, Sharrol Bachas, and John Froehlig for their support and involvement in his project.

Bryan Kohrs is a junior from Half Moon Bay, California.

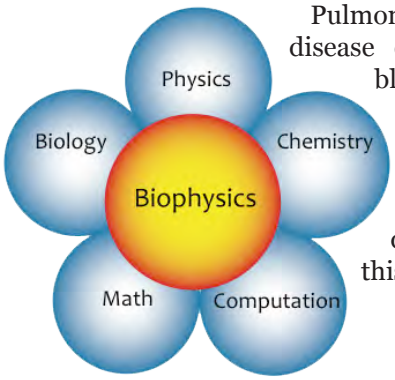
Phillip Baddoura, Biophysics '13

Biophysics of Pulmonary Hypertension

Phil says, "*When I began looking for research, I felt overwhelmed. The number of opportunities in different fields seemed endless, and I could not possibly pick one. However, I knew that I could turn to my good friends and the superb faculty in biophysics for help.*"



After receiving great advice from, Dr. Sarah Woodson, his biophysics faculty advisor, as well as from some friends in the major, Phil found Dr. Paul M. Hassoun's laboratory at the Johns Hopkins Bayview Medical Center. Dr. Hassoun is the directory of the Pulmonary Hypertension Program, and Phil found that the lab incorporated a lot of what he learned in biophysics and applied it to studying complications of pulmonary hypertension at the protein level. As a pre-med biophysics student, he felt this combination of studying protein function in relation to disease progression was an ideal marriage of his interests and long-term goals.



Pulmonary hypertension is a disease characterized by increased blood pressure in the vasculature surrounding the lungs. Having various causes such as connective-tissue disorder scleroderma, lung disease and genetic factors, this disease progresses aggressively and quickly causes death from heart failure. Understanding the molecular and genetic factors involved in the disease progression is key to developing treatments.

In the Pulmonary Hypertension (PH) lab, he quickly gained experience performing basic laboratory techniques such as running western blots, quantitative polymerase chain reactions (PCR), ELISA's (enzyme linked immunosorbent assay), gel electrophoresis and much more.

Each researcher in the lab studies a unique disease modifier, which is a protein or factor that influences disease severity and susceptibility. Once he gained enough experience he began researching his own modifier, and he currently studies collagen 18A1. It is a non-fibrillar proteoglycan found in the extracellular matrix of various organs, including the heart. This protein is cleaved to form endostatin, a potent inhibitor of tumor angiogenesis. The 3'UTR untranslated region of the collagen 18A1 encoding gene is bound by micro RNAs. These miRNAs decrease expression of target genes, such as endostatin, by suppressing translation or by reducing mRNA abundance. It is believed that increased levels of endostatin in pulmonary hypertension patients are caused by mutations in this 3'UTR region. High levels of endostatin are associated with worsened disease progression, so understanding how these mutations affect endostatin abundance is key.

By inducing a single nucleotide polymorphism (SNP) at a known site of mutation on the 3'UTR region of collagen l8A1 (using PCR), levels of endostatin can be measured with respect to a control in vitro. This is achieved by using a 3'UTR luciferase reporter gene construct, where one can analyze the activities of secreted gaussia luciferase against a control, secreted alkaline phosphatase, using a luminescence assay. *In vitro* assays can be run with the SNP collagen 18A1 construct, measuring differences in hypoxic (low oxygen, model for induced PH) and normoxic levels of gene expression (endostatin). Preliminary experiments have shown that this mutant collagen 18A1 increases levels of gene expression, and accordingly endostatin. Future experimentation in the disease model will confirm if this mutated collagen-encoding gene significantly increases protein expression and thus disease severity in pulmonary hypertension.

Phillip Baddoura is a senior from Ridgewood, NJ.

Jeffrey Granat, Biophysics '13

Energetics of Nucleosome Stability

Jeff has been doing research for the past two years in the laboratory of Dr. Gregory Bowman in the T. C. Jenkins Department of Biophysics. Dr. Bowman's lab studies a class of enzymes called chromatin remodelers, which are protein complexes that alter the structure of chromatin in eukaryotic cells. Specifically, chromatin remodelers are able to assemble, disassemble, and slide nucleosomes – the fundamental structural unit of chromatin. These proteins serve an important epigenetic role in eukaryotic cells, regulating which genes are expressed by controlling the spatial positioning of nucleosomes. Genes that are occluded by nucleosomes are less likely to bind the necessary proteins that are essential for transcription, while unobstructed genes are able to be turned "on." Improper regulation of DNA accessibility can result in aberrant gene expression, leading to diseases like cancer.

To define the principles that underlie DNA accessibility, the biophysical properties of the nucleosome must be understood. The nucleosome contains an octameric core of proteins called histones wrapped by approximately two loops of DNA. The interactions between the histone octamer and DNA are essential for stability of the nucleosome. If histone-DNA contacts are disrupted, the nucleosome can shift position, exposing new segments of DNA that can now be "read" to express the proteins they encode. Insight into these interactions is central to understanding nucleosome stability and will provide further insight into the mechanism by which chromatin remodelers alter the structure of DNA.

Jeff's goal is to better understand nucleosome stability by indentifying important histone-DNA interaction locations in the nucleosome. Using the nucleosome crystal structure as a guide, he mutated selected histone residues to determine how much they contribute to nucleosome stability. Of particular interest to his project are the arginine residues of the histone octamer located near and within the minor grooves of the surrounding DNA. Arginine has a positively charged side chain at physiological pH, and the electrostatic interaction between the arginine side chains with the negatively charged DNA phosphate-sugar backbone is thought to contribute significantly to the stability of the nucleosome.

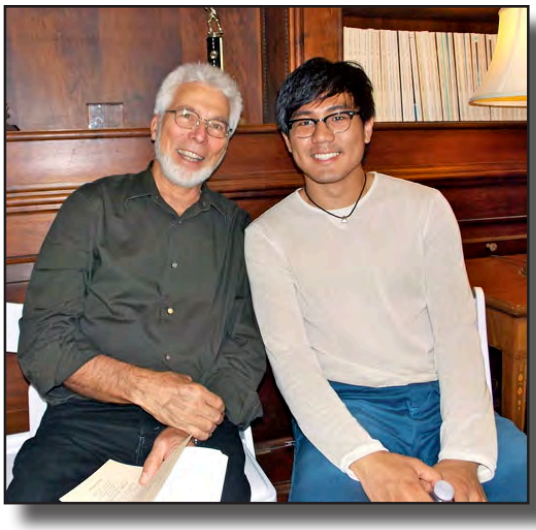
Two ways of determining the contribution of arginine residues to nucleosome stability include restriction enzyme cutting and nucleosome repositioning assays. Comparing the rate that restriction enzymes cut DNA at mutated regions in the nucleosome can help reveal changes in how tightly the DNA binds the octamer. Mutation of a residue that is significant for histone-DNA interactions will have increased accessibility and show increased cutting compared to the wild-type nucleosome. Additionally, the nucleosome repositioning assay will help reveal which histone-DNA interactions must be destabilized by the remodeler to alter chromatin structure. This experiment is used to assess the rate at which a chromatin remodeler can reposition mutant and wild-type nucleosomes, allowing Jeff to determine if the mutation introduced is an important histone-DNA contact point that is normally destabilized by the remodeler.

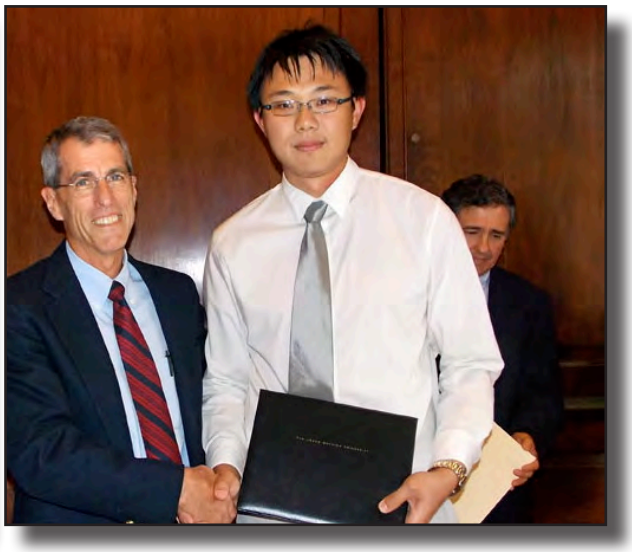
> *"Being able to see the things that you learn about in the classroom happen before your eyes is a very rewarding experience."*
>
> *Brian Kohrs,*
>
> *JHU Biophysics '14*

Jeff thanks Dr. Bowman for all of his advice and support with the project, and he also thanks Ilana Nodelman, Kyle Horvath, Ashok Patel, and other members of the Bowman lab for devoting time to helping him learn and for always lending a helpful hand.

Jeffrey Granat is a senior from Livingston, NJ.

Congratulations JHU Biophysics Class of 2012!

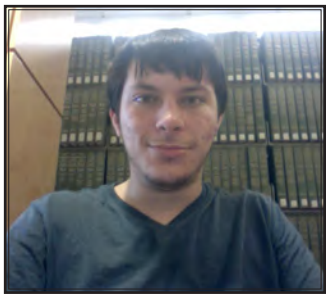




Photos by Alexias Ebert. Additional photos from the 2012 graduation reception can be found at: http://biophysics.jhu.edu/Undergrad_Reception_2012

Sam Chirtel, Biophysics '14

Membrane Protein Origami



Membrane proteins in all organisms must fold into their native conformation and insert themselves into their cells' membranes before they can function. The thermodynamic stability of membrane proteins' folded states can provide clues to their biological function, evolutionary history, and biotechnological application potential. A major driving force behind the stability of membrane proteins is the poor solubility in water compared to nonpolar solvents of many amino acids' side chains. This property is known as hydrophobicity. Since Spring 2010, Sam has been working with Dr. Karen Fleming to investigate the influence of hydrophobicity on the stability of Outer Membrane Protein W (OmpW), a β-barrel outer membrane protein in *E. coli.*

Using a different *E. coli* outer membrane protein named Outer Membrane Phospholipase A (OmpLA), Dr. Fleming developed a method to estimate the hydrophobic contribution of a single amino acid side chain to the energetic stability of a membrane protein resulting in the Moon-Fleming Hydrophobicity Scale. Sam's research goal is to test the findings of this scale using OmpW. To do this he first purified wild type OmpW and thermodynamically characterized it using Tryptophan fluorescence spectroscopy to obtain an estimate the protein's free energy of folding. He was excited to find that results were in close agreement with earlier work done by a graduate student in the lab. Next, Sam mutated the plasmid expressing OmpW to produce nineteen sequence variants, each with a different amino acid residue substituted in the same position (residue 138, naturally a Valine). Sam chose this position because it is close to the center of the phospholipid bilayer and thus fully buried in the membrane.

After expressing and purifying these variants, Sam is currently carrying out their thermodynamic analysis. If the values he obtain with OmpW closely agree with those the lab previously calculated from experiments with OmpLA, this result will provide strong evidence that the Moon-Fleming scale's values are largely independent of the sequence context of OmpLA and instead describe the inherent energetic properties of the amino acids. If they are significantly different, then those differences will hopefully provide clues about how a protein's overall structure affects the energetics of bilayer insertion. In the future Sam hopes to extend this project to study amino acid substitutions at different positions in OmpW and in other membrane proteins.

Sam Chirtel is a junior from Virginia.

Course Notes

Writing Credits

Tired of writing poetry or rehashing Nabokov?? Want to write about science instead? Did you know you can fill 6 credits of writing requirements right here in the Biophysics Department? Two of our electives that count towards the major also have a writing component: the spring Advanced Seminar in Biophysics course as well as the fall Molecular Interactions Laboratory both meeting writing requirements.

New Biophysics Courses

250.253 Protein Engineering Laboratory

Taught by Dr. Carolyn Fitch

Reviewed by Karen Woods, JHU Biophysics '15

Protein Engineering Laboratory is a great intro-level lab that teaches many techniques that are commonly used in Biophysics research such as electrophoresis, polymerase chain reaction (PCR), circular dichroism scans, and temperature melts. Lab etiquette is also strongly emphasized so that students can gain marketable "lab hands" that would be a great asset when joining your first lab. We learn the nuances and tricks to become experts at pipetting, measuring accurate masses and volumes, centrifugation, and other skills that transform beginners into a strong member of a lab.

Throughout the semester, we purified a protein with a mutation chosen by ourselves, as well as observe the changes our mutation made to the stability of the protein and see if our hypotheses about our protein's stability were correct. Dr. Fitch never misses an opportunity to help students understand the properties of proteins and why we perform each task that we do.

I was at first a little intimidated to join this class as a freshman. To be honest, I didn't know the first thing about proteins or working in a laboratory setting. However, I found that the laid back atmosphere of this class was perfect for me. Everything was explained to me by Dr. Fitch or other students, and I never felt embarrassed about being a beginner. Any questions were always addressed until Dr. Fitch was sure everyone was on the same page. This lab class was the perfect way for me to start off my lab experience in the Biophysics field.

Protein Engineering Laboratory has no pre-requisites and is offered in the spring semester. It is designed for freshmen and sophomores.

Undergraduate biophysics majors in the Spring 2012 *Protein Engineering Laboratory* class. Brittany Ashe '14, Sam Chirtel '14, Karen Woods, '15 and Josh Riback '13.

250.205 Introduction to Computing

Taught by Dr. Carolyn Fitch

Reviewed by Nirvan Sengupta, JHU Biophysics '14

Introduction to Computing is new course offered by the Biophysics department. The course is a survey of three widely used and useful programming languages: Unix shell scripting (Bash), Python and Matlab. While this course is a programming class, Dr. Fitch also briefly gives some historical perspective on computing and a gives a technical perspective on the hardware and electronics of computers to help demystify the nature of computing.

Dr. Fitch has designed the course to be challenging and maybe even frustrating at times, but the effort is well worth it. She expects students to work out their problems on their own via google search, books, online forums, etc. At the same time, Dr. Fitch's goal is to teach programming so she encourages students to make use of her office hours and inbox--she's always responded helpfully to all my questions. But because of the expectation of independence, I refined my ability to deal with programming problems on my own. I spent many long and frustrating hours behind this class but it was worth it; I think it's good preparation for later life (*e.g.* graduate school, medical school, workforce) where you've got to figure out the solutions to your problems by yourself. I left the class with a definite feeling of empowerment.

Introduction to Computing has no pre-requisites and is offered both spring and fall semesters for students of all levels and especially freshmen and sophomores.

Keep in touch with the JHU Biophysics Group at Linked-In

Join the JHU Undergraduate Biophysics group on Linked-In to connect with Biophysics majors, past and present. The group was created to facilitate networking connections between current majors and alumni and for staying in touch with Biophysics once students graduate. This group is for current students and Alumni. Be sure to register before you graduate. Check it out online at:

http://www.linkedin.com/groups?gid=1776717&trk=hb_side_g