I am fascinated by the process of evolution—that natural selection can act on mutations in a linear sequence of DNA and alter the structure and thus the function of proteins. Though this fascination has held for many years, my research experiences thus far have been diverse, and reflect my flexibility and readiness to approach this topic from different angles. Through the process of discovering and refining my interests, I have gained a deep respect for several modes of scientific inquiry—evolutionary analysis, genetics, structural biology, and biochemistry—a breadth of experience that has equipped me to ask truly interdisciplinary questions.

My first experience in a lab was in the summer of 2007, when I was an NSF REU fellow in Howard Ochman's lab at the University of Arizona. There I explored the hypothesis that linear chromosomes have evolved because they promote recombination. To this end, I worked with postdoctoral fellow Pradeep Reddy Marri, investigating recombination rates between twenty strains of *A. tumefaciens*, the only species of bacteria known to have both a linear and a circular chromosome. We employed a multilocus sequence typing approach to look for a correlation between chromosome geometry and genetic stability. Our analysis showed that there is not significantly more recombination on the linear chromosome, suggesting that increased recombination is not the reason bacteria have evolved linear chromosomes. I not only learned to amplify divergent genes and manually check sequencing data, but also tested computer programs to detect recombination within and between genes on the linear and circular chromosomes. After the summer, I was thrilled to keep up correspondence with Professor Ochman, watching my results develop into a manuscript that would be published in *Genetics* the following year.

When I returned to Amherst in the fall, I began work in Caroline Goutte's lab, using genetic approaches to examine vulval development in *C. elegans*. The projects I worked on were focused on Sao-1, a new mutation identified by the Goutte lab as a suppressor of Aph-1, a component of the Notch pathway that interacts with genes homologous to human presenilins—proteins linked to early-onset Alzheimer's disease. I used fluorescence microscopy and performed classical genetic crosses and temperature shift experiments to observe the effects of Sao-1 both alone and in conjunction with other well-studied mutations in an effort to further elucidate its function. I enjoyed working in the Goutte lab, and learned much about designing experiments and thinking analytically. However, this experience with classical genetics led me to the realization that I desire a more molecular understanding of biology, and I consequently chose to spend the summer of 2008 working in an X-ray crystallography lab.

As a SURF fellow at the Rockefeller University, I worked in Seth Darst's lab with graduate student Joe Osmundson. Over the summer, I subcloned, expressed, purified, and set up crystallization trials for the RNA polymerase α-subunit that is encoded in the plastid of *P. falciparum*, the causative agent of malaria. Over one million people die of malaria each year, the majority of them women and children in sub-Saharan Africa, and parasite resistance to current anti-malarial drugs is a growing problem. The RNA polymerase from the *P. falciparum* plastid closely resembles bacterial RNA polymerase, probably because the plastid itself is the relic of an ancient event of endosymbiosis, and is a promising drug target because there already exist clinically approved antibiotics known to bind bacterial RNA polymerase. These drugs, however, are only mildly effective at combating malaria. We wanted to determine the structure of this protein in order to explore how it has diverged from bacterial RNA polymerase, perhaps gaining insights that could aid in anti-malarial drug design. By the end of the summer, I had two crystal hits and was able to collect preliminary diffraction data on them. Unfortunately, both hits were salt crystals. Being at Rockefeller was empowering and eye-opening, allowing me to participate in research at the forefront of biology. Yet even more significantly, I realized that while

exploring the basic biological question I find fascinating—how the structures of proteins change over time—I could also focus my energies on a project that had the potential to benefit millions.

For my senior thesis, I worked with Amherst chemistry professor Anthony Bishop on developing a yeast-based assay to test inhibitors of protein tyrosine phosphatases (PTPs), signaling proteins that are involved in many essential cellular processes, including growth and differentiation, and linked to diseases such as diabetes and cancer. The assay monitors the ability of yeast cells to grow when v-Src, a tyrosine kinase known to inhibit yeast growth by over-phosphorylation, is co-expressed with a phosphatase domain in the presence of different inhibitors. If the phosphatase is active in yeast cells, it rescues the cells from v-Src-induced growth arrest, but if the phosphatase is inhibited, it is unable to rescue growth, allowing a high-throughput readout of phosphatase inhibition. The motivation for developing such an assay was to test the *in vivo* inhibition of several human PTPs the Bishop lab has specifically sensitized to inhibition by the small molecule FlAsH. The ultimate goal is to use this chemical tool to obtain otherwise inaccessible information about signaling pathways and drug target validation. Starting in the summer of 2009 and continuing through the summer of 2010, I refined the yeast assay, using western blots to demonstrate that every PTP that is expressed in yeast is able to rescue growth, and that this rescue is correlated to a global reduction in the amount of phosphotyrosine. However, the growth curves did not prove sensitive enough to respond to changes in the dosage of different known PTP inhibitors or to FlAsH. I then tried using anti-phosphotyrosine western blots to detect inhibition, but the variation between lanes in the westerns was too high to be conclusive. My work illuminated the limitations of this system, and suggested future approaches that could prove more successful at detecting the *in vivo* inhibition of phosphatases. The culmination of my research was a 114-page thesis for which I received top honors from my department. As the only person working with yeast in the Bishop lab, I learned independence—researching techniques and troubleshooting protocols on my own—but also the importance of seeking help from others, both within and outside my department.

For graduate school, I chose to apply to biophysics programs. A biophysical accounting of how protein structure and function evolve is a powerful approach for understanding how modern-day proteins came to be and is potentially critical in the field of enzyme design—helping to improve current methods of evolving existing enzymes to catalyze new chemical reactions—with the potential to impact the areas of alternative energy, environmental remediation, health, and others. I chose to come to Stanford to work with Daniel Herschlag, whose interest in protein evolution and widely recognized expertise in the field of mechanistic enzymology represent an ideal match for my research interests. In his lab, I have begun to characterize the physical adaptations that underlie the ability of proteins from the same superfamily to utilize similar catalytic mechanisms while specializing in different overall reactions. I have long wanted to directly examine the ways in which evolution acts on protein structure and function; with the expertise and resources in the Herschlag lab, I am now in the perfect position to do so.

Publications & Posters

- Marri, P. R., **L. K. Harris**, K. Houmiel, S. C. Slater, and H. Ochman (2008). **The effect of chromosome geometry on genetic diversity**. *Genetics*. 179: 511-516.
- Harris, L. K. **A Cell-Based Assay for Inhibition of Divergent Protein Tyrosine Phosphatases**. BA thesis. Amherst College, 2010.
- Rockefeller University Summer Undergraduate Research Fellowship Program (08/2008). Poster: **Determining the structure of the C-terminal domain of the plastidic RNA polymerase α-subunit in *Plasmodium falciparum***.
- NSF REU Summer Research Program at the University of Arizona (08/2007). Poster: **The Effect of Chromosome Geometry on Bacterial Genetic Diversity**.