

## Personal Statement – Will Sharpless, October 2020

Evolution magnetized me as a kid. When I was 11, I was blown away by a pre-2000, *National Geographic* animation that depicted how fish evolved into monkeys, and it became my go-to presentation topic in middle school. Despite a classmate describing my presentation as “the work of the devil”, I thought it was so cool that I presented it three times before the eighth grade. My friends of course thought this was hilarious, and it’s shocking that I am still taken aback to be called nerdy. Back then though, I would not have said I was interested in ‘science’ or becoming a ‘scientist’. Even beginning college, I was reluctant to become a scientist because of their aloof reputation. As I have aged, however, I have come to appreciate the tremendous benefit science can have on a society – made even more clear by recent public health events – and the responsibility scientists have to explain discovery despite backlash and skepticism.

When I look toward my future, I question how my work will help people and make society better, and why its worth doing. I am proud that both of my parents worked as public doctors and I aspire to match their service in my own manner. Given that perspective, I am interested in studying scientific topics of clear value to the public, with an eye towards the creation of tangible products. In addition, my enthusiasm for biology is married to a love of math, and I see bioengineering as a gateway to fulfill public service through science. As a kid, I was fascinated by evolution because it proved that life is malleable, and today, I am thrilled by the application of systems and feedback engineering to construct biological machines and augmentations of the living. We are in an age when designing life is not only possible, but laden with solutions grander than the effect of a single doctor or hospital.

I applied to join Dr. Jay Keasling’s lab my sophomore year of college because I was wowed by the synthesis of artemisinin in yeast and its global implications, and because I wanted to learn genetic engineering. In that lab, I worked with Dr. Mitchell Thompson to increase production titers of several valuable, industrial compounds in *Pseudomonas putida* (Pp). To recapitulate the vision of metabolic engineering, our goal was to design a microbial chassis that could transform waste into value more efficiently and economically than inorganic chemistry might. However, that vision is complicated by its own nature: often hosts which are best at degrading recalcitrant wastes will devour the valuable molecules they are engineered to produce. This principle governed many of the projects on which I worked.

My first project in the Keasling lab under Dr. Thompson was to precisely inhibit the degradation of valerolactam by *Pseudomonas putida* (Pp) with minimal deterioration of its faculties. Lactams are precursors of nylon produced in excess of one million tons per year, and valerolactam had been produced in *Escheria coli* (Ec) with Pp-friendly enzymes. Furthermore, Pp is a more attractive host than Ec because of its native ability to degrade aromatic compounds. Growth curves demonstrated that the organism readily grew on both lactams in minimal medium. We hypothesized that the bacterium might be hydrolyzing the molecule, often a first step in catabolism, which would open the lactam cycle and make the molecule 5-aminovalerate (SAVA), an intermediate of the lysine metabolism. First, we conducted Random Barcode-Transposon Insertion (RBTnSeq) analysis with pools of Pp mutants grown on glucose, lysine and the lactam in minimal medium to identify genes which would be necessary for growth on the lactams, but not vital. The assay demonstrated that transposon insertions in genes responsible for downstream Pp lysine catabolism, indeed yielded 1 to 2 log reduction in fitness on the lactam (and not glucose) and in general, the two conditions produced similar fitness deficiency profiles, with knock-outs downstream of SAVA in the lysine catabolism performing identically. However, the method did not yield any hydrolases or first-step enzymes, desirable for our engineering goals of minimal alteration.

Interestingly, the RB-TnSeq assay yielded several genes which were not reported in the lysine pathway but presented promise for connecting Pp lysine catabolism to central metabolism which was undefined. This tangential project was enticing because lysine metabolism can produce multiple commodity chemicals and one of the enzymes found contained a domain-of-unknown-function (DUF) highly conserved in the amino acid catabolism of plants, fungi and several bacterial phyla. We began by knocking out each of the genes individually via a double selection method in which one selects for the integration event with an antibiotic resistance and then selects for the recombination event with the suicide gene SacB. I learned how to write Python scripts to design these integration plasmids in silico and assemble the vectors with

Golden Gate and Gibson cloning. I made 20 knock-out mutants in the 18 months I worked in the Keasling lab and troubleshooting various steps of the method taught me how to plan strategically for economic uses of my time. Aided by domain homology, we elucidated the function of four novel enzymes by characterizing the lysed supernatant of the deletion mutants grown on the metabolites as sole carbons sources with liquid chromatography and mass spectrometry for which I was taught to do the analyses. To confirm the findings, we designed, assembled and transformed the overexpression plasmids and purified the proteins for in-vitro characterization of enzymatic activity and specificity on various metabolites in the pathway. We proved the DUF protein to be a novel Fe(II)-dependent decarboxylase, and I was a coauthor on the complete work published in MBio in June, 2019.

We returned to the original project in the hopes of engineering Pp for enhanced production of the lactams, particularly with our greater understanding of the catabolism pathway. We realized that the first step might involve a secreted enzyme and, thus, single transposon insertions would be ineffective for eroding fitness in a pool of mutants assayed in parallel ('cheaters' might survive by group metabolism). To circumvent this potential problem, we isolated and filtered the supernatant of wild type Pp grown on glucose or the lactams and compared results of shotgun proteomic analyses. The two subunits of 5-oxoprolinase, an enzyme implicated in *P. jessenii* catabolism of caprolactam, were amongst the most abundant of enzymes uniquely expressed on the lactams. We knocked out *oplBA* in wild-type Pp via the SacB method and confirmed that growth on valerolactam as a sole carbon source halted and that the compound was not consumed when the mutant was grown in a rich medium. We made a double knock-out of *oplBA* and the 5AVA downstream gene to show an increase of production titers by 200-fold when the mutant Pp was grown on lysine, and I was a coauthor of a publication of the findings in Metabolic Engineering Communications in December, 2019.

I highlight these works from the Keasling lab because both the subjects and processes were instructive for me, a young scientist who thought that success was direct in research. So many of my knock-outs failed. I detail the shotgun proteomic assay succinctly but we tried it for three months without success. Only after returning a year later did we find the oxoprolinase. My work with Drs. Thompson and Keasling was instrumental to my understanding that science is not only riddled with failure but also indiscernible noise. Perseverance is not suggested for scientific achievement; it is the baseline. The long hours spent in the Joint BioEnergy Institute were never a chore because I understood the value of the education in modern genetic engineering, a variety of biochemical methods, how to approach science and how to creatively reconceive an obstacle. Moreover, I take great pride in these projects because of their impacts for industrial production by shedding light on greener and cheaper chemistry. Although I enjoyed my time working with that group on these important topics, I concluded my work in the Keasling lab to pursue a bioengineering project that involved the math that fascinated me.

I applied to join Dr. Adam Arkin's research group after taking one of his classes. I was magnetized by the systems theory that he applied to bacteria and genetic 'parts' because it presented an interface for control theory that I studied out of interest. In the Arkin lab, I work with Post-Doctoral researcher Dr. Kyle Sander on designing microbial consortia for enhancing the growth of rice, funded by a NASA grant called the Center for the Utilization of Biological Engineering in Space. I particularly like this topic because it provides a platform for me to think about how to control biology in a mathematical manner: microbial consortia involve complicated networks of interactions and nonintuitive inputs, which result in cascades of impact, are required to drive the state of the microbiome toward a favorable composition. Furthermore, the project is thrilling because of its far-reaching potential; a microbiome, or a method for building one, that enhances rice growth could be beneficial to nations around the world. I am indebted to Drs. Sander and Arkin who pushed me to explore questions of my own in this space and supported my proposal of an independent project in my senior year that I strive to publish in a peer-reviewed journal as a first-author.

When modeling microbial consortia, researchers strive for accurate interaction parameters which capture relationships between constituents. I hypothesized that these relationships might not be constants but rather functions of their molecular environment. For example, it is well known that the natural production of antibiotics in *Actinomyces* is generally induced by resource scarcity. If strategies like this were abundant in a set of organisms, in a limited environment we might expect increased competition to

alter the community dynamics and the dominating species. Counterintuitively, this might be a tool: last year, researchers demonstrated a control framework for microbial communities that wielded a Model Predictive Controller to steer a minimal set of “driver species,” a subset of the community that had particular influence because of their position in the network. Changing the environment, which is likely to alter the interactions between species, might present network topologies that have more desirable sets of driver species and are, thus, easier to control. Furthermore, we might control the environment of a given microbiome, through a person’s diet or the feed to a batch reactor, to provide a dimension for changing the dominating members without having to resort to direct manipulation of species (e.g. like with prebiotics or targeted antibiotics). The simple question “do the interactions for a given community vary on an environmental gradient,” therefore, holds a lot of power.

After some debate, Dr. Arkin liked the idea and supported my investigations in this area. Toward that end, I have written three grants, and the most recent entitled “Environmental Control of Microbiomes” was funded by UC Berkeley. I settled on a seven-member community chosen from the isolate library that Dr. Sander and I had extracted from rice plants, selecting for organisms that spanned two traits: reported plant growth effect and growth rate (which often determines dynamics). I chose to separately vary pH and carbon quantity as these molecules would fluctuate in an agricultural plot naturally and might be controlled in validation experiments. The work has been an exercise in reasoning through experimental decisions without knowledge of what will work; I realize how practicality and contingency plans rightfully govern science. Furthermore, to assay all the pairwise interactions and growth rates, in triplicate and at each environmental gradient point, I decided to learn to operate the Biomek robot to pipette autonomously. I also adapted a library sequencing pipeline written in Python, used for previous Arkin lab work, to my project, and wrote a Matlab scheme for optimizing the parameters of interactions based on what I found other researchers to be doing in the field. Just as I started the major interaction assay in March, the work was interrupted by COVID-19. During the pandemic, I finished the post-processing code to interpolate the environment-interaction relationship and model how the community dynamics fluctuate in different environments, but I have not yet been allowed to return to finish the wet-lab work. Nonetheless, the endeavor bettered me as a scientist because it taught me to persevere in defending my ideas, in dodging digressions on “what-ifs,” and in teaching myself new skills.

Despite the pandemic, my scientific education has been highly fruitful: the skills I have learned – particularly genetic engineering and model-based control – have prepared me to advance the field of bioengineering. Today, genetic circuits lack the required fidelity to be housed in machines that naturally replicate and distribute themselves. Simple if-then logic suffers in cacophonous biochemical systems due to randomness, non-orthogonality and noise. In the last decade however, the field of robotics has generated robust control schemes for safely maneuvering stochastic systems as they employ their designs in the real world. The integration of these mathematical schemes into bioengineering will improve precision, thus, allowing for additional layers of orchestrated complexity. The beauty of researching control is its power to advance all forms of bioengineering, and this is why the application of the same algorithms that land a *Space X* rocket or drive a *Waymo* car will usher in the next generation of medicine, bioremediation and industrial production.

The prospect of becoming an independent scientist at a time when so many inventions in bioengineering have become possible is thrilling, and I am grateful to be in a position to contribute. I owe it to the many people who have shaped me and impressed the power of productive science upon me: my family and their unwavering support, the professors who donated their time to develop my independent projects, the mentors who spent painstaking hours guiding me through failures. Most of all, I am thankful to live in a diverse community – both locally and nationally – with people of all backgrounds and cultures because I am a more insightful and understanding human being. I owe it to those who face adversity through poverty, racism, sexism and other unjust hardships to take advantage of my position and improve our lives as a people. I hold dearly the sentiment that there is no purpose in science, academia, industry, or the self if naught for one's peers. “The community stagnates without the impulse of the individual; the impulse dies away without the sympathy of the community,” (William James).