

I was six when I learned twins do not have to be identical. I was *incredibly* relieved. My mother had to paint my younger twin brothers' fingernails to tell them apart while they were infants - one red and one blue - and six-year-old me did not want to rely on nail polish for the rest of our lives. As fraternal twins, my brothers grew into their distinct features, and I was fascinated by how different they looked. How could they be born at the same time yet look nothing alike? How closely did I relate to them as their sister but not a twin? Growing up, these questions stuck with me. Further interested in studying how genetics impacts our lives, I chose to pursue a major in Biology at Iowa State University to seek answers from a genetics standpoint. Once at Iowa State, I became interested in investigating how gender roles affect a woman's ability to perform in scientific environments, and thus also pursued a minor in Women's and Gender Studies. Diversity within science can be examined through many lenses, from how female-identifying scientists shape their work environments to the genetic mechanisms that cause humans to have unique phenotypes.

What genetic mechanism made my brothers different? Learning that humans possess practically identical genomes, with just a fraction of a percent generating an incomprehensible assortment of characteristics, pushed me to seek research opportunities studying biological diversity. Because of my previous knowledge of genetics and my excitement for conducting research, I was awarded a position in the Science Undergraduate Laboratory Internship (SULI) program through the Department of Energy. There, I worked with Dr. Larry Halverson to improve our understanding of the molecular interactions between plants and microbes, and amongst microbes, in the rhizosphere. We created a synthetic microbiome that produces amino acids or ammonia that could be detected with a novel imaging device, which would help us understand these molecular interactions. I started my summer by sonicating maize roots to isolate bacterial strains, followed by streaking them onto agar plates to grow colonies for 16S sequencing. The 16S sequencing gave us an understanding of the relative abundance of the different strains within this community. Using these sequences, I created phylogenetic trees to visualize the relative abundance and the relationships between environmental isolates. Based on this sequence analysis, I examined the ability of select bacterial isolates to secrete extracellular proteases to identify which strains to use with the ammonia and amino acid imaging device. This assay also allowed us to find the dynamic range of secretion by the bacterial strains. These data would inform later work for determining the optimal soil conditions for the imaging device. By the end of my internship, I had identified and cataloged over 30 unique bacterial strains in the maize rhizosphere and determined secretion activity for over 20 of those isolates. My work laid the foundation for the development of the synthetic microbial community and will be used during the production of the novel imager. Working in Dr. Halverson's lab challenged my biological knowledge and critical thinking skills, and I discovered that I thrived in a research environment. There were even more biological questions than I could have dreamed of, and I was ecstatic about the diversity of topics I could pursue in future research endeavors.

My rewarding experience with Dr. Halverson gave me a well-rounded set of research skills and empowered me to pursue another advanced research project for my senior year of undergraduate studies. I spent my year working with Dr. Bryan Bellaire, whose lab investigated nanoparticles (NP) as a means for vaccine delivery. Dr. Bellaire's lab sought to determine what specific polyanhydride nanoparticle formulations would be able to transfect into cells and how nanoparticle transfection influenced cell viability. In RAW264.7 cells, a murine macrophage cell line, I performed lactate dehydrogenase (LDH) assays to determine cell viability following transfection, since these cells release LDH when damaged or apoptosing. Following viability experiments, I measured the metabolic activity of surviving cells with resazurin to determine if the presence of nanoparticles inhibited the metabolic activity of these cells. The impact of nanoparticles on metabolic activity would influence how a particular NP formulation would be utilized in future technology development projects. While my overarching project was cut short by the start of the COVID-19 pandemic, I was still able to determine that some of the nanoparticle formulations we had on hand were indeed toxic to this particular cell line. If I had been able to continue this work, I would have liked to test delivery within different mammalian cell lines to determine its generalizability.

Through both my undergraduate experiences, I built a strong research foundation and I was confident that upon graduation I would succeed in a full-time research position. I developed molecular

skills in the Halverson lab and became proficient in cell culture in the Bellaire lab; next, I wanted to find an opportunity that would allow me to use those skills to explore the consequences of genetic diversity in human genes. This led me to Dr. Doug Fowler's lab in the Department of Genome Sciences at the University of Washington, where I was hired as a staff Research Scientist. In this lab, we focus on the effects of missense variants on protein function through Deep Mutational Scanning (DMS), a method that leverages high-throughput DNA sequencing to experimentally evaluate the functional consequences of tens of thousands of variants of a protein simultaneously. My current research efforts are geared towards understanding how missense variation in coagulation Factor IX (FIX) leads to Hemophilia B (HB) in patients. Hemophilia B is a recessive X-linked disorder that results in an inability to form blood clots, leading to excessive, and sometimes spontaneous, bleeding in patients. However, despite the strong genetic association of FIX with HB, the majority of missense variants in FIX are considered "variants of uncertain significance" (VUS). A VUS designation means that, although the variant may be associated with the disease in question, there is insufficient genetic, molecular, and population-based evidence to report that variant back to a patient in the clinic. In fact, variants in FIX are often classified as VUS because they lack evidence that the variant directly impacts FIX function.

To generate the direct functional evidence required to reclassify FIX VUS, I am working with a senior graduate student in the lab to perform DMS on FIX. Early in the project, we realized that FIX would not work with traditional DMS approaches since FIX is a secreted protein, making us unable to determine a phenotype for each given variant. This biological mechanism directed us to re-establish the genotype-phenotype linkage required for DMS. We developed a cell surface display system to tether the FIX protein to the cell surface, where it can be stained with different FIX-specific antibodies and sorted by antibody fluorescence intensity using Fluorescence Activated Cell Sorting (FACS). To date, we have stained and collected replicate data for 8,525 out of 8,740 (97.5%) possible missense variants in FIX using five different antibodies. These antibodies are directed against a C-terminal strep II tag, FIX itself, and a necessary post-translational modification of FIX. We have used these data to identify biochemical sequence motifs in the secretion and propeptide domains that are used by binding partners during intracellular processing. We plan to reinterpret FIX VUS to determine pathogenicity by using these data in conjunction with publicly available clinical sequencing data on HB patients.

While collecting data for this scan, I wondered about the generalizability of the assay and if our display system would need to be tailored for every secreted protein. Following this curiosity, I cloned constructs for five other secreted proteins that are associated with disease in humans, expressed these proteins in our cell surface display system, and stained the cells with the C-terminal tag antibody. I observed increased fluorescence for these five secreted proteins compared to our controls, proving that our secreted protein surface display system is generalizable. This display technology will open up the possibility to perform functional assays on the ~10% of the human genome that encodes secreted proteins, all of which were previously inaccessible to DMS. These display system experiments were my first endeavor in the technology development side of research, and it has been inspiring to see how much creativity is involved with solving a technology-based problem.

Through my experience in the Fowler lab, I have learned to implement assays to directly study the breadth of coding variation for individual human genes and seen firsthand how the development of new technologies can help answer previously intractable questions in human genetics. The scientific work I've done here has impacted the direction I'd like to pursue next. Yet, the biggest discovery I've made during my time at the Fowler lab is how I am truly happiest and most fulfilled when I am in a research environment. Pursuing a Ph.D. would allow me to continue that streak while also developing my problem solving abilities and leadership skills. The Department of Genome Sciences has already been wonderful to me as a staff member, so much so that I can picture myself here as a Ph.D. student for the next several years. It's not often that someone can directly see what being a graduate student in a specific department is like, and I know for certain that I would be happy as a Genome Sciences student. I know I made a great choice when I chose to work here, and I hope the department will continue to invest in me and my professional development as a graduate student.

Based on my enthusiasm about genetic variation and recent excitement about clinical implications of data, I am particularly interested in working with Dr. Maitreya Dunham, Dr. Robert Bradley, and Dr. Andrew Stergachis. The Dunham lab's research fascinates me as someone wanting to dig deeper into impacts of genetic variation, and it would be a fantastic opportunity for me to build upon my current foundation with brand-new skills and ideas. Her lab's work on transposons is intriguing to me since transposons are mobile in the genome, which is a very different mechanism than the DMS approaches I am familiar with. I am interested in seeing how we can utilize transposons to study genetic variation. Additionally, Dr. Robert Bradley's research on how mutations affect RNA splicing factors sounds particularly interesting to me, as I have mostly focused on how mutations affect proteins thus far. Diving into RNA research would grant me the opportunity to apply what I have already learned in my current position while gaining new expertise in a related area. The exposure to computational biology would also be immensely helpful for my development since I would like to improve my computational skill set. Overall, I feel that I would be a good match for Dr. Bradley's group. Furthermore, Dr. Stergachis's lab's research is appealing to me as well. His lab's work on noncoding sequences as a basis for disease would be an interesting mirror to my current research since I have been working exclusively on genes that encode proteins. The drive to integrate genomics into the clinic aligns with my recent research interests as well. I have seen the innovative approaches developed in Genome Sciences to answer problems at the edge of our understanding of human genetics, and I know I would be happy studying in almost any lab in our department.

While research efforts are undoubtedly the main focus during any graduate program, I also aim to prioritize diversity, equity, and inclusion during my graduate career. During my undergraduate studies, I earned a Minor in Women's and Gender Studies by exploring the socially constructed paradox of being both a woman and being a scientist. We know that women and gender-nonconforming people have historically been dissuaded or excluded from the sciences, but during my studies I learned that this rift between "male" and "not male" in STEM fields is taught during childhood education. In order to combat this social construct, I would invest time into outreach efforts that show middle and high school students from underrepresented populations to teach them anyone can succeed in science and diversity in science is a necessary strength. If accepted into the Genome Sciences Ph.D. program, I would immediately start volunteering with the Genome Hackers program. I also plan to continue my membership in both Women in Genome Sciences (WiGS) and Genome Sciences Association for the Inclusion of Minority Students (GSAIMS) in order to continue the important inclusion efforts by both organizations. I would also like to get on the board for both groups if possible, as I have experience with administrative tasks, event organization, member retention, and outreach efforts from previous academic memberships during my undergraduate career. When science is more diverse and inclusive, it benefits us all, and I want to continue shaping our environment from a trainee perspective.

I have been very fortunate to have such impactful research experiences that allowed me to develop as a molecular and cellular biologist. My experiences have fostered a genuine enjoyment of mastering new skills and solving problems creatively. Enrolling in the Genome Sciences Ph.D. program would empower me to take the next steps by working towards developing independent problem-solving and leadership skills. Every scientist started their training somewhere, and it is clear to me that my scientific advancement should begin here. As I think back on my career path up to this point, I am thankful for my initial childhood curiosity and the scientific opportunities available to me as a first-generation woman in science. While my brothers are now adults and excelling in their own careers, the questions I formed while watching them grow up still inspire my drive to pursue a career in genetics research.