

## DOCTORAL DISSERTATION AND RESEARCH EXPERIENCE

**Undergraduate and Post-Baccalaureate.** Prior to entering the Medical Scientist Training Program (MSTP) at the University of Pittsburgh, I worked for four years (2010-2014) on a new therapeutic approach for GM3 Synthase Deficiency, a devastating neurological disease afflicting the Old Order Amish. This disease stems from an inability to make gangliosides, which are glycosphingolipids that mediate axon-glia interactions essential for myelination in the developing nervous system. The disease is unremittingly progressive and nearly always fatal by adolescence, and no effective treatment is available. I worked to develop ganglioside replacement therapy as a therapeutic option for these patients. During my first summer (2010), I developed an assay for blood levels of gangliosides to monitor the therapy under the mentorship of **Drs. Ryan Mehl** and **Ken Hess**. Since the lack of gangliosides causes the disease phenotype in these patients, we needed reliable quantification to track the course of the disease and to titrate dosages of gangliosides. Gangliosides are difficult analytes, however. For one, their amphiphilic, detergent-like structure causes them to aggregate into micelles in solution. Also, they neither absorb light or fluoresce, making them difficult to quantify using conventional techniques. With these considerations in mind, I developed a three-step assay. First, I expressed a recombinant *endo*-glycoceramidase from the leech *Macrobdella decora*, and used it to release the glycans from these glycolipids. Second, I developed a step to conjugate the glycans to fluorophores. Third, I developed a chromatographic method to quantify the fluorescently-labeled ganglioside sugars. This was my first independent research experience, and it introduced me to molecular biology techniques, organic synthesis, and analytical chemistry, and resulted in a poster presentation at the 2010 Franklin & Marshall (F&M) College Fall Research Fair (see **Applicant Biosketch**). Meeting the families afflicted by this disease, who are counting on translational medicine for their childrens' lives and well-being, motivated me to pursue training so I could spend my career finding solutions to unsolved problems in human health.

Having developed the assay for blood gangliosides, I switched my focus to obtaining gangliosides for therapeutic use starting in 2011. At the time of writing, Matreya LLC, the cheapest commercial supplier, sells ganglioside GM3 for \$175 per milligram. Each patient, however, will likely require seven to ten grams per year, which, at this price point, would cost \$1.2 to \$1.8 million annually. At first, I extracted ganglioside GM3 from natural sources. Mammalian brain tissue and milk contain the richest amounts, but they only contain part-per-thousand levels. Recovering these gangliosides in high amounts also presents a challenge. Gangliosides' amphiphilic behavior complicates the isolation. They associate with the other components during separations, and—as radioisotopic tracing shows—tightly adhere to every beaker, column, filter, and silica gel particle with which they come into contact. Despite these challenges, I developed a four-step process to extract pure gangliosides from powdered bovine buttermilk, resulting in a poster presentation at the 2011 F&M College Fall Research Fair (see **Applicant Biosketch**). I learned a good bit of natural product isolation and chemistry in the process. As yield topped out at 13%, and as the extraction cost \$400 per gram of GM3 and left the product laden with harmful organic solvents, however, this approach was inappropriate for clinical use. Additionally, my mass spectrometry data indicated that the bovine gangliosides I recovered had a longer fatty acid chain than their human counterparts, which could limit their pharmacologic utility. In light of these issues, I changed approaches and devised a synthetic route to prepare ganglioside GM3.

At this point in the project, it was 2012 and I was a rising senior. I had the opportunity to work in **Dr. Vladimir Muzykantov's** lab at the University of Pennsylvania for the summer, and jumped at the chance. I switched my focus from chemistry to pharmacology, and I learned about an entirely new field: targeted nanotherapeutics for vascular pathologies. I made a focused effort to develop an enzyme-linked immunosorbent assay (ELISA) to quantify vascular cell adhesion molecule (VCAM) as a marker of vascular injury in mice, and to cross-validate it using Western blot. This experience, in addition to building my skills as an experimentalist, kindled my interest in translational vascular biology. Additionally, working with Dr. Muzykantov exposed me to idea of selectively delivering therapeutics to sites of vascular injury in high local concentrations, setting the stage for the present proposal. I gave two oral presentations on my work (see **Applicant Biosketch**), and returned to F&M College to start work on the synthesis of GM3 for treating GM3 Synthase Deficiency.

After scouring the literature, I proposed a fifteen-step synthesis to prepare GM3 from commercially-available materials. I began work on it during my last semester as an undergraduate in 2013 under the mentorship of **Dr. Ken Hess**. I made a bit of progress, and fortunately, after I graduated, I was appointed a Research Fellow at the Clinic for Special Children for one year with **Dr. Kevin Strauss** as a mentor. I also was appointed a Research Assistant at the F&M College Department of Chemistry, which allowed me to make substantial progress towards making GM3. As a post-baccalaureate researcher, I completed the key step in which the two arms of the

synthesis converge using a model system. I also mentored four other undergraduates, who have since prepared 3 batches of GM3 using my route in larger and larger scales (up to 100 milligrams). This work is expected to lead to clinical trials at the Clinic for Special Children shortly. Additionally, further work has followed me to Pittsburgh; clinicians at Pittsburgh Children's Hospital are working with our team to determine how ganglioside replacement therapy will mesh with other treatments (hematopoietic stem cell or liver transplantation). Working on this project inspired me to become a clinician-scientist and gave me the skills in organic synthesis that I have already used to generate pilot data for the present proposal (in addition to my other work in graduate school). The synthesis of GM3 resulted in two oral presentations, the first of which took place at a national conference (see **Applicant Biosketch**).

**Graduate.** When I matriculated to the MSTP, I did my first research rotation in 2014 in **Dr. Patrick Pagano's** lab. I dissected cell signaling downstream of NADPH oxidase (Nox) using isoform-specific Nox inhibitors. This experience further improved my skills in pharmacology, familiarized me with biochemical assays for reactive oxygen species (ROS), and exposed me to literature that painted a picture of Noxes as double-edged swords. On the one hand, Nox activity is required for homeostasis and certain isoforms may promote healing from vascular injury, while on the other hand, Nox1 and Nox2 activity in particular can produce harmful levels of ROS that account for large components of various vascular injuries. This rotation resulted in one poster (see **Applicant Biosketch**) and introduced me to cell culture.

Next, I returned to the field of chemistry as I began my next research rotation in 2015 with **Dr. Alexander Deiters**. During this rotation, I worked on a novel method of knocking down gene expression in zebrafish with light. This entailed preparing a photocleavable linker that could form bonds with the two ends of an antisense oligonucleotide. The resulting circularized oligo had backbone geometry that was too curved and distorted to bind the target efficiently. Exposing the oligo to light with the proper wavelength, however, cleaved the linker and linearized the oligo, activating its function. My synthetic work resulted in a poster (see **Applicant Biosketch**) and laid the foundation for my colleagues to demonstrate temporally-controlled gene knockdown in zebrafish embryos after I had returned to medical school.

When I started work in my thesis lab with **Dr. Alexander Deiters** in 2016, I immediately started work on more projects to develop new chemical tools for spatial and temporal control of biological processes. My work centered on the Staudinger reduction, an exquisitely bioorthogonal (yet characteristically sluggish) reaction that we were developing to control protein and nucleic acid function with a high degree of spatial and temporal control. My work on the protein aspect of this project has led to a manuscript in preparation (see **Applicant Biosketch**) on the conditional control of protein SUMOylation, an important posttranslational modification that has come under active investigation. This work has honed my skills in molecular biology, cell culture, and organic synthesis even further, and has made me a more independent scientist. The most important result, however, was an intellectual connection I made throughout the various threads of my research experience.

At this point, I have developed a motivation for using translational research to investigate novel therapeutic approaches for unsolved problems based on my experience developing ganglioside replacement therapy for GM3 Synthase Deficiency. My work with Dr. Muzykantov at UPenn aroused an interest in vascular biology in me, and made me think for the first time about carefully-targeted therapeutics that honed in on sites of injury. After working with Dr. Pagano, I understood that the Nox field could benefit immensely from spatially-defined, isoform-specific inhibitors, both for basic research and for developing therapeutics for the many conditions in which Noxes are implicated (e.g., neurodegenerative disease, diabetic kidney injury, and ischemia-reperfusion injury). At the same time, I realized that the expertise in spatial control of biologically-active molecules I was developing by working in the Deiters lab could produce such an inhibitor. I then realized that our lab's recent advancements in H<sub>2</sub>O<sub>2</sub>-responsive chemistry was the ideal tool for the job. Connecting these observations with the unique skill set I have begun to develop in my position at the intersection of chemistry and vascular biology led to the present proposal.

I believe that I am ideally suited to carry out the proposed research based on my past experience and current position, although I will still learn many techniques in doing so (e.g., advanced cell culture models, animal surgery, and hemodynamics). The project has significant scientific and training potential, and—if supported by this fellowship—it will help me attain my goal of becoming a translational physician-scientist working on new treatment approaches for unaddressed problems in cardiology.