APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

A. 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-glial 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 ampiphilic, 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_2O_2-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.

B. GOALS FOR FELLOWSHIP TRAINING AND CAREER

My overall career goal is to carry out translational research at the intersection of chemistry and vascular biology as an independent investigator, care for patients with a clinical practice in cardiology, and teach at an academic medical center. Upon completing my MD/PhD training, I plan to pursue a residency in internal medicine followed by a fellowship in cardiology, and I will seek out a residency program that offers substantial protected time for research. Residency and fellowship will prepare me for a faculty position at an academic medical center, where I will use the majority of my time to run a research lab, and will also see patients and teach. I hope to align my research and clinical work to carry out experiments based on my clinical experience and to use my research as the basis for novel diagnostics and therapeutic interventions in the clinic. Additionally, I hope to help bridge the gap between clinicians and scientists and help form new collaborations that could not take place without my training. I have tailored the training plan in this fellowship proposal to give me the skills and knowledge I will need to meet these career goals, taking advantage of the exceptional training environment of the Deiters lab and the University of Pittsburgh-Carnegie Mellon University Medical Scientist Training Program (MSTP).

The proposed experiments I will carry out in this proposal will give me a unique opportunity to learn techniques from chemical biology and vascular biology that I will rely on as the foundation for my career. First, I will develop my skills in organic synthesis. Few investigators in translational cardiology are able to use organic synthesis to prepare new molecules to be used in their research. I believe that this skill will be incredibly useful for my future work. Dr. Deiters is an extremely respected synthetic chemist with well over 100 publications in top journals in the field, and the larger environment of Pitt's Department of Chemistry is well-established and well-equipped as a leader in organic, biological, and medicinal chemistry, making this a superb training environment. Second, I will develop my skills in probing redox biology with innovative cell culture models by working with Dr. Weber's group. Dr. Weber has developed several unique assays to understand redox biology in living tissue. I am excited to learn to use his recently-developed superfusion system for modeling ischemia/reperfusion (I/R) injury in stroke that is capable of producing nearly instantaneous changes in tissue oxygen tension. I will apply this system for the first time to myocardial I/R, and I believe that working on this novel model will be an invaluable experience for my training. I am also looking forward to expanding my knowledge of confocal microscopy that I have built up from my coursework in imaging, which will build on my prior experience with epifluorescence microscopy. Third, I will learn how to investigate my therapeutic approach as a treatment for myocardial I/R under Dr. Wang's group's expert guidance. Specifically, I will learn to perform coronary ligation surgery on rats, and how to phenotype the animals afterwards using histological staining, echocardiography, and PV catherization. Concomitantly, I will complete a Longitudinal Clinical Clerkship (LCC) with Dr. Frederick Crock, an accomplished cardiologist and expert in echocardiography, who will teach me about the clinical ramifications of my results and will provide advice on the experiments.

The experiments, coursework, seminars, and conferences outlined in this proposal will also cultivate my intellectual development as an investigator. By working closely with Dr. Deiters, the other members of our group, and the team of consultants and collaborators I have assembled, I will gain more experience in the intellectual skills I will need to carry out the scientific method: forming hypotheses, designing experiments, analyzing data with statistics, and critically interpreting results in the context of the scientific literature, among other skills. I will bolster these skills by presenting my results at group meetings, VMI Research in Progress meetings, biological chemistry division seminars, and semiannual thesis committee meetings. Apart from my own research, this proposal comprises many activities that will enhance my knowledge of principles of chemical and vascular biology, of the latest findings in the field, and of how they apply to clinical practice (see **Sponsor and Co-Sponsor Information**). These critical skills and concepts will give me the foundation I need for my future career as a physician-scientist.

For my professional development, I will present my work at multiple conferences with diverse audiences, including the American Chemical Society National Meeting and the NOX Family (NADPH Oxidases) Gordon Reseearch Conference. These conferences will help me form critical connections with clinicians and scientists at other institutions and will give me experience presenting my work to audiences with different areas of expertise. Overall, I believe that my proposed training plan and research project are ideally suited to help me reach my goal of becoming a translational researcher in cardiology with an active, complementary clinical practice.

C. ACTIVITIES PLANNED UNDER THIS AWARD

The following table shows percent effort to be devoted to the various activities planned under this award:

Year	Research	Coursework/Professional Development	Clinical	Other
Pre-Funding	80%	Courses and Milestones (19%)	0%	1%
1	80%	Professional Development (14%)	5%	1%
2	85%	Professional Development (9%)	5%	1%
3	90%	Professional Development (4%)	0%	1%
4	85%	Professional Development and Milestones (10%)	4%	1%

Research: I will allocate the majority of my time to research during the fellowship. In the lab, I will continue mastering organic synthesis, *in vitro* testing of molecular function, and cell culture. I will also gain experience working with a co-culture hypoxia-reoxygenation model under the expert guidance of Dr. Weber's group. These cell culture experiments give me an opportunity to build on my knowledge about confocal microscopy from my coursework and learn the techniquie in practice. Finally, the proposed animal experiments will give me the opportunity to learn several new techniques, including animal surgery (specifically, coronary artery ligation), histology, echocardiography, and hemodynamics (PV loops) with the expert guidance of Dr. Wang's group and the Vascular Medicine Institute Small Animal Hemodynamic Core.

I will collect and analyze my own data, and I will write up all manuscripts on my own and revise them with Dr. Deiters's guidance. I will continue my standing weekly meeting with Dr. Deiters, and will have a conference call with Drs. Deiters, Weber, and Wang every two months to discuss research progress. After completing my comprehensive exam in Summer 2018, I will start meeting with my thesis committee semiannually. I will also continue semiannual meetings with my career advisor, Dr. Don DeFranco, to update him on my progress, to discuss career goals, and to get an impartial second opinion on scientific or personal matters that arise during my training. I will present my work at lab meetings, the biological chemistry division seminar series, VMI Research in Progress meetings, and the American Chemical Society National Meeting. In 2020, I will apply for a travel award to present my work at the Nox Family (NADPH Oxidases) Gordon Research Conference.

Coursework and Professional Development: I will complete all required coursework by the beginning of the fellowship. The Chemistry Graduate Student Advising Committee helped me design a customized course of study suited to my needs as a physician-scientist in training. I have already completed a personalized medicine course and an advanced molecular biology course, and I audited a synthesis course based on FDA-approved drugs. Currently, I am taking Advanced Biological Chemistry 2 and Imaging Cell Biology in Living Systems.

My professional development will continue through the MSTP, building off of the foundation I have built from the first three MSTP professional development courses and the relevant courses in the School of Medicine. I will continue to attend monthly workshops discussing ethics and career development, and will continue to plan one such workshop every year. Additionally, I will take the MSTP Ethics for Medical Scientists course in April-May 2017. I will attend professional development workshops through the Dietrich School of Arts and Sciences as well (including workshops on grant writing, presentations, and an upcoming Computational Biophysics workshop at the Pittsburgh Supercomputing Center on the rational design of drugs and protein engineering).

Clinical: I will complete two Longitudinal Clinical Clerkships (LCCs), each 20 weeks long and consisting of a half-day each week of seeing patients one-on-one with an attending physician. I have already planned one with Dr. Frederick Crock, a consultant on this proposal who is a seasoned cardiologist with particular expertise in echocardiography, which will take place in Fall 2017. I will complete a second LCC in Fall 2018. As I prepare to return to medical school, I will take the MSTP Clinical Reentry course, which is a case-based review of skills in physical diagnosis, medical decision-making, and treatment planning led by master clinicians.

Other: I will devote time to community outreach and committee involvement through the medical school, chemistry program, and MSTP. My lab participates regularly in community outreach to teach underprivileged youth about science, particularly through DNAZone, and I have made arrangements to run several sessions at the Center of Life Camp this summer. Additionally, I regularly volunteer to help provide healthcare to uninsured Americans through the Birmingham Clinic and the Guerilla Eye Service via the School of Medicine. I currently serve as my class's representative for building improvement in the medical school. Lastly, In the MSTP, I currently serve on the Interviewing Committee and co-chair the Annual Scientific Retreat Committee.