OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED | **Application Identifier**

5. APPLICANT INFORMATION **Organizational DUNS*:** 0045143600000

Legal Name*: UNIVERSITY OF PITTSBURGH AT PITTSBURGH
Department:
Division:
Street1*: UNIVERSITY OF PITTSBURGH
Street2: OFFICE OF RESEARCH
City*: PITTSBURGH
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 152132303

Person to be contacted on matters involving this application
Prefix: Dr. First Name*: Jennifer Middle Name: E Last Name*: Woodward Suffix: Ph.D
Position/Title: Associate Vice Provost for Research Operation
Street1*: 123 University Place
Street2:
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 15213-2303
Phone Number*: 412-624-7400 Fax Number: Email: offres@offres.pitt.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1250965591A6

7. TYPE OF APPLICANT* X: Other (specify)

Other (Specify): private, non-profit, state-related educ inst
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction

12. PROPOSED PROJECT

Start Date*	Ending Date*
09/01/2017	08/31/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
PA-014

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Joshua Middle Name: Last Name*: Wesalo Suffix:
Position/Title: MSTP Trainee
Organization Name*: UNIVERSITY OF PITTSBURGH AT PITTSBURGH
Department:
Division:
Street1*: UNIVERSITY OF PITTSBURGH
Street2: OFFICE OF RESEARCH
City*: PITTSBURGH
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 152130000
Phone Number*: (443) 798-0037 Fax Number: Email*: JSW51@pitt.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$216,968.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$216,968.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Jennifer Middle Name: E Last Name*: Woodward Suffix: Ph.D
Position/Title*: Associate Vice Provost for Research Operation
Organization Name*: University of Pittsburgh
Department: Office of Research
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Street1*: 123 University Place
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Country*: USA: UNITED STATES
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Phone Number*: 412-624-7400 Fax Number: Email*: offres@offres.pitt.edu

Signature of Authorized Representative*
Stacey Barron

Date Signed*
04/10/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	UNIVERSITY OF PITTSBURGH AT PITTSBURGH
Duns Number:	0045143600000
Street1*:	UNIVERSITY OF PITTSBURGH
Street2:	OFFICE OF RESEARCH
City*:	PITTSBURGH
County:	
State*:	PA: Pennsylvania
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	152132303
Project/Performance Site Congressional District*:	PA-014

Additional Location(s) File Name:

Funding Opportunity Number: PA-16-305. Received Date: 2017-04-10T14:49:40.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00118

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary_Wesalo.pdf
8. Project Narrative*	Project_Narrative_Wesalo.pdf
9. Bibliography & References Cited	References.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources.pdf
11.Equipment	Equipment.pdf

Funding Opportunity Number: PA-16-305. Received Date: 2017-04-10T14:49:40.000-04:00

PROJECT SUMMARY

Despite substantial progress in treatments over the past few decades, myocardial infarctions (MIs) kill 610,000 Americans every year, making MIs the number one cause of mortality and morbidity in the US. Currently, the best available treatment is prompt removal of the clot, which restores blood flow to the area. Although the heart muscle requires blood flow to survive, reperfusion with blood paradoxically induces an injury that accounts for roughly half of the final size of the infarct. This reperfusion injury stems largely from the activity of NADPH oxidase 2 (Nox2), an enzyme which functions exclusively to produce reactive oxygen species (ROS). These ROS damage biomolecules and induce cell death in the heart. In addition to contributing to the acute injury, Nox2 also drives tissue remodeling that leads to arrhythmias and heart failure in the weeks following MIs. Consequently, Nox2 inhibitors are an attractive target for treating MIs. Nox2, however, is widely expressed and maintains redox balance important for cell signaling in uninjured tissues. Current anti-ROS approaches to treating reperfusion injury have seen little success in the clinic. Existing approaches react with ROS too slowly and do not target ROS with any specificity for injured areas, resulting in off-target perturbations in cell signaling. This proposal comprises a novel approach to treating myocardial reperfusion injury using a boronate-based mechanism to trigger a Nox2 inhibitor with unprecedented specificity for tissues accruing pathologic levels of ROS. The proposed treatment will reduce ROS via two mechanisms during activation, then will inhibit Nox2. First, a series of boronate-caged Nox2 inhibitors will be synthesized (Sub-Aim 1A), and then will be optimized for rapid activation (Sub-Aim 1B). Second, a novel cell culture model that simulates the rapid changes in oxygen tension that occur in myocardial ischemia/reperfusion injury will be set up. Using this model, inhibitor activation, cellular ROS production, and cell survival will be quantified (Sub-Aim 1C). Third, Nox2 activity, infarct size, and cell death will be measured in mice subjected to reperfusion injury and treated with the proposed inhibitors (Sub-Aim 2A). Fourth, the specificity of the approach will be assessed by quantifying off-target perturbations to cell signaling in these animals (Sub-Aim 2B). Finally, cardiac function one month after injury will be assessed in treated mice (Sub-Aim 2C). These experiments will test an innovative strategy for addressing the unmet need for targeted treatments of reperfusion injury in MI. Further, the approach lays a foundation for treating other forms of reperfusion injury, such as ischemic stroke, peripheral artery disease, and transplant surgery.

PROJECT NARRATIVE

In myocardial infarctions—the leading cause of death in the US—treatment by promptly restoring blood flow to the heart induces a paradoxical reperfusion injury with no effective treatments to date. Existing approaches are not targeted to the site of injury and do not shut off the source of damaging reactive oxygen species (ROS). The current proposal expands the scope of innovative ROS-sensing chemistry to develop a targeted approach for treating reperfusion injury.

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FACILITIES AND OTHER RESOURCES

The environment at the University of Pittsburgh and specifically the Deiters, Wang, and Weber laboratories provide an ideal environment for the proposed research on developing a new treatment approach for myocardial ischemia/reperfusion injury using hydrogen peroxide (H_2O_2)-responsive Nox2 inhibitors.

All equipment required for the synthesis and characterization of the H_2O_2-responsive Nox2 inhibitors is available in the Deiters lab and the Department of Chemistry at Pitt: several shared and staff-supervised facilities exist in the department and provide services at no cost, including a Mass Spectrometry Facility, an NMR Facility, and a Biological Core Instrumentation Facility. The University's Center for Biological Imaging and Cytometry Facility augment these services.

The department provides administrative support for facilities, IT, purchasing, payroll, grant administration, and clerical tasks. The chemistry department is home to three independent shops (electronics, machine, and glass) and design, fabricate, and repair equipment and instrumentation. Eight full-time technical personnel (an electrical engineer, three electronics technicians, three machinists, and a glassblower) staff the shops. The department's stockroom supplies commonly-used chemicals, gasses, glassware, office supplies, and general lab consumables.

Deiters Laboratory. Dr. Deiters has a combined total of 3,500 sq. ft. of lab space in the Chevron Science Center in the Department of Chemistry at Pitt, including two laboratories specifically designated for biochemical work with radioisotopes and a tissue culture laboratory (BSL2). The BSL2 lab is equipped with two Type A2 (Class II) biological safety cabinets. The synthetic laboratories are equipped with eight 2-person fume hoods, including individual nitrogen/vacuum lines, and the instrumentation necessary for all chemistry (solution, solid-phase, and microwave) experiments. Josh has a 2-person fume hood to himself, in addition to a 6-foot bench on both the chemistry and biology sides of the laboratory.

Wang Laboratory. Dr. Wang has over 2,000 sq. ft. of lab space in the Department of Bioengineering at Pitt, with dedicated labs for chemistry, materials characterization, animal procedures, microscopy, and cell culture. The cell culture lab is equipped with two 5' and two 6' BSL2 laminar flow hoods.

Weber Laboratory. The Weber lab consists of 3,150 sq. ft. of modern lab space, including a darkroom in which the group has constructed a microfluidic system dedicated to the quantification of thiols to study redox biology. The superfusion system for the near-instantaneous manipulation of oxygen levels in cultured tissue is set up in Langley Hall, which is 2 blocks away from Chevron. The apparatus is set up with the tissue chamber on the stage of a Leica TCS SP5 II Broadband Confocal microscope.

Clinical. Not applicable.

Animal. Dr. Wang and Dr. Weber have access to shared animal facilities through the Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh. Rats will be maintained at this facility, which has been fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) since 1971 (Unit Number 000496). The University of Pittsburgh is registered with the USDA (23-R-0016) and OLAW, NIH (#A3187-01). All animal facilities are under the direction of full-time veterinarians. The University of Pittsburgh complies with all provisions of the Animal Welfare Act in addition to all other applicable federal, state, and local laws. Rats are housed in pairs and monitored daily by a member of the veterinary staff and/or animal care staff and by the PI. Veterinary services are available twenty-four hours a day, seven days a week for animal care issues. The facility has procedure rooms available for all treatments in this proposal.

Computer. Dr. Deiters' laboratories and offices are equipped with 6 networked computers, 3 printers, and 2 scanners. The computers include software for graphics, word, and data processing, chemical drawings, gel imaging, DNA analysis, and statistical analysis. Through Pitt, LAN access is provided to the electronic versions of all major scientific journals and important databases (including Reaxys and SciFinder). All computer equipment is networked and supported by a trained systems administrator. All data is backed up locally on a RAID array and remotely via LabArchives. Drs. Wang and Weber also have several computers with all necessary software to support the proposed research, in addition to scanners, printers, and digital cameras.

Office. Dr. Deiters' office (~150 sq. ft.) is about 25 ft. from the laboratories and is equipped with a networked computer and a printer. Josh has his own desk among the 18 desks in the lab that are available for technicians, post-docs, graduate, and undergraduate students. Dr. Deiters shares an administrative assistant with four other faculty members. Dr. Weber's office is located 7 floors below in the same building, and Dr. Wang's is 2 blocks away in Benedum Hall.

Other. The Deiters lab routinely uses instrumentation in shared facilities within the department (Biological Instrumentation Cluster, Mass Spectrometry Facility, NMR Facility) and the Pitt campus (Biomedical Mass Spectrometry Center at the University of Pittsburgh: http://www.bioms.pitt.edu; Genomics and Proteomics Core Laboratories (GPCL) at the University of Pittsburgh: www.genetics.pitt.edu; the University of Pittsburgh Cancer Institute Cytometry Facility: https://upci.upmc.edu/cytometry/). See Major Equipment section for more details.

Proposed imaging experiments may be completed through the Center for Biologic Imaging (CBI: http://www.cbi.pitt.edu), which is housed in the University of Pittsburgh Medical School South BST with approximately 5,500 sq. ft. of space. The CBI is a leading center for applying cellular imaging methods with a particular specialization in live cell fluorescence applications. Dr. Wang maintains an active collaboration with Donna Stolz, PhD, the associate director of the CBI. This dedicated, state-of-the-science imaging center has fully-equipped microscopy suites, computer labs, and wet and dry bench space for light and electron microscopy preparations. Apart from the office space for the director and faculty, desk areas are provided for the 12 full-time research specialists, post-doctoral fellows, and students who work in the facility. Importantly, there is sufficient undedicated bench space within the facility for users to conduct several concurrent projects. Most of the light microscopy suites can be reserved online and are available to trained users twenty-four hours a day, seven days a week.

The shared animal facility has common procedure rooms with three surgical areas equipped with thermoregulated surgical tables, isoflurane vaporizers, dissection microscopes, ventilators, all necessary instrumentation.

Contribution of the Scientific Environment to Success. The University of Pittsburgh consistently ranks among the top 10 recipients of NIH funding and has recently risen to the fifth-highest recipient. The Department of Chemistry is one of the nation's largest and most well-funded, and has awarded of 1,000 PhDs, including one to Nobel laureate Paul Lauterbur. The department is located in the Chevron Science Center (236,768 sq. ft.) and Eberly Hall (56,051 sq. ft.).

The research carried out in the Vascular Medicine Institute (VMI) will take place in the Thomas E. Starzl Biomedical Science Tower adjacent to UPMC Presbyterian, the flagship hospital of our academic medical center where Dr. Crock (Josh's cardiology mentor) practices. The VMI is amidst 4.2 million sq. ft. of research, academic, and administrative space in several connected buildings. The VMI works to determine the molecular mechanisms underlying cardiovascular disease and develops novel therapeutic interventions for diseases such as pulmonary hypertension, sickle cell disease, atherosclerosis, hypertension, and reperfusion injury. In addition to six core laboratories (nitric oxide metabolomics, reactive oxygen species measurement, animal phenotyping, human translational vascular biology, genomics, and confocal microscopy), the VMI offers numerous seminars, grand rounds, and research conferences. The Department of Chemistry and the VMI provide an exceptional research and training environment for the proposed studies.

EQUIPMENT

DEITERS LABORATORY:

Equipment for molecular biology work in the Deiters lab includes two 37 °C shakers, three 37 °C incubators, four Eppendorf microcentrifuges, one Thermo Legend X1R tabletop centrifuge, one Sorvall Lynx 6000 floor centrifuge, three Bio-Rad T100 PCR thermocyclers, one Bio-Rad T1000 CFX96 qRT PCR thermocycler, one Bio-Rad ChemiDoc MP imaging system, one Bio-Rad GenePulse Xcell electroporator, one Nanodrop spectrophotometer, and several gel boxes with power supplies. Two fully equipped Tecan M1000 plate-readers and one Tecan M200 plate-reader are available and can perform a wide range absorbance, fluorescence, and luminescence assay. Equipment for cell biological work includes two 6-foot biosafety cabinets; three Forma II CO_2 incubators; and a Zeiss AxioObserver Z1 inverted epifluorescent microscope equipped with a CCD camera, a Tokai Hit WSKM Stage Top Incubator, and an Ametek TMC CleanBench vibration isolation table. Two –80 °C and two –20 °C freezers are available for storage of organic compounds, DNA/RNA, and bacterial cells. Two liquid nitrogen dewars with temperature monitors are available for long-term storage of mammalian cells.

Equipment for chemical synthesis includes fifteen 5-foot fume hoods, six 3-foot fume hoods, synthetic chemistry glassware, rotary evaporators equipped with Thermo Scientific ThermoChill II Recirculating Chillers, vacuum pumps, balances, two drying ovens, four refrigerators/freezers, a Teledyne Isco CombiFlash Rf automated flash chromatography systems, an Agilent 1200 HPLC system, and a Shimadzu LC20 HPLC system. Three CEM Discover microwave synthesizers are available for microwave-mediated chemistry. An ABI 394 DNA/RNA synthesizer and a SpeedVac are used for oligonucleotide syntheses.

Major equipment relevant for this research is available within the Department of Chemistry at the University of Pittsburgh in the same building as the Deiters laboratory:

The department mass spectrometry facility houses six instruments, including a Bruker ultrafleXtreme MALDI-TOF mass spectrometer, a Micromass Ultima Q-TOF API, a Thermo Scientific Q-Exactive Oribtrap, and a Shimadzu LCMS system available for walk-on student use. The chemistry department NMR facility has seven Bruker spectrometers (two 300 MHz, two 400 MHz, a 500 MHz, a 600 MHz, and a 700 MHz). Each magnet is equipped with a state-of-the-art console capable of automated tuning, locking, and shimming. A 300 MHz, 400 MHz, and 500 MHz instrument comes equipped with a Bruker SampleXpress Lite autosampler for high-throughput automated data acquisition, and the 700 MHz instrument has microprobe capabilities that can enhance sensitivity for low-concentration samples. A Horiba/Jobin-YvonFluoroMax 3 Fluorescence Spectrophotometer, a VeecoNanoscope III atomic force microscope, a Brookhaven 90 Dynamic Light Scattering Spectrometer, and a Wyatt DAWN EOS Multi-Angle Light Scattering Spectrometer are open for shared use in the department's Materials Characterization Lab. Autoclaves, incubators, centrifuges, a tissue culture hood, and ancillary equipment related to bacterial and mammalian cell culture are available in the shared departmental Biological Instrument Cluster, which is managed by a PhD-level scientist (Dr. Gillespie).

Major equipment relevant to this research is available at the Biomedical Science Tower 3, only four blocks from the Department of Chemistry:

We routinely use the Biomedical Mass Spectrometry Center at the University of Pittsburgh for a wide range of proteomics needs, including protein identification analysis via tryptic digests, sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS), and reassembly the identified peptides into proteins. The BioMS facility is equipped with two ThermoFisher LTQ XL linear ion trap mass spectrometers, one ThermoFisher LTQ XL linear ion trap mass spectrometer with a multiplexed LC interface, two ThermoFisher triple stage quadrupole (Ultra and Quantiva) mass spectrometers, two ThermoFisher LTQ Orbitrap mass spectrometers (Velos and XL), ThermoFisher LTQ Velos Orbitrap with ETD, a Bruker microTOF ESI-TOF with HPLC, and several MALDI mass spectrometers (not relevant for this research). The facility is led by Dr. Yates and supported by three PhD-level scientists (Drs. Zeng, Wendell, and Mullett) and six technicians.

WEBER LABORATORY:

Equipment for the superfusion system includes a Watson-Marlow Sci-Q 400DM2 two-channel peristaltic pump, Warner Instruments SH-27B inline solution heater with TC-324B temperature controller, Living Systems Instrumentation OX miniature gas exchange oxygenator, Dwyer Instruments OMA-1 gas flowmeter, and a Unisense OX-25 oxygen sensor with a BASi LC-4C amperometric detector connected to a National

Instruments NI USB-6008 digital logger. Imaging is done with a Leica TCS SP5 II Broadband Confocal Microscope equipped with an HCX PL FLUOTAR 5x objective (N.A. = 0.15) and an HCX APO L U–V–I water immersion 63x objective (N.A. = 0.90). To prepare primary cells for culture, a dedicated Baker Edgeguard Laminar Hood with a stereomicroscope equipped with all necessary surgical instruments is available for animal surgery. Two biological safety cabinets (Hareaus Herasafe and Nuaire) are available for cell culture, along with two VWR CryoPro liquid nitrogen cell storage dewars and two dedicated HeraCell CO_2 incubators. This equipment is located in the Department's Biological Instrument Cluster, which is managed by a PhD research scientist. This equipment is available in a secured BSL2 laboratory adjacent to a space with autoclaves, a –80 ºC freezer, and additional equipment for bacterial and mammalian cell culture.

WANG LABORATORY:
Equipment for the preparation and purification of biomaterials in the Wang lab includes four Schlenk lines, a Carver press, a Biotage Isolera One automated flash purification system, three NE-1000 syringe pumps, a Labconco Freezezone 4.5 freeze dryer, a Blue M vacuum oven, a Tousimis SAMDRI-PVT-3B critical point dryer, an Eppendort 5417 C centrifuge, two ES30P-5W high voltage power supplies, and a MasterFlex 8-channel water-resistant peristaltic pump with remote digital controller. An MBraun Unilab inert atmosphere glovebox is available for work in the exclusion of oxygen and moisture. For characterization of biomaterials, equipment includes a Malvern Viscotek GPCmax with RI and RALS detector, Malvern Zetasizer Nano ZS90, Nicolet iS10 FTIR spectroscope, Robbins Model 2000 Hybridization Incubator, BioTek Synergy MX microplate reader, Keyence laser micrometer and camera, an MTS Insight Mechanical Analyzer with Bionix Envirobath, and a Nikon TiE motorized inverted microscope for DIC and fluorescence imaging with Retiga-SRV digital camera. Equipment for cell culture includes a Sanjo MCO18AICUV air-jacketed incubator, four Thermo Heracell air-jacked incubators, and a Locator 4 liquid nitrogen cell storage tank with liquid nitrogen level monitor.

Major equipment relevant for this research is available within the Center for Biologic Imaging (CBI) at the University of Pittsburgh in the South Biomedical Science Tower 2 blocks away:

We maintain an active collaboration with the CBI, which allows us to reserve time on a Leica TCS NT Confocal Microscope (inverted), a Leica TCS-SL Confocal Microscope (upright), one of three Olympus FV1000 Confocal Microscopes (inverted), an Olympus FV500 Confocal Microscope (upright), a Zeiss 510 Meta Confocal Microscope (inverted), a Perkin Elmer Spinning Disk Confocal Microscope (inverted), an Olympus DSU Spinning Disk Confocal Microscope (inverted), a Noran OZ (retrofitted to PC platform) Confocal Microscope (inverted), a Nikon Sweptfield confocal microscope (TSI inverted), one of two Optiscan Confocal Endoscope/Microscopes, an Olympus FluoView MPE Multiphoton Microscope, one of three Multimode microscopes (Automated XY, Ratioing, FRET; two of which have microinjection capabilities), one of two Multimode Widefield/TIRF microscopes (one with microinjection capabilities), an Olympus Multimode (3D, 2D fluorescence, high speed) dissecting microscope, an Olympus SXZ12 Dissecting Microscope, an Olympus SXZ-ILLD100 Dissecting Microscope, an Olympus BX51 light microscope (Brightfield, darkfield, epifluorescence DIC), one of two Olympus Provis light microscopes (Brightfield, darkfield, epifluorescence DIC), a Nikon Eclipse 800 microscope (Brightfield, darkfield, epifluorescence DIC), one of two Nikon FX Series microscopes (Brightfield, darkfield, epifluorescence DIC), a JEOL 1011CX Transmission Electron microscope with high resolution AMT digital camera, a JEOL 1210 Transmission Electron microscope with high resolution AMT digital camera, and a JEOL 9335 Field Emission Gun SEM with backscatter detector.

The following equipment is available for surgery and hemodynamic measurements via the Vascular Medicine Institute (VMI) Cardiopulmonary Phenotyping Core 2 blocks from my lab:

An Ohmeda Isotec 2 Anesthetic Vaporizer, Hugo Sacks Murine Mini Vent, two Kent Scientific Genie Plus Infusion Systems, a World Precision Instruments Operating Microscope, Geiger Thermal Cautery Units, a Scisense Advantage Derived Volume/Pressure Measurement System, two Scisense Mouse Pressure Catheters, a Doppler Signal Processor 200 (www.instruments.com), two dedicated Dell computers for data acquisition and analysis, and a Moore Instruments LDI2 laser doppler system for tissue and arterial blood flow analysis are available at the VMI.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:	First Name*: Joshua	Middle Name	Last Name*: Wesalo	Suffix:

Position/Title*:	MSTP Trainee
Organization Name*:	UNIVERSITY OF PITTSBURGH AT PITTSBURGH
Department:	
Division:	
Street1*:	UNIVERSITY OF PITTSBURGH
Street2:	OFFICE OF RESEARCH
City*:	PITTSBURGH
County:	
State*:	PA: Pennsylvania
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	152130000

Phone Number*: (443) 798-0037 Fax Number:

E-Mail*: JSW51@pitt.edu

Credential, e.g., agency login: jwesalo

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Wesalo_Biosketch_F30.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-305 . Received Date: 2017-04-10T14:49:40.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: ALEXANDER Middle Name Last Name*: DEITERS Suffix:

Position/Title*: Professor
Organization Name*: University of Pittsburgh
Department:
Division:
Street1*: 219 Parkman Ave
Street2:
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 152600000

Phone Number*: 4126245515 Fax Number:

E-Mail*: deiters@pitt.edu

Credential, e.g., agency login: alexdeiters

Project Role*: Other (Specify) Other Project Role Category: Sponsor

Degree Type: PHD Degree Year:

Attach Biographical Sketch*: File Name: Deiters_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Yadong Middle Name Last Name*: Wang Suffix:

Position/Title*: WK Whiteford Professor
Organization Name*: University of Pittsburgh
Department:
Division:
Street1*: 3700 O'Hara St.
Street2: Department of Bioengineering
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 152610000

Phone Number*: 4126247196 Fax Number:

E-Mail*: yaw20@pitt.edu

Credential, e.g., agency login: yadongwang

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PHD,MS Degree Year:

Attach Biographical Sketch*: File Name: Wang_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Patrick Middle Name J Last Name*: Pagano Suffix:

Position/Title*: Professor
Organization Name*: University of Pittsburgh
Department:
Division:
Street1*: 200 Lothrop St.
Street2:
City*: Pittsburgh
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 152610000

Phone Number*: 412-383-6505 Fax Number:

E-Mail*: pagano@pitt.edu

Credential, e.g., agency login: ppagano1

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: PHD,MS,BA Degree Year:

Attach Biographical Sketch*: File Name: Pagano_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: STEPHEN Middle Name G. Last Name*: WEBER Suffix:

Position/Title*: PROFESSOR
Organization Name*: University of Pittsburgh
Department:
Division:
Street1*: UNIVERSITY OF PITTSBURGH
Street2: DEPT OF CHEMISTRY
City*: PITTSBURGH
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 152600000

Phone Number*: (412) 624-8520 Fax Number: (412) 624-8611

E-Mail*: SWEBER@PITT.EDU

Credential, e.g., agency login: sweber

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PHD Degree Year:

Attach Biographical Sketch*: File Name: Weber_SG_NIH_Biosketch.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Wesalo, Joshua

eRA COMMONS USER NAME (credential, e.g., agency login): JWesalo

POSITION TITLE: MSTP Trainee

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date	FIELD OF STUDY
Franklin & Marshall College, Lancaster, PA	BA	08/2009	05/2013	Chemistry, Economics (Minor: Applied Mathematics)
University of Pittsburgh School of Medicine, Pittsburgh, PA	MD, PhD	06/2014	05/2023	Medicine, Chemistry

A. Personal Statement

I am a first-year graduate student in the MD/PhD program at the University of Pittburgh with the goal to become a translational physician-scientist in cardiology. My ultimate career goal is to run an NIH-funded research program, practice cardiology, and teach at an academic institution. During my dual-degree training, I will work at the intersection of chemical biology and vascular biology. My thesis mentor, Dr. Alexander Deiters, is an expert in developing new chemical approaches to solving persistent biological projects. I will complete my thesis work in Dr. Deiters' laboratory studying spatially defined, H_2O_2-responsive inhibition of NADPH oxidase activity as a novel treatment approach for ischemia-reperfusion injury. Aside from Dr. Deiters, I have assembled a complementary team of mentors in vascular biology, taking advantage of the University of Pittsburgh Vascular Medical Institute's strong training environment. My team of mentors will enhance my skills as an experimentalist, communicator, and clinician. I am confident that this proposed project, my team of mentors, and the integrative training of the Medical Scientist Training Program (MSTP) will launch my career as an independent physician-investigator with a strong foundation of skills and scientific principles.

My foundation of past research experiences and achievements has prepared me well to complete my training. Prior to medical school, I focused on developing a new treatment approach for GM3 Synthase Deficiency, a rare neurological disease affecting the Amish population. This project taught me how to design experiments at the interface of biology and chemistry in developing a treatment approach while keeping clinical applicability in mind. Additionally, the project helped build my skills as a synthetic chemist, which I have relied upon to generate pilot data for the present proposal. Through my work, I had the opportunity to work with outstanding physician-scientists and to get to know several patients and families afflicted with this debilitating disease, which inspired me to seek dual-degree training to become a physician-scientist. This project resulted in four poster presentations and two talks, one of which took place at a national conference.

Since joining the MSTP in June 2014, I have completed the preclinical years of medical school (capped off by a clerkship in neurology and psychiatry), the United States Medical Licensing Examination (USMLE) Step 1, and three graduate school research rotations that have broadened my technical skills and my scientific understanding. With the strong mentoring and rich resources available to me at between the department of chemistry and the VMI as outlined in my training plan, this fellowship will unequivocally enable to execute my proposed experiments and to fulfill my goal of becoming a physician-investigator with a research program at the intersection of chemical biology and vascular biology.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Graduate Student Researcher	09/16	08/21	Chemistry	University of Pittsburgh	Alexander Deiters, PhD

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Graduate Rotation 3	09/15	09/16	Chemistry	University of Pittsburgh	Alexander Deiters, PhD
Graduate Rotation 2	06/15	08/15	Chemistry	University of Pittsburgh	Alexander Deiters, PhD
Medical Student	08/14	05/23	Medicine	University of Pittsburgh School of Medicine	N/A
Graduate Rotation 1	06/14	08/14	Pharmacology	University of Pittsburgh	Patrick Pagano, PhD
Research Assistant	05/13	05/14	Chemistry	Franklin & Marshall College	Ken Hess, PhD
Research Fellow	05/13	05/14	Chemistry	Clinic for Special Children	Kevin Strauss, MD
Calculus Tutor	08/10	05/13	Mathematics	Franklin & Marshall College	Robert Gethner, PhD
Summer Undergraduate Researcher	06/12	08/12	Pharmacology	University of Pennsylvania	Vladimir Muzykantov, MD, PhD
Undergraduate Researcher	01/12	05/13	Chemistry	Franklin & Marshall College	Ken Hess, PhD
Undergraduate Researcher	06/11	08/11	Chemistry	Franklin & Marshall College	Ryan Mehl, PhD and Ken Hess, PhD
Undergraduate Researcher	06/10	08/10	Chemistry	Franklin & Marshall College	Ryan Mehl, PhD and Ken Hess, PhD
High School Volunteer Research	06/09	08/09	Neuroscience	Kennedy Krieger Institute	Mary E. Blue, PhD
High School Volunteer Research	06/08	08/08	Bioinformatics	Kennedy Krieger Institute	Jonathan Pevsner, PhD

Other Experience and Professional Memberships

2014 - Allegheny County Medical Society
2014 - American Medical Association
2015 - American Chemical Society

Selected Honors

2013 - Phi Beta Kappa Society
2013 Magna Cum Laude
2013 Theodore Alexander Saulnier, Jr. Prize in Chemistry— to the student majoring in Chemistry who in the opinion of the faculty demonstrates an especially searching mind in probing the frontiers of the unknown.
2013 Theodore E. Woodward, MD Healing Arts Prize—to the outstanding senior who will be pursuing post-graduate study in a health professions field.
2013 Isaac E. Roberts Prize in Biology— student with the highest grades in biology.
2009-2013 Honor's List
2009-2013 National Merit Scholarship
2009-2013 John Marshall Scholarship
2010 Eric C. Rackow, MD Endowed Achievement Scholarship for the Pre-Healing Arts

2005 and 2006 Carson Scholars Fund Scholarship

C. Contributions to Science

1. **Development of methods for ganglioside GM3 production and analysis for the treatment of GM3 Synthase Deficiency** (Mentors: Ryan A. Mehl, PhD; Kenneth R. Hess, PhD; Kevin Strauss, MD).
One in one-hundred Amish individuals carry a loss-of-function mutation in GM3 synthase (*Siat9*) that can cause GM3 Synthase Deficiency in their children. Affected children lose control of their movements, lose their vision and hearing, and suffer from profound developmental delay, typically dying before adolescence. No treatment approaches to date have been successful. I worked with a team of clinicians and scientists based at the Clinic for Special Children and Franklin & Marshall College on a new approach: ganglioside replacement therapy.

   Once I started work on this project, I developed an assay for quantifying serum gangliosides. As gangliosides are challenging analytes, this required enzymatic digestion followed by conjugation to a fluorophore and detection with HPLC, so I learned molecular biology, organic synthesis, and analytical chemistry techniques as I developed the assay. Next, I worked on methods to obtain ganglioside GM3 for the therapy. At first, I developed an arduous protocol for extracting milligram quantities of GM3 from buttermilk. Next, I switched my efforts to synthesis of multigram quantities of this complex natural product. Researchers I trained have completed my work and are planning clinical trials through the Clinic for Special Children soon. Further work is underway at Pittsburgh Children's Hospital to determine how ganglioside replacement therapy will fit in with efforts to treat the condition with hematopoietic stem cell or liver transplantation.

   a. **Wesalo, J. S.** Oral presentation. "Laboratory Synthesis of GM3: A Bridge to the Cure." *GM3 Synthase Deficiency Research Summit*. 11 Oct 2016. Children's Hospital of Pittsburgh, Pittsburgh, PA.

   b. **Wesalo, J. S.** Oral presentation. "GM3 Synthase Deficiency." *Genomic Medicine and the Plain Populations of North America.* 17 Jul 2013. Franklin & Marshall College, Lancaster, PA.

   c. **Wesalo, J. S.**; Mehl, R. A.; Hess, K. R. Poster presentation. "From Making Pancakes to Saving Lives: The Quest for a Cure for GM3 Synthase Deficiency in Buttermilk." *Franklin & Marshall College Autumn Research Fair*. 23 Sept 2011Franklin & Marshall College, Lancaster, Pa.

   d. **Wesalo, J. S.**; Mehl, R. A.; Hess, K. R. Poster presentation. "Hot on the Ganglioside Trail: Towards a Practical and Reproducible Assay for Serum Ganglioside Levels in GM3 Synthase Deficiency Patients." *Franklin & Marshall College Autumn Research Fair*. 24 Sept 2010. Franklin & Marshall College, Lancaster, Pa.

2. **Small-molecule control of protein function** (Mentor: Alexander Deiters, PhD).
Conditional control over protein function in living systems is an extremely useful tool for dissecting the molecular mechanism of biological processes. Our group recently pioneered a method for activating protein function using phosphines as a small-molecule trigger (Luo et al., *Nat. Chem.*, 2016). The method uses site-specific incorporation of unnatural amino acids that are activated through a bioorthogonal Staudinger reduction. Currently, this process requires over an hour for protein activation, which limits its applications. I have synthesized a new amino acid that has shown significantly faster activation in an *in vitro* assay, and I am currently preparing a manuscript on its application in conditionally controlling protein SUMOylation (Luo, J.; **Wesalo, J. S.**; Morihiro, K.; Deiters, A. "Enhanced small molecule switches for protein function: conditional control of SUMOylation." *Journal of the American Chemical Society*. Manuscript in preparation).

3. **Other presentations:**
   a. **Wesalo, J. S.** Oral presentation. "Targeting nanotherapeutics to injured vascular endothelium and quantifying VCAM, a marker of endothelial injury." *CTSA Summer Internship Program Symposium*. 27 Jul 2012. University of Pennsylvania, Philadelphia, PA.

   b. **Wesalo, J. S.** Oral presentation. "Targeting nanotherapeutics to injured vascular endothelium and quantifying VCAM, a marker of endothelial injury." *Franklin & Marshall College Autumn Research Fair*. 19 Oct 2012. Franklin & Marshall College, Lancaster, PA.

c. **Wesalo, J. S.**; Vázquez-Maldonado, L. A.; Liu, J.; Tsang, M.; Deiters, A. Poster presentation. “Light-activated circularized morpholino oligonucleotides for RNA knockdown and gene silencing in zebrafish.” *Pitt-CMU MSTP Annual Scientific Retreat*. 19 Aug 2016. University of Pittsburgh, Pittsburgh, PA.

d. **Wesalo, J. S.**; Meijles, D. N.; Cifuentes-Pagano, E.; Pagano, P. J. Poster presentation. “Isoform-specific NADPH oxidase inhibitors for the investigation of the role of NADPH oxidase in tumor necrosis factor-α-induced endothelial dysfunction.” *Pitt-CMU MSTP Annual Scientific Retreat*. 21 Aug 2016.

D. Additional Information: Research Support and/or Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE
	Franklin & Marshall College	
2009	General Chemistry 1	A
2009	Calculus 3	A
2010	Evolution, Ecology, and Heredity	A
2010	General Chemistry 2	A
2010	Linear Algebra and Differential Equations	A
2010	Principles of Physiology and Development	A
2010	Organic Chemistry 1	A
2010	Probability and Statistics 1	A-
2011	Organic Chemistry 2	A-
2011	Probability and Statistics 2	B+
2011	Cell Biology	A
2011	Fundamental Physics 1	A-
2011	Chemical Analysis	A
2011	Thermodynamics and Kinetics	B+
2012	Introduction to Biochemistry	A
2012	Directed Study–Chemistry	A
2012	Fundamental Physics 2	A
2012	Independent Study–Chemistry	A
2013	Inorganic Chemistry	B+
2013	Medicinal Chemistry	P
2013	Chemistry of Solar Energy Conversion	P
2013	Advanced Biochemistry	A
2013	Independent Study–Chemistry	A

YEAR	OTHER COURSE TITLE	GRADE
	Franklin & Marshall College	
2009	Microeconomics (AP Credit)	S
2009	Macroeconomics (AP Credit)	S
2009	English Language/Composition (AP Credit)	S
2009	English Literature/Composition (AP Credit)	S
2009	US Government and Politics (AP Credit)	S
2009	US History (AP Credit)	S
2009	World History (AP Credit)	S
2009	Calculus 2 (AP Credit)	S
2009	Mortality & Meaning	A
2009	Mathematics of Art	A
2009	Low Brass	A-
2009	Orchestra	NC
2010	Intro to Economic Perspectives	A
2010	Altered States	A
2010	Low Brass	A
2010	Orchestra	NC
2010	Value and Distribution	B
2010	Music Theory 1	A
2011	Intermediate Microeconomics	A
2011	Low Brass	A
2011	Orchestra	A
2011	Game Theory	A-
2011	Elementary Chinese 1	A
2012	Intermediate Macroeconomics	A
2012	Low Brass	A
2012	Orchestra	A
2012	Musicianship 1	A
2012	Environmental History	A
2012	Money and Banking	A
2012	History of Economic Thought	A
2013	Political Economy of Health	A

YEAR	SCIENCE COURSE TITLE	GRADE

YEAR	OTHER COURSE TITLE	GRADE
2013	Low Brass	A
2013	Orchestra	A

YEAR	SCIENCE COURSE TITLE	GRADE
	University of Pittsburgh School of Medicine	
2014	Human Anatomy	P
2014	Biochemistry	P
2014	Genetics	P
2014	Cellular Pathology 1	P
2015	Cellular Pathology 2	P
2015	Immunology	P
2015	Medical Microbiology	P
2015	Neuroscience	P
2015	Psychiatry	P
2015	Cardiology	P
2015	Nephrology	P
2015	Pulmonology	P
2015	Digestion & Nutrition	P
2015	Skin & Musculoskeletal System	P
2016	Hematology and Oncology	P
2016	Endocrinology	P
2016	Reproduction & Developmental Biology	P
	Graduate School Coursework	
2016	Basics of Personalized Medicine	A
2016	Molecular Biology	A+

YEAR	OTHER COURSE TITLE	GRADE
	University of Pittsburgh School of Medicine	
2014	Ethics, Law, & Professionalism	P
2014	Medical Decisionmaking	P
2014	Medical Interviewing	P
2014	Introduction to Physical Examination	P
2015	Advanced Physical Examination	P
2015	Behavioral Health	P
2015	Population Health	P
	MSTP Coursework	
2014-2016	Professional Development 1, 2, and 3	P
2014-2016	Research Basis of Medical Knowledge 1 and 2	P

Grade Legend:

“P” – Pass

1. Franklin & Marshall College: Letter grades of “A” to “C-” are converted to Pass for courses that are taken as Pass/Fail.
2. University of Pittsburgh School of Medicine and MSTP: All courses are graded Pass/Fail.

“S” – Satisfactory

Franklin & Marshall College awards an “S” for AP credits if a student earns a 4 or 5 on the AP exam and elects to receive credit for it.

MCAT: 37S
USMLE Step 1: 257

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Deiters, Alexander

eRA COMMONS USER NAME (credential, e.g., agency login): alexdeiters

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Münster, Münster	BS	10/1995	Chemistry
University of Münster, Münster	PhD	10/2000	Organic Chemistry
The University of Texas at Austin, Austin, TX	Postdoctoral Fellow	08/2002	Organic Chemistry
The Scripps Research Institute, La Jolla, CA	Postdoctoral Fellow	06/2004	Chemical Biology

A. Personal Statement

My group is developing innovative chemical tools to address biological problems and close persisting methodology gaps. We have a particular focus on developing conditional control over pharmacological inhibitors, oligonucleotides, and proteins. While a major emphasis of our work is on the optical control of cellular processes through light-triggered removal of so called "caging groups", we also have significant experience in the hydrogen peroxide-triggered activation of small molecules and gene function in human cells. Thus, my group is perfectly situated to support Josh Wesalo's F30 application with extensive expertise and infrastructure that we have in place. My students and postdocs routinely conduct the synthesis of complex organic molecules and have experience in a wide range of laboratory techniques required for Josh's proposed studies on the H_2O_2-triggered control of Nox function, such as mammalian cell culture, fluorescence microscopy, Western blots, qPCR, reporter assays, cell viability assays, etc.

My ability to advise and support an interdisciplinary research group working in chemistry and biology is documented by over 100 peer-reviewed publications and by external funding from public and private agencies. Currently, I am supervising a group of 12 graduate and 4 undergraduate students who come from very diverse research backgrounds, ranging from synthetic organic chemistry, to chemical engineering, biochemistry, biophysics, and functional genomics. This diversity creates synergistic effects that further facilitate Josh's successful completion of his project at the interface of chemistry and cardiovascular biology.

1. Luo J, Liu Q, Morihiro K, **Deiters A**. Small-molecule control of protein function through Staudinger reduction. Nat Chem. 2016 Nov;8(11):1027-1034. PubMed PMID: 27768095; PubMed Central PMCID: PMC5119652.
2. Hanna RD, Naro Y, **Deiters A**, Floreancig PE. Alcohol, Aldehyde, and Ketone Liberation and Intracellular Cargo Release through Peroxide-Mediated α-Boryl Ether Fragmentation. J Am Chem Soc. 2016 Oct 12;138(40):13353-13360. PubMed PMID: 27636404.
3. Govan JM, Uprety R, Thomas M, Lusic H, Lively MO, **Deiters A**. Cellular delivery and photochemical activation of antisense agents through a nucleobase caging strategy. ACS Chem Biol. 2013 Oct 18;8(10):2272-82. PubMed PMID: 23915424; PubMed Central PMCID: PMC3856437.
4. Govan JM, McIver AL, Riggsbee C, **Deiters A**. Hydrogen peroxide induced activation of gene expression in mammalian cells using boronate estrone derivatives. Angew Chem Int Ed Engl. 2012 Sep 3;51(36):9066-70. PubMed PMID: 22855386.

B. Positions and Honors

Positions and Employment

2004 - 2009 Assistant Professor, North Carolina State University
2009 - 2012 Associate Professor, North Carolina State University
2012 - 2013 Professor, North Carolina State University
2013 - Professor, University of Pittsburgh

Other Experience and Professional Memberships

2013 - Member, University of Pittsburgh Cancer Institute
2014 - Member, Molecular Biophysics & Structural Biology Program, University of Pittsburgh
2014 - Member, Center for Nucleic Acids Science & Technology, Carnegie Mellon University
2015 - Member, Medical Scientist Training Program, University of Pittsburgh School of Medicine
2016 - Member, Editorial Advisory Board, ChemBioChem (Wiley-VCH)
2016 - Member, Editorial Board, Scientific Reports (Nature Publishing Group)

Honors

2006 Basil O'Connor Scholar Award, March of Dimes Foundation
2007 Faculty Research Award, Sigma Xi
2007 Cottrell Scholar Award, Research Corporation
2007 Beckman Young Investigator Award, Arnold and Mabel Beckman Foundation
2007 MJ Collins Award, CEM Corporation
2009 Faculty Early Career Development (CAREER) Award, National Science Foundation
2009 Teva USA Scholar Award, American Chemical Society
2010 Thieme Chemistry Journals Award, Thieme Medical Publishers
2010 Research Scholar Award, American Cancer Society
2011 Alumni Association Outstanding Research Award, North Carolina State University
2014 New Initiative Research Award, Charles E. Kaufman Foundation

C. Contribution to Science

1. **Optical control of oligonucleotide function.** My group has developed a synthetic, generally applicable approach to the optical control of morpholino, DNA, and RNA molecules and has applied these reagents in the regulation of gene expression in cells and aquatic embryos. Using light as an external trigger enables the activation and deactivation of oligonucleotide function with high spatial and temporal resolution. Our nucleobase-caging approach has been patented (Publication No. US2010099159) and our caged thymidine phosphoramidite is commercially available from Glen Research and Berry & Associates. Caged oligonucleotides based on this methodology can be synthesized by any RNA/DNA facility.

   a. Hemphill J, Liu Q, Uprety R, Samanta S, Tsang M, Juliano RL, **Deiters A**. Conditional control of alternative splicing through light-triggered splice-switching oligonucleotides. J Am Chem Soc. 2015 Mar 18;137(10):3656-62. PubMed PMID: 25734836.

   b. Yamazoe S, Liu Q, McQuade LE, **Deiters A**, Chen JK. Sequential gene silencing using wavelength-selective caged morpholino oligonucleotides. Angew Chem Int Ed Engl. 2014 Sep 15;53(38):10114-8. PubMed PMID: 25130695; PubMed Central PMCID: PMC4206551.

   c. Hemphill J, Govan J, Uprety R, Tsang M, **Deiters A**. Site-specific promoter caging enables optochemical gene activation in cells and animals. J Am Chem Soc. 2014 May 14;136(19):7152-8. PubMed PMID: 24802207; PubMed Central PMCID: PMC4333597.

   d. Govan JM, Uprety R, Hemphill J, Lively MO, **Deiters A**. Regulation of transcription through light-activation and light-deactivation of triplex-forming oligonucleotides in mammalian cells. ACS Chem Biol. 2012 Jul 20;7(7):1247-56. PubMed PMID: 22540192; PubMed Central PMCID: PMC3401312.

2. **Optical control of protein function.** Genetically encoded light-regulation of protein function has led to "optogenetics" as an emerging new research area. My group, based on fundamental work by the Schultz lab, has developed genetic code expansion methodologies for the site-specific incorporation of photocaged amino acids into proteins. We have applied genetically encoded caged amino acids to the optical control of cell signaling, protein translocation, gene editing, gene expression, gene silencing, and other biological processes. The installation of light-removable protecting groups with exquisite residue-specificity into proteins enables spatial and temporal control over a wide range of cellular functions, which we are currently applying to the dissection of cell signaling networks.

   a. Hemphill J, Chou C, Chin JW, **Deiters A**. Genetically encoded light-activated transcription for spatiotemporal control of gene expression and gene silencing in mammalian cells. J Am Chem Soc. 2013 Sep 11;135(36):13433-9. PubMed PMID: 23931657; PubMed Central PMCID: PMC4188981.
   b. Engelke H, Chou C, Uprety R, Jess P, **Deiters A**. Control of protein function through optochemical translocation. ACS Synth Biol. 2014 Oct 17;3(10):731-6. PubMed PMID: 24933258; PubMed Central PMCID: PMC4210160.
   c. Luo J, Uprety R, Naro Y, Chou C, Nguyen DP, Chin JW, **Deiters A**. Genetically encoded optochemical probes for simultaneous fluorescence reporting and light activation of protein function with two-photon excitation. J Am Chem Soc. 2014 Nov 5;136(44):15551-8. PubMed PMID: 25341086; PubMed Central PMCID: PMC4333581.
   d. Hemphill J, Borchardt EK, Brown K, Asokan A, **Deiters A**. Optical Control of CRISPR/Cas9 Gene Editing. J Am Chem Soc. 2015 May 6;137(17):5642-5. PubMed PMID: 25905628; PubMed Central PMCID: PMC4919123.

3. **DNA computation.** Cells and computers have in common that both process diverse patterns of input signals in order to create defined responses. The emerging field of DNA computation uses oligonucleotide-based logic gates for the assembly of complex computation circuits. My group reported the first examples of interfacing DNA computation with optical inputs and the first examples of triggering DNA logic gates with endogenous inputs in live cells. These developments will expand the utility of DNA computation within biological systems to new diagnostic and therapeutic applications. In addition, we are currently developing DNA logic gates that activate and release small molecules through proximity-enabled Staudinger reductions.

   a. Prokup A, Hemphill J, **Deiters A**. DNA computation: a photochemically controlled AND gate. J Am Chem Soc. 2012 Feb 29;134(8):3810-5. PubMed PMID: 22239155.
   b. Hemphill J, **Deiters A**. DNA computation in mammalian cells: microRNA logic operations. J Am Chem Soc. 2013 Jul 17;135(28):10512-8. PubMed PMID: 23795550.
   c. Prokup A, **Deiters A**. Interfacing synthetic DNA logic operations with protein outputs. Angew Chem Int Ed Engl. 2014 Nov 24;53(48):13192-5. PubMed PMID: 25283524.
   d. Prokup A, Hemphill J, Liu Q, **Deiters A**. Optically Controlled Signal Amplification for DNA Computation. ACS Synth Biol. 2015 Oct 16;4(10):1064-9. PubMed PMID: 25621535.

4. **Discovery of small molecule modifiers of microRNA function.** miRNAs are very important regulators of gene function and have been linked to a wide range of diseases, most importantly cancer. My group was the first to report small molecule inhibitors of specific miRNAs (miR-21 and miR-122). Since then, several other labs have adapted our discovery approach and have identified additional new miRNA inhibitors. We are using our molecules to probe miRNA biology and to study their therapeutic utility against cancer and HCV infection in pre-clinical models.

   a. Naro Y, Thomas M, Stephens MD, Connelly CM, **Deiters A**. Aryl amide small-molecule inhibitors of microRNA miR-21 function. Bioorg Med Chem Lett. 2015 Nov 1;25(21):4793-6. PubMed PMID: 26220158.
   b. Connelly CM, Thomas M, **Deiters A**. High-throughput luciferase reporter assay for small-molecule inhibitors of microRNA function. J Biomol Screen. 2012 Jul;17(6):822-8. PubMed PMID: 22412086; PubMed Central PMCID: PMC3758890.

c. Young DD, Connelly CM, Grohmann C, **Deiters A**. Small molecule modifiers of microRNA miR-122 function for the treatment of hepatitis C virus infection and hepatocellular carcinoma. J Am Chem Soc. 2010 Jun 16;132(23):7976-81. PubMed PMID: 20527935.

d. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, **Deiters A**. Small-molecule inhibitors of microrna miR-21 function. Angew Chem Int Ed Engl. 2008;47(39):7482-4. PubMed PMID: 18712719; PubMed Central PMCID: PMC3428715.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 GM112728-01A1
DEITERS, ALEXANDER (PI)
09/01/15-07/31/19
Chemically Triggered Morpholino Antisense Oligonucleotides
Role: PI

KA2014-73921, Kaufman Foundation
DEITERS, ALEXANDER (PI)
09/01/14-08/31/17
Expanding the Genetic Code of Zebrafish
Role: PI

R01 GM108952, National Institute of General Medical Sciences (NIGMS)
CHEN, JAMES (PI); DEITERS, ALEXANDER (CO-I)
08/01/14-07/31/18
Development of Lariat-Shaped Caged Morpholinos for Optochemical Gene Regulation
Role: Co-Investigator

CHE-1404836, National Science Foundation
DEITERS, ALEXANDER (PI)
07/01/14-06/30/17
Control of Protein Dimerization Through Light-Regulated Rapamycin
Role: PI

MCB-1330746 , National Science Foundation
DEITERS, ALEXANDER (PI)
09/01/13-08/31/17
Optogenetic Dissection of Protein Kinase Networks
Role: PI

CCF-1617041, National Science Foundation
DEITERS, ALEXANDER (PI)
07/01/16-06/30/19
DNA Computation in Cells
Role: PI

CBET-1603930, National Science Foundation
DEITERS, ALEXANDER (PI)
08/01/16-07/31/19
Near-natural Amino Acid Mutagenesis for the Engineering and Study of Protein Function
Role: PI

R21 HD085206, National Institutes of Health (NICHD)
DEITERS, ALEXANDER (PI)
09/23/16-08/31/18
Optical Control of Translation and Gene Editing in Zebrafish Embryos
Role: PI

Completed Research Support

120130RSG1106601RMC, American Cancer Society
DEITERS, ALEXANDER (PI)
11/01/13-12/31/15
Small Molecule Regulation of microRNAs to Understand and Treat Cancer
Role: PI

50181-TEV, Teva USA Scholar Grant
DEITERS, ALEXANDER (PI)
09/01/09-08/31/13
Small Molecules as new Probes and Therapeutics for Liver Diseases
Role: PI

CHE-0846756, National Science Foundation
DEITERS, ALEXANDER (PI)
06/01/09-05/31/14
CAREER: Solid-Supported Cyclotrimerizations - A Library Approach to Research and Teaching
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Yadong Wang	William Kepler Whiteford Professor of Bioengineering, Surgery, Chemical Engineering, and Mechanical Engineering and Materials Science
eRA COMMONS USER NAME (credential, e.g., agency login) YADONGWANG	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Kansas State University	M.S.	1995	Chemistry
Stanford University	Ph.D.	1999	Chemistry
Massachusetts Institute of Technology	Postdoctoral	2002	Biomedical Engineering

A. Personal Statement

My passion is design and application of biomaterials in regenerative medicine. I am an adjunct faculty in 3 departments in engineering and medicine, and a core member of 3 institutes. This facilitates the interdisciplinary approach my laboratory takes in problem solving. We have been collaborating with scientists, engineers, and clinicians in the last 14 years in an effort to solve key challenges in biomaterials. We have published 107 peer-reviewed journal publications with >4000 citations, and 8 issued and 6 pending patents.

B. Positions and Honors

Positions and Employment

- 2002-2003 Research Associate, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA
- 2003-2008 Assistant Professor, Department of Biomedical Engineering, Georgia Institute of Technology/Emory University, Atlanta, GA
- 2008- Associate Professor, Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA
- 2008- Core member, McGowan Institute for Regenerative Medicine
- 2011- Associate Professor, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA
- 2012- Associate Professor, Department of Chemical and Petroleum Engineering, University of Pittsburgh, Pittsburgh, PA
- 2013- Professor, Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA
- 2013- William Kepler Whiteford Professor, Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA
- 2014- Adjunct professor, Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA
- 2014- Core member, Clinical and Translational Science Institute
- 2015- Core member, Vascular Medicine Institute

Awards and Other Professional Experiences

1998-1999	Franklin Veatch Memorial Scholarship, Stanford University
2005	Finalist, the INDEX: Award, Copenhagen, Denmark
2007	American Heart Association Scientist Development Award
2007	Best Professor Award, BMES Georgia Tech Chapter
2007	Hunter Chair Lecture, Clemson University

2007	SAIC Outstanding Research Paper Award
2007-	Member, Advisory Board, Lifeboat Foundation
2008	Best Undergraduate Advisor Award, BMES Georgia Tech Chapter
2012	American Heart Association Established Investigator Award
2012	Randall Family Big Idea Competition, 1st Place Winner (Mentor)
2012	CMU Summit New Venture Competition, 1st Place Winner (Mentor)
2013	Dutch Heart Foundation Lecture, Leiden, The Netherlands
2013	Keynote Speaker, TERMIS-AP Conference, Shanghai, China
2014	Fellow, American Institute for Medical and Biological Engineering
2014	Grand Prize, Pitt Innovation Challenge
2014	Burroughs Wellcome Fund Collaborative Research Travel Grant
2014	Keynote Speaker, International Symposium of Materials on Regenerative Medicine Conference, Taoyuan, Taiwan
2014	Keynote Speaker, TERMIS-AP Conference, Daegu, Korea
2015	Carnegie Science Award in Life Sciences
2016	Grand Prize winner, Pitt Innovation Challenge
2016	Pitt Innovator Award
2007-	Reviewer for: *National Institutes of Health, National Science Foundation, DOD (CDMRP), Canada Foundation of Innovation, Technology Foundation (STW, NWO), the Netherlands*, *National Research Foundation, South Africa*, and *Hong Kong Research Grants Council.*
2016-2020	Member of the Biomaterials and Biointerfaces Study Section, CSR, NIH.
2004	Invited speaker, the 133rd TMS Annual Meeting and Exhibition, Charlotte, NC, March 2004.
2006	Invited speaker, Thermec'2006 International Conference on Processing and Manufacturing of Advanced Materials. Vancouver, Canada, July, 2006.
2008	Invited speaker, the 21st Annual and International Meeting of the Japanese Association for Animal Cell Technology, Fukuoka, Japan, November, 2008.
2009	Invited speaker, ACS National Meeting, Washington, DC. August, 2009.
2013	Track organizer-Tissue Engineering 2013 BMES Annual Meeting, Seattle, WA, September 2013.
2013	Invited speaker, the Elastin, Elastic Fibers & Microfibrils Gordon Research Conference, Biddeford, ME, July 2013.
2013	Keynote speaker, TERMIS-AP 2013 Annual Conference, Shanghai, China, October 2013.
2014	Invited speaker, the 5th International Conference on Tissue Engineering, Kos, Greece. June 2014.
2014	Keynote speaker, TERMIS-AP 2014 Annual Conference, Daegu, Korea, September 2014.
2014	invited speaker, American Society for Matrix Biology Biennial Meeting, Cleveland, October 2014.
2015	Invited speaker, MRS Fall Meeting, Boston, November 2015.
2016	Invited speaker, BMES Annual Meeting, Minneapolis, MN. October 2016.
2017	Invited speaker, SFB annual meeting, Minneapolis, MN. April 2017

2017 Plenary speaker, the12th International Symposium on Frontiers In Biomedical Polymers. Seoul, Korea. July 2017.

2017 Keynote Speaker, the 4th International Symposium of Materials on Regenerative Medicine, Taoyuan, Taiwan, August 2017.

C. Contributions to Science

Selected from 107 peer-reviewed journal publications (cited >5000 times) and 8 issued patents

I invented poly(glycerol sebacate) (PGS) when I was a postdoc in the Langer lab at MIT. It is now used in over 20 academic and industrial laboratories worldwide. This filled the void of biomedical elastomers for applications such as tissue engineering and regenerative medicine. The publication of poly(glycerol sebacate) in 2002 sparked great interests and rapid increase in development of biodegradable elastomers for regenerative medicine. The polymer is licensed and marketed by Secant Medical, LLC under the trade mark Regenerez™. Clinical use of PGS is approved in Europe as a major component of bone cement. Our translational work with PGS has been focused on in-situ tissue engineering of arteries.

1. **Wang Y,** Ameer GA, Sheppard BJ, and Langer R. A Tough Biodegradable Elastomer. *Nat. Biotechnol.*, 20: 602-606, 2002.
2. Lee K-W, Stolz DB, and **Wang Y.** Substantial Expression of Mature Elastin in Arterial Constructs. *Proc. Natl. Acad. Sci. U.S.A.* 108:2705-10, 2011. PMC3041142
3. Wu W, Allen R, and **Wang Y.** Fast degrading elastomer enables rapid remodeling of a cell-free synthetic graft into a neo-artery. *Nat. Med.*18, 1148–1153, 2012. (Featured in **Editors' Choice** in *Science Translational Medicine* and *Nature Medicine* **Podcast**.)
4. Lee K-W, Johnson N, Gao J, and **Wang Y**. Human Progenitor Cell Recruitment via SDF-1alpha Coacervate-laden PGS Vascular Grafts. *Biomaterials.* 34 (38): 9877-9885, 2013.

Proteins can be very potent drugs. However, they typically have very short half-lives in vivo. Inspired by how extracellular matrix uses heparan sulfates to anchor and stabilize proteins, a synthetic polycation, heparin, and heparin-binding proteins are combined to form a complex coacervate. The heparin-based coacervate system solves a long term challenge of maintaining the bioactivity of the proteins. This is, to my knowledge, the first report of using complex coacervate as a controlled release vehicle. The coacervate has been used for wound healing, therapeutic angiogenesis, and treating myocardial infarction.

5. Chu H, Johnson NR, Mason NS, and **Wang Y.** A [polycation:heparin] complex releases growth factors with enhanced bioactivity. *J. Control. Release*, 150, 157-163, 2011.
6. Chu H, Gao J, Chen C-W, Huard J, and **Wang Y.** An injectable FGF2 coacervate for persistent angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 13444-13449, 2011.
7. Johnson N and **Wang Y**. Controlled delivery of heparin-binding EGF-like growth factor yields fast and comprehensive wound healing. *J. Control. Release*, 166, 124-9. 2013. PMID: 23154193
8. Johnson N and **Wang Y**. Coacervate delivery systems for proteins and small molecule drugs. Expert Opin. Drug Deliv. 11(12):1829-1832, 2014.

Minimally invasive drug delivery will further increase the utility of controlled drug delivery. To this end, we created reverse thermal gels (polymer solution that gels upon heating up) that can be modified with biological molecules and gels at body temperature. To my knowledge, this is the first reverse thermal gel that carries functional groups allowing bio-functionalization.

9. Park D, Wu W, and **Wang Y.** A functionalizable reverse thermal gel based on a polyurethane / PEG block copolymer. *Biomaterials*, 32:777-86, 2011. PMC2991555
10. Park D, Shah V, Rauck B, Friberg TR, and **Wang Y.** An anti-angiogenic reverse thermal gel as a drug delivery system for age-related wet macular degeneration. *Macromol. Biosci.* 13, 464–469, 2013.

11. Ritfeld GJ, Rauck B, Novosat TL, Park D, Patel P, Roos RAC, **Wang Y***, and Oudega M*. The effect of a polyurethane-based reverse thermal gel on bone marrow stromal cell transplant survival and spinal cord repair. *Biomaterials.* 35(6): 1924-1931, 2014.
12. Rauck BM, Friberg TR, Mendez CAM, Park D, Shah V, Bilonick RA, and **Wang Y.** Biocompatible reverse thermal gel sustains the release of intravitreal bevacizumab in vivo. *Invest. Ophthalmol. Vis. Sci.* 55, 469-476, 2014.

Switchable adhesives allow the adhesion between skin and adhesive bandage to be significantly reduced upon applying an external trigger. This will greatly reduce the skin trauma caused by medical adhesives such as fixation device for indwelling catheter, ostomy bag, and adhesive drapes of VAC therapy. A new switchable adhesive technology that is triggered by applying rubbing alcohol is the cornerstone for Global Biomedical Technologies, LLC. The reduction in tissue damage improves the quality of life and is particularly important for patients with chronic wounds. As the scientific founder of the company, I co-authored 4 patents and 1 journal article.

13. Rosing H and **Wang Y.** Selectively-releasable adhesives. US 7745562. Issued Jun29 ,2010.
14. Rosing H and **Wang Y.** Articles incorporating preparing selectively-releasable adhesives. US 7887915. Issued Feb 15, 2011.
15. Rosing H and **Wang Y.** Methods for preparing selectively-releasable adhesives. US 7888451. Issued Feb 15, 2011.
16. Robertson F**, Wang Y**, Rosing H. An oligomeric switch that rapidly decreases the peel strength of a pressure sensitive adhesive. *Int. J. Adhes. Adhes.* 55: 64-8, 2014.

Nervous system controls many functions of the body. Functional restoration and regeneration is extremely limited for the central nerve and limited for long peripheral nerve damages. We are the first lab that investigated the use of neurotransmitters as guidance cues for polymeric materials that facilitate nerve regeneration. We continue to explore the use of these polymers as nerve guides.

17. Gao J, Kim YM, Coe H, Zern B, Sheppard B, and **Wang Y.** A Neuro-inductive Biodegradable Material Based on Dopamine. *Proc. Natl. Acad. Sci. U.S.A.*, 103: 16681-16686, 2006. PMC1636515
18. Gumera C and **Wang Y.** Modulating neuronal responses by controlled integration of acetylcholine-like functionalities in biomimetic polymers. *Adv. Mater.* 19: 4404-4409, 2007.
19. Gumera C, Rauck B, and **Wang Y**. Materials for Central Nervous System Regeneration: Bioactive Cues. *J. Mater. Chem.* 21, 7033 – 7051, 2011. (featured as 'hot article' by JMC on Apr. 20, 2011)
20. Jeffries E and **Wang Y.** Incorporation of parallel electrospun fibers for improved topographical guidance in 3-D nerve guides. *Biofabrication*. 5: 035015. doi: 10.1088/1758-5082/5/3/035015, 2013. (Featured as "Article of particular interest" by *Biofabrication*)

D. Research Support

Ongoing Research Support

2R01HL089658-07 (Wang) 8/1/2012 – 7/31/2017
National Heart, Lung, and Blood Institute
Biodegradable Synthetic Vascular Graft
Goal: To investigate host remodeling of biodegradable synthetic vascular grafts as interposition implant in rat abdominal aorta. The foci are specific bi-layered graft design, type of cells that participate in the remodeling, phenotype comparison of the ECs and SMCs in the remodeled graft with their native counterparts, and in vivo vasoactivities of the remodeled graft with native rat aorta as the control.

1 R01 NR16436-01 (Wang) 7/1/2016-6/30/2021
NIH
Extended release of bioactive factors to treat refractory wounds

The goal of this research is to apply a controlled growth factor release technology to heal chronic wounds, particularly diabetic ulcer. This combines the expertise of 4 groups in nursing, pathology, surgery, and bioengineering in order to translate a benchtop discovery to clinical benefits.

1R01HL128602-01 (MPI: Breuer, Humphrey and Wang) 7/1/2015 – 6/30/2019
NIH
Computational Model Driven Design of Tissue Engineered Vascular Grafts
The primary goals of this work are threefold: to perform limited series in vivo tests to inform and validate a computational model, to use this model to perform myriad parametric studies to identify optimal scaffold parameters that warrant experimental testing, and to fabricate and test the model identified scaffold in for a (high pressure) arterial graft.

Selected Completed Research Support in the Last 3 Years

12EIA9020016 Wang (PI) 1/1/2012 – 12/31/2016
American Heart Association
Established Investigator Award
Biomimetic Coacervates for Cardiac repair and regeneration
Goal: Examine the potential synergistic effect of multiple growth factors and cytokines on cardiac repair and regeneration.

IIP-1444774 Wang (PI) 8/15/2014 -1/31/2016
National Science Foundation
PFI:AIR - TT: Accelerate wound healing via biomimetic protein therapy
Goal: Clinical translation of HB-EGF coacervate as a treatment for hard to heal wounds.

DMR- 1005766 Wang (PI) 7/1/2010 – 6/30/2013
National Science Foundation
Design and Application of Biocompatible Polycations
Goal: To determine the principles of designing biocompatible polycations and its use in controlled release.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Patrick J Pagano

eRA COMMONS USER NAME (credential, e.g., agency login): ppagano1

POSITION TITLE: Professor, Vice-Chair for Graduate Education
Department of Pharmacology & Chemical Biology
Vascular Medicine Institute
University of Pittsburgh

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Binghamton University-Harpur College, Binghamton, NY.	B.A.	05/1985	Chemistry
New York Medical College, Valhalla, NY.	M.Sc.	05/1988	Pharmacology
New York Medical College, Valhalla NY.	Ph.D.	05/1991	Pharmacology
Boston University Medical Center, Vascular Biology Unit, Boston, MA.	Postdoc.	05/1994	Vascular Biology

A. Personal Statement

A vascular biologist by training, my laboratory explores multiple aspects of cardiovascular cell biology as they relate to tissue dysfunction. The laboratory's primary focus has been the vascular effects of reactive oxygen species. Among the first to identify NADPH oxidase (Nox) in the vasculature, we subsequently cloned phagocyte-like p67^{phox} in cells from the vascular adventitia and demonstrated upregulation of this and catalytic subunits of this complex enzyme in response to the pro-hypertensive hormone angiotensin II. Major research interests of the laboratory include paracrine effects of adventitial Nox-derived reactive oxygen species (ROS) on cell hypertrophy, proliferation and vascular tone dysfunction. My group has had many years of experience in *in vivo* models of systemic and pulmonary hypertension and oxidative stress-induced disease as well as detection of tissue ROS. To elucidate the role of Nox signaling in cardiovascular disease, major emphasis has been placed on novel isoform-specific peptidic and small molecule inhibitor development. Finally, a track record of achievement and extensive collaborative experience over the past 2 decades have afforded me the required skills to lead and co-manage this project. The truly outstanding environment at the University of Pittsburgh provides myriad opportunities and state-of-the-art facilities serving to greatly enhance the success of the research plan.

B. Positions and Honors

Positions and Employment

1996-1998 Assistant Professor of Medicine (tenure track), Boston University School of Medicine
1998-2008 Senior Staff investigator, Hypertension & Vasc. Res. Division, Henry Ford Hospital
2003-2008 Director, Vascular Biology Research, Hypertension & Vasc. Res. Division, Henry Ford Hospital
2004-2008 Assistant Professor of Medicine (tenure track), Case Western Reserve University
2004-2008 Associate Professor of Physiology, Wayne State University
2008- Professor, Dept. of Pharmacology & Chemical Biology, Univ. of Pittsburgh School of Medicine
2010- Director, Graduate Program, Molecular Pharmacology. Univ. of Pittsburgh, School of Medicine

2012- Vice Chair, Graduate Education, Dept. of Pharmacology & Chemical Biology, Univ. of Pittsburgh School of Medicine

Other Experience and Professional Memberships

American Heart Association
American Physiological Society
American Association for the Advancement of Science
Society for Free Radical Biology and Medicine (formerly The Oxygen Society)
Inter-American Society of Hypertension (Conference Organizing Committee)

Honors

2011- Standing Member, Hypertension & Microcirculation Study Section, NIH NHLBI
2012- Associate Editor, *Clinical Science*
2015- Editorial Board, *Circulation Research*
2005-2010 Established Investigator Award, American Heart Association
2006- Editorial Board, American Journal of Physiology
2006- Editorial Board, Cardiovascular Research
2002 Invited International Participant, Cold Spring Harbor Laboratory, First International Nox Symposium, "Oxidases in Inflammation and Cellular Signaling" symposium
2001- Fellow, American Heart Association
1996-2001 NIH F.I.R.S.T. Award
1990 Pharmaceutical Manufacturers' Foundation, Advanced Pre-doctoral Fellowship
1991-94 Cardiovascular Training Fellowship, Boston University
1987, 1988 National Science Foundation Honorable Mention
1985 Academic honors, Harpur College, Binghamton University

B. Contributions to Science [84 peer-reviewed publications].

The major focus of the research in the Pagano lab is the study of NADPH oxidases, a complex family of proteins that catalyze the production of reactive oxygen species (ROS). Our research interests have centered on the investigation of the pivotal role this enzyme class plays in physiological cell signaling as well as under pathological conditions. Since the deleterious role of high levels of ROS (a.k.a. oxidative stress) has been described for many disorders in the cardiovascular system as well as in cancer and neurodegenerative disease, great efforts have been dedicated to the development of isoform-specific inhibitors of NADPH oxidases that, in turn, can be used as (a) tools to delineate the role of this class of enzymes in normal and pathological cellular signaling pathways; and (b) therapeutic agents and scaffolds for the development of new drugs.

1. Identification of NADPH oxidase in the vasculature.

NADPH oxidase(s), major source(s) of superoxide in a variety of tissues, was first described in neutrophils as involved in the respiratory burst. The following publications from our group are among the first in the field to describe and characterize Nox in a non-phagocytic cell. These findings constitute the basis of what is now known to be an important proximal signaling pathway and a major culprit in the development of myriad diseases.

1a. Al Ghouleh I, Meijles DN, Mutchler S, Zhang Q, Sahoo S, Gorelova A, Henrich Amaral J, Rodríguez AI, Mamonova T, Song GJ, Bisello A, Friedman PA, Cifuentes-Pagano ME, **Pagano PJ**. Binding of EBP50 to Nox organizing subunit p47^{phox} is pivotal to cellular reactive species generation and altered vascular phenotype. *Proc Natl Acad Sci U S A.* 2016; 113 (36):E5308-17. PubMed PMID: 27540115, PMCID: PMC5018796

1b. Pagano PJ, Ito Y, Tornheim K, Gallop PM, Tauber AI, Cohen RA. An NADPH oxidase superoxide-generating system in the rabbit aorta. *Am J Physiol.* 1995; 268: H2274-80.PubMed PMID: 7611477

1c. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proc Natl Acad Sci U S A.* 1997, 94(26):14483-8.PubMed PMID: 9405639, PMCID: PMC25029

1d. Pagano PJ, Chanock SJ, Siwik DA, Colucci WS, Clark JK. Angiotensin II induces p67phox mRNA expression and NADPH oxidase superoxide generation in rabbit aortic adventitial fibroblasts. *Hypertension.* 1998; 32(2):331-7. PubMed PMID: 9719063

2. First-in-class Nox inhibitor and development of other isoform-selective Nox inhibitors.

The understanding of the specific role of each Nox isoform in a signaling pathway or pathophysiological process has been hindered by the paucity of specific Nox inhibitors. A major focus of our research has been the development of isoform-specific inhibitors, both peptidic as well as small molecules. Nox2ds-*tat,* was the first Nox inhibitor rationally designed to specifically inhibit Nox2-oxidase activity (**2a, 2b**) and to date is arguably the most widely used not only in the cardiovascular field but also in the study of numerous diseases where Nox2-derived ROS are involved, including neurodegenerative disease and cancer. *Nox2ds-tat* is a peptidic inhibitor designed to mimic the docking sequence on Nox2 that is important for its interaction with $p47^{phox}$ and that contains a short amino acid region corresponding to HIV-tat protein, this provides our inhibitor with the capacity to cross plasma membrane and block subunit assembly and thus superoxide generation. A related yet distinct strategy was used to develop a Nox1-specific inhibitor (**2c**). In this case, the peptide *NoxA1ds* blocks the interaction of Nox1 with NoxA1 necessary for superoxide anion production. In the case of small molecule inhibitors, we have identified two compounds that specifically inhibit Nox2, using high throughput screening, rational design and stringent biochemical assays (**2d**).

2a. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, **Pagano PJ**. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O_2^- and systolic blood pressure in mice. *Circ Res.* 2001;89(5):408-14. PubMed PMID:11532901

2b. Csányi G, Cifuentes-Pagano E, Al Ghouleh I, Ranayhossaini DJ, Egaña L, Lopes LR, Jackson HM, Kelley EE, **Pagano PJ**. Nox2 B-loop peptide, Nox2ds, specifically inhibits the NADPH oxidase Nox2. *Free Radic Biol Med.* 2011; 51(6):1116-25. PubMed PMID: 21586323, PMCID: PMC3204933

2c. Ranayhossaini DJ, Rodriguez AI, Sahoo S, Chen BB, Mallampalli RK, Kelley EE, Csanyi G, Gladwin MT, Romero G, **Pagano PJ**. Selective recapitulation of conserved and non-conserved regions of putative NOXA1 protein activation domain confers isoform-specific inhibition of Nox1 oxidase and attenuation of endothelial cell migration. *J Biol Chem*. 2013; 288(51):36437-50. PubMed PMID: 24187133, PMCID: PMC3868757

2d. Cifuentes-Pagano E, Saha J, Csányi G, Ghouleh IA, Sahoo S, Rodríguez A, Wipf P, **Pagano PJ**, Skoda EM. Bridged tetrahydroisoquinolines as selective NADPH oxidase 2 (Nox2) inhibitors. *Med Chem Comm.* 2013; 4(7):1085-1092. PubMed PMID: 24466406, PMCID: PMC3897123

3. Paracrine role of the vascular adventitia

In the early days of vascular biology research, the adventitia (the outermost layer of a blood vessel) was essentially ignored for its effect on vascular wall biology. It was largely considered to play a structural role in maintenance of vessel tensile strength and as a matrix from which innervating neurons and the vasa vasorum subserved large vessel function. Our seminal research in this area helped bring to light a paracrine signaling role for the adventitia in vascular homeostasis by demonstrating cross-talk between adventitial NADPH oxidase-derived ROS and remote cellular signaling effecting tone and medial hypertrophy and proliferation.

3a. Di Wang H, Hope S, Du Y, Quinn MT, Cayatte A, **Pagano PJ**, Cohen RA. Paracrine role of adventitial superoxide anion in mediating spontaneous tone of the isolated rat aorta in angiotensin II-induced hypertension. *Hypertension.* 1999; 33(5):1225-32. PubMed PMID: 10334816

3b. Rey FE, Li XC, Carretero OA, Garvin JL, **Pagano PJ**. Perivascular superoxide anion contributes to impairment of endothelium-dependent relaxation: role of $gp91^{phox}$. *Circulation.* 2002; 106(19):2497-502. PubMed PMID: 12417549

3c. Weaver M, Liu J, Pimentel D, Reddy DJ, Harding P, Peterson EL, **Pagano PJ**. Adventitial delivery of dominant-negative $p67^{phox}$ attenuates neointimal hyperplasia of the rat carotid artery. *Am J Physiol, Heart Circ Physiol.* 2006; 290(5): H1933-41. PubMed PMID: 16603705

3d. Cascino T, Csanyi G, Al Ghouleh I, Montezano AC, Touyz RM, Haurani MJ, **Pagano PJ**. Adventitia-derived hydrogen peroxide impairs relaxation of the rat carotid artery via smooth muscle cell p38 mitogen-

activated protein kinase. *Antioxid Redox Signal.* 2011; 15(6):1507-15. PubMed PMID: 21126185, PMCID: PMC3151421

4. Role of NADPH oxidase in cardiovascular diseases

With the discovery of non-phagocytic NADPH oxidase in the vasculature and other tissues and the development of specific inhibitors, our lab has pioneered exploration of the role of NADPH oxidase in cardiopulmonary diseases including systemic and pulmonary hypertension.

4a. Cifuentes ME, Rey FE, Carretero OA, **Pagano PJ**. Upregulation of $p67^{phox}$ and $gp91^{phox}$ in aortas from angiotensin II-infused mice. *Am J Physiol Heart Circ Physiol*. 2000 Nov; 279(5):H2234-40. PubMed PMID: 11045958

4b. Jacobson GM, Dourron HM, Liu J, Carretero OA, Reddy DJ, Andrzejewski T, **Pagano PJ**. Novel NADPH oxidase inhibitor suppresses angioplasty-induced superoxide and neointimal hyperplasia of rat carotid artery. *Circ Res.* 2003; 92(6):637-43. PubMed PMID: 12609967

4c. Liu J, Yang F, Yang XP, Jankowski M, **Pagano PJ**. NADPH oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. *Arterioscler Thromb Vasc Biol.* 2003; 23(5):776-82. PubMed PMID: 12637340

4d. Quesada IM, Lucero A, Amaya C, Meijles DN, Cifuentes ME, **Pagano PJ,** Castro C. Selective inactivation of NADPH oxidase 2 causes regression of vascularization and the size and stability of atherosclerotic plaques. *Atherosclerosis*. 2015, 242(2):469-75. PubMed PMID: 26298737, PMCID: PMC4818577

4e. Sahoo S, Meijles DN, Al Ghouleh I, Tandon M, Cifuentes-Pagano E, Sembrat J, Rojas M, Goncharova E, **Pagano PJ .** MEF2C-MYOCD and Leiomodin1 Suppression by miRNA-214 Promotes Smooth Muscle Cell Phenotype Switching in Pulmonary Arterial Hypertension. *PLoS One.* 2016,11(5):e0153780. PubMed PMID: 27144530, PMCID: PMC4856285

5. NADPH oxidase and TSP1

Despite increasing evidence supporting the role of matricellular proteins, such as thrombospondin 1 (TSP1) in vascular disease, their potential to stimulate ROS in vascular tissue and their pathological significance in oxidative stress−mediated vascular dysfunction remained unknown. Data from our group suggested that this protein could act as a ligand for NADPH oxidase activation through the specific engagement of integrin-associated protein CD47. This interaction represents a highly regulated process of ROS stimulation and blood flow regulation promoted through the direct TSP1/CD47-mediated activation of Nox1.

5a. Bauer EM, Qin Y, Miller TW, Bandle RW, Csanyi G, **Pagano PJ**, Bauer PM, Schnermann J, Roberts DD, Isenberg JS. Thrombospondin-1 supports blood pressure by limiting eNOS activation and endothelial-dependent vasorelaxation. *Cardiovasc Res*. 2010; 88(3):471-81. PubMed PMID: 20610415, PMCID: PMC2972685

5b. Csányi G, Yao M, Rodríguez AI, Al Ghouleh I, Sharifi-Sanjani M, Frazziano G, Huang X, Kelley EE, Isenberg JS**, Pagano PJ**. Thrombospondin-1 regulates blood flow via CD47 receptor-mediated activation of NADPH oxidase 1. *Arterioscler Thromb Vasc Biol.* 2012 Dec; 32(12):2966-73. PubMed PMID: 23087362, PMCID: PMC4394361

5c. Rogers NM, Sharifi-Sanjani M, Csányi G**, Pagano PJ**, Isenberg JS. Thrombospondin-1and CD47 regulation of cardiac, pulmonary and vascular responses in health and disease. *Matrix Biol*. 2014; 37:92-101. PubMed PMID: 24418252, PMCID: PMC4096433

5d. Yao M, Rogers NM, Csányi G, Rodriguez AI, Ross MA, St Croix C, Knupp H, Novelli EM, Thomson AW, **Pagano PJ**, Isenberg JS. Thrombospondin-1 activation of signal-regulatory protein-α stimulates reactive oxygen species production and promotes renal ischemia reperfusion injury. *J Am Soc Nephrol.* 2014; 25(6):1171-86. PubMed PMID: 24511121, PMCID: PMC4033366

Complete List of Published Work:
http://www.ncbi.nlm.nih.gov/sites/myncbi/patrick.pagano.1/collections/47859398/public/

D. Research Support

Ongoing Research Support (Selected)

The proposed research project has no overlap with any of my past, current or pending research support.

R01 HL079207-A1, NIH Pagano (PI) 08/14/2015 – 5/31/2019
Reactive Oxygen Species in Vascular Disease
This project aims to open a new field of inquiry by (a) identifying novel pathways involving pulmonary vascular oxidant production leading to vascular thickening and occlusion; and (b) testing promising therapies aimed at abolishing these pathways and alleviating PH and RV failure. **Role: PI.**

R01 HL112914, NIH Pagano/Isenberg (PIs) 8/13/13- 5/31/17
TSP-1 and ROS: CD47 and SIRP-alpha as Mediators of Vascular Dysfunction
This project focuses on the novel activation of NADPH oxidases by thrombospondin1 (TSP1) in vascular smooth muscle and endothelial cells via CD47 and/or SIRP-alpha. The first and second aims characterize the mechanisms by which CD47- and SIRP-alpha mediate cellular ROS production and dysfunction via Nox1 and Nox4, respectively. **Role: Co-PI**

P01 HL103455, NIH Gladwin (PI) 04/01/11 – 03/31/16
Vascular Subphenotypes of Lung Disease
Project #2 – ROS signaling and NOS uncoupling in Pulmonary Vascular Disease
We hypothesize that sub-phenotypes of common diseases, including pulmonary arterial hypertension (PAH), have a profound influence on outcome and responsiveness to therapy. The overarching translational goal of this program is to define common mechanistic and therapeutic pathways for PAH in the context of major lung and systemic diseases, such as COPD and HIV. Our proposed three major projects and two cores, are designed to integrate and synergize fundamental translational research addressing major current and high impact problems in the PAH and advanced lung disease field.
Role: Project Investigator – Project 2

T32 GM08424-21 Pagano (PI) 07/01/2015 – 06/30/2020
Predoctoral Training in Pharmacological Sciences
The purpose of this Predoctoral Training Program is to provide a broad education in areas relevant to the pharmacological sciences and to train students in the techniques, strategies and philosophy of modern biological research.
Role: PI

Completed Research Support (Past 3 years)

R01 HL079207, NIH/NHLBI Pagano (PI) 04/01/07 – 03/31/13
Reactive Oxygen Species in Vascular Disease
This grant focuses on the development of specific isoforms of major vascular NADPH oxidase isoforms (Nox1 and 4) and applies them in the characterization of cyclic stretch induced reactive oxygen species production with respect to balloon angioplasty-induced neointimal proliferation. In essence, this grant is expected to develop novel Nox inhibitors to act as conditional suppressors of individual Nox proteins in the process leading to neointimal growth.
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: WEBER, STEPHEN G

eRA COMMONS USER NAME (credential, e.g., agency login): sweber

POSITION TITLE: Professor of Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Case Western Reserve University, Cleveland, OH	BA	06/1970	Biology and Chemistry
McGill University, Montreal, Quebec	PHD	05/1979	Chemistry

A. Personal Statement

We want to understand chemical events related to normal and pathological brain. Our laboratory develops, refines, and applies new and quantitative methods to learn more about biological systems. The primary goals are (1) the determination of neurotransmitters by microdialysis with fast, capillary, online liquid chromatography (2) the investigation of neuropeptide processing in the extracellular space and (3) to empower others to make better measurements using our methods. We are experienced in capillary chromatography, electrochemical detection, chromatographic theory, mass transport phenomena, electroosmostic flow in brain, small sample handling, peptide chemistry, and live animal experiments. We also have experience in microscopy, organotypic culturing as well as chemical synthesis and purification, statistical methods, and standard biochemical techniques. Our emphasis is on critical assessment of what information can be obtained from a measurement, and how to obtain more accurate information. We have collaborated with the Michael group for several years in the pursuit of better understanding of the limitations of microdialysis. We use that information to develop ideas of how to avoid the limitations. The best of those ideas become new methodological improvements providing more and better information about the brain.

1. Gu H, Varner EL, Groskreutz SR, Michael AC, Weber SG. In Vivo Monitoring of Dopamine by Microdialysis with 1 min Temporal Resolution Using Online Capillary Liquid Chromatography with Electrochemical Detection. Anal Chem. 2015 Jun 16;87(12):6088-94. PubMed PMID: 25970591; PubMed Central PMCID: PMC4835028.
2. Zhang J, Jaquins-Gerstl A, Nesbitt KM, Rutan SC, Michael AC, Weber SG. In vivo monitoring of serotonin in the striatum of freely moving rats with one minute temporal resolution by online microdialysis-capillary high-performance liquid chromatography at elevated temperature and pressure. Anal Chem. 2013 Oct 15;85(20):9889-97. PubMed PMID: 24020786; PubMed Central PMCID: PMC3899587.
3. Zhang J, Liu Y, Jaquins-Gerstl A, Shu Z, Michael AC, Weber SG. Optimization for speed and sensitivity in capillary high performance liquid chromatography. The importance of column diameter in online monitoring of serotonin by microdialysis. J Chromatogr A. 2012 Aug 17;1251:54-62. PubMed PMID: 22771067; PubMed Central PMCID: PMC3419010.
4. Liu Y, Zhang J, Xu X, Zhao MK, Andrews AM, Weber SG. Capillary ultrahigh performance liquid chromatography with elevated temperature for sub-one minute separations of basal serotonin in submicroliter brain microdialysate samples. Anal Chem. 2010 Dec 1;82(23):9611-6. PubMed PMID: 21062014; PubMed Central PMCID: PMC3008768.

B. Positions and Honors

Positions and Employment

1970 - 1974	Hospital Corpsman (E5), United States Navy, Great Lakes, IL
1979 - 1985	Assistant Professor, University of Pittsburgh, Pittsburgh, PA
1985 - 1994	Associate Professor, University of Pittsburgh, Pittsburgh, PA
1991 - 1992	Visiting Scientist Fellow, Swedish Medical Research Foundation, Goteborg
1994 -	Professor of Chemistry, University of Pittsburgh, Pittsburgh, PA
1999 - 2001	Associate Chair, University of Pittsburgh, Pittsburgh, PA
2001 -	Director of Graduate Studies, University of Pittsburgh, Pittsburgh, PA
2009 -	Professor, Clinical and Translational Science, University of Pittsburgh, Pittsburgh, PA

Other Experience and Professional Memberships

1989 -	Contributing Editor, Trends in Analytical Chemistry
1989 - 2004	Editorial Board, Journal of Pharmaceutical and Biomedical Analysis
1991 - 1997	Editor, Comprehensive Analytical Chemistry
2001 - 2004	Editorial Advisory Board, Analytical Chemistry
2001 - 2004	Editorial Board, Talanta
2010 -	Editorial Board, Journal of Chromatography A
2011 -	Editorial Board, Chemical Analysis
2016 - 2019	Editorial Advisory Board, Analytical Chemistry

Honors

1991	Visiting Scientist Fellowship, Swedish Medical Research Council
1998	Fisher Lectureship, Bucknell University
2005	Gold Medal of Assiut University, Assiut University
2008	Frontiers Lectureship, Wayne State University
2008	Pittsburgh Award of the American Chemical Society, American Chemical Society
2012	Excellence in Mentoring, University of Pittsburgh
2015	Palmer Award, Minnesota Chromatography Forum
2016	Dal Nogare Award, Chromatography Forum of the Delaware Valley

C. Contribution to Science

1. Recent work has focused on the development and application of techniques to understand neurochemical function in vitro (organotypic hippocampal slice cultures, OHSCs) and in vivo. We have developed the idea that electroosmotic flow is a better and more controllable method for tissue perfusion than pressure flow. We are applying that to the determination of ectopeptidase activity and thiol metabolism in OHSCs.

    a. Guy Y, Muha RJ, Sandberg M, Weber SG. Determination of zeta-potential and tortuosity in rat organotypic hippocampal cultures from electroosmotic velocity measurements under feedback control. Anal Chem. 2009 Apr 15;81(8):3001-7. PubMed PMID: 19298057; PubMed Central PMCID: PMC2736137.

    b. Xu H, Guy Y, Hamsher A, Shi G, Sandberg M, Weber SG. Electroosmotic sampling. Application to determination of ectopeptidase activity in organotypic hippocampal slice cultures. Anal Chem. 2010 Aug 1;82(15):6377-83. PubMed PMID: 20669992; PubMed Central PMCID: PMC2920223.

    c. Wu J, Sandberg M, Weber SG. Integrated electroosmotic perfusion of tissue with online microfluidic analysis to track the metabolism of cystamine, pantethine, and coenzyme A. Anal Chem. 2013 Dec 17;85(24):12020-7. PubMed PMID: 24215585; PubMed Central PMCID: PMC3899583.

    d. Ou Y, Wu J, Sandberg M, Weber SG. Electroosmotic perfusion of tissue: sampling the

extracellular space and quantitative assessment of membrane-bound enzyme activity in organotypic hippocampal slice cultures. Anal Bioanal Chem. 2014 Oct;406(26):6455-68. PubMed PMID: 25168111; PubMed Central PMCID: PMC4184924.

2. Capillary liquid chromatography, cLC, is ideally suited to the small, complex samples obtained from tissue culture perfusion or microdialysis in vivo. However, improvements can make it operate faster, with higher sensitivity and greater flexibility.

   a. Groskreutz SR, Horner AR, Weber SG. Temperature-based on-column solute focusing in capillary liquid chromatography reduces peak broadening from pre-column dispersion and volume overload when used alone or with solvent-based focusing. J Chromatogr A. 2015 Jul 31;1405:133-9. PubMed PMID: 26091787; PubMed Central PMCID: PMC4488902.
   b. Liu Y, Zhang J, Xu X, Zhao MK, Andrews AM, Weber SG. Capillary ultrahigh performance liquid chromatography with elevated temperature for sub-one minute separations of basal serotonin in submicroliter brain microdialysate samples. Anal Chem. 2010 Dec 1;82(23):9611-6. PubMed PMID: 21062014; PubMed Central PMCID: PMC3008768.
   c. Zhang J, Liu Y, Jaquins-Gerstl A, Shu Z, Michael AC, Weber SG. Optimization for speed and sensitivity in capillary high performance liquid chromatography. The importance of column diameter in online monitoring of serotonin by microdialysis. J Chromatogr A. 2012 Aug 17;1251:54-62. PubMed PMID: 22771067; PubMed Central PMCID: PMC3419010.
   d. Groskreutz SR, Weber SG. Temperature-assisted on-column solute focusing: a general method to reduce pre-column dispersion in capillary high performance liquid chromatography. J Chromatogr A. 2014 Aug 8;1354:65-74. PubMed PMID: 24973805; PubMed Central PMCID: PMC4100596.

3. Collaboration with Prof. Adrian Michael, who is working on minimizing the impact of microdialysis-induced probe damage on microdialysis determinations of neurotransmitter concentrations in vivo in combination with our development of fast and sensitive capillary liquid chromatography will lead to better, more informative neurochemical investigations.

   a. Jung MC, Shi G, Borland L, Michael AC, Weber SG. Simultaneous determination of biogenic monoamines in rat brain dialysates using capillary high-performance liquid chromatography with photoluminescence following electron transfer. Anal Chem. 2006 Mar 15;78(6):1755-60. PubMed PMID: 16536408; PubMed Central PMCID: PMC1488825.
   b. Jaquins-Gerstl A, Shu Z, Zhang J, Liu Y, Weber SG, Michael AC. Effect of dexamethasone on gliosis, ischemia, and dopamine extraction during microdialysis sampling in brain tissue. Anal Chem. 2011 Oct 15;83(20):7662-7. PubMed PMID: 21859125; PubMed Central PMCID: PMC3193568.
   c. Zhang J, Jaquins-Gerstl A, Nesbitt KM, Rutan SC, Michael AC, Weber SG. In vivo monitoring of serotonin in the striatum of freely moving rats with one minute temporal resolution by online microdialysis-capillary high-performance liquid chromatography at elevated temperature and pressure. Anal Chem. 2013 Oct 15;85(20):9889-97. PubMed PMID: 24020786; PubMed Central PMCID: PMC3899587.
   d. Gu H, Varner EL, Groskreutz SR, Michael AC, Weber SG. In Vivo Monitoring of Dopamine by Microdialysis with 1 min Temporal Resolution Using Online Capillary Liquid Chromatography with Electrochemical Detection. Anal Chem. 2015 Jun 16;87(12):6088-94. PubMed PMID: 25970591; PubMed Central PMCID: PMC4835028.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 GM044842-25 WEBER, STEPHEN G. (PI) 05/01/91-04/30/19
Sensitive and Selective Detection of Peptides

Role: PI

R01 MH104386-02 WEBER, STEPHEN G. (PI) 08/15/14-05/31/18
Fast Online Microdialysis/Liquid Chromatography for Monoamine Neurotransmitters
Role: PI

1608757, National Science Foundation WEBER, STEPHEN G (PI) 08/01/16-07/31/19
Expanding the Use of Liquid Chromatography Through Active Temperature Control
This project is funded by the Chemical Measurement and Imaging Program of the Division of Chemistry at the National Science Foundation. Professor Stephen Weber and colleagues at the University of Pittsburgh address limitations of liquid chromatography. The team is improving the ability of liquid chromatography techniques to distinguish one substance from another, as well as improving the ability to measure trace concentrations in a variety of samples. Chromatography is based on the principle of partitioning, the tendency of a substance to prefer an oily substance to water-based solution. The outcomes of this project include obtaining more and better information from samples with lower cost and lower environmental burden than has been previously realized. Collaborations with scientists in the fields of proteomics (for example, health, pharmaceuticals, and biochemistry research) and two-dimensional liquid chromatography (for example, pharmaceuticals, chemical industry, agriculture, and the environment) demonstrate the effectiveness of the project goals. The broader impacts are evidenced in that improvements in chromatographic processes that are useful across many fields and applications. Liquid chromatography is used in virtually every industry, for example, environmental, pharmaceutical, nutritional, forensic, toxicology, polymers, chemicals, and cosmetics. A Girl Scout merit badge project is also being developed by Dr. Weber to explore chromatography methods and its uses. The project uses temperature control of the column to achieve greater solute focusing and to control retention in a predictable way. Retention enthalpies, which control the sensitivity of a particular compound to changes in temperature, are determined for a number of related organic compounds. These data are used to determine molecular fragment enthalpies. The temperature dependence of the retention of novel compounds are then predicted. Software to predict chromatographic behavior in the face of changing column temperature aids in chromatographic method development. The temperature control, when used to aid solute focusing, significantly improves concentration detection limits.
Role: PI

Completed Research Support

R01 GM066018-12 WEBER, STEPHEN G. (PI) 07/01/03-07/31/16
Single Cell Electroporation
Role: PI

R21 MH083134-02 WEBER, STEPHEN G. (PI) 04/01/08-02/28/11
Serotonin Transporter Kinetics In Vivo by Microdialysis/Capillary UPLC
Role: PI

R41 GM067325-01 WEBER, STEPHEN G. (PI) 02/03/03-02/02/06
Supported Fluorous Lipids for Triphasic Reactions
Role: PI

PHS Fellowship Supplemental Form

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction

1. Introduction
(RESUBMISSION)

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training*	Applicant's_Background_and_Goals_for_Fellowship_Training.pdf

Research Training Plan Section

3. Specific Aims*	Specific_Aims.pdf
4. Research Strategy*	Research_Strategy.pdf
5. Respective Contributions*	Respective_Contributions.pdf
6. Selection of Sponsor and Institution*	Selection_of_Sponsor_and_Institution.pdf
7. Progress Report Publication List (RENEWAL)	
8. Training in the Responsible Conduct of Research*	Training_in_the_Responsible_Conduct_of_Research.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	Sponsor_and_Co-Sponsor_Information.pdf
10. Letters of Support from Collaborators, Contributors and Consultants	Letters_Wesalo.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	Additional_Educational_Information.pdf

Other Research Training Plan Section

Human Subjects

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? ☐ Yes ☑ No

12. Human Subjects Involvement Indefinite?

13. Clinical Trial?

14. Agency-Defined Phase III Clinical Trial?

15. Protection of Human Subjects

16. Data Safety Monitoring Plan

17. Inclusion of Women and Minorities

18. Inclusion of Children

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? ☑ Yes ☐ No

19. Vertebrate Animals Use Indefinite? ☐ Yes ☑ No

Funding Opportunity Number: PA-16-305. Received Date: 2017-04-10T14:49:40.000-04:00

PHS Fellowship Supplemental Form

20. Are vertebrate animals euthanized?	☑ Yes ☐ No
If "Yes" to euthanasia Is method consistent with American Veterinary Medical Association (AVMA) guidelines?	☑ Yes ☐ No
If "No" to AVMA guidelines, describe method and provide scientific justification	
21. Vertebrate Animals	Vertebrate_Animals.pdf

Other Research Training Plan Information

22. Select Agent Research

23. Resource Sharing Plan

24. Authentication of Key Biological and/or Chemical Resources

PHS Fellowship Supplemental Form

Additional Information Section

25. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ☐ Yes ☑ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

26. Alternate Phone Number: 4103562605

27. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):
OTH: Other	MD/PhD	05/2023

28. Field of Training for Current Proposal*: 539 Chemistry, Other

29. Current Or Prior Kirschstein-NRSA Support?* ☐ Yes ☑ No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)

30. Applications for Concurrent Support?* ☐ Yes ☑ No

If yes, please describe in an attached file:

31. Citizenship*

U.S. Citizen — U.S. Citizen or Non-Citizen National? ☑ Yes ☐ No

Non-U.S. Citizen
- ☐ With a Permanent U.S. Resident Visa
- ☐ With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, please also check here. ☐

32. ☐ Change of Sponsoring Institution — Name of Former Institution:*

PHS Fellowship Supplemental Form

Budget Section

All Fellowship Applicants:

1. Tuition and Fees*:

☐ None Requested ☑ Funds Requested

Year 1	$26,666.00
Year 2	$26,666.00
Year 3	$26,666.00
Year 4	$26,666.00
Year 5	$0.00
Year 6 (when applicable)	$0.00
Total Funds Requested:	$106,664.00

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary: Amount | Academic Period | Number of Months

3. Stipends/Salary During First Year of Proposed Fellowship:

a. Federal Stipend Requested: Amount | Number of Months

b. Supplementation from other sources: Amount | Number of Months

Type (sabbatical leave, salary, etc.)

Source

Appendix

Tracking Number: GRANT12384848 Funding Opportunity Number: PA-16-305. Received Date: 2017-04-10T14:49:40.000-04:00

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.

SPECIFIC AIMS

Despite substantial progress in treatment over the past few decades,[1] myocardial infarctions (MIs) kill 120,000 Americans every year.[2] The best available treatment today is timely reperfusion of the heart using percutaneous intervention (PCI).[1,3] Upon reperfusion, however, the return of oxygenated blood stuns the myocardium, induces arrhythmias, and destroys additional myocytes. [4] This injury, known as ischemia-reperfusion (I/R) injury, explains roughly half of infarct size.[5,6] Molecularly, the pathology stems from a sudden burst of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$) and its rapidly formed dismutation product, hydrogen peroxide (H_2O_2).[7,8] Acutely, ROS damage biomolecules and induce cell death via multiple signaling pathways.[9] Chronically, they induce remodeling that leads to heart failure.[10–12] Antioxidants could ameliorate the damage, but trials of antioxidants have proven disappointing,[13] as they fail to target the cause of the injury effectively[14,15] and may perturb cell signaling in uninjured tissues.[9,16]

My long-term goal is to address the lack of effective therapies for MI and to develop an approach that mitigates damaging ROS production in the reperfused myocardium without perturbing redox balance elsewhere. The overall objective of this proposal is to develop a novel anti-ROS approach in which pathologic levels of H_2O_2 activate small molecule inhibitors of ROS generation exclusively in infarcted areas. Much of the H_2O_2 in I/R injury (and in subsequent heart failure) comes from NADPH oxidase 2 (Nox2) activity.[10,11,17,18] When activated, the proposed agents will inhibit Nox2. To achieve selectivity in drug activation, I will leverage my group's expertise in conditional control of cellular processes using "caging groups." These are nontoxic protecting groups that block a pharmacophore's function until removed via a chemical trigger. My proposed molecules use boronate ester caging group chemistry for selective activation in cells with elevated H_2O_2 levels. The activation process reduces H_2O_2 to water, and generates only non-toxic by-products. **Completion of the following specific aims will test my hypothesis that a novel boronate-based triggering mechanism will allow development of tissue-specific Nox2 inhibitors that selectively target cells accruing pathologic H_2O_2 levels.**

Specific Aim 1. Development and cell-based testing of H_2O_2-responsive Nox2 inhibitors. To ameliorate I/R injury in the heart without perturbing redox balance elsewhere, I will cage known Nox2 inhibitors to become active only in infarcted areas containing high levels of H_2O_2. **Sub-Aim 1A**: Synthesize a series of boronate-caged benzoisothiazolone Nox2 inhibitors. **Sub-Aim 1B**: Determine the kinetics of decaging and activation of the caged inhibitors. **Sub-Aim 1C:** Quantify inhibitor activation, ROS production, and cell survival/cardiac enzyme release upon treatment with caged inhibitors. Successful completion of Aim 1 will afford rapidly-activated Nox2 inhibitors that selectively inhibit ROS production in cells containing pathologic levels of H_2O_2.

Specific Aim 2. Evaluate caged Nox2 inhibitors in preventing acute and chronic injury via targeting injured tissue in a rat model of myocardial I/R injury. To validate this targeted therapy for I/R injury, I will determine the extent to which boron-caged Nox inhibitors protect the heart from excessive ROS production and resulting injury. **Sub-Aim 2A**: Measure Nox2 activity, infarct size, and apoptosis in the ischemic area in a rat model of myocardial I/R with and without caged Nox inhibitor treatment. **Sub-Aim 2B**: Quantify the extent of reductive stress in the uninjured myocardium in animals treated with the caged prodrug or with the active compound in order to assess specificity of the developed approach. **Sub-Aim 2C**: Assess cardiac function 6 weeks post-I/R injury in treated rats. Successful completion of Aim 2 will gauge the potential for caged Nox2 inhibitors to selectively reduce acute myocardial injury and to prevent subsequent heart failure.

Overall Impact: Although treatments are available to minimize damage from ischemia in MI, no treatments *selectively* target damage from I/R injury. As a first step in developing such treatments, I am using a novel strategy that targets injured cells by applying a nontoxic H_2O_2-triggered protecting group. If successful in the proposed studies, these compounds could reduce acute injury and chronic sequelae of MI without perturbing the redox balance in uninjured tissues. This work could provide a generally applicable starting point for treating other conditions involving I/R injury, including stroke, peripheral artery disease, and transplant surgery.

Contribution to training: The proposed research will build on my experience synthesizing caged biologically-active compounds and allow me to learn how to work with cell culture and animal models of I/R injury through my network of expert collaborators and consultants. Through this project, I will learn to develop new approaches to treating unmet clinical needs and to evaluate data from clinically-relevant cellular and animal models, which will prepare me for a career as a physician-scientist at the interface of chemistry and cardiology.

RESEARCH STRATEGY

A. SIGNIFICANCE

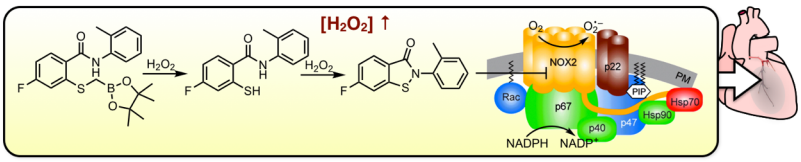


Fig. 1. Approach. A hydrogen peroxide (H_2O_2)-responsive prodrug will be synthesized. In the reperfused myocardium, which has high levels of H_2O_2, the drug will become activated in a two-step sequence, consuming two equivalents of H_2O_2 in the process. The active drug will inhibit Nox2 by perturbing subunit assembly and inhibiting translocation to the plasma membrane (Smith et al., 2012), turning off a major source of ROS production that drives I/R injury. In contrast, the prodrug will remain inactive unaffected tissues. Active Nox2 complex image adapted from Altenhöfer et al., 2015.[80]

Despite tremendous advances in treatment for myocardial infarction over the past few years,[1] mortality has held constant[19] at a rate approaching 10%,[20] and nearly one-fourth of survivors develop heart failure in the ensuing months.[6] Reperfusion, the standard-of-care, paradoxically induces an injury that accounts for up to 50% of infarct size[6] because rapid reintroduction of oxygen to ischemic tissue results in a burst of enzymatic overproduction of harmful reactive oxygen species (ROS).[7,8] The resulting ROS can oxidize proteins, lipids, carbohydrates, and nucleic acids, resulting in irreversible functional losses.[9] Although several enzymes[21] (and the mitochondrial electron transport chain)[22] produce ROS as a by-product, the NADPH oxidase (Nox) family comprises the only enzymes that produce ROS as the primary function,[23,24] implicating Nox proteins as key targets to reduce ROS-mediated tissue damage in ischemia-reperfusion (I/R) injury.

The Nox2 isoform in particular is a key player in I/R injury. At the moment of reperfusion, high Nox2 activity in humans increases the risk that the coronary microvasculature remains obstructed (the “no-reflow” phenomenon)[25,26] by promoting a thromboinflammatory state.[27] Afterwards, Nox2 produces high, sustained levels of ROS for several hours post-reperfusion,[17] leading to further oxidative damage.[28] When Nox2 is knocked out, less vascular oxidative stress occurs in restenosis models,[29] and pharmacologic Nox inhibitors reduce oxidative stress in models of vascular disease.[30] Further, humans with loss-of-function mutations in Nox2 are protected from I/R-induced endothelial dysfunction that contributes to myocardial death.[31] Taken together, these findings suggest that Nox2 is an attractive target for inhibition to ameliorate acute myocardial I/R injury in patients.

Not only does Nox2 drive acute I/R injury, it also drives the chronic injury in the ensuing weeks. Persistent ROS generation from Nox2 in cardiac myocytes causes chronic remodeling that compromises ventricular function and contributes to arrhythmias after MIs.[10–12] Arrhythmias and heart failure in the months following an MI contribute to much of the morbidity and mortality.[32] Current therapeutics for heart failure implicate Nox in the pathophysiology. Angiotensin-converting enzyme (ACE) inhibitors decrease Nox activity in in the vasculature,[33] which may underlie their benefits to heart failure patients.

Clinicians need effective treatments that reduce ROS levels after MI, but no therapies for I/R injury have been successfully translated to the clinic.[15] Although many antioxidant therapies have proven successful in animals, results in humans have been disappointing.[15] Why have these therapies failed? Exogenous antioxidants may reduce ROS levels too slowly imprecisely for clinical benefit. Endogenous antioxidant enzymes (superoxide dismutases, catalase, peroxidases, and peroxiredoxins) have such high activity levels that exogenous antioxidants are unlikely to lower steady-state ROS levels.[14] Further, antioxidant compounds react with ROS too slowly to prevent damage,[15] so even targeted antioxidants[34–36] are not likely to yield clinical benefit on their own. Inhibiting ROS generation in the first place, on the other hand, will likely prove more effective.[37] But since ROS play important homeostatic roles throughout the body,[9] including cell signaling,[38] regulation of cerebral vascular caliber,[39,40] regulation of metabolism,[41] and innate immunity,[37] *an approach that selectively blocks ROS generation in the reperfused myocardium without perturbing cell signaling elsewhere is needed.* We are developing a targeted approach to treating myocardial reperfusion injury by synthesizing H_2O_2-triggered small molecule inhibitors of Nox2, a ROS producer that drives I/R injury both acutely[17,29,5] and in the chronic phase.[10,11]

Existing Nox inhibitors suffer from nonspecificity, off-target effects, and problems in drug delivery.[42] Since Nox enzymes play important homeostatic roles throughout the body, and since ROS from Nox4 may be cardioprotective in I/R injury[43,44], the ideal Nox inhibitor should be isoform-specific for Nox2. Peptidic Nox inhibitors, such as Nox2ds-tat,[45,46] improve isoform specificity, but suffer from poor ADME (absorption, distribution, metabolism, and excretion) characteristics that hamper their clinical use,[47] particularly for a treatment lasting several weeks post-MI. Thus, ideal therapeutics would be small molecules that can be site-specifically activated in injured tissue with isoform specificity that excludes Nox4. **THR101** is a promising starting point. **THR101** inhibits Nox2 with sub-micromolar potency (EC_{50} = 300 nm); its potency is 10-fold lower for Nox1 and >27-fold lower for Nox4,[48] indicating favorable isoform specificity. **THR101** and analogues, however, inhibit off target enzymes (PHOSPHO1[49] and phosphomannose isomerase[50]) with μM potency, so keeping activation inside injured tissues takes on added importance. **THR101** will serve as the starting point for our approach.

The approach proposed here will selectively target tissues with pathologic levels of H_2O_2 (>100 μM),[51–54] as is found in reperfused myocardium[55] in a pattern that correlates well with the infarct.[56] Away from this area, the nanomolar concentrations of H_2O_2 present under physiological conditions[57] will not trigger Nox inhibition. We will achieve specificity by developing a chemical functionality responsive to H_2O_2 at pathologic levels. H_2O_2 will release the Nox2 inhibitor. Additionally, the H_2O_2-sensitive functionality will consume two equivalents of harmful H_2O_2 in the process, and will release only non-toxic by-products. The resulting locally- and temporally-defined Nox inhibition will greatly enhance the therapeutic window of existing inhibitors.

Our proposal builds on several reports[58–61] that use boronates to trigger drug release in the presence of H_2O_2, including a report from our group.[62] Existing chemistries, however, generate toxic quinone methide[63,64] or acrolein[65] by-products, precluding clinical use. Our lab—in collaboration with the Floreancig lab—recently developed a facile method for preparing boron-masked functionalities that react rapidly with H_2O_2 in oxidatively-stressed cells to release alcohols, aldehydes, and ketones without producing toxic by-products.[59]

In this proposal, we will expand the scope of our approach to mask the thiol of **THR101**.[48] SAR studies have demonstrated that **THR101** requires divalent sulfur to be in a benzisothiazolone motif for inhibitory activity;[48] hence, we will cage the sulfur with a boronate ester to prepare a prodrug that will be deprotected and oxidatively cyclized[66] in injured tissues accumulating harmful levels of H_2O_2. Prodrug activation will consume two equivalents of H_2O_2 and the released inhibitor will block Nox2 activity, thereby preventing any further production of tissue-damaging ROS in I/R injury. **The proposed research is significant because it extends cutting-edge chemistry to develop a targeted therapy for I/R injury, arrhythmogenesis, and heart failure after MI**.

B. APPROACH

B.1. Preliminary Results

B.1.1. Synthesis and testing of model compound. H_2O_2-responsive **THR101** analogue **4a** was prepared from commercially-available starting materials (**Fig. 2**). Next, model compound **4a** was treated with low millimolar concentrations of H_2O_2, and cyclization kinetics were determined using nuclear magnetic resonance spectroscopy (NMR, **Fig. 3**).[59,67] NMR showed complete consumption of starting material to form cyclized product **5** in under 20 minutes, presumably through a hemithioacetal intermediate. This assay is still under development, and the yield of **5** has yet to be determined. **5** is the predominant product, however, and as the intermediate hemithioacetal was not observed, it is not so stable as to preclude prodrug activation. These preliminary results suggest that the proposed inhibitors are likely to become activated rapidly in the presence of pathophysiologically-relevant levels of H_2O_2.

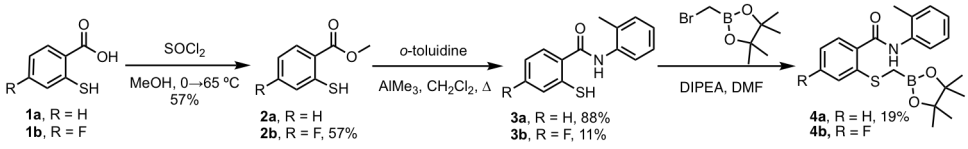


Fig. 2. Preparation of model compound 4a and caged THR101 (4b). Esterification of commercially-available carboxylic acid **1a** with thionyl chloride in the presence of methanol affords methyl ester **2b**. Treatment of commercially-available **2a** and **2b** with *o*-toluidine in the presence of trimethylaluminum generates amides **4a** and **4b**. Finally, treatment of **3b** with $BrCH_2Bpin$ in the presence of DIPEA affords model compound **4a**. A similar sequence is used to prepare caged **THR101** (**4b**).

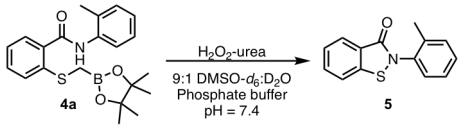


Fig. 3. Determination of activation kinetics of a model compound. Boronate ester **1** was treated with H_2O_2, and the activation of the compound was monitored by ^{1}H-NMR. Full activation took <20 min.

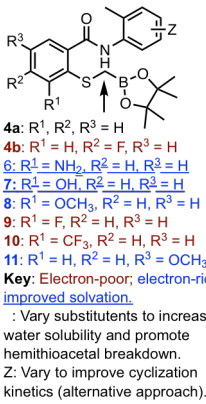
B.1.2. Synthesis of the caged analogue of published Nox2 inhibitor THR101. With the model compound in-hand, further work has been directed to the preparation of caged **THR101** (**4b**) (**Fig. 2**), which is nearly complete. During the first reaction in this sequence, the thiol **2b** and its corresponding disulfide were formed in a 42:58 ratio, suggesting that fluorination *meta* to the thiol substantially increases the propensity of these compounds to oxidize. Consequently, it is likely that **4b** and analogues are likely to oxidatively cyclize even faster than **4a**, making them more useful treating the acute reperfusion injury induced by percutaneous intervention.[68]

B.2. Specific Aim 1: Development and cell-based testing of H_2O_2-responsive Nox2 inhibitors.

B.2.1. Sub-Aim 1A: Synthesize a series of boronate-caged benzoisothiazolone Nox2 inhibitors. *Rationale*: To treat I/R injury in the acute setting, we need a Nox2 inhibitor that rapidly responds to H_2O_2. The initial lead compound **4b** may not have optimized solubility and cyclization kinetics. The first step of activation—oxidation of the boronate ester to the hemithioacetal—proceeds to completion in under 5 minutes for a variety of substrates,[67] consuming one equivalent of H_2O_2 in the process. This step was similarly rapid for model compound **4a** (see **C.1** and **Fig. 3**). By contrast, the second step, oxidative cyclization to the active Nox inhibitor, takes 90 minutes rapidly at room temperature in the literature.[66] Although this is likely to proceed nearly four times as fast at body temperature, this may be too slow for treating acute I/R injury. Since rapid activation kinetics are essential

for use in the acute setting, we will explore several analogues likely to cyclize even faster while maintaining inhibitory potency and isoform selectivity for Nox2.

Experimental Design: We hypothesize that substitution near to the thiol—positions highly tolerant of substitution without diminishing potency[48]—could enhance cyclization kinetics through 3 potential mechanisms. As our synthesis (**Fig. 2**) is highly modular, different commercially-available or easily-prepared[69,70] analogues of **1a** may be used to vary the substitution here to test the three hypotheses. First, improved transition state alignment of the thiol with an H_2O_2 oxygen atom can enhance the rate by several orders of magnitude.[71] Thus, positioning a hydrogen bond donor nearby to stabilize H_2O_2, as in proposed compounds **6** and **7**, is likely to improve cyclization kinetics (**Fig. 4**). Second, theoretical calculations show that thiol solvation plays a key role in the bimolecular rate-determining step of the oxidation.[72,73] Consequently, adding hydrophilic substituents to promote solvation (as in compounds **6** and **7**) is likely to increase the rate of cyclization. Third, modulating the electronics of the thiol could improve reactivity. Lowering the pK_a via the inductive effect, as in compounds **4b**, **9**, and **10**, will promote ionization to the more reactive thiolate,[73] which promotes sulfenic acid formation[74,16] and therefore cyclization. On the other hand, addition of electron donors to the pi system, as in **6**, **7**, **8**, and **11**, is will increase the nucleophility of the thiol, which could speed up its reaction with H_2O_2. A control compound with a methylene group replacing the amide nitrogen will be prepared to establish the role of cyclization.



4a: R^1, R^2, R^3 = H
4b: R^1 = H, R^2 = F, R^3 = H
6: R^1 = NH_2, R^2 = H, R^3 = H
7: R^1 = OH, R^2 = H, R^3 = H
8: R^1 = OCH_3, R^2 = H, R^3 = H
9: R^1 = F, R^2 = H, R^3 = H
10: R^1 = CF_3, R^2 = H, R^3 = H
11: R^1 = H, R^2 = H, R^3 = OCH_3
Key: Electron-poor; electron-rich; improved solvation.
: Vary substitutents to increase water solubility and promote hemithioacetal breakdown.
Z: Vary to improve cyclization kinetics (alternative approach).

Fig. 4. Proposed analogues of caged THR101.

Anticipated Results, Possible Pitfalls, and Alternative Approach: We anticipate that several compounds in this series will have enhanced cyclization kinetics over model compound **4a** and parent compound **4b**, though it is difficult to speculate about which of the three competing theoretical hypotheses will be empirically correct. In the unlikely possibility that none of the proposed modifications enhances the kinetics, modifications to promote nucleophilic attack of the amide nitrogen on the sulfur atom by altering the attached ring can be made without diminishing inhibitory potency.[48] A pitfall so far has been the low yield obtained for coupling ester **2b** to *o*-toluidine to afford amide **3b**. Alternative conditions have been validated for this transformation in the literature.[75,76]

B.2.2. Sub-Aim 1B: Determine the kinetics of decaging and activation of the caged inhibitors. *Rationale:* To select the best compound for biological testing, we will quantify the kinetics and yield of the activation of the caged Nox2 inhibitors synthesized in Sub-Aim 1A. The resulting data will illustrate the relationship between the electronics of the thiol and the rate of oxidative cyclization, providing information about the hotly-debated[71,77] and biologically-important[16] mechanism by which H_2O_2 oxidizes thiols.

Experimental Design: Kinetics will be determined using NMR as in **B.1.1**.[59,67,78] ^{1}H NMR spectra will be acquired every 32 scans (every 3'24"). Compounds **4b**, **6**-**11**, and the negative control compound will be dissolved in 9:1 DMSO-d_6:D_2O with pH 7.4 phosphate buffer. After acquiring a baseline, repeated measurements will begin after adding H_2O_2. Final concentrations will be 20 mM for the compounds being tested and 100 μM for H_2O_2, as is found in the reperfused myocardium.[55] We have observed significant downfield shifts in the aryl hydrogens upon cyclization, making it easy to measure Nox inhibitor activation. Activation will be confirmed using ^{11}B NMR.[67]

Anticipated Results, Possible Pitfalls, and Alternative Approach: We expect that some of the proposed inhibitors **6**-**11** will cyclize faster in with greater yields than parent compound **4b**, though it is difficult to predict which hypothetical mechanism of rate enhancement will prevail (see **B.2.2**). The pitfall of limited aqueous solubility became evident during preliminary NMR experiments (see **B.1.1**), which necessitated 90% DMSO to dissolve the model compound **4a**. To get around this limitation, we envision two approaches. First, heating the sample and spectroscope bore to 37 ºC is likely to improve solubility (and is also more physiologically relevant than room temperature). Second, we predict that adding a polar ammonium or sulfonate group on a short alkyl chain at the α-boryl methylene position will enhance water solubility (**Fig. 4**, arrow). This modification is likely to increase the rate of hemiacetal collapse (and, consequently, the rate of Nox2 inhibitor activation), improving the compound for use in the acute setting.

B.2.3. Sub-Aim 1C: Quantify inhibitor activation, ROS production, and cell survival/cardiac enzyme release upon treatment with caged inhibitors. *Rationale*: These cell culture experiments are crucial next steps in determining the spatial control and therapeutic potential of our approach. I/R injury may be modeled by subjecting cells to hypoxia-reoxygenation. Hypoxia-reoxygenation increases Nox2's ROS-generating activity in cultured myocytes,[43] making this a good model for I/R injury. Further, Nox2 knockdown has been shown to increase cell survival during I/R; by contrast, knockdown of both Nox2 and Nox4 *decreases* survival,[43,44] underscoring the need for a targeted Nox inhibitor that is selective for Nox2 and activated only in tissues exposed to pathologic levels of H_2O_2.[47,79,80]

Cultured cardiac myocytes[81,82] and endothelial cells (ECs)[83] are useful, well-validated models for studying pathophysiology. ECs, which are key players in reperfusion injury, express high levels of Nox2[84] that generates a great deal of diffusible H_2O_2 in reperfusion injury.[55] As both ECs and myocytes are involved in the Nox2-driven component of cardiac reperfusion injury, a co-culture of ECs and cardiac myocytes[85] will provide a sophisticated and relevant cell culture model for testing our approach (**Fig. 5A**).

Typical tissue culture hypoxia-reoxygenation models do not recapitulate the rapid changes in tissue oxygen tension that occur in myocardial I/R. The ischemic injury results from cause sudden obstructions to coronary blood flow due to vasospasms, hemorrhage into plaques, and/or luminal thrombi,[87,88] resulting in acute myocardial oxygen deprivation.[68] Reperfusion injury results when PCI rapidly restores oxygen by physically recanalizing the stenotic vessel. Unlike real-life I/R injury, existing hypoxia-reoxygenation models change oxygen levels slowly and gradually (**Fig. 5A**). We propose taking the ischemic stroke I/R injury apparatus from our collaboration with Dr. Weber and applying it to myocardial I/R (**Fig. 5B**). This setup changes tissue oxygen levels nearly instantaneously.[89] *We hypothesize that our proposed inhibitors will activate specifically in cultured cells exposed to hypoxia-reperfusion, where they will downregulate ROS production, improve cell survival, and decrease cardiac enzyme release*

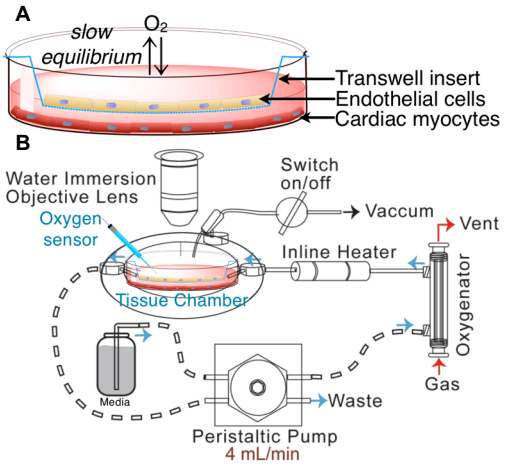


Fig. 5. Proposed hypoxia-reoxygenation model. Primary cardiac myocytes will be isolated from Sprague Dawley rats.[86] Extrapolating from growth curves, 2 rats will be needed to obtain the 1 to 2 x 10^8 cells required for each experiment.[81] Coronary artery ECs will be obtained from American Type Culture Collection (ATCC PCS-100-020). Published hypoxia-reoxygenation co-culture models[85] (**A**) change tissue oxygen tension by changing the gas composition in the incubator, resulting in gradual equilibration between the gas, culture medium, and cells that slowly changes oxygen tension, in contrast to rapid changes in real-life I/R. Our proposed model (**B**) takes the Weber group's recent system for modeling I/R injury in stroke[89] and applies it to myocardial I/R. This system can change tissue oxygen tension from >700 mmHg to <20 mmHg in under 30 seconds, recapitulating real-life I/R with unprecedented accuracy[89]. This system pumps media through a microfluidic oxygenator. The media is warmed and gently superfused over cultured tissue, and its oxygen level is constantly monitored. Vacuum aspiration can rapidly remove media to allow for rapid changes in oxygen tension. Mindful that shear stress from short-term fluid flow can activate endothelial Nox2,[114] we will minimize flow rates over the endothelial cell layer during steady-state perfusion to reduce this source of Nox2 activation that is extraneous to the I/R injury model. Image adapted from Yin et al. 2015.

Experimental Design: Three experiments will be run using our EC-cardiac myocyte co-culture system: we will quantify prodrug activation, ROS production, and cell survival/cardiac enzyme release upon treatment with caged inhibitors. Our group recently published a fluorophore that becomes activated in cells stimulated to produce ROS via Nox2 (**Fig. 6**).[67] The fluorophore is activated by H_2O_2-induced boronate oxidation in a mechanism identical to proposed Nox2 inhibitors. First, we will quantify inhibitor activation in our rapid hypoxia-reoxygenation model by using confocal microscopy and quantifying signal from the fluorescent probe. Second, we will measure the extent to which **6** (or the best inhibitor identified in Sub-Aim 1B) lowers ROS production in hypoxia-reoxygenation. Superoxide levels will be measured using the SOD-inhibitible cytochrome c assay[45,46,90] and H_2O_2 levels with Amplex Red.[90,91] Third, we will evaluate our inhibitors' abilities to protect cardiac myocytes from injury. We will quantify survival using CellTiter Blue. As an additional clinically-relevant marker of myocyte injury,[92,93] we will measure the amount of creatine kinase-MB and lactate dehydrogenase[94] that dying cells release. We will test positive control compound **5** to gauge the importance of the boronate cage.

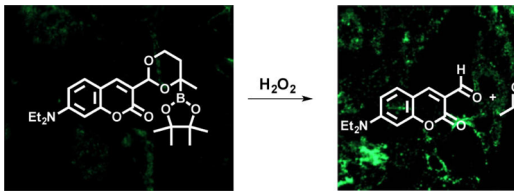


Fig. 6. H_2O_2-responsive fluorophore activation. This fluorophore responds to H_2O_2 generated by HeLa cells treated with phorbol 12-myristate 13-acetate (PMA), a well-known activator of Nox2. Image adapted from Hanna et al. 2016.

Anticipated Results, Potential Pitfalls, and Alternative Approach: We anticipate rapid Nox2 inhibitor activation, decreased ROS production, improved survival, and decreased cardiac enzyme leakage in cells treated with the proposed inhibitors and subjected to hypoxia-reoxygenation. As an alternative approach, in the unlikely situation that our cell culture model does not induce inhibitor activation, a well-validated line of COS-22 cells stably transfected with Nox2 and all subunits required for activity is available

from Dr. Pagano.[46] These cells may be used in place of ECs, as they produce high levels of diffusible[23] H_2O_2 in response to hypoxia-reoxygenation. They also are expected to have a lesser response to shear stress from fluid flow than ECs. As another pitfall, if the laser in confocal microscopy is found to induce excessive cellular damage, one of the two spinning disk confocal microscopes at the Center for Biologic Imaging will be used instead.

B.3. Specific Aim 2: Evaluate caged Nox2 inhibitors in preventing acute injury and chronic dysfunction via targeting injured tissue in a rat model of myocardial I/R injury.

B.3.1. Sub-Aim 2A: Measure Nox2 activity, infarct size, and apoptosis in the ischemic area in a rat model of myocardial I/R. *Rationale*: First, we will test our inhibitors in the acute setting. Since Nox2 has been shown to drive substantial ROS production in the hours after I/R,[17,29] and since Nox2 knockout has been shown to decrease infarct size in I/R injury,[31,43] we hypothesize that our targeted approach to Nox2 inhibition will reduce infarct size in a rat model of I/R injury.

Experimental Design: We will induce I/R injury by ligating and later reopening the left anterior descending (LAD) coronary artery. Ligating the LAD produces the largest infarcts in animal models,[95] is easiest to reproduce,[96] and is the most common site of occlusion in humans.[97] We will carry through the best compound from Sub-Aim 1B and use it for this experiment as well. Based on pharmacokinetic data for the related compound **Jm-77c**[50] and potency data for **THR101**,[48] our lead compound will be dosed at 71.3 mg/kg to achieve a high level of Nox2 inhibition (90%). The compound will be administered in a way that mimics our approach's application to PCI in humans: we will inject the compound (or saline) into the tail vein immediately before reperfusion.

Myocardial I/R will be modeled using a well-validated surgical technique,[43,96,98] which merely adds a reperfusion step to the ligation procedures routinely carried out in the Wang lab.[99–102] Briefly, a left thoracotomy will be made at the level of the fourth intercostal space, and the pericardium will be gently pulled apart. Under a dissecting microscope, polypropylene suture will be passed under the LAD 2 mm distal to the tip of the left auricle. A loose double knot will be tied, through which a short length of PE-10 tubing will be placed, and the loop will be secured with a slipknot. Occlusion will be verified by confirming pallor in the LAD's territory. Ischemia will be maintained for 60 minutes. Afterwards, the knot will be untied and the tubing removed. The suture will be left in place for determination of infarct size and area-at-risk (AAR) by triphenyltetrazolium chloride (TTC) and Alcian blue staining.[103] After 24 hours of reperfusion, the animals will be reanesthetized and the chest reopened. Analysis of the infarct will be completed after intracardiac injection of potassium chloride and excision of the heart.

We will measure infarct size and area-at-risk (AAR) using triphenyltetrazolium chloride (TTC) and Alcian blue staining. In the ischemic area, the extent of apoptosis will be determined using a Western blot for cleaved Caspase-3 and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).[43] Lastly, efficacy of the inhibitor in reducing ROS generation in the AAR will be determined by measuring H_2O_2 levels in homogenized tissue using Amplex Red.[43] These readouts will be compared for rats injected with the compound versus with saline. We performed a power analysis to determine the minimal number of rats necessary to detect an effect. Assuming an effect size and variance for infarct size/AAR consistent with Nox2 knockout,[43] finding a significant difference with $\alpha = 0.05$ powered at 80% necessitates a sample size of 11 animals per treatment group.

Anticipated Results, Possible Pitfalls, and Alternative Approach: We anticipate significant reductions in infarct size/AAR, apoptosis, and H_2O_2 production in the infarcted area comparing animals treated with the caged inhibitor to vehicle-treated animals. In the event that the reperfusion period is suboptimal, timing can be adjusted to 90 minutes of ischemia with >1 hour of reperfusion, which is well-validated for this method.[98]

B.3.2. Sub-Aim 2B: Assess specificity of caged Nox2 inhibitor activation for the injured area. *Rationale*: Due to the important homeostatic roles that ROS from Nox2 play throughout the body[9] and the potential off-target effects of **THR101** analogues,[49,50] it is essential that our approach inhibits Nox2 in tissues accruing pathologic levels of H_2O_2. To determine our approach's selectivity for such tissues, we will compare inhibitor activation and markers of excessive Nox2 inhibition between the infarcted myocardium and unaffected areas of the heart and other organs.

Experimental Design: First, to determine the spatial distribution of inhibitor activation, we will administer our published H_2O_2-responsive fluorophore (**Fig. 6**)[67] to rats subjected to myocardial I/R. The organs will be removed, treated with catalase to prevent further fluorophore activation, subjected to 3 freeze-thaw cycles with liquid nitrogen, lysed, and homogenized.[101] For the heart, the infarct and the uninjured areas will be carefully dissected and treated separately. Fluorophore activation in the organ homogenate will be measured using a fluorimeter. Fluorescence will be compared between animals treated with the caged and uncaged (active) fluorophore, and between animals subjected to I/R injury and sham-operated controls. We used power analysis to calculate the

minimum number of animals needed (α = 0.05, 1-β = 0.8) based on the compound's response to H_2O_2 generated by PMA-stimulated cells.[67] In each of the 4 treatment groups, 2 animals are necessary.

Second, we will quantify markers of off-target Nox2 inhibition in animals treated with our lead compound and subjected to I/R injury. In previous studies, Nox2 knockout was found to perturb cell signaling by increasing Erk2 and Stat3 phosphorylation in the myocardium.[31,104] This illustrates an important role of Nox2 in regulating key cell signaling pathways.[105] Erk2 and Stat3 phosphorylation will be probed using Western blot, comparing the injured and uninjured portions of the myocardium. Additionally, pStat3 will be measured in the lungs, adipose tissue, and thymus *ex vivo*, where it is highly expressed,[106] and pErk2 will be measured in the liver, where it is highly expressed.[107] Erk3 and Stat2 phosphorylation will be compared in animals treated with the caged compound, the corresponding active compound, or saline, across I/R and sham-operated treatment groups. Once again, we performed a power analysis to determine the necessary sample size. Using literature values for Erk2 phosphorylation in a Nox2 knockout mouse,[31] 5 animals will be needed for each of the 6 treatment groups.

Anticipated Results, Possible Pitfalls, and Alternative Approach: We anticipate spatially-controlled inhibitor activation specifically in the area of infarction. Additionally, we anticipate that the active compound will significantly increase off-target Erk2 and Stat3 phosphorylation compared to the caged prodrug and to the saline control. Although our caged fluorophore has proven to be quite sensitive to ROS generated in cell culture,[67] two pitfalls may arise for its use in live animals. First, the fluorophore is not particularly bright (brightness ≈ 10^4 M^{-1} cm^{-1}),[108] so the resulting signal may fall below the limit of quantification. Second, the activated fluorophore is small and lipophilic, so it may diffuse between tissue compartments in animals, compromising our ability to detect tissue-specific activation. As an alternative approach, caged fluorescein (**Fig. 7**) could be used instead as a facile application of our phenol-caging chemistry.[67] Fluorescein is significantly brighter,[108] can be detected with commercially-available antibodies, and will stay in cells after decaging,[109] solving these potential problems.

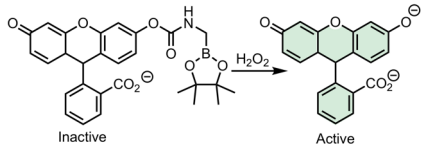


Fig. 7. Alternative H_2O_2 fluorophore strategy. Here fluorescein is caged with a self-immolative spacer.[93] Oxidation will release the phenol, which is an efficient fluorophore (brightness ≈ 10^5 M^{-1} cm^{-1}).[108] Anti-fluorescein antibodies are available commercially for sensitive quantification by Western blot or ELISA. The decaged fluorophore is a large and highly charged molecule which is likely to remain trapped in cells.[109]

B.3.3 Sub-Aim 2C: Assess cardiac function 1 month post-I/R injury in treated rats. *Rationale*: Nox2 drives the chronic sequelae of I/R injury[10–12] responsible for much of the morbidity and mortality.[32] Thus, our approach's potential to preserve cardiac function in the weeks following I/R injury is of significant public health importance. We hypothesize that caged Nox2 inhibitors will lessen the extent of cardiac dysfunction secondary to I/R injury.

Experimental Design: Because our proposed inhibitors are likely to be bioavailable orally,[50] which is preferable for a long-term treatment, we will treat mice post-I/R with caged Nox2 inhibitors by mouth (PO). We will divide animals into 5 groups: saline, treated acutely (tail vein injection at the time of reperfusion), treated chronically (PO after reperfusion), treated acutely and chronically, and aline/sham-operated (stopping after opening the pericardium). For 50% inhibition with a bioavailability of 35%,[49] our lead compound will be dosed at 36 mg/kg.

Transthoracic echocardiography provides a reproducible, noninvasive method for measuring clinically relevant hemodynamic parameters in the rat.[110] To this end, echocardiographic readings will be taken for all rats in the experiment at five time-points (pre-I/R baseline, 3 days, 7 days, 14 days, and 28 days). These readings will be used to calculate fractional area change (FAC),[102] which closely approximates ejection fraction, an important parameter for monitoring the progression of heart failure.[111] After the final echocardiographic reading, a pressure-volume (PV) catheter will be placed. Catheterization with a pressure-volume conductance catheter is a well-established method widely considered to be the gold standard for measuring cardiac function in live animals.[112] Once again, power analysis (α = 0.05, 1-β = 0.8) was carried out to determine the number of minimum animals required. Based on literature values for left ventricular (LV) dP/dt_{max} (normalized to end-diastolic volume, EDV) in Nox2 knockout mice,[12] 9 animals per treatment group are needed. To compensate for attrition, however, based on our past experience,[102] we will have 13 animals in each of the 5 treatment groups.

Anticipated Results, Potential Pitfalls, and Alternative Approach: We anticipate that FAC values and hemodynamic parameters from PV catheterization will be ranked as follows: saline < acute treatment only < chronic treatment only < acute and chronic treatment ≈ untreated/sham-operated. In the rare scenario that technical issues preclude the recording of PV loops, we will obtain hemodynamics measurements using the Langendorff preparation instead. The Langendorff (isolated perfused) heart is well-validated model with over a century of use,[113] and the VMI uses this technique extensively. We will measure measure left ventricle end diastolic pressure (LVEDP) and ventricular contractility (dP/dt_{max})[43] while obtaining electrocardiographic recordings directly from the epicardium so that mechanical and electrical cardiac function may be coordinated.[113]

RESPECTIVE CONTRIBUTIONS

This proposal is the result of several months of dialogue between the sponsor, Dr. Deiters, and the applicant, Josh Wesalo. Josh wrote all sections of this proposal, except for the Sponsor and Co-Sponsor Information section, which Dr. Deiters wrote. Dr. Deiters read the application in full and helped to revise it to improve the writing and experimental design.

The proposed fellowship work entails closely-mentored research encouraging independence in designing and carrying out experiments, but with frequent advice from the sponsor. Josh will share results weekly with Dr. Deiters at weekly one-on-one meetings and at group meetings. Josh will also draft all manuscripts from his data, and Dr. Deiters and all co-authors will review them prior to submission to a journal. Dr. Deiters will oversee Josh's progress on the training plan laid out in this proposal, and he will serve as a mentor to aid in Josh's development as a physician-investigator.

SELECTION OF SPONSOR AND INSTITUTION

I aspire to become a physician-scientist who runs an NIH-funded basic science laboratory, practices cardiology, and teaches. The mentorship of my sponsor, Dr. Alexander Deiters, in the context of the University of Pittsburgh-Carnegie Mellon University Medical Scientist Training Program (MSTP) will provide me with an exceptional training project to carry out my proposed research and build a strong foundation for my career.

I chose to work with Dr. Deiters based on his superb record of training pre-doctoral students, his superb reputation as an expert in chemical biology, and his dedication to mentoring his students as his top priority. Despite it being relatively early in his career, Dr. Deiters has already trained 24 graduate students and 11 post-doctoral fellows, and he currently runs a dynamic lab comprised of 12 graduate students, another MSTP trainee, several undergraduates, and myself. His trainees publish prolifically (5 to 6 papers on average) in the top journals in the field (including *Nat. Chem.*, *J. Am. Chem. Soc.*, and *Angewandte Chemie*). Alumni from our group have gone on to post-doctoral fellowships at top-notch institutions (including the NCI and Scripps) and have started NIH-funded research labs. The group has several collaborations within the department, the university, and the nation making it an exciting and dynamic place to work.

Dr. Deiters himself is a brilliant chemical biologist with over 100 publications and over a dozen patents who—despite his many commitments—makes active mentoring his top priority. His office door inside our lab area is always open, and he regularly responds to emails between 7 AM and 12:30 AM. We have a standing weekly one-on-one meeting at 4:00 PM to discuss results, milestones, the latest developments in the field, the responsible conduct of research, and our new ideas. As a trainee in his lab, I have had the opportunity to write all of my own manuscripts, posters, and grant applications (including the present proposal and an aim for an R01 proposal), and Dr. Deiters consistently works with me through several rounds of revisions until each document is quite polished. Just as I have had independence in writing, I have taken on independence in managing my projects as well. Several of the projects I have taken on have stemmed from my own ideas (with Dr. Deiters' expert guidance), allowing me to grow into a more independent scientist as I become more advanced in my training. Additionally, Dr. Deiters constantly receives emails from undergraduates eager to work in his lab; he lets us interview candidates and choose students to mentor, which has given me the rare opportunity to gain experience in managing lab personnel followed by experience mentoring them. Dr. Deiters has also supported and advised me as I independently spearheaded the new collaborations in this project. The collaborations will take the new treatment approach we're developing and give me the opportunity to test it in an innovative new tissue culture model and in animals, all while obtaining input from an expert cardiologist. In sum, I am confident that the superb training environment of Dr. Deiters' group will allow me to complete my proposed research and will help me develop into an independently-funded physician-scientist.

The University of Pittsburgh Medical Center (UPMC) and the School of Medicine have consistently ranked among the best hospital systems and medical schools in the nation. The University receives the fifth-highest amount of NIH support of any institution. Pitt and CMU are across from one another in Pittsburgh's Oakland neighborhood, an area packed densely with UPMC's academic hospitals. Thanks in part to the physical proximity, scientists and clinicians collaborate much more often than comparable institutions. Investigators have a culture of sharing resources (e.g., tissue specimens and research compounds) that has facilitated the collaborations I have set up for this project and that makes Pitt a great place to train to become a physician-scientist.

Our MSTP integrates clinical training and research through innovations in our curriculum. When I interviewed, I was impressed that I would have the opportunity to do three lab rotations, three semesters of journal club synchronized with the medical school curriculum, and three semesters of professional development courses all within the first two years of the program. These classes have built up key skills I will rely on during my career, including statistical analysis, manuscript writing, and grant writing. Our program invented Longitudinal Clinical Clerkships, which are one-on-one experience with a clinician during graduate school that will strengthen my clinical acumen in cardiology and reinforce the translational aspects of my research (see **Additional Educational Information**). To keep me immersed in research at the end of my clinical training, the program will fund a five-month "mini post-doc" fellowship when I complete medical school.

On a more personal note, I selected the Pitt-CMU MSTP because of the people in it. The program director, Dr. Richard Steinman, gets to know every trainee and their research, and is a fierce advocate for us as we navigate our dual degree training. The other students are some of the kindest and brightest people I have ever met, and I have greatly enjoyed working on MSTP committees and talking about science with them. I could not be more pleased with my choice to matriculate at the Pitt-CMU MSTP and to join the Deiters lab.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Prior Training and Instruction in the Responsible Conduct of Research:

1) **University of Pittsburgh School of Medicine: Ethics, Law, and Professionalism Course** (8/2014 - 12/2014). This course was the second installment in the Patient, Physician, and Society series of courses. The course met for 1 hour and 40 minutes each week for 20 weeks. Every time the course met, I attended a 50-minute lecture by a physician, lawyer, or bioethicist, followed by a 50-minute faculty-led small group discussion. We discussed ethics in human subjects research, patient care, conflicts of interest, and emerging technologies.

2) **University of Pittsburgh Online Training Courses** (6/2014 and 6/2015). In 2014, I completed the University of Pittsburgh "Use of Laboratory Animals in Research and Education" and "Purpose Bred Mice and Rats in Research, Testing and Teaching" online training modules. I completed the Collaborative Institutional Training Initiative Biomedical Responsible Conduct of Research and Conflict of Interest online training courses in 2014 and 2015. These are hour-long training courses that I will re-complete as required by the University.

3) **MSTP Workshops** (since 9/2014). The MSTP holds monthly workshops for the entire student body. Each session lasts 90 minutes and involves small- or large-group discussions (often with a panel of experts) in addition to lectures. Topics vary widely, but we emphasize the ethics of biomedical research and clinical practice at each session. So far, I have attended 26 workshops, 3 of which I led:

a) **2015** - Seminar on ethical concerns with microscopy with Dr. Donna Stolz (Associate Director of the University of Pittsburgh Center for Biologic Imaging).
b) **2016** - Small- and large-group discussion of four bioethics case studies (clinical research risks/benefits, conflicts about authorship, and two cases about peer review).
c) **2017** - Seminar on Big Data with Dr. Shyam Visweswaran (director of the MSTP's biomedical informatics program).

4) **MSTP Professional Development Course 2: Methods and Analysis** (6/2015 - 8/2015). This course met for 1.5-2 hours every week for 10 weeks. It combined lectures and small group discussions led by MSTP Director Dr. Richard Steinman and outside experts (notably 3 sessions on biostatistics by Dr. Daniel Normolle, Director of the University of Pittsburgh Cancer Institute Biostatistics Facility). Topics included authorship, reproducibility, and the appropriate use of statistics for designing experiments and testing hypotheses.

5) **Basics of Personalized Medicine Course** (8/2016 - 12/2016). This course met for two hours twice a week for 20 weeks. Each class entailed a lecture and small-group discussion led by an expert in an aspect of personalized medicine. Topics included patient privacy, disclosure of incidental findings, and informed consent.

Training to be Completed During the Fellowship Period:

1) **MSTP Workshops** (since 9/2014). See above.

2) **MSTP Professional Development Course 4: Ethics for Medical Scientists** (04/2017 - 05/2017). This course will meet four times for 2-hour small-group discussions led by Dr. Richard Steinman and Dr. Karen Schmidt (Director of the University of Pittsburgh Responsible Conduct of Research Center). Topics will include plagiarism, conflicts of interest, and research misconduct.

3) **University of Pittsburgh Responsible Conduct of Research Training Center** (09/2017 - 08/2021). The Clinical and Translational Sciences Institute (CTSI) at the University of Pittsburgh offers weekly, 1-hour lecture and case-study centered workshops on a wide variety of topics taught by University of Pittsburgh faculty. I will attend 8 of these classes during my graduate training.

Role of Dr. Alexander Deiters in Responsible Conduct of Research Training: Throughout my graduate school training, I have had, and will continue to have, discussions with Dr. Deiters about ethical issues in the biomedical research. Dr. Deiters is conscientious about these matters and brings them up frequently during his classes, during group meetings, and in one-on-one conversations. It is part of our lab's culture to be up-front and transparent about documentation and decisions about authorship. As an accomplished medicinal chemist who has consulted for several pharmaceutical companies, received 14 issued patents as an inventor or co-inventor, commercialized his lab's optical nucleic acid triggering technology, and received a 2009 grant from Teva Pharmaceuticals (see **Sponsor Biosketch**), Dr. Deiters is quite knowledgeable about the relationship between academia and industry, and intellectual property considerations and conflicts of interest frequently find their way into our discussions.

SECTION II – SPONSOR AND CO-SPONSOR INFORMATION

Alexander Deiters, PhD, Primary Sponsor/Mentor

Dr. Deiters is a Professor of Chemistry and a member of the University of Pittsburgh Cancer Institute, the Molecular Biophysics and Structural Biology Program, the Center for Nucleic Acids Science & Technology at Carnegie Mellon University, and the Pitt-CMU Medical Scientist Training Program. He runs a multidisciplinary research program combining small molecule synthesis, medicinal and organometallic chemistry, cell and molecular biology, protein engineering, nucleic acid chemistry, natural product chemistry, and photochemistry. He is known for his work in controlling protein and gene function using various triggers, including light, chemical triggers, and reactive oxygen species. Dr. Deiters will mentor Josh throughout his training. He will guide Josh to foster his career development as an independent physician-investigator.

A. RESEARCH SUPPORT AVAILABLE

The following table summarizes all current external support in the Deiters lab. In addition, Dr. Deiters has access to internal funds at the University of Pittsburgh, including start-up funds from his move in 2013.

Source	Grant #	Title	PI	Dates	Annual Direct
Charles E. Kaufman Foundation	KA2014-73921	Expanding the Genetic Code of Zebrafish	Deiters, Alexander	09/01/14-08/31/17	$68,182
NSF	MCB-1330746	Optogenetic Dissection of Protein Kinase Networks	Deiters, Alexander	09/01/13-08/31/17	$72,157
NSF	CHE-1404836	Control of Protein Dimerization through Light-Regulated Rapamycin	Deiters, Alexander	07/01/14-06/30/17	$81,381
NSF	CCF-1617041	DNA Computation in Cells	Deiters, Alexander	07/01/16-06/30/19	$88,295
NSF	CBET-1603930	Near-natural Amino Acid Mutagenesis for the Engineering and Study of Protein Function	Deiters, Alexander	08/01/16-06/30/19	$68,829
NSF	CHE-1625002	MRI: Acquisition of a MALDI-TOF/TOF Mass Spectrometer to Enable Research and Education at the Interface of Chemistry, Biology, and Materials Science	Horne, Seth	08/01/16-07/31/19	$454,257
Carnegie Mellon University	131RA01	yPNA Delivery and Application in Mammalian Cells	Armitage, Bruce	06/01/16-05/31/17	$20,000
NIH	R01 GM108952	Development of Lariat-Shaped Caged Morpholinos for Optochemical Gene Regulation	Chen, James	08/01/14-07/31/18	$120,571
NIH	R01GM112728-01A1	Chemically Triggered Morpholino Antisense Oligonucleotides	Deiters, Alexander	09/01/15-07/31/19	$158,849
NIH	R21HD085206-01A1	Optical control of translation and gene editing in zebrafish embryos	Deiters, Alexander	01/07/13-01/31/17	$150,000
NIH	R43GM121129	High Throughput RNA and DNA analysis and detection by MALDI-MS	Yu, Marvin	04/01/17-03/31/18	$75,000

B. SPONSOR'S PREVIOUS FELLOWS/TRAINEES

Dr. Deiters has trained 24 graduate students and 11 post-doctoral fellows. He currently trains 12 graduate and 2 MSTP students.

5 Representative Trainees:

Douglas Young, PhD (2004-2009) Dr. Young finished his PhD in 2009, during which he received 3 predoctoral fellowships (the GAANN Biotechnology Fellowship, the ACS Medicinal Chemistry Predoctoral Fellowship, and the Burroughs Wellcome Fellowship). As a student, he published a book chapter and 33 research articles, including 4 *JACS* papers and 3 *Angewandte Chemie* papers. He won several awards, most notably winning the Gordon Research Conference on Medicinal Chemistry Poster Session in 2007. After graduating in 2009, he completed a post-doctoral fellowship at Scripps (La Jolla, CA) in Peter Schultz's lab funded with a Ruth L. Kirschstein Postdoctoral Research Fellowship. In 2011 he started his independent career at the College of William & Mary, where he is an Associate Professor of Chemistry, running an R15-funded research lab.

James Hemphill, PhD (2010-2015) Dr. Hemphill, a Mellon Graduate Fellow, gave 2 talks and 3 posters at national meetings and published 10 articles, including 6 *JACS* papers, as a graduate student. He currently holds a position as a Scientist in Molecular Engineering at Unum Therapeutics in Cambridge, MA.

Jeane Govan, PhD (2008-2013) Dr. Govan finished her PhD training in 2013, during which she published 10 articles (4 of which were in *JACS* or *Angewandte Chemie*) and a book chapter. She also presented 6 posters and gave 1 talk at national meetings. At present, Dr. Govan is a Senior Clinical Studies Coordinator in Investigational Cancer Therapeutics at the University of Texas MD Anderson Cancer Center.

Chung-jung Chou, PhD (2010-2012) Dr. Chou completed his post-doctoral fellowship in 2012, during which he presented 5 posters and published 9 articles (5 of which were in *JACS*, *Angewandte Chemie*, or *Nat. Chem.*). He received an Outstanding Poster Award at the 2nd Biennial Chemical Insights into Biological Processes Symposium at the NCI. Currently, he is an Assistant Professor at Chung Yuan Christian University in Taiwan.

Colleen Connelly, PhD (2008-2014) Dr. Connelly finished her PhD in 2014, during which she received an NIH Molecular Biotechnology Training Program Fellowship and a GlaxoSmithKline Graduate Fellowship, published 9 articles and a book chapter, and presented 4 posters and gave 2 talks at national meetings. Her awards include the RNA Society of North Carolina Symposium on RNA Biology VIII Poster Presentation Travel Award, the Dennis W. Wertz Award for Excellence in Teaching, and the BASF Innovative Graduate Research Award. Currently, she is completing a post-doctoral fellowship with Dr. John "Jay" Schneekloth, Jr. at the NCI.

C. TRAINING PLAN, ENVIRONMENT, RESEARCH FACILITIES

C.1. RESEARCH TRAINING PLAN (Primary Sponsor's Overview):

Introduction. Josh and I have worked closely to design a comprehensive training plan tailored to prepare him for an exceptional career as a physician-investigator at an academic medical center. Training in my laboratory will make him an expert in the field of chemical biology. Through the network of collaborators, consultants, and mentors that he has assembled, he will further receive training and advice in cardiology and vascular biology, as it relates to his proposed work. This will prepare him for a successful career balancing basic science research and clinical practice in cardiovascular medicine.

My laboratory develops new chemical tools to study biological function and we leverage organic synthesis to engineer proteins, nucleic acids, and therapeutics. Recently, we built on our experience in conditionally controlling biomolecules by preparing H_2O_2-responsive molecules to release alcohols, ketones, and aldehydes without generating any toxic by-products, and we demonstrated the utility of this approach in cell culture. Josh has cleverly proposed an application of this work to develop a new approach to treating reperfusion injury in myocardial infarction. Leveraging his unique position, Josh has shrewdly selected the interface of organic chemistry and vascular biology for his studies, as it is a greatly underdeveloped area that will provide him with long-term opportunities beyond his studies in my group. The training plan outlined here will teach Josh how to use new approaches to tackle open problems in treating cardiovascular disease, which will help Josh to complete his proposed research and will lay the foundation for his career as an independent investigator.

Research Experience. In addition to developing a new treatment approach, Josh's project will expand our understanding of redox biology, and will further broaden the scope of our lab's H_2O_2-responsive chemistry. The project marries chemistry with vascular biology, and will create novel reagents in developing a highly specific treatment approach. This is an excellent project for a dual-degree student and since the scientific premise for the project is strong, the chance of success is high.

Josh's work will start from chemical synthesis and cell culture work, and will progress to animal experiments. He will soon complete the synthesis of the boronate-caged inhibitors and establish a cardiac myocyte-endothelial cell co-culture model of hypoxia-reoxygenation to test them in our lab. These experiments will reinforce his skills in designing and carrying out biochemical assays with a high level of rigor. Josh already

acquired extensive cell culture experience since starting at Pitt, from his previous work in Dr. Pagano's lab combined with further experience in my group. He will develop the cell-based model under the guidance of a post-doc from our collaboration with Dr. Weber's group, who has published extensively on hypoxia-reoxygenation in cell culture to simulate ischemia-reperfusion. Josh has made it clear that he would like to gain more experience with animal work; he will rely on skills in this area throughout his career. By working with Dr. Wang's group, Josh will gain invaluable experience with surgery in rodent models.

Between his previous work and the proposed research, I expect Josh to publish two first-author high-profile papers in journals with an impact factor of at least 10, in addition to collaborative papers with his colleagues in mid-tier journals. Students in my group average 5-6 papers in their PhD. Josh will draft all manuscripts and posters and we will work together extensively on revisions.

Laboratory Environment. My senior graduate students are skilled at all pertinent laboratory techniques, and will assist Josh as needed. Several funded collaborations make the lab a multidisciplinary and exciting environment for a future physician-scientist. Josh has established an impressive network of collaborators and contributors listed in the table below that will provide excellent training opportunities, allowing him to complete his proposed aims.

Name	Primary Appointment	Role on Grant	Role in Project
Frederick W. Crock, MD	Medicine (Cardiologist)	Consultant	1. Consult about clinical relevance, echocardiography. 2. Clerkship with Josh during grad school.
Patrick J. Pagano, PhD	Pharmacology & Chemical Biology	Other Significant Contributor	1. Expert in NADPH oxidase pharmacology. 2. Liason with Vascular Medicine Institute (VMI).
Stephen G. Weber, PhD	Chemistry	Collaborator	Expert in tissue culture models of hypoxia-reoxygenation.
Yadong Wang, PhD	Bioengineering	Collaborator	Expert in rodent models of myocardial ischemia.

At the Vascular Medicine Institute (VMI), which is within a short walk of my lab and with whom Dr. Pagano will serve as a liaison, Josh has the support of several core facilities to help him complete his proposed aims. The Biostatistics Core there works exclusively with data from research in cardiology and cardiothoracic surgery, and thus is very familiar with the field. Additionally, the Institute has a Free Radical and ROS Core Facility with which Josh worked in the past, and that will provide expert assistance with the cell-based assays. Dr. Wang maintains an appointment with the VMI as well, and his lab has published extensively using rodent models of myocardial infarction taking advantage of the VMI's Small Animal Hemodynamics Core. Dr. Wang's lab will transition to Cornell later in 2017, which will provide Josh with the opportunity to attend a weeklong training program with Transonic in Ithaca, NY, the leading manufacturer of pressure-volume catheters for rodent experiments. Afterwards, he will complete his proposed aims while working with Dr. Wang. Several of his lab members are proficient with rodent models of myocardial ischemia; they had four publications in this area over the past 3 years, and have agreed to work with Josh on his proposed experiments. Overall, the network of mentors that Josh has assembled is uniquely suitable to train Josh in the techniques and scientific principles that he needs to succeed as a physician-investigator.

Mentorship. I have set aside Mondays at 4:00 PM to meet with Josh every week to go over progress and to discuss the direction of his work. During these meetings, we will discuss experimental design, the scientific method, the latest developments in vascular biology and in chemical biology, balancing time as an investigator, writing, responsible conduct of research, and mentoring undergraduates.

In addition to my mentoring, Josh has assembled an outstanding team of physicians, scientists, and physician-scientists to serve as mentors. First, Richard Steinman, MD, PhD, the Director of the MSTP, has already provided extensive training in scientific writing and presenting scientific data, and in bioethics. Dr. Steinman will continue advising Josh, and will help Josh should any difficulties arise as he navigates graduate school while balancing clinical responsibilities. Second, Josh will continue to meet semi-annually with his career advisor, Don DeFranco, PhD (Professor, Pharmacology & Chemical Biology). Dr. DeFranco continues to track Josh's progress, helps him set goals using an Individual Development Plan, and will give him an impartial second opinion on any scientific or personal matters involved in the research. Third, Josh is planning to complete a clerkship with Frederick Crock, MD (Clinical Director of Inpatient Cardiology at UPMC Presbyterian) during graduate school. Dr. Crock is a seasoned cardiologist who has won 6 teaching awards and who sees many patients recovering from myocardial infarctions. He is an echocardiography expert, which is germane to Josh's

proposed experiments. Josh first learned cardiology as a medical student through Dr. Crock's small group instruction. Continuing this relationship will teach him about the clinical aspects of his proposal. Josh has already observed several cardiac catheterizations as an undergraduate, and Dr. Crock will arrange for Josh to visit the cath lab at UPMC Presbyterian so he can learn about reperfusion from local expert cardiologists.

Aside from receiving mentorship from the team of scientists and clinicians that he has assembled, Josh has made it clear to me that he wants the opportunity to mentor other trainees throughout his training. Before starting at Pitt, Josh mentored 4 undergraduates. Josh has been mentoring an undergraduate in my lab (Gabe Rajkovic) since Fall 2016. Josh has built up Gabe's technical skills and understanding of the field to the point of independence in the laboratory, allowing Josh to delegate many responsibilities to him.

Presentations. In addition to the aforementioned presentations at group meetings and annual progress reports, Josh will participate in the biological chemistry division's weekly seminar series, giving him outside feedback from faculty and students in the department. Outside the department, Josh will present at VMI Research in Progress seminars, the annual MSTP retreat, American Chemical Society national meetings, and international vascular biology conferences. He will apply for independent travel awards for these conferences.

Graduate Coursework. Josh has worked with Graduate Student Advising Committee (GSAC) to design a streamlined and personalized course of study. Specifically, he has worked with our collaborator Dr. Weber, Director of Graduate Studies (who also has experience in mentoring MSTP students and is the chemistry liaison for the program) to design his coursework. The department has exempted him from teaching requirements so he can devote his full effort to research. So far, he has audited my CHEM 2370 synthesis course on the construction and medicinal chemistry of FDA-approved drugs. He has also completed a Basics of Personalized Medicine course and an advanced molecular biology course, receiving top marks in each. The former course complemented the dual nature of his training by teaching him about state-of-the-science advancements in precision medicine in addition to the bioethical, financial, and political landscape; the latter course developed in him a keen eye for critiquing the literature, which I have noticed in interacting with him in our meetings and in the course I'm teaching. Currently, he is taking my Advanced Biological Chemistry 2 course (CHEM 2820), in which he performs in the top 5% based on exams, homework assignments, and class participation. He is also taking Imaging Cell Biology in Living Systems through our world-renowned Center for Biologic Imaging, which will give him in-depth background for the proposed imaging experiments. Aside from the coursework, Josh will attend regular seminars that the department hosts. Additionally, he will attend cardiology grand rounds, VMI seminars, and the Laureate Lecture Series, in which the Dean of the School of Medicine invites physician-investigators who are believed to be on the shortlist for Nobel prizes to speak. The coursework and seminars Josh attends in his unique position at the interface of the chemistry department and the VMI will prepare Josh to carry out the research he is proposing, and will help him develop the skills he needs to become a physician-investigator and to develop ideas for his own independent career.

Professional Development. Josh has already completed the first three courses in a four-part series on professional development through the MSTP (see **Additional Educational Information**). He led two hour-long journal club sessions during the first course. During the second course, Methods and Analysis, Josh learned extensively about research ethics and biostatistics. In my lab's culture, rigorous laboratory practices involving documentation, ethics, and statistics are of the utmost importance and ensure high reproducibility of the obtained results. I reiterate these principles in our labs' standard operating procedures, in lab meetings, and in email reminders, and I'm pleased that Josh was well-prepared thanks to this course. Josh has worked closely with the University to help implement electronic lab notebooks via several conference calls with IT officers.

Josh recently completed the third MSTP professional development course (Grantwriting), and will begin the fourth (Ethics for Medical Scientists) this spring. The MSTP also provides monthly student-run workshops and a training course on research ethics. Josh has run three workshops on so far: a seminar on ethics in imaging, a discussion-based bioethics workshop, and a seminar on "big data" in biomedical research. In summary, as Josh completes his training in my lab and in the MSTP, he will grow to become an excellent scientific writer, presenter, and mentor, and will expand upon his keen awareness of bioethics.

Clinical Experience. Josh has already completed intensive clinical training integrated into the preclinical years of medical school, and capped off this experience with a full eight-week clerkship in neurology and psychiatry, earning top marks and receiving exceptional feedback from his attending physicians and residents.

To continue his clinical training throughout graduate school, in addition to grand rounds, Josh will attend cardiology conferences (consistent with his clinical interests). Additionally, Josh will complete two Longitudinal Clinical Clerkships (LCCs) starting next fall. LCCs involve one-on-one mentoring with a master clinician, one

half-day per week for 20 weeks. This weekly schedule will prepare him for the 80:20 research:practice split typical of most physician-investigators. The LCCs will allow Josh to discuss the translational aspects of his work with practicing physicians, and will refine his clinical skills in preparation for a return to medical school. He has planned the first LCC in Fall 2018 with Dr. Frederick Crock, the aforementioned cardiologist consulting on this proposal. The LCCs will give Josh more opportunities for advising and networking as he prepares for residency. After Josh returns to medical school, several research months and an MSTP-funded "mini-post-doc" will allow him to tie up loose ends with lab research in a capstone scientific experience while Josh finishes medical school. This training will prepare Josh for an exceptional career as a clinician-investigator.

C.2. ENVIRONMENT

Department of Chemistry. The chemistry department, Josh's home department, is recognized internationally for excellence in chemical research and education, particularly in organic, biological, and medicinal chemistry. It is housed in Pitt's Chevron Science Center and consists of 39 primary faculty, 231 grad students and postdocs, and 35 staff members. The department enjoys strong collaborations throughout the university, the nation, and world-wide. Among chemistry departments, Pitt's is exceptionally well-equipped with a large instrument facility containing full-time PhD-level directors and staff members to support Josh's research.

University of Pittsburgh School of Medicine and Vascular Medicine Institute (VMI). Ongoing interactions with the University of Pittsburgh School of Medicine and the collaboration with the VMI that Josh started enhance the research and training potential of my lab. Pitt is recognized as a leading academic medical center in the U.S., commanding the 5th-largest share of NIH funding of any institution. My group's focus on engineering new tools that enable hypothesis-driven biomedical research places us at the forefront of the University's research and training. The University has also demonstrated a commitment to MD/PhD training epitomized by our strong MSTP, which receives generous funding from NIGMS and the School of Medicine.

Within the School of Medicine, the VMI is poised as an exceptional interdisciplinary center with strong financial support and training potential. The VMI comprises investigators from a wide variety of clinical and scientific disciplines, with appointments in Pulmonary, Allergy, and Critical Care Medicine; Hematology/Oncology; Cardiology; Pathology; and Pharmacology & Chemical Biology. The institute's multidisciplinary approach brings together human subjects research, physiology, biochemistry, molecular biology, pharmacology, and bioinformatics to understand mechanisms of disease and to develop therapeutics while training the next generation of translational researchers. The aforementioned core facilities, seminars, and grand rounds within the institute will support Josh's training as he continues to grow into an independent physician-investigator.

C3. RESEARCH FACILITIES

My lab occupies a combined total of 3,500 sq. ft. in the Chevron Science Center at the University of Pittsburgh. This includes a laboratory designed for cell biological work (BSL2) equipped with two Type A2 (Class II) biological safety cabinets. The synthetic laboratories are equipped with fifteen 2-person fume hoods, including individual nitrogen/vacuum lines, and the instrumentation necessary for all chemistry experiments. Josh has his own fume hood, and a bench on both the chemistry and biology sides of the laboratory. Dr. Weber's and Dr. Wang's group will provide Josh with access to the equipment he will need to complete his proposed cell culture and animal experiments, respectively. Also, Josh has full access to the core facilities at the VMI, with both Drs. Pagano and Wang as liaisons. Full details are in the "Facilities and Other Resources" section.

D. NUMBER OF FELLOWS/TRAINEES SUPERVISED DURING FELLOWSHIP

During the fellowship—in addition to Josh—I anticipate training another MD/PhD student, Wesley Brown. The group currently consists of Josh, 12 graduate students, and 4 undergraduates. On average, my group size is around a dozen graduate students plus 1-2 postdocs.

E. APPLICANT'S QUALIFICATIONS AND POTENTIAL FOR A RESEARCH CAREER

I am thrilled to have Josh join my laboratory to undertake this project. I met Josh when he first interviewed for Pitt's MSTP and I was immediately impressed by his grasp of scientific principles, his enthusiasm, and his maturity. I gave him my most enthusiastic recommendation to the admissions committee, and corresponded with him to try to recruit him. Fortunately, he matriculated to the program, and ultimately joined my lab. Overall, Josh is an exceptional student and I would rank him in the top 2% of all 1st year graduate students that I have interacted with. This is based on his academic track record (biosketch and transcripts), his enthusiasm for research (he started to read about developing and applying conditional switches of biological function months

before he started in lab), his diligence in lab (he always includes all necessary controls in his experiments and always systematically screens experimental parameters), his creativity (he offered several new ideas for the project he initially started in my lab – a new external triggering approach for gene silencing), his work-ethic (he regularly works 60-70 h weeks), and his ability to communicate (his weekly progress reports are excellent and his final summer report after his initial lab rotation was one of the best that I had seen from any student).

Josh had a nearly perfect academic record at Franklin & Marshall College, despite the institution's reputation for grade *deflation*. This is all the more impressive given his challenging course of study (he completed a double major in chemistry and economics with a minor in applied mathematics). For these accomplishments, he was invited into Phi Beta Kappa, and received several honors upon graduating. As expected, his performance on the MCAT was exemplary (a 37S, which is at the 98th percentile). He continues to distinguish himself academically as an MSTP student. He passed all of his medical school coursework with flying colors, capping it off with an outstanding performance on Step 1 of the U.S. Medical Licensing Exam. Josh earned a 257, which qualifies him for the most competitive residencies in any specialty and places him between the 96th and 91st percentiles for allopathic medical students. In graduate school, he maintains a perfect GPA. Josh upheld this exceptional performance while competing on Pitt's club cross-country team and leading a variety of school committees (Pitt Med Health and Wellness, MD/PhD Welcoming Committee, MD/PhD Interview Committee, and MD/PhD Retreat Planning Committee), proving himself to be an excellent, well-rounded student. I have found him to have outstanding scientific abilities in my research group, and his outstanding performance in research and laboratory work correlates well with his stellar performance in Pitt Med's challenging curriculum. Just as he has always been a great team player as a scholar-athlete, he has been a team player in the lab too, always willing to take on extra duties and to help out with tasks to keep the lab running, even if they are completely unrelated to his own work. Despite him only being in the early stage of his graduate career, he is a well-respected lab member who other students seek out for advice and support.

The proposed project will build on Josh's considerable skills as a young investigator. Josh entered the MSTP with an exceptionally broad and deep portfolio of research experience. Before matriculating at Pitt, Josh spent four years synthesizing an investigational new drug with the potential to treat GM3 Synthase Deficiency, a rare genetic disorder afflicting the Amish population. As I understand it, he worked independently on this project for all four years—the last year full-time—and mentored four other students to follow in his footsteps. In addition to his background from this project, Josh also helped optimize an in-house blood amino acid assay for the Amish clinic where he worked. He also has experience in pharmacology from a lab rotation here and from a summer at UPenn. With this background, Josh had a strong footing when he entered my lab, allowing him to hit the ground running from the first week in a way that few of my beginning students can do. He started in my lab with several projects centered around caged molecules. During his first rotation, he made substantial progress on the synthesis of a nucleic acid linker molecule to develop light-activated gene knockdown in zebrafish. Since starting his graduate school research in my lab, he has quickly and thoughtfully optimized reagents for use in chemically-triggered protein and nucleic acid activation, and is preparing an impressive manuscript applying this tool to the conditional control of sumoylation, an important post-translational modification that is currently under investigation. Josh's minor in applied mathematics from his undergraduate education (plus his medical school and MSTP training in biostatistics) also prepared him extremely well for statistical analysis of his data. I am consistently impressed with his initiative in bringing statistical rigor to his presentations in lab meetings. Josh's previous experience developing conditionally activated nucleic acids and proteins in my lab lays the foundation for H_2O_2-activated Nox inhibitors and the corresponding novel therapeutic approach proposed here. Thus, the project Josh is proposing builds on a solid foundation of knowledge.

Josh's proposed research is both ambitious intellectually and challenging technically, and he has developed a strong network of collaborators to tackle it. To my knowledge, no therapies for reperfusion injury have been successfully translated to the clinic. This proposal represents a promising approach well-grounded in basic biology and chemistry. It stands at the intersection of chemical biology and vascular biology, which is an underdeveloped area that will provide Josh with many opportunities for his career.

In summary, Josh is an exceptional applicant who possesses all the qualities and skills to become an independent physician-scientist. Few students are as well rounded as he is: great scientific knowledge, excellent experimental skills, and an outstanding communicator/writer; however, his strongest assets are his razor-sharp intellect and his phenomenal drive! Josh is a highly qualified candidate who shows great potential even at this early time in his career and is on a steep upward trajectory. The proposal outlined here will give Josh world-class training. Therefore, I recommend Josh for the NRSA Award with no reservations and with my greatest enthusiasm.

University of Pittsburgh
Swanson School of Engineering
Department of Bioengineering

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March 18, 2017

Dear Josh,

I am happy to collaborate with you on your project "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction." Treatment of ischemia-reperfusion injury is a major unsolved program for biomedical research, and your proposed approach represents a novel and very promising idea.

As you know, my lab has worked extensively on vascular regeneration and cardiac repair after myocardial infarction (MI), and thus we have substantial experience with rodent models of MI (*Biomaterials*, 2017 and 2015; *Sci. Adv.* 2016; *J. Controlled Release* 2015). During the collaboration, you'll be working with Daniel Long, who you have met already. Daniel has carried out all of our MI model work since 2016, and has begun training others in the technique. He has also become quite skilled with echocardiography. This collaboration will help you complete your proposed experiments from Aim 2. Also, Daniel and other members of our lab have extensive experience in obtaining rat myocytes for primary culture. We will be happy to add you to our protocol and assist with your proposed cell culture experiments in Sub-Aim 1C.

I should mention that my lab is relocating to Cornell University in late July. We are happy to continue the collaboration after the move. Notably, our location in Ithaca, NY will facilitate your training in how to use pressure-volume (PV) catheters for your proposed experiments in Sub-Aim 2C. Transonic is the leading manufacturer of PV catheters for rodent experiments. At their global headquarters in Ithaca, NY, they host weeklong training seminars on recording PV loops with their devices. PV loops are the gold standard for measuring hemodynamic parameters in the rodent heart, and I expect that you and your proposed work will benefit enormously from the this training opportunity.

I look forward to working with you on this exciting collaboration. As you know, my lab is less than a block away from yours, and you are welcome to stop by my office whenever you would like to discuss progress or find solutions to road blocks. Please feel free to talk to me at any time should you have any questions about myocardial injury and repair, rat models, or treatment approaches for MI.

Sincerely,

Yadong Wang, PhD
William Kepler Whiteford Professor of Bioengineering
University of Pittsburgh

University of Pittsburgh

Kenneth P. Dietrich School of Arts and Sciences
Department of Chemistry

Stephen G. Weber
Professor of Chemistry
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March 31, 2017

Joshua S. Wesalo
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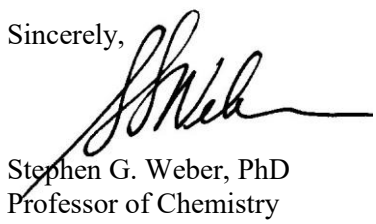
Dear Josh,

I am happy to serve as a collaborator on your project "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction." You have made a strong case for H_2O_2-responsive Nox2 inhibitors based on an extensive evaluation of the clinical and basic science literature. I believe that this approach has significant potential for treating the glaringly unaddressed problem of myocardial ischemia-reperfusion injury.

My group will collaborate with you on your proposed cell culture experiments in Sub-Aim 1C. We have developed techniques to model ischemia-reperfusion in tissue culture by inducing changes in oxygen tension that occur nearly instantaneously. The technique has grown out of my longstanding work devoted to understanding redox biology in living tissue subjected to ischemic injury (see, for example, Wallin et al. 1999, Li et al. 1999, Mitala et al. 2008, Jaquins-Gerstl et al. 2011, Yin et al. 2015, and Yin et al. 2017). Unlike previous approaches, we developed an online oxygenator with microfluidic channels containing a gas-permeable membrane. Applying a vacuum allows for rapid removal of perfusion solution from the tissue chamber. Our system has shortened the time required for tissue oxygen tension to equilibrate from over 12 minutes in the standard setup to below 30 seconds for our system. Consequently, this approach models ischemia-reperfusion much more realistically than previous approaches, as it changes oxygen tension nearly instantaneously. So far, my group has demonstrated this in our model of ischemic stroke, and this collaboration is a great opportunity to expand the scope of our technique to myocardial ischemia-reperfusion. I am excited to collaborate with you and apply our innovative and highly effective superfusion setup to the evaluation of your new approach for treating myocardial reperfusion injury.

My office is a mere 30 second elevator ride from yours, and my door is always open. I am pleased to field any questions you may have. Please note that our group has also extensive experience monitoring cellular redox status using H_2O_2- and GSH-responsive readouts, and we will be happy to answer questions that come up as you use these approaches to complete your proposed experiments. As you know, as the Director of Graduate Studies, I am also happy to help and provide general advice during your graduate training. I am particularly attuned to the unique training requirements you face as an MSTP trainee, having mentored one myself (Amir Faraji, who is currently a PGY4 neurosurgery resident at the University of Pittsburgh and whom you have shadowed). Best wishes as you continue your experimental work, and feel free to stop by any time!

Sincerely,



Stephen G. Weber, PhD
Professor of Chemistry
Director of Graduate Studies
Professor of Clinical Translational Science

UPMC LIFE CHANGING MEDICINE

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March 25, 2017

Dear Josh,

I would be thrilled to serve as a consultant on your project “Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction.” I’m glad that you’re devoting your research efforts to developing a novel approach for treating reperfusion injury. I feel that you are in a unique position to leverage your skills as a chemist and knowledge of vascular biology for preclinical testing of your approach, and I’m excited to work with you and see your results.

I have been a practicing cardiologist at UPMC for 33 years. I run a busy outpatient general cardiology clinic in addition to directing the inpatient Cardiac Pavilion Service at UPMC Presbyterian, which is our medical center’s flagship hospital. As you know, I have always made teaching a high priority throughout my career, and I have earned six awards for teaching medical students, residents, and fellows. I very much enjoyed having you as a student in my small “problem-based learning” group during your second-year cardiology course. A few years ago, I was lucky enough to get to train an MSTP student, Beth Oczypok, when she did a Longitudinal Clinical Clerkship (LCC) with me as she completed her graduate school training. I think Beth got a lot out of it, and I would be thrilled to take you on as an LCC student. As many of my patients have a history of MI and have undergone reperfusion by thrombolytic therapy or percutaneous intervention, I think it will be a good experience that shows you the clinical aspects of your research. During the LCC, you’ll certainly become familiar with the diagnosis and management of the heart failure, valvular pathology, and electrical abnormailities that arise as sequelae of MI and are driven—at least in part—by reperfusion injury. Additionally, I will make arrangements for you to see the cath lab so that you can see reperfusion in action.

Echocardiography is a major component of my practice, and I have published several articles on the subject. On your LCC, I expect that you’ll gain a lot of experience interpreting echos and will come to understand their role in diagnosis and treatment. Your clinical experience with echocardiography will complement the echocardiographic readings you’ll obtain in your animal experiments.

Please don’t hesitate to contact me to discuss the clinical ramifications of your research as you complete your experiments. As you are learning during your dual-degree training, close cooperation of clinicians and basic scientists has yielded some really exciting results and discoveries over the years, and I’m excited to see where it takes us.

Sincerely,

Frederick W. Crock, MD

Fred Crock, MD
Assistant Professor of Medicine
University of Pittsburgh

University of Pittsburgh

Kenneth P. Dietrich School of Arts and Sciences
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Kay M. Brummond, PhD
Professor and Chair

April 5, 2017

Joshua Wesalo
1314S Chevron Science Center
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Pittsburgh, PA 15260

Dear Josh,

I am writing to confirm that the Department of Chemistry supports your F30 proposal entitled "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction." It is excellent to see that you're working on your grant writing skills early-on in your career. Your proposal, in particular, exemplifies the collaborative approach our department takes that leverages our unique position near the medical center to carry out research that would be very difficult to do anywhere else.

I remember meeting you during your second look visit three years ago and was hoping you would come to Pitt. I am thrilled to have more MD/PhD students in the department, since we have a history of successfully training MSTP students. Most recently, Amir Faraji earned his PhD in Steve Weber's group (with whom you're collaborating as I understand it), and is now a PGY4 resident in neurosurgery at our medical center – on track for a career as an academic surgeon-scientist. We also trained Justin Baca, who did his thesis work with Sandy Asher and is now a physician-scientist in the emergency medicine department at the University of New Mexico. You have laid out an excellent training plan and will receive top-notch training working in the Deiters group. Alex is exceptionally well-funded and has established a track record of success in applying for grants from the NIH and from other agencies.

I want to reiterate my enthusiasm for this highly collaborative project that combines medicinal chemistry with vascular biology, and I think the proposed research will help you grow as a scientist. The department pledges its full support. I wish you continued success.

Sincerely,

Kay M. Brummond

University of Pittsburgh
School of Medicine
Vascular Medicine Institute

Patrick J. Pagano, PhD
Professor & Vice Chair for Graduate Education,
Pharmacology & Chemical Biology
Director, Molecular Pharmacology Program

March 29, 2017

Dear Josh,

I am pleased to serve as a consultant on NADPH oxidase (Nox) inhibitors and biochemical reactive oxygen species (ROS) assays for your project "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction". The treatment approach you are proposing is highly innovative, and represents a promising strategy for inhibiting Nox2, a key producer of harmful ROS in the area-at-risk, without perturbing redox balance elsewhere. The localized inhibition of Nox2 in response to pathologic hydrogen peroxide inhibition will be transformative for the field, as it presents a clever solution to the numerous off-target effects that are observed with systemic Nox2 inhibition.

As you know from your rotation in my laboratory, my group has made significant progress in preparing and testing isoform-specific Nox inhibitors and investigating their therapeutic potential. We were one of three labs to first to identify that Nox was found outside phagocytes (in the vascular wall). Subsequently, we were the first group to publish an isoform-specific Nox inhibitor, and we also were the first to clone p67phox, the activating subunit of Nox2. Our work has led to several fundamental discoveries about the roles specific Nox isoforms play in cardiovascular disease. I have 25 years of experience working on ROS and Nox inhibition and my group has published over 90 papers and reviews in the field. Additionally, Dr. Eugenia Cifuentes from my group co-directs the Free Radical and Reactive Oxygen Species Core Facility. She is an expert in the biochemical assays that you are proposing for measuring ROS production, and will be happy to consult with you on performing the assays and analyzing the data, just as she did when you rotated in the lab.

As you described in your training plan, the Vascular Medicine Institute will serve as a key resource for you, both for completing your experiments and for your training at the intersection of chemistry and vascular biology. I encourage you to take advantage of the VMI's core facilities in Biostatistics and Small Animal Hemodynamics, and I look forward to seeing you at grand rounds and VMI seminars. I am happy to act as your liaison with the VMI.

Please feel free to contact me at any time if you have any questions regarding Nox inhibitors or biochemical assays for ROS production. As you know, my lab and office are just a short walk from your lab, and you're welcome to stop by any time.

Sincerely,

Patrick J. Pagano, PhD, FAHA
Professor and Vice-Chair, Graduate Education, Department of Pharmacology and Chemical Biology
Program Director, Molecular Pharmacology Graduate Program

ADDITIONAL EDUCATIONAL INFORMATION (by Richard Steinman MD PhD, Director MSTP, Steinman@pitt.edu)

A. University of Pittsburgh-Carnegie Mellon University MSTP Structure. MSTP students in Pittsburgh complete a MSTP-specific enrichment curriculum beyond the standard courses in medical and graduate school. This consists of 3 summer research rotations, 3 summer professional development courses, a 3-semester weekly journal club featuring research papers consistent with the coincident SOM curriculum, a 4-week case-based ethics course, a monthly program-wide workshop, a 40-week longitudinal clinical clerkship (1/2 day/week) during the graduate years, and yearly special events such as the two-day MSTP Scientific Retreat.

B. Laboratory Research Rotations. Research rotations begin the summer prior to the start of medical school. In addition to developing manuscripts and presenting at scientific meetings based on their rotation results, all students turn in a written scientific report that is reviewed by MSTP leadership and present their work at the annual MSTP Scientific Retreat. The choice of thesis laboratories by students is informed by their rotation history and by discussion with their individual Career Advisors (who follow them longitudinally in the program).

C. Professional Development. Students take three successive 10-week long Professional Development Courses during summers prior to starting graduate school. The first course (PD1) focuses on scientific writing and introduces students to biomedical software and to key methods used by different disciplines to approach scientific problems. The PD2 course focuses on scientific design and career development strategies, with particular emphasis on reproducibility and biostatistics. The PD3 course focuses on grant review and writing.

D. Training in Reproducibility in Science. The PD2 course focuses on optimizing reproducibility of findings, to power experiments, and analyze data with appropriate statistical testing. Topics for classes include problems arising from non-reproducible work, optimal experimental and reagent documentation and handling, the ARRIVE guidelines for animal work, measurement validity and sources of error, robust hypothesis testing, and a series of sessions on biostatistics including customized problem solving tied to student data.

E. Biomedical and ethical expertise. During MS1 and 2 years, students build biomedical knowledge through a 3-semester MSTP literature review course in which students present papers after formal consultation with local faculty experts in the field of that paper. During the G1 year of graduate school, MSTP students take a month-long, weekly, case-based research ethics course. Throughout both medical school and graduate school, all MSTP students meet monthly for student-arranged seminars that pose scientific, logistical, clinical and/or ethical dilemmas. These workshops are presented by students and/or guest faculty experts.

F. Clinical and Research Integration. This is a central focus to better model the physician scientist career.

F.1 Clinical Activities During the Research Years: Prior to starting graduate school, all of our MSTP students complete 8 weeks of required clinical core clerkships. This front-loads requirements once students re-enter medical school post thesis and enables research engagement in MS3 and 4. MSTP students are required to complete a (credited) minimum of two 20-week long Longitudinal Clinical Clerkships during graduate school. For each LCC, students spend a half day per week with a clinician scientist receive one-on-one clinical mentoring by a clinician scientist in an area of interest chosen by the student with guidance from the MSTP LCC director, Paul Monga, MD. Student objectives for the LCC and write-ups at the end are reviewed by MSTP leadership.

F.2 Transition from Graduate to Clinical Years. During our MSTP Clinical Reentry elective, a master clinician mentors the returning students once weekly over the month prior to reentry as they examine, discuss, diagnose and plan treatment for surrogate patients presenting with common outpatient or inpatient ailments.

F.3 Research During Clinical Years. Our students continue their research focus after re-entry to medical school generally in four ways: (1) MS3 and MS4 students continue to plan and execute MSTP Workshops that feature research topics and research challenges to be discussed with MSTP peers. (2) Students complete formal reflective and goal-oriented self-assessment evaluations during twice-yearly Career Advisor meetings. (3) Students average 2.8 new publications during the MS3 and MS4 years (at least one first authored), averaging 5-7 papers upon graduation. (4) Most students elect to take 1-2 Research Elective months during their MS4 year to extend findings of thesis work and/or to build skillsets in a translational area. Another novel feature of our MSTP, the Postdoctoral Fellowship, provides support for 5 months of postdoctoral research prior to residency for MSTP students graduating in December (25% of graduates in recent years). Applications address research hypotheses and aims, career development aims, planned deliverables, mentor fit, and intellectual goals.

G. Monitoring and Evaluating Student Progress. Prior to matriculation, the Program Director assigns each new student a Career Advisor based on matching research interests who helps orient and guide the student

throughout their career. Most of a trainee's time in the graduate program is spent in research training under the guidance of their research mentors, program leadership, and eventually their doctoral dissertation committee. To customize advice and resource allocation, all MSTP students complete and share *individual development plans* with the Director and with their Career Advisor. The form allows students to identify specific skills that they want to develop; to set technical, intellectual and professional goals; and to identify how goals will be achieved and measured. Resources to reach goals and obstacles that could compromise success are enumerated and discussed. Progess toward goals is regularly reviewed with the Advisor and new goals are set.

H. Career Counseling. To better reflect the student's educational experience to prospective residency programs, the MSTP creates an executive summary which describes student evaluations, honors, presentations and participation in the combined degree training, rewarding students who altruistically give their time and demonstrate prowess in working in groups. Six months to one year before completing their doctoral program, students meet with the Program Director and the Career Advisory committee to discuss postgraduate training, residencies, fellowships, and faculty positions and non-academic based positions. Many of the faculty are MD/PhDs and are capable of participating in career planning for third and fourth year medical students.

I. Program Duration and Outcomes. Over the past 5 years, our time from enrollment to graduation has averaged 7.7 years (8.1 years in the prior 5-year period). The Pittsburgh MSTP has 170 alumni. 89% of graduates from the past 15 years are in the academic pipeline (either still in training or in academic positions). Graduating MSTPs in 2011-6 averaged 6.2 papers/student (3 first authored) and 47% had obtained F-grants.

J. Joshua Wesalo is a stellar member of our MSTP who matriculated into the MSTP program in June 2014 and is in his G1 year as a graduate student in the Chemistry graduate program. He is pursuing his doctorate in the laboratory of Dr. Alex Deiters, a highly respected expert in synthetic and opto-chemical chemistry applied to biological systems. Josh's outstanding performance in the MSTP to date is described in the Letter of Recommendation from the MSTP Director. Josh completed his MS1 and MS2 coursework and passed USLME Step 1 in April 2016. He completed the MSTP Professional Development courses, 3 laboratory rotations (2 in the laboratory of Dr. Deiters), Research Basis of Medical Knowledge courses, and an 8-week clinical rotation in neurology and psychiatry prior to beginning graduate training. Josh formally entered the Chemistry program in 2016. Over the past 5 years, he has given 5 oral presentations at 5 meetings, including *Genomic Medicine and the Plain Populations of North America*. A co-authored manuscript on the chemically-triggered control of protein SUMOylation is under preparation.

The coursework for Chemistry consists of a total of four courses. Josh has completed two courses and is completing the next two this semester. The Graduate Student Advising Committee has worked with Josh to design a unique course of study that suits the needs of a physician-scientist in training.

The Chemistry PhD has several program milestones with timelines adjusted for expeditious completion by MSTP students. Josh expects to take his Comprehensive Exam in the summer of 2018, where he will prepare a report describing his research findings thus far, and will discuss the findings and field questions from pre-selected training faculty that will eventually become his thesis committee members. Josh plans to schedule his Thesis Proposal immediately after completing his Comprehensive Exam**,** where he will propose the research aims that he will complete to fulfill his PhD. After completing his milestones, Josh will be a formal PhD candidate and his progress will be monitored at regular, biannual committee meetings as well as in biannual MSTP Career Advisor Meetings where his updated Individualized Development Plans will be reviewed. Josh expects to complete Longitudinal Clinical Clerkships in Fall 2017 and Fall 2018 during graduate school. He expects to defend his PhD and research proposal and return to medical school in Summer 2021, putting him on track to complete his remaining medical school clerkships and graduate from our program in Spring 2023. For the terms of the fellowship proposed, Josh plans to complete an additional 48 months of research, followed by clinical work for 18 months. Should Josh elect the route in which he graduates medical school in December of 2022, he will then undertake the 5 month MSTP Postdoctoral Fellowship which is not included in the time of covered support requested in the current application.

Richard Steinman MD, PhD
Director, Medical Scientist Training Program
Steinman@pitt.edu

Vertebrate Animals

Aim 2 of this proposal requires *in vivo* experiments, and Aim 1C requires isolation of primary cells from vertebrate animals; all other aims will be accomplished using *in vitro* studies.

1. **Description of Procedures.** 6-7 week old Sprague Dawley rat pups will be used for the cell culture studies in the proposed aims. One-year-old male (550–650 g) and female (350-450 g) Sprague Dawley rats will be used for the animal studies.

   TOTAL RATS: 131 RATS

   Sub-Aim 1C. Number of rats (6 rats): Primary cardiac myocytes will be isolated from 6-7 week old Sprague Dawley rats using the Worthington Cardiomyocyte Isolation System (Worthington Biochemical Corporation, Lakewood, NJ). Hearts will be excised from rats deeply anesthetized with isoflurane. 2 rats are required for each experiment. As three cell culture experiments are proposed, 6 total animals are required.

   Sub-Aim 2A. Number of rats (22 rats): One-year-old male (550-650 g) and female (350-450 g) Sprague Dawley rats will be used to assess the therapeutic benefit of the proposed approach. Power indicates that 11 animals will be required for each of the two treatment groups, treated vs. untreated. A left thoracotomy will be made at the level of the fourth intercostal space, and the pericardium will be gently pulled apart. Under a dissecting microscope, the left anterior descending (LAD) coronary artery will be visualized. A no. 4 ½ circle taper point needle will be used to pass 6–0 polypropylene suture underneath the LAD at a point 2 mm distal to the tip of the left auricle. A loose double knot will be tied, through which a short length of PE-10 tubing will be placed, and the loop will be tightened and secured with a slipknot. Occlusion will be verified by confirming pallor in the LAD's territory. Ischemia will be maintained and skin will be clamped together for 60 minutes. Afterwards, the knot will be untied and the tubing removed. The suture will be left in place for determination of infarct size and area-at-risk (AAR) by triphenyltetrazolium chloride (TTC) and Alcian blue staining. After 24 hours of reperfusion, the animals will be reanesthetized and the chest reopened. Analysis of the infarct will be completed after intracardiac injection of potassium chloride and excision of the heart.

   Sub-Aim 2B. Number of rats (38 rats): First, our fluorescent H_2O_2 probe (caged or active/uncaged) will be injected into in animals subjected to I/R injury or to sham surgery. After euthanasia, fluorescence will be quantified in organ homogenates. Power analysis indicates that 2 animals will be necessary for each of the 4 treatment groups. Second, in animals treated with either the active drug, caged compound, or saline and subjected to I/R injury or sham surgery, Erk2 and Stat3 phosphorylation will be compared between injured and uninjured tissues by Western blot. Based on power analysis, 5 animals will be needed for each of the 6 treatment groups. Overall, 38 rats will be needed.

   Sub-Aim 2C. Number of rats (65 rats): To determine the extent to which the proposed treatment approach reduces chronic injury after ischemia-reperfusion in Sub-Aim 2C, rats will be monitored for 28 days post-surgery. At five time points (pre-surgery baseline, 3 days, 7 days, 14 days, and 28 days), the rats will be anesthetized for echocardiography. After the final echocardiographic reading, anesthesia will be deepened, and hemodynamics will be further assessed by PV catherization. Based on power analysis, 9 animals per treatment group will be necessary. Based on the Wang group's past experience with a similar model, 13 animals will be used in each treatment group to compensate for attrition and to ensure a large enough sample size for adequate power at the end of the experiment. Five treatment groups (vehicle, acute treatment, acute and chronic treatment, chronic treatment, and vehicle/sham-operated) will be present, necessitating 65 animals total. The sham surgery will stop after dissection of the pericardium.

   Housing. Animals will be housed at the Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh. Rats will be housed in pairs with unrestricted access to food and water, with bedding provided in each cage. Staff veterinarians and veterinary technicians in addition to the PI will monitor the animals' health at least once per day; staff will recommend treatment with antibiotics and analgesics as necessary to minimize pain in the animals. Veterinary staff members are available during business hours, and are always on-call in case of emergencies. The staff can also intervene or suggest euthanasia for any animals showing signs of distress, which, for this model, include hunched, immobile posture; spiked coat; self-mutilation; aggressive behavior; vocalizations; and signs of excessive inflammation at the surgical site.

2. **Justifications.** Live organisms are necessary for the proposed work because cell culture experiments alone do not accurately recapitulate myocardial ischemia-reperfusion in a way that allows assessment of clinically-important parameters (infarct size/area-at-risk and hemodynamics). Additionally, cultured cells cannot be used to detect perturbations of cell signaling pathways in uninjured organs. Current mathematical models and computer simulations are insufficient for this purpose. Rats are a well-accepted myocardial ischemia model, and accepted models are not available in lower species (e.g., invertebrates). Sprague Dawley rats were chosen because they have been well-established as a model organism for myocardial ischemia-reperfusion, and are widely considered to be a docile, easy-to-handle strain.
3. **Minimization of Pain and Distress.** For surgical procedures, anesthesia with isoflurane will be accompanied by analgesia and sedation with ketamine and xylazine (100 mg/kg i.p. and 7.5 mg/kg i.p., respectively). Anesthesia will be induced using 2-5% isoflurane in oxygen and maintained using 1-3% isoflurane in oxygen. After surgery, EMLA cream will be applied to the surgical site as a local anesthetic. Post-operative rats will recover in an oxygen-rich chamber, and will not be returned to their housing until fully awake and mobile. Buprenorphine hydrochloride (0.1 mg/kg SC BID) and ketorolac (5 mg/kg SC BID) will be given for three days to minimize pain. Antibiotics will be given if the surgical site becomes infected. This surgery has a risk of pneumothorax, and animals will be euthanized if discomfort arises from this condition. Buprenorphine will be given as needed if any signs of pain or distress are observed in the animals. If any animals have a severe negative reaction to surgery, characterized by aggression, vocalization, and/or hunched posture, veterinary staff will be consulted, and animals will be euthanized if necessary.
4. **Euthanasia.** Euthanasia will be performed by cardiac injection of potassium chloride (KCl) in deeply anesthetized rats. This method is necessary to ensure that the heart is in diastole when it is excised, which is essential for the proposed experiments. The American Veterinary Medical Association (AVMA) *Guidelines for the Euthanasia of Animals* approves this method as a special case if the animal is fully anesthetized. Isoflurane will be used to ensure full anesthesia before KCl injections.