

## SPONSOR'S RESEARCH PROJECT ENVIRONMENT

The environment at the University of Pittsburgh and specifically the Deiters, Dutta, 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<sub>2</sub>O<sub>2</sub>)-responsive Nox2 inhibitors.

All equipment required for the synthesis and characterization of the H<sub>2</sub>O<sub>2</sub>-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.

All equipment and necessary support for the proposed animal experiments is available in the Vascular Medicine Institute (VMI) via the Dutta lab. As core faculty of the VMI, Dr. Dutta has the full support of this comprehensive research infrastructure, access to multiple shared research resources, and collegial interactions with other funded investigators with diverse interests in cardiovascular research.

**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.

**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.

**Dutta Laboratory.** The laboratory of Dr. Dutta is located on the 17th floor of the main Biomedical Science Tower (Starzl). Dr. Dutta has dedicated space with full access to shared space for acute and chronic animal surgical procedures (600 sq. ft.) and a cell culture room (150 sq. ft.). All investigators, as members of the Vascular Medicine Institute (VMI), have free access to extensive common equipment and over 2200 sq. ft. of common research space, including tissue culture rooms, animal procedure suites, cold rooms, chemical hoods, flow

cytometry, and microscopy suites. Dr. Dutta has access to university-wide imaging, histology, gene expression, and proteomics core facilities at the University of Pittsburgh. All resources relevant to the proposed research plan are housed in the University of Pittsburgh. Dr. Dutta's cell culture hoods are required to have a biosafety level 2 rating when working with human cells, which is in compliance with the Environmental Health and Safety board at the University of Pittsburgh.

**Clinical.** Not applicable.

**Animal.** Dr. Dutta and Dr. Weber have access to shared animal facilities through the Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh. Animals will be maintained in the animal facilities located at the 10th floor vivarium adjacent to Dr. Dutta's laboratory space in the East Biomedical Science Tower. The facility 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 (Animal Welfare Assurance No. D16-00118). 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. Mice are housed 4-5 per cage, rats in pairs, and all animals are monitored daily by a member of the veterinary staff and/or animal care staff and by the PI. Full veterinary consultant 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. Dutta and Weber also have several computers with all necessary software to support the proposed research, in addition to scanners, printers, and digital cameras. All major pieces of equipment are controlled with dedicated desktop computers.

**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. Dutta's is 2 blocks away in the VMI. The VMI provides full administrative support to Dr. Dutta and offers two conference rooms for private discussions or lab meetings that are wired with state-of-the-art projectors.

**Major Equipment.** 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 of absorbance, fluorescence, and luminescence assays. Equipment for cell biological work includes two 6-foot biosafety cabinets; three Forma II CO<sub>2</sub> incubators; and two Zeiss AxioObserver Z1 inverted epifluorescence microscopes on Ametek TMC CleanBench vibration isolation tables equipped with an Andor Zyla sCMOS camera, a Tokai Hit WSKM Stage Top Incubator, a custom-built desktop computer, Excelitas X-Cite 120LEDBoost, and a Zeiss Definite Focus unit. 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 Orbitrap, and a Shimadzu LCMS system available for walk-on student use. This facility is managed by a PhD-level scientist (Dr. Godugu). 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. The NMR facility is managed by a PhD-level scientist (Dr. Achary) and a technician. A Horiba/Jobin-Yvon FluoroMax 3 Fluorescence Spectrophotometer, a Veeco Nanoscope 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. The facility is led by Dr. Yates and supported by three PhD-level scientists (Drs. Zeng, Wendell, and Mullett) and six technicians.

*Major equipment relevant to this research available in the Weber Laboratory and Chemistry Department Biological Instrument Cluster (7 floors and 2 floors below the Deiters lab, respectively):*

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 Nuairé) are available for cell culture, along with two VWR CryoPro liquid nitrogen cell storage dewars and two dedicated HeraCell CO<sub>2</sub> incubators. This equipment is located in the Department's Biological Instrument Cluster. This equipment is available in a secured BSL2 laboratory adjacent to a space with autoclaves, a -80 °C freezer, and additional equipment for bacterial and mammalian cell culture.

*Major equipment relevant to this research available at the In Vivo Imaging Facility at the University Pittsburgh Cancer Institute (with which Dr. Deiters is affiliated) a short walk away:*

Equipment for preclinical optical imaging includes an IVIS 2000 system (Xenogen, Perkin Elmer) with full bioluminescence and fluorescence imaging capabilities and a Lumina XR system (Perkin Elmer) with bioluminescence, fluorescence, and x-ray imaging capabilities. The preclinical ultrasound service offers three Vevo 770 small animal ultrasound (Visual Sonics) with three transducers designed for use in different rat or mouse models.

*Major Equipment Available at the Vascular Medicine Institute, Department of Medicine 2 blocks away (<http://www.vmi.pitt.edu/centers-cores.html>):*

A. Cell Analysis (<http://www.immunology.pitt.edu/flow-cytometry/instrumentation>)

5 Phase contrast and fluorescent microscopes

2 LSR II flow cytometers (equipped with 3-4 lasers and have up to 12 color fluorescent cytometry capability)

3 FORTESSA flow cytometers (equipped with 4-5 lasers and have up to 16 color fluorescent cytometry capability)

2 Aria I/ II cell sorters (equipped with 4-5 lasers and have up to 16 color fluorescent cytometry capability)

ImageStream Mark II (equipped with 6 lasers and 20X, 40X and 60X magnification lenses, has combined ability of flow cytometry and microscopy)

Miltenyi cell separator

## B. Cell Culture

- 6 cell culture rooms (150-350 sq. ft. each)
- 15 Laminar flow hoods
- 20 Water-jacketed CO2 incubators
- 5 Liquid nitrogen storage tanks
- 2 Ultra-pure water purification systems
- 4 Autoclave facilities
- 3 EVOS XL Core microscopes

## C. Live animal imaging facility (<http://www.cbi.pitt.edu> )

### Microscopes:

- 2 Olympus FV 1000 Confocal microscopes (inverted)
- 1 Olympus FV1000 Confocal Microscopes (upright)
- 1 Olympus FV500 Confocal Microscope (Upright)
- 1 Zeiss 510 Meta Confocal Microscope (Inverted)
- 1 Perkin Elmer Spinning Disk Confocal Microscope (Inverted)
- 1 Olympus DSU Spinning Disk Confocal Microscope (Inverted)
- 6 Nikon Sweptfield confocal microscopes
- 1 Optiscan Confocal Endoscope/Microscope
- 2 Nikon A1R confocal microscopes
- 1 Nikon N-Storm system
- 1 Nikon SIM system
- 1 Nikon A1R multiphoton microscope (with resonant scanning)
- 3 Multimode microscopes ((Automated XY, Rationing, FRET) (two with microinjection capabilities)
- 2 Multimode widefield/TIRF microscopes (one with microinjection capabilities)
- 1 Olympus multimode (3D, 2D fluorescence, high speed) dissecting microscope
- 1 Olympus SXZ12 dissecting microscope
- 1 Olympus SXZ-ILLD100 dissecting microscope
- 1 Olympus BX51 light microscope (brightfield, darkfield, epifluorescence, DIC)
- 3 Olympus Provis light microscopes (brightfield, darkfield, epifluorescence, DIC)
- 1 Nikon Eclipse 800 microscope (brightfield, darkfield, epifluorescence, DIC)
- 1 Nikon FX Series microscope (brightfield, darkfield, epifluorescence, DIC)
- 2 Nikon 90i Upright microscopes (Automated XYZ, brightfield, darkfield, epifluorescence DIC)
- 1 JEOL 1011CX Transmission Electron microscope with high resolution AMT digital camera
- 1 JEOL 1400 Transmission Electron Microscope with high resolution AMT digital camera
- 1 JEOL 9335 Field Emission Gun SEM with backscatter detector

The microscopes are equipped with Ti:Sapphire lasers, automatically adjustable lenses, adjustable prechirping, and variable backfilling. They are equipped for small animal *in vivo* imaging and are coupled to the following visible lasers for confocal mode: 488, 532, 560, and 660 nm.

### Microtomes:

- 3 Microm cytostats
- 6 Reichert Ultracut (R.S.E. Ultramicrotome 1 with cryokit)

### Image analysis:

- 2 scanners (flat bed and slide)

58 Pentium-based PCs with MetaMorph and Photooshop, and other software packages (including Imaris, Amira, Image Pro, and NIS Elements)

4 Large Network servers and online storage (150 terabytes)

Miscellaneous:

Multiple CCD cameras for light microscopes

Multiple (15) live cell chambers and syringe pumps for media perfusion

Miscellaneous small light (inverted phase) microscopes

PCR thermocycler

Assorted protein and nucleic acid gel running apparatuses and power supplies.

Tissue culture facilities include 2 tissue culture hoods and 3 incubators.

#### D. Microscopy and Digital Imaging at the Vascular Medicine Institute

2 Nikon 90i microscopes (with motorized X-Y-Z stage, 6 Position Fluorescence Turret and Digital Imaging Head driven by Nikon Elements Advance Research Software)

3 Olympus Provis microscopes (equipped for high resolution Brightfield, DIC, Darkfield and Fluorescence imaging)

3 Nikon Eclipse Ti (an inverted microscope for live cell imaging, equipped with a unique Perfect Focus System)

2 JEM Transmission Electron Microscope (equipped with elemental mapping with large-area SDD detectors, cryomicroscopy, 3D tomography and montaging).

3 EVOS XL fluorescent microscopes and imaging systems

#### F. Molecular Biology

2 Applied Biosystems Pro-flex PCR systems

8 PCR thermal cyclers

3 Applied Biosystems QuantStudio 6 Real Time PCR machines

4 NanoDrop spectrophotometers

4 Thermo Scientific E-gel electrophoresis imaging systems

3 Water purification systems

4 Spectrophotometers

4 Chemiluminometers

8 ELISA plate readers

8 Bio-rad Electrophoresis systems

10 Mettler Toledo pH meters

4 Electroporation apparatuses (Cellzap II)

1 Bio-Rad Gel Doc EZ System

2 Odyssey CLx Imaging Systems

3 Bio-Rad semi-dry transfer systems

4 SDS gel separation systems

#### G. Pathology Facilities

2 Thermo Scientific Sahnidon Excelsior ES tissue processors

4 Tissue-Tek TEC embedding stations

3 Leica RM2235 tissue sectioning apparatuses

2 Microm HM550 cryostat tissue sectioning apparatuses

#### H. Surgical Facilities (<http://www.vmi.pitt.edu/hemodynamic.html> )

2 Small animal surgery rooms (600 sq. ft.)

4 Stereo Zoom microscopes with halogen dissection lights

2 Small animal respirators  
1 Advantage Pressure Volume Measurement Systems (Transonic)  
1 Systemic blood pressure monitor (AD Systems)  
1 Millar 1.4-F Mikro-Tip catheter connected to a pressure-control unit and interfaced with a Gould signal amplifier and recorder  
1 Acuson C256 ultrasonographic system  
1 High resolution digital echocardiography system (Vero770, Visualsonics)  
1 Telemetry blood pressure system model TA11PA-C20  
1. Radio telemetry system from DSI outfitted for measuring right ventricular systolic pressure, heart rate and activity in 12 mice simultaneously in real-time using PA-C10 transmitters.  
2 Kent Scientific Genie Plus infusion Systems  
1 Doppler Signal Processor 200  
20 Rodent hypoxia chambers  
4 isoflurane vaporizers  
2 warming pads  
2 glass bead sterilizers

#### I. Shared research cores available to VMI researchers

Genomics Research Core (<http://www.genetics.pitt.edu>)

Microscopy and Imaging Facility (<http://www.biology.pitt.edu/facilities/microscopy-facility> )

PET-CT imaging facility (<http://www.rad.pitt.edu>)

Magnetic Resonance Research Center (<http://www.rad.pitt.edu/?research/mrrc.html>)

STI research cores (<http://www.biology.pitt.edu/facilities/microscopy-facility>)

Health Science Tissue Bank (<http://www.tissuebank.pitt.edu>)

**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](http://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. 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. Enhancing the resources available to the PI is a state-of-the-art Free Radical and Reactive Oxygen Species Core in the VMI, equipped for

measuring oxygen consumption (including Seahorse Bioanalyzers), EPR (Electron Paramagnetic Resonance) spectroscopy, hydroethidine oxidation (DHE), Immunospin Trapping, and high-throughput assays for precise characterization and quantification of cell, tissue and animal ROS. An in-house transgenic animal core facility is also readily available to the investigators. In close proximity to the investigators lab space are the facilities of the University of Pittsburgh Drug Discovery Institute (UPDDI) headed by Dr. L. Taylor. The UPDDI's HTS/HCS houses equipment specific for high throughput screening including, and not limited to, a compound storage and retrieval system which maintains > 225,000 compounds, automated liquid handling systems, robotic plate handlers, multiple plate readers, and data analysis software. Additionally, Dr. Dutta has immediate access to the NO Metabolomics Core facility of the VMI for quantification of nitric oxide (NO), nitrite, nitrate, S-nitrosothiols, NOS expression, and NOS activation. In collaboration with Dr. St. Croix, Dr. Dutta has access to the core facilities of the Center for Biologic Imaging (CBI). The CBI is housed in the biomedical research facility of the University of Pittsburgh Medical School South BST in approximately 5,500 sq ft. of space, equipped with state-of-the-art facilities for both light and electron microscopy, for digital image creation and handling, and for conventional photography and film development. The suite houses both scanning and transmission electron microscopes as well as a complete system for laser scanning confocal microscopy with 3D image reconstruction using a graphics workstation.

University of Pittsburgh biomedical core facilities also include: FACS analysis, DNA sequencing, SNP genotyping, RNA/DNA extraction, purification and QC services, Affymetrix and Illumina gene expression micro-arrays, Luminex technology-based protein, gene and transcription factor expression profiling, isotyping, mRNA expression, and signal transduction, which are paid services available at a discounted rate for university researchers.

**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. 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.

In summary, the state-of-the-art facilities, multi-faceted faculty, collaborative research and training environment, and strong institutional commitments to investigators make the Department of Chemistry, the VMI, and the University of Pittsburgh a world-class environment for Josh's AHA Predoctoral Fellowship.