

Facilities and Other Resources

Laboratory: The laboratory of Dr. Chan is located on the 17th floor of the main Biomedical Science Tower (Starzl). Dr. Chan has 1000 ft² of dedicated space, with full access to shared space for acute and chronic animal surgical procedures (600 ft²) and a cell culture room (150 ft²). Miranda has been supplied with her own laboratory bench space, fully equipped with her own set of pipettes and assorted supplies. Miranda has access open access to this space as well as extensive common equipment and over 2200 ft² of common research space in the Vascular Medicine Institute, including tissue culture rooms, animal procedure suites, cold rooms, chemical hoods, flow cytometry, and microscopy suites. Miranda, in conjunction with her sponsor, 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.

Animal: Mouse colonies are maintained at the animal facilities that are located at the 10th floor vivarium of the South Biomedical Science Tower at the University of Pittsburgh School of Medicine, which is directly adjacent to Dr. Chan's laboratory space in the East Biomedical Science Tower. All animal facilities at the University of Pittsburgh are under the direction of full time veterinarians and have been fully accredited by the American Association for Accreditation of Laboratory Animal Care since 1971. The University of Pittsburgh, School of Medicine complies with the NIH policy on the Animal Welfare Act, and all other applicable federal, state, and local laws. Mice are housed 4-5 per cage, and monitored daily by a veterinarian as well as by the P.I. Full veterinary consultant services are available 24hrs/day for animal care issues.

Office: The office space is directly adjacent to the laboratory space and Miranda has been provided with individual desk space and a network computer with free access to electronic journals, and research databases. Software to perform all necessary operations (word processing, data and statistical analysis, image analysis, stereology, graphing etc.) is readily available.

Other: Miranda has access Free Radical and Reactive Oxygen Species Core of the VMI, providing oxygen consumption (including Seahorse Bioanalyzers), EPR (Electron Paramagnetic Resonance) spectrometry, hydroethidine oxidation (DHE), Immunospin Trapping, High-Throughput Assay Systems for precise characterization and quantification of cell, tissue and animal ROS species. For *in vivo* work, the laboratory has common procedure rooms that house three mouse surgical areas including thermoregulated surgical tables, isoflurane vaporizers, surgical dissection microscope, ventilators, and all necessary surgical equipment for chronic instrumentation. For biochemical analyses, there are centrifuges, -80° and -20° freezers and all necessary equipment and supplies for Western, Northern, and qPCR analysis. State-of-the-art cell culture facilities with phase-contrast, fluorescence, standard bright-field microscopes are also available. There is also an NO Metabolomics Core facility of the VMI for state of the art quantification of nitric oxide (NO), nitrite, nitrate, s-nitrosothiols, NOS expression and NOS activation. Miranda has been trained by and has full 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. The CBI prides itself on being a leading center in the application of cellular imaging methods, from single molecules to the whole animal, particularly specializing in live cell fluorescence applications in both basic and applied studies. Other available core facilities at the University of Pittsburgh biomedical core facilities 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 are also available on a pay basis.

Scientific Environment: The University of Pittsburgh has a strong research record and consistently ranks among the top 10 recipients of NIH funding. This project will be carried out in the VMI at the University of Pittsburgh. The VMI is located in the Thomas E. Starzl Biomedical Science Tower on the main campus, adjacent to Presbyterian Hospital, the flagship hospital of the University of Pittsburgh Medical Center, and in the midst of 4.2 million gross square feet of research, academic, and administrative space in various buildings.

The mission of the VMI is to determine the molecular mechanisms underlying clinically important biomedical problems of cardiovascular biology, hemostasis, thrombosis, and transfusion medicine and the development of novel and rationally designed therapies for diseases such as pulmonary hypertension, sickle cell vasculopathy, atherosclerosis, hypertension, and heart disease. The VMI has published more than 210 peer-reviewed manuscripts since its 2008 inception. The VMI has six core laboratories that offer specialized techniques to enhance research in vascular biology: nitric oxide metabolomics, reactive oxygen species measurement, animal phenotyping, a human translational vascular unit, a genomics core facility, and confocal microscopy. The VMI offer numerous seminars, grand rounds, and research conferences. The exceptional and highly collaborative research environment in the VMI and the Department of Medicine at the University of Pittsburgh provides an ideal research environment for the studies that are proposed.