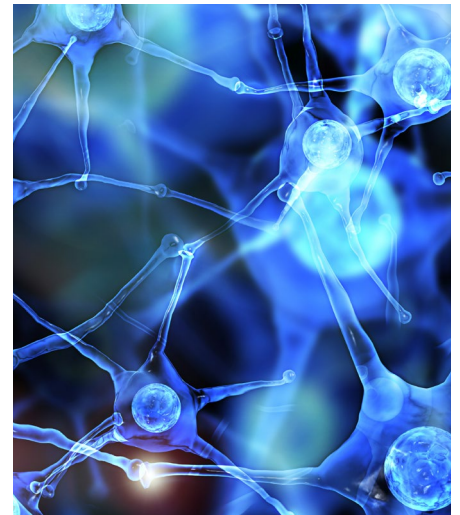


# ADVANCED TECHNOLOGY CORES



*Catalog 2019*

Baylor  
College of  
Medicine

ADVANCED  
TECHNOLOGY  
CORES







# ADVANCED TECHNOLOGY CORES



I am pleased to present the BCM Advanced Technology Cores catalog for 2019. This publication is designed to help you access the high-end instrumentation and specialized technologies you need for your research. The Advanced Technology Cores (ATC) at BCM expand the research capabilities of all researchers and essentially create unlimited research opportunities.

Each of the cores is staffed by a faculty level academic director, core directors and dedicated research technicians with highly specialized expertise in the technologies provided. A range of research support services are provided such as access to shared instrumentation, analysis of research samples provided by investigators and experiments with Core personnel performing specialized portions of the project. In addition to technical procedures, Cores provide consultation on experimental design, data analysis and training.

This catalog provides an introduction to each of the Cores including services and major instrumentation/technology platforms, core leadership, contact information and examples of scientific research supported by core. For more information about any of the Cores, visit [www.bcm.edu/research/corelabs.cfm](http://www.bcm.edu/research/corelabs.cfm).

On behalf of all the faculty and staff in the Cores, we look forward to working with you to advance science across all areas at BCM.

— **Mary E. Dickinson, PhD**  
Vice President and Dean of Research

## CORE LEADERSHIP



**Dean P. Edwards, PhD**  
*Executive Director*

Dr. Edwards provides scientific oversight and guidance and establishes policies for governance and funding.



**Jennifer McCullough, MBA**  
*Director of Business Operations*

Ms. McCullough administers financial and accounting policies, and provides strategic planning and guidance for business operations.

# ACKNOWLEDGEMENTS

**Financial support to subsidize Core operations is provided by the following Institutional sources and extra-mural grants.**

## **INSTITUTIONAL SUPPORT**

Dan L Duncan Comprehensive Cancer Center

Baylor College of Medicine Seed and Capital Funds

Office of Research: Advanced Technology Cores unit

## **GRANT SUPPORT**

NCI P30 Cancer Center Support Grant (CCSG)

NIH P30 Digestive Disease Center (DDC)

NIH U54 Intellectual & Development Disabilities Research Center (IDDR)

Cancer Prevention and Research Institute of Texas (CPRIT) Core Facility Support Awards

NEI P30 Instrumentation Module Center

NIH UM1 Consortium for large-scale production and phenotyping of knockout mice

NIH S10 Shared Instrument Grants





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# ANTIBODY-BASED PROTEOMICS

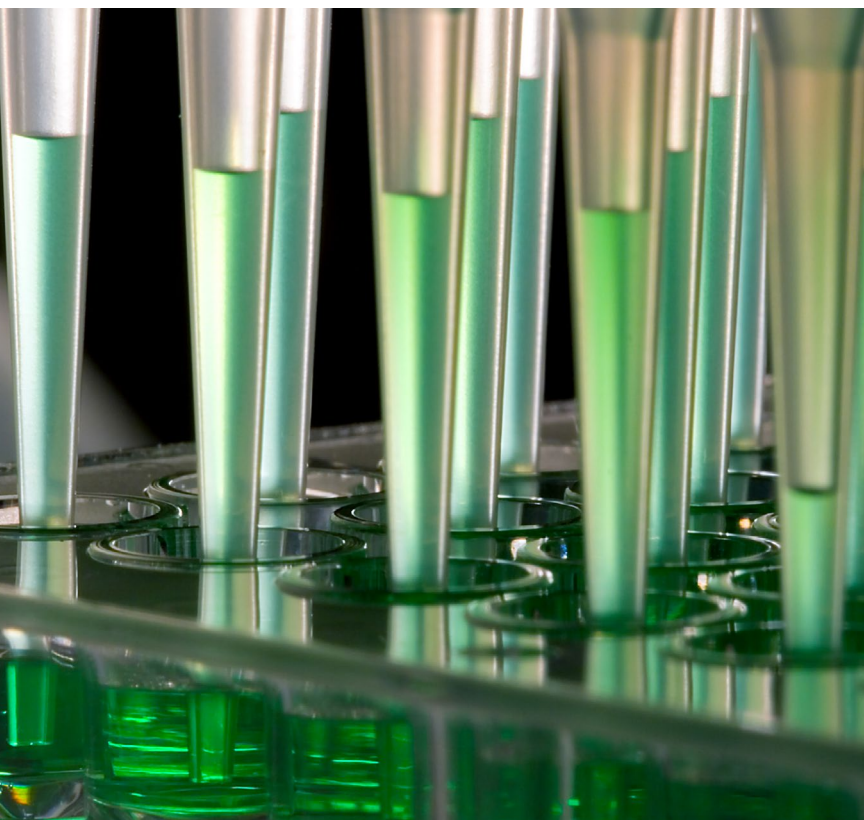
This Core provides customized services for protein profiling by antibody-based affinity platforms. These platforms provide targeted quantitative assays both for validation and protein biomarker discovery research, particularly for low abundance regulatory proteins and activation states of proteins with antibodies to specific phosphorylation sites. Services provided include reverse phase protein arrays (RPPA) and Luminex bead technology for multiplex quantitative analyses of intracellular and extracellular signaling proteins.

## MAJOR EQUIPMENT

- Bio-Plex 200 Luminex bead reader (Bio-Rad)
- Luminex bead washer (Bio-Tek ELx405)
- Aushon 2470 protein arrayer
- Dako Autostainer Link 48
- Axon Array Scanner 4200AL and GenePix software (Molecular Devices)
- TissueLyzer II (Qiagen)
- Molecular Devices Spectramax 340PC Plate Reader

## SERVICES

- Reverse Phase Protein Array (RPPA). High density microarrays spotted with researchers' protein lysates and probed with specific antibodies (>240 antibodies to proteins and phosphoproteins of multiple functional groups).
- Luminex bead assays (Luminex xMAP technology) for highly sensitive quantitative measurement with very small protein lysate or serum samples.
- Image analyses of protein/antibody microarrays.
- Data analysis
- Protein sample preparation.
- Consultation and experimental design.



## CORE LEADERSHIP



**Shixia Huang, PhD**

*Academic Director  
Associate Professor,  
Department of Molecular  
& Cellular Biology*

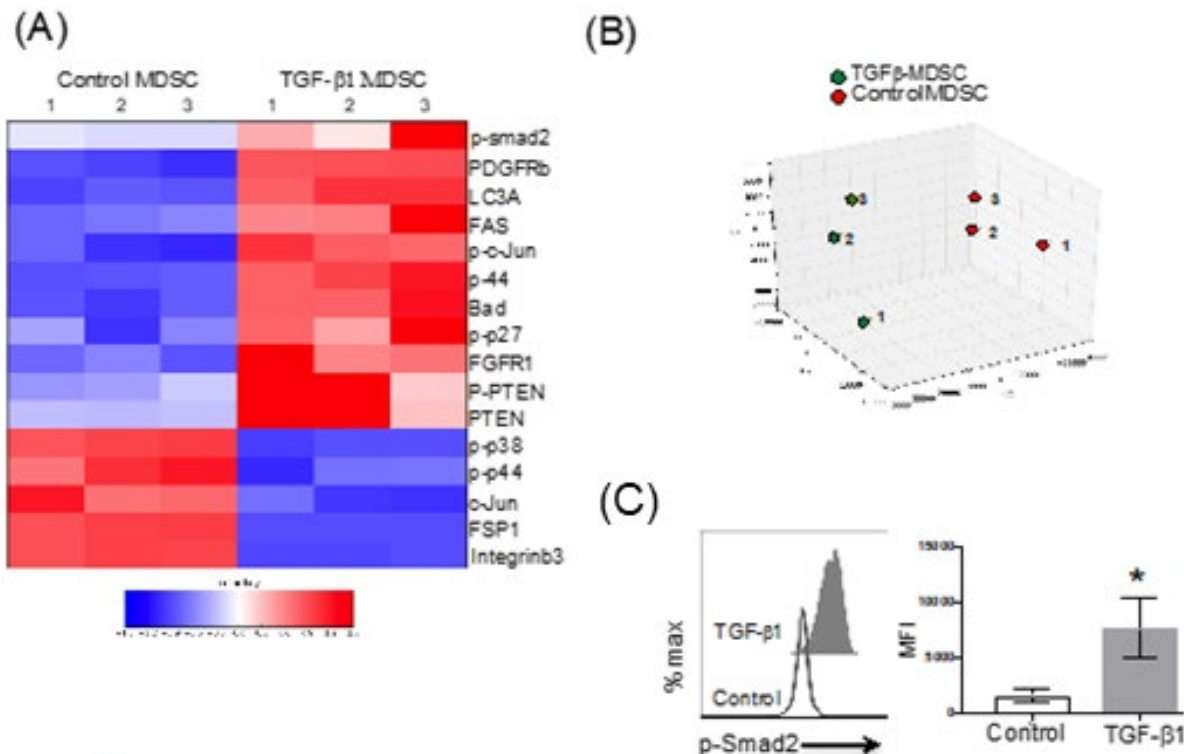
713.798.8722

shixiah@bcm.edu



## TGF- $\beta$ 1 programmed myeloid-derived suppressor cells (MDSC) acquire immune-stimulating and tumor killing activity capable of rejecting established tumors in combination with radiotherapy.

Myeloid-derived suppressor cells (MDSC) are induced from bone marrow precursor cells by cancer-associated inflammatory mediators, and play an important role in tumor immune evasion. TGF- $\beta$ 1 is a highly pleiotropic cytokine abundantly expressed in the tumor microenvironment; while primarily immunosuppressive effects of TGF- $\beta$ 1 on tumor, lymphocytes, and macrophages are well-described, little is known about the direct effects of TGF- $\beta$ 1 on MDSC. Reverse phase protein array (RPPA) analysis was used to determine the regulatory TGF- $\beta$ 1 protein signaling pathways in MDSCs. RPPA identified that canonical signaling molecules downstream of TGF- $\beta$  receptor, including phosphorylated SMAD2, phosphorylated c-JUN and p44MAPK, were upregulated. Dr. Sikora's group also observed that TGF $\beta$ -MDSC derived from human PBMC, with either tumor supernatants or cytokines in the presence of TGF- $\beta$ 1 also developed tumor killing activity and lost immunosuppressive function, associated with downregulation of PD-L1. In summary, induction in the presence of TGF- $\beta$ 1, causes myeloid precursor cells otherwise destined to become immunosuppressive MDSC, to acquire a novel phenotype, with loss of ability to suppress T cell proliferation and acquisition of FAS-dependent killing activity capable of durable tumor control in combination with radiotherapy.



**Figure Legend:** Characterization of TGF- $\beta$ 1 MDSC protein expression and pathway analysis by RPPA. Protein lysates from control or TGF- $\beta$ 1 treated MDSC were compared via RPPA. A) Heatmap of proteins (n=16) differentially expressed between control and TGF  $\beta$ 1-MDSC subjected to supervised hierarchical clustering (p-value < 0.05, fold change > 1.5 or < 0.8). B) Principal Component analysis (PCA) of the RPPA data. x, y, and z axes represent three major principal components. Control MDSC samples are represented as red dots; TGF  $\beta$ 1-MDSC samples as green dots. C) Representative histogram and summary data showing p-Smad2 expression as detected by flow cytometry in control and TGF- $\beta$ 1MDSC (n= 9) \*p  $\leq$  0.05.

Jayaraman P, Parikh F, Newton JM, Hanoteau A, Rivas C, Krupar R, Rajapakshe K, Pathak R, Kanthaswamy K, MacLaren C, Huang S, Coarfa C, Spanos C, Edwards DP, Parihar R, Sikora AG. TGF- $\beta$ 1 programmed myeloid-derived suppressor cells (MDSC) acquire immune-stimulating and tumor killing activity capable of rejecting established tumors in combination with radiotherapy. *Oncoimmunology*. 2018 Jul 26;7(10). PubMed PMID: 30288358; PubMed Central PMCID: PMC6169570.



# BIOENGINEERING

The goal of the Bioengineering Core is to provide investigators custom scientific instrumentation needed to conduct elegant experiments and ask truly cutting-edge research questions, and also to provide clinicians custom, one-of-a-kind, medical devices to create innovative solutions for health care. The core is staffed with an experienced bioengineer and a machinist who can work with investigators and clinicians to design complex devices, identify suitable off-the-shelf devices, manufacture custom parts, and integrate the apparatuses/instruments into the research work flow or clinical practices.

## MAJOR EQUIPMENT

- Hermle 5-axis CNC (Computer Numerical Control) Milling machine center capable of cutting solid materials such as metal, plastics, and wood into parts with complex geometries up to a size of 24" x 18" x 18".
- Haas CNC Lathe – capable of machining custom cylindrical parts up to 14" diameter and 14" long.
- Hardinge manual precision lathe.
- Bridgeport manual milling machine.
- Vertical band saw and horizontal cutoff saw.
- Epilog Laser cutter – capable of cutting plastic, wood, or paper sheets up to 32" x 20" with 3/4" thickness and engraving plastic, leather, metal, and glass.
- Stratasys 3D printer –capable of printing ABS plastics and supporting material up to a size of 8" x 8" x 6".
- Thorlabs optical workstation equipped with vibration isolation optical table, laser diode mount, laser controller, and power meter allowing design and tests of optical devices.

## SERVICES

- Customized instrumentation design and manufacture.
- Customized electronics/optics design and manufacture.
- High precision mechanical manufacture.
- 3D design and printing.
- Laser cutting and engraving.
- Stockroom of fasteners and raw materials such as aluminum, stainless steel, and plastics.
- Consultation for biomedical engineering projects.

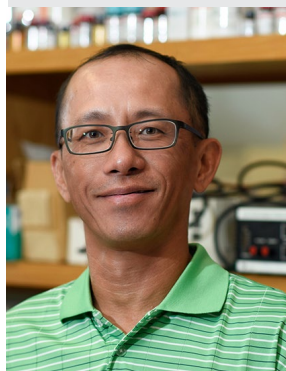


## CORE LEADERSHIP



### **Fabrizio Gabbiani, PhD**

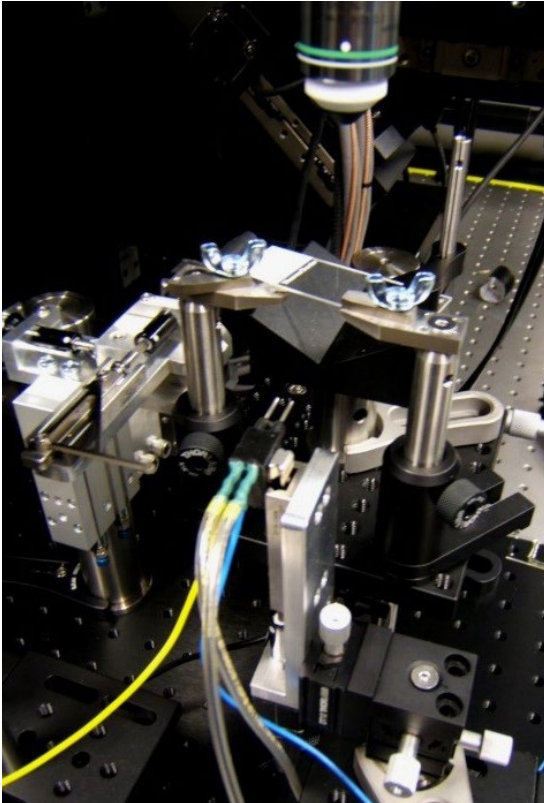
*Academic Director  
Professor, Department  
of Neuroscience*  
713.798.1849  
gabbiani@bcm.edu



### **I-Chih Tan, PhD**

*Core Director/Engineer  
Assistant Professor,  
Department of  
Neuroscience*  
713.798.9168  
itan@bcm.edu

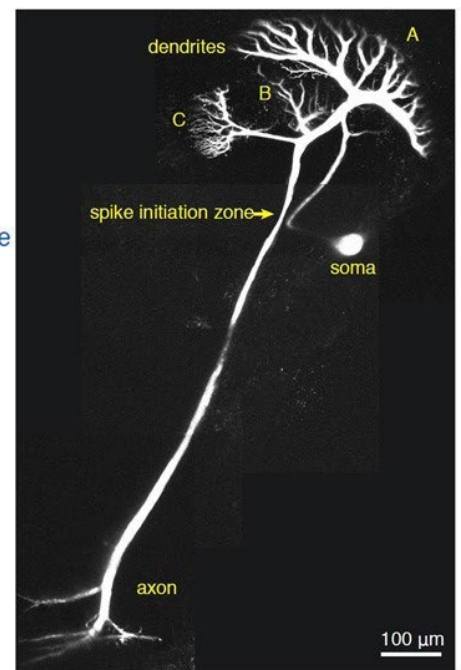
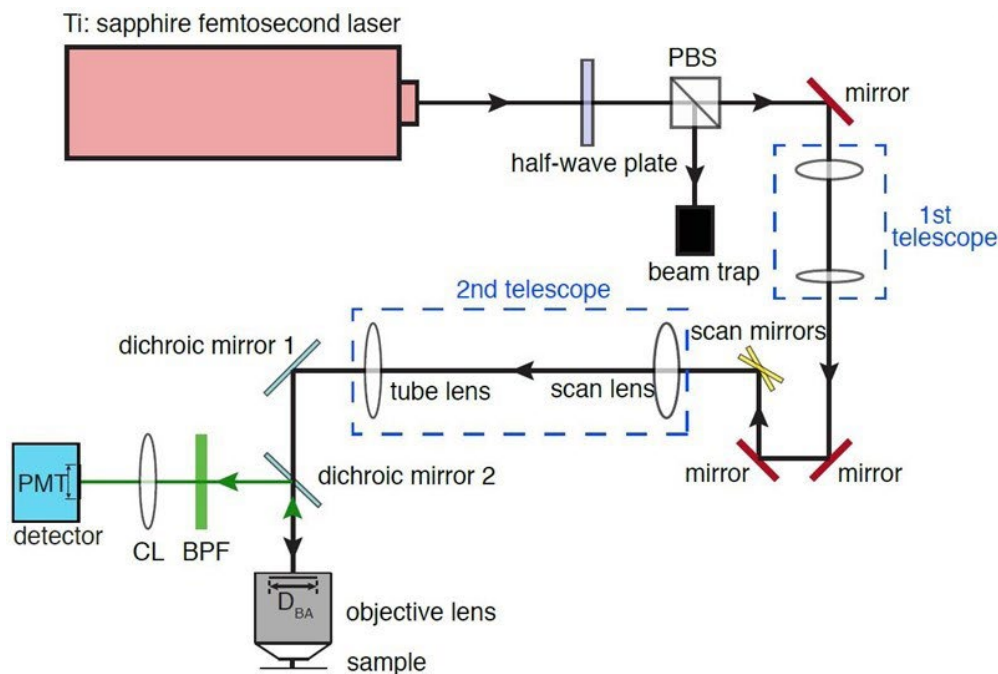
## CORE SUPPORTED RESEARCH



The Core has produced custom parts for several two-photon microscopes used to study neural information processing from the single cell to the network level.

Picture of an imaging setup used for studying motor-related activities in mice.

*Gao Z, Davis C, Thomas AM, Economo MN, Abrego AM, Svoboda K, De Zeeuw CI, Li N (2018) A cortico-cerebellar loop for motor planning. Nature 563:113-116.*



Left: Schematic of the configuration of a two-photon microscope. Right: Two-photon micrograph of an entire lobula giant movement detector neuron in the locust visual system stained with calcium indicator Oregon Green BAPTA-1. The labels A, B, and C denote the three dendritic fields.

*Zhu Y, Gabbiani F (2018) Combined two-photon calcium imaging and single-ommatidium visual stimulation to study fine-scale retinotopy in insects. In: Extracellular Recordings Approaches (Sillitoe R, ed), Neuromethods, Vol. 134. Springer, New York.*



# BIostatISTICS AND INFORMATICS SHARED RESOURCE

The goal of this Core is to provide state of the art biostatistical, bioinformatics, multi-omic analysis and computational support for clinical, translational and basic science research.

## MAJOR EQUIPMENT

Highly-available cluster with >900 physical CPUs in a single compute node architecture with a 10 Gb Ethernet connection to 495 TB of extensible Tier 1 direct-attached, rapid I/O data storage. Queues are managed with the PBS scheduler. The system is maintained by an expert HPC system administrator in a Tier 3 data center under standard governance structures.

## SERVICES

- **Biostatistics and Analytics:** Experimental design; assistance with design and conduct of clinical trials; data analysis, including integrative bioinformatic analyses
- **Multi-Omics Bioinformatics:** Data analysis for 'omics' core facilities including downstream integrative bioinformatic analyses
- **High Performance Computing (HPC):** cluster management and storage allocation; user training, central software library maintenance; troubleshooting
- **Other:** Assistance with grant applications; education; statistical review for the Protocol Review and Monitoring and Data Review Committees; deposition of 'omics-scale' datasets

### Investigators needing assistance with the following, please use the indicated contacts:

- HPC Cluster (cluster-help@breastcenter.tmc.edu)
- Biomedical Informatics & Research IT (dowst@bcm.edu)
- OnCore®, & Clinical Trials Data Management (oncore-support@breastcenter.tmc.edu )
- Acquire and Biobanking Data Management (acquire-support@breastcenter.tmc.edu biobank-support@breastcenter.tmc.edu )
- Software licensing for Oncomine™, Ingenuity®, SAS®, and SPSS® (licensing@breastcenter.tmc.edu)

## CORE LEADERSHIP



**Susan Hilsenbeck, PhD**

*Director*

*Professor, Department of Medicine, Smith Breast Center, Duncan Comprehensive Cancer Center*

713.798.1632

sgh@bcm.edu



**Cristian Coarfa, PhD**

*Co-Director (Multi-Omics Bioinformatics)*

*Associate Professor, Molecular & Cell Biology, Duncan Comprehensive Cancer Center*

713.738.7938

coarfa@bcm.edu



**Chad Creighton, PhD**

*Co-Director (Cancer Bioinformatics)*

*Associate Professor, Department of Medicine, Duncan Cancer Center*

713.798.2264

creight@bcm.edu



**Tao Wang, PhD**

*(Cancer Biostatistics) Assistant Professor*

*Department of Medicine, Duncan Comprehensive Cancer Center*

713.798.5388

taow@bcm.edu



**Charles Minard, PhD**

*(Biostatistics) Assistant Professor*

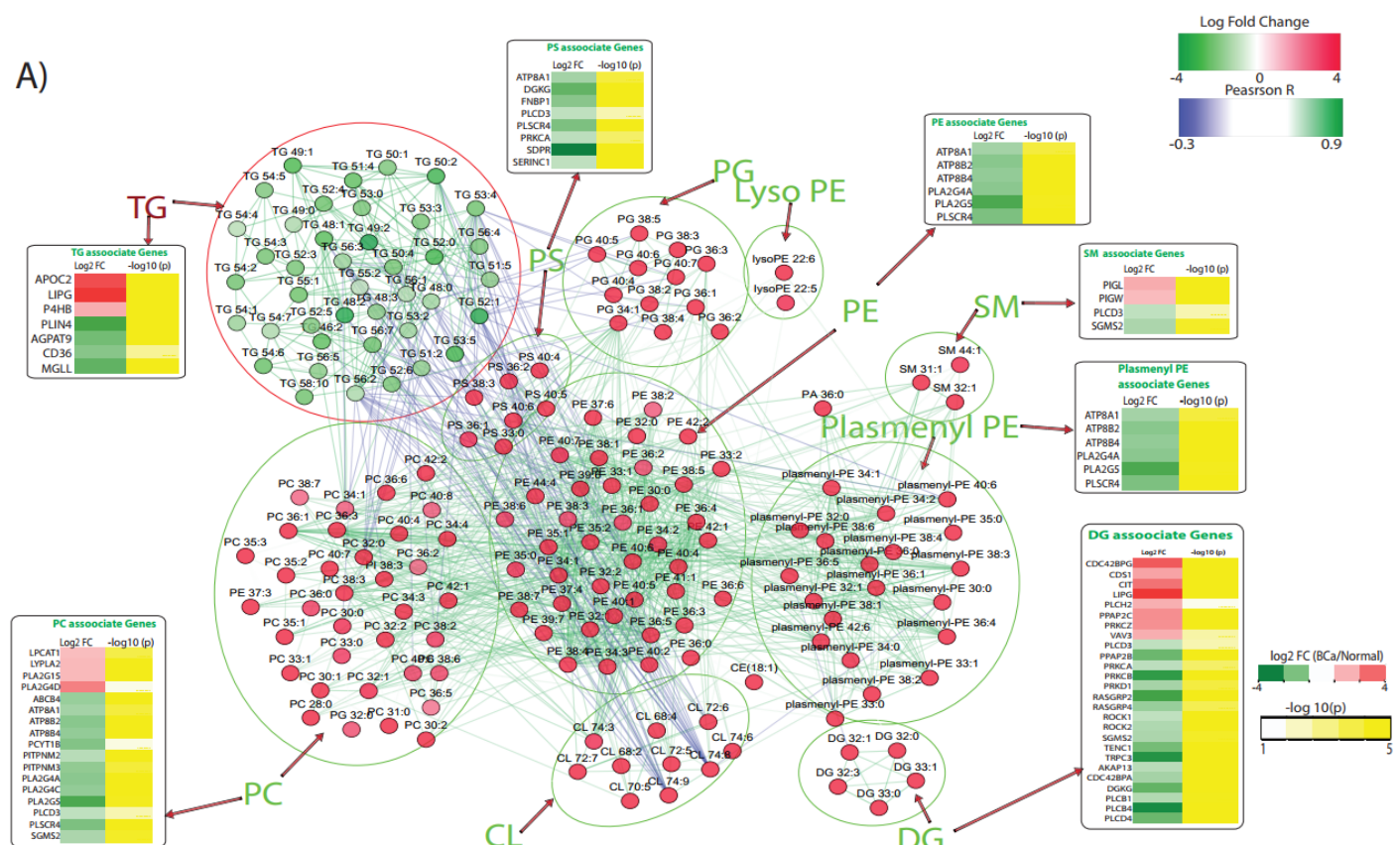
*Department of Medicine, Institute for Clinical and Translational Research*

713.798.23538

minard@bcm.edu

## Lipidomic profiles associated with urothelial cancer of the bladder (UCB) and its clinical stages associated with progression

Pathologically confirmed 165 bladder-derived tissues (126 UCB, 39 benign adjacent or normal bladder tissues). UCB tissues included Ta (n=16), T1 (n=30), T2 (n=43), T3 (n=27), and T4 (n=9); lymphovascular invasion (LVI) positive (n=52) and negative (n=69); and lymph node status NO (n=28), N1 (n=11), N2 (n=9), N3 (n=3), and Nx (n=75). UCB tissues have higher levels of phospholipids and fatty acids, and reduced levels of triglycerides compared with benign tissues. A total of 59 genes associated with altered lipids in UCB strongly correlate with patient survival in an UCB public dataset. Within UCB, there was a progressive decrease in the levels of phosphatidylserine (PS), phosphatidylethanolamines (PEs), and phosphocholines, whereas an increase in the levels of diacylglycerols (DGs) with tumor stage. Transcript and protein expression of phosphatidylserine synthase 1, which converts DGs to PSs, decreased progressively with tumor stage. Levels of DGs and lyso-PEs were significantly elevated in tumors with LVI and lymph node involvement, respectively. Lack of carcinoma *in situ* and treatment information is the limitation of our study. To date, this is the first study describing the global lipidomic profiles associated with UCB and identifies lipids associated with tumor stages, LVI, and lymph node status. Our data suggest that triglycerides serve as the primary energy source in UCB, while phospholipid alterations could affect membrane structure and/or signaling associated with tumor progression.



Piyarathna DWB, Rajendiran TM, Putluri V, Vantaku V, Soni T, von Rundstedt FC, Donepudi SR, Jin F, Maity S, Ambati CR, Dong J, Gödde D, Roth S, Störkel S, Degener S, Michailidis G, Lerner SP, Pennathur S, Lotan Y, Coarfa C, Sreekumar A, Putluri N. Distinct Lipidomic Landscapes Associated with Clinical Stages of Urothelial Cancer of the Bladder. *Eur Urol Focus*. 2017 Apr 20.



# CORE FOR ADVANCED MAGNETIC RESONANCE IMAGING (CAMRI)

The Core for Advanced Magnetic Resonance Imaging (CAMRI) is a state-of-the-art resource for the Houston research community that makes possible advanced imaging studies of the function, physiology and anatomy of humans and animals, with special expertise in human blood-oxygen level dependent functional MRI (BOLD fMRI). Conveniently located in the heart of BCM main campus, the center houses two cutting edge MR imaging systems.

## MAJOR EQUIPMENT

- Two Siemens 3 Telsa MRI Scanners. One Magnetom Trio and one Prisma<sup>fit</sup> with 80/200 gradients.
- A wide variety of equipment for functional brain imaging studies, including sensory stimulation devices, response buttons, eye trackers, and MR-compatible transcranial magnetic stimulation (TMS).
- Additional space available for animal preparation, TMS, behavioral testing, and stimulus recording.
- Weekly journal club and seminar series, details on our wiki at <http://openwetware.org/wiki/CAMRI>
- Flywheel scientific data management system to make data easily accessible and shareable.

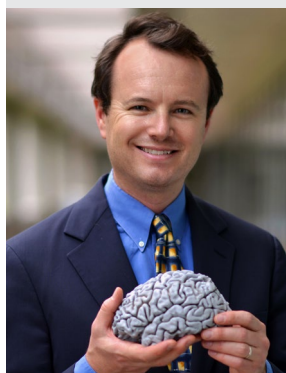
## SEQUENCES

- Functional MRI (fMRI), including multiband acceleration
- Diffusion tensor imaging (DTI)
- Single and multi-voxel magnetic resonance spectroscopy (MRS)
- Arterial spin labeling (ASL), both pulsed and continuous
- High-resolution structural imaging: FLASH, TSE, FLAIR, etc.

## SERVICES

- Imaging technologist available to assist in data collection
- Analysis Support: Includes consultation and protocol development time, data management, and general collaborations on MRI projects
- Pilot Time Program: Principal Investigators may apply for scanner time in order to collect preliminary data for grant proposals
- Operator training available free-of-charge to enable safe use of MRI equipment by new users
- Access to the instruments for fully trained users is available 24/7, facilitating subject recruitment and retention

## CORE LEADERSHIP



**Michael S. Beauchamp, PhD**

*Academic Director*

*Professor, Department of Neurosurgery and Neuroscience*

713.798.3175

[Michael.Beauchamp@bcm.edu](mailto:Michael.Beauchamp@bcm.edu)



**Krista Runge, MS, RT(R)(MR)**

*Operations Director*

713.798.3046

[krunge@bcm.edu](mailto:krunge@bcm.edu)



**Meghan Robinson, PhD**

*Technical Director*

*Assistant Professor, Department of Neurosurgery*

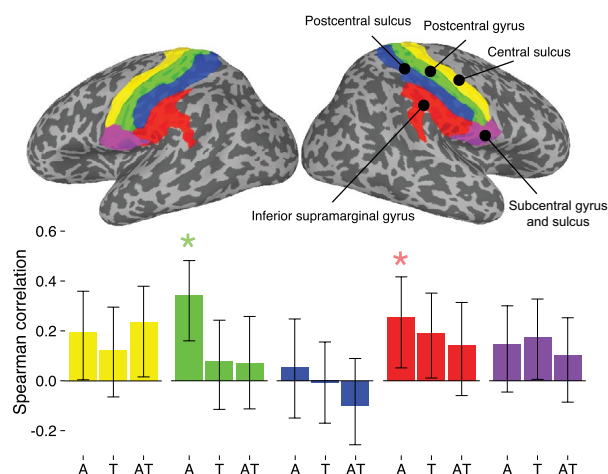
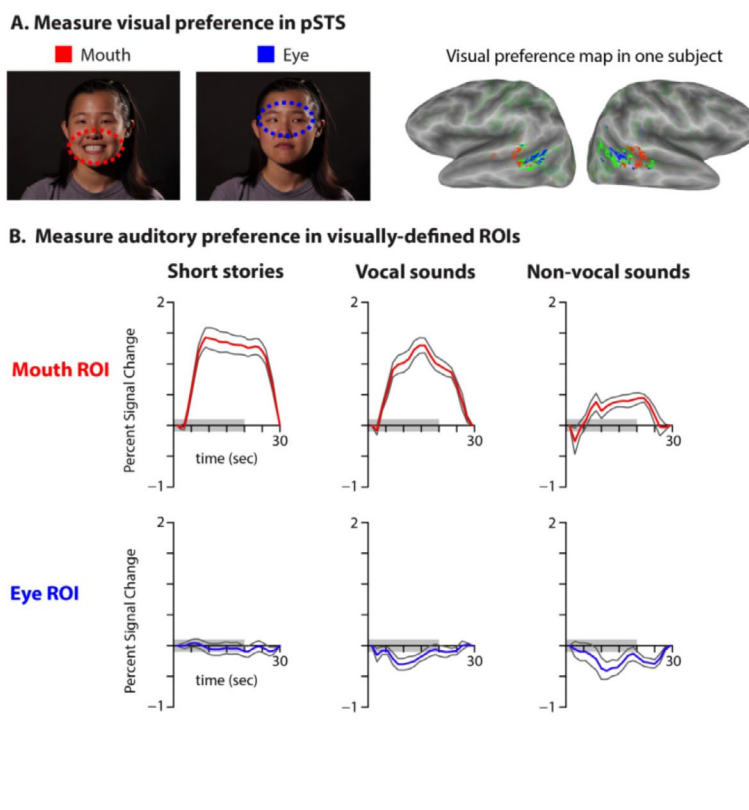
713.798.9039

[meghan.robinson@bcm.edu](mailto:meghan.robinson@bcm.edu)

## CORE SUPPORTED RESEARCH

Work by Dr. Michael Beauchamp examines the application of fMRI to the human ability to communicate face-to-face using speech. Speech consists of both auditory information from the talker's voice and visual information from the talker's face. Working with MD/PhD student Lin Zhu, Dr. Beauchamp has discovered a new organizing brain principle underlying multisensory speech perception. Using CAMRI's Trio scanner, Lin found that the natural statistics of human speech, in which voices co-occur with mouth movements, are reflected in the neural architecture of the superior temporal sulcus: different subregions prefer visually-presented faces containing either a moving mouth or moving eyes, but only mouth-preferring subregions respond strongly to voices. This discovery may help treat stroke patients who have lost the ability to understand speech and children who have language learning deficits.

Figure adapted from Zhu LL, Beauchamp MS. Mouth and Voice: A relationship between visual and auditory stimulus selectivity in the human superior temporal sulcus. *Journal of Neuroscience* 2017, 37(10): 2697-2708.



Work by Dr. Jeff Yau and colleagues examines representations of both auditory and tactile stimuli in the brain, specifically finding auditory representations in somatosensory cortices. In the manuscript "Auditory Frequency Representations in Human Somatosensory Cortex," published in *Cerebral Cortex* in 2018 by lead author Alexis Pérez-Bellido, the group applied an advanced fMRI analysis strategy called representational similarity analysis to identify regions where similar patterns of brain activation correspond to similar stimulus types. They further explored where in somatosensory cortex these representations were strongest, demonstrating representations in postcentral and supramarginal gyri (see Figure).

Figure from Pérez-Bellido A, Anne Barnes K, Crommett L E, Yau JM. Auditory frequency representations in human somatosensory cortex. *Cerebral Cortex* 2018 Nov 1;28(11):3908-3921.



# CELL-BASED ASSAY SCREENING SERVICE (C-BASS)

C-BASS strives to provide cutting-edge technologies and the latest genomic tools to BCM researchers in their cell-based functional genomics studies, and to aid their investigation of individual gene function, pathway identification, and large-scale genome-wide screens. The cell-based services we offer are built upon interconnected and complementary technology platforms of RNAi-based functional genomics and CRISPR/Cas9-mediated genome editing. Services include generating knockout (KO) and knock-in (KI) cell lines using CRISPR/Cas9, providing cDNA and shRNA vectors individually or as custom libraries, and consultation and expert advice on genome-wide or subgenome-targeted genetic screens. Through education and on-going improvement and optimization, we aim to enable BCM researchers to carry out drug discovery screens using a variety of platforms available in the core.

## MAJOR RESOURCES

- A lentivirus-based shRNA library that targets the human genome, in both arrayed and pooled format
- A lentivirus-based shRNA library that targets the mouse genome, in arrayed format
- A human cDNA library in a Gateway® compatible vector
- A mouse cDNA library
- A lentivirus-based CRISPR sgRNA library that targets the human genome, in arrayed format
- A vector collection for CRISPR/Cas9-mediated genome editing and other functional applications

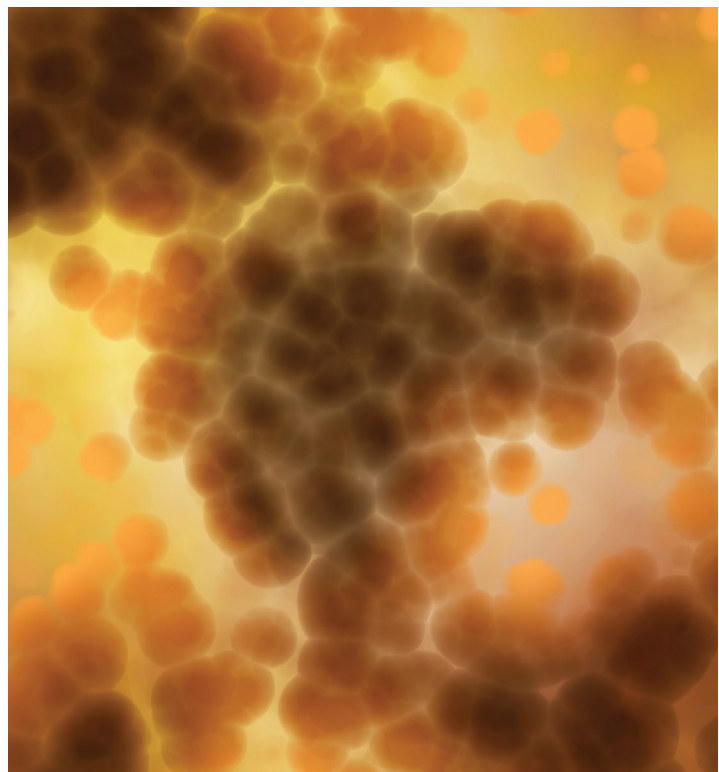
## SERVICES

### *RNAi/cDNA vector resources*

- Individual vectors
- Pre-assembled shRNA sublibraries (e.g., kinase, transcription factors, etc.)
- Pre-assembled CRISPR sgRNA sublibraries (e.g., kinase, transcription factors, etc.)
- Custom sublibraries (gene collection designed by investigator)
- Whole-genome shRNA/cDNA collection (human and mouse)
- Lentiviral production and infection (individual or 96-well format)

### *CRISPR/Cas9*

- Consultation and experimental design for genome editing
- Vector design, construction, and testing
- KO and KI cell line generation and validation



## CORE LEADERSHIP



### **Dan Liu, PhD**

*Core Director*

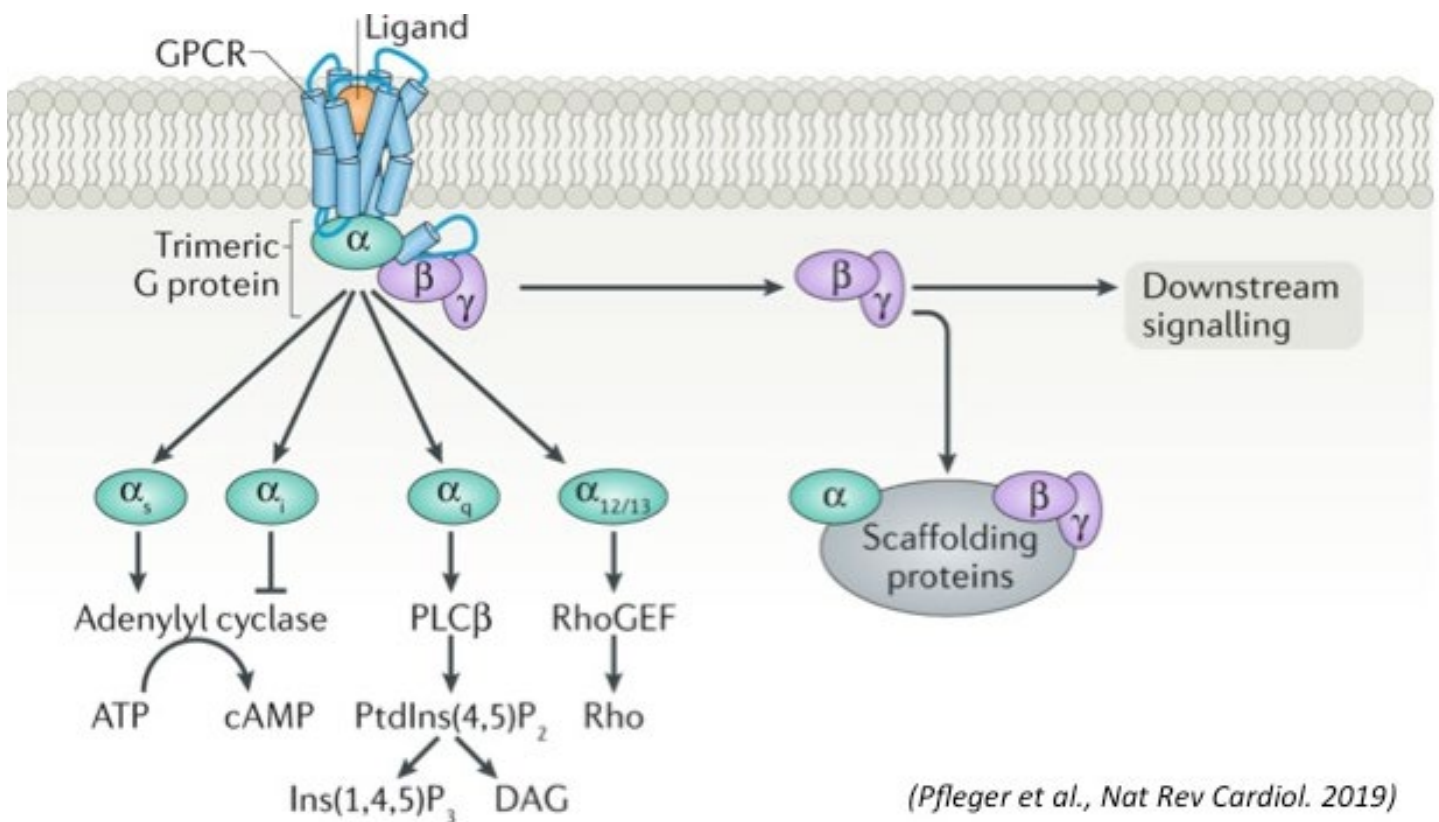
*Associate Professor,  
Department of  
Biochemistry and  
Molecular Biology*

713.798.8032

[danl@bcm.edu](mailto:danl@bcm.edu)

## $\beta_2$ -Adrenergic receptor activation mobilizes intracellular calcium via a non-canonical cAMP-independent signaling pathway

Beta adrenergic receptors ( $\beta$ ARs) are G-protein-coupled receptors essential for physiological responses to the hormones/neurotransmitters epinephrine and norepinephrine, which are found in the nervous system and throughout the body. They are the targets of numerous widely used drugs, whose ligands are used for asthma and cardiovascular disease. In addition to signaling through  $G_{\alpha_s}$  G-proteins and via activation of adenylyl cyclase and cAMP-dependent protein kinase, they may also signal through alternative downstream pathways that could be important for understanding their normal physiological function and disruptions in disease. To test for the role of  $\beta_2$ AR in the observed  $Ca^{2+}$  release by a genetic approach, the C-BASS core created an ADRB2 gene deletion using the CRISPR/Cas9 system in HEK-293 cells. This KO line was transfected with control or HA- $\beta_2$ AR-expressing constructs and tested for  $Ca^{2+}$  response to isoproterenol (ISO). In the absence of  $\beta_2$ AR, no detectable  $Ca^{2+}$  response to ISO up to its  $EC_{50}$  in wild-type cells was observed. Furthermore, the response was completely rescued by direct expression of  $\beta_2$ AR. These data demonstrate that potent ISO stimulation of  $Ca^{2+}$  release requires a functional  $\beta_2$ AR gene. Combining such gene KO experiments with fluorescence-based  $Ca^{2+}$  flux assays and pharmacology methods, the investigators discovered a previously unrecognized endogenous pathway in HEK-293 cells whereby  $\beta_2$ AR activation leads to robust  $Ca^{2+}$  mobilization from intracellular stores via activation of phospholipase C and opening of inositol trisphosphate ( $InsP_3$ ) receptors. This newly uncovered mechanism for  $Ca^{2+}$  mobilization by  $\beta_2$ AR has broad implications for adrenergic signaling, crosstalk with other signaling pathways, and the effects of  $\beta$ AR-directed drugs.



Galaz-Montoya M, Wright SJ, Rodriguez GJ, Lichtarge O, Wensel TG.  $\beta(2)$ -Adrenergic receptor activation mobilizes intracellular calcium via a non-canonical cAMP-independent signaling pathway. *J Biol Chem.* 2017 Jun 16;292(24):9967-9974.



The Cryo Electron Microscopy (CryoEM) Core is a state-of-the-art resource for near-atomic resolution 3-D analysis of the structure and dynamics of macromolecules and assemblies, either purified or within cells. This includes the established technique of single particle analysis, whereby images of tens of thousands to millions of isolated macromolecules are reconstructed to produce one or more 3-D structures at resolutions as high as 0.2 nm (near atomic resolution), as well as *in-situ* electron cryotomography which permits the 3-D study of cells or regions of cells at resolutions 100x better than optical microscopy. Single particle analysis is a direct alternative to X-ray crystallography, and can provide additional information about dynamics and compositional variability, which crystallography cannot access. We can also work with users to optimize specimens and provide preliminary data to gain free access to the new 'beamline' style CryoEM facilities sponsored by the NIH.

## MAJOR EQUIPMENT

- JEOL-3200FSC - 300 keV instrument with a field-emission gun, energy filter and a K2 summit direct detector. Capable of single particle reconstructions beyond 3 Å resolution, and nanometer resolution cellular tomography of thin specimens. Fully automated for 24 hour operation.
- JEOL-2200FS - 200 keV instrument with a field-emission gun, phase plate, energy filter, Gatan CCD camera and a DE-20 direct detector. Workhorse instrument for single particle reconstruction at subnanometer resolution, able to look at particles smaller than the 300 keV instrument.
- JEOL-2100 - 200 keV instrument with Gatan CCD camera and a DE-12 direct detector. This is our primary screening instrument.
- FEI Mark IV Vitrification Robot with 2-sided blotting for specimen preparation.
- Leica EMGP automatic plunge freezer with 1-sided blotting for specimen preparation.
- Fischione Model 1070 Nanoclean plasma cleaner for grid preparation.
- PELCO easiGlow™ Glow Discharge Cleaning System.

## SERVICES

- CryoEM/CryoET project consultation
- Near-atomic resolution CryoEM Single particle analysis. We can support all stages of the pipeline from specimen preparation through computer reconstruction.
- Cellular CryoET to provide 3-D structure of intact cellular material ~5 nm resolution in bulk leading to ~1 nm after averaging. Limited to thin cells or regions of cells.
- Screening and optimizing new specimens for CryoEM.
- Training students and staff in all aspects of the CryoEM/CryoET pipeline.

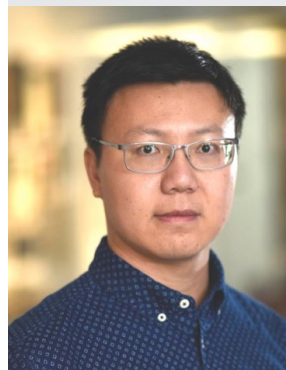


## CORE LEADERSHIP



### Steven Ludtke, PhD

*Academic Director*  
*Professor, Department of*  
*Biochemistry*  
 713.798.9020  
 sludtke@bcm.edu



### Zhao Wang, PhD

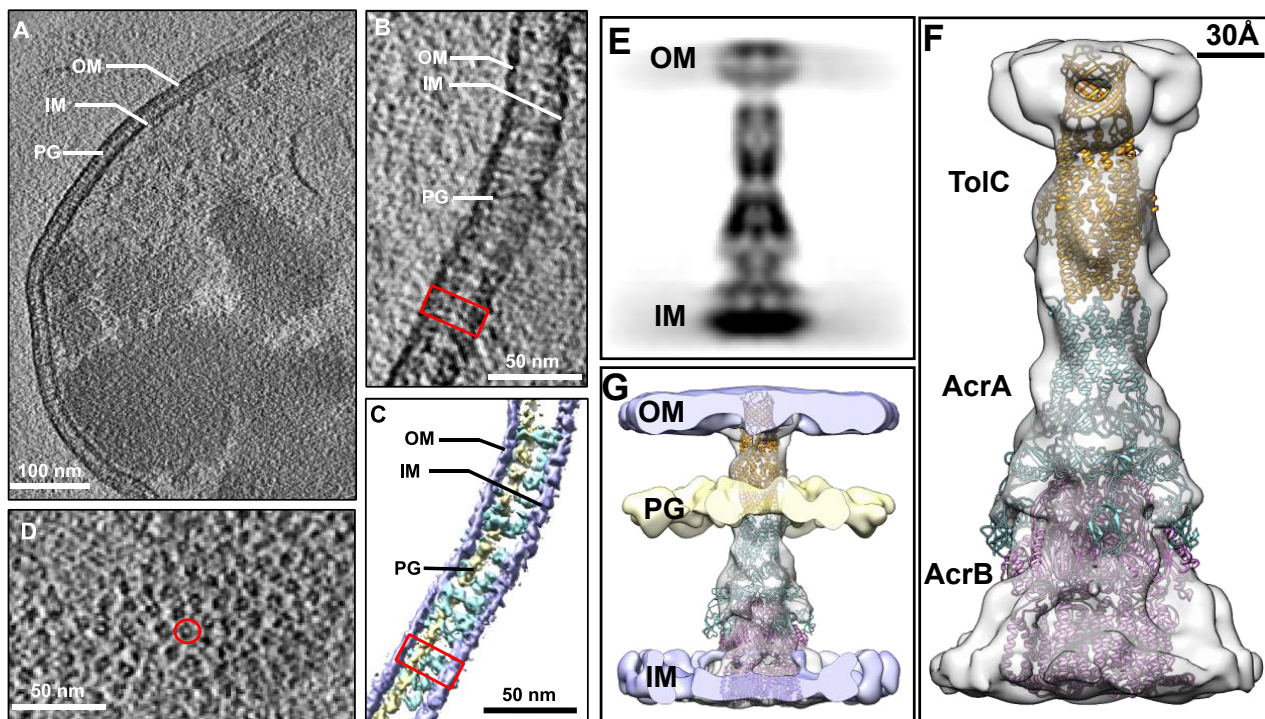
*Core Co-Director*  
*Assistant Professor,*  
*Department of*  
*Biochemistry*  
 713.798.6989  
 zhaow@bcm.edu

## Drug Efflux Pumps via CryoEM and CryoET

Drug efflux pumps play important roles in intrinsic or acquired drug resistance to a wide variety of currently available antimicrobial agents. In Gram-negative bacteria, AcrAB-TolC is a RND-based tripartite efflux pump, comprised of the outer membrane protein TolC, the periplasmic membrane fusion protein AcrA, and the inner membrane transporter AcrB. Using CryoEM single particle analysis, we were able to solve a series of structures of this large complex at 3.6 – 3.9 Å resolution. We followed this by performing CryoET of *E. coli* with the pump overexpressed for *in situ* structural studies. By classifying the individual pump assemblies from the in situ 3-D reconstruction of the cell, we can observe intermediate states in the assembly process, and gain new insights into the formation of this complex assembly. This work provides detailed structural information, such as interactions between each component that accounts for functional consequences of mutations and bound substrate structure and multiple functional states. The structural organization of the complex suggests a mechanism for transporting drugs from the periplasm to the extracellular matrix through coordinated conformational switch of the protein components. By combining the high resolution structural information from CryoEM Single Particle Analysis with the lower resolution but in situ information provided by CryoET, we obtain a much more complete picture of the assembly and function of the pump than could be achieved using any other method.

**Legend:** Visualizing the AcrAB-TolC efflux pump in the *E. coli* cell envelope. a A single slice from a tomogram of *E. coli*. The condensed materials shown inside of the cell are inclusion bodies resulting from membrane protein overexpression. b Zoomed inside view of the cell envelope containing the AcrAB-TolC pump which is indicated by the red rectangle. c Corresponding three-dimensional annotation of b showing the outer membrane (OM; blue), the inner membrane (IM; blue), peptidoglycan (PG; yellow), and the AcrAB-TolC pump (cyan). d Top view of the cell envelope containing the AcrAB-TolC pumps which appear as ring-shaped densities (indicated by the red circle). e The side-view projection of the subtomogram average of the pump in presence of antibiotics. f Isosurface rendering of a fitted with high resolution cryo-EM model (PDB: 5V5S). g Isosurface rendering of b overlaid with density map of the cell envelope

Wang Z, Fan G, Hryc CF, Blaza JN, Serysheva II, Schmid MF, Chiu W, Luisi BF, Du D. An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump. *Elife*. 2017, Mar 29;6: e24905.





# CYTOMETRY AND CELL SORTING

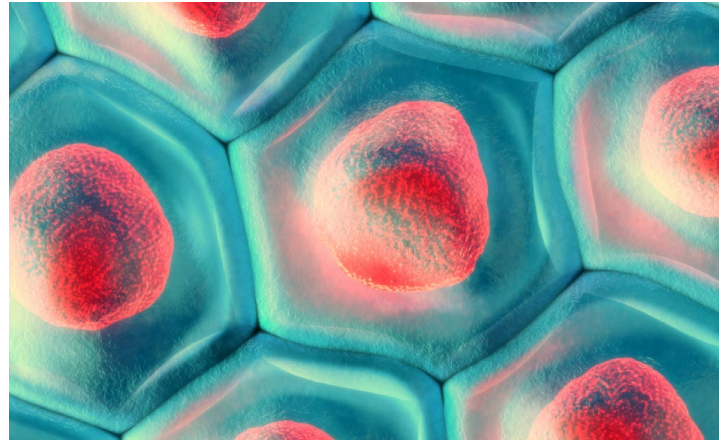
Cytometry is an integral part of the research of BCM faculty across all disciplines. The technology, including flow, mass and image cytometry, continues to develop at a rapid pace driven by advances in instrumentation, labeling reagents, and computational capabilities. The Core provides state-of-the-art instrumentation, technologies, and exceptional specialized expertise and training in cytometry. Services include analysis by mass cytometry, image cytometry and flow cytometry as well as fluorescence-activated cell sorting (FACS) supported by an in-house bank of validated antibodies. Additionally, services include large particle sorting, magnetic cell separation, automated cell counting and viability, consultation, data analysis and training. Access to instruments in the facility for fully trained users is 24 hours and 7 days a week.

## MAJOR EQUIPMENT

- Fluidigm Helios Mass Cytometry with Hyperion mass imaging platform
- BD Symphony A5 30+ Parameter Flow Cytometer
- Amnis ImageStreamX MKII, a 4 laser imaging cytometer providing a multispectral image for every cell
- Seven Flow Cytometric Cell Analyzers; two 5 laser BD LSRs, one 4 laser LSRII, and a 3 laser LSRII, two 3 laser BD Canto IIs (one violet and one yellow-green), and 4 laser Invitrogen Attune NxT
- High Through-put Flow Cytometric Analysis; High through-put systems available on flow cytometric analyzers
- Three Flow Cytometric Cell Sorters; two 5 laser BD SORP Aria IIs and a 4 Laser BD Aria IIu
- Union Biometric BioSort Large Particle Cell sorter; 30 – 300um objects using Blue and YG lasers
- Viability Analyzer; Beckman Coulter Vi-CELL
- Magnetic Cell Separator; Miltenyi AutoMACS Pro
- Cell Tissue Dissociator; Miltenyi gentleMACS Octo Tissue Dissociator
- Two Computer Work Stations; both Mac and PC

## SERVICES

- Cellular Analysis: Assisted and unassisted flow cytometric and viability analysis using up to 5 separate lasers and 20 parameters for multiple assays including small particles.
- Cell Sorting: Assisted and unassisted flow cytometric and magnetic cell sorting services that include parity with analyzers so any project capable of analysis can be moved to cell sorting.
- Mass and Fluorescent Antibody Bank for high-parameter cytometry
- Data Analysis: Assisted and unassisted data analysis including a dedicated server for data storage, workstations for data analysis and a FlowJo software site license available to investigators.
- Training: Didactic Flow Analyzer course as well as individual training on cell sorting and other instrumentation, software or equipment updates.



## CORE LEADERSHIP



### **Christine Beeton, PhD**

*Academic Director*

*Associate Professor,  
Department of Molecular  
Physiology and Biophysics*

713.798.5030

beeton@bcm.edu



### **Joel M Sederstrom, MS**

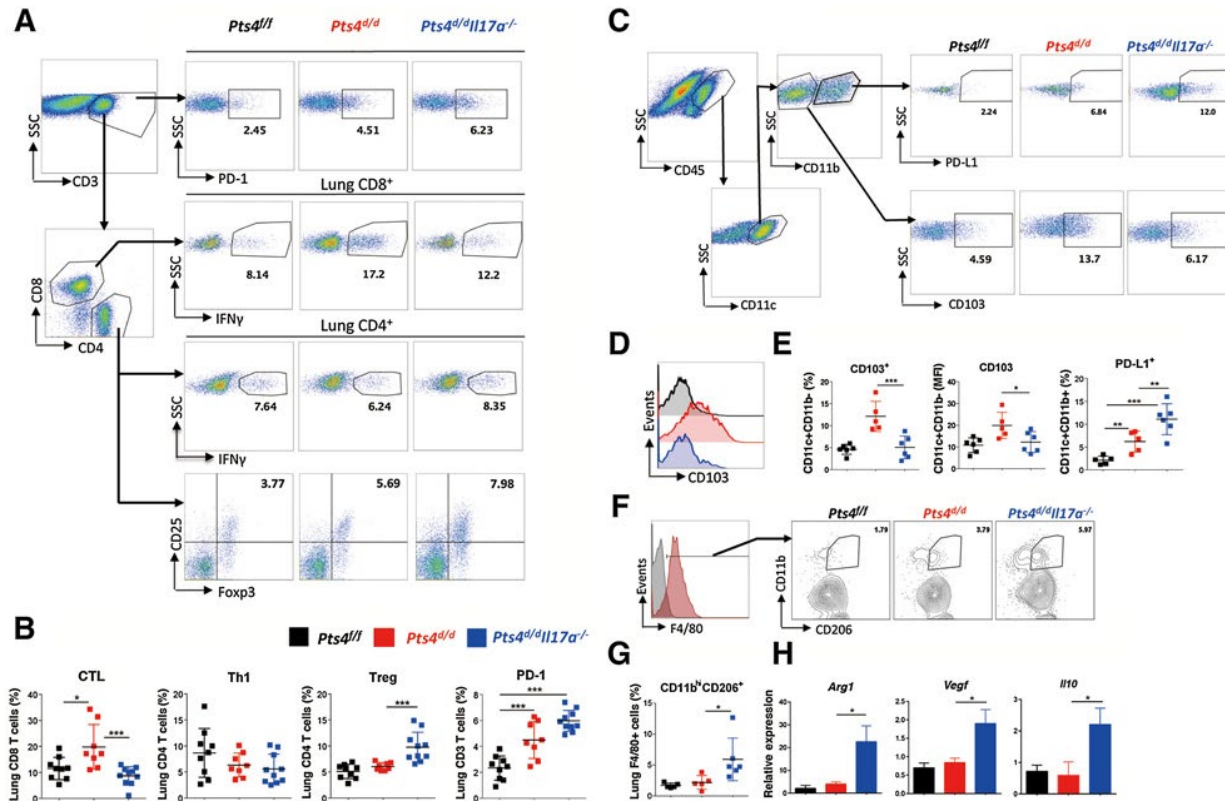
*Core Director*

Lab: 713.798.3868

Office: 713.798.3774

sederstr@bcm.edu

## IL17A Regulates Tumor Latency and Metastasis in Lung Adeno and Squamous SQ.2b and AD.1 Cancer



The CCSC performed a comprehensive immunophenotyping of 7-month-old Pts4<sup>d/d</sup>Il17a<sup>-/-</sup> mice for a study on the role of IL17A in lung cancer. Representative flow gating, and ICC **(A)** and cumulative analysis **(B)** for lung IFN $\gamma$ -expressing CD8<sup>+</sup> cells (CTLs), CD4<sup>+</sup> (Th1), foxp3<sup>+</sup>-expressing (Tregs), and PD-1 expression in 7-month-old age-matched mice: WT (Pts4<sup>f/f</sup>), Pts4<sup>d/d</sup>, and Pts4<sup>d/d</sup>Il17a<sup>-/-</sup> (data pooled from two independent experiments; n = 9 per group). C, Representative flow gating and relative abundance of lung CD103<sup>+</sup> DCs in CD45<sup>+</sup>CD11c<sup>+</sup>CD11b<sup>-</sup> lung cells and PD-L1 expression in CD11b<sup>+</sup> DCs in the same groups of mice. D, Representative histogram and **(E)** cumulative relative abundance of CD103 expression, and MFI, and PD-L1 expression in CD11c<sup>+</sup>CD11b<sup>-</sup> DC subset described in C (n = 5 or 6 per group). F, Representative histogram and **(G)** cumulative expression of CD206 on F4/80 TAMs in whole-lung single-cell homogenates in the indicated groups of mice. H, Quantitative expression of the Arg1, Vegf, and Il10 genes using qPCR in bronchoalveolar lavage (BAL) cells isolated from the same groups of mice (n = 5 or 6 per group)

You R, DeMayo FJ, Liu J, Cho SN, Burt BM, Creighton CJ, Casal RF, Lazarus DR, Lu W, Tung HY, Yuan X, Hill-McAlester A, Kim M, Perusich S, Cornwell L, Rosen D, Song LZ, Paust S, Diehl G, Corry D, Kheradmand F. IL17A regulates tumor latency and metastasis in lung adeno and squamous SQ.2b and AD.1 Cancer. *Cancer Immunol Res.* 2018, 6(6):645-657.



# GENE VECTOR

The Gene Vector Core (GVC) assists investigators with the production of gene transfer vectors, which can be used for studying gene function by over-expression, ectopic expression, gene silencing, or gene editing. Recombinant viral vectors retain the native features of viruses which have been tested in nature for millions of years, and are among the most efficacious. The GVC has undertaken a variety of activities aiming at increasing productivity cutting cost, improvement/development of quality control assays, improving existing services and expanding the repertoire of viral vector-based research tools. The core offers a number of popular viral vector platforms and has extensive experience in the production of viral vectors including adeno-associated virus (AAV), helper-dependent adenovirus (HDAd), lentivirus (LV) and Rabies virus (RV). Viruses have evolved for their survival not to accommodate our needs. The improvement of viral vectors for research needs is an active research area. Our core is vigilant on recent advances in viral vectors, provides appropriate advice and works together with investigators.

## SERVICES

- Packaging and purification of AAV (serotype 1, 2, 5, 6, 7, 8 and 9, 10, DJ, DJ8, PHP.B, PHP.eB, and AAV2Retro), in half-scale, regular-scale or in high quality large scale.
- Rescue, and/or amplification/purification of HDAd (serotype 2, 5 and 5/35, and custom targeted vectors).
- Note: FGAd production is suspended.
- Packaging and concentration/purification of VSVG-pseudotyped integrating or non-integrating LV with 2nd or 4th generation packaging systems.
- Packaging G-deleted Rabies virus.
- Subcloning into viral transfer vectors and preparation of plasmids for viral vector production.
- Titration for infectivity.
- Customer provides transfer vectors for transfection. Packaging plasmids or helper viruses are provided by the Core.
- Off-the-shelf packaged vectors are available on catalogue.
- Common viral transfer plasmids vectors developed by the Core have been deposited to Addgene [https://www.addgene.org/Kazuhiro\\_Oka/](https://www.addgene.org/Kazuhiro_Oka/). These plasmid DNAs are available from the Core.



## CORE LEADERSHIP



**Kazu Oka, PhD**

*Core Director*

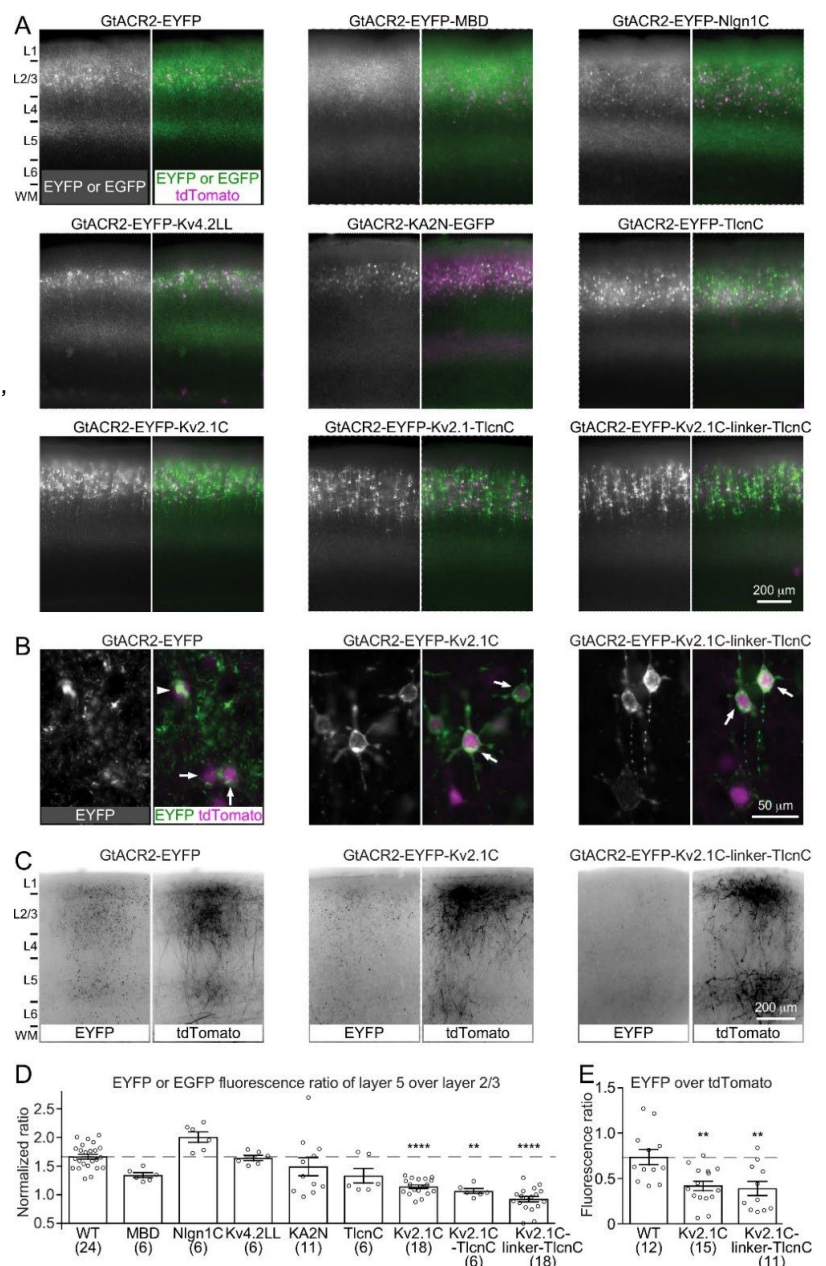
*Associate Professor,  
Department of Molecular  
and Cellular Biology*

713.798.7381

## Cortical Layer-Specific Neuromodulation

Targeted manipulation of neuronal activity is a powerful technique in neuroscience that provides fundamental insights into the roles of specific neurons in brain. Optogenetics involves introducing light-gated ion channels in the outer membrane of a target neuron. When light excites the cell, these pore-like proteins open their channels allowing ions to move into or out of neurons. Messier et al. inhibited the activity of neurons in mouse brain slices using a light-gated chloride channel called GtACR2 packaged in AAV by the Gene Vector Core. Activating the chloride channel inhibited the cells body while it had the opposite effect within the axon. Further studies revealed that the concentration of chloride ions is higher inside the axon than the cell body, explaining the observed opposite effects. To reduce the excitatory effect of GtACR2, Messier et al. screened a panel of somatodendritic targeting motifs to reduce the trafficking of GtACR2 to the axon and presynaptic terminals and created a hybrid motif (Kv2.1Clinker-TlcnC) that is most effective in concentrating GtACR2 in the somatodendritic domain. Thus, the authors created a powerful new inhibitory optogenetic tool.

**Figure legend:** Reducing the trafficking of light-gated chloride channels into the axon and presynaptic terminals should reduce or eliminate their depolarizing action. Messier et al. restricted GtACR2 expression by fusing the channel with somatodendritic targeting motifs including a 26-amino acid Myosin Va-binding domain of Melanophilin (MBD), a 32-amino acid cytoplasmic C-terminal motif of Neuroligin 1 (Nlgn1C), a 16-amino acid dileucine-containing motif of potassium channel Kv4.2 (Kv4.2LL), the N-terminal 150 residues of kainite receptor subunit 2 (KA2A), the C-terminal 17 residues of Telencephalin (TlcnC), and a 65-amino acid cytoplasmic C-terminal motif of potassium channel Kv2.1 (Kv2.1C). The GtACR2 variants were tagged with EYFP or EGFP compared with dTomato expressed in layer 2/3 pyramidal neurons to evaluate the GtACR2 variant targeting. TlcnC and Kv2.1C motifs were the most effective in targeting GtACR2 to the soma and dendrites.



Cai Z, Chen H, Messier JE, Xue M. Targeting light-gated chloride channels to neuronal somatodendritic domain reduces their excitatory effect in the axon. *Elife*. 2018, August 9;7: e38506

# GENETICALLY ENGINEERED MOUSE (GEM)

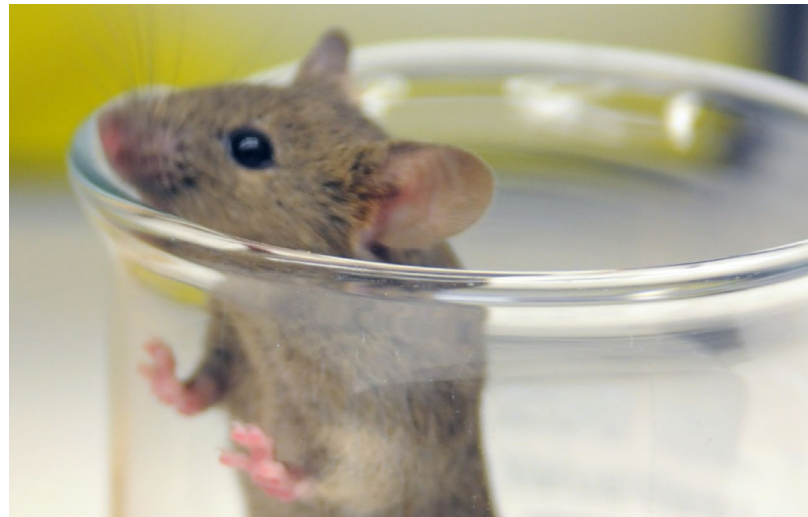
The Genetically Engineered Mouse (GEM) Core possesses specialized expertise and equipment for producing genetically modified mice essential for investigators working with mouse experimental models. The GEM core performs microinjection of mouse ES cells, DNA transgene constructs and CRISPR sgRNA/Cas9/donor DNA into mouse embryos to generate genetically engineered founder mice. Also provided are services for cryopreservation of mouse embryos and sperm for long-term storage, mouse line re-derivation to remove pathogen contamination, re-establishment and expansion of mouse colonies, help with importation of mouse lines into BCM facilities and *in vitro* fertilization. Although the GEM Core will accept ES cells and CRISPR sgRNA/Cas9/donor DNA samples directly from investigators for microinjection, the preferred process is to use genetically manipulated ES cells and CRISPR sgRNA/Cas9 reagents designed as services by the Mouse ES Cell Core. The GEM and the Mouse ES Cell Cores work closely to coordinate these services.

## MAJOR EQUIPMENT

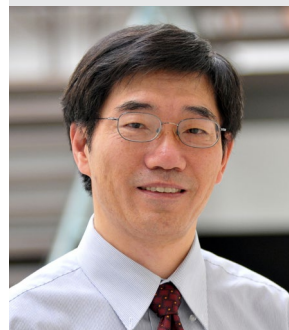
- Nikon Eclipse Te300 Microscopes with Hoffman objective lenses
- Nikon Diaphot inverted microscopes
- Narishige micromanipulators
- SMZ 800 and 1000 dissecting microscopes
- Sutter P-97 horizontal pull
- Cryosafes SSBA LN2 storage units
- Osmometer
- BioCool IV40 controlled-rate freezer

## SERVICES

- Generation of transgenic mice by DNA microinjection (Traditional, BAC, and Lentiviral)
- Mouse embryonic stem cell microinjection using C57BL/6J blastocysts from super ovulation
- Mouse embryonic stem cell microinjection using Albino C57BL/6J (also known as B6 albino with mutated tyrosinase) blastocysts from natural mating
- Mouse embryo cryopreservation
- Re-derivation of mouse lines
- Importing and exporting mouse lines
- Mouse line colony expansion
- Mouse sperm cryopreservation
- *In vitro* fertilization
- Generation of transgenic mice on an existing mutant backgrounds
- Microinjection of CRISPR gRNA/Cas9/donor DNA
- Storage of BCM PI's cryopreserved embryos and sperm



## CORE LEADERSHIP



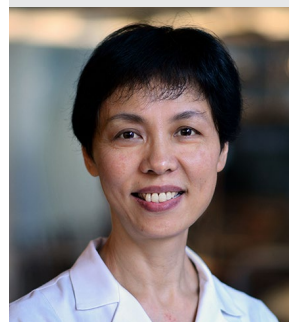
### **Jianming Xu, PhD**

*Academic Director*

*Professor, Department of Molecular and Cellular Biology*

713.798.6199

[jxu@bcm.edu](mailto:jxu@bcm.edu)



### **Lan Liao, M.S.**

*Core Director*

*Assistant Professor, Department of Molecular and Cellular Biology*

713.798.5278

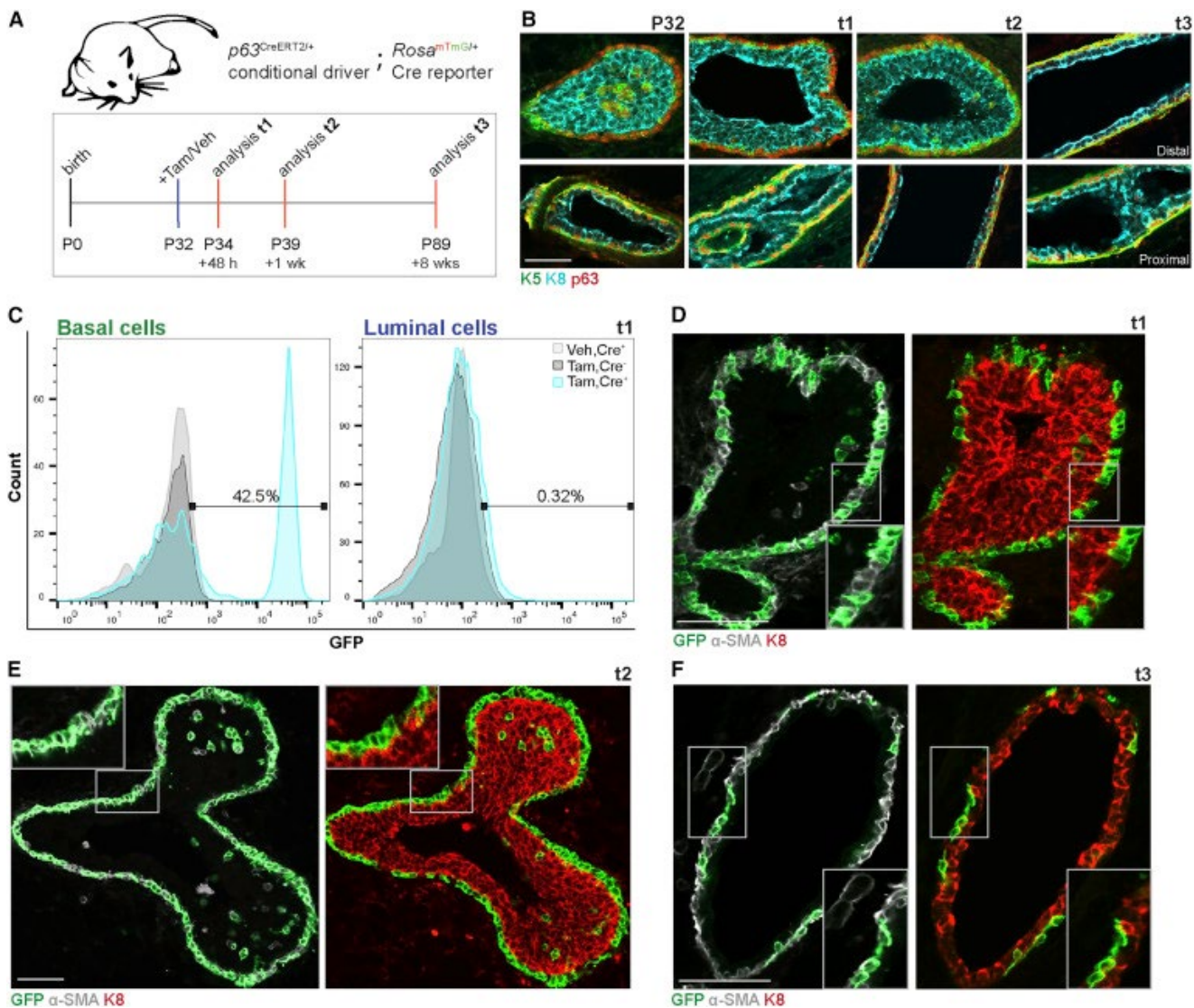
[lliao@bcm.edu](mailto:lliao@bcm.edu)



## CORE SUPPORTED RESEARCH

By using the GEM Core generated p63-CreERT2 knock-in mouse line, Sreekumar et al., demonstrate that WNT pathway-mediated regulation of FOXO cellular localization dictates proliferative versus apoptotic fates in pubertal mammary stem cells, termed cap cells.

**Figure 1.** Basal Lineage-Specific Fate Mapping Identifies Cap Cells as Unipotent. **(A)** Timeline used for basal lineage fate mapping using p63CreERT2/+;RosamTmG/+ mice. **(B)** Representative IF images depicting total TP63 localization (red) relative to K5+ cap/basal cells (green) and K8+ body/luminal cells (cyan) at time points of interest for lineage tracing. **(C)** Representative histograms depict distribution of recombined, GFP+, and unrecombined, GFP- basal (left) and luminal (right) cells upon initial labeling +Tam in p63CreERT2/+;RosamTmG/+ mice (+Tam, Cre+; cyan histogram), +Tam in RosamTmG/+ mice (+Tam, Cre-; dark gray histogram), or +Veh in p63CreERT2/+;RosamTmG/+ mice (+Veh, Cre+; light gray histogram) at t1. **(D)** Representative IF images characterizing GFP+ cells relative to  $\alpha$ -SMA+ cap/basal cells (gray) and K8+ body/luminal cells (red) at t1. **(E)** Representative IF images characterizing GFP+ cells relative to  $\alpha$ -SMA+ cells (gray) and K8+ (red) at t2. **(F)** Representative IF images characterizing GFP+ cells relative to  $\alpha$ -SMA+ cells (gray) and K8+ (red) at t3.



Sreekumar A, Toneff MJ, Toh E, Roarty K, Creighton CJ, Belka GK, Lee D-K, Xu J, Chodosh LA, Richards JS, Rosen JM. WNT-mediated regulation of FOXO1 constitutes a critical axis maintaining pubertal mammary stem cell homeostasis. *Dev. Cell* 2017, 43:436-448.

# GENOMIC & RNA PROFILING (GARP)

The mission of the Genomic & RNA Profiling (GARP) Core Facility is to provide the most cutting edge genomic technologies to research investigators with an emphasis on personal service and quality. In order to provide whole genome profiling we offer multiple next generation sequencing platforms (Illumina iSeq 100, NextSeq 500 and NovaSeq 6000) and targeted NanoString nCounter assays.

## MAJOR EQUIPMENT

- Illumina NovaSeq 6000 Sequencer
- Illumina NextSeq 500 Sequencer
- Illumina iSeq 100 Sequencer
- SMARTer Apollo NGS Library Prep System (Clontech)
- Nanostring nCounter Digital Quantification System
- ABI ViiA7 Real Time PCR/qPCR instrument
- Agilent Bioanalyzer
- Covaris Ultrasonicator

## SERVICES

- Next generation sequencing
  - » Sequencing only
  - » RNA-seq (polyA, whole transcriptome, small RNA)
  - » DNA-seq (whole exome, whole genome and targeted capture)
  - » ChIP-seq (protein-DNA interactions)
  - » Whole Genome Bisulfite Sequencing
- Nucleic Acid Shearing
- Targeted NanoString nCounter assays (up to 800 multiplexed assays/sample)
- Sample quality check
- Consultation



## CORE LEADERSHIP



### **Lisa D. White, PhD**

*Academic Director  
Associate Professor,  
Department of Molecular  
& Human Genetics*  
713.798.7607  
lisaw@bcm.edu

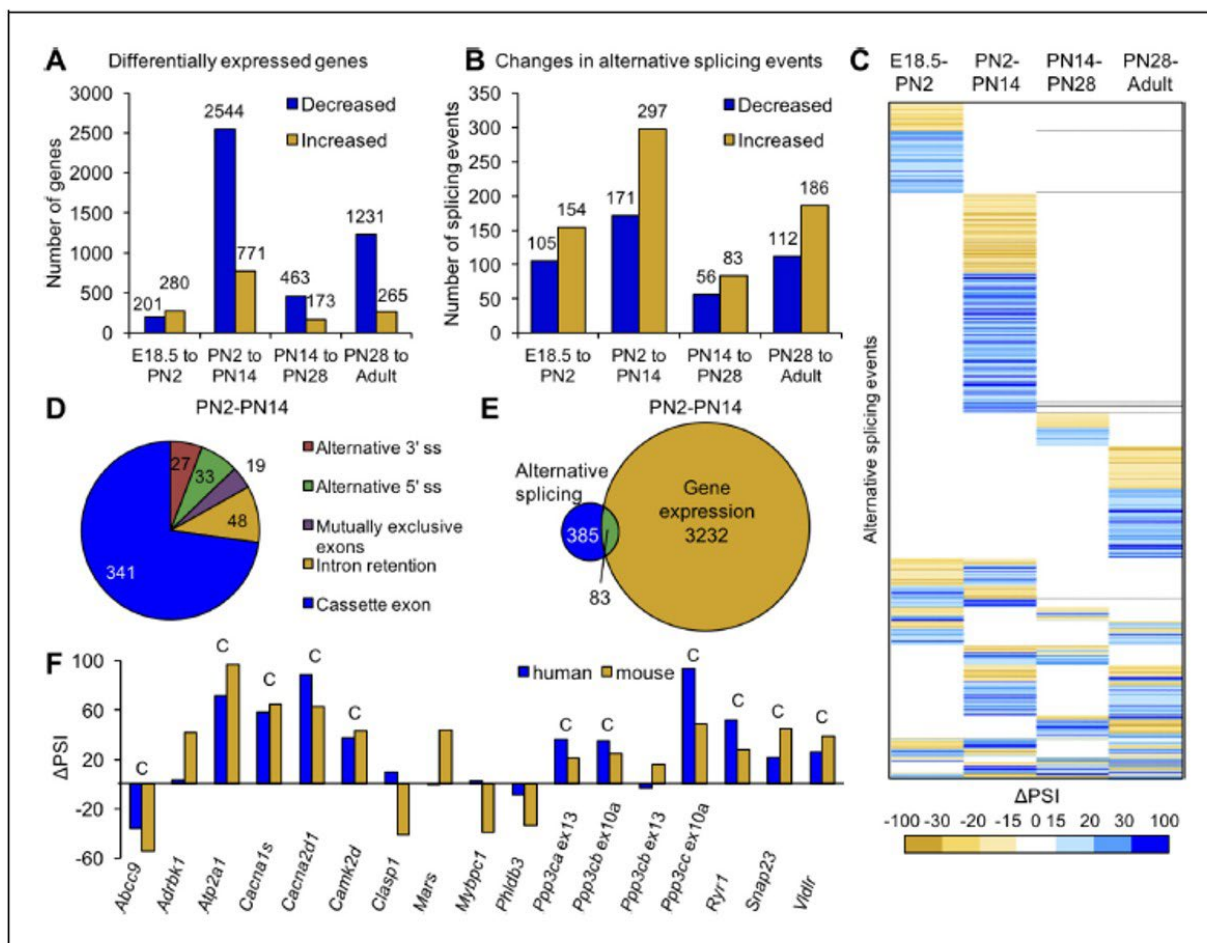


### **Daniel C. Kraushaar, PhD**

*Core Director  
Instructor, Department  
of Molecular & Cellular  
Biology*  
713.798.7787  
Daniel.Kraushaar@bcm.edu

## Postnatal gene expression and alternative splicing transitions in mouse skeletal muscle

- Number of differentially expressed genes ( $> 2$  fold increase or decrease) at various developmental stages of postnatal skeletal muscle development.
- Alternative splicing events at various developmental stages of skeletal muscle development. Decreased indicates more skipping of an alternative splicing event and increased indicates more inclusion of an alternative splicing event.
- Heat map of alternative splicing transitions between four time intervals. Most splicing transitions occur between PN2 and PN14.
- Splicing patterns of events between PN2 and PN14.
- Venn diagram of genes with gene expression changes ( $>2$ -fold) compared to alternative splicing transitions ( $> 15\%$   $\Delta$ PSI).
- Conservation of splicing transitions during mouse and human skeletal muscle development. The  $\Delta$ PSI between PN2 to PN28 mouse gastrocnemius samples were compared to the  $\Delta$ PSI between gestation week 22 to adult human skeletal muscle by RT-PCR. Events showing a  $15\%$   $\Delta$ PSI or greater in the same direction in mouse and human samples were scored as conserved (indicated by C).



Brinegar, AE; Xia, Z; Loehr, JA; Li, W; Rodney, GG; Cooper, TA. Extensive alternative splicing transitions during postnatal skeletal muscle development are required for calcium handling functions. *eLife* 2017(6), e27192.

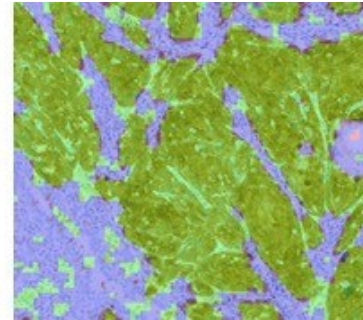
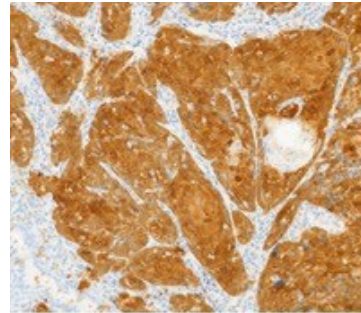


# HUMAN TISSUE ACQUISITION AND PATHOLOGY (HTAP)

The Human Tissue Acquisition and Pathology (HTAP) Core provides services for processing of tissues for various histological and immunohistochemical (IHC) assays for research projects of investigators working with either human specimens or animal models. Services are performed by expert technical staff with assistance of pathologists who provide consultation and review of slides and images. Histology, tissue microarray development, immunohistochemistry, RNAScope, laser capture microdissection and image analysis are available on a fee-for-service basis. HTAP is also involved in tissue acquisition and tissue banking. The tissue bank functions provide human tissue to BCM researchers that have IRB protocols in place. Tissue requests can be made by contacting the Core Director which are then reviewed and approved by committee.

## MAJOR EQUIPMENT

- Shandon Excelsior ES Tissue Processor
- Shandon HistoCentre Embedding System
- Sakura TissueTek SCA Coverslipper
- Shandon Varistain Gemini Slide Stainer
- Microm HM 315 Microtome
- Microm 505 E Cryostat
- Nuance FX multispectral imaging system
- Arcturus XT Laser Capture Microdissection instrument
- Vectra imaging system with inForm software
- Nikon slide scanning and imaging system



## SERVICES

- Histology - Tissue processing, embedding, cutting, and staining of human and animal tissues
- Immunohistochemistry (IHC) and TUNEL Assays - IHC for proliferation and apoptosis are performed using optimized methods and antibodies provided by the Core. Investigator supplied antibodies are used for other IHC assays which are optimized for performance.
- RNAScope and BaseScope - Advanced Cell Diagnostics Technology for detection of RNA in paraffin tissue.
- Laser capture microdissection (LCM)-Isolation of specific cell types or group of cells from frozen or paraffin tissue sections to be used for RNA, DNA or protein analyses.
- Digital imaging - State-of-the-art imaging of tissue sections or TMAs using the Nikon slide scanner or Vectra imaging system with Nuance FX multispectral camera. Image analysis using inForm software or Nikon Elements for pattern recognition analysis and quantitative scoring.
- Tissue microarray (TMA) - TMAs are developed using the Cores archival formalin fixed paraffin embedded sections or tissues provided by researchers.
- Consultation with pathologists. Experienced pathologists will assist with review of stained slides.

## CORE LEADERSHIP



**Michael Ittmann, MD, PhD**

*Academic Director*

*Professor, Department of  
Pathology & Immunology*

713.798.6196

mittmann@bcm.edu



**Patricia Castro, PhD**

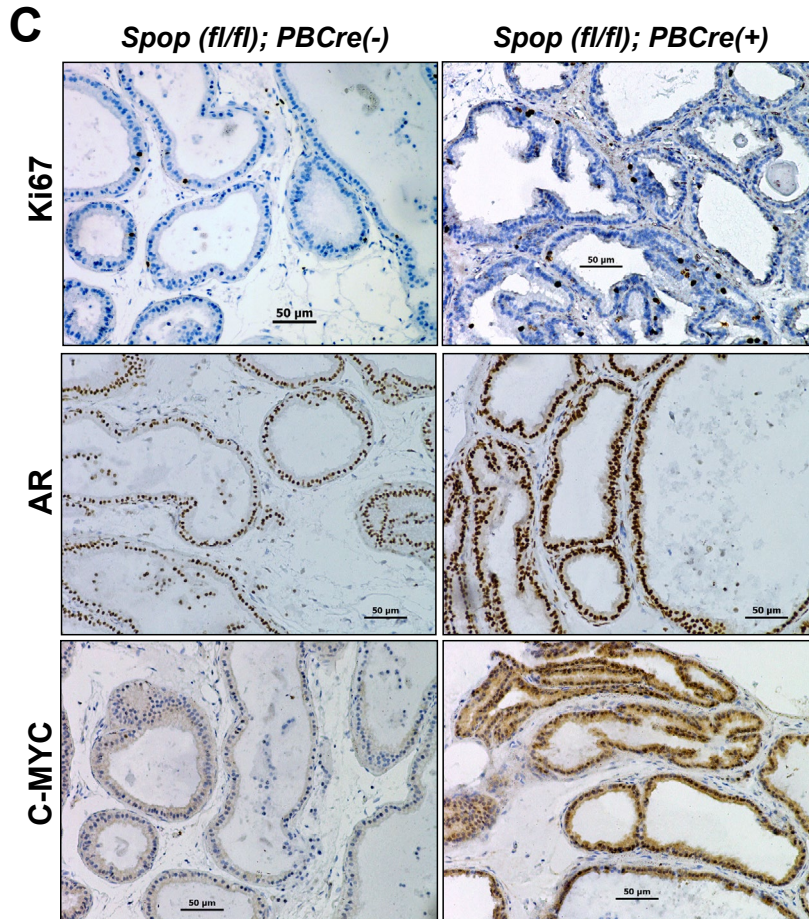
*Core Director*

*Assistant Professor,  
Department of  
Pathology & Immunology*

713.798.6795

pcastro@bcm.edu

## SPOP regulates prostate epithelial cell proliferation



The above figure is taken from Dr. Mitsiades *Oncogene* paper cited below. *Spop* mRNA is frequently decreased in human prostate cancer, indicating that it has a significant role in prostate biology. Complete knockout of the *Spop* gene results in neonatal lethality and so Dr. Mitsiades group developed a prostate specific conditional knockout using Cre recombination. The figure shows that knocking out *Spop* in mouse prostates, *Cre*(+), results in significantly increased prostate mass compared to *Cre*(-) littermates. Additionally, there was marked increase in luminal epithelium as shown by Ki-67 immunohistochemistry. *Cre*(+) prostates exhibited significantly increased expression of AR and c-MYC, two important cell cycle regulators in prostate biology.

The mouse tissues used for these studies were processed and sectioned by the HTAP histology lab. The Ki-67, AR and c-myc IHC were also done by the HTAP core lab.

Geng C, Kaochar S, Li M, Rajapakshe K, Fiskus W, Dong J, Foley C, Dong B, Zhang L, Kwon OJ2, Shah SS, Bolaki M, Xin L, Ittmann M, O'Malley BW, Coarfa C, Mitsiades N. *SPOP* regulates prostate epithelial cell proliferation and promotes ubiquitination and turnover of c-MYC oncoprotein. *Oncogene* 2017, 36(33):4767-4777.



# HUMAN STEM CELL

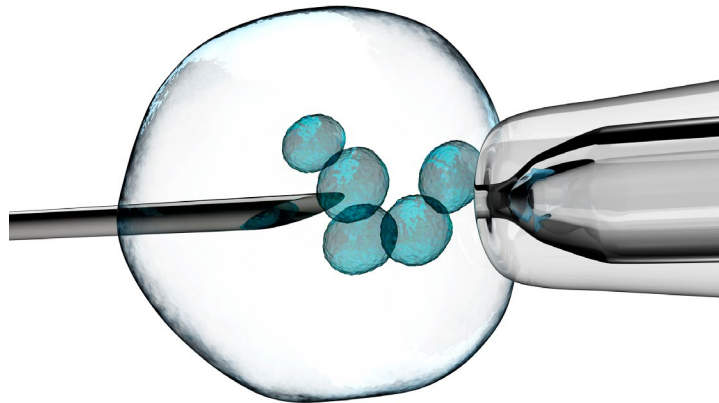
The Human Stem Cell Core (HSCC) provides a wide range of products and services related to human pluripotent stem cell (hPSC) research, as well as hands-on training classes for basic and advanced stem cell culture techniques. We offer cost-effective solutions to generate and characterize new induced pluripotent stem (iPS) cells for *in vitro* disease modeling, employing non-integrating vector technologies such as Sendai virus-based and episomal vector-based reprogramming. We also offer customized research support for experimental design and validation assays, as well as genome editing of hPSCs using CRISPR/Cas9.

## MAJOR EQUIPMENT

- EVOS XL and FL inverted microscope systems
- Lonza 4D-Nucleofector transfection system
- NuAire In-VitroCell CO2 Incubators with O2 control
- Beckman Coulter Allegra X-14R centrifuge
- ABI StepOnePlus Real-Time PCR system
- MVE TEC 3000 LN2 cryostorage system

## SERVICES

- Hands-on training classes and workshops
- Human pluripotent stem cell (hPSC) culture services
- Generation of induced pluripotent stem (iPS) cell lines
- Stem cell line characterization (karyotyping and trilineage differentiation)
- Mycoplasma testing
- Consultation on experimental design
- Customized genome editing of hPSCs using CRISPR/Cas9



## CORE LEADERSHIP



**Jean J. Kim, PhD**

*Core Director*

*Assistant Professor,  
Department of  
Molecular & Cellular  
Biology*

713.798.7471

[jean.kim@bcm.edu](mailto:jean.kim@bcm.edu)

**Margaret Goodell, PhD**

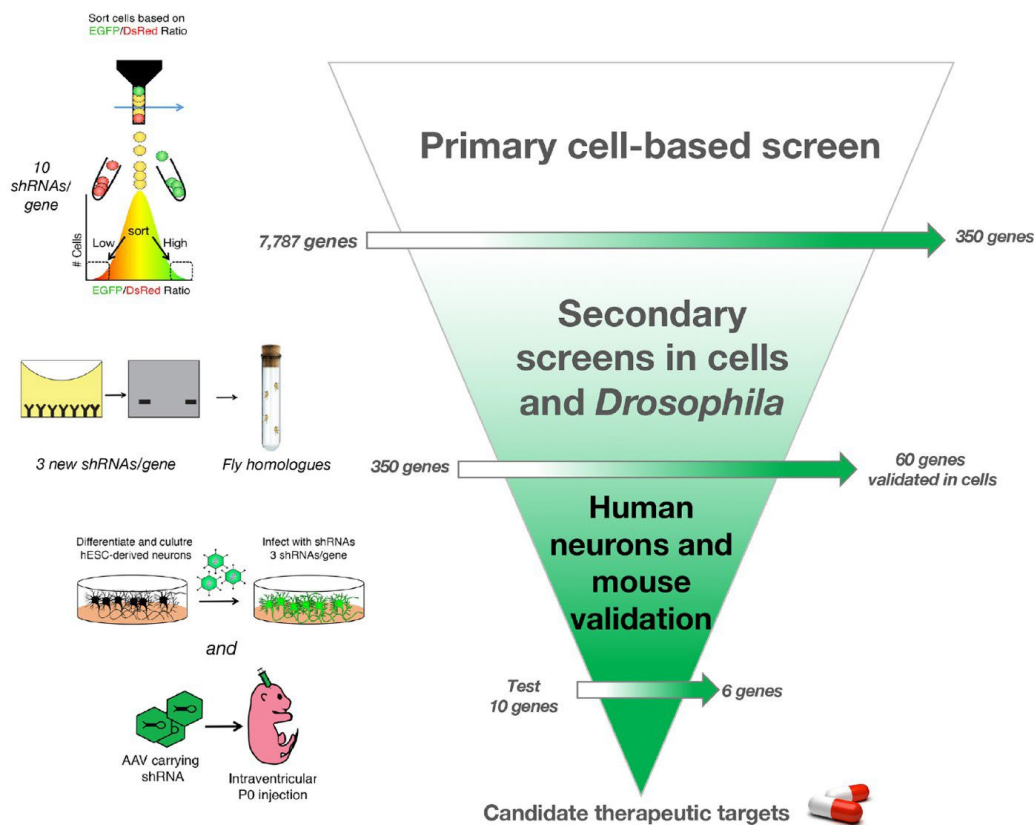
*Senior Faculty Advisor*

*Professor, Department of Pediatrics-  
Hematology*



## A Druggable Genome Screen Identifies Modifiers of $\alpha$ -Synuclein Levels via a Tiered Cross-Species Validation Approach.

Accumulation of  $\alpha$ -Synuclein ( $\alpha$ -Syn) protein causes Parkinson's disease (PD) and other synucleopathies. Increased  $\alpha$ -Syn levels are strongly implicated in PD pathogenesis and underscore the importance of identifying the factors that regulate its levels. In this study, Dr. Zoghbi's lab established a pooled RNAi screening approach and validation pipeline to probe the druggable genome for modifiers of  $\alpha$ -Syn levels and identified 60 promising targets. Using a cross-species, tiered validation approach, her group validated 6 strong candidates that modulate  $\alpha$ -Syn levels and toxicity in cell lines, *Drosophila*, human ES cell-derived neurons, and mouse brain of both sexes. More broadly, this genetic strategy and validation pipeline may be applied for the identification of therapeutic targets for disorders driven by dosage-sensitive proteins.



The diagram above depicts the pipeline for identifying potentially druggable modulators of  $\alpha$ -Syn levels. A primary cell-based screen was performed using pooled libraries of shRNAs targeting ~7500 genes. Secondary screens in HEK293T cells and *Drosophila* help narrow down the list and highlight strong modulators of  $\alpha$ -Syn levels and toxicity. Validation in human ES cell-derived neurons, performed by HSCC, and mouse brain, ensures robustness of hits and validates targets for preclinical nomination.

Rousseaux MWC, Vazquez-Velez GE, Al-Ramahi I, Jeong HH, Bajic A, Revelli JP, Ye H, Phan ET, Deger JM, Perez AM, Kim JY, Lavery LA, Xu Q, Li MZ, Kang H, Kim JJ, Shulman JM, Westbrook TF, Elledge SJ, Liu Z, Botas J, Zoghbi HY. A Druggable Genome Screen Identifies Modifiers of alpha-Synuclein Levels via a Tiered Cross-Species Validation Approach, *J Neurosci*, 2018, 38(43):9286-9301.

# INTEGRATED MICROSCOPY

The Integrated Microscopy Core (IMC) is a state-of-the-art imaging, training and assay development resource to support live and fixed cell confocal, deconvolution and super-resolution microscopy, and fully-automated high throughput microscopy. A full suite of image analysis, statistics and reporting software is available for data mining and management.

## MAJOR EQUIPMENT

- Nikon A1-Rs laser scanning spectral confocal microscope
- GE Healthcare DeltaVision deconvolution microscope with large sCMOS camera
- Nikon Ci-L upright brightfield microscope with color camera
- Vala Sciences IC-200 high throughput microscope
- Sartorius IncuCyte S3 long term live imager
- GE Healthcare OMX Blaze super-resolution instrument (SIM) with ultrafast camera for live imaging and TIRF capabilities
- Biotek Cytation 5 microscope-in-a-box (fluorescence, color, slide scanning, plastic plates, live imaging, hypoxia), plus plate reader (fluorescence, absorbance, luminescence)
- NEW: Bruker Vutara 352 STORM biplane super-resolution microscope with microfluidics
- NEW: Optical Biosystems StellarVision SV20 synthetic aperture optics high resolution/large field of view high throughput microscope

## SERVICES

- One-on-one training for all instruments and assisted use
- Assay development and project consultations
- Fully automated and assisted high throughput microscopy for 96/384 well plates
- Image Analysis, custom or pre-set (i.e., cell count, subcellular localization, spot counting, translocation, cell cycle, toxicity, live/dead, apoptosis)
- Full service for sample prep and imaging for STORM super-resolution microscopy
- Training in immunofluorescence and RNA FISH protocols

## CORE LEADERSHIP



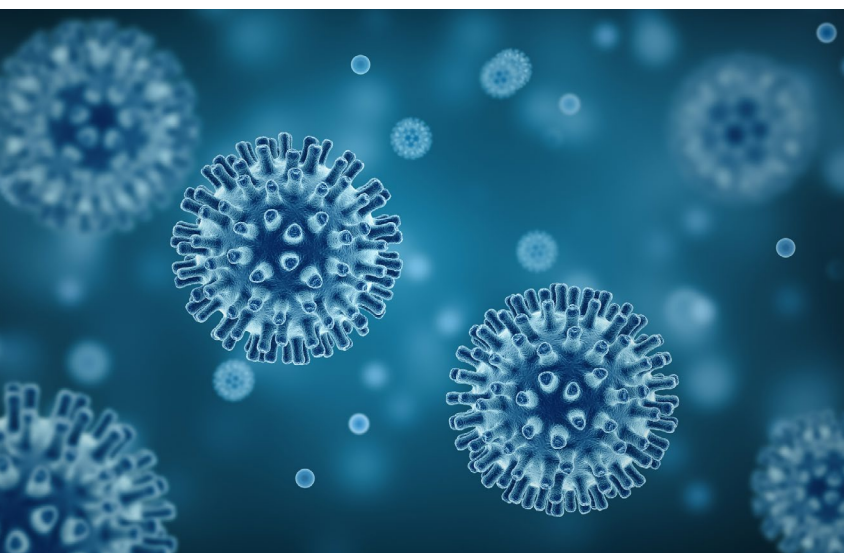
**Michael Mancini, PhD**

*Academic Director*  
*Professor, Department of Molecular and Cellular Biology*  
713.798.8952  
mancini@bcm.edu



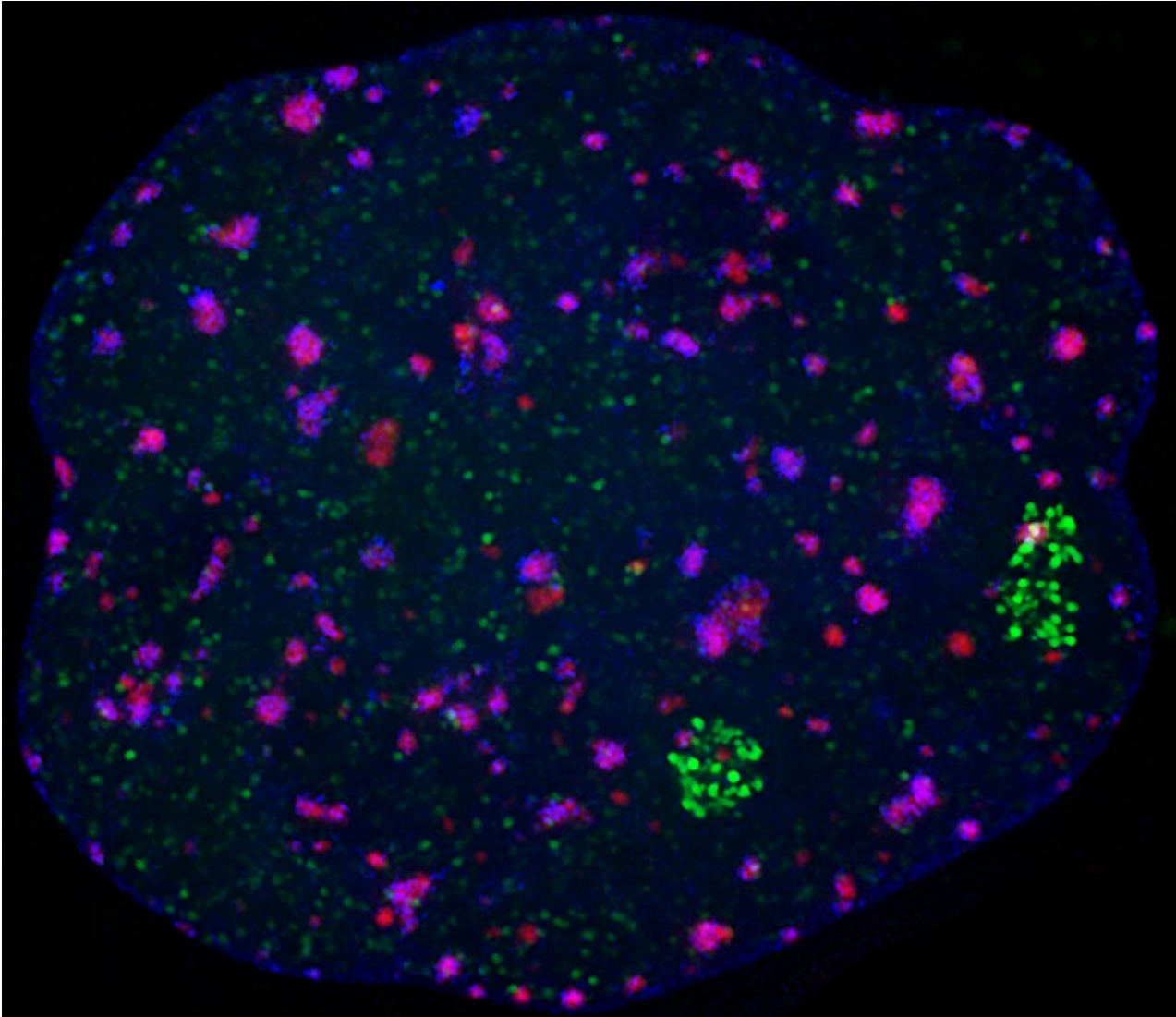
**Fabio Stossi, PhD**

*Technical Director*  
*Associate Professor, Department of Molecular and Cellular Biology*  
713.798.6940  
stossi@bcm.edu



## CORE SUPPORTED RESEARCH

### High resolution imaging of chromatin domains



**Figure Legend:** High resolution fluorescent microscopy (100x/1.4, GE Healthcare DVLIVE image restoration deconvolution microscope) revealing GFP-estrogen receptor-targeted transcription sites (Green), post-translational modification of heterochromatin (Red), and DNA (Blue). The two large GFP-positive clusters (with sub-structures) represent engineered loci rich in estrogen response elements, and the PTM marker is labeling dense DNA (heterochromatin). Michael A. Mancini (unpublished)

*Unpublished data courtesy of Dr. Alexandre Carisey and Dr. Jordan Orange, Center for Human Immunobiology, Baylor College of Medicine, Texas Children's Hospital.*



# MASS SPECTROMETRY PROTEOMICS

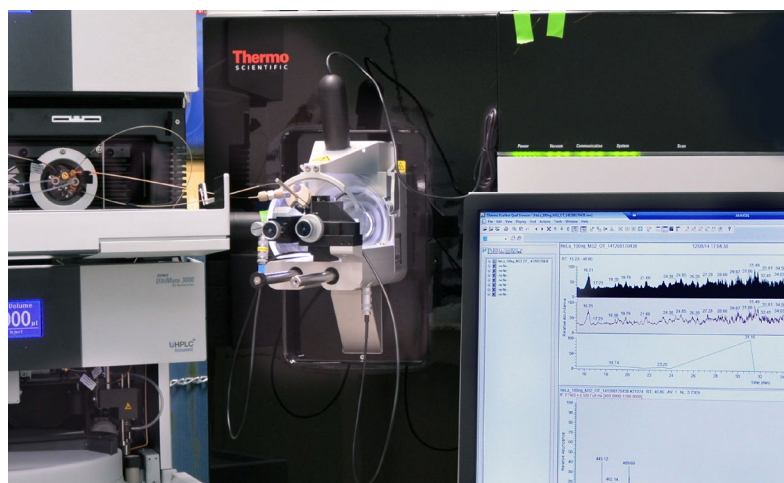
The Mass Spectrometry Proteomics Core offers services for quantitative proteome-wide profiling of cells and tissues, isolation and characterization of protein complexes, post-translational modification (PTM) analysis, and routine identification of purified proteins. We specialize in providing comprehensive project-based support that includes project design, optimization of biochemical procedures for sample preparation, state-of-the-art mass spectrometry technology, and custom data analysis to address specific challenges of different proteomics approaches.

## MAJOR EQUIPMENT

- Thermo Scientific Mass Spectrometers:  
Q-Exactive Plus  
Orbitrap Fusion Tribrid  
Orbitrap Lumos ETD Tribrid  
Orbitrap Exploris 480
- EASY-nLC1200 and Dionex Ultimate 3000  
RSLCnano UHPLC Systems

## SERVICES

- 365 Proteome Profiling service combines efficient non-detergent sample preparation procedure with dual reverse phase fractionation procedure and optimized mass spectrometry acquisition methods to allow identification and label-free quantification of up to 6,000 proteins from as little as 100,000 cells or 20 micrograms of tissue lysate.
- IP/MS (antibody-based affinity purification followed by MS identification) packages offer characterization of protein complexes and their extended interaction networks. The core's unique emphasis is in purification of endogenous complexes. Custom data analysis against BCM's own complexome database and filtering of non-specific precipitants is included in this package service.
- Post-translational modification (PTM) analysis includes identification and quantification of phosphorylation, ubiquitination or acetylation sites on purified proteins.
- The core offers routine MS sequencing of purified protein samples for single-protein identification or verification.
- Consultation, experimental design and data analysis.



## CORE LEADERSHIP



**Anna Malovannaya, PhD**

*Academic Director*

*Assistant Professor,  
Department of Biochemistry  
and Molecular Biology*

713.798.8699

[anna.malovannaya@bcm.edu](mailto:anna.malovannaya@bcm.edu)



**Sung Yun Jung, PhD**

*Core Director*

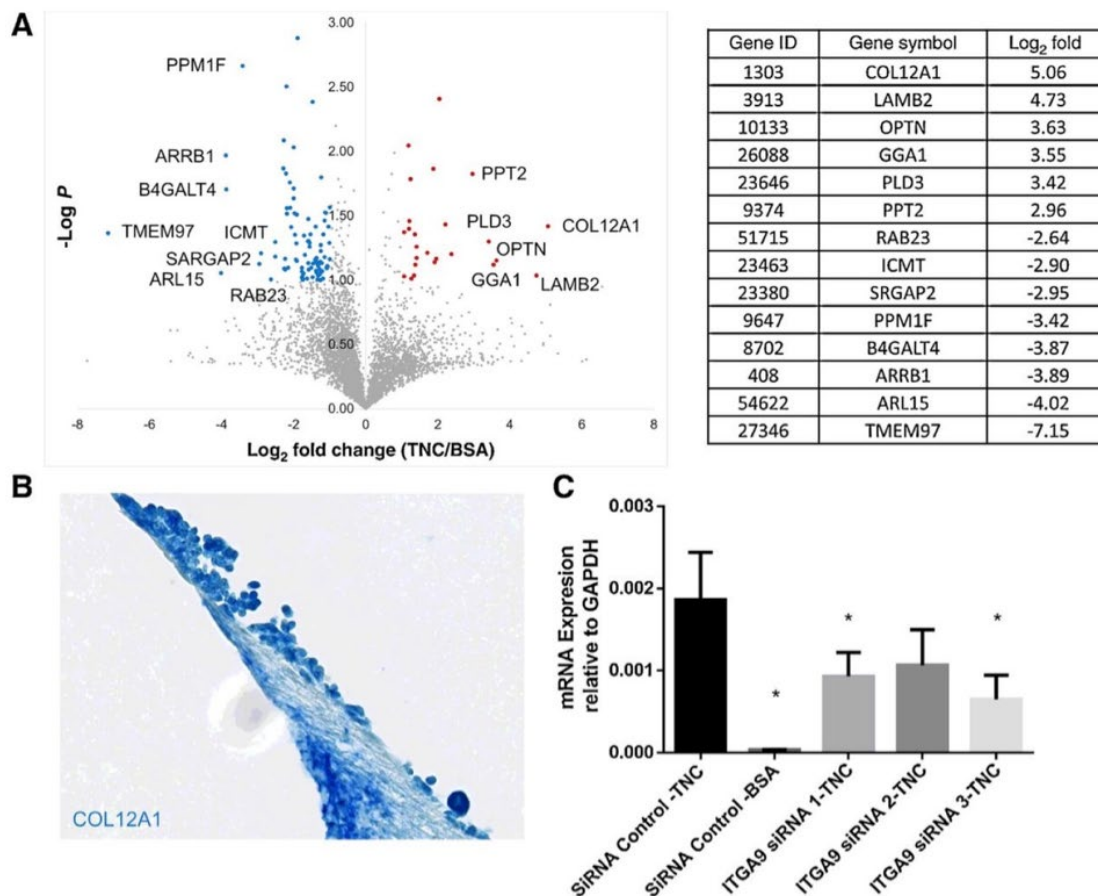
*Associate Professor,  
Department of Biochemistry  
and Molecular Biology*

713.798.7231

[syjung@bcm.edu](mailto:syjung@bcm.edu)

## Tenascin-C and Integrin $\alpha 9$ Mediate Interactions of Prostate Cancer with the Bone Microenvironment

Dr. David Rowley's (BCM, Department of Molecular and Cellular Biology) laboratory reported that tenascin C is expressed in the bone endosteum and is associated with formation of prostate bone metastases. Their group used the Mass Spectrometry Proteomics Core to assess potential downstream effectors of tenascin-C-induced biology by "365" Proteome Profiling of VCaP cells cultured on tenascin-C-coated Osteo Assay plates. An increase in production of several proteins including a distinct 30-fold upregulation of collagen12 (COL12A1) protein was noted and subsequently confirmed in VCaP grown on 3D osteogenic organoids using IHC. Ablation of adhesion to tenascin-coated osteo plates via siRNA knockdown of integrin  $\alpha 9$  resulted in a decrease of the transcript for COL12A1 in VCaP cells cultured on tenascin-C-coated osteo-mimetic surfaces, suggesting a direct link between cell binding and this osteogenic collagen production by the epithelial cell.



**Differential protein expression in VCaP cells cultured in tenascin-C-osteoplate.** A, Mass spectrometry reveals enhanced expression of collagen 12A and laminin b2 subunit in VCaP because of culture on tenascin-C-coated osteo surfaces. B, VCaP that associate with the osteogenic organoid express COL12A1. IHC COL12A1 (blue). Methyl green, counterstain. C, Ablation of adhesion via integrin  $\alpha 9$  knock out decreases expression of COL12A1 in VCaP when cells are cultured on tenascin-C-coated osteo-mimetic surfaces. Summary of three independent RT-PCR studies on the expression of COL12A1 upon ITGA9 knockout. \*,  $P < 0.05$

San Martin R, Pathak R, Jain A, Jung SY, Hilsenbeck SG, Piña-Barba MC, Sikora AG, Pienta KJ, Rowley DR. Tenascin-C and Integrin  $\alpha 9$  Mediate Interactions of Prostate Cancer with the Bone Microenvironment. *Cancer Res.* 2017, 77(21):5977-5988.

# METABOLOMICS

The Metabolomics Core provides targeted and unbiased metabolic profiling for discovery and validation of biomarkers of various diseases with state-of-the-art high throughput mass spectrometry as the main platform. Metabolites can be measured in tissue samples, cell lines, fecal and biofluids including urine. The entire process starting from sample preparation to mass spectrometry is monitored using spiked isotopic standards that have been characterized for their chromatographic behavior as well as fragmentation profile. Biostatisticians are available for further analysis of the resulting output data.

## MAJOR EQUIPMENT

- Agilent 6490 triple quadrupole (QQQ) mass spectrometry
- Agilent 6495 triple quadrupole (QQQ) mass spectrometry
- Agilent 6550 QTOF UHD Accurate-Mass spectrometry
- AB SCIEX 5600 Triple TOF Mass Spectrometer
- 1290 and 1260 Series HPLC Systems

## SERVICES

**Targeted metabolite steady-state profiling:** The Core has the capability of identification, quantification and, characterization of over 500 metabolites using the targeted multiple reaction monitoring approaches (MRM) developed for different chemical classes of compounds. Data can be reported either in absolute concentrations or as intensity ratios to internal standards.

**Metabolic Flux:** Isotope flux and metabolite profiling to help formulate and test hypotheses about the metabolic consequences of various changes, in order to guide further integrative systems biology analyses of the underlying mechanisms in disease. The Core has the capability of characterizing  $[^{13}\text{C}]$  Glutamine and  $[^{13}\text{C}]$  Glucose flux using LC-QQQ Mass Spectrometry.

**Lipidomics:** Using an ABSCIEX 5600 triple TOF MS, identification of lipids is accomplished by data-dependent production (MS/MS) information of human plasma, tissues, and urine in both positive and negative ionization modes. MS/MS acquisition provides information on the nature of the lipid head group and/or neutral loss of the head group from the molecular ion adducts. Information on the fatty acid composition of the lipids is obtained in the negative mode.

### Data Analysis:

- Pathway mapping using OCM, GSA or NETGSA
- Developing classification models
- Integration with other OMICS datasets



## CORE LEADERSHIP



### Nagireddy Putluri, PhD

*Academic Director*

*Associate Professor,  
Department of Molecular  
& Cellular Biology*

713.798.3139

putluri@bcm.edu

### Arun Sreekumar, PhD

*Senior Faculty Advisor*

*Professor, Department of Molecular &  
Cellular Biology*



## CLASSES OF METABOLITES MEASURED BY MRM ANALYSIS

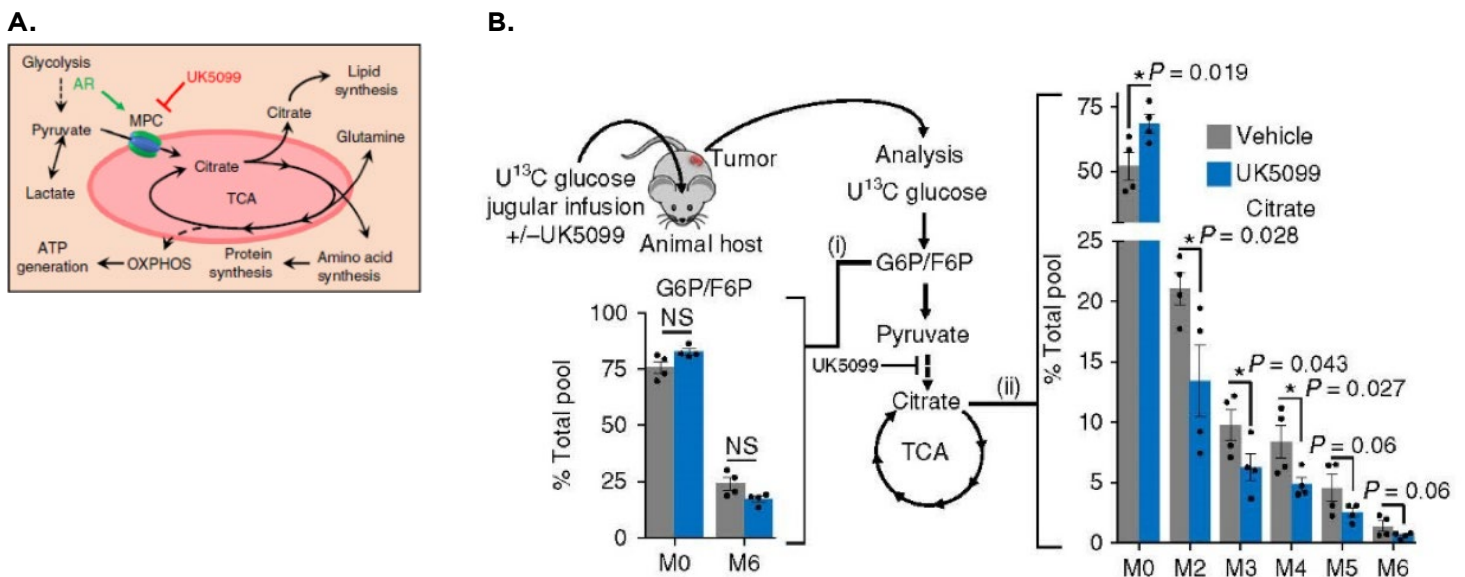
- |                                        |                                      |                                      |
|----------------------------------------|--------------------------------------|--------------------------------------|
| 1. Amino Sugars                        | 6. TCA metabolites                   | 11. Bile acids                       |
| 2. Amino acids and related metabolites | 7. CoA's and Carnitines (Long chain) | 12. Short-Chain Fatty Acids          |
| 3. Prostaglandins                      | 8. Sugars                            | 13. Methylated Metabolites           |
| 4. Carnitines (Short chain)            | 9. Nucleotides / Nucleic acid        | 14. Polycyclic aromatic hydrocarbons |
| 5. Polyamines                          | 10. Vitamins and Steroids            | 15. Miscellaneous/Other metabolites  |

## CORE SUPPORTED RESEARCH

### Mitochondrial pyruvate import is a metabolic vulnerability in androgen receptor-driven prostate cancer.

In a study published by the Dr. Sean E. McGuire lab (BCM, Department of Molecular Genetics), the mitochondrial pyruvate carrier (MPC) was shown to have an important role in prostate cancer (**Figure 1A**). MPC inhibition blocked the entry of glucose-derived carbon into the TCA cycle, resulting in increased glutamine uptake as a carbon source for the TCA cycle. The contribution of the Metabolomics Core to this study was to perform metabolic flux analysis using  $^{13}\text{C}$  labeled glucose to demonstrate the change in glucose incorporation into the TCA cycle in living cells. Glucose incorporation into TCA metabolite pools was quantified by pretreating ABL prostate cancer cells for 2 h (vehicle or UK5099), then adding U $^{13}\text{C}$  glucose for 48 h. Individual isotopomeric distributions are shown for G6P/F6P (i), citrate (ii),  $\alpha$ -kg (iii) and oxaloacetate (iv) (**Figure 1B**). Collectively, these findings characterize the MPC as a facultative component of tumor metabolism and support further examination of the MPC as a potential therapeutic target in additional tumor types.

**Figure 1. A)** Model depicts metabolic and biosynthetic outputs of the TCA cycle in relation to glycolysis and the MPC. **B).** Glucose incorporation into TCA metabolite pools was quantified by pretreating ABL cells for 2 h (vehicle or UK5099), then adding U $^{13}\text{C}$  glucose for 48 h.



Bader DA, Hartig SM, Putluri V, Foley C, Hamilton MP, Smith EA, Saha PK, Panigrahi A, Walker C, Zong L, Martini-Stoica H, Chen R, Rajapakshe K, Coarfa C, Sreekumar A, Mitsiades N, Bankson JA, Ittmann MM, O'Malley BW, Putluri N, McGuire SE. Mitochondrial pyruvate import is a metabolic vulnerability in androgen receptor-driven prostate cancer. (*Nat Metab.* 2019 Jan;1(1):70-85. doi: 10.1038/s42255-018-0002-y. Epub 2018 Nov 19). PMID: 31198906

# MHC TETRAMER

MHC Tetramer technique has become a “gold standard” for the quantification of T cell immune responses. Joining multiple copies of the MHC/antigen complex into a single probe resolves the difficulties presented by the low affinity of the class I MHC molecule for the CD8 receptor. By offering exquisite antigen specificity and sensitivity, this unique technique is suitable for basic and clinical studies in a number of applications, including cancer prevention, cancer therapy, cell and gene therapy, immunotherapy, and non-cancer related immunology research. The mission of the core is to provide BCM investigators with customized MHC/peptide tetramers for identification of antigen specific T lymphocytes by flow cytometry.

## SERVICES

- MHC Class I Tetramers
- We offer more than fifty human, mouse, macaque, and chimpanzee alleles for customized production of class I MHC reagents with desired epitopic peptides. Researches will also have two fluorescent labels choices: R-phycoerythrin (PE) or allophycocyanin (APC).
- Biotinylated Monomers
- For customers who intend to try to label tetramers with small molecule fluorophores or require longer storage life
- Unbiotinylated Monomers can be used in various applications such as ELISA or ELISPOT; using unbiotinylated monomers coat the plate to present the peptide
- Special reagents
  - » MHC Allele Inclusion Bodies
  - » CD8 binding-deficient MHC Class I tetramers: Containing mutations in the MHC  $\alpha 3$  domain that ablate CD8 binding; can be used to quantitate and to sort CD8-independent T cells.
  - » Chimeric Tetramer: Containing MHC Human/Mouse chimeric heavy-chain
  - » MHC Monomers for generating TCR-like antibodies
  - » MHC Class I monomers and tetramers designed for ligand exchange
  - » Cytotoxic Saporin-conjugated tetramers (Tet-SAP)



## CORE LEADERSHIP



**Lily Wang, MS**

*Core Director*

*Advanced Technology  
Cores*

713.798.3918

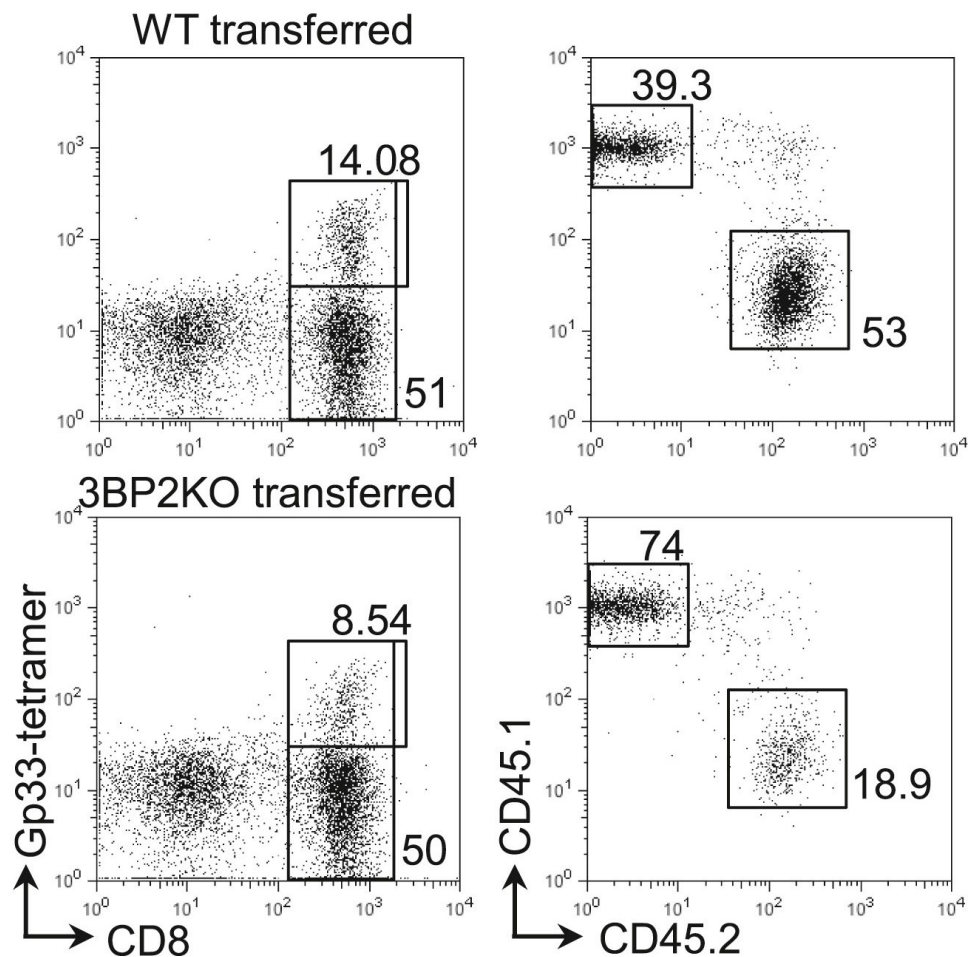
## CORE SUPPORTED RESEARCH

The study below focused on the role of 3BP2 in sustaining CD8+ T cell expansion and differentiation.

Successful anti-viral response requires the sustained activation and expansion of CD8+ T cells for periods that far exceed the time limit of physical T cell interaction with antigen-presenting cells (APCs). The expanding CD8+ T cell pool generates the effector and memory cell populations that provide viral clearance and long-term immunity, respectively. This study showed that induction of the adaptor molecule 3BP2 is a sensor of TCR signal strength and is critical for sustaining CD8+ T cell proliferation and regulating effector and memory differentiation. Our tetramer reagent, H-2D(b)/LCMV.GP33-41, was used to assess the antigen-specific CD8+ T cells at the peak of the anti-viral response on day 8 LCMV post-infection using flow cytometry.

Figure below showed the investigation of development of 3BP2<sup>-/-</sup> cytotoxic CD8+ T cell responses using an *in vivo* model of viral infection. 3BP2<sup>+/+</sup> or 3BP2<sup>-/-</sup> P14+ T cells (CD45.2<sup>+</sup>) were purified and transferred into wild-type recipient mice (CD45.1<sup>+</sup>), respectively. The two groups of mice were inoculated with 2000 plaqueforming units (PFU) of the Armstrong LCMV strain. At the peak of the primary anti-viral response on day 8 post-infection, the spleens of the infected mice were harvested, and the antigen-specific P14+ T cell responses were quantified by flow cytometry using H-2D(b)/LCMV.GP33-41 tetramers.

Representative fluorescence-activated cell sorting (FACS) plots showed percentage of CD8+Gp33+ tetramer-stained cells in the spleens of virus infected mice (left panel) and percentages of endogenous (CD45.1<sup>+</sup>) or transferred (CD45.2<sup>+</sup>) antigen-specific cells (right panel). The results demonstrated that 3BP2 is critical for the expansion, memory generation, and memory response of P14+ T cells upon antigen stimulation *in vivo*.



Dimitriou ID, Lee K, Akpan I, Lind EF, Barr VA, Ohashi PS, Samelson LE, Rottapel R. Timed Regulation of 3BP2 Induction Is Critical for Sustaining CD8+ T Cell Expansion and Differentiation. *Cell Reports*, 2018, Vol. 24:1123, PMID: 30067970



# MOUSE EMBRYONIC STEM CELL CORE

The mission of the Mouse Embryonic Stem Cell Core is to provide BCM investigators with standardized, high quality services for precise engineering of the mouse genome. Procedures for modifying the genome of mouse embryonic stem (ES) cells and the subsequent construction of mice from manipulated ES cells are intensive and technically demanding. It is a goal of the Core to make such experiments accessible to a wide range of BCM investigators. The Core focuses on the manipulation of embryonic stem cells for further studies *in vitro* with primary mouse ES cell lines and *in vivo* with mice generated with targeted ES cells. In addition, the Core provides centralized, highly specialized expertise in the design and rapid production of reagents for *in vivo* CRISPR/Cas9 genome engineering in mice and for *in vitro* CRISPR/Cas9 mutagenesis in mouse ES cells. The Mouse ES Cell Core works closely with the Genetically Engineered Mouse (GEM) Core by providing targeted ES cells for blastocyst microinjections and CRISPR/Cas9 reagents for microinjection of pronuclear stage embryos.

## SERVICES

- Advice with construct design for gene targeting in ES cells
- Electroporation of mouse ES cells (129 or B6-derived) with targeted vectors provided by investigators
- Isolation of ES cell colonies for genotyping
- Expansion of mouse ES cell clones from single wells of 96 well plates
- Cryopreservation of mouse ES cells
- Transgene knock-ins at the Rosa26 and Hprt loci
- Advice with KOMP/EUCOMM vectors and ES cells
- Generation of new mouse ES cell lines from blastocysts
- Murine virus testing of ES cells
- *In vivo* CRISPR/Cas9 mutagenesis project design and reagent production for both nonhomologous end joining and homology directed repair in mice (and other model organisms such as rats)
- CRISPR/Cas9 mutagenesis in mouse ES cells
- CRISPR/Cas9 on target and off target genotyping



## CORE LEADERSHIP



**Jason Heaney, PhD**

*Academic Director  
Assistant Professor,  
Department of  
Molecular and  
Human Genetics*

713.798.1778



**Isabel Lorenzo**

*Core Director  
Instructor,  
Department of  
Molecular and  
Human Genetics*

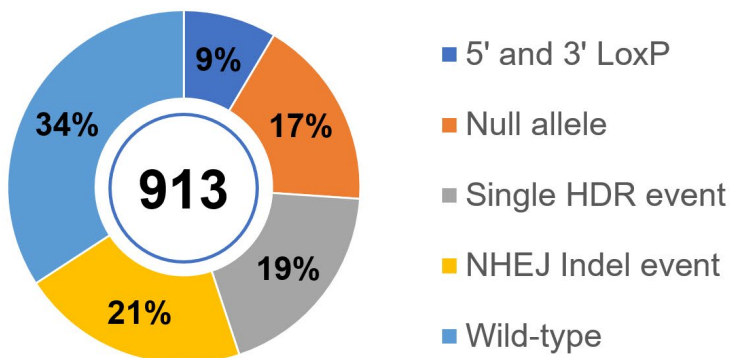
713.798.1981

## CORE SUPPORTED RESEARCH

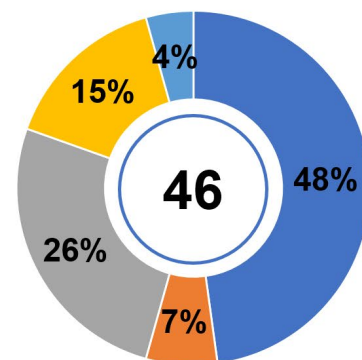
### Long single-strand DNA donors are more efficient than pairs of short single-stranded oligodeoxynucleotides for producing conditional knockout alleles in mice

Conditional knockout alleles for the BCM Knockout Mouse Phenotyping Project (KOMP2) and BCM investigators were generated using CRISPR/Cas9-mediated homology directed repair (HDR) with (A) pairs of loxP containing short single-stranded oligodeoxynucleotides (ssODNS) or (B) long-single stranded DNAs (lssDNAs) harboring loxP-flanked exon(s). Each donut chart represents the summation of each allele type for all founder mice genotyped. In the center of the chart is the total number of live-born mice genotyped. Percentages of each allele type are indicated. 5' and 3' loxP: Includes animals genotyped for both 5' and 3' loxP sites, irrespective of the presence of any additional alleles (e.g., animals with 5' loxP, 3' loxP and a null allele detected); Null allele: Includes animals genotyped for a null allele, which may also have a single HDR and/or NHEJ indel event; Single HDR event: Includes animals genotyped for a single HDR event with or without additional indel events; NHEJ Indel event: Animals in which only indel alleles were observed. (C) HDR efficiency is influenced by the efficiency of sgRNA-guided, Cas9-mediated DSB production. Data plotted based on number of founder mice with an HDR event (x-axis) versus any evidence for a DSB generated at the respective sgRNA site such as a NHEJ indel, HDR event, or the formation of a null allele (y-axis). Pearson correlation coefficients were calculated and analyzed.

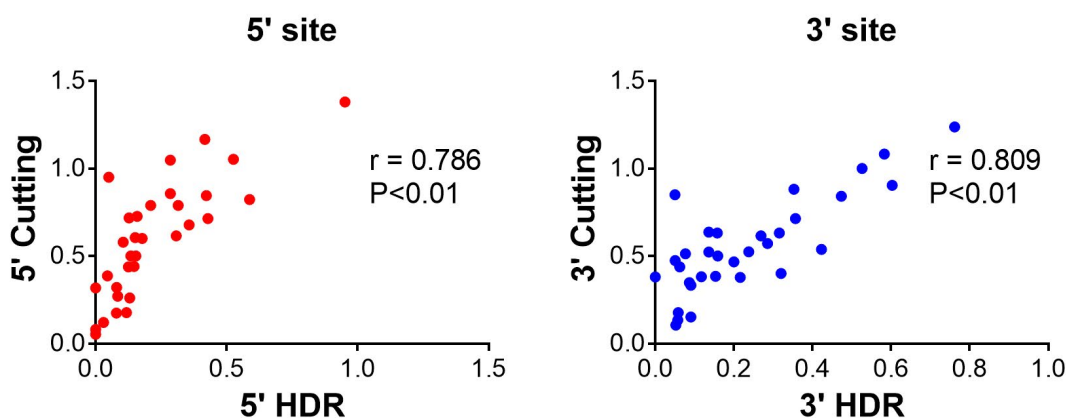
**(A) Conditional by ssODN**



**(B) Conditional by lssDNA**



**(C)**



Lanza DG, Gaspero A, Lorenzo I, Liao L, Zheng P, Wang Y, Deng Y, Cheng C, Zhang C, Seavitt JR, DeMayo FJ, Xu J, Dickinson ME, Beaudet AL, Heaney JD. Comparative analysis of single-stranded DNA donors to generate conditional null mouse alleles. *BMC Biol.* 2018; 16(1):69.

# MOUSE METABOLISM AND PHENOTYPING CORE

The Mouse Metabolic and Phenotyping Core (MMPC) is a comprehensive phenotyping core that provides investigators with a wide variety of state-of-the-art equipment and techniques for testing rodent models from embryo to adult. The mission of the MMPC is to provide equipment, services, and resources for the expert characterization of whole animal and organ systems phenotypes within rodent models. The MMPC has standardized key methodologies and can expedite comprehensive research analyses on diseases related to cancer, cardiovascular dysfunction, metabolic disorders, rodent models of human disease and drug studies. The MMPC also provides advanced analysis of metabolic pathways and related physiological and biochemical parameters in mice and rats, as well as in isolated/cultured cells in vitro. Workstations for image reconstruction and data analysis are also available within the core. MMPC personnel provide consultation on selecting the appropriate tests and procedures, and the interpretation of data.

## MAJOR EQUIPMENT

- Vevo 2100 Ultrasound (Visulasonics)
- 7.0T Pharmascan MRI (Bruker)
- eXplore CT 120 (TriFoil Imaging)
- Ms-FX Pro Optical Imager and X-Ray (Bruker)
- Unrestrained Whole Body Plethysmography (Buxco)
- Oxymax FAST Indirect Calorimetry System (Columbus Instruments)
- Comprehensive Lab Animal Monitoring System [CLAMS-HC] (Columbus Instruments)
- UltraFocus [X-Ray and Body Composition Analyzer] (Faxitron)
- EchoMRI-100™ [Body Composition Analyzer] (EchoMRI)
- Non-Invasive Blood Pressure (IITC Life Sciences)
- Blood Pressure and ECG Telemetry (DSI)
- 6-lane treadmill (Columbus Instruments)
- Running wheels (Minimitter)
- Metabolic cages (Techniplast)
- ECG-Mouse Monitor (Indus Instruments)
- Pulse oximetry (Indus Instruments)
- Grip strength meter (Columbus Instruments)
- XFe96 Seahorse Analyzer (Agilent)
- XF24 Seahorse Analyzer (Agilent)
- Versamax System (Accuscan)
- Vessel Doppler (Indus Instruments)
- Rectal probe for body temp measurement
- Isoflurane anesthesia stations

## SERVICES

- Hyperinsulinemic-euglycemic clamp in conscious mice: direct quantification of the role of specific organs and tissues in glucose-insulin homeostasis
- Cellular oxygen consumption & glycolysis monitoring of cultured cells by use of the Seahorse instrument
- Metabolic monitoring of food intake, energy expenditure and real-time body temperature for small animals (mouse) after 72 hours of acclimation using the Comprehensive Laboratory Animal Monitoring System (HC-CLAMS)
- Home cage activity monitoring
- Hypoxia chamber animal housing
- Plasma/Blood parameter Analysis
- Lipid Metabolism
- Glucose metabolism
- Glucose tolerance test
- Insulin tolerance test
- Pyruvate tolerance test (gluconeogenesis)
- Transverse Aortic Constriction (TAC)
- Telemetry Device Implantation
- Osmotic Pump Implantation
- Cecal Ligation

## CORE LEADERSHIP



**Christopher S. Ward, PhD**  
*Director*  
*Instructor, Department of Molecular Physiology & Biophysics*  
713.798.5040  
[christopher.ward@bcm.edu](mailto:christopher.ward@bcm.edu)



**Pradip K. Saha, MS, PhD**  
*Co-Director*  
*Assistant Professor, Department of Molecular & Cellular Biology*  
713.798.3704  
[psaha@bcm.edu](mailto:psaha@bcm.edu)

### Susan L. Hamilton, PhD

*Senior Faculty Advisor*

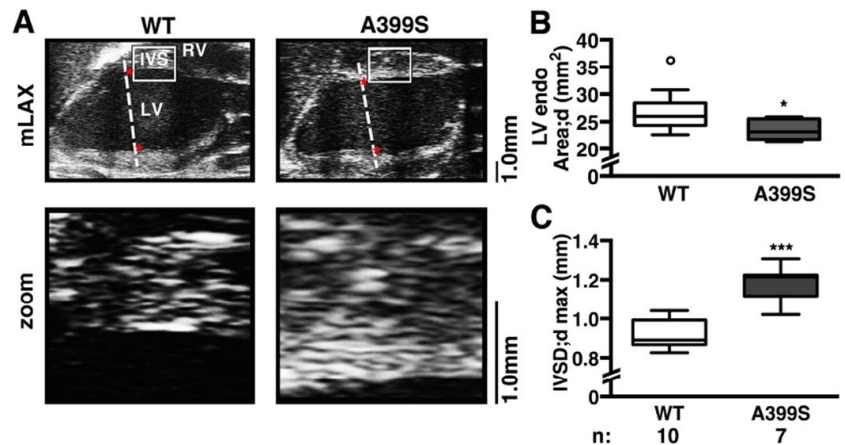
*Professor and Chair, Department of Molecular Physiology & Biophysics*



## Standard echocardiographic analysis of A399S mice

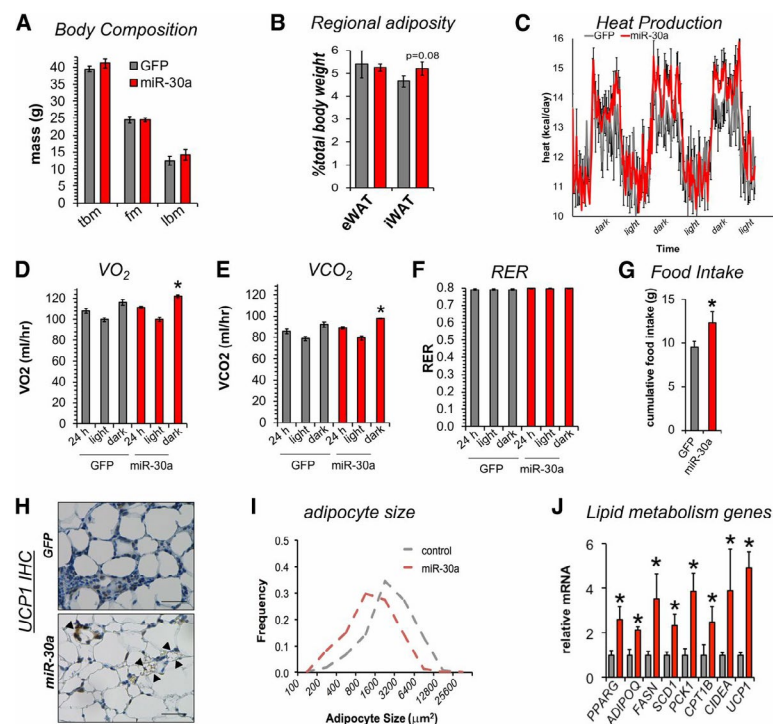
**Figure 1.** (A) Representative echocardiograms of traditional mouse short axis (SAX) mid-level B-modes; asterisks mark papillary muscles with corresponding standard M-mode tracings. Quantifications revealed no differences in (B) left ventricular outer diastolic diameter (LVOD;d) or (C) ejection fraction (EF) between A399S and WT mice. N = number animals. Circles in box plots represent outliers. IVS: intraventricular septum; LV: left ventricle; SAX: short axis.

Quick AP, Landstrom AP, Wang Q, Beavers DL, Reynolds JO, Barreto-Torres G, Tran V, Showell J, Philippen LE, Morris SA, Skapura D, Bos JM, Pedersen SE, Pautler RG, Ackerman MJ, Wehrens XH. Novel junctophilin-2 mutation A405S is associated with basal septal hypertrophy and diastolic dysfunction. *JACC Basic Transl Sci.* 2017, 2(1):56-67.



## miR-30a expression in iWAT of DIO mice improves energy balance

**Figure 2.** Body composition (A) and fat depot mass (normalized to body weight) (B) of DIO mice ectopically expressing Adv-GFP or Adv-miR-30a in iWAT. C: Energy expenditure (heat) during two complete 12-h light-dark cycles 11 days after local expression of Adv-GFP or Adv-miR-30a in iWAT of DIO mice. Average oxygen consumption (VO<sub>2</sub>) (D), carbon dioxide production (VCO<sub>2</sub>) (E), and respiratory exchange ratio (RER) (F) during the 12-h light-dark cycles were determined by Comprehensive Lab Animal Monitoring System (CLAMS). G: Cumulative food intake during CLAMS experiments (n = 5). H: Histology showing UCP1 immunostaining in iWAT of Adv-GFP or Adv-miR-30a DIO mice. Arrowheads indicate UCP1-positive cells. Scale bars, 50 μm. Adv-miR-30a expression in iWAT remodels adipocyte size (I) and restores expression of lipid metabolism genes (J) (n ≥ 4). All data are expressed as mean ± SEM. \*P < 0.05. All metabolic cage measurements are presented on a per mouse basis. eWAT, epididymal WAT; fm, fat mass; IHC, immunohistochemistry; lbm, lean body mass; tbm, total body mass.



Koh EH, Chernis N, Saha PK, Xiao L, Bader DA, Zhu B, Rajapakshe K, Hamilton MP, Liu X, Perera D, Chen X, York B, Trauner M, Coarfa C, Bajaj M, Moore DD, Deng T, McGuire SE, Hartig SM. miR-30a Remodels Subcutaneous Adipose Tissue Inflammation to Improve Insulin Sensitivity in Obesity. *Diabetes.* 2018, 67(12):2541-2553.

# NMR AND DRUG METABOLISM

The Nuclear Magnetic Resonance (NMR) and Drug Metabolism Core offers tools to support the discovery, synthesis, screening, identification, metabolism and pharmacokinetics of small molecules. Expert NMR services are available to determine small molecule identity or conformation, to identify and quantify metabolites that may serve as biomarkers, to determine macromolecular structure or detect structural perturbations upon ligand binding, or to screen small molecule compound libraries against purified macromolecular targets. The Core also investigates the metabolism and pharmacokinetics of small molecular weight compounds using liquid chromatography-mass spectrometry (LC-MS and MS/MS) and metabolic stability in liver microsomes by reaction phenotyping assays with CYP450s. Core personnel provide advice on the use of the supported methods for a wide variety of applications, and assistance is available in project experimental design and data analysis. NMR spectrometers are available for unassisted use by trained and qualified users, and user training in simple 1D and 2D NMR data acquisition and analysis is available.

## MAJOR EQUIPMENT

- 800 MHz Bruker Avance HD III spectrometer
- 800 MHz Bruker QCI Cryoprobe
- SampleJet automated sample changer
- 600 MHz Bruker Avance HD III spectrometer
- SampleXpress automated sample changer
- Thermo Q Exactive Hybrid Quadrupole-Orbitrap LC-MS system
- Thermo Quantis Triple Quadrupole LC-MS/MS system
- Thermo Scientific Multidrop Combi
- HP D300 Digital Dispenser
- SensiQ SPR

## SERVICES

- Compound identification and quality control
- Small molecule conformational analysis
- Ligand/target screening
- Chemical shift perturbation mapping of protein/ligand interactions
- Feasibility studies for NMR structure determination
- NMR user training
- Drug quantification in fluids or tissues
- Drug metabolite identification
- Microsomal stability assays
- Reaction phenotyping
- Pharmacokinetic profiles

## CORE LEADERSHIP



**Kevin MacKenzie, PhD**

*Academic Director*  
*Associate Professor,*  
*Department of Pathology*  
*& Immunology*  
713.798.3952  
kevin.mackenzie@bcm.edu



**Feng Li, PhD**

*Co-Director*  
*Assistant Professor,*  
*Department of Molecular*  
*and Cellular Biology*  
713.798.3623  
feng.li@bcm.edu

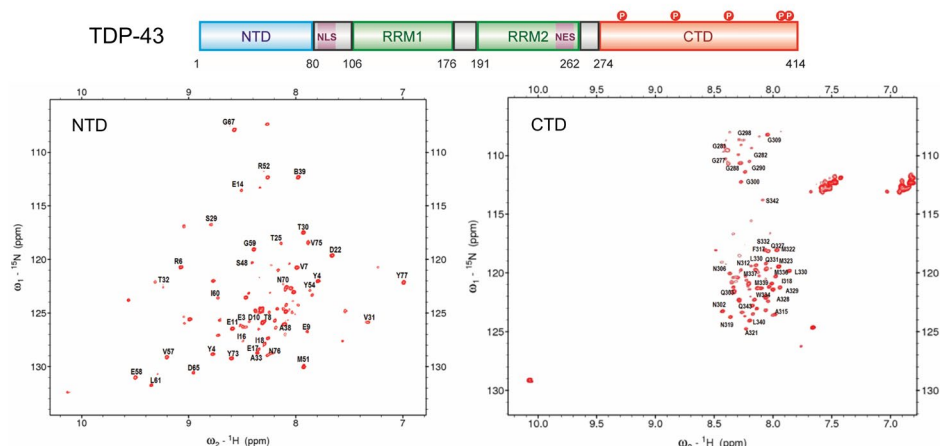


**Kimberly Kerlec, BS**

*Operations Manager*  
*Advanced Technology*  
*Cores*  
713.798.6830  
kimberly.kerlec@bcm.edu

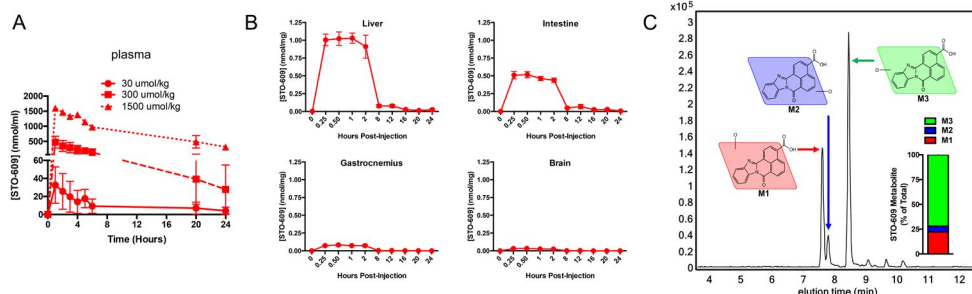
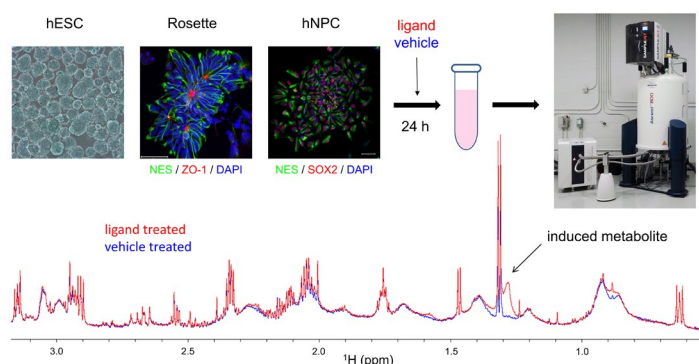
### Figure 1. NMR Spectroscopy.

TDP-43 contains folded and unfolded domains. TDP-43 comprises an N-terminal domain (NTD), two RNA recognition motifs (RRM1 and RRM2) and the intrinsically disordered C-terminal (CTD) domain, which can be phosphorylated (top). Strong  $^1\text{H}$  and  $^{15}\text{N}$  chemical shift dispersion of the resonances in the 2D  $^1\text{H}/^{15}\text{N}$  HSQC spectrum of the isolated NTD (left) indicate that it is well structured, whereas limited shift dispersion of the isolated CTD (right) indicate that it is unfolded.



Tsoi PS, Choi KJ, Leonard PG, Sizovs A, Moosa MM, MacKenzie KR, Ferreon JC, Ferreon ACM. The N-Terminal Domain of ALS-Linked TDP-43 Assembles without Misfolding. *Angew Chem Int Ed Engl.* 2017, 56(41):12590-12593.

**Figure 2.**  $^1\text{H}$  NMR identifies a metabolite perturbed by a given ligand. To examine the effects of a given ligand on the human neuroprogenitor cell (hNPC) metabolome, human embryonic stem cells (hESC) were induced toward neuroprogenitor lineage and treated with a ligand or vehicle. 24 hrs later,  $\sim 5 \times 10^5$  cultured hNPCs were collected and  $^1\text{H}$  NMR spectra acquired at 800 MHz. Differences between spectra reflect differences in metabolites; treatment induced a single metabolite change in the hNPCs (arrow). Additional NMR spectra can be used to identify this species. Unpublished data courtesy of Dr. Aleksandar Bajić and Dr. Mirjana Maletić-Savatić (Jan and Dan Duncan Neurological Research Institute).



**Figure 3.** Pharmacokinetics, tissue distribution, and metabolite identification of STO-609, a CamKK2 antagonist, in mice and liver microsomes. A. Pharmacokinetics of STO-609 at different doses in mice; the half-life is  $\sim 3$  hours. B. Tissue distribution of STO-609 in mice. Liver and intestine have

higher concentrations than muscle or brain. C. LC-MS chromatogram and relative abundance of STO-609 metabolites in liver microsomes. The three major Phase I metabolites are three distinct single oxygen adducts that can be resolved by liquid chromatography, though they all give the same mass.

York B, Li F, Lin F, Marcelo KL, Mao J, Dean A, Gonzales N, Gooden D, Maity S, Coarfa C, Putluri N, Means AR. Pharmacological inhibition of CaMKK2 with the selective antagonist STO-609 regresses NAFLD. *Sci Rep.* 2017, 7(1):11793.



# OPTICAL IMAGING AND VITAL MICROSCOPY (OIVM)

The mission of the Optical Imaging & Vital Microscopy (OIVM) Core is to provide state-of-the-art instrumentation and cutting edge imaging and image analysis tools for the research applications of a broad range of BCM investigators. This core is dedicated to vital and intravital imaging of processes within cells, intact tissue explants, developing embryos and functioning organs within the live animal. Our users are focused on a variety of applications such as understanding cell migration, optimizing angiogenic therapies, how blood flow influences development and cancer, immune cell recruitment, stem cell-niche interactions and cancer metastasis.

## MAJOR EQUIPMENT

- Zeiss LSM 880 with AiryScan FAST – High Speed Super Resolution/Confocal point scanning microscope
- Zeiss LSM 780 – Spectral Confocal point scanning microscope
- Zeiss Lightsheet Z.1 – Light-sheet fluorescence microscope
- Bruker Skyscan 1272 – X-ray  $\mu$ CT
- Zeiss LSM 7 MP – Two-photon point scanning microscope
- Leica TCS SP8 – Confocal and two-photon microscope
- Zeiss LSM 5 LIVE – Confocal line scanning microscope
- Zeiss Axio Zoom.V16 Stereomicroscope
- Optical Projection Tomography (OPT) Microscope
- Logos Biosystems X-Clarity Tissue Clearing System
- Bioptigen Optical Coherence Tomography (OCT) Microscope
- Dell Precision T7600/T7610 Workstations
- Dell PowerVault data storage array

## SERVICES

- Independent or Assisted Imaging
- Expert training/instruction on core instrumentation
- 24/7 Core Access for Trained Users
- Tissue Clearing with CLARITY protocol provided on a per sample basis
- Super Resolution Microscopy with AiryScan detection system
- Confocal microscopy with spectral array detection for imaging multiple fluorophores (up to 8) simultaneously
- Automated 3D tile scanning of image large fields with high resolution
- LightSheet fluorescence microscopy for 3D imaging of thick tissues cleared with Scale, CLARITY, etc.
- Two-photon and second harmonic generation (SHG) intravital imaging
- Live imaging of tissue/organ development in embryos and live animals with environmental control of the stage and anesthesia support of live animals
- High speed imaging of blood and fluid flow such as fluorescent beads, fluorescently labeled erythroblasts, etc.
- Live imaging of mouse cornea with the OCT microscope
- Imaging and 3D rendering of embryos, organs, bioengineered gels, etc. using the X-ray microCT scanner
- Imaging and 3D rendering of optically cleared (BABB) embryos, mammary glands, etc. using the OPT microscope
- Quantitative analysis of cellular dynamics and cell tracking
- 3D/4D Image Rendering using high end workstations equipped with latest image visualization/analysis software

## CORE LEADERSHIP



**Jason M. Kirk, BS**

*Co-Director*

713.798.6486

[jmkirk@bcm.edu](mailto:jmkirk@bcm.edu)



**Chih-Wei Logan Hsu, PhD**

*Co-Director*

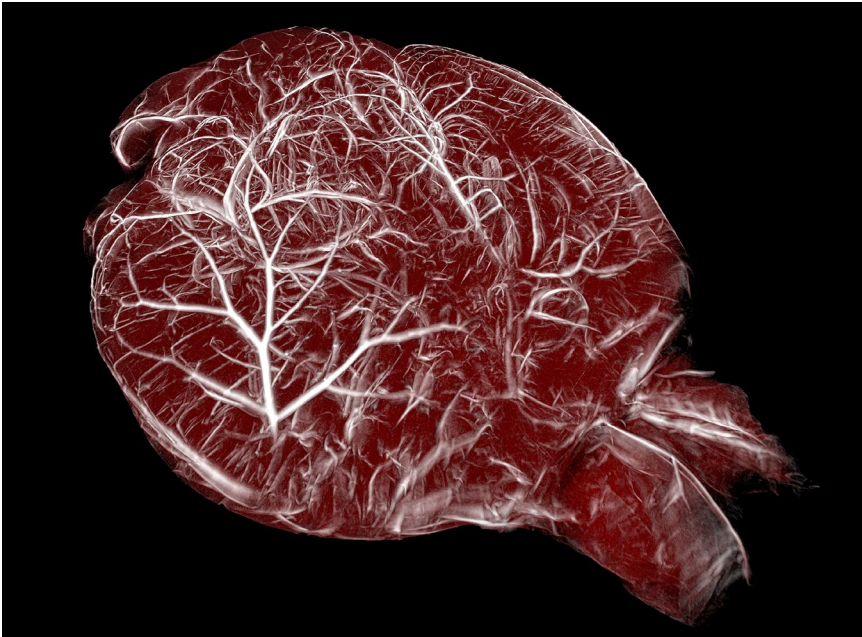
*Instructor,  
Department of  
Molecular Physiology  
and Biophysics*

**Mary E. Dickinson, PhD**

*Senior Faculty Advisor*

*Professor, Department of Molecular  
Physiology and Biophysics*

## CORE SUPPORTED RESEARCH



Adult mouse brain perfused with  $\text{MgSO}_4$  as a vascular contrast agent. 3D computed tomography collected by microCT shows vasculature (white) overlaid with surface reconstruction of intact brain (red). Unpublished data courtesy of Joshua D. Wythe, PhD, Baylor College of Medicine and Sean P. Marrelli, PhD, University of Texas Health Sciences Center.



Confocal image of *Pdgfra-Cre* expressed in cranial neural crest of an E10.5 mouse embryo. Unpublished data courtesy Annita Achilleos, PhD, Ross Poché Lab, Baylor College of Medicine.

# PATIENT-DERIVED XENOGRAPH AND ADVANCED *IN VIVO* MODELS CORE

The Patient-Derived Xenograft and Advanced *In Vivo* Models (PDX-AIM) Core of Baylor College of Medicine is divided into two independent but closely interacting functional units, a PDX Development Unit created to facilitate establishment and use of PDXs using immunocompromised mice as the host species, and an Advanced *In Vivo* Models Unit created to facilitate establishment and use of patient material grown on the chorioallantoic membrane (CAM) of the chicken egg. The Advanced *In Vivo* Models Unit also employs the CAM model to establish non-tumorigenic spheroids, 3-D tumors from existing cancer cell lines, and creation and optimization of custom bioassays to assess tumor neovascularization, invasion, and metastasis. Recent acquisition of the IVIS lumina series III instrument has facilitated a more precise and quantitative assessment of tumor growth in PDX/AIM models.

A primary focus of the core is to develop, and provide to the Baylor PDX community, computational and bioinformatics infrastructure to support large-scale generation, characterization, and use of PDX and CAM-PDX models for breast, head and neck, pediatric cancers, pancreas, brain, and other cancer types of interest. The core will also provide expertise in transplantation and animal handling to those wishing to generate PDX from various cancer types. Finally, the core will coordinate, and assist with, the evaluation of experimental therapeutics using the PDX and CAM-PDX *in vivo* platforms in conjunction with those investigators maintaining PDX collections for each organ/disease type.

All PDX work involving animals is conducted in dedicated housing and surgical suites in the Transgenic Mouse Facility of BCM, a fully AAALAC-accredited animal care and housing facility. Work is supported by the Center for Comparative Medicine (CCM), which administers the facility. CCM provides full veterinary care, administrative and regulatory oversight, and assistance with animal husbandry. All CAM-PDX work is conducted in dedicated space in the Neurosensory Tower.

## MAJOR EQUIPMENT

- IVIS Lumina III luminescence/fluorescence imager
- Tissue Cassette Labeler
- Computation and Bioinformatics Infrastructure for managing clinical and PDX associated data elements

## SERVICES

### MOUSE PDX:

- Development of computational/bioinformatics infrastructure to support PDX-based research
- Assist with, or facilitate, the generation of PDX models
- Facilitate *in vivo* treatment experiments with investigational drugs with PDX models or cell lines. Provide training for PDX related procedures upon request
- Coordinate provision of snap frozen tissue, viably frozen tissue, serum/plasma, and FFPE blocks/slides from PDX models from PDX program leads
- Coordinate provision of molecular derivatives of PDX models from PDX program leads
- Provide excess immunocompromised SCID/Bg mice from our breeding colony to BCM investigators

### CAM-PDX:

- Conversion of cancer cell lines into 3D vascularized tumors
- Establish Patient Derived Xenografts (PDX) on the chicken egg chorioallantoic membrane (CAM-PDX)
- Custom bioassays including angiogenesis, invasion, and metastasis
- Drug sensitivity screening on 3D vascularized tumors and PDX
- Assistance in end-point assays (Flow cytometry, DNA/RNA purification, IHC)
- Imaging of luciferase expressing tumor cells on CAM with the IVIS Lumina III instrument
- Investigator access to IVIS Lumina III instrument for *in vitro* and *ex vivo* experiment imaging

## MOUSE PDX MODELS AVAILABLE

- 53 Breast Cancer Models
- 8 Pancreatic Cancer Models
- 4 Glioblastoma Multiforme Cancer Models

## CORE LEADERSHIP



### Michael Lewis, PhD

*Academic Director*

*Mouse-based PDX Development Unit*

*Professor, Breast Center*

713.798.3296

mtlewis@bcm.edu



### Lacey Dobrolecki, MS

*Core Director*

*Mouse-based PDX Development Unit*

*Research Associate, Breast Center*

713.798.9024

dobrolec@bcm.edu



### Andrew Sikora, MD, PhD

*Academic Director*

*CAM-based PDX and Advanced In Vivo Models Unit*

*Associate Professor, Otolaryngology*

713.798.3909

Andrew.Sikora@bcm.edu



### Hugo Villanueva, PhD

*Co-Director*

*CAM-based PDX and Advanced In Vivo Models Unit*

713.798.8609

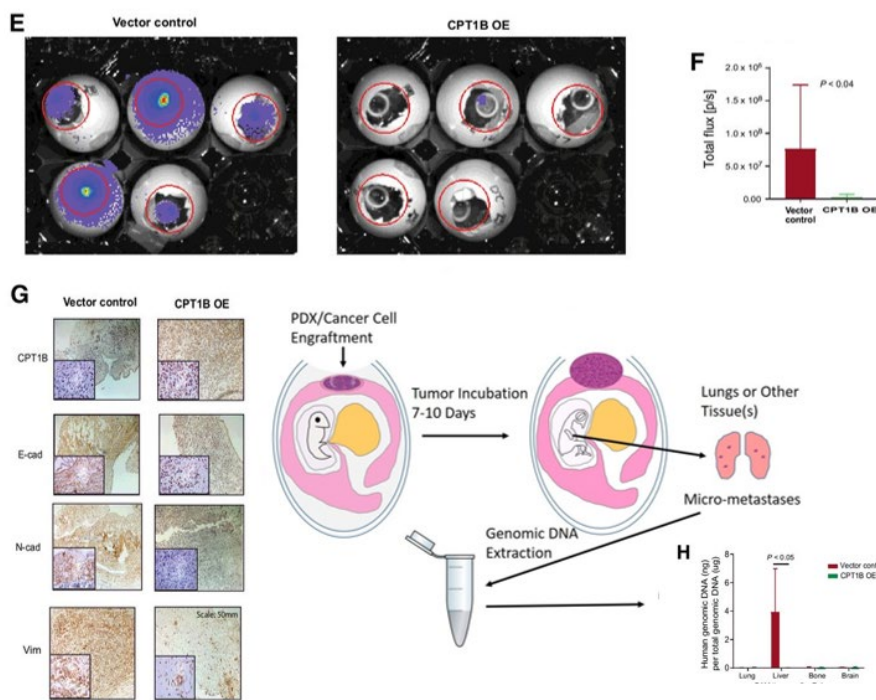
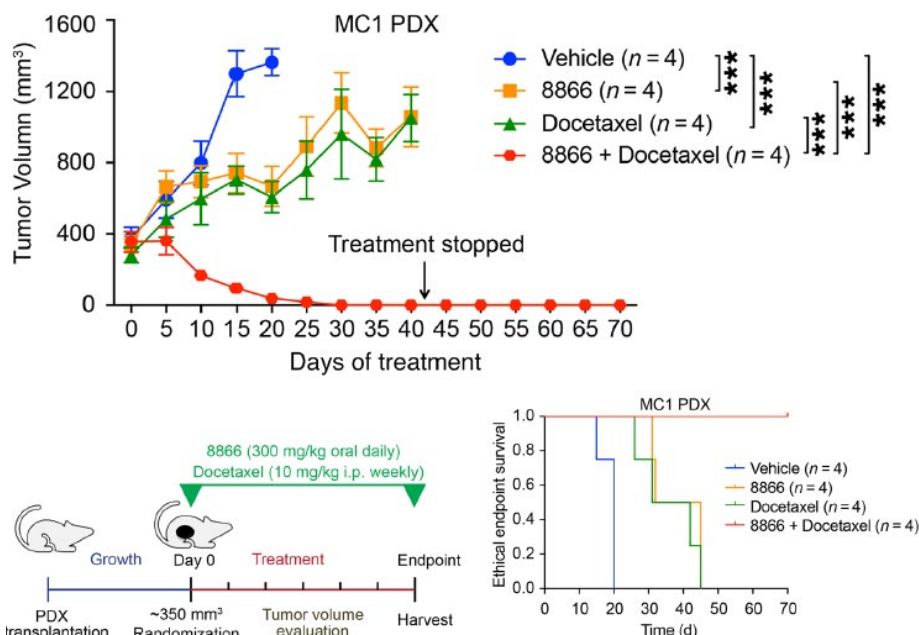
hugov@bcm.edu



## Pharmacological targeting of MYC-regulated IRE1/XBP1 pathway suppresses MYC-driven breast cancer

Zhao N, Cao J, Xu L, Tang Q, Dobrolecki LE, Lv X, M, Lu Y, Wang X, Hu DZ, Shi Q, Xiang Y, Wang Y, Liu X, Bu W, Jiang Y, Li M, Gong Y, Sun Z, Ying H, Yuan B, Lin X, Feng XH, Hartig SM, Li F, Shen H, Chen Y, Han L, Zeng Q, Patterson JB, Kaiparettu BA, Putluri N, Sicheri F, Rosen JM, Lewis MT, Chen X. *J Clin. Invest.* 2018, 128(4):1283-1299

**Figure 1.** 8866 enhances MYC-overexpressing PDX and GEM tumor response to docetaxel chemotherapy. Top: tumor growth curve after treatment. Bottom: treatment strategy and Kaplan-Meier survival plot.



## Multi-omics integration analysis robustly predicts high-grade patient survival and identifies CPT1B effect on fatty acid metabolism in bladder cancer

Vantaku V, Dong J, Ambati CR, Perera D, Donepudi SR, Amara CS, Putluri V, Ravi SS, Robertson MJ, Piyaathna DWB, Villanueva M, von Rundstedt FC, Karanam B, Ballester LY, Terris MK, Bollag RJ, Lerner SP, Apolo AB, Villanueva H, Lee M, Sikora AG, Lotan Y, Sreekumar A, Coarfa C, Putluri N. *Clin. Cancer Res.* 2019 Jun12;25(12):3689-3701

**Figure 2.** Effects of CPT1B in high grade bladder cancer cells on the CAM chick embryo model. Bioluminescence imaging. (E) and flux (F) of Vector and CPT1B overexpressing bladder cancer cells on CAM. IHC marker panel staining on CAM (G). qPCR analysis on chick embryo visceral tissue DNA to detect human alu repeats (H).

# POPULATION SCIENCES BIOREPOSITORY (PSB)

The Population Sciences Biorepository (PSB) serves as a resource for centralized cost-effective biospecimen processing and storage for epidemiological, translational, and clinical studies. The PSB also provides risk factor and clinical data collection. Services are available for individually funded investigators as well as for clinical centers that require prospective banking of specimens from patients for future research projects. The PSB team will consult with you to plan for data collection and specimen processing and storage needs for your projects. In addition, the PSB has a banked collection of annotated samples from a variety of cancer types that are available for individual investigator use. Contact the PSB to learn how to gain access to these important samples.

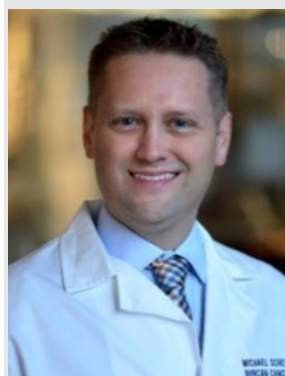
## MAJOR EQUIPMENT

- CryoBioSystem MAPI high-security straw system
- QIAcube robotic workstation
- Chemagic Prepito-D extraction system
- Perkin Elmer Janus automated workstation
- Nano-drop 1000
- MVE 1536P LN2 vapor freezers
- VWR -80°C mechanical freezers
- Thermo Scientific VisionMate scanner
- Thermo Scientific 8-channel decapper
- Barcode printers and scanner system

## SERVICES

- Patient consenting, phlebotomy, and data collection
- Questionnaire development and administration
- Full fractionation and aliquoting for blood and urine samples
- DNA extraction from whole blood, buffy coat, plasma, or saliva
- RNA extraction from whole blood or buffy coat
- DNA Quantitation (absorbance and pico-green fluorescence)
- Whole Genome Amplification
- Long-term specimen archival

## CORE LEADERSHIP



**Michael Scheurer, PhD**

*Academic Director*  
*Professor, Department of Pediatrics, Section of Hematology-Oncology*  
713.798.5547  
scheurer@bcm.edu

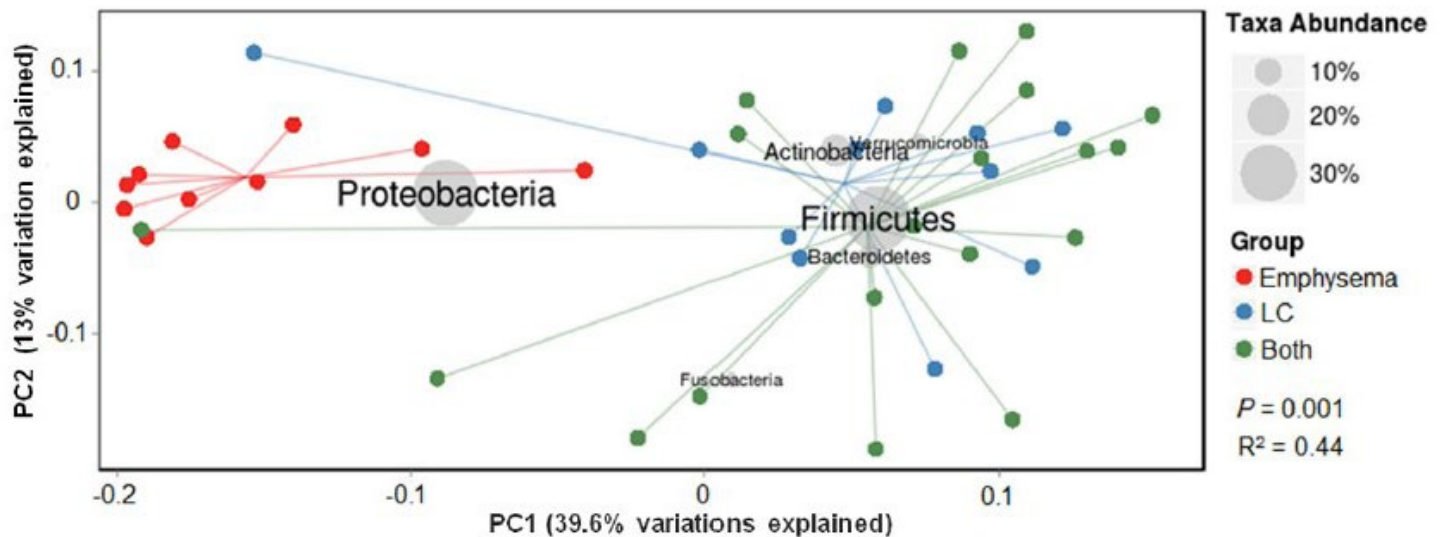


**Marlisa Hardy, DrPH, MPH**

*Co-Director*  
*Research Director, Advanced Technology Cores*  
713.798.3927  
mshardy@bcm.edu

## A genome-wide association study of LCH identifies a variant in SMAD6 associated with susceptibility.

Langerhans cell histiocytosis (LCH) is characterized by lesions with pathologic dendritic cells (DCs) harboring universal mutually exclusive somatic activating mutations in *MAPK* pathway genes. The role of inherited genetic variation in the pathogenesis of LCH is largely unknown. The first family-based genome-wide association study for LCH was conducted ( $n = 118$  case-parent trios/duos) and identified a single nucleotide polymorphism located on chromosome 15 in *SMAD6* associated with LCH risk that results in suppression of *SMAD6* protein expression. Replication and joint analysis in an independent set of cases ( $n = 132$ ) and controls ( $n = 1,645$ ) yielded a genome-wide significant association [summary odds ratio (OR) = 3.48; 95% confidence interval (CI): 2.26-5.36;  $P = 1.51 \times 10^{-8}$ ]. These results provide evidence for the influence of inherited genetic variation on LCH risk and highlight a probable pathogenic role for *SMAD6*.



**Figure:** Principal coordinate analysis of lung bacterial communities based on weighted Unifrac distances. Each symbol represents a sample. The proportion of variance explained by each principal component is denoted in the corresponding axis label. These two components explained 52.6.1% of the variance. The lung bacterial communities of the LC patients, with (green circles) or without (blue circles) emphysema, were clustered together, and distinctly separated from the emphysema-only patients (red circles). Both P values from the Kruskal-Wallis test (comparison among three groups) and the Wilcoxon rank-sum test (emphysema-only versus LC patients), were 0.001.

To describe and contrast lung tissue-associated microbial signatures in smokers with lung cancer and/or emphysema, Dr. Liu et al. employed pyrosequencing of 16S rRNA gene hypervariable V4 region and compositional analysis in non-malignant lung tissue samples obtained from 40 heavy smokers, including 10 with emphysema-only, 11 with lung cancer-only, and 19 with both lung cancer and emphysema. The emphysema-only group presented a lower bacterial community evenness defined by a significantly lower Shannon diversity index compared to the lung cancer patients with or without emphysema ( $P = 0.006$ ). Furthermore, community compositions of lung cancer patients with or without emphysema were characterized by a significantly lower abundance of Proteobacteria (primary the genera *Acinetobacter* and *Acidovorax*) and higher prevalence of Firmicutes (*Streptococcus*) and Bacteroidetes (*Prevotella*), compared to emphysema-only patients.

Liu Y, O'Brien JL, Ajami NJ, Scheurer ME, Amirian ES, Armstrong G, Tsavachidis S, Thrift AP, Jiao L, Wong MC, Smith DP, Spitz MR, Bondy ML, Petrosino JF, Kheradmand F. Lung tissue microbial profile in lung cancer is distinct from emphysema. *Am J Cancer Res.* 2018 Sep 1;8(9):1775-1787. PubMed PMID: 30323970; PubMed Central PMCID: PMC6176189.



# PROTEIN AND MONOCLONAL ANTIBODY PRODUCTION

The Protein and Monoclonal Antibody Production Core (PMAPC) provides investigators with high quality mouse monoclonal antibodies (MAbs) and purified recombinant proteins to facilitate their research programs. The Core has experience with intact proteins, synthetic peptides, and subcellular fractions as immunogens in generating MAbs that perform for a wide range of applications including, but not limited to immunoblotting, immunoprecipitation, ELISA, immunocytochemistry, and IHC. Expression and purification of recombinant proteins of interest is a parallel service that involves consultation on design and construction of expression vectors, protein overexpression in the baculovirus insect cell system, *E. coli* or mammalian cells, and purification of the expressed protein. Characterization of protein products for purity and other quality controls is provided to assure suitability for biochemical and structure analysis studies.

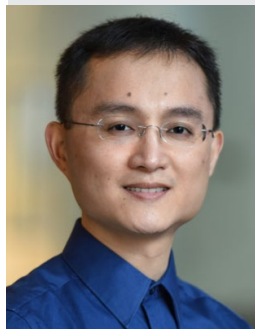
## MAJOR EQUIPMENT

- HAMILTON ClonaCell EasyPick for robotic hybridoma cell cloning
- GE Healthcare ÄKTA Pure FPLC systems for efficient purification of antibodies and recombinant proteins
- Nexcelom Cellometer Automated T4 Cell Counter
- FiberCell Hollow Fiber Bioreactors for mass production of monoclonal antibodies in culture
- Bioreactors for large scale insect and mammalian cell cultures
- Microfluidizer LM20 High Shear Fluid Processor
- Thermo MaxQ HP Incubated and refrigerated console shaker for multi-later scale bacterial

## SERVICES

- Generation of mouse monoclonal antibodies (MAbs) using standard hybridoma technology. Immunization of mice, cell fusion, screening, and cloning of hybridomas and cryopreservation of clones.
- Production & purification of monoclonal antibodies from existing hybridomas (up to gram-scale).
- Generation of recombinant baculovirus expression vectors for protein production in insect cells.
- Over-expression and purification of recombinant proteins in three systems.
  - » Insect cells using baculovirus vectors
  - » *E. coli*
  - » Mammalian cells (HEK293)
- Purification of recombinant proteins of interest from any of the three expression systems.
- Analysis and Q/C of purified proteins and MAbs.
- Consultation and project design for MAbs and recombinant protein production and purification.

## CORE LEADERSHIP



**Yingmin Zhu, PhD**

*Core Director (Protein Production and Purification)*

*Instructor, Department of Molecular & Cellular Biology*

713.798.5012

yingmin.zhu@bcm.edu



**Kurt Christensen, BS**

*Lab Director (MAb & Baculovirus Production)*

713.798.2325

krchrist@bcm.edu

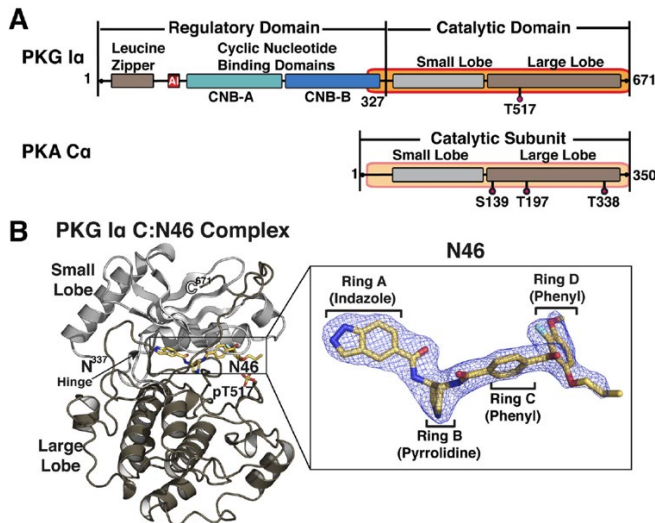
**Dean P. Edwards, PhD**

*Senior Faculty Advisor*

*Professor, Department of Molecular & Cellular Biology and Pathology & Immunology*

## Structural Basis for N46-Mediated Selective Inhibition of Human PKG I $\alpha$

The Core constructed a recombinant baculovirus for a fragment of PKG I $\alpha$  C-domain and performed its expression in high five insect cells with a yield of 10 mg of protein from a 5-liter bioreactor. Dr. Kim's lab was able to generate co-crystals with inhibitor N46 of core-provided protein to determine the structure at high resolution of 1.98 Å. N46 binds the active site of PKG I $\alpha$  with its external phenyl ring, specifically interacting with the glycine-rich loop and the C helix. This research provided a starting point for structure-guided design of selective PKG I $\alpha$  inhibitors.



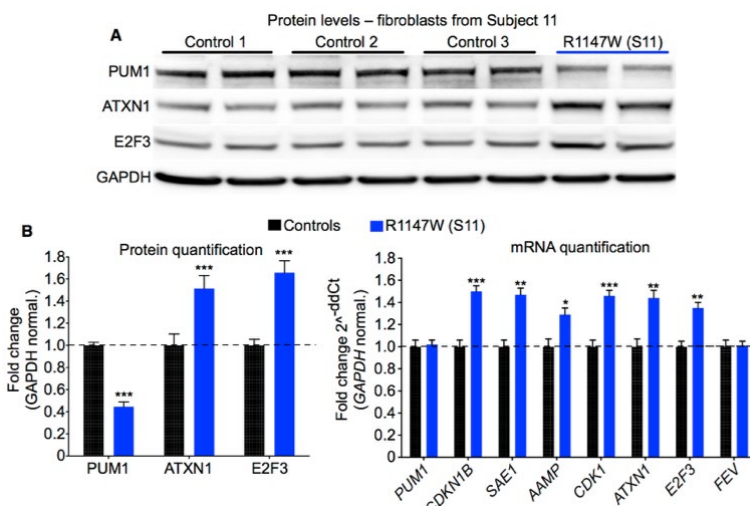
A) The domain organizations of PKG I $\alpha$ . The catalytic domains used for crystallization are shaded in orange and labeled with the corresponding residue numbers. B) overall structures of the PKG I $\alpha$ -N46 (C) complexes

Qin L, Sankaran B, Aminzai S, Casteel DE, Kim C. Structural basis for selective inhibition of human PKG I $\alpha$  by the balanol-like compound N46. *J Biol Chem.* 2018, 293:10985-10992.

## Missense Mutations Decrease Pum1 Stability and Increase Expression of Ataxin1 in Paddas Patient-Derived Cells.

An increase in expression of wild type ATXN1 in the cerebellum due to mutations and loss of a repressor, PUM1, can cause progressive neurological degeneration in mice that is reminiscent of spinocerebellar ataxia type 1 (SCA1). The core generated a mouse monoclonal antibody to Ataxin1 that was used in a study by Dr. Huda Y. Zoghbi to detect the Ataxin1 protein in fibroblasts and lymphoblastoid cells from patients with adult-onset PUM1-associated developmental disability, ataxia and seizure (PADDAS).

A) Representative immunoblots and B) quantification of protein levels of PUM1 and Ataxin1 in patient-derived fibroblast cells from a PADDAS subject and three age matched fibroblast control cell lines. PUM1 levels are 50-60% lower and Ataxin1 levels are 49-53% higher than in healthy controls.



Gennarino VA, Palmer EE, McDonell LM, Wang L, Adamski CJ, Koire A, See L, Chen CA, Schaaf CP, Rosenfeld JA, Panzer JA, Moog U, Hao S, Bye A, Kirk EP, Stankiewicz P, Breman AM, McBride A, Kandula T, Dubbs HA, Macintosh R, Cardamone M, Zhu Y, Ying K, Dias KR, Cho MT, Henderson LB, Baskin B, Morris P, Tao J, Cowley MJ, Dinger ME, Roscioli T, Caluseriu O, Suchowersky O, Sachdev RK, Lichtarge O, Tang J, Boycott KM, Holder JL Jr, Zoghbi HY23. A mild PUM1 Mutation is Associated with Adult-Onset Ataxia, whereas Haploinsufficiency Causes Developmental Delay and Seizures. *Cell* 2018, 172(5):924-936.

# RNA IN SITU HYBRIDIZATION

The Core performs non-radioactive RNA in situ hybridization (ISH) on tissue sections. A unique high-throughput technology developed by the Core (Yaylaoglu MB, Titmus A, Visel A, Alvarez- Bolado G, Thaller C, Eichele G. Dev Dyn. 2005 Oct;234(2):371-86) is used to determine gene expression patterns on sections, with an emphasis on tissues from rodent experimental models. The Core provides a full service that includes collection of rodent tissue specimens, preparation of frozen sections, preparation of RNA probes from customer templates, conducting high-throughput ISH and documentation and quantification of expression patterns by microscopy.

## MAJOR EQUIPMENT

- Tecan EVO Genepaint robot (for automated RNA in situ hybridization)
- Three cryostats (Leica)
- Autostainer (Leica)
- Automated coverslipper
- Zeiss Axio Scan.Z1 slide scanner (brightfield and fluorescence)

## SERVICES

- RNA in situ hybridization on tissue sections-brightfield or fluorescence development
- Tissue processing and embedding (frozen tissue)
- Sectioning (frozen tissue)
- Preparation of non-radioactive RNA in situ probes (DIG- or FITC-labeled)
- X-gal staining (sections)
- Imaging (slide scanner –automated mosaic images)
- Automated quantification of in situ hybridization signals, brightfield only (gene expression levels and cell counts)



## CORE LEADERSHIP



**Cecilia Ljungberg, PhD**

*Academic Director*

*Assistant Professor  
Department of Pediatrics  
– Neurology*

832.824.8873

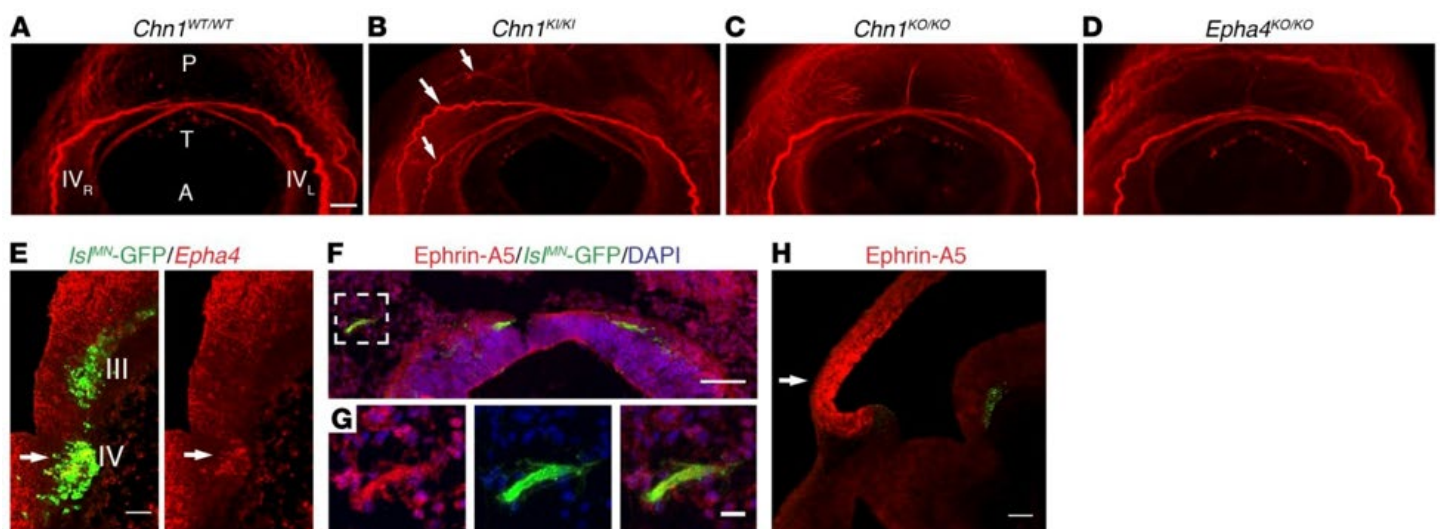
[cecilial@bcm.edu](mailto:cecilial@bcm.edu)



### *Chn1*<sup>KI/KI</sup> trochlear nerve branching abnormalities are unaltered by *Epha4*<sup>KO/KO</sup> allele. (A-D)

Transverse view of crossing trochlear nerves (IV) in E11.5 *Chn1*<sup>WT/WT</sup> (A; n = 4), *Chn1*<sup>KI/KI</sup> (B; n = 4), *Chn1*<sup>KO/KO</sup> (C; n = 4), and *Epha4*<sup>KO/KO</sup> (D; n = 4) embryos. P, posterior; A, anterior; T, tectum; IV<sub>R</sub> and IV<sub>L</sub>, right and left trochlear nerves; arrows, aberrant trochlear branches; red, neurofilament. Scale bars: 100  $\mu$ m. (E) *Epha4* expression in trochlear nucleus (arrow); red, *Epha4*; green, *Isl*<sup>MN</sup>-GFP; III, oculomotor; IV, trochlear nuclei. Scale bar: 50  $\mu$ m. (F and G) Ephrin-A5 protein in peripheral trochlear axons. Scale bar: 100  $\mu$ m. White box: enlargement in (G) with 20  $\mu$ m scale bar. Red, Ephrin-A5; green, *Isl*<sup>MN</sup>-GFP; blue, DAPI. (H) Ephrin-A5 (red) and *Hb9*-GFP (green) fluorescence ISH on sagittal E11.5 WT brainstem. Arrow, tectum.

The *Chr1*, *Epha4*, *Ephna5* and *EGFP* ISH in this study was performed by the RNA ISH Core.



Nugent AA, Park JG, Wei Y, Tenney AP, Gillette NM, DeLisle MM, Chan WM, Cheng L, Engle EC. Mutant  $\alpha$ 2-chimaerin signals via bidirectional ephrin pathways in Duane retraction syndrome. *J Clin Invest*. 2017, 127(5):1664-1682. doi: 10.1172/JCI88502. PMID: 28346224

# SINGLE CELL GENOMICS CORE

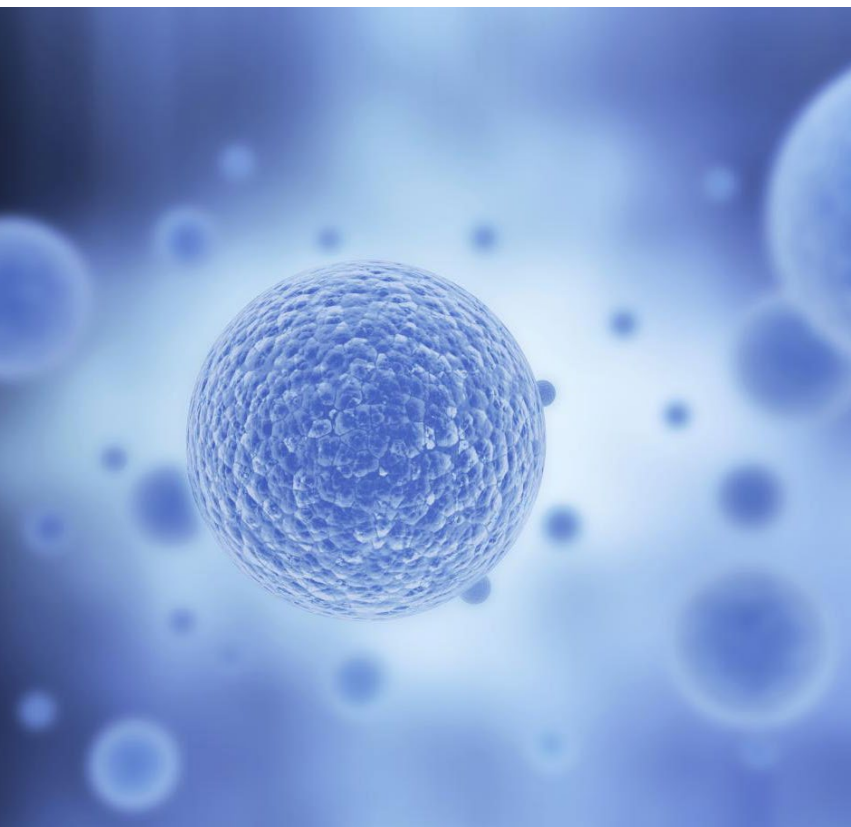
Single Cell Genomics Core (SCGC) provides services to conduct high throughput genome profiling, including DNA, RNA, and epigenetics profiling, on a single cell or a small number of cells.

## MAJOR EQUIPMENT

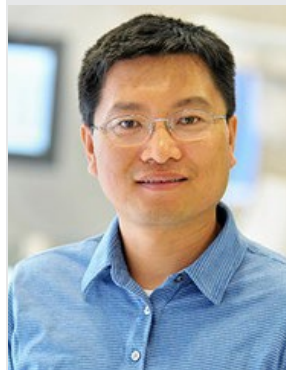
- **10x Genomics Chromium:** Droplet based system capable of profiling the transcriptome of up to 10,000 cells.
- **Takara iCELL8 system:** A MultiSample NanoDispenser (MSND) system that is capable of isolating up to 1,800 cells of mix types and sizes on each chip for RNA-Seq.
- **Fluidigm BioMark HD System:** A fully integrated real-time PCR system that enables analysis of gene expression, genotyping, mutant detection, and absolute quantification of nuclei acid sequences.
- **C1 Single Cell Auto Prep:** The C1 Single Cell Auto Prep enables the isolation and preparation of individual cells for single-cell gene expression analysis in a streamlined fashion.

## SERVICES

- **Single cell capture and 3' RNAseq:** Provide service for single cell capture and 3' RNAseq using chromium from 10x Genomics and ICELL8 from takara.
- **Single cell capture and V(D)J/5' RNAseq:** Provide service for single cell capture, VDJ profiling and 5' RNAseq using 10x Genomics.
- **Single cell capture and ATACseq:** Provide service for single nuclei capture and ATACseq using 10x Genomics.
- **Low input RNAseq:** RNAseq from 1-1000 cells or 10pg-10ng RNA using Takara SMART-seq v4 ultra low input RNA kit.
- **Low input bisulfite sequencing:** Methylation profiling for 20ng of genomic DNA using Tagmentation based protocol.



## CORE LEADERSHIP



**Rui Chen, PhD**

*Academic Director  
Professor, Department  
of Molecular & Human  
Genetics*

713-798-5194

ruichen@bcm.edu



**Yumei Li, PhD**

*Technical Core Director  
Assistant Professor,  
Department of Molecular  
& Human Genetics*

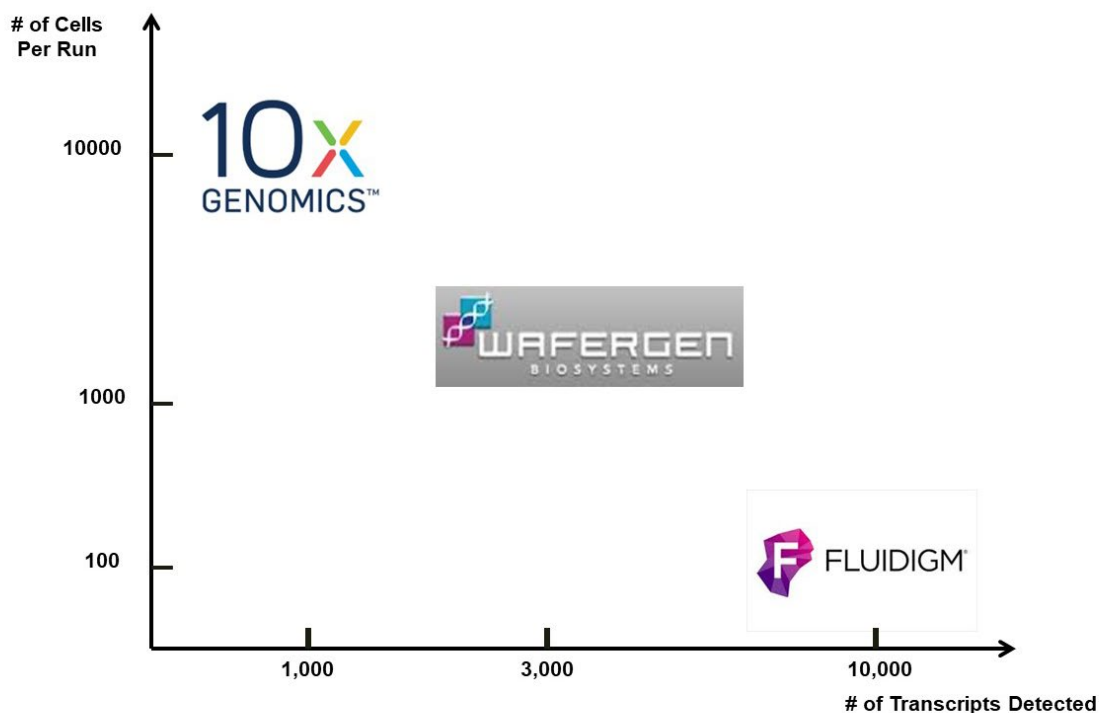
713.798.7565

yumeil@bcm.edu

## CORE SUPPORTED RESEARCH



tSNE plot based on single cell RNA-Seq using the Chromium system from 10x Genomics. Mouse retina is dissociated into single cell and transcriptome of individual cells. Cell subtypes can be readily classified by single cell RNA-Seq. Each color represents a different cell type.



The performance of three single cell platforms. The Chromium system from 10x Genomics can profile up to 10,000 cells with about 1000 transcripts detected per cell. In contrast, up to 10,000 transcripts can be detected by the Fluidigm system which can only profile several hundred cells per run. Finally, the iCELL8 system falls between these two platforms and profiles 1500 cells with 2500 transcripts per cell detected.



# SMALL ANIMAL MRI

The goal of this Core is to provide investigators with access to high quality *in vivo* and *in vitro* magnetic resonance imaging (MRI) for imaging and spectroscopy to accommodate their research projects. MRI offers unprecedented *in vivo* and longitudinal access to anatomical and physiological processes. Molecular imaging allows access to imaging some sub-cellular processes. Additionally, it is possible in many scenarios to image migrating labeled cells with MRI. Additionally, high resolution scans on the order of 20 microns can also be achieved in fixed samples ranging from fixed mouse embryos to white matter tractography in fixed pig brains. MRI is an outstanding resource for both *in vivo* and also *in vitro* phenotyping.

## MAJOR EQUIPMENT

- 9.4T, 20 cm bore, Bruker Biospin imaging system
- **\*\*NEW** We will be upgrading the hardware to AV NEO and Paravision 360 through our recently funded NIH S10 Shared Instrument grant.
- 1H volume and surface coil for rat, mouse, chicken and mouse embryos and fixed samples
- Heteronuclear coils are also available.
- Workstations for image processing.

## SERVICES

A state-of-the-art Bruker BioSpec® 9.4T horizontal bore MRI scanner is equipped to perform a wide variety of magnetic resonance imaging and spectroscopy studies on small animals (mice and rats) for non-invasive, high resolution longitudinal imaging for translational research. This elegant system allows for the following -- if it is not listed, we can work to incorporate additional features upon request.

- Pristine *in vivo* anatomical assessments
- Cerebral blood flow
- Diffusion tensor imaging (DTI) for white matter tractography
- Perfusion imaging
- <sup>31</sup>P spectroscopy of metabolites
- <sup>1</sup>H spectroscopy of metabolites
- MRI contrast agent assessments
- Diffusion imaging
- Amyloid beta plaque imaging
- Longitudinal tumor volume assessments
- Angiography
- Muscle imaging
- Magnetization transfer contrast (MTC) to assess white matter damage
- In Utero Imaging in Rats and Mice
- CEST Imaging — Chemical Exchange Saturation Transfer
- Cardiac Imaging — EDV, ESV and EF Assessments
- Cardiac Imaging — Anatomical Assessments
- Cardiac Imaging — Stress/Strain Calculations
- Cardiac Imaging — *In vivo* Ca<sup>2+</sup> influx changes in Myocardium
- Fat Assessment
- <sup>19</sup>F MRI — Imaging Inflammation
- <sup>19</sup>F MRI — Imaging tagged cells (e.g. lymphocytes)
- Dynamic Contrast Enhancement (BBB, tumor and placenta permeability)
- Resting state functional MRI in the rat and mouse brain



## CORE LEADERSHIP



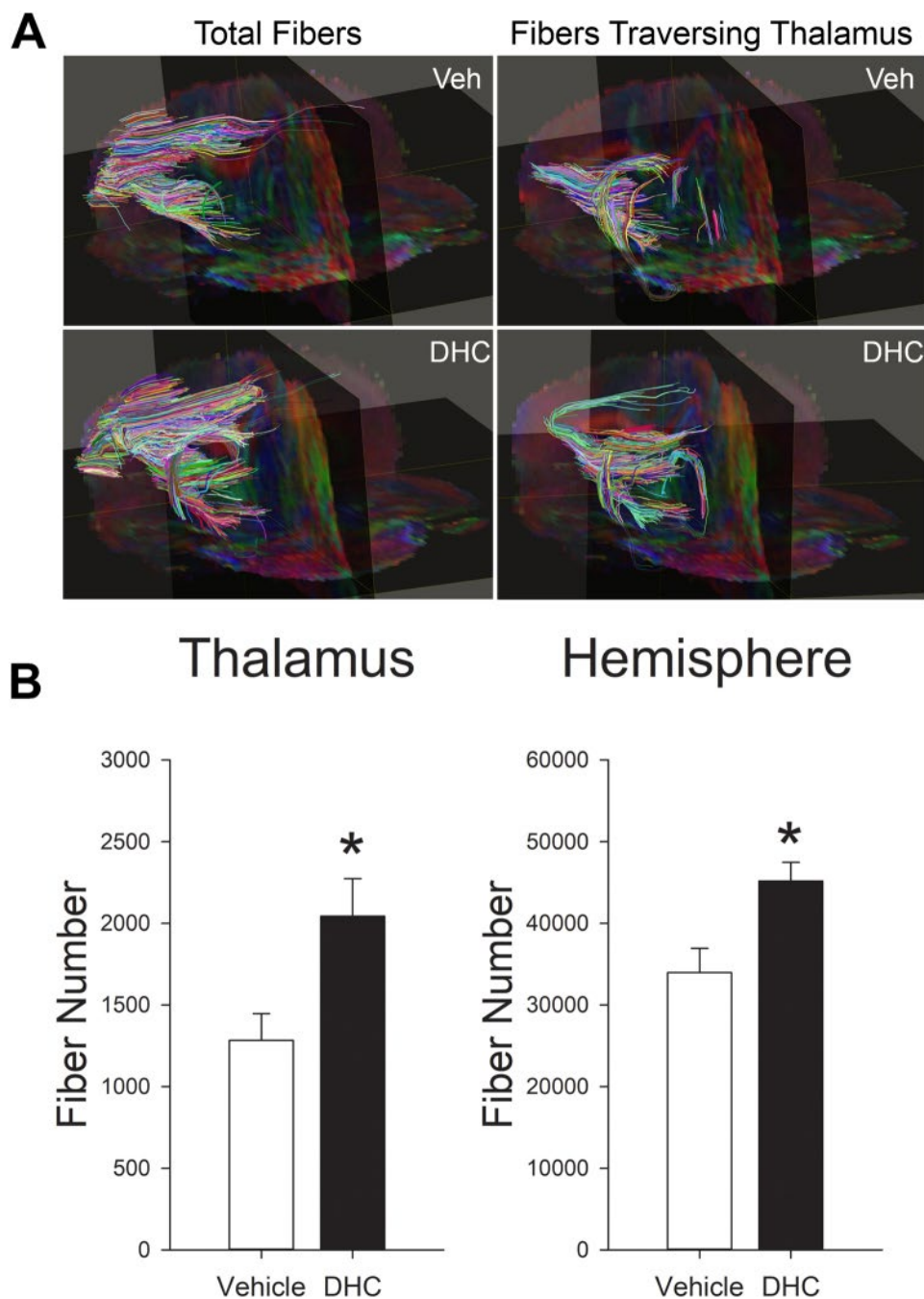
**Robia G. Pautler, PhD**

*Academic Director*

*Associate Professor,  
Department of Molecular  
Physiology and  
Biophysics*

713.798.3892

[rpautler@bcm.edu](mailto:rpautler@bcm.edu)



**Figure Legend:** DHC-induced hypothermia protects against fiber loss in ipsilateral hemisphere and thalamus after one month post-stroke. (A) Representative diffusion tensor imaging (DTI) fiber tract map of stroke injury hemispheres from vehicle treated and DHC treated mice at one month post-stroke. Left side panels reflect total fibers from the ipsilateral hemisphere. Right side panels reflect only those fibers traversing the injury region of the thalamus. (B) Quantitative analysis of fiber density in ipsilateral thalamic regions and whole brain hemisphere between Stroke/vehicle and Stroke/DHC groups (t test, \* $P < 0.05$ ,  $n = 4/\text{group}$ , data are expressed as mean  $\pm$  SEM).

Cao Z, Balasubramanian A, Pedersen SE, Romero J, Pautler RG, Marrelli SP. TRPV1-mediated pharmacological hypothermia promotes improved functional recovery following ischemic stroke. *Sci Rep.* 2017, 7(1):17685.



## CORE DIRECTORY

### Antibody-Based Proteomics

One Baylor Plaza, Houston, TX 77030  
Alkek Building for Biomedical Research, Room R507  
[shixiah@bcm.edu](mailto:shixiah@bcm.edu) | 713.798.8722

### BioEngineering Core

One Baylor Plaza, Houston, TX 77030  
Taub Research Building, Room T130  
[I-Chih.Tan@bcm.edu](mailto:I-Chih.Tan@bcm.edu) | 713-798-9168

### Biostatistics and Informatics

One Baylor Plaza, Houston, TX 77030  
Cullen Building, 3rd and 4th Floors  
[sgh@bcm.edu](mailto:sgh@bcm.edu) | 713-798-1632

### Core for Advanced MR Imaging (CAMRI)

One Baylor Plaza, Houston, TX 77030  
Smith Building, Room S104AF  
[krunge@bcm.edu](mailto:krunge@bcm.edu) | 713.798.3046

### Cell Based Assay Screening Services (C-BASS)

One Baylor Plaza, Houston, TX 77030  
Taub Research Building Room T328  
[c\\_bass@bcm.edu](mailto:c_bass@bcm.edu) | 713.798.8987

### CryoEM Core

One Baylor Plaza, Houston, TX 77030  
Alkek Building, Room 420  
[sludtke@bcm.edu](mailto:sludtke@bcm.edu) | 713-798-9020

### Cytometry and Cell Sorting

One Baylor Plaza, Houston, TX 77030  
Taub Tower of Main Campus, T103 & T105  
DeBaKey Building of Main Campus, M901.3  
[ccsc@bcm.edu](mailto:ccsc@bcm.edu) | 713.798.3868

### Genomic and RNA Profiling (GARP)

One Baylor Plaza, Houston, TX 77030  
Anderson Building, Lab 325E, Office 333E  
[microarray@bcm.edu](mailto:microarray@bcm.edu) | 713.798.7699

### Genetically Engineered Mouse (GEM)

One Baylor Plaza, Houston, TX 77030  
Margaret Alkek Biomedical Research Building, R223  
[lliao@bcm.edu](mailto:lliao@bcm.edu) | 713.798.4684

### Gene Vector

One Baylor Plaza, Houston, TX 77030  
Margaret Alkek Biomedical Research Building, R650  
[Shuyun.Deng@bcm.edu](mailto:Shuyun.Deng@bcm.edu) | 713.798.4409

### Human Tissue Acquisition and Pathology (HTAP)

One Baylor Plaza, Houston, TX 77030  
ABBR Histology Service, R536; Immunocytochemistry.R531D  
[PathologyCore@bcm.edu](mailto:PathologyCore@bcm.edu) | 713.798.6795

### Human Stem Cell (HSCC)

One Baylor Plaza, Houston, TX 77030  
Ben Taub Research Center, Suite T143  
[jean.kim@bcm.edu](mailto:jean.kim@bcm.edu)  
713.798.7471 Office | 713.798.8211 Lab

### Integrated Microscopy

One Baylor Plaza, Houston, TX 77030  
Cullen Building Room 123A  
[stossi@bcm.edu](mailto:stossi@bcm.edu) | 713.798.6940

### Mass Spectrometry Proteomics

One Baylor Plaza, Houston, TX 77030  
Jones Wing, Alkek Center for Molecular Discovery  
Rooms 108CB, 112C & 113C  
[malovann@bcm.edu](mailto:malovann@bcm.edu) | 713.798.8699

### Metabolomics

One Baylor Plaza, Houston, TX 77030  
Jones Wing, Alkek Center for Molecular Discovery  
Rooms 109C & 112C  
[metabolomicscore@bcm.edu](mailto:metabolomicscore@bcm.edu) | 713.798.3139

### MHC Tetramer

One Baylor Plaza, Houston, TX 77030  
Smith Building, Room S230  
[lxwang@bcm.edu](mailto:lxwang@bcm.edu) | 713.798.3918





#### Mouse Embryonic Stem Cells (ES)

One Baylor Plaza, Houston, TX 77030  
Alkek Building for Biomedical Research  
Room R.761

[heaney@bcm.edu](mailto:heaney@bcm.edu) | 713.798.1778

#### Mouse Metabolic & Phenotyping Core

One Baylor Plaza, Houston, TX 77030  
Taub Animal Facility, Room T001.T002

[clreynolds@bcm.edu](mailto:clreynolds@bcm.edu) | 713-798-5040

ABBR, Room R618

[psaha@bcm.edu](mailto:psaha@bcm.edu) | 713-798-3704 | 713.798.3704

#### NMR and Drug Metabolism

1250 Moursund Street, Houston, TX 77030  
Jan and Dan Duncan Neurological Research Institute  
NMR: Suite N.O220  
Drug Metabolism: Suite N.0600.01C

[kevin.mackenzie@bcm.edu](mailto:kevin.mackenzie@bcm.edu) | 713-798-3952

#### Optical Imaging and Vital Microscopy (OIVM)

One Baylor Plaza, Houston, TX 77030  
Cullen Building, Rooms 112A & 114A

[oivm@bcm.edu](mailto:oivm@bcm.edu) | 713.798.5040

#### Patient-Derived Xenograft Core

One Baylor Plaza, Houston, TX 77030  
Mouse-PDX — Albert B. Alkek Building, Room N1210

[dobrolec@bcm.edu](mailto:dobrolec@bcm.edu) | 713.798.9024

CAM-PDX — Neurosensory Building, Room NA516

[hugov@bcm.edu](mailto:hugov@bcm.edu) | 713-798-8609

#### Population Sciences Biorepository

Texas Children's Hospital  
1102 Bates Ave. Suite 1160  
Houston, Texas 77030

[Scheurer@bcm.edu](mailto:Scheurer@bcm.edu) | 713.798.5547

#### Protein & Monoclonal Antibody Production

One Baylor Plaza, Houston, TX 77030  
Alkek Building for Biomedical Research, Room R551E

[krchrist@bcm.edu](mailto:krchrist@bcm.edu) | 713.798.2325

#### RNA in Situ Hybridization

1250 Moursund Street, Houston, TX 77030  
Jan and Dan Duncan Neurological Research Institute  
Suite N1325

[cecilial@bcm.edu](mailto:cecilial@bcm.edu) | 832.824.8873

#### Single Cell Genomics

One Baylor Plaza, Houston, TX 77030  
Albert B. Alkek Building Room N1409

[ruichen@bcm.edu](mailto:ruichen@bcm.edu) | 713-798-5194

#### Small Animal MRI

One Baylor Plaza, Houston, TX 77030  
M.D. Anderson Hall Room 103G

[rpautler@bcm.edu](mailto:rpautler@bcm.edu) | 713-798-3892

### Advanced Technology Cores Administration Offices

#### Dean P. Edwards, PhD

One Baylor Plaza, Houston, TX 77030  
Alkek Building for Biomedical Research, Room R505

[deane@bcm.edu](mailto:deane@bcm.edu) | 713.798.2326

#### Jennifer McCullough, M.B.A.

One Baylor Plaza, Houston, TX 77030  
Alkek Building for Biomedical Research, Room R510

[jmmccull@bcm.edu](mailto:jmmccull@bcm.edu) | 713.798.6992

WWW.BCM.EDU/CORELABS • ATC@BCM.EDU • 713.798.6992

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College of  
Medicine

ADVANCED  
TECHNOLOGY  
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