Department of Cellular and Integrative Physiology
Experimental Expertise

Listed by Investigator

Updated 6/15/05

David Basile

1. Small animal surgery (rats and mice): induction of acute renal failure by ischemia, blood pressure measurements using femoral artery catheters, blood flow using ultrasonic Doppler probes; intravenous delivery of compounds; chronic instrumentation of blood pressure (telemetry); chronic instrumentation for repeated intravenous delivery of drugs or peptides.

2. Immunohistochemistry and in situ hybridization using paraffin or frozen sections in tissue samples; double labeling using chromogenic or fluorescence labeling techniques. In vivo labeling of blood vessels; microvascular casting and analysis.

3. Morphometric analysis of stained structures using Metamorph software; used to evaluate cell proliferation, blood vessel density tissue fibrosis etc.

4. Basic biochemical and molecular biology techniques; RNA isolation from tissues, Western blot analysis, real-time PCR, also ribonuclease protection assays and generation of templates for anti-sense generation of riboprobes.

5. Isolation of mitochondria from tissues; functional respiration analysis of mitochondria using oxygen sensitive electrodes.

6. Basic renal physiology measurements including creatinine assays, and evaluation of proteinuria.

Glenn Bohlen — **Regulation of the microscopic blood vessels in normal and diabetic conditions.**

1. Rat and mouse anesthesia, surgery, and vital sign monitoring such as arterial blood pressure, blood gases without having to withdraw blood, and mechanical ventilation to improve the physiological status of the animal.

2. Morphological image analysis techniques. Using Metamorph Software, be able to find objects in an image and quantitate their number, size, orientation, and shape characteristics.

3. Oxygen tension measurements with microelectrodes. Any thing from a single cell to a group of cells in an in vivo organ can be used for oxygen tension measurements. The changes in oxygen tension with a constant supply of oxygen reflect changes in aerobic metabolism. In in vivo preparations, measurements of blood flow coupled with oxygen

tension measurements allow studies of physiological regulation both of oxygen and control systems that influence oxygen availability to cells. The data are given in real time, with precise spatial capability and very good resolution.

4. Nitric oxide measurements with microelectrodes. Any thing from a single cell to a group of cells in an in vivo organ can be used for Nitric Oxide concentration measurements. The changes in NO concentration reflect increased or decreased production of NO or alterations in destruction of NO.

5. Blood flow measurements in organs using Doppler ultrasound measurements that require a flow probe be placed on an artery entering the organ. Vessels comparable in size to a 25 guage needle can be used for the flow measurements. The measurement is not absolute, but does allow accurate relative changes to be measured during an experiment.

6. Blood flow measurements in single microvessels with optical Doppler systems. This can provide accurate flow and flow velocity for microvessels in living tissues.

7. Microiontophoretic application of chemicals or drugs. Any charged molecule than can be dissolved in distilled water can be applied to single cells under very controlled conditions. The technique is best used for conditions where relative changes in response to increasing amounts of the chemical are useful.

8. Microinjection techniques. Pressurized application of known volumes or flows of a chemical dissolved in a liquid vehicle to individual cells or groups of cells. This allows very precise amounts of fluid with known concentrations of a given substance to be applied cells or tissues.

9. In vivo microscopy of small intestine, various skeletal muscles, the liver, the cerebral cortex and mesentery. Observations of any sized vessels in small mammals (mice, rats, rabbits, etc) are fairly straightforward and allow changes in vascular structure and function to be evaluated in real time.

10. Per cent saturation of hemoglobin measurements in microvessels. This is an optical technique for measurement of the oxygen content of blood in small blood vessels. It provides evidence of oxygen delivery and use changes in an in vivo environment.

Matthias Clauss **Angiogenic factors and endothelial activation**

1. General techniques:
    a. Monoclonal antibody production and purification.
    b. Cloning, expression and purification of recombinant proteins in yeast (Pichia pastoris).
    c. Receptor expression and binding studies.
    d. Site directed mutagenesis of receptor tyrosine kinases for linking signal transduction pathway analysis with growth factor function**.**

2. Cell biology and tissue culture:
    a. Isolation and cultivation of endothelial cells from different organs.
    b. Directed migration of monocytes, vascular endothelial and progenitor cells to test repair mechanism *in vitro*.
    c. Assessment of barrier function of endothelial cell monolayer as an *in vitro* model for vascular permeability.
    d. Proliferation, survival, and differentiation of progenitor and endothelial cells.
    e. In vitro assays for sprouting angiogenesis.

3. Signal transduction:
    a. Determination of MAP kinase activation by Western blot analysis and immunohistochemistry using phosphospecific antibodies.
    b. Online measurements of intracellular signal transduction pathways (such as Calcium mobilization and reactive oxygen species generation) by using fluorescent reagents and the Flex Station.

4. Techniques *in vivo*:
    a. Analysis of angiogenic factors by using the Chicken choreoallantois membrane assay in the fertilized chicken egg.
    b. Analysis of angiogenic mechanisms with the Matrigel plug assay and assessment of tumor angiogenesis by determination of vessel density using immunohistochemistry (in the mouse).
    c. Induction of vascular injury and ischemia in the mouse.
    d. Determination of vascular leakage in skin and brain by using fluorochromic compounds.

5. Transgenic animals:
    a. Construction and analysis of transgenic animals expressing tissue specific and inducible genes.

Jeffrey Elmendorf **Molecular mechanisms by which insulin regulates glucose transport in muscle and fat cells**

1. Adipocyte culture
    a. Culture and differentiation of 3T3-L1 adipocyte cells.
    b. Isolate primary fat cells from fat pads.
    c. Growth of mammalian cell lines including, 3T3-L1, C2C12, and L6 cells.

2. Protein Compartmentalization
    a. Examine subcellular localization of cellular molecules using subcellular fractionation.

b. Display cellular compartmentalization using co-labeling confocal imaging techniques.
c. Quantitatively determine amount of protein in the endosomal recycling compartment using an endosomal ablation technique.
d. Nuclear localization of proteins and transcription factors such as SREBP using subcellular fractionation and nuclear extract preparation techniques.

3. Protein-Protein Interactions
   a. Display potential protein-protein interactions using co-labeling confocal imaging techniques.
   b. Identify direct protein-protein interactions using co-immunoprecipitation methods.
   c. Determine globular actin polymerization/depolymerization using biochemical and microscopy techniques.

4. Membrane Lipid Analyses
   a. Add and remove plasma membrane cholesterol from cells using methyl-b-cyclodextrin.
   b. Examine phosphoinositide (such as phosphatidylinositol 4,5-bisphosphate; PIP_2, and phosphatidylinositol 3,4,5-trisphosphate; PIP_3) changes in the cell or isolated membrane using immunofluorescence and enhanced green fluorescent protein tags displaying high/specific affinity for PIP_2 and PIP_3.
   c. Measure amount of membrane lipids (in particular plasma membrane sphingolipids and cholesterol) using thin layer chromatography and cholesterol oxidase assays.
   d. Prepare highly purified plasma membrane fragments for immunofluorescent analysis of 3T3-L1 adipocyte membrane proteins.
   e. Visualize caveolar lipid raft domains using immunofluorescence microscopy.

5. Protein Activity Assays
   a. Assay the activities of membrane transport proteins (in particular glucose transporters).
   b. Measure protein kinase activity directly using immunoprecipitation and *in vitro* activity assays, and indirectly by determining protein modification associated with activation.

Patricia Gallagher **Cytoskeletal regulation of cell death.**

1. General cDNA cloning.
   a. Isolation of cDNA encoding your favorite protein and generation of expression vectors.
   b. Alteration of proteins by mutagenesis of expression vectors.
   c. Expression of proteins in mammalian cells in culture.
   d. Generation and use of adenovirus to express proteins in mammalian cells and tissues.
   e. Express and purify recombinant proteins from bacteria.

2. Cell Culture techniques.
   a. Growth of mammalian cell lines including MDCK, COS, HEK 3T3

3. Protein trafficking Studies
    a. Biotin labeling of cell surface proteins

4. Apoptosis analysis
    a. DNA fragmentation using FACs analysis
    b. Caspase activity assays

5. Protein expression analysis.
    a. Identify and quantify expression of specific proteins (in particular smooth muscle proteins) in cells and tissues by western blotting.
    b. Characterize the cellular or intracellular distribution of proteins by immunofluorescence microscopy.
    c. Determine the cellular or tissue localization of mRNAs by Northern blotting, RNase protection assays or in situ hybridization.
    d. Knock-down expression of your favorite protein using plasmid and adenoviral based siRNA.

6. Analysis of Protein-Protein interactions.
    a. Identifying protein-protein interactions using yeast two-hybrid screens.
    b. Characterize protein-protein interactions in mammalian cells using mammalian two-hybrid assays or FRET analysis.
    c. Identify direct protein–protein interactions using GST-pull down assays.

7. Protein kinase analysis
    a. Kinase activity assays in vitro

Susan Gunst **Regulation of smooth muscle contractility by the cytoskeleton.**

1. Tissue transfection using plasmids and expression of recombinant proteins in intact smooth muscle tissues in vitro – techniques may be applicable to other tissue types.
    a. "Reversible permeabilization" or "chemical loading" technique for introducing cDNA plasmids, purified proteins, antisense, or siRNA into intact tissues at high efficiency without altering physiologic properties.
    b. Organ culture techniques for muscle tissues
    c. Analysis of expression of recombinant proteins in tissues

2. Analysis of physiologic functions of smooth muscle tissues.
    a. mechanical properties of muscle including contractile force, shortening velocity, and stiffness.
    b. Quantitative measurement of changes in intracellular Ca^{2+} in vivo in muscle tissues during contraction and relaxation using fura or other fluorescent probes.
    c. Myosin ATPase activity in muscle tissues in vivo during contraction and relaxation using NADH-coupled fluorescence assay.
    d. Permeabilization of muscle tissues using α-toxin, β-escin or Triton and evaluation of contraction in permeabilized tissues with controlled intracellular environment.
    e. Use of PTI Fluorescence Spectrofluorimeter

3. Enzymatic dissociation of smooth muscle tissues, studies of freshly dissociated cells, primary culture of smooth muscle cells.

4. Evaluation of effects of physiologic stimuli on protein localization in cells and tissues.
    a. Use of immunofluorescence and confocal imaging to view fluorescently labeled proteins in smooth muscle cells.
    b. Digital Image analysis and deconvolution analysis to analyze protein localization in primary cells and tissues.
    c. Imaging of living cells and real time visualization of protein localization.

5. Knockdown of protein expression in intact tissues using antisense or siRNA.
    a. Introduction of antisense into tissues and protein depletion
    b. Analysis of knockdown on protein expression and muscle function

6. Measurement of myosin light chain phosphorylation using urea gel electrophoresis and Western blotting

7. Assay of G and F actin content and actin polymerization in tissues and cells by fractionation techniques or DNAse assay.

8. Biochemical approaches for studying effects of physiologic stimuli on protein phosphorylation, cell localization, and protein associations, protein kinase activation.
    a. Western blotting
    b. Immunoprecipitation and co-immunoprecipitation
    c. Cell fractionation
    d. Protein Kinase activity assays

9. Physiological assessments of properties and responses of isolated airways.

Paul Herring **Regulation of smooth muscle development.**

1. General cDNA cloning.
    a. Isolation of cDNA encoding your favorite protein and generation of expression vectors.
    b. Alteration of proteins by mutagenesis of expression vectors.
    c. Expression of proteins in mammalian cells in culture.
    d. Generation and use of adenovirus to express proteins in mammalian cells and tissues.
    e. Express and purify recombinant proteins from bacteria.

2. Cell Culture techniques.
    a. Growth of mammalian cell lines including, A10 (VSM), 10T1/2(embryonic fibroblast), COS, HEK. Also available but not routinely growing: A7r5(VSM), MDCK (kidney), C2C12 (skeletal muscle), L6(skeletal muscle), Sol8 (slow skeletal muscle), 3T3, LI (colonic SMC).
    b. Primary cultures of smooth muscle cells from mice, or pigs.

c. Culture and in vitro differentiation of mouse embryonic stem cells.

3. Gene Regulation Studies
4. Analyzing the function of putative gene regulatory regions using reporter gene assays in vitro in cultured cells or in vivo in transgenic mice.
5. Analyze expression of β-galactosidase or GFP reporter genes in organs and cells.
6. Investigating the ability of proteins to bind to DNA both in vitro (using gel mobility shift assays) and in vivo (using chromatin immunoprecipitation assays).
7. Identify proteins that bind to specific genetic elements by yeast one hybrid and lambda southwestern screens.
8. Examine chromatin structure using chromatin IP assays.
9. Generate single copy transgenes that are targeted to a specific locus (HPRT)

10. Protein expression analysis.
    a. Identify and quantify expression of specific proteins (in particular smooth muscle proteins) in cells and tissues by western blotting.
    b. Characterize the cellular or intracellular distribution of proteins by immunofluorescence microscopy.
    c. Determine the cellular or tissue localization of mRNAs by Northern blotting, RNase protection assays or in situ hybridization.
    d. Knock-down expression of your favorite protein using plasmid and adenoviral based siRNA.

11. Analysis of Protein-Protein interactions.
    a. Identifying protein-protein interactions using yeast two-hybrid screens.
    b. Characterize protein-protein interactions in mammalian cells using mammalian two-hybrid assays or FRET analysis.
    c. Identify direct protein–protein interactions using GST-pull down assays.

12. Useful reagents/techniques available.
    a. Express proteins *in vivo* in mice specifically in smooth muscle cells.
    b. Mice that express GFP specifically in smooth muscle cells (primarily visceral).

Chiu Shuen Hui **Mechanisms of excitation-contraction coupling in skeletal muscle.**

1. Voltage-clamp of skeletal muscle fibers:
   This technique is used to control membrane potential and to measure electrical current through the membrane and/or intracellular calcium release
   Voltage clamp accomplished with microelectrodes
   Voltage clamp accomplished with Vaseline seals

2. Global calcium transient in skeletal muscle fibers:
   Calcium release is monitored by loading the fibers with absorbance dyes
   The global changes in absorbance resulted from calcium binding are recorded with a group of photodetectors which serve collectively as a photometer

3. Local calcium transient in skeletal muscle fibers:

Calcium release is monitored by loading the fibers with fluorescence dyes
The localized changes in fluorescence resulted from calcium binding are recorded with a laser scanning confocal microscope which provides sub-micron resolution

Steve Kempson **Kidney epithelial cell membrane transport.**

Subah Packer **Smooth muscle mechanics and biochemistry.**

1. Functional studies of contractility and relaxation and of compliance/stiffness in both isometric and isotonic modes with force transducers, an electromagnetic lever system in intact or permeabilized muscle strips or with a video dimension analyzing system for studies in isolated resistance vessels or airways.

2. Pharmacological studies of stimulation and of inhibition of contraction/relaxation.

3. Simultaneous measurements of changes in intracellular free calcium and/or pH using fluorescent indicator dyes and of contraction/relaxation.

4. Gel electrophoresis and western blot technique for investigating shifts in contractile protein isoforms and for studying regulatory proteins and kinase cascades (e.g. MAPK, PKC, MLCK) in signal transduction pathways associated with contraction/relaxation including time course studies of regulatory protein activity.

5. Tail cuff plethysmography of rodents for blood pressure studies.

6. Assays for oxidation of low density lipoprotein cholesterol.

7. Science/Medical Education
a. OSCE case writing/evaluation
b. Modeling of inquiry-based learning and equity in the classroom
c. Incorporation of health care communication into problem-based learning in medical school

Fredrick Pavalko **Mechanisms of signal transduction through cell adhesion molecules and the cytoskeleton in regulation of cellular function.**

1. Bone and blood cell isolation and tissue culture:

   a. Isolation of osteoblasts from rats and mice.
   b. Culture of primary and immortalized bone cells.
   c. Isolation of blood cells including leukocytes and platelets.

2. Application of fluid shear stress to cultured cells:

   a. Application of tissue culture cells to fluid shear stress using custom apparatus designed to apply either unidirectional or oscillatory fluid flow.

b. Application of fluid shear stress to cells in biological or artificial blood vessels.

3. Analysis of cells:

a. Fluorescence microscopy, digital imaging of cytoskeletal proteins.
b. Cell adhesion assays.
c. Immunoprecipitation and co-immunoprecipitation of cellular proteins.
d. Labeling of cell surface proteins and analysis by Western blotting.

4. Cell biology techniques:

a. Microinjection of single cells.
b. Enzyme linked assays for detection of prostaglandins.
c. Detection and analysis of cellular apoptosis markers.
d. Adenoviral protein expression in cultured cells.
e. Analysis of protein interactions using GST-pull down assays.

Daniel Peavy Medical and graduate education

Simon Rhodes **Transcriptional regulation of pituitary development.**

1. General cDNA and genomic cloning.
    a. Isolation of cDNA or gene encoding your favorite protein and generation of expression vectors by library screening.
    b. Alteration of proteins by mutagenesis of expression vectors (to study e.g. nuclear localization signal, phosphorylation sites, & other functional domains/sequences).
    c. Expression of proteins in mammalian cells in culture.
    d. Express and purify recombinant proteins from bacteria.

2. General molecular biology and gene mapping/analysis.
    a. Gene structure analysis - Southern blots, PCR.
    b. Gene expression analysis - Northern blots, real time quantitative PCR, RNAse protection, standard RT-PCR.
    c. DNA-binding protein analysis - Southwestern blots.
    d. Mapping of human, murine, porcine, bovine, ovine genes.
    e. Screening of patients for genomic mutations associated with diseases.

3. Cell Culture techniques.
    a. Growth of mammalian cell lines including HEK293, HEK293T, LbetaT2 (gonadotrope). Also available but not routinely growing: P19, alphaT3, GC, GH3, GH4, CV-1, HeLa.

4. Gene Regulation Studies
    a. Analyzing the function of putative gene regulatory regions using reporter gene assays in vitro in cultured cells or in vivo in transgenic mice.
    b. Analyze expression of beta-galactosidase, luciferase, or GFP reporter genes in organs and cells.

c. Investigating the ability of proteins to bind to DNA both in vitro (using gel mobility shift assays, DNAse footprinting, Southwesterns) and in vivo (using chromatin immunoprecipitation assays).
d. Identify proteins that bind to specific genetic elements by lambda southwestern expression screens.
e. Examine chromatin structure using chromatin IP assays.
f. Determination of transcription factor binding sites by site selection ("SAAB assays' or "casting").

5. Protein expression analysis.
   a. Identify and quantify expression of specific proteins in cells and tissues by western blotting.
   b. Characterize the cellular or intracellular distribution of proteins by immunocytochemistry (immunofluorescence, GFP/YFP/CFP fusions, and DAB) microscopy.
   c. Determine the cellular or tissue localization of mRNAs by Northern blotting, RNase protection assays or in situ hybridization.

6. Analysis of Protein-Protein interactions.
   a. Identifying protein-protein interactions using yeast two-hybrid screens.
   b. Characterize protein-protein interactions in mammalian cells using mammalian two-hybrid assays.
   c. Identify direct protein–protein interactions using GST-pull down assays and His-tag pull-down assays.

7. Overexpression of proteins in transgenic mice
   a. Target expression to specific tissues or at specific times
   b. Analysis of the resulting physiology
      i. Hormone assays
      ii. Growth and development parameters
      iii. Reproductive physiology

Michael Sturek **Ion transport in vascular smooth muscle and endothelium; in vivo diabetes, hyperlipidemia, exercise training.**

1. In vivo physiology of conscious animals (mainly miniature swine)
   a. intravenous glucose tolerance tests to determine insulin secretion by the pancreas in response to glucose injection
   b. insulin sensitivity tests to more directly determine the effect of injected insulin on ability of skeletal muscle to take up glucose
   c. post-prandial (post feeding) determination of hormone, glucose, and lipid responses
   d. treadmill exercise testing and training of miniature swine
   e. selective breeding of miniature swine to derive optimal features of the metabolic syndrome that leads to type 2 diabetes
   f. electrocardiography, with emphasis on determination of diabetic autonomic neuropathy
   g. induction of insulin-dependent diabetes in swine using beta cell toxins
   h. non-invasive measurement of blood pressure
   i. percutaneous vascular access for acute and short term blood sampling or drug injection

j. surgical implantation of vascular access ports for long-term blood sampling or drug injection
k. low-stress restraint of swine using a specialized sling
l. non-invasive transcutaneous ultrasound measurement of vascular diameter, structure, and blood flow velocity in peripheral vessels
m. non-invasive echocardiographic measures of cardiac dimensions, muscle function, and blood ejection
n. virtually any acute or chronic diet manipulation to induce, e.g. hyperlipidemia, nutrient therapy or deprivation, etc.
o. pharmacokinetic studies of drug absorption into the blood from the route of administration
p. acute and chronic drug therapy studies
q. imaging studies involving magnetic resonance, computerized tomography, and positron emission tomography to determine, e.g. fat distribution (in collaboration with the department of radiology)
r. tissue biopsy on conscious swine for subsequent biochemical analysis

2. In vivo physiology of anesthetized animals (mainly miniature swine). Our main emphasis is on heart and coronary vascular function and structure, but virtually any blood vessel can be monitored.

a. coronary blood flow velocity measures using intravascular Doppler flow wires
b. measurement of coronary conduit artery function and atherosclerosis using intravascular ultrasound
c. direct cardiac catheterization
d. angiographic visualization of vascular structure and function, e.g. coronary angiography
e. direct measurement of arterial, atrial, and ventricular blood pressure
f. measurement of coronary artery pressure and flow velocity to diagnose flow-limiting coronary atherosclerosis or restenosis
g. coronary angioplasty and stent placement
h. measurement of effects of drug-eluting stents and effects of other drugs and exercise therapy on coronary or other vascular disease
i. effects of different types of anesthesia on physiological responses

3. In vitro vascular reactivity – measurement of contraction and relaxation of isolated blood vessel segments in response to vasoactive hormones and drugs

4. Analysis of histological sections
   a. quantitative digital imaging of standard histological stains provides highly sensitive measurement of vascular and cardiac disease progression and effects of drug and exercise therapy
   b. immunohistochemical determination of molecular expression of ion transporters, e.g. sarcoplasmic reticulum calcium pump provides information of tissue level distribution
   c. DNA synthesis rates are determine by bromodeoxyuridine uptake by cells and incorporation into DNA

5. Organ culture techniques

a. blood vessel segments can be maintained in typical incubator conditions to preserve the differentiated function of the native blood vessel freshly harvested from the animal or
b. mimicry of atherosclerosis can be elicited by long-term maintenance in organ culture or after exposure to lipids, high glucose concentrations, or hormones present in vascular disease
c. phenotype of cells are monitored by marker proteins such as smooth muscle alpha actin, smooth muscle myosin heavy chain, smoothelin, desmin
d. protein and DNA synthesis rates are measured
e. gene expression is assessed with real-time (quantitative) polymerase chain reaction (PCR)

6. Cell culture techniques
   a. endothelial or smooth muscle cells isolated from different blood vessels, e.g. coronary artery vs. aorta are maintained in multiple passages in monlayer culture
   b. gene regulation, intracellular signaling are studied

7. Patch clamp of vascular smooth muscle and endothelial cells
   a. enzymatic isolation of single cells from blood vessels
   b. whole-cell recordings of Ca, K, Cl currents
   c. membrane potential measurements
   d. single ion channel measurements

8. Single cell fluorescence imaging
   a. immunocytochemical determination of subcellular distribution of proteins (e.g. ion transporters) or other molecules (e.g. phosphorylated protein species)
   b. intracellular free calcium measures with fura-2 at rest and during stimulation with hormones and drugs
   c. subcellular free calcium measures with confocal microsopy, e.g. nuclear calcium
   d. DNA synthesis
   e. 3-dimensional reconstruction of confocal images to yield full spatial orientation of molecules
   f. deconvolution microscopy of widefield epifluorescence images to yield a “digital confocal” images that have superb resolution

9. Molecular biology
   a. real-time (quantitative) polymerase chain reaction (PCR)
   b. cloning of plasma membrane receptors, i.e. P2 nucleotide receptors, adenosine receptors
   c. overexpression of genes in cells cultured in monolayer preparations
   d. in vivo gene delivery methods are being developed for selective modulation of genes and proteins

George Tanner **Kidney function in health and disease.**

1. Kidney micropuncture
   This approach is used to analyze functions at the single nephron level in anesthetized rats and salamanders.

2. Renal clearance
   This method is used to evaluate whole kidney function (glomerular filtration rate, renal blood flow, tubular transport) in anesthetized rats and salamanders.

3. Kidney microdissection
   This classical method is used to examine the structure of normal and cystic nephrons.

4. Gene delivery into single nephrons, combined with in vivo two-photon microscopy
   Using kidney micropuncture and an adenovirus vector, we can express proteins labeled with a green fluorescent protein and observe protein dynamics in vivo in tubular and endothelial cells in the rat. (Tanner GA, RM Sandoval, BA Molitoris, JR Bamburg, SL Ashworth. Micropuncture gene delivery and intravital two-photon visualization of protein expression in rat kidney. Am J Physiol Renal Physiol, in press, available Online).

Frank Witzmann **Proteomic applications in toxicology, alcoholism and cardiovascular physiology.**

1. Sample preparation for protein analytical studies using various proteomic platforms.
   b. Protein extraction from various tissues and cells for 2DE analysis.
   c. Protein mixture isolation from various tissues and cells for in-solution isoelectric focusing.
   d. Protein reduction and alkylation before 2DE.
   e. Desalting and/or concentrating of protein mixtures for 2DE analysis using ultra-filtration techniques.
   f. Protein extraction from various tissues and cells for LC-MS proteomic analysis.
   g. Protein assays in the presence of reducing agent sand detergents.

2. Sample complexity reduction using various pre-fractionation techniques.
   h. Centrifugation-based subcellular fractionation of various tissues and cells to yield cellular organelles and compartments
   i. Protein fractionation by isoelectric point using in-solution isoelectric focusing and free-flow electrophoresis.
   j. Isolation of sub-proteomes using affinity and/or depletion columns.

3. Two-dimensional gel electrophoresis and image analysis of 2D patterns.
   a. Analyzing differentially expressed proteins in the proteome or the sub-proteome in tissues, cells or body fluid as a function of a particular state (e.g. differentiation, developmental stage, disease state) or under the influence of a xenobiotic or physical stimulus.
   b. Performing 1st dimensional IEF and 2nd dimensional SDS-PAGE in a highly parallel manner enabling the throughput and reproducibility.
   c. Staining 2D gels with various types of staining techniques, including but not limited to colloidal Coomassie Blue stain, silver stain, Sypro Ruby™ stain, and ProQ Diamond™ stain.
   d. 2D image capture and analysis of 2D patterns.

4. Large format 1-dimensional SDS-PAGE for complex mixtures and high MW proteins.

5. Identification and characterization of proteins using peptide mass fingerprinting and/or tandem mass spectrometry.
   k. Excision of protein spots/bands from 2D or 1D gels, robotically or manually.

l. Performing reduction and alkylation before peptide mass fingerprinting.
m. In-gel (and solution) proteolytic digestion of proteins and resulting peptide extraction.
n. Peptide mixture desalting for peptide mass fingerprinting.
o. Performing MS analysis using MALDI-TOF or LC-MS/MS mass spectrometry.
p. Performing mass spectral interrogations and database searches for protein identification.

6. Western blot analysis
   q. Confirmation of protein identities determined by 2DE/MS based proteomic analysis.
   r. Confirmation of protein chemical modification observed in 2-DE/MS based proteomic analysis, (e.g. posttranslational modifications such as phosphorylation, nitration etc.).

Associate Faculty

Robert M. Bigsby **Ob/gyn** **rbigsby@iupui.edu**

1. Animal models available in the lab:

   a. Knockouts: estrogen receptor-alpha, ERaKO
      Progesterone receptor, PRKO
      Prolactin receptor, PRLR-KO

   b. Cre/Lox: floxed-Rb
      floxed-p53
      floxed-Rb/floxed-p53
      floxed-STOP-oncogenic K-RasG12D (LSL-KRasG12D)
      floxed-p53/LSL-KRasG12D
      floxed-androgen receptor (floxed-AR)

   NB: These very valuable colonies are in need of financial support to maintain. I am actively seeking funded collaborations.

2. Cell culture models available:

   a. Estrogen-responsive cell lines:
      MCF-7 (breast CA)
      TD-47 (breast CA)
      BG-1 (ovarian CA)
      BG-1-luc, stable transfectant with estrogen-responsive luciferase reporter

   b. Others:
      MDA-MB-231 (breast CA)
      HeLa (cervical CA)
      Hey C2 (ovarian CA)
      Hey A8 (ovarian CA)
      SKOV-3 (ovaian CA)

SKOV-3x (ovaian CA, adapted to ip graft in athymic mice)
OVCA (ovarian CA)
CAOV (ovarian CA)

3. Rodent models of estrogen and progesterone action:
   Ovariectomy, cell proliferation indices, gene expression
   Carcinogen-induced mammary tumors –rat
   Slow-release capsules for hormone treatments

4. Xenograft models:
   Breast CA cells – subcutaneous, fat pad
   Ovarian CA cells – sc, ip
   Developing tissues (uterus, vagina, prostate) – sub-renal capsule

5. Tissue recombinations:
   Separation and recombination of mesenchymal and epithelial components of developing organs to study tissue interactions involved in hormone action; recombined tissues of different genotypes are grown as grafts (sub-renal capsule); host's hormonal milieu is manipulated

6. Cataractogenesis models (rat): alkylating agent- or radiation-induced

7. Mouse models of ovarian cancer using Cre/Lox systems – intrabursal inoculation of adeno-Cre to induce mutation of floxed genes

Bonnie Blazer-Yost **Biology** **bblazer@iupui.edu**

Won Kyoo Cho **Medicine/Gastroenterology** **wkcho@iupui.edu**

1. Cystic Fibrosis Mouse Colony
   a. Locally housed and cared in Roudebush VAMC
   b. Readily available CF-/- and CF+/- mouse organs

2. Animal Surgery and Cell Isolations and Perfusion Studies
   a. Rat and mouse surgeries and organ harvest.
   b. Isolation of hepatocytes, bile duct cells, etc. using various enzymatic and immunomagnetic isolations.
   c. Bile duct ligation surgeries.
   d. In situ and isolated liver perfusion studies for rat and mouse.

3. Cell Culture Techniques.
   a. Growth of mammalian cell lines including human cholangiocarcinoma cell line, human normal and cystic fibrosis (CF) bile duct cell lines, mouse and rat bile duct cell lines.
   b. Primary cultures of hepatocytes and bile duct cells from rat and mouse.

4. Quantitative Videomicroscopic Studies
    e. Time-lapse videomicroscopy using OpenLabs software – automated programs to control motorized X-Y stage to register the location of the cells or objects and take time-lapse images.
    f. Quantitate the number, size, area, orientation, and shape characteristics of cells or objects.

5. In Situ Quantitative and Qualitative Fluorescent Studies of live and fixed cells.
    a. Using various fluorescent dyes, measure changes in intracellular pH, calcium, chloride, potassium, sodium, membrane potential, nitric oxide, etc. in live cells.
    b. 3 Dedicated quantitative/qualitative fluorescent microscope set-ups – Leica (has monochromator excitation and emission filterwheel using Perkin-Elmer program); Olympus (excitation and emission filterwheels using OpenLabs program); Nikon (excitation filterwheel using MetaMorph) attached to patch-clamping set up.
    c. Capable to use single as well dual ratio fluorescent measurements – can measure 2-4 simultaneous fluorescent measurements (i.e. calcium (Fura-2 340/380)+pH (490/440)).

6. UltraView Fast Live Cell Confocal Microscopy
    a. Suitable for fast live cell confocal imaging studies.
    b. Has thermostated perfusion chamber for live cell imaging.
    c. Useful to measure local or global changes in intracellular calcium, pH, NO, etc. in live cells.
    d. Capable of live cell fluorescent and fixed cell immunofluorescent confocal imaging.
    e. Capable of 3-D image reconstruction using Volocity program.
    f. Capable of Colocalization study.

7. Patch-clamp and Ussing Chamber Electrophysiologic Studies
    a. Has patch-clamping set up for studying chloride and potassium channels in bile duct cells.
    b. Set up for Ussing chamber studies available.

8. Microinjection and Microperfusion
    a. Complete microinjection and microperfusion pump and set up available.
    b. Microinjection or microperfusion of chemicals or fluorescent dyes into the cells or lumen or cell clusters.

9. General Molecular Biological Techniques
    a. RT-PCR, Western blot, etc.
    b. Have used proteomics techniques in collaboration with Proteomics Core.

Robert Considine Medicine/Endocrinology rconsidi@iupui.edu

Loren Field **Medicine/Pediatrics** **ljfield@iupui.edu**

1. Standard molecular techniques to study expression of protein (Western blot) or RNA (Northern blot, RT-PCR)

2. Standard molecular analyses to screen for the presence of specific sequences (Southern blot, PCR)
3. Standard assays to look for protein-protein interactions (immune precipitation/Westerns)
4. Generation and husbandry of transgenic and knock-out mice
5. In vivo imaging of cardiomyocyte intracellular calcium transients
6. Protein isolation for sequence analysis
7. Induction of myocardial infarction in mice
8. Induction of cardiac hypertrophy in mice
9. Analysis of cell cycle progression in cardiomyocytes in vivo
10. Embryonic stem cell differentiation

Lincoln Ford Medicine/Cardiology lieford@iupui.edu

Richard Friedman **Surgery** **rfriedma@iupui.edu**

1. Electrophysiological studies of nerve and muscle.
    a. Crayfish neuromuscular junction (pre- and post-synaptic intracellular recording)
        -Specifically, studies of intracellular Ca^{2+} role in neurotransmitter release.
    b. Frog sciatic nerve-gastroc muscle preparation.
    c. Brain slice recording (intra- and extracellular).
    d. Rat sciatic nerve.
    e. Impact lesion of rat spinal cord (long-term maintenance and evaluation).
    f. Studies of hyperbaric-induced spinal cord injury.
    g. Recording of magnetic fields that arise from conducted electrical activity in nerve and muscle.
    h. Clinical deep brain electrophysiological cell characterization to guide surgical implants of deep brain stimulators.
    i. Sympathetic muscle potential recording.
    j. Recording signals from functionally vestigial muscles as command signals for controlling assistive devices (alternate control).
2. Aerospace physiological studies in high gravitational fields.
3. Test construction.

Janice Froehlich **Medicine** **jcfroehl@iupui.edu**

1. Determination of hormone content in tissue and body fluids via radioimmunoassay and immunoradiometric assays.
2. Determination of ethanol content in tissue and body fluids via gas chromatography. Microinjection of drugs into specific brain sites for in vivo studies in rats.
3. Implantation of indwelling carotid and jugular cannulae for in vivo repeated blood sampling in rats.
4. Automated chronic monitoring of heart rate, blood pressure, temperature, eating, drinking, activity in rats.
5. Assessment of acoustic startle reactivity, mechanical and thermal pain threshold, electrical brain stimulation theshold in rats.

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Alon Harris	Ophthalmology	alharris@iupui.edu
Gary Hutchins	Radiology	gdhutchi@iupui.edu
John Kincaid	Neurology	jkincaid@iupui.edu
Edward Mannix	**Medicine/Pulmonary**	**emannix@iupui.edu**

1. Use a computerized metabolic cart to measure O2 uptake, CO2 production, minute ventilation and caloric expenditure at rest and during exercise. The system relies on an open circuit, indirect calorimetry technology for its determination of caloric expenditure. During exercise, one is able to determine the integrity of the pulmonary and cardiovascular systems.

2. Use computerized spirometry to measure airflow and volume characteristics. This is used to determine if an obstruction to airflow is present and also if a restrictive lung defect are operative.

3. Use a 12-lead EKG for determination of arrhythmias or ischemia at rest and during exercise.

4. Use pulse oximetry to measure O2 Sat and manual sphygmomanometry for BP determinations.

Keith March	**Medicine/Cardiology**	**kmarch@iupui.edu**

1. Cell culture techniques.
    a. isolation and culture of primary cells from human and animal tissue specimens
    b. cells include adipose stromal, vascular smooth muscle, vascular endothelial, peripheral blood mononuclear and endothelial progenitor cells
    c. animal species include mouse, rat, rabbit and pig

2. Histology
    a. immunohistochemistry and immunofluorescence
    b. antibodies to vascular and blood cell surface markers
    c. both paraffin and cryofixed samples

3. Rodent ischemic hindlimb surgeries
    a. creation of unilateral blood flow insufficiency
    b. injection of material either by IV or IM routes
    c. laser Doppler imaging for measurement of blood flow restoration at multiple survival points
    d. contrast imaging

4. Rat acute myocardial infarction model
    a. thoracotomy-based access with temporary ligation of the LAD
    b. delivery of agent through either direct injection or iv at pre- or post-occlusion
    c. 2D echocardiography
    d. invasive LV pressures using Millar catheters

5. Rat chronic ischemia / heart failure model
    a. as above but with permanent occlusion
    b. iv or direct intramyocardial injection of agent
    c. 2D echocardiography
    d. invasive LV pressures using Millar catheters

6. Minimally invasive porcine model of acute myocardial infarct
    a. catheter-based balloon occlusion of coronary arteries
    b. occlusion of up to 45 minutes to produce a transmural infarct of ~ 30% of the LV

7. Stenting of porcine coronary and peripheral arteries
    a. X-ray guided catheterization for placement
    b. intravascular ultrasound (IVUS) to monitor neointima formation

8. Porcine model of restenosis
    a. catheter-guided, balloon overstretch model
    b. overstretch damages endothelium without denuding the lumen

9. Porcine chronic ischemia model
    a. surgical placement of an ameroid occluder on the LAD and LCx
    b. gradual occlusion of coronary artery to minimize infarction
    c. allows partial restoration of blood supply through collateralization

10. Multiple methods of delivery to the porcine heart
    a. agents include formulated plasmid DNA, viruses, small molecule and cells
    b. direct intramyocardial injection through epicardium after thoracotomy or endocardial access using specialized catheters
    c. intracoronary infusions using balloon catheters
    d. retrograde venous infusion using end-hole balloon catheters
    e. percutaneous, transmyocardial, and direct delivery into the pericardium

11. Measurement of porcine heart function and blood perfusion status
    a. left ventriculography for assessment of ejection fraction, volumes and wall motion using clinical assessment software.
    b. coronary angiography and quantitative coronary analysis (QCA) utilizing digital software.
    c. microsphere analysis of blood perfusion
    d. percutaneous intravascular blood pressure and flow analysis

12. High-resolution ultrasound based micro-imaging system (37.5 to 82.5 MHz)
    a. EKV (ECG based kilohertz reconstruction) provides visualization and measurement of the beating adult mouse heart at up to 1,000 frames/sec.

b. B-Mode (30Hz), M-mode and Doppler capabilities.
c. image-guided needle injections; deep embryonic or cardiac.
d. abdominal, embryonic, vascular and cardiac imaging with up to 30 mm resolution.
e. microcirculatory and cardiovascular blood flow assessment

Jim Marrs **Medicine/Nephrology** **jmarrs@iupui.edu**

1. Zebrafish:
   a. cDNA cloning/PCR cloning to characterize expressed genes.
   b. Antibody production to facilitate characterization of protein expression and localization.
   c. In situ hybridization to examine gene expression during development Immunofluorescence to examine protein distribution.
   d. Confocal and 2 photon microscopy to examine immunofluorescence labeling.
   e. Morpholino oligonucleotide knockdown to block gene expression during embryogenesis and organogenesis.
2. Cell Culture Model:
   a. Immunoblot/immunofluorescence/immunoprecipitation to examine protein expression, distribution and protein-protein complexes.
   b. Northern blot/PCR to examine gene expression.
   c. Antibody production to facilitate characterization of protein expression and localization.
   d. Immunofluorescence to examine protein distribution.
   e. Confocal and 2 photon microscopy to examine immunofluorescence labeling.

Richard Meiss **Ob/Gyn** **igeq100@iupui.edu**

1. The ability to measure muscle contraction under a variety of mechanical conditions (isometric, isotonic, and combinations of these) while continuously measuring the stiffness of the muscle. This capability allows the determination of the relationship between force and stiffness of a contracting muscle and can reveal the presence of internal crossbridge activity that is not expressed as external force.

2. Instrumentation
   -design, construction, and implementation.
   The lab has had long experience with electrical/mechanical approaches to many physiological problems and can approach them in a way that might be different from the solutions provided by the departmental electronic and machine facilities.

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