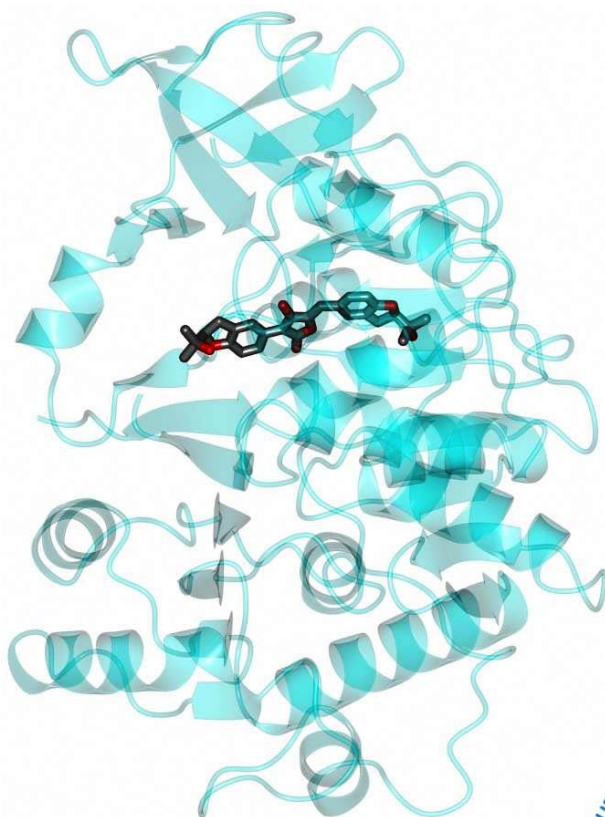




NIH Center of Biomedical Research Excellence (COBRE) in Protein Structure and Function

Principal Investigator: Robert P. Hanzlik, Ph.D.



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National Institute of General Medical Sciences.**



The Center of Biomedical Research Excellence in Protein Structure and Function (COBRE-PSF) at the University of Kansas conducts health-related basic research in protein structure and function. The Center, established in 2002, now is supported by a Phase III grant from the National Institute of General Medical Sciences (1 P30 GM110761) at the National Institutes of Health. The objectives of the COBRE program are:

- 1) To strengthen an institution's biomedical research infrastructure through establishment of a thematic multi-disciplinary center,
- 2) To continue growing a critical mass of new and continuing investigators focused on the broad theme of protein structure and function, and
- 3) To strengthen our existing Core labs by expanding their capabilities and use.

The result of this will be a strong cadre of successful mid-career biomedical research faculty.

The Center now supports six Pilot Project Leaders from six departments at three of our four participating Kansas universities: the University of Kansas, Kansas State University, Wichita State University, and the University of Kansas Medical Center. The Pilot Project program continues a successful research support program by advertising competitions for awards broadly across the four participating institutions, obtaining external reviews of all applications, and consulting with the Center's External Advisory Committee to identify new projects to support. Since its inception, COBRE-PSF has supported 41 junior and senior faculty investigators by providing research support and a professional development program with mentoring by accomplished senior faculty researchers.

Our investigators address significant problems in protein structure and function from biological and chemical perspectives in an integrated, multi-disciplinary way. An important feature of the Center is that biologically-oriented scientists work with chemically-oriented scientists, and vice versa, resulting in cross-disciplinary broadening of new and continuing faculty.

These Center objectives have resulted in a self-sustaining group of Core Laboratories that support research in protein structure and function and are available to regional researchers. The laboratories are dedicated to protein purification, protein X-ray crystallography, and biomolecular NMR spectroscopy. They complement other core laboratories at the University of Kansas and other Kansas Board of Regents universities, and create a substantial and continuing impact on biomedical research.

Cover: Crystal structure of firefly luciferase (cyan ribbons and wires) containing bound aspulvinone J-CR (sticks). The aspulvinone family of compounds, identified via high throughput screening of natural product extracts, are potent inhibitors of luciferase.



Robert P. Hanzlik
Program Director

COBRE Pilot Project Leaders

The COBRE-PSF continues to add new Center members. Several are supported through its Pilot Project Program. The goals of the program are to both help investigators obtain research results to use to attain external support, and to expand the client base of the Center's core laboratories.

Phase III Faculty Pilot Project Leaders (2014-2019)



Ryan Altman (2015-2016)

Assistant Professor, Medicinal Chemistry, University of Kansas
Inhibition of bacterial aminoglycoside resistance enzymes using a fragment-based drug design approach



Fariba Behbod (2015-2016)

Associate Professor, Cancer and Developmental Biology, University of Kansas Medical Center
Role of BCL9 in STAT3 signaling and DCIS invasive progression



Cory Berkland (2015-2016)

Professor, Pharmaceutical Chemistry & Chemical Engineering, University of Kansas
Antigen-drug conjugates as potent antigen-specific immunotherapies



Carey Johnson (2015-2016)

Professor, Chemistry, University of Kansas
Tracking nitric oxide synthase conformations and dynamics



Jed Lampe (2015-2016)

Assistant Professor, Pharmacology, Toxicology and Therapeutics
University of Kansas Medical Center
Defining hydrophobic and electrostatic interactions between CYP3A and CYPb5



Ping Li (2015-2016)

Assistant Professor, Chemistry, Kansas State University
Engineering human NRMT1 for its substrate profiling



Robin Maser (2015-2016)

Associate Professor, Clinical Laboratory Science, University of Kansas Medical Center
Heterotrimeric G Protein Binding by Polycystin-1, an Atypical GPCR



Jakob Moskovitz (2015-2016)

Associate Professor, Pharmacology & Toxicology, University of Kansas
Effect of methionine substitution and oxidation on structure-function of COMT



Hao Zhu (2015-2016)

Associate Professor, Clinical Laboratory Science, University of Kansas Medical Center
Atomic structure of a multi-domain redox enzyme Ncb5or implicated in diseases

Phase III Pilot Project Leaders (cont)

Moriah Beck (2014-2015)

Assistant Professor, Chemistry, Wichita State University
Regulation of Palladin Structure and Function

Jeremy Chien (2014-2015)

Assistant Professor, Cancer Biology, University of Kansas
Medical Center
Structure and Function of RABL3 in Paclitaxel Resistance

Raymond Perez (2014-2015)

Professor, Medicine, University of Kansas Medical Center
Rational Design of SPRY2-Cbl Inhibitors as Potential Anticancer Drugs

Mario Rivera (2014-2015)

Professor, Chemistry, University of Kansas
Design Protein/Protein Interaction Modulators to Perturb Bacterial Iron Metabolism

Liskin Swint-Kruse (2014-2015)

Associate Professor, Biochemistry & Molecular Biology
University of Kansas Medical Center
The Cra-FruK Complex Alters Regulation of Central Metabolism of γ -proteobacteria

Liang Xu (2014-2015)

Associate Professor, Molecular Biosciences, University of Kansas
Fragment-based Drug Discovery for Inhibitors of RNA-binding Protein HuR

Phase II Pilot Project Leaders (2008-2013)

Katsura Asano (2012-2013)

Associate Professor, Biology, Kansas State University
Molecular Basis of Control of Translational Initiation in Eukaryotes

Roberto De Guzman (2008-2010)

Associate Professor, Molecular Biosciences, University of Kansas
Interactions of Salmonella Needle and Tip Proteins

Revathi Govind (2012-2013)

Assistant Professor, Biology, Kansas State University
Structure-function Studies on Clostridium Difficile Anti-sigma Factor TcdC

P. Scott Hefty (2012-2013)

Associate Professor, Molecular Biosciences, University of Kansas
Structural and Functional Genomics for Chlamydial Hypothetical Proteins

Wonpil Im (2008-2010)

Associate Professor, Molecular Biosciences, University of Kansas
NMR and Computational Studies of Protein Translocation in Bacterial Needles

John Karanicolas (2009-2011; ARRA)

Associate Professor, Molecular Biosciences, University of Kansas
Toward Novel Inhibitors of OX40L-OX40: A Dominant Negative Approach

Phase II Pilot Project Leaders 2008-2013 (cont.)

Ping Li (2012-2013)

Assistant Professor, Chemistry, Kansas State University

Expression and Purification of an Obesity-Important Enzyme hGOAT in *E. coli*

Kristin Michel (2009-2011; ARRA)

Associate Professor, Biology, Kansas State University

Structure and Target Protease of Anopheles SRPN6, an Inhibitor of Malaria Parasite Infection

Alexander Moise (2009-2011; ARRA)

Assistant Professor, Pharmacology and Toxicology, University of Kansas

Structural Characterization of Lecithin:Retinol Acyltransferase

Mario Rivera (2008, 2010-2012, 2012-2013)

Professor, Chemistry, University of Kansas

Probes to Disrupt Iron Homeostasis in *Pseudomonas Aeruginosa*

Emily Scott (2010-2012)

Associate Professor, Medicinal Chemistry, University of Kansas

Structure and Function of Cyp17A1, a Critical Enzyme in Human Androgen Biosynthesis

Liang Tang (2008-2010; 2010-2012)

Associate Professor, Molecular Biosciences, University of Kansas

Mechanisms of Genome Packaging in DNA Viruses

Kandatege Wimalasena (2009-2012)

Professor, Chemistry, Wichita State University

Structure-Activity Relationship Studies of Dopamine beta-Monooxygenase

Phase I Pilot Project Leaders (2002-2007)

James Bann (2005-2007)

Associate Professor, Chemistry, Wichita State University

Structure and Mechanism of CS1 Pilus Assembly

Xue-Wen Chen (2004-2007) (*now Professor, Computer Science, Wayne University*)

Assistant Professor, Electrical Engineering & Computer Sciences, University of Kansas

Computational Proteomics: Protein Interaction Prediction

David Eichhorn (2004-2007)

Professor and Chair, Chemistry, Wichita State University

Synthesis of Model Complexes for Nitrile Hydratase

Jianwen Fang (2004-2007)

Research Assistant Professor, Center for Bioinformatics

(*now Staff Researcher, Biometric Research Branch, National Cancer Institute*)

Computational Proteomics: Protein Interaction Prediction

Weijun Huang (2004-2007) (*now researcher at Zhongshan School of Medicine, Sun Yat-Sen University, China*)

Director, Protein Structure Laboratory, University of Kansas

Structural and Functional Studies on D52 Tumor Proteins from Human Tissue

Phase I Pilot Project Leaders 2002-2007 (cont.)

Shiguang Liu (2004-2007) (*now researcher at Sanofi-Genzyme R&D Center*)
Research Assistant Professor, Kidney Institute, University of Kansas Medical Center
Identification of Inhibitors and Substrates of Phex

Silvia Mora (2004-2006) (*now researcher, Molecular Physiology, Univ. of Liverpool, UK*)
Assistant Professor, Biology, Kansas State University
Protein Interactions that Regulate Leptin Secretion

Robert R.R. Rowland (2004-2006)
Professor, Diagnostic Medicine and Pathobiology, Kansas State University
Structure/Nucleolar Function of SARS N Protein

Irina Smirnova (2005-2007)
Associate Professor, Physical Therapy-Rehabilitation Sciences, University of Kansas
Medical Center and Director, Ph.D. in Rehabilitation Science program
Role of Posttranslational Protein Modifications in Diabetic Cardiomyopathy

Qize Wei (2005-2007) (*now Associate Professor of Biochemistry, Fordham University*)
Assistant Professor, Biochemistry, Kansas State University
Roles of MyoGEF and its Interacting Partners in Cell Division and Cell Signaling

Asma Zaidi (2004-2007) (*now Professor of Biochemistry, Kansas City University of Medicine & Biosciences*)
Research Assistant Professor, Pharmacology & Toxicology, University of Kansas
Trafficking of Plasma Membrane Ca²⁺-ATPase to Rafts

COBRE Research Investigators

In Phases I and II of the COBRE-PSF Center, faculty selected to become COBRE research investigators were early in their careers and were establishing research programs with significant emphasis on protein structure and function. Investigators received research support and mentoring by a senior faculty advisor. They set specific goals for developing and submitting proposals for independent external research support.

Phase II Research Investigators (2008-2013)

James Bann (2008-2011)
Associate Professor, Chemistry, Wichita State University
Structural Characterization of the Anthrax Toxin Protective Antigen

Moriah Beck (2011-2013)
Assistant Professor, Chemistry, Wichita State University
Palladin and Regulation of Actin Dynamics

Christopher Fischer (2008-2011)
Associate Professor, Astronomy and Physics, University of Kansas
Remodeler Translocation Along DNA

Phase II Research Investigators 2008-2013 (cont.)

P. Scott Hefty (2008-2010)

Associate Professor, Molecular Biosciences, University of Kansas
Mechanism and Role of a Novel Chlamydial Transcriptional Regulator, CHXR

Todd Holyoak (2008-2012)

Associate Professor, Biochemistry and Molecular Biology, University of Kansas Medical Center (*associate professor, biology, University of Waterloo, Canada*)
The Role of Dynamics in PEPCK Mediated Catalysis

John Karanicolas (2011-2013)

Associate Professor, Molecular Biosciences, University of Kansas
Structure-based Chemical Rescue of Enzyme Activity

Alexander Moise (2011-2013)

Assistant Professor, Pharmacology & Toxicology, University of Kansas
Structure-function Studies of Adipose Phospholipase

Jeroen Roelofs (2010-2013)

Assistant Professor, Biology, Kansas State University
Mechanism of Chaperone-assisted Assembly of Proteasome Regulatory Particle

Phase I Research Investigators (2002-2007)

Brian Blagg (2002-2005)

Professor, Medicinal Chemistry, University of Kansas
Identification of Hsp90 Cochaperones, Immunophilins, and Client Proteins

George Bousfield (2002-2005)

Professor, Biological Sciences, Wichita State University
Structural Characterization of Glycoproteins using Mass Spectrometry

Roberto De Guzman (2005-2008)

Associate Professor, Molecular Biosciences, University of Kansas
Structure and Dynamics of Bacterial Needle Proteins

Heather Desaire (2002-2005)

Professor, Chemistry, University of Kansas
Structural Characterization of Glycoproteins using Mass Spectrometry

Susan Egan (2002-2005)

Professor, Molecular Biosciences, University of Kansas
Protein-Protein Interactions Required for Transcription Activation by RhaR

Audrey Lamb (2004-2007)

Associate Professor, Molecular Biosciences, University of Kansas
Structural Biology of Pyoverdinin Biosynthesis

Jennifer Laurence (2005-2007)

Associate Professor, Pharmaceutical Chemistry, University of Kansas
Redox Effects on Structure and Dynamics of PRL-1

Julian Limburg (2004-2007; *deceased*)

Assistant Professor, Chemistry, University of Kansas
Structure and Function of Bacterial Prolyl-4-hydroxylase

Phase I Research Investigators (2002-2007)

William Picking (2002-2005)

Professor, Pharmaceutical Chemistry, University of Kansas
Subversion of Eukaryotic Cell Function by Shigella

Emily Scott (2004-2007)

Associate Professor, Medicinal Chemistry, University of Kansas
Structure-Function of Mammalian Cytochromes P450

Jeffrey Staudinger (2002-2005)

Professor, Pharmacology and Toxicology, University of Kansas
Crystallization of Pregnane X Receptor Splice Variants

Liskin Swint-Kruse (2004-2007)

Associate Professor, Biochemistry and Molecular Biology
University of Kansas Medical Center
Elucidating Structural Elements that Fine-tune Function of Members in a Genetic Regulatory Protein

Anna Zolkiewska (2002-2004)

Associate Professor, Biochemistry, Kansas State University
Structure and Function of Cell Adhesion Domain of ADAM12

Core Laboratories

The COBRE Center supports three **Core Laboratories**, each directed by a highly experienced Ph.D. scientist. These fee-for-service facilities are available to qualified researchers in academia and industry. Each director provides consultation in experimental design, assistance preparing grant applications and manuscripts, training in specialized research techniques, access to state-of-the-art research equipment (for experienced users), and a menu of research services. The laboratories are housed in the 44,000 sq. ft. Shankel Structural Biology Center (SBC) on the west campus of the University of Kansas, which is devoted to advancing biomedical and bioscience research.



Shankel Structural Biology Center

Our **fragment screening program** merges the services of all three core labs. The Center has prepared a starter set of 288 carefully selected low-molecular weight compounds that represent ‘fragments’ of numerous drugs and other bio-active compounds. Core laboratories’ staff are ready to screen the collection against any protein of interest using STD-NMR, SPR (surface plasmon resonance) or even X-ray crystallography. Our core laboratories also interface effectively with both computational and synthetic chemistry service labs to carry a project seamlessly from screening for hits to lead development and improvement.



Protein Production Group

Director: Fei Philip Gao, Ph.D.

Assistant Researcher: Anne Cooper

Del Shankel Structural Biology Center, Room 1095

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The COBRE Protein Production Group (PPG) focuses on the cloning, expression and purification of prokaryotic and eukaryotic proteins. The laboratory maintains a variety of equipment to support the production of properly folded proteins in quantities suitable for structural studies (X-ray and NMR), functional studies (catalytic or biological), label-free binding studies (SPR) and/or high throughput (HTP) screening studies. The PPG employs *E. coli*, yeast, insect, mammalian and cell-free expression systems. It is equally at home with routine as well as complex procedures for expressing challenging proteins. The PPG also conducts label-free protein binding studies using a Biacore 3000 instrument; the 288-compound Zenobia fragment library is available for fragment-based drug discovery studies. The PPG also provides advice and consultation on project design, technical training, access to equipment, and custom services in many aspects of protein production, starting with cloning. **The laboratory offers the following services:**

Activities in the Protein Production Laboratory

- Construct design
- Isotope-labeled proteins
- Scale-up purification
- Large scale protein expression in *E. coli*, yeast, insect or mammalian cells
- Label-free binding studies
- Cloning, expression & solubility testing
- Protein characterization and analysis
- Protein production in cell-free systems
- Protein refolding and reconstitution

High Throughput Cloning

- Ligation independent cloning used to clone genes into a pET vector without restriction enzyme digestion and ligation
- Targeted gene can be cloned in ~one day with low background
- Protein can be purified using one-step affinity chromatography

Expression of Challenging Proteins

Various fusion proteins are used to increase the expression level, solubility and/or stability of the protein, or to drive the protein into inclusion bodies. The same PCR product can be inserted into all the fusion vectors. All tags and fusion proteins are cleavable.

Major Equipment

- ÅKTAexpress, ÅKTA purifier and FPLC systems
- Biacore 3000
- High capacity centrifuges
- Gradient thermocycler
- Shaker incubators and fermentors
- Hydraulic cell disruptors, thermocyclers
- Freezer Mill, French Press and sonicator



Protein Structure Laboratory

Director: Scott Lovell, Ph.D.

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Email: swlovell@ku.edu

The mission of the Protein Structure Laboratory (PSL) is to provide investigators with state-of-the-art instrumentation, facilities, and expertise for protein crystallization, X-ray data collection, data analysis, and structure solution and refinement from an à la carte menu.

Laboratory staff members provide advice and consultation, training, access to facilities for experienced crystallographers, and a range of services from crystal growth to full structure solution and refinement. The PSL staff will perform or assist investigators with any of these functions:

High Throughput Crystallization Screening

- 96-well screen setup in five to 10 minutes, using 50mL of concentrated protein per 96-well screen
- Extensive sampling of crystallization space (~672 conditions)
- Co-crystallization and soaking experiments to obtain protein-ligand complexes

In-house and Synchrotron X-ray Data Collection

- Rigaku rotating anode generator, R-axis IV++ image plate detector and X-stream 2000 cryostream
- Screen crystals for diffraction and collect data for structure solution
- Collaboration with Advanced Photon Source (APS) IMCA-CAT for synchrotron data collection
- Ship samples to APS, where data are collected and transferred to the PSL in real time

Computational Crystallography

- Molecular replacement, MIR and SAD/MAD phasing methods
- Model building, refinement and analysis of structures
- Preparation of a publication-quality crystal structure report
- Preparation of materials for grant submissions involving structural biology work



Biomolecular NMR Laboratory

Director: Asokan Anbanandam, Ph.D.

Office: Del Shankel Structural Biology Center, Room 1011

Telephone: 785-864-3746; Fax: 785-864-8141

Email: asokan@ku.edu

The Biomolecular NMR Laboratory maintains two high field solution state NMR spectrometers in support of structural and dynamics studies of proteins, peptides, and their complexes.

- Bruker Avance 800 MHz with TCI cryoprobe
- Bruker Avance III 600 MHz with TXI-RT probe, Broad Band probe, and 24 sample autosampler.

Capabilities include determining high resolution structures of proteins, peptides and protein-peptide complexes; performing chemical shift perturbation studies of protein-protein, protein-nucleic acid, protein-peptide, protein-drug interactions; and studying the fast- and slow-motion dynamics of proteins and their complexes.

The BNMR Laboratory also provides consultation, training, assistance and service to investigators. The staff is also responsible for implementation of new NMR pulse sequences, and assisting local and remote users with technical problems.

Laboratory Capabilities and Services

- From 1D to 4D NMR data acquisition
- NMR data processing, analysis, and interpretation
- High resolution structure determination of peptides, proteins, etc.
- ^{15}N - T_1 , ^{15}N - T_2 , ^{15}N -{1H}-NOE-NMR experiments for fast motion dynamics studies
- Micro to millisecond motion dynamics studies by CPMG NMR
- Chemical Shift Perturbation based NMR titration measurements
- Protein-protein, protein-DNA, protein-ligand interaction studies
- Small molecule-protein interaction studies by STD-NMR and SPR



Anbanandam loading sample into the Bruker Avance III 600 MHz.

Participating Institutions

The Center of Biomedical Research Excellence (COBRE) in Protein Structure and Function is hosted in the Department of Medicinal Chemistry at the University of Kansas. The KU Higuchi Biosciences Center provides the Center with its administrative support. Other participating institutions are Kansas State University, Wichita State University and the University of Kansas Medical Center.



University of Kansas



Kansas State University
Manhattan, Kansas



Wichita State University
Wichita, Kansas



University of Kansas
Medical Center
Kansas City, Kansas

For more information contact:

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