COBRE Core Labs Enhance Research Efforts by Scientists

Our three core laboratories, the Protein Production Group, the Protein Structure Laboratory and the Bio-NMR Laboratory, have assisted 53 research groups from across the United States in the past year. Our primary mission is to help researchers solve problems and accelerate research productivity. Our services include technical training for all researchers—faculty, staff and students—in state-of-the-art research facilities. If you think we might be too expensive, we invite you to contact us and be pleasantly surprised.

PROTEIN PRODUCTION GROUP

Establishment of the Insect Cell Lab
Besides using *E. coli* as the workhorse system for protein expression, the PPG is now establishing insect cell systems to express proteins with post translational modification. A cloning method has been established to insert genes into five different insect cell expression vectors. The insect laboratory, housed in 1008 SBC, is equipped with an Orion ET-22 Cryo liquid nitrogen tank from MVE Cryogenics, a Class II Biological Safety Cabinet with UV light from Safeaire, an Infors Ecotron Shaker incubator with sticky tape from ATR, and a Cellometer Auto T4 cell counter from Nexcelom.

New Research Grants
“Innate Immune Receptors and Adjuvant Discovery,” HHSN (PI: S.A. David, Co-I: F.P. Gao) National Institutes of Health/NIAID. The PPG is producing large amounts of Flagellin from *E. coli*, Semenella and Shigella in the first two years of the grant. “Design of a Stabilized Ricin Vaccine,” (PI: J. Karanicolas; Co-I: F.P. Gao) Institute for Advancing Medical Innovation (IAMI). The PPG is expressing and purifying multiple mutant proteins designed by computational techniques to develop a stable antigen.

Recently Completed Projects
- Construction and purification of Calmodulin-GST fusion protein
- Subcloning and expression of Ricin vaccine
- Expression & purification of beta-galactosidase from insect cells
- Cloning human DBH into 5 insect cell expression vectors
- Subclone tLRAT in 3 bacterial expression vectors, screening and purification of soluble protein
- Purification of cytochrome b5 reductase
- Purification of MBP-huntingtin from *E. coli*
- Anealing, ligation and purification of fluorescent tagged DNA
- Subcloning, expression and purification of human Hsp90
- Construction and purification of 4 Calmodulin mutants
- Cloning human DBH into 5 insect cell expression vectors
- Construction and purification of 1mM 0.5ml uniformly $^{15}$C$^{15}$N ubiquitin

PROTEIN STRUCTURE LABORATORY

Projects Recently Completed in the Protein Structure Laboratory
PSL Director Scott Lovell assisted Mario Rivera (professor of chemistry, KU) in solving numerous structures of the spherically shaped iron storage protein bacterioferritin (BfrB) from *Pseudomonas aeruginosa* in apo and iron bound states. The protein forms a $\sim$440 kDa complex with molecules arranged in a 24-mer manner. Structures obtained from apo crystals soaked in solutions containing iron revealed four iron binding sites that allowed determination of the mechanism for iron transfer from the protein exterior to the core. An article on the work was published in *Biochemistry*; structures are available from the Protein Databank (PDB IDs: 3IS7, 3IS8, 3ISE, 3ISF).

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Newly Updated Equipment Available This Summer

Asokan Anbanandam and Bio-NMR Laboratory clients are looking forward to later this summer when the laboratory’s decade-old Varian Inova 600 MHz NMR instrument will be upgraded to the latest generation, state-of-the-art Bruker AVANCE-III with toppsin software. The system is located on the first floor of the Multidisciplinary Research Building on The University of Kansas’s (KU) West Campus, 2030 Becker Drive. The upgrade has been financed through a grant from the U.S. Small Business Administration. A strong feature of the instrument will be a 5mm inverse detection triple resonance probe, which will be used mainly for protein work.

Recently Completed Projects

- Assisted Susan Egan, (professor of molecular biosciences, KU) in acquiring a 2D-1H-15N-HSQC spectrum of DNA binding domain of RhaS. NMR data collected was included as preliminary data for a NIH grant proposal submitted in March 2010. The information will also be used in a publication in preparation.
- Worked with Scott Hefty (assistant professor of molecular biosciences, KU) to conduct 3-dimensional structure determination of a transcriptional regulatory protein from chlamydia, by solution NMR methods. The sequence specific resonance assignment was successfully completed and the high resolution solution NMR structure solved. Two manuscripts are being written.
- Dr. Anbanandam recently assigned 120 residues of the anthrax toxin protective antigen (143 residues total) for a project by James Bann (assistant professor of chemistry, WSU). A manuscript planned for the Journal of Biomolecular NMR will provide assignments as well as comments on the structural heterogeneity. The structure will be used as a template for assignments of domain 4 within the context of the full-length 83 kDa PA, as well as the structure of domain 4 bound to capillary morphogenesis protein. The latter will aid in designing therapeutics to block interactions between PA and the host cellular receptors. This information will be used in a July NIH R01 submission.

The KU NIH Center of Biomedical Research Excellence in Protein Structure and Function (COBRE-PSF) at the University of Kansas began in October 2002. It is intended to i) synergize with other regional efforts in proteomics and protein chemistry and biology, ii) recruit, support and mentor new faculty intending to establish competitive research programs in protein structure and function, and iii) enhance the overall infrastructure for state-of-the-art research in protein structure and function through the establishment of Core Laboratories. The COBRE-PSF and its core research laboratories are supported by the National Institutes of Health National Center for Research Resources through the Institutional Development Award (IDeA Program).