PSL helps researchers work toward successful control of viral diseases

Protein Structure Core Laboratory Director Scott Lovell and his staff worked last winter in collaboration with Kyeong-Ok Chang (KSU) and Bill Groutas (WSU) to determine the structures of the Norovirus, Poliovirus and Transmissible Gastroenteritis Coronavirus (TGEV) 3C or 3CL proteases in complex with a single inhibitor. Chang and Groutas have shown that compounds possessing a common dipeptidyl residue with different “warheads”, aldehyde, bisulfite adduct (GC376) and α-ketoamide are potent inhibitors against viruses belonging to the picornavirus-like supercluster.

Viruses in the supercluster include classical and emerging human and animal pathogens: noroviruses (Norwalkvirus [NV] and MD145virus); human rhinovirus (HRV), polio-virus (PV), foot-and-mouth disease virus (FMDV), hepatitis A virus (HAV), and porcine teschovirus (PTV) in the Picornaviridae family; and human coronavirus 229E, transmissible gastroenteritis virus (TGEV). Great efforts have been made to discover effective preventive and therapeutic measures, including vaccines and antiviral agents, against these viruses.

A common feature amongst the 3C and 3CL proteases is a nucleophilic cysteine residue in the active site. The structures revealed that compound GC376 forms a covalent complex with the active site cysteine of each protease as shown in Figure 1.

This work highlights the potential of these compounds to serve as inhibitors for a broad range of viral targets and illustrates the significance of obtaining the crystal structures of protein:inhibitor complexes to facilitate drug discovery efforts. A manuscript about this work was recently published (Kim, Y. et al., Journal of Virology, 2012, 86, 11754-11762).

Techniques Available in the Protein Structure Laboratory

**Protein Crystallization** High throughput crystallization screening of protein samples conducted in a climate-controlled environment.

**X-Ray Data Collection** Inhouse and/or Synchrotron.

**Structure Solution** X-ray diffraction data obtained in-house or from a synchrotron source is used for structure solution by molecular replacement, MIR, SAD or MAD phasing methods. The PSL maintains Linux workstations that contain crystallographic software for X-ray data processing, structure solution, structure refinement, model building and preparation of publication quality graphics.
The Protein Production Group (PPG) provides services at all stages of the protein expression and purification processes including cloning and mutagenesis. It routinely prepares high quality proteins for functional, binding, structural, and high throughput screening (HTS). PPG is able to expand existing purification schemes for large scale preparations. We can also train your lab personnel in any of the techniques they use. Some of the special capabilities of the laboratory include:

**Ligation Independent Cloning.** Insertion of desired genes into custom pET vectors without use of restriction digestion or ligation; target genes can be cloned in one day with low background.

**Mutagenesis.** Site specific mutations, fusions or deletions are introduced by sequential or Multi-Site Quickchange PCR, Inverse PCR, Nested/Multi-Step PCR, or Overlapping PCR.

**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 for subsequent refolding into soluble protein.

**Surface Plasmon Resonance.** Protein-Protein, protein-ligand interactions by SPR (Biacore 3000).

**Automated Purification.** Most preparations and purifications are conducted with automated AKTA systems to ensure speed, efficiency and reproducibility.

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KU Core Labs collaborate to assist KSU scientist

The PPG Laboratory and a fellow University of Kansas core laboratory have collaborated to assist a Kansas State University professor’s research efforts. The KU High Throughput Screening Laboratory asked the PPG staff if they could generate a stable cell line of HeLa cells (human cervical carcinoma) so that sufficient sample quantities could be produced for testing and evaluation using high throughput screening (HTS). The HeLa cell line was used to constitutively express luciferase with a promoter of interest. The laboratories were working with KSU professor of biochemistry Gerald Reeck. Reeck’s areas of specialty include the biochemistry and molecular biology of the structure and function of proteinases and proteinase inhibitors. The project’s goal was to screen a drug that inhibits expression of ARMET gene.

Mammalian cells can be used to produce proteins that need post-translational modifications not available in other expression hosts or in which the researcher is planning to conduct live cell studies. The cells can be transiently or stably transfected, depending on whether a homogeneous population is needed; the protein can be either constitutively expressed or inducibly expressed. PPG efforts resulted in initial transfected cells showing luciferase activity increasing up to 16000-fold above vector baseline (Figure 2). This result indicated the cells were not only expressing the luciferase using the promoter of interest, but that they were expressing the luciferase at high levels, which will be helpful for high throughput screening later. PPG staff member Anne Cooper then established homogeneity by selection with puromycin and subsequent rounds of clonal selection.

Inverse PCR for difficult vectors

The PPG has developed a robust method to clone inserts of interest into vectors that will not linearize as desired. There are many reasons that certain vectors may prove difficult to use for cloning; desired restriction sites may be missing or are too close in proximity and double digests aren’t working efficiently, among others (Figure 3). To overcome these obstacles, the PPG staff carefully design primers to amplify the entire vector of choice. The primers can be designed to add or remove restriction sites as desired. The inverse PCR method linearizes vectors with desired restrictions sites intact. The PCR product is treated with DpnI for a short time to digest methylated and hemi-methylated template DNA, leaving the unmethylated PCR product intact. The digest greatly reduces the background of vector-only colonies from the ligation mixtures, increasing the likelihood of obtaining plasmids containing the desired insert (Figure 4).
BNMR Lab helps characterize new chaperones involved in proteasome assembly

The Bio-Molecular NMR (BNMR) Core Laboratory helps non-NMR experts to apply NMR to their research problems. In this case Jeroen Roelofs, assistant professor of biology from Kansas State University, was interested in characterizing two newly identified chaperons involved in assembly of a multi-protein complex called the proteasome. These chaperones (Nas2 and Hsm3) bind to a small region of AAA+ATPases known as C-domain of Rpt1-Rpt6 proteins. No structure-function analysis of Nas2 and Rpt5-C domain protein had been conducted previously, so the BNMR staff assisted Roelofs to learn more about how these proteasome assemble.

One goal was to understand how a chaperone such as Nas2 binding impacts the ability of Rpt proteins to interact with other partners in the proteasome subunits. Once the BNRM staff determined that the sizes of Nas2 (24.8 kDa) and Rpt5-C domain protein (10 kDa) were amenable for NMR studies, the Protein Production Group Laboratory expressed, purified and isotopically (15N and 15N, 13C) enriched Nas2 and rpts-C-domain proteins independently. A 200 uM concentration of 15N labeled Nas2 in 500 uL volume was added to an NMR tube. Using the BNMR 800 MHz NMR instrument the staff ran a 20-minute 2D-1H-15N-HSQC NMR experiment to check the structure (protein folding) and stability of the purified protein. In another NMR tube the staff added 500 uL of 300 uM concentration of 15N labeled Rpt5-C domain protein. As in the case of Nas2 the staff ran the same 2D-1H-15N-HSQC NMR experiment to check its structural stability. Excellent preliminary results were obtained with the initial NMR screening. In the next step, the staff successfully solved the high resolution solution structure of Rpt5-C domain by biological solution NMR approach (Figure 5). Roelofs included these NMR results in a recent NIH grant application.

Bio-Molecular NMR Laboratory Services

This laboratory enables both the neophyte and the expert to use NMR to answer important questions about protein structure, dynamics and intermolecular interactions. It provides a full range of training and services in high-field NMR, including consultation in experimental design and data interpretation, training in instrument use, access to instruments for qualified users, and data acquisition for pilot studies. The laboratory has two instruments capable of performing virtually any advanced experiment:

- Bruker Avance 800 MHz: TCI cryoprobe/TXI-RT probe
- Bruker Avance III 600 MHz: TXI-RT probe/13C,15N,31P– 19F and all the way to silicon NMR Broad Band probe

Our staff can provide assistance from 1D to 4D- NMR data acquisition; high resolution structure determination in solution; nD-NMR data processing, analysis, and interpretation; H/D exchange measurements by NMR; NMR titration measurement; protein–protein, protein-DNA, protein-peptide interaction studies; fast motion dynamics by 15N-T1, 15N-T2 and 15N-{1H}NOE and micro second to milli second dynamics by relaxation dispersion NMR experiments, and consultation on proposal preparation.

At a Glance: COBRE Core Laboratories Publications

BioNMR Laboratory


PPG Laboratory


PSL Laboratory


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 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) (5P20RR017708-10) and the National Institute of General Medical Sciences (8P20GM103420-10).