



Protein Structure Laboratory

**The University of Kansas
Protein Structure Laboratory (PSL)**

Dr. Weijun Huang, Director

www.psl.ku.edu

A Core Laboratory of the
Center of Biomedical Research Excellence in
Protein Structure and Function

Robert P. Hanzlik, PI

050215

1



Protein Structure Laboratory

PSL Contact Information:

Dr. Weijun Huang

115 Structural Biology Center

office: 785-864-3772

lab: 785-864-3787

weijun@ku.edu

www.psl.ku.edu

Dr. Robert P. Hanzlik

Director, COBRE-PSF

4048 Malott Hall

tel. 785-864-3750

rhanzlik@ku.edu

2



Protein Structure Laboratory

The PSL will perform or assist investigators with any or all of the following functions:

- Attempt crystallization
- I solate and mount crystals
- Assess crystal quality
- Collect a complete data set locally and/or at synchrotron
- Phase determination
- Solve the structure
- Build protein model
- Refine structure
- Presentation graphics

Important note: Individual investigators are responsible for purification and concentration of their own protein to make it ready for crystallization. For assistance in large-scale protein expression and purification, contact COBRE Core C Protein Purification Group.

www.medchem.ku.edu/COBRE/Core_C_brochure_050215.pdf

3



Protein Structure Laboratory

In addition the PSL will provide:

- Advice on design of protein constructs for expression and crystallization.
- Training of students and/or lab personnel in any or all crystallographic and data analysis techniques available in the PSL.
- Assistance in the preparation of manuscripts and grant applications dealing with crystallography and structure determination.
- In short, this lab is designed and equipped to handle every job from training neophytes to tackling large complex protein structure problems.

4



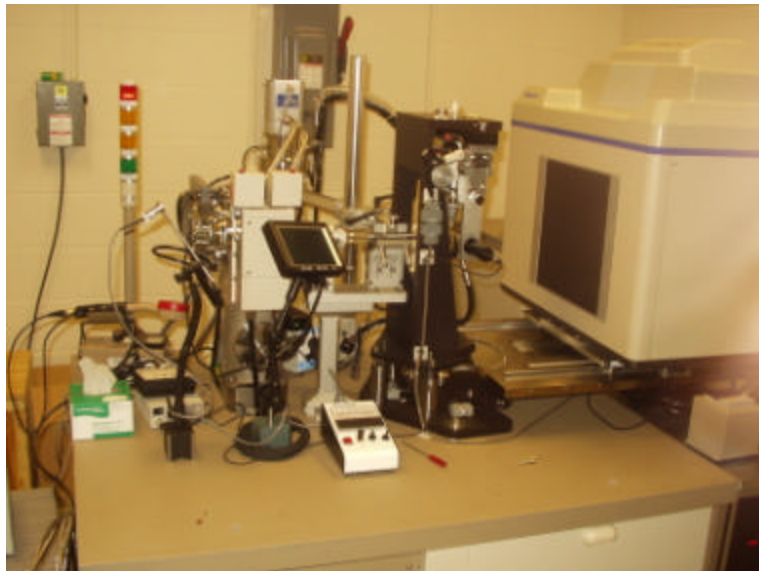
Protein Structure Laboratory

Major equipment holdings of the PSL:

- 18 kW high brilliance rotating anode generator (Rigaku RU-H3RHB)
- Blue Max-Flux (Osmic) confocal optical system
- Two-theta stage and inverse Phi axis
- R-Axis IV++ imaging plate system
- X-stream cryogenic system
- Dell Workhorse workstation to operate the diffractometer and collect data. CrystalClear Software for data collection/analysis.
- Two Dell Server Workstations with crystallographic and modeling software for data processing and model building and refinement (CCP4, CNS, O, and others) and a Color Laser Printer.

5

Rotating Anode Generator - 2-Theta Stage - Image Plate Detector



6



Protein Structure Laboratory

Additional PSL Equipment:

- Crystallization incubators (2) and Stereo microscopes (2)
- Mini-centrifuge for concentrating protein
- Crystallization kits, reagents, pipettors
- Cary 50 UV-Vis spectrophotometer
- Access to shared Dynamic Light Scattering detector for monitoring protein aggregation during concentration.
- Crystallography Reference Library

In short, we are a well-equipped lab!

7



Protein Structure Laboratory

Who can access PSL services? **Everyone!**

The KU-PSL is here to make protein structure determination accessible to everyone. Let us help you bring (protein) structure to your life!

How can I access PSL services?

The first step is to contact the Director to discuss the following:

| | |
|---|--|
| project goals literature background materials needed work required division of responsibilities | timeline and milestones deliverables publication plan likely costs of project |
|---|--|

8



Protein Structure Laboratory

Is there a cost for PSL services?

The PSL and its staff are supported in part by a COBRE Grant from NIH-NCRR; other costs must be recovered from clients served.

See www.medchem.ku.edu/COBRE/ for current rate information.

Every attempt is made to keep the fees low and the service of high professional quality. Thus all users are billed for 1) consumables, 2) maintenance of the X-ray generator, and 3) professional staff time. Rates for generator and staff time are subsidized by COBRE-PSF.

COBRE participants and other junior faculty researchers may be able to receive additional cost subsidies for their use of the PSL (subject to availability of funds; please enquire).

9



Protein Structure and Function

What does it cost to determine a protein structure?

The cost is proportional to several factors:

- A. The number of amino acid residues in the protein.
- B. The number of protein molecules per asymmetric unit.
- C. The resolution afforded by the crystal. Better crystals give better data which require *less* effort to analyze and yield *better* final results.
- D. The number of data sets needed to solve the structure. Totally new structures usually require several independent data sets, but structures related to known structures can usually be solved quickly from one good data set.

For specific examples see the next two frames.

10



Protein Structure and Function

Example 1. Methionine aminopeptidase (265 aa).

Five different structures of MetAP crystallized with various metal ions and small molecule inhibitors were determined over a 6 month period using a total of 203 crystallization plates, 336 hours of X-ray generator time and 105 hours of PSL Staff time for a total cost of ca. \$9800 plus protein (ca. 150 mg protein used). For further details of one structure determined at 1.52Å resolution see: Ye Q., Xie S., Huang M., Huang W., Lu J., Ma Z. (2004) Metalloform-Selective Inhibitors of E. coli Methionine Aminopeptidase and X-ray Structure of a Mn(II)-Form Enzyme Complexed with an Inhibitor *J. Am. Chem. Soc.*, **126**, 13940.

11



Protein Structure and Function

Example 2. S-Adenosylhomocysteine Hydrolase. SAHH (438 aa) was crystallized with NAD plus a small molecule inhibitor. The crystals had 2 molecules of protein (876 aa) per asymmetric unit. To obtain the structure by molecular replacement required one good crystal from 10 crystallization plates, 100 hours of X-ray beam time and 63 hours of PSL staff time for a total cost of \$5300 plus protein (ca. 7 mg in this case). The structure was solved to a resolution of 1.93Å and coordinates were deposited to the Protein Data Bank. Manuscript in preparation. The overall project required about 2 months time.

12