

Biological  
X-ray Absorption  
Spectroscopy and  
Non-crystalline Diffraction  
at the BioCAT beamline at the  
Advance Photon Source

Raul A. Barrea  
BioCAT/APS,  
Illinois Institute of Technology



BioCAT

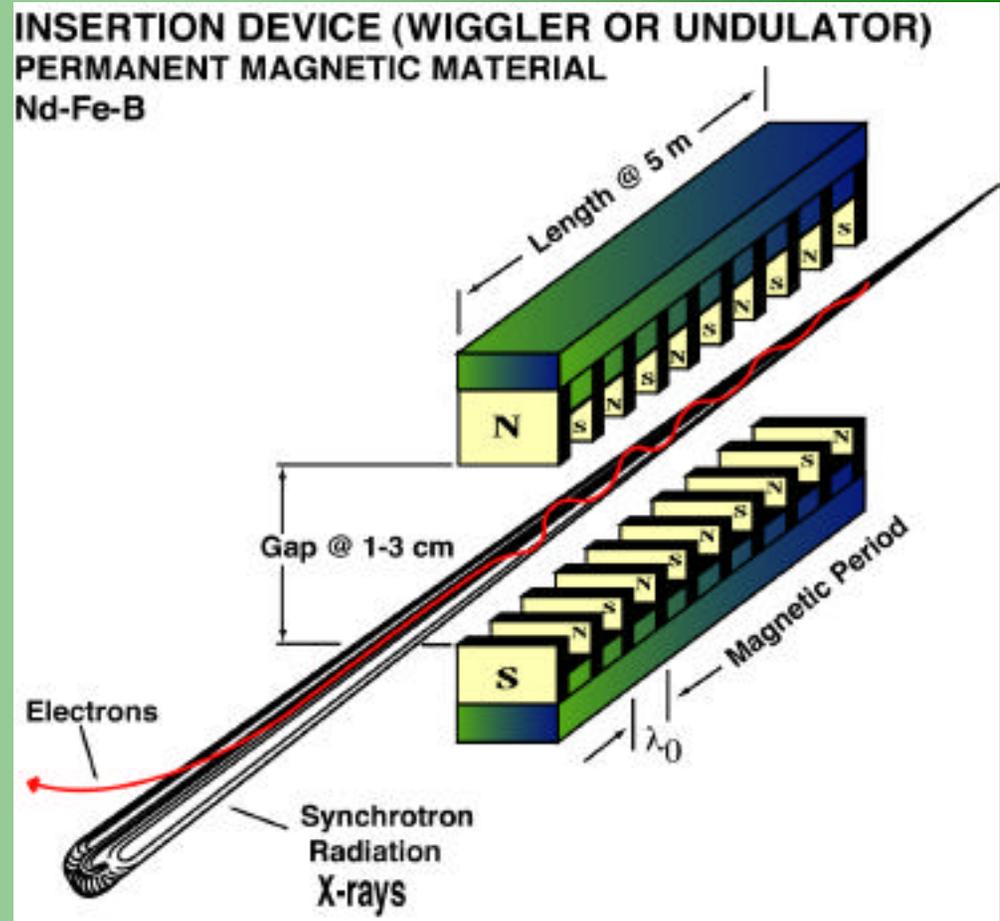
A NIH Supported Research Center

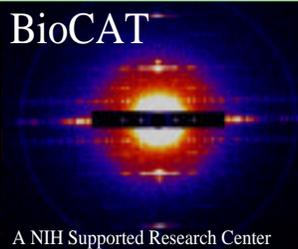
# The Advanced Photon Source



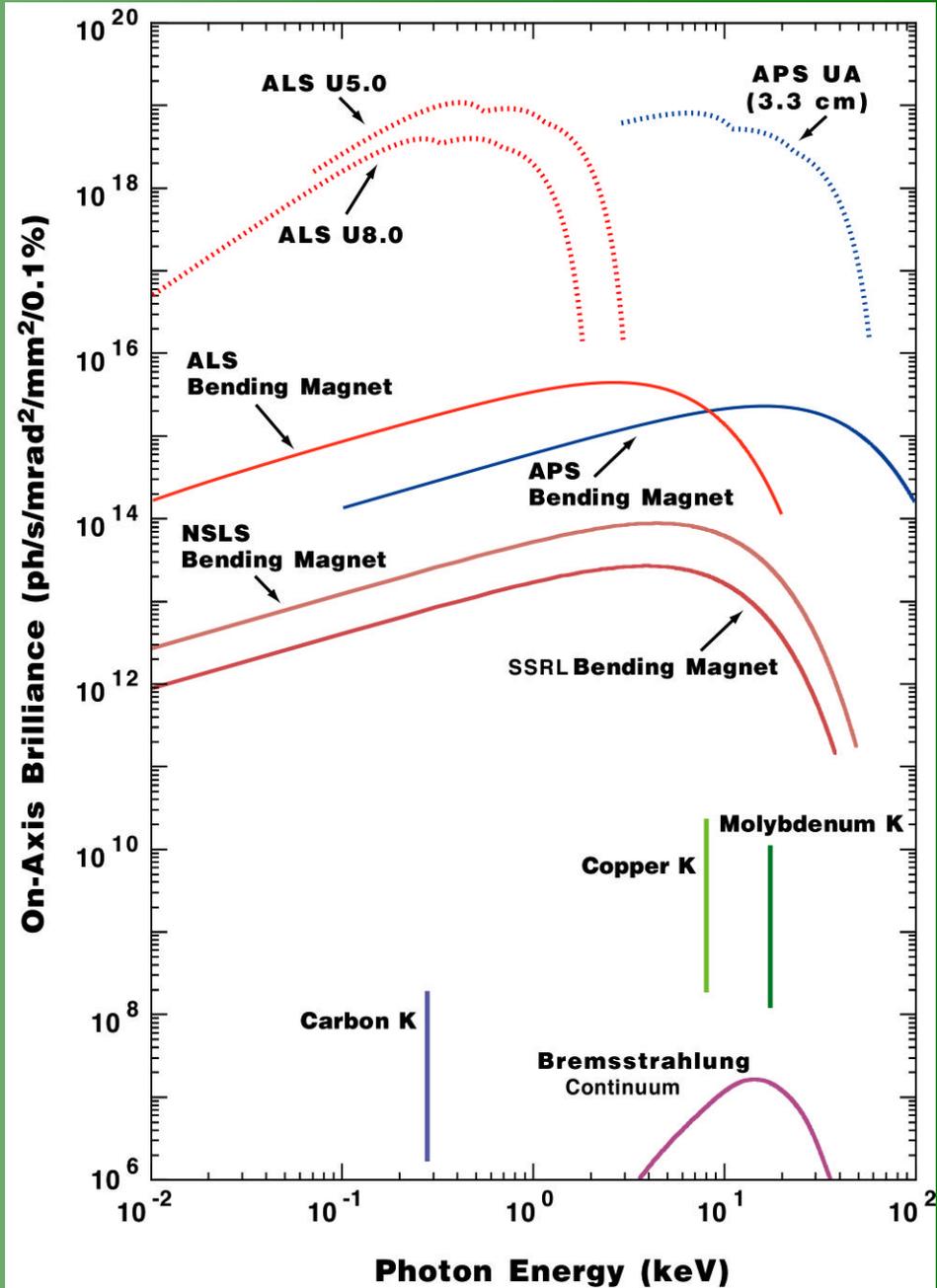
# The APS is Optimized for Producing Undulator Radiation

- Total X-ray flux  $1-6 \times 10^{13}$  photons/s
- Source size is  $9.7 \times 220 \mu\text{m}^2$

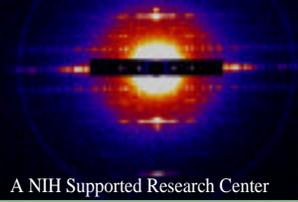




# Undulators are Very Bright Sources of X-rays

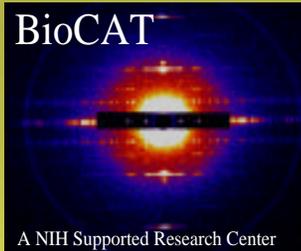


BioCAT



# Why Is APS Undulator Radiation Good for Biological Studies?

- Wide energy range available for spectroscopy
- High flux for time resolved applications
- Very low beam divergence for high quality diffraction patterns
- Can focus to very small beams to examine small samples or regions within samples



# What is BioCAT?

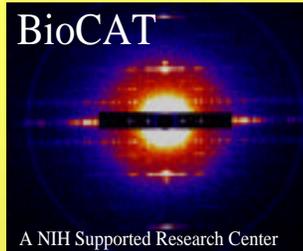
- » A NIH-supported research center for the study of partially ordered and disordered biological materials
- » Comprises an undulator based beamline (18-ID), associated laboratory and computational facilities.
- » Available to all scientists on basis of peer-reviewed beamtime proposals

BioCAT

A NIH Supported Research Center

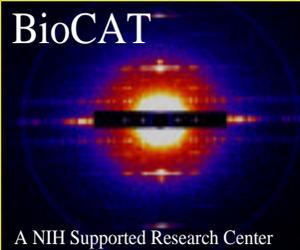
## Supported Techniques:

- X-ray absorption spectroscopy
- Small angle scattering from macromolecules in solution
- Diffraction from membrane systems
- Biomedical imaging
- Fiber Diffraction of complex biological tissues



# The BioCAT Sector at the APS





# Beamline Optics

Cryogenically cooled  
Si (111) or Si (400)  
first crystal

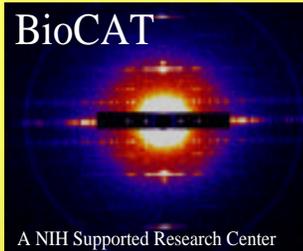
Sagittally focusing second crystal 7:1  
maximum source demagnification.

Fixed Exit .

**Energy range** - 3.5 keV – 39 keV,  
resolution -  $2 \times 10^{-4}$ .



Elliptically bent vertical focusing mirror  
11:1 maximum source demagnification



# Beamline Facilities



6 m flight tube camera



Experimental Table



Huber Goniometer



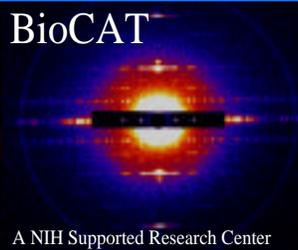
CCD detector



VME Crates



Displex closed cycle cryostat

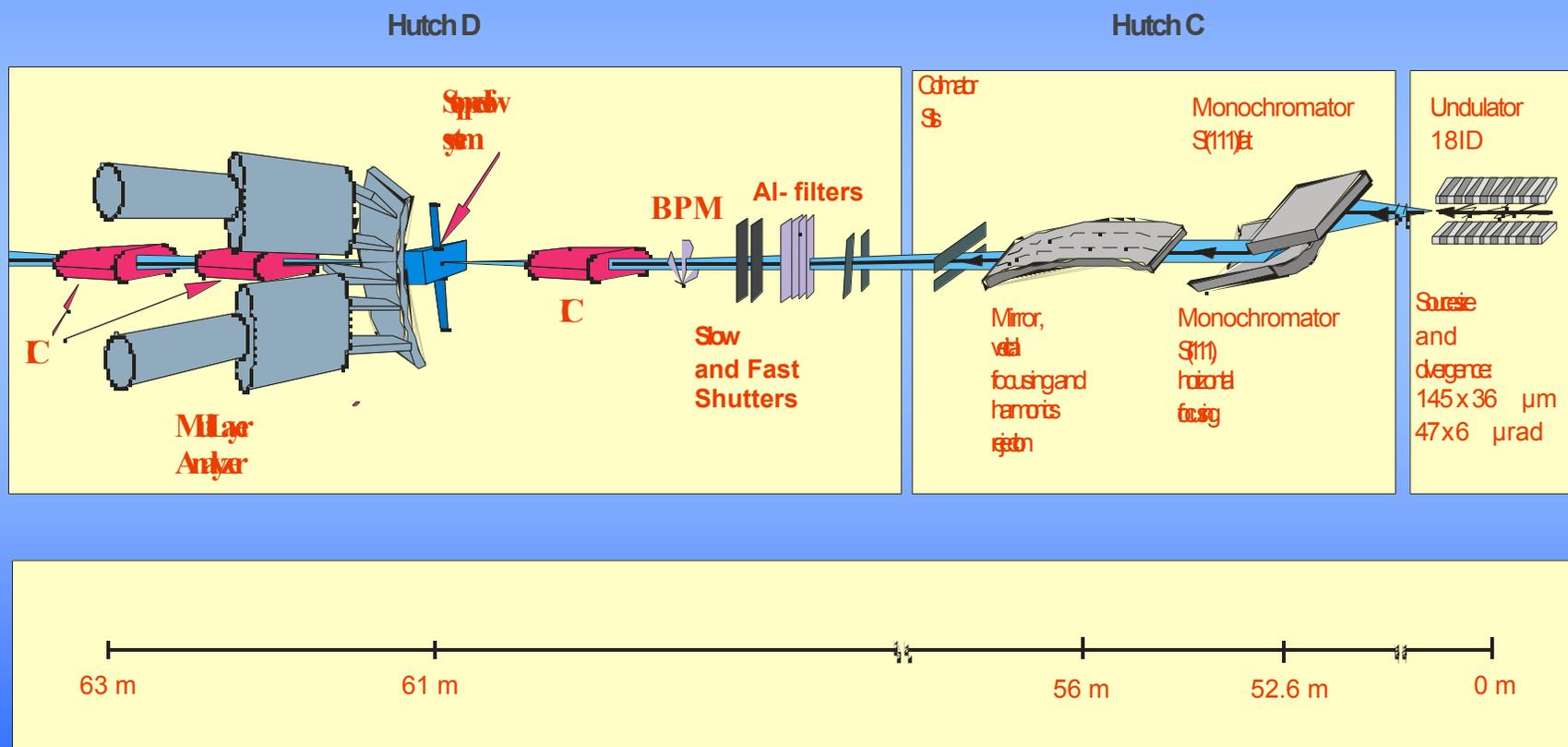


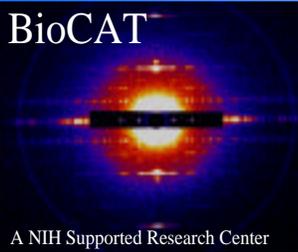
# X-ray Absorption Spectroscopy

BioCAT XAS contact: Tom Irving, Director  
Ke Zhang, Beamline Scientist  
Raul Barrea, Beamline Scientist

XAS user groups: James Penner-Hahn et al. (UMichigan)  
Steven Cramer et al. (LBL)  
Jeffrey Zaleski et al. (IndianaU)  
Timothy L. Stemmler et al., Wayne State University  
Chanoch Carmeli et al., (Tel Aviv Univ, Israel)  
Irit Sagi, Weizmann Inst. Of Science, Israel  
and others.

## XAS Set up at the BioCAT 18ID Undulator Beamline

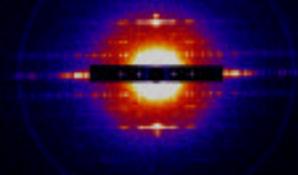




# Advantages for XAS at BioCAT

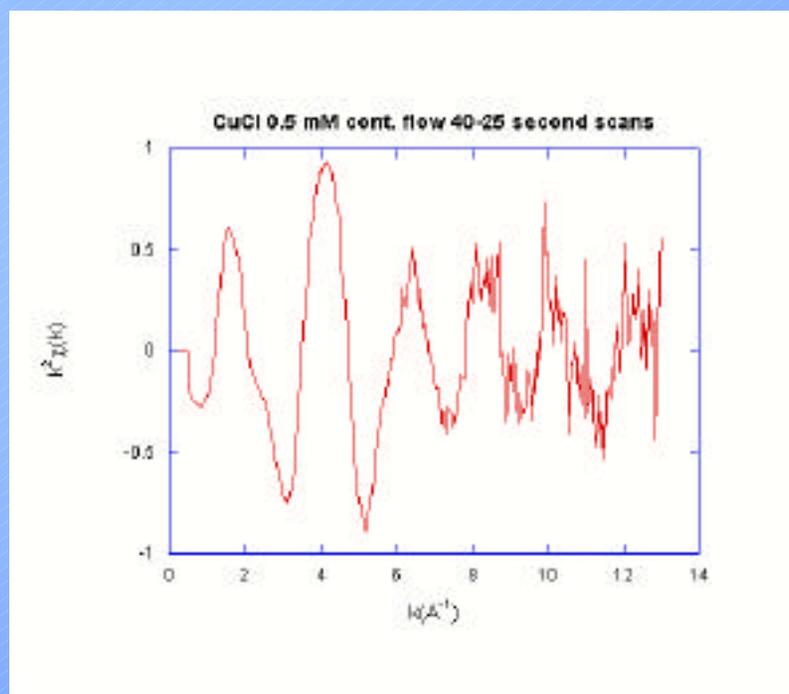
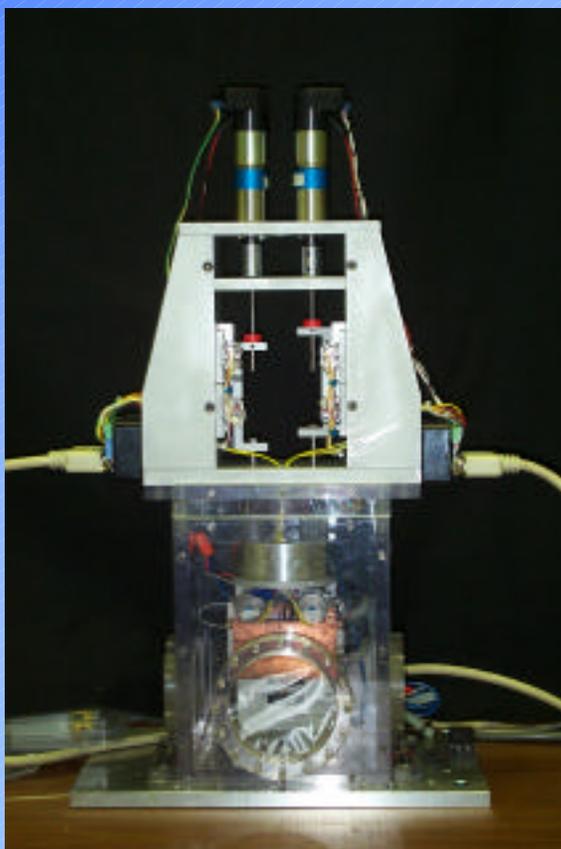
- Double focused beam (v:100umx h:200um)
- Beam stability (40umx40um) (10umx10um under development)
- Sample concentration 100uM-1mM
- Small sample volumes (100uL)
- Displex close cycle cryostat (30 °K)
- Stop-flow system (20-40uL)
- Multilayer analyzer, Bent Laue Analyzer, Lytle detector.
- Training is provided for new users.

BioCAT

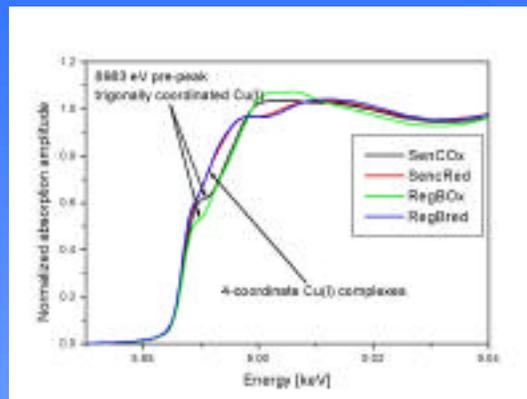


A NIH Supported Research Center

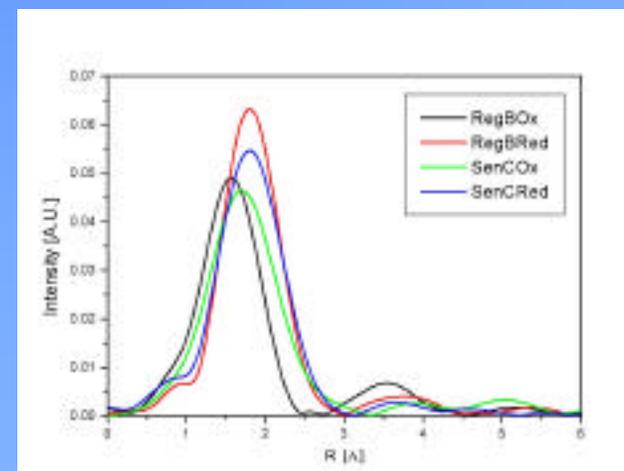
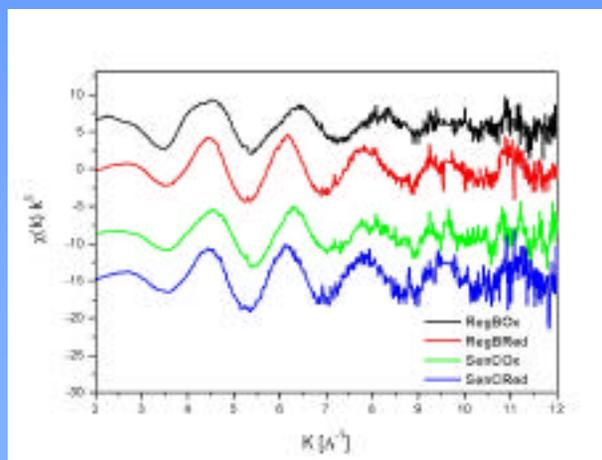
# Stop-flow device for flow mode XAS at room temperature

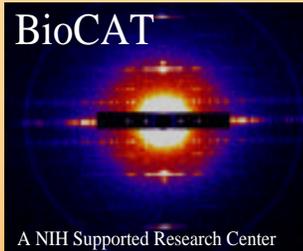


# Autophosphorylation Mechanism of the Copper binding proteins RegB and SenC.



RegB and SenC are considered responsible of redox controlled synthesis of the bacterial photosystem, respiratory chain, carbon and nitrogen fixation enzymes.





# Small Angle Scattering

**BioCAT SAXS contact: Tom Irving, Director**

**Elena Kondrashkina, Beamline Scientist**

SAXS user groups: Nick Menhart et al. (IIT)

Tobin Sosnick et al. (UOC)

Pappannan Thiyagarajan et al. (ANL/IPNS)

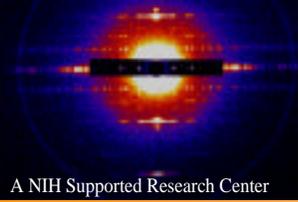
Lee Makowski et al. (ANL)

Jill Trewhella et al. (LANL)

Susan Taylor et al. (UC)

Steven Almo (AECOM)

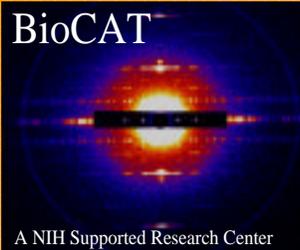
Braian Shilton et al. (UWO) and others.



# Why SAXS?

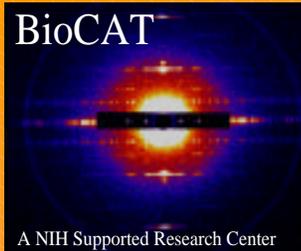
- ❖ Powerful technique for extracting large-scale structural parameters from macromolecules in solution
- ❖  $R_g$  ratio of gyration, distance distribution  $P(r)$  function can be used to test hypotheses concerning large scale changes in solution.
- ❖ Potential for low resolution 3-D electron density maps
- ❖ High flux allows time resolved experiments on ms time scales or less





# BioCAT Facilities for SAXS

- ✓ **Working size of doubly focused beam** –  $145 \times 40 \mu\text{m}^2$  (FWHM), divergence -  $0.19 \times 0.16 \mu\text{rad}^2$ .
- ✓ **Temperature controlled sample flow cell** with mixing provided by two-syringe pump. Stopped flow device.
- ✓ **Scattering chambers** length of 5 m ( $0.0004 < q < 0.1 \text{ \AA}^{-1}$ ), 2 m ( $0.006 < q < 0.23 \text{ \AA}^{-1}$ ), 0.25 m ( $0.07 < q < 2 \text{ \AA}^{-1}$ ), combined data covering  $q$  range  $0.0004 \text{ \AA}^{-1} < q < 2 \text{ \AA}^{-1}$ .
- ✓ **Beam stop contains** a pin-diode for measuring transmitted beam intensity.
- ✓ **CCD detector:**  $50 \times 90 \text{ mm}^2$  working area,  $50 \mu\text{m}$  spatial resolution,  $\sim 1$  X-ray photon sensitivity.



# Advantages for SAXS at the BioCAT

## Beam characteristics:

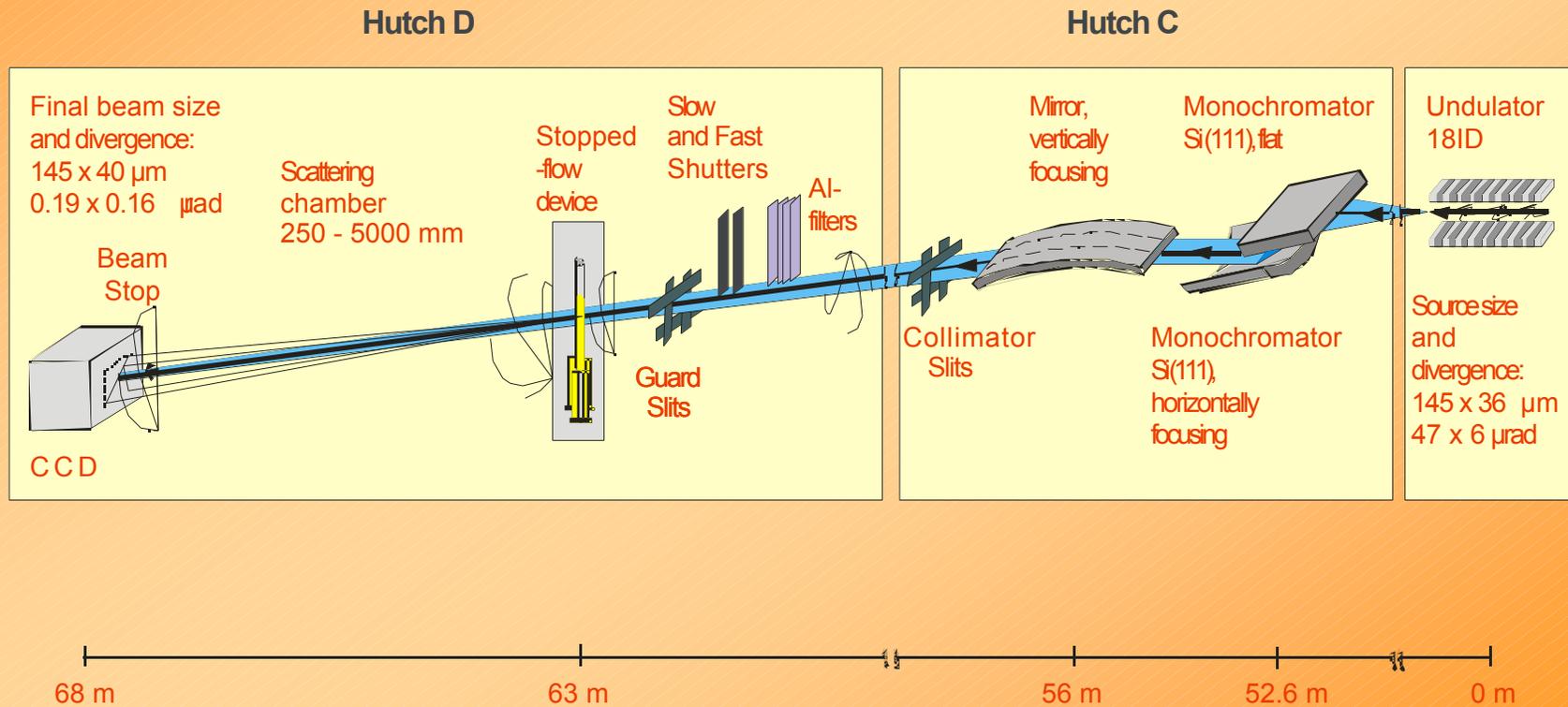
- **High brilliance** ( $10^{13}$  photons/s on the sample)
- **Doubly-focused beam**
- **With excellent positional stability.**

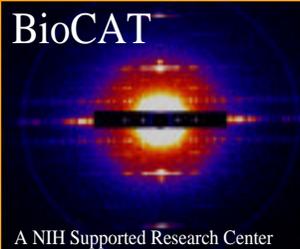
## Protein experiment characteristics:

- Possibility to study **low concentration** of proteins  $\sim 1$  mg/ml.
- **Low quantity of sample** required - 100  $\mu$ l.
- Sample flow reducing radiation damage.
- **Short exposure times** -  $\sim 1$  s.
- Time-resolved measurements in **millisecond scale** using stopped flow device are in progress.

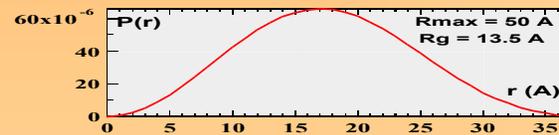
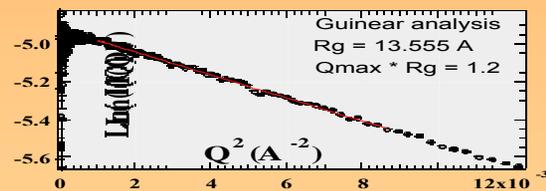
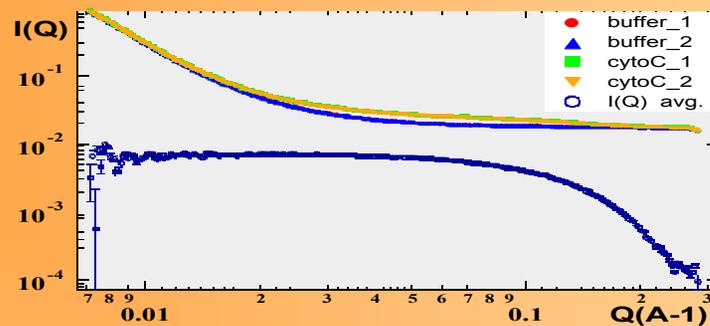
## Workshops

## SAXS Set up at the BioCAT 18ID Undulator Beamline





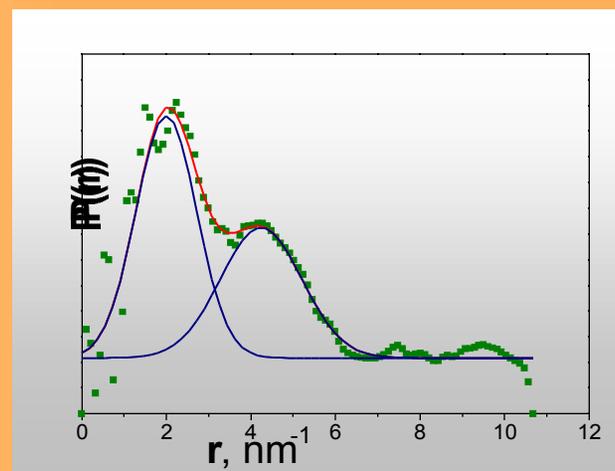
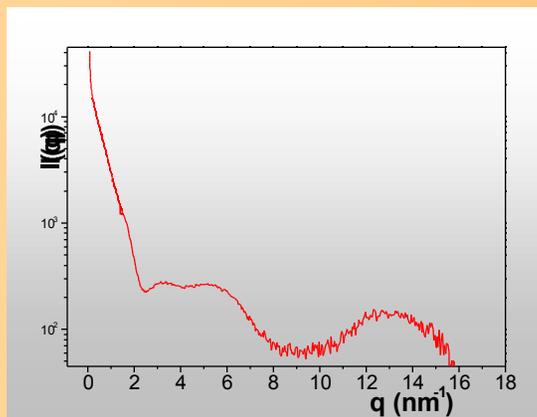
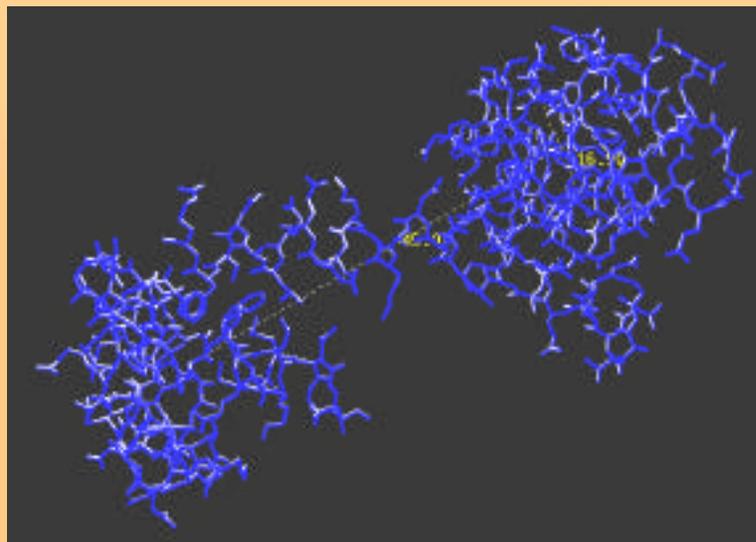
# Cytochrome C



BioCAT

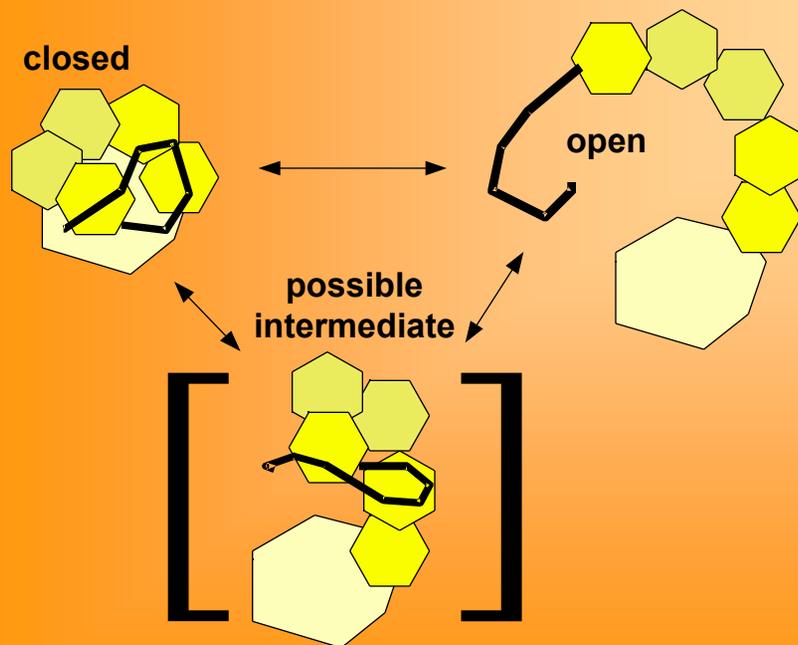
A NIH Supported Research Center

# Troponin C



# Plasminogen

*A multi-domain zymogen present in the blood that undergoes a large scale conformational rearrangement. The kringle domains are known to bind lysine and lysine analogues such as *e*-aminocaproic acid, EACA, that induce a so-called “open” form of Pg. Previous SAXS work shown that EACA changes the  $R_g$  of Pg from  $\sim 30\text{\AA}$  to  $\sim 50\text{\AA}$ .*

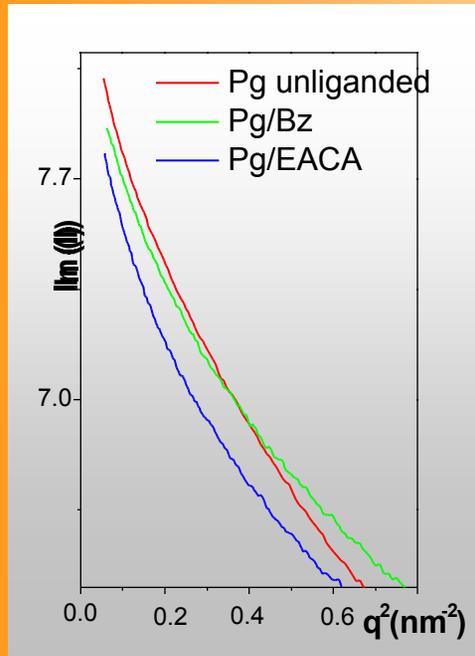


*A ligand specific for only the fifth kringle, benzamidine, Bz was also shown to induce a form with  $R_g \sim 40\text{\AA}$ . On the other hand, if Bz merely perturbs the equilibrium between the open and closed forms so that the equilibrium constant is near unity, the Bz data set should be a linear function of the open and closed forms.*

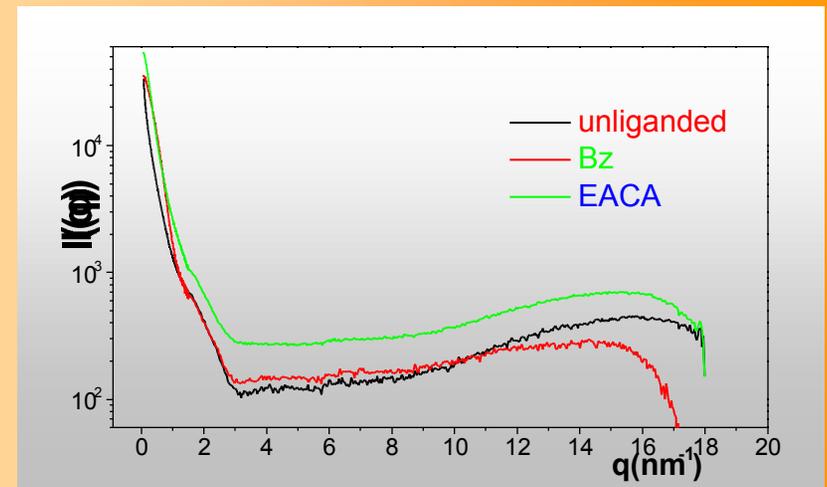
# Plasminogen samples

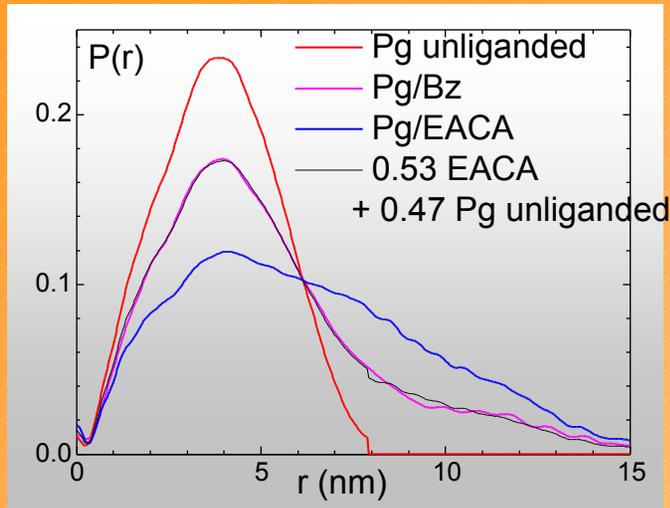
*Plasminogen samples at ~3 mg/ml in the presence or absence of EACA and Bz (30mM) were measured.*

*Guiner analysis: although the unliganded Pg yielded a fairly linear plot of  $\ln(I)$  vs.  $q^2$ , and an  $R_g$  of ~31 Å, the liganded form curves were substantially non-linear, indicating that the sample was either heterogeneous or non-spherical.*



*$P(r)$  analysis confirmed that the liganded forms were indeed more asymmetrical, having significant high tails. The  $R_g$ 's obtained from these  $P(r)$  curves were 29.5, 42.1 and 50.4 Å for the unliganded, Bz and EACA containing forms.*



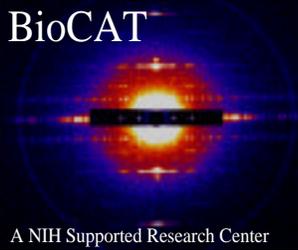


*In order to assess the uniqueness of the Bz form, we attempted to simulate it as a linear combination of  $r(r)$  unliganded and  $r(r)$  EACA. It appears that this is possible, raising the likelihood that this state is an equilibrium mixture, and not a true unique conformer.*

*The data is consistent with the Bz liganded form of Pg being a combination the open and closed forms. A unique shape cannot so far be detected.*

*Since Bz is specific for only one kringle, addition of excess Bz does not further shift the equilibrium since the other kringles are insensitive to this ligand. This may mimic a unique conformer, but is a distinct situation.*

BioCAT



A NIH Supported Research Center

# Muscle Diffraction

BioCAT Muscle Diffraction contact: Tom Irving, Director

Muscle Diff. Users:

D. Maughan, U. Vermont,

Andrew Miller, Univ. Stirling

Andrew Hammersley, ESRF

Matthias Aurich, Rush Univ.

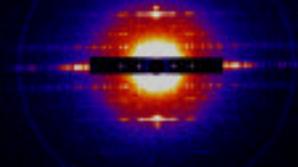
Juergen Mollenhauer, Rush Univ.

Joseph Orgel, Univ. Stirling

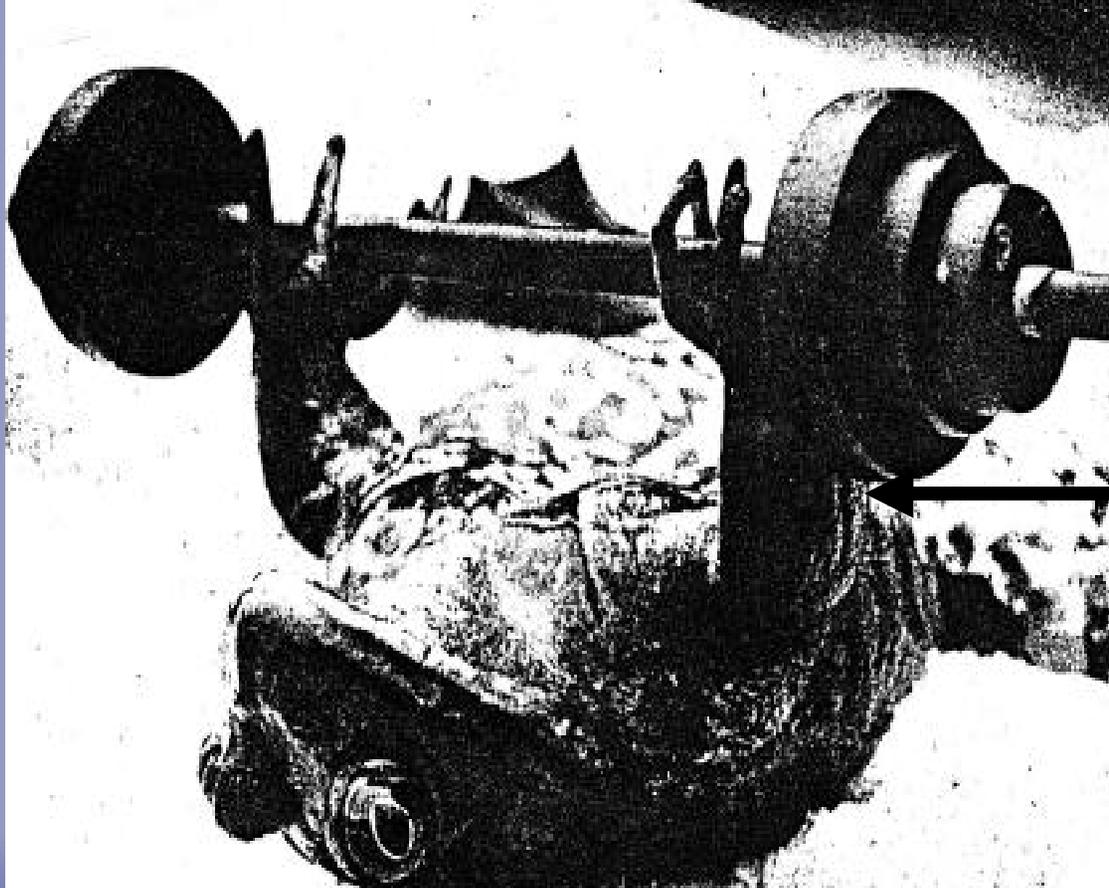
Tim Wess, Univ. Stirling

P.deTombe, Univ. Illinois at Chicago

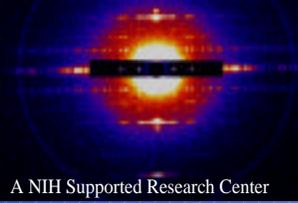
BioCAT



A NIH Supported Research Center

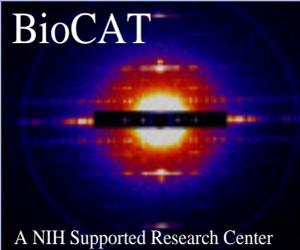


X-ray  
Beam

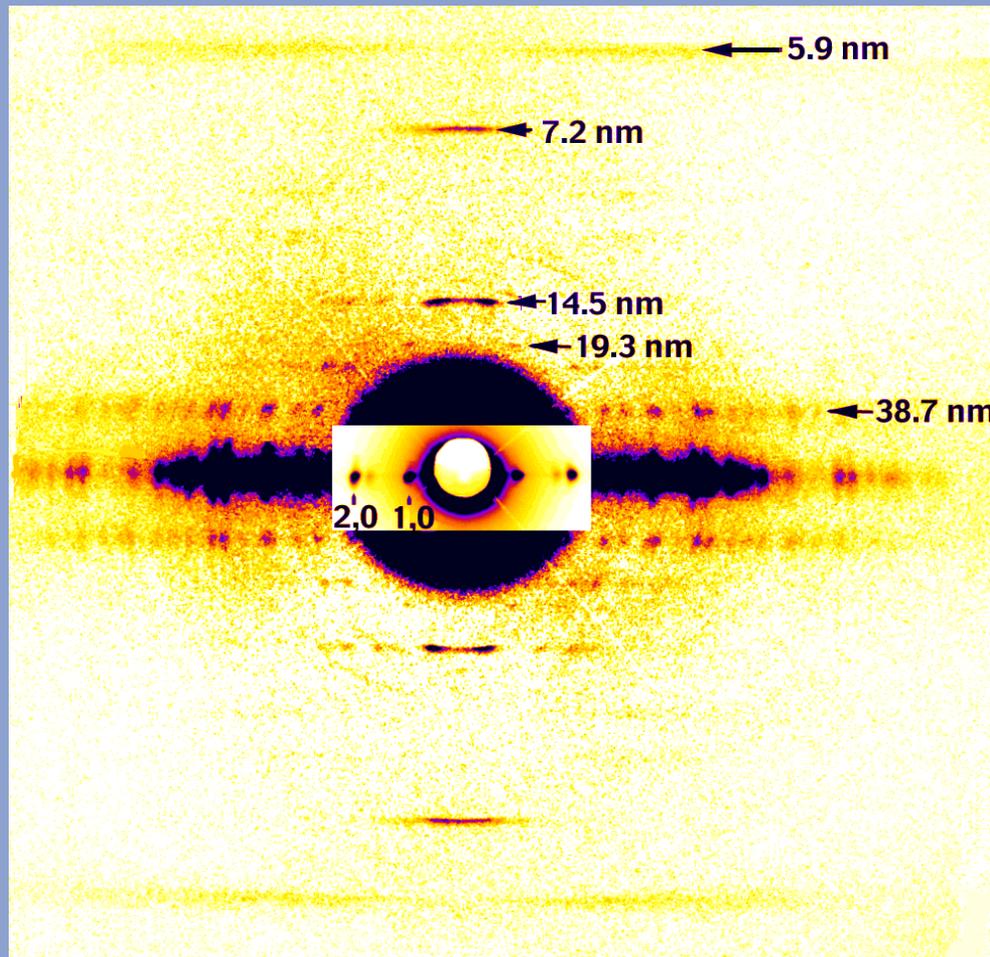


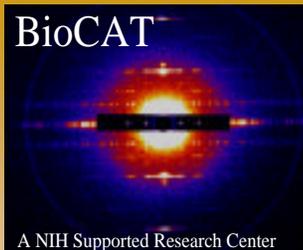
# Time resolved X-ray diffraction from live *Drosophila* during tethered flight

- *Drosophila* wing beat frequency  $\sim 200$  Hz
- Were able to obtain informative X-ray fiber patterns at the top and the bottom of the wing beat cycle ( 1 ms time resolution)



# First 2-D diffraction patterns from living *Drosophila* IFM





# BioCAT is funded by the:



NIH



NCR



IIT



CSRRI



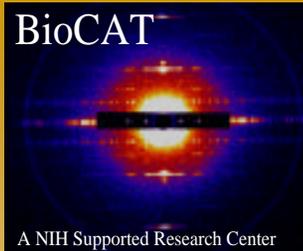
APS



ANL

**Home page <http://www.bio.aps.anl.gov/>**

Funding by NIH RR08630



# BioCAT staff



Tom Irving (director)



David Gore



Elena Kondraskina



Raul Barrea



Ke Zhang



Rich Heurich



Claren Krolik



Grant Bunker (former director)