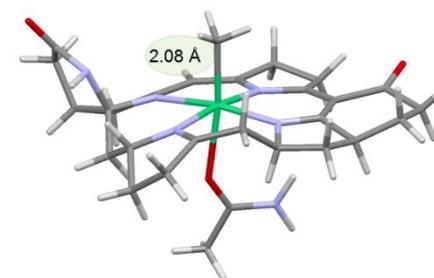
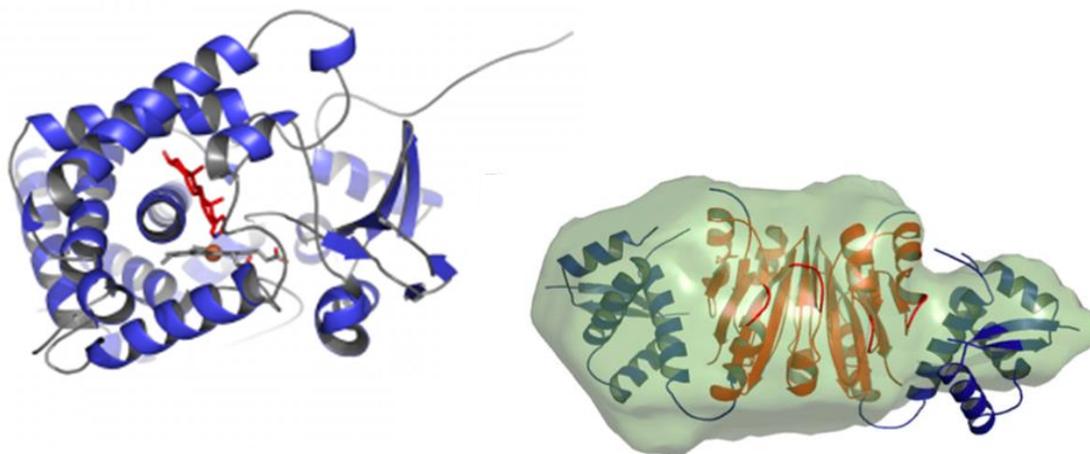


# Workshop on Small-Angle X-ray Scattering and Diffraction Studies

March 28-30, 2016

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**Britt Hedman**  
Professor and SSRL Science Director

*The SMB program is funded by DOE-BER, NIH-NIGMS, PRT and Collaborative Partners*

# Program

## MONDAY, MARCH 28, 2016

MONDAY, MARCH 28, 2016		
8:00AM	Refreshments	
8:30AM	Welcome	Thomas Weiss (Stanford/SSRL)
8:45AM	Introduction to the SSRL - SMB Program	Britt Hedman (Stanford/SSRL)
9:30AM	Biological Small Angle Scattering	Thomas Weiss (Stanford/SSRL)
10:30AM	Break and Refreshments	
10:45AM	Synchrotron SAXS Instrumentation	Ivan Rajkovic (Stanford/SSRL)
11:30AM	BioSAXS: Practical Considerations	Thomas Weiss (Stanford/SSRL)
12:15PM	Lunch Break	
1:15PM	Visit to SSRL BL4-2 & start of remote data collection	BL4-2 staff
3:00PM	Break and Refreshments and Software Installation	
3:30PM	Data Reduction and Preliminary Analysis	Ivan Rajkovic (Stanford/SSRL)
4:15PM	How to Judge Data Quality	Tsutomu Matsui (Stanford/SSRL)
5:15PM	Adjourn	

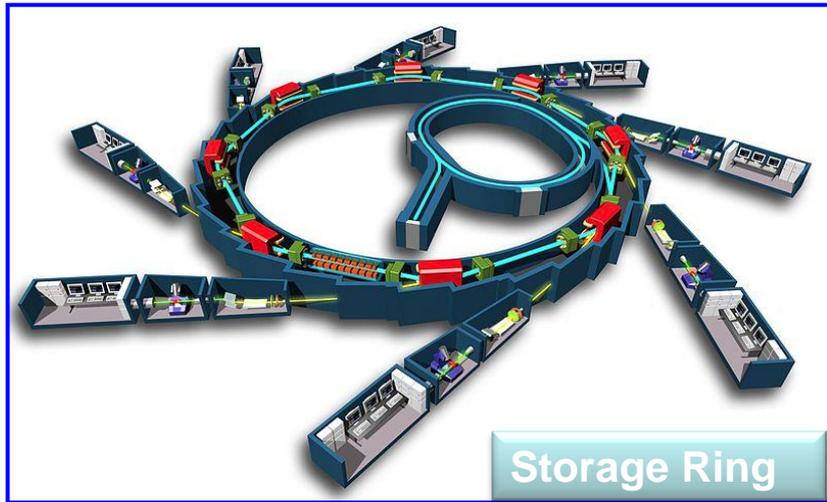
# Program

TUESDAY, MARCH 29, 2016		
8:00AM	Refreshments & Announcements	
8:30AM	Introduction to Analytical software: What Can You Do Using BioSAXS?	Tsutomu Matsui (Stanford/SSRL)
9:30AM	Integrative Structural Modeling with Small Angle X-ray Scattering Profiles	Seung Joong Kim (UC San Francisco)
10:30AM	Break and Refreshments	
10:45AM	Introduction to Time-Resolved SAXS	Thomas Weiss (Stanford/SSRL)
11:45AM	SAXS, NMR and Crystallography Reveal the Activation Mechanism	Allyn Schoeffler (Spring Hill College)
12:00PM	Lunch Break	
1:00PM	Better Data with SEC-SAXS	Tsutomu Matsui (Stanford/SSRL)
2:00PM	MultiFoXS: Modelling Heterogeneous Samples with SAXS Data	Daniel Saltzberg (UC San Francisco)
3:00PM	Break and Refreshments	
3:30PM	Data Analysis and Q/A	BL4-2 Staff
5:30PM	Adjourn	
6:30pm	Dinner	

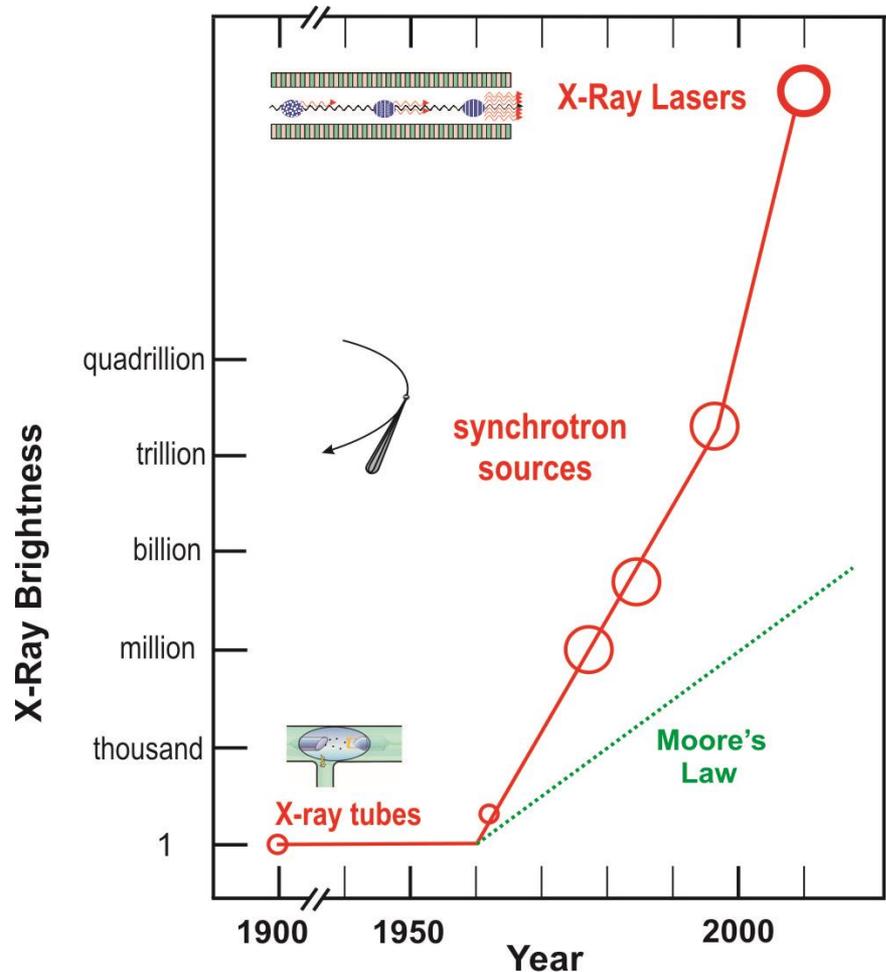
# Program

WEDNESDAY, MARCH 30, 2016		
8:00AM	Refreshments and Announcements	
8:30AM	Structure and Dynamics of Adapter Proteins the Interface of ell Membrae and Actin Cytoskeleton	Zimei Bu (City College of NY)
9:30AM	Uncovering Macromolecule Conformation Ensembles with X-ray Scattering Interferometry	Xuesong Shi (Stanford)
10:30AM	Break and Refreshments	
10:45AM	US-SOMO HPLC-SAXS Module: Dealing with Capillary Fouling, and Extraction of Pure Component Patterns from Poorly Resolved SEC-SAXS Data	Alexey Savelyev (UT San Antonio)
12:30PM	Lunch Break	
1:30PM	Creating Structural Models from SAXS Data Using the FoXS Suite (tutorial)	Daniel Saltzberg (UC San Francisco)
3:00PM	Break and Refreshments	
3:30PM	Data Analysis, Q&A	BL4-2 Staff
5:00PM	Close of Workshop	

# Synchrotron Radiation – A Revolution in X-ray Science (1974 ⇒ )

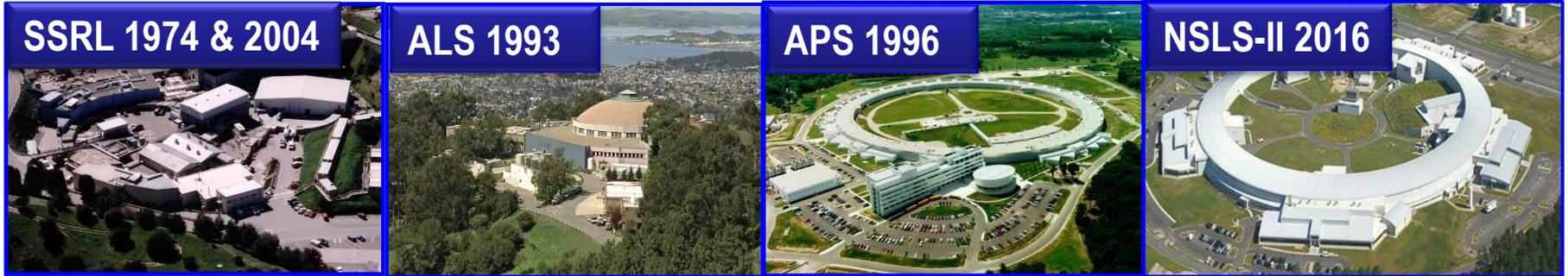


- Circular electron storage rings
- Wide x-ray wavelength range and high average brightness, picosecond pulses
- Properties make synchrotron radiation (SR) uniquely useful for structural biology research



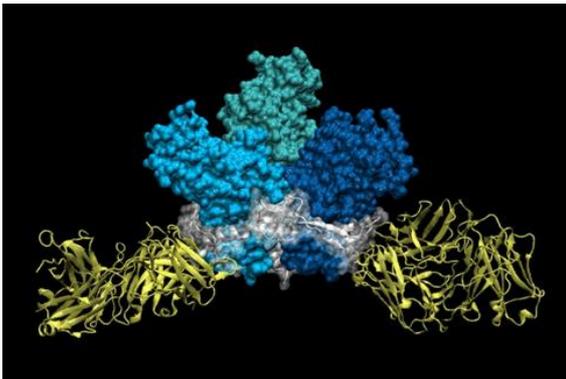
# Synchrotron Lightsources and Structural Biology

## - a Revolution for Life Science



- Four operating DOE BES-funded synchrotron lightsources in the U.S. (of 47 total worldwide)
- Structural biology instrumentation at all 4 lightsources – NIH, DOE-BER, non-federally funded
- Cooperation to provide R&D and access for biology studies to the science community

### *Synchrotron x-rays and the life sciences*



Ebola virus glycoprotein-AB structure  
(Sapphire and collaborators, TSRI)

- Extreme brightness enables study of biological structures and bioimaging with multiple techniques/methodologies
- About 35% of research done at U.S. SRs is in the life sciences
- About 90% of the structures in the Protein Data Bank were solved using SR
- DOE SR facilities had ~3500 (1/3 of total) life sciences investigators in FY15 (includes LCLS x-ray laser to be discussed later)
- Extensive robotics, automation and remote access enable high throughput from home institutions

# Synchrotron Radiation - How is it Practically Produced and Used for Research?

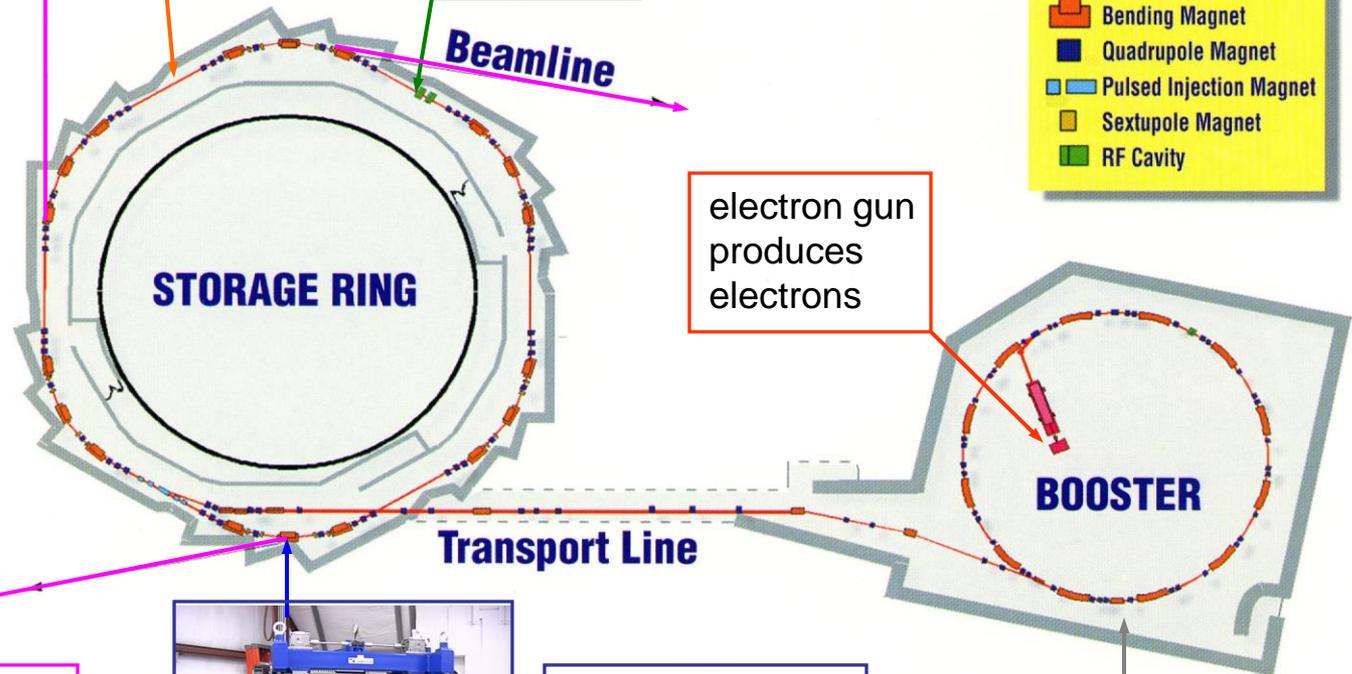


the storage ring circulates electrons and where their path is bent - synchrotron radiation is produced

klystrons generate high power radiowaves to sustain electron acceleration, replenishing energy lost to synchrotron radiation



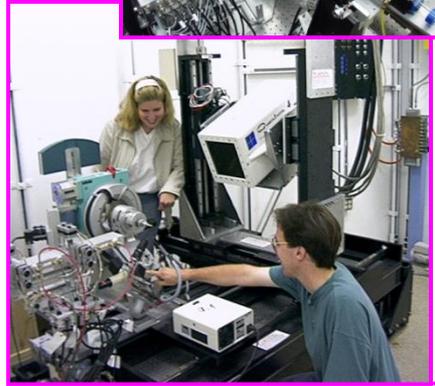
- Bending Magnet
- Quadrupole Magnet
- Pulsed Injection Magnet
- Sextupole Magnet
- RF Cavity



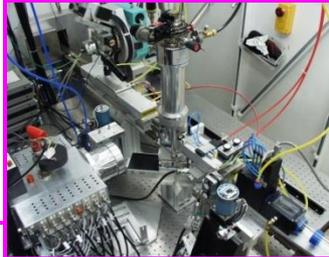
electron gun produces electrons

linear accelerator/booster accelerate  $e^-$  which are transported to storage ring

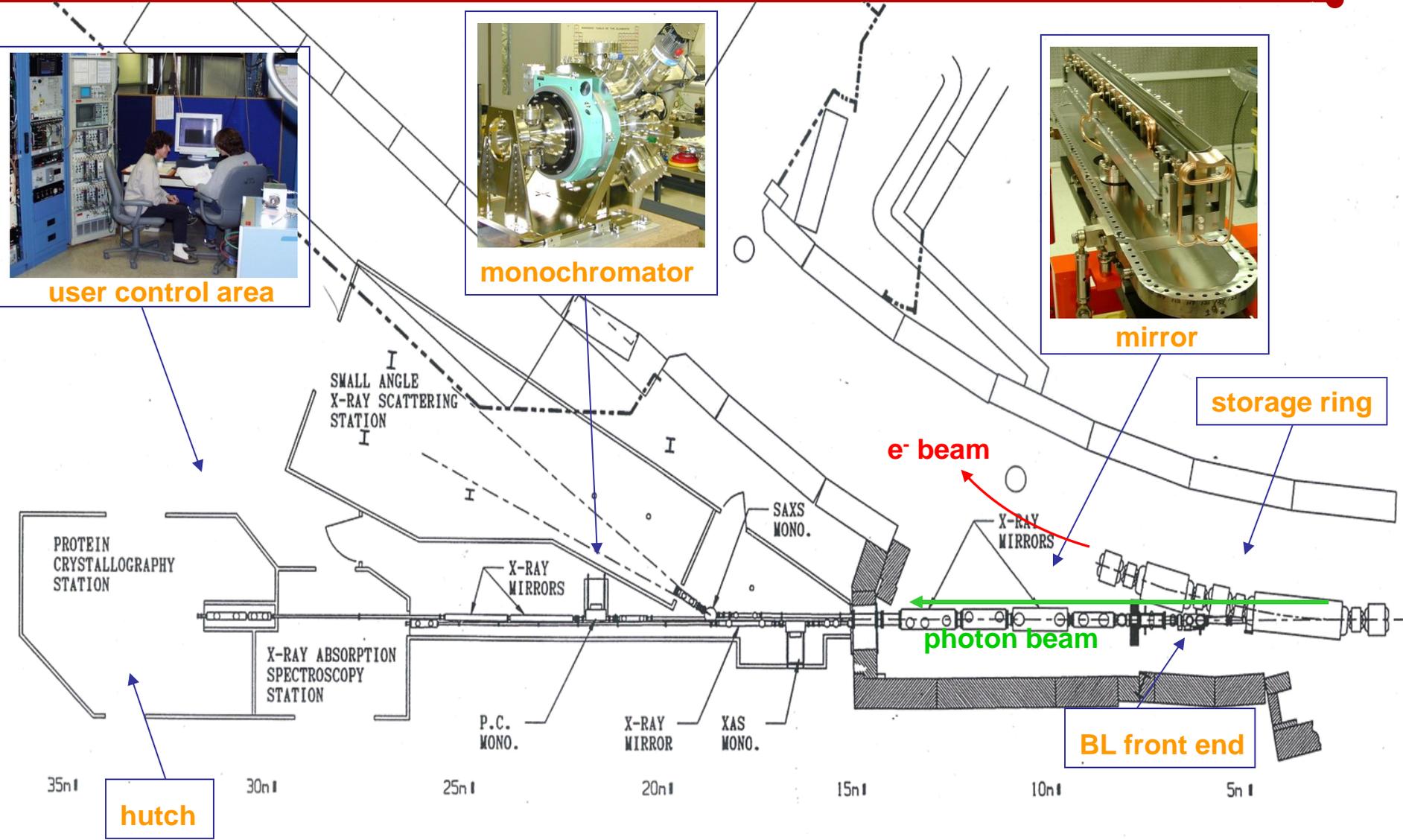
special "wiggler" insertion devices used to generate x-rays



beam lines transport radiation into "hutches" where instrumentation is available for experiments

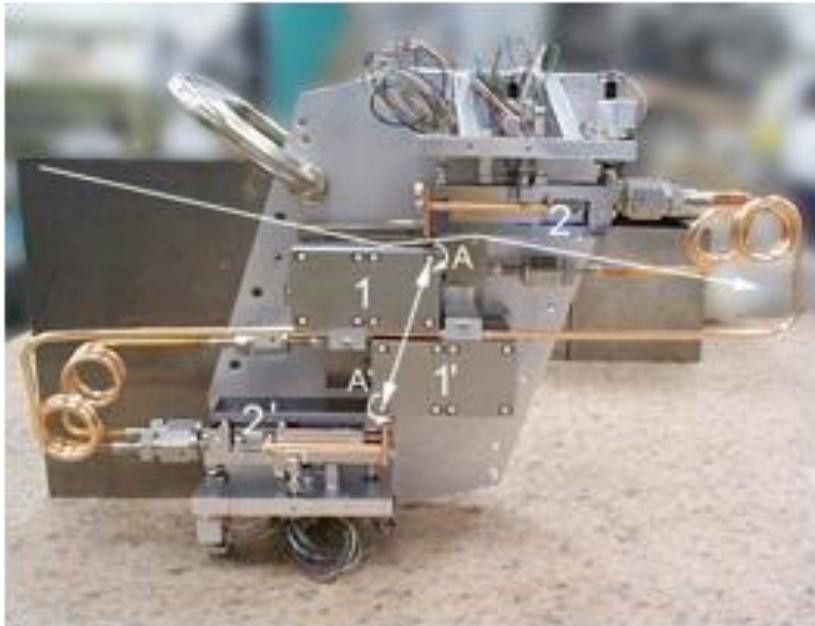
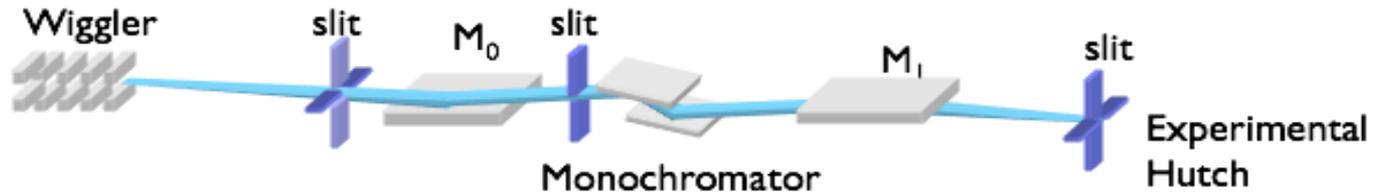


# Beam Lines - Delivering the Photons to the Experimenters - What Are They?

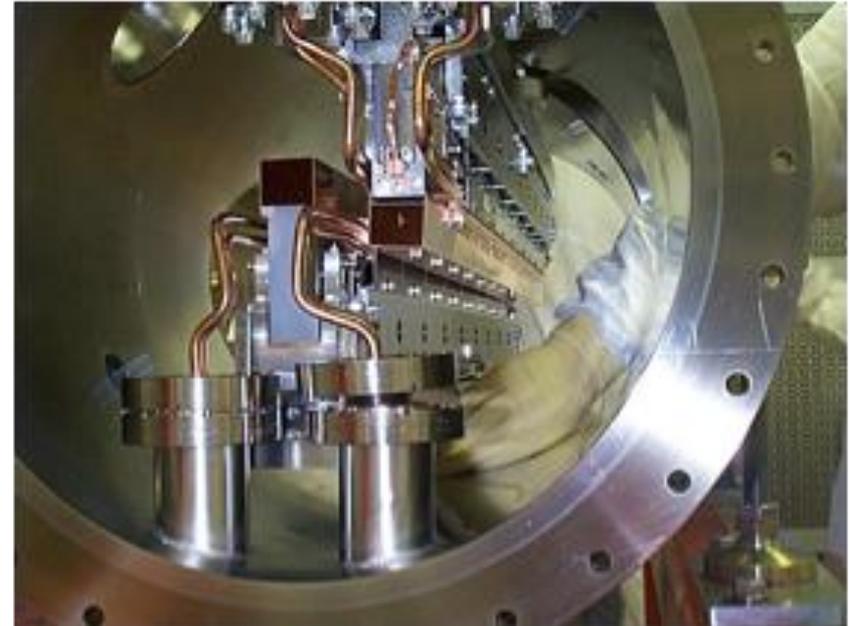


Typical wiggler beam line with multiple (3) branches

# Beam Line Components

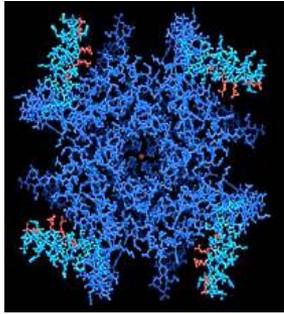


SSRL BL9-3 LN<sub>2</sub>-cooled Si crystal monochromator



SSRL BL9-2 and BL9-3  $M_0$  water-cooled Si mirrors

# Synchrotron X-ray Methodologies Combine to Provide Unique and Powerful SMB Insights

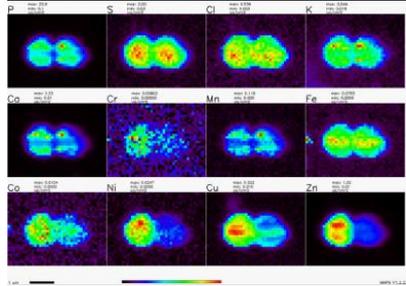
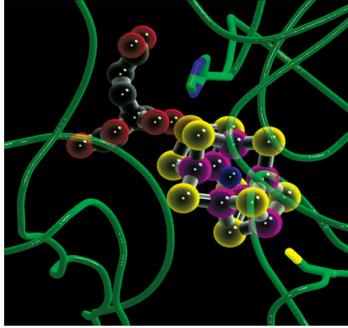


**X-ray  
Crystallography**

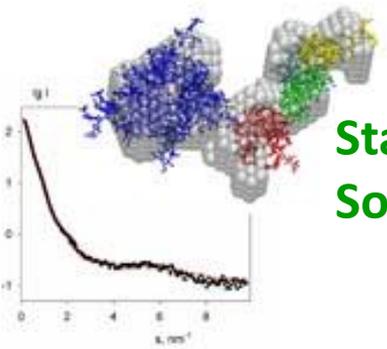


**Time-resolved  
X-ray Crystallography**

**X-ray Spectroscopy**

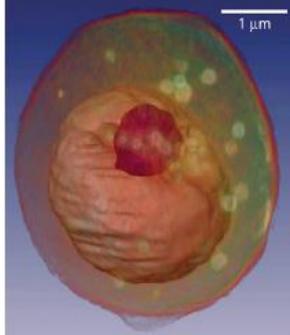
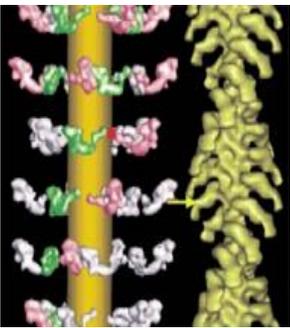


**X-ray Fluorescence  
Spectroscopy Imaging**



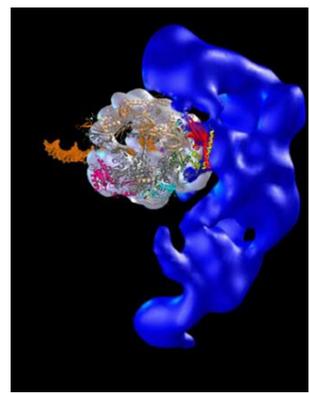
**Static and Time-resolved  
Solution X-ray Scattering**

**X-ray  
Fiber  
Diffraction**

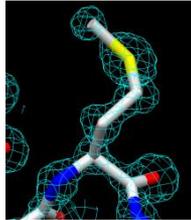
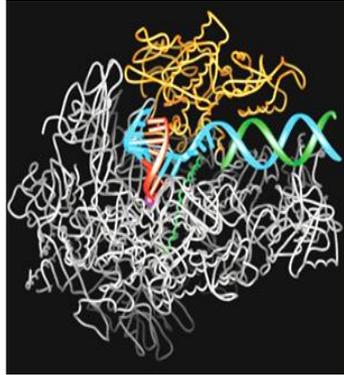


**X-ray Microscopy**

**Integrated X-ray and  
other Methodologies**



# SR X-rays - a Seminal Tool in the Study of Structure and Function



*With structural biology, as with many other systems -*

- **Function** follows **Form**
- In biology - study of shapes (form or structure) and how it changes - leads to understanding of how living things work (function)
- X-rays are one of primary means to obtain direct insight into the atomic-level structure of proteins and other macromolecules
- X-rays provide information about the electronic structure in active sites
- Synchrotron radiation, beginning in the mid seventies, enabled a revolution in this field

## **Small Angle X-ray Scattering**

- Protein folding & conformational changes
- Complex structures in solution
- Low resolution

## **Macromolecular Crystallography**

- Atomic resolution, 3-D structure of macromolecules and complexes
- Structure/function but in crystalline state - no dynamics – but coming

## **X-ray Absorption/Emission Spectroscopy**

- Metal centers in proteins
- Reaction intermediates
- Electronic structure
- Imaging - spatial co-location
- Dynamics

High brightness 3<sup>rd</sup> generation undulator sources ⇒ **strong growth in imaging and spectromicroscopy**

# Brief Background of the SSRL Structural Molecular Biology (SMB) Program

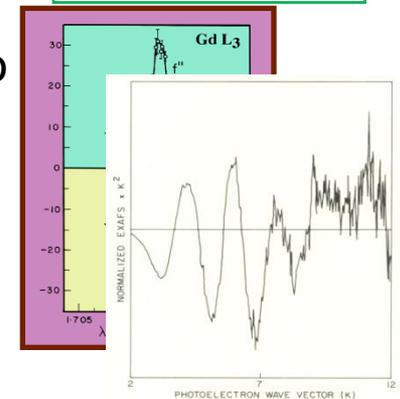


- SSRL began operation in 1974 - the first storage ring-based x-ray SR user facility in the world
- Early on - pioneering methodology developments for SMB & first applications in several areas:



First SSRL "hutch"

- Fundamentals on anomalous dispersion techniques - evolving to MAD phasing in crystallography
- Development of x-ray absorption spectroscopy and the study of metallo-enzyme active site structure



- Led to establishment at SSRL of first SR-based US Structural Biology Center
- Scientific thrusts today in **macromolecular crystallography, small angle x-ray scattering, x-ray absorption/emission spectroscopy, x-ray imaging**
- Developments at SSRL and elsewhere have catalyzed explosive growth worldwide of SR-based SMB science over past two decades

# Structural Biology – Early Synchrotron Success (1<sup>st</sup>/2<sup>nd</sup> Generation)



Reprinted from  
*Proc. Nat. Acad. Sci. USA*  
Vol. 73, No. 1, pp. 128–132, January 1976  
Biophysics

1974-75

## Applications of synchrotron radiation to protein crystallography: Preliminary results

(x-ray diffraction/anomalous dispersion/rubredoxin/azurin/nerve growth factor/glutaminase-asparaginase)

JAMES C. PHILLIPS, ALEXANDER WLODAWER, MARGUERITE M. YEVITZ, AND KEITH O. HODGSON\*

Department of Chemistry and Stanford Synchrotron Radiation Project, Stanford University, Stanford, California 94305

Communicated by Richard H. Holm, October 23, 1975

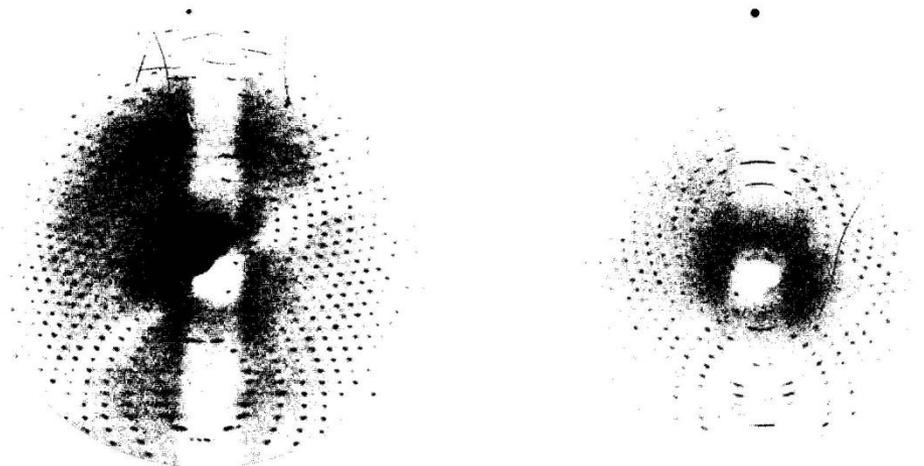


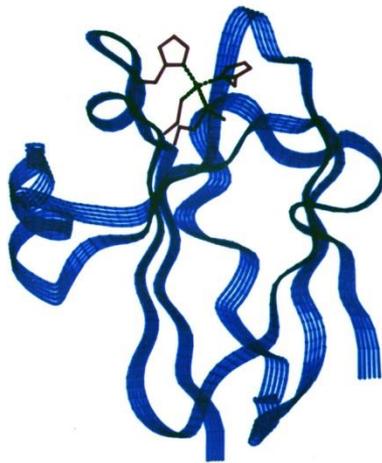
FIG. 3. Cone-axis oscillation photographs of the same azurin crystal. Precession angle  $6.5^\circ$ , oscillation angle  $20^\circ$ . (left) Synchrotron source,  $E = 3.7 \text{ GeV}$ ,  $I = 40 \text{ mA}$ , only electrons present,  $\lambda = 1.740 \text{ \AA}$ , exposure time 10 min. (right) Philips fine-focus sealed Cu anode tube, operated at 40 kV, 30 mA, exposure time 6 hr, Ni filter.

- Measured much shorter data collection times -  $60x <$  fine focus tube
- Reported successful use of smaller crystals
- Observed anomalous scattering effects at Fe (rubredoxin) and measured effects of good collimation
- Reported striking observation that higher intensity of SR offered significant advantages with regard to recording higher quality data to higher resolution

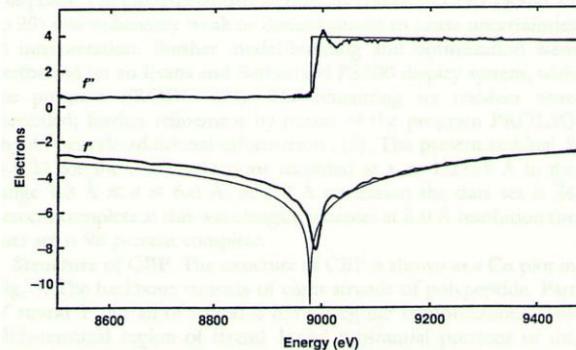
# Structural Biology – Early Synchrotron Success (1<sup>st</sup>/2<sup>nd</sup> Generation)

1985-87

Reprint Series  
12 August 1988, Volume 241, pp. 806–811



SCIENCE



**Fig. 1.** Energy dependence of the anomalous dispersion terms  $f''$  and  $f'$  in the region of the CuK absorption edge. Values of  $f''$  and  $f'$  are in electrons. Experimental values for  $f''$  (heavy line) were obtained from x-ray fluorescence from a single crystal of CBP; ideal  $f''$  values (thin line) for atomic Cu are from (58). Experimental values for  $f'$  are derived by numerical integration from the  $f''$  spectrum with the Kramers-Kroenig relation; ideal  $f'$  values (thin line) are from Hönl theory (59). Derivation of the experimental  $f''$  and  $f'$  values was performed with an in-house program DISCO (60).

## Phase Determination by Multiple-Wavelength X-ray Diffraction: Crystal Structure of a Basic “Blue” Copper Protein from Cucumbers

J. MITCHELL GUSS, ETHAN A. MERRITT,\* R. PAUL PHIZACKERLEY, BRITT HEDMAN, MITSUO MURATA,† KEITH O. HODGSON, AND HANS C. FREEMAN

- One of earliest successes in high resolution “de novo” MAD structure using synchrotron radiation
- MAD data collection at SSRL BL1-5 with MWPC – 4 wavelengths – required **8 days beam time!**
- Phasing of the 10kDa protein using native Cu atom gave excellent map at  $\sim 2.5$  Å resolution

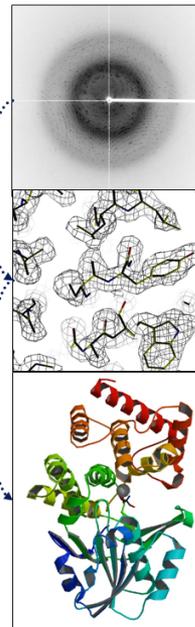
# Macromolecular Crystallography – from Four Decades Ago to Today



## – from Five Decades Ago (x-ray tubes) to Current (SR)



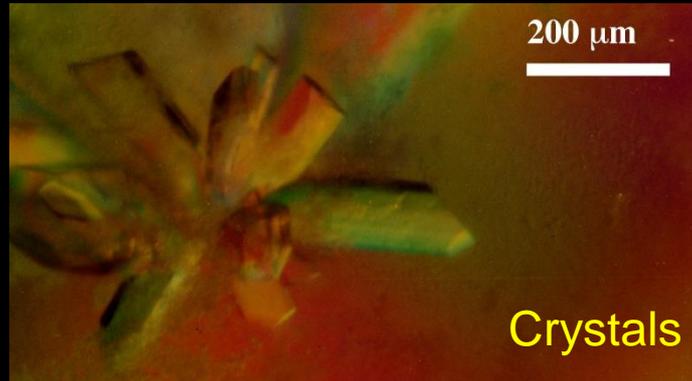
- David Phillips presents the 3-D structure of lysozyme to the Royal Society in 1965
- A single protein structure was the project taking many years
  - ⇒ 1959 Myoglobin
  - ⇒ 1965 Lysozyme
  - ⇒ 1967 Chymotrypsin, Ribonuclease, Carboxypeptidase



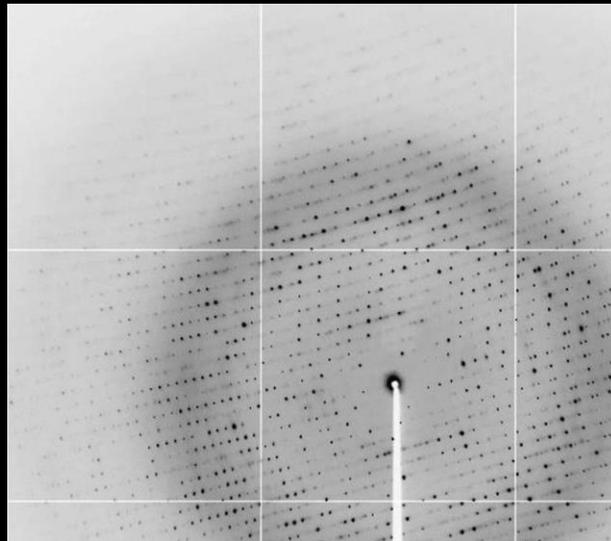
Time (mins)	Event
0.00	Mount crystal
4.00	Collect test shot
35.00	Determine initial orientation matrix + strategy
45.00	Perform fluorescence scan and CHOOCH
55.00	Determine exposure time
1.08.00	Start MAD collection to 2.0Å resolution
1.35.00	Finish collection of inflection point wavelength
1.36.00	Process inflection point wavelength data
1.55.00	Start SHAKE-AND-BAKE
2.06.00	Finish collection of remote wavelength
2.22.00	Process remote wavelength data
2.29.00	Cross-check SHAKE-AND-BAKE results
3.03.00	Start MLPHARE job
3.06.00	Finish MLPHARE and DM job
3.44.00	Check bones and maps in O
3.45.00	Start WARPNTTRACE to 2.0 Å resolution
3.50.00	Kill MAD data collection experiment
5.40.00	Finish data collection to 1.5 Å resolution
11.14.00	Complete trace of 360 / 364 amino acid residues

- Today** - Key elements enabling this development
- ⇒ scientific techniques and technologies
  - ⇒ instrumentation and automation
  - ⇒ computational advances
  - ⇒ training and dissemination into the community

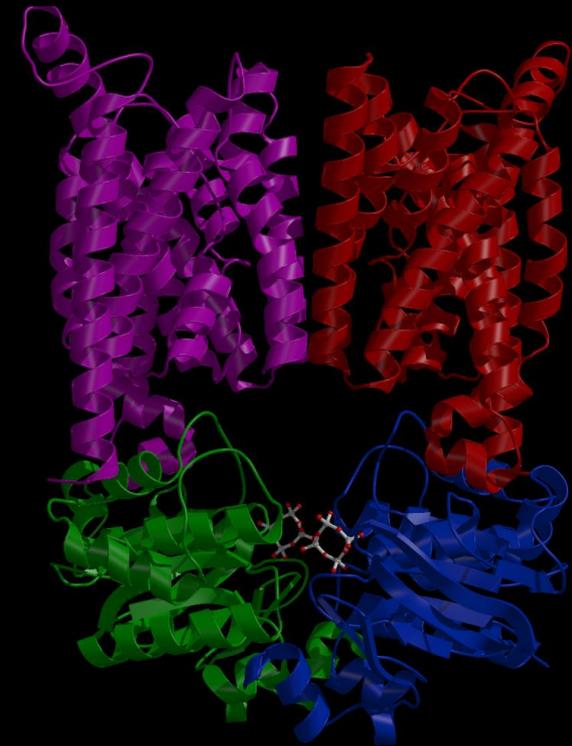
# Macromolecular Crystallography for Structure Determination of Increasingly Complex and Challenging Systems



↓ Synchrotron data collection



→ Solve, trace and refine

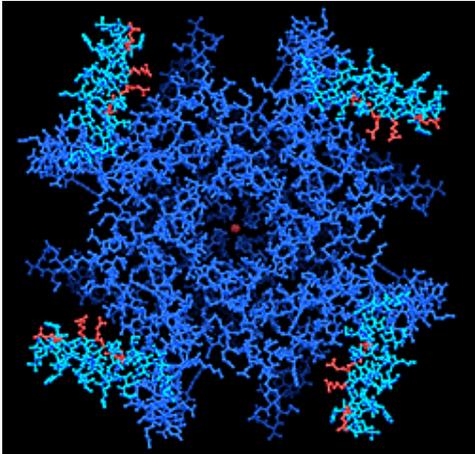


Atomic-resolution structure

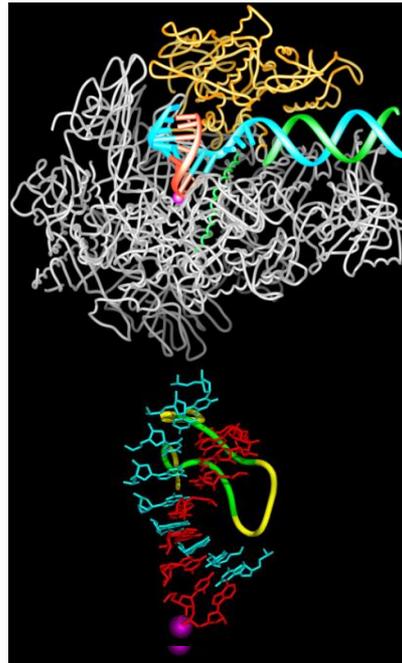
# Impact of Synchrotron Structural Biology - Enabling Nobel Prize Winning Research



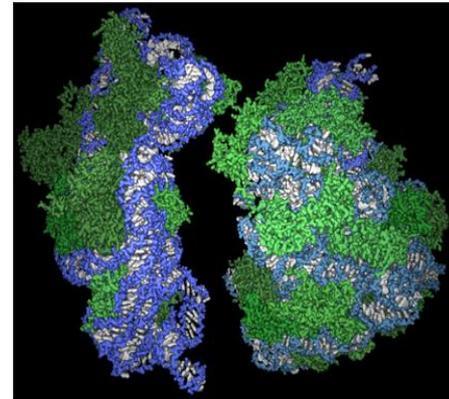
## Membrane Channels, Polymerase, Ribosome, GPCRs



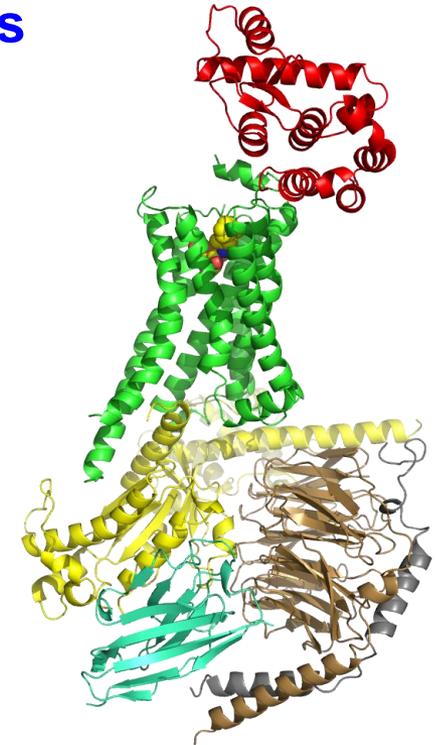
*Roderick MacKinnon and  
Peter Agre – 2003 –  
K<sup>+</sup> channel (KcsA)*



*Roger Kornberg – 2006 –  
RNA Polymerase II*



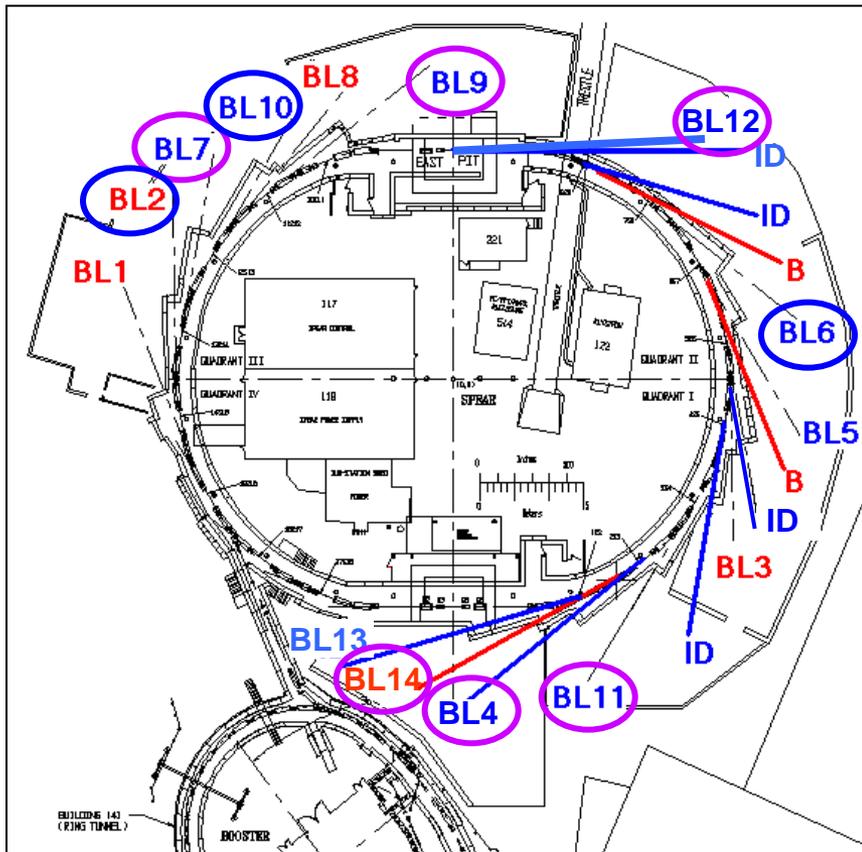
*Venki Ramakrishnan, Thomas  
Steitz and Ada Yonath – 2009  
– Ribosome*



*Brian Kobilka and Robert  
Lefkowitz – 2012 –  $\beta_2$ AR –  
beta adrenergic receptor*

**Most challenging class of problems (often requiring significant effort and time) but yielding remarkable 'atomic level/chemical' insights into some of the most important processes in biology)**

# SSRL Structural Molecular Biology Beam Lines

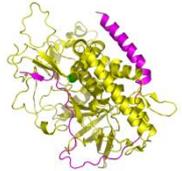


- **SSRL has 8 beam line experimental stations in use and one in construction (BL12-1) devoted primarily to structural molecular biology studies (PX, XAS, SAXS)**
- **An additional 5 stations are used on a shared basis for SMB XAS research**

*Three scientific focus areas*

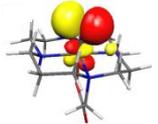
## Macromolecular Crystallography (5)

- high resolution structures
- large-complex assemblies
- structure/function relationships
- high throughput methodologies
- structural genomics
- microcrystal diffraction



## X-ray Absorption Spectroscopy (2+n)

- electronic & geometric active site structure
- reaction intermediates
- $\mu$ XAS imaging
- low concentration, high- $k$
- single crystal XAS
- emission and time-resolved



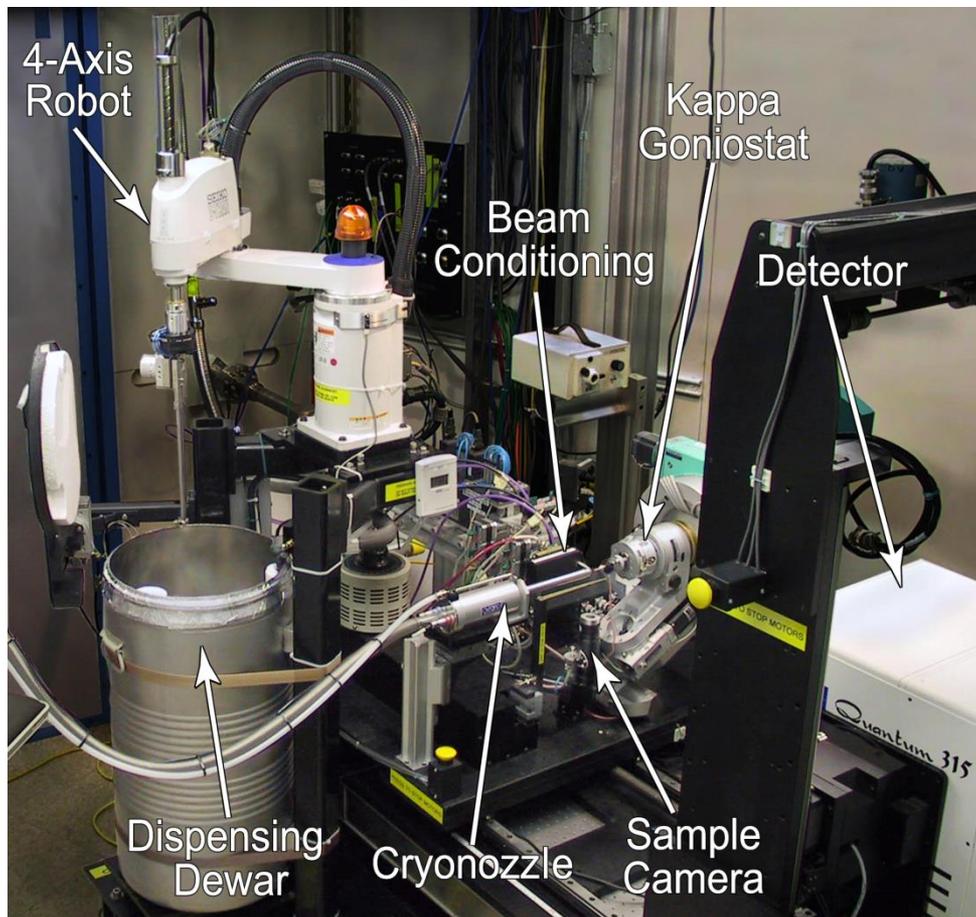
## Small Angle X-ray Scattering/Diffr. (1)

- protein folding
- oligomer assembly
- conformational changes
- time-resolved studies
- low resolution virus structures
- fiber diffraction



# Macromolecular Crystallography

## Automated Data Collection – Remotely Accessed

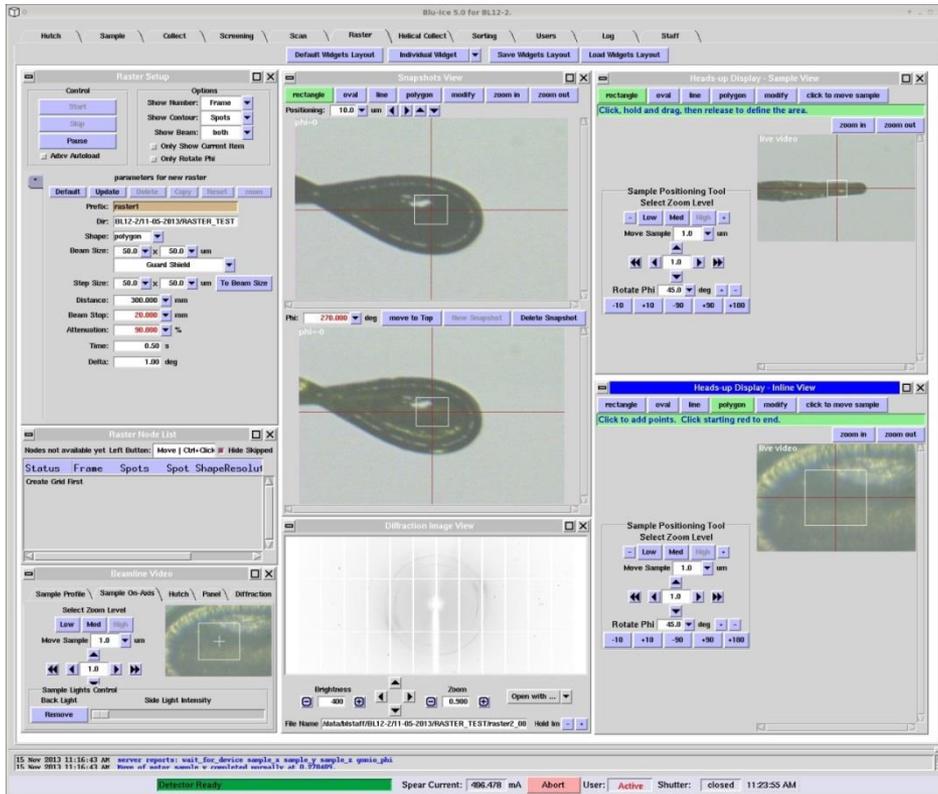
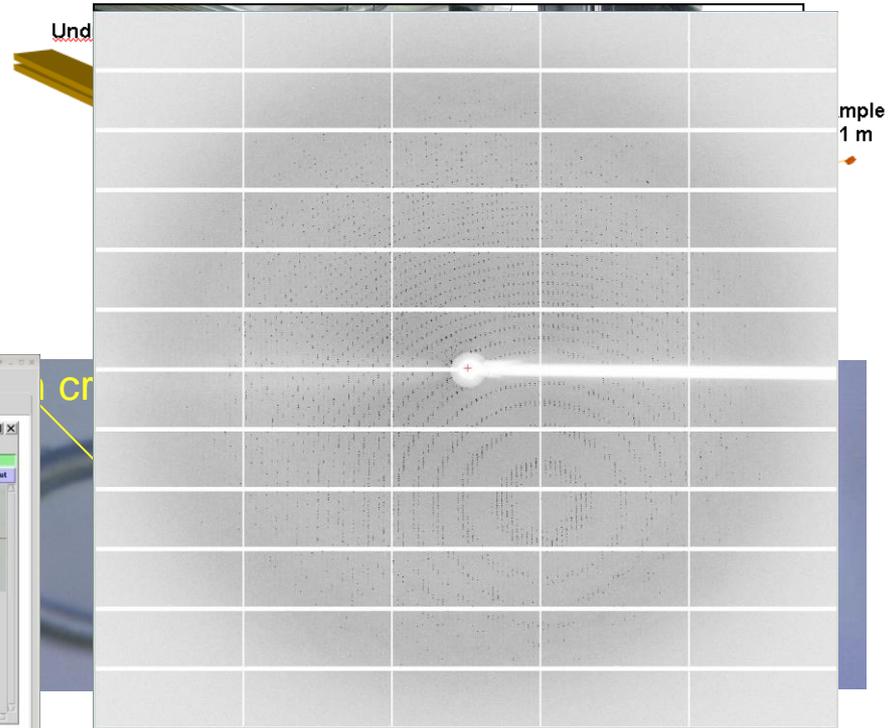


**Complete Automation of Experiment**  
Robotics - Stanford auto-mounting (SAM) system



# Micro-focus Beams for Low $\mu\text{m}$ -Size Crystals

- Micron sized crystals - require
  - Beam size - down to  $5 \times 5 \mu\text{m}$
  - High flux -  $3 \times 10^{12}$  p/s
  - Beam stability to  $<1 \mu\text{m}$  rms over 1-2 hrs
  - Large-area, fast, high-performing detector

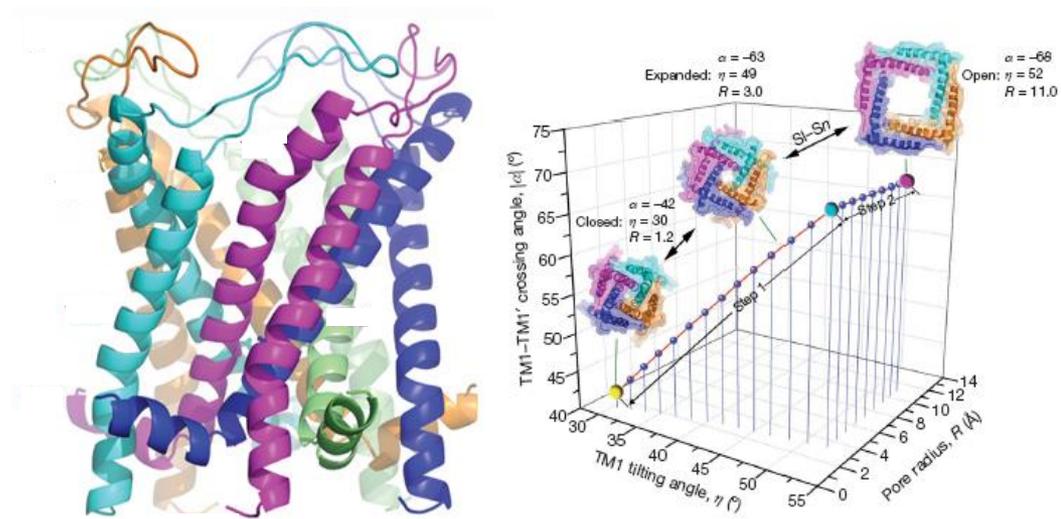


- Finding and aligning micron sized crystals challenging
- Automatic rastering with low-dose beam
- A full data set on SSRL BL12-2 micro-focus beam line with Pilatus 6M PAD is recorded in **30 sec - 2 min**

# BL12-2 – Micro-beam Enables Use of SMALL Crystals

## Intermediate-State Structure of a Mechanosensitive Membrane Channel

- Rees Group (Caltech) solved a structure of a membrane protein to 3.8 Å resolution in an intermediate “gating” state - data from BL12-2
- Gating channels in cell membranes act as “relief valves” which protect bacteria and other organisms from changes in surrounding conditions
- The structure represents an intermediate state between the “open” and “closed” gating states of the channel
- A two-step helix pivot has been proposed for the gating mechanism

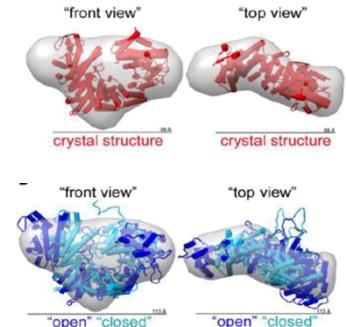
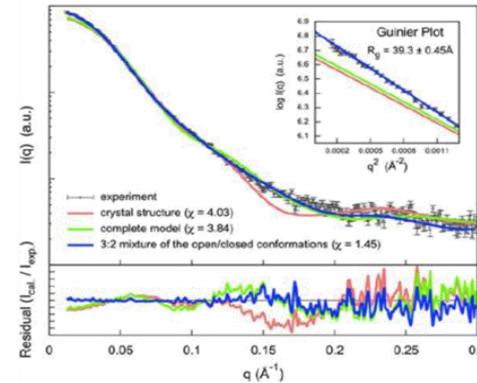


(A) Side view of the “expanded” mechanosensitive channel. (B) The gating model: top view of the resting “closed” state, the “expanded” intermediate state and the “open” state plotted as a function of the helix tilt and helix crossing angles ( $\alpha$  and  $\eta$ , respectively) and the pore opening radius ( $R$ ).

# So What If No Crystals – or Want to Know More?

## Small / Wide Angle X-ray Scattering (SAXS / WAXS)

- Protein closer to physiological state
- Protein folding & conformational changes
- Complex formations between biomolecules
- Dynamics
- Lower structural resolution

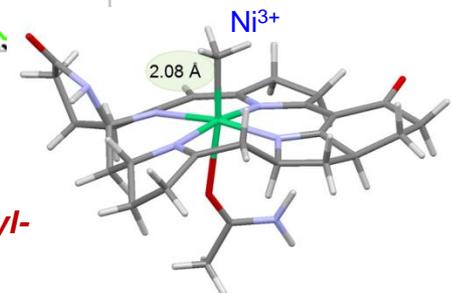
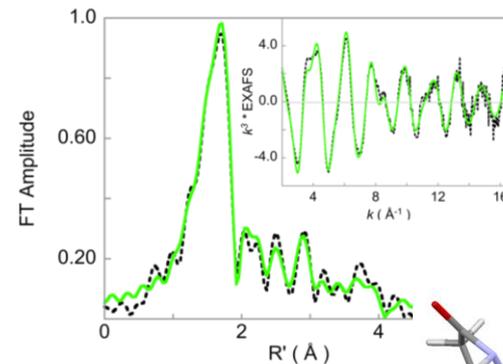


### Nuclear Pore Complex: Nup192

Sampathkumar, *et al. Structure* 4, 560 (2013)

## X-ray Spectroscopy (Absorption / Emission)

- Geometric and electronic structure of metal centers / active sites in proteins
- High structural resolution – local structure
- Reaction intermediates
- Dynamics

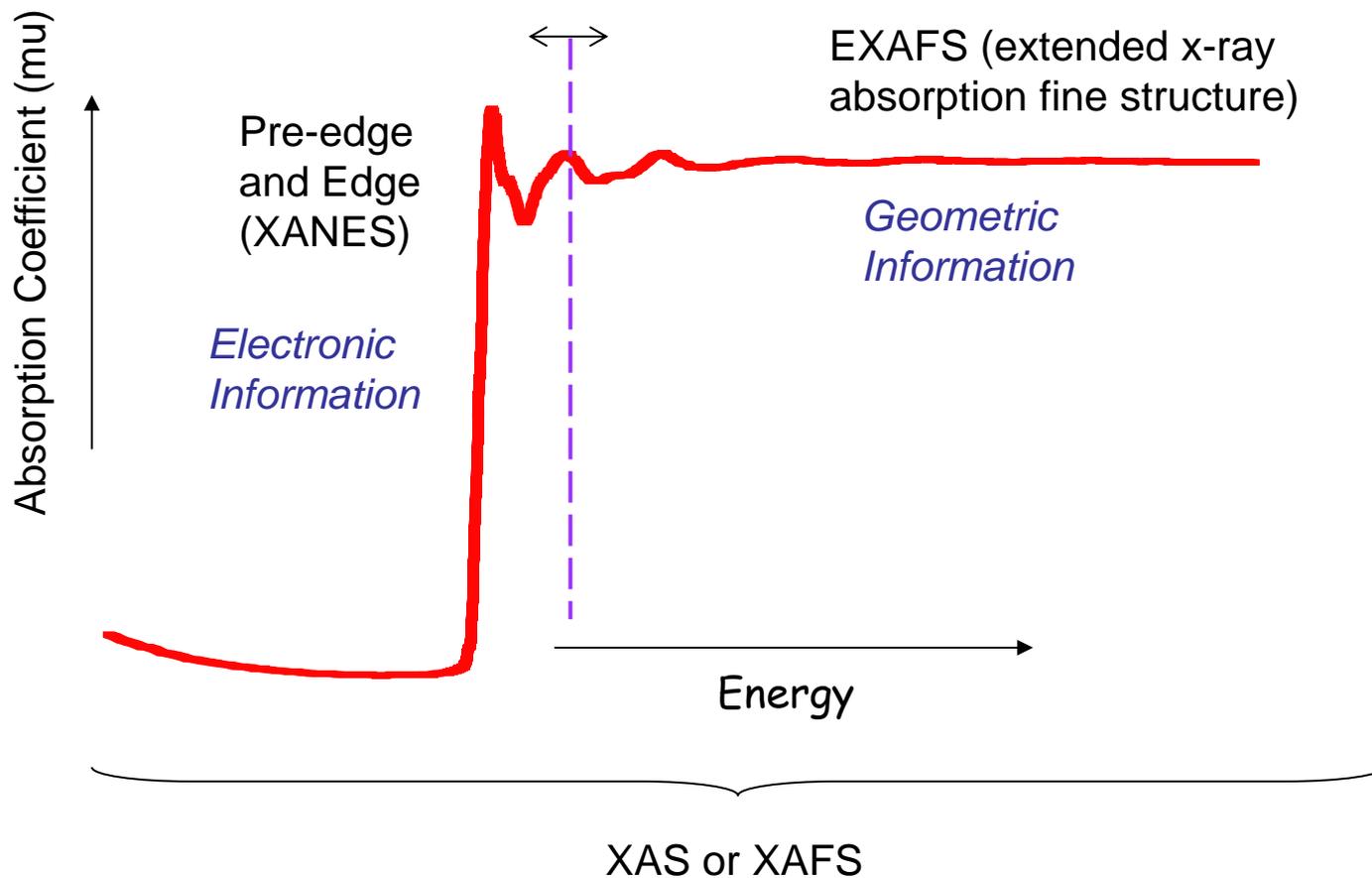


### Ni(III)-Me Intermediate in Methyl-Coenzyme M Reductase

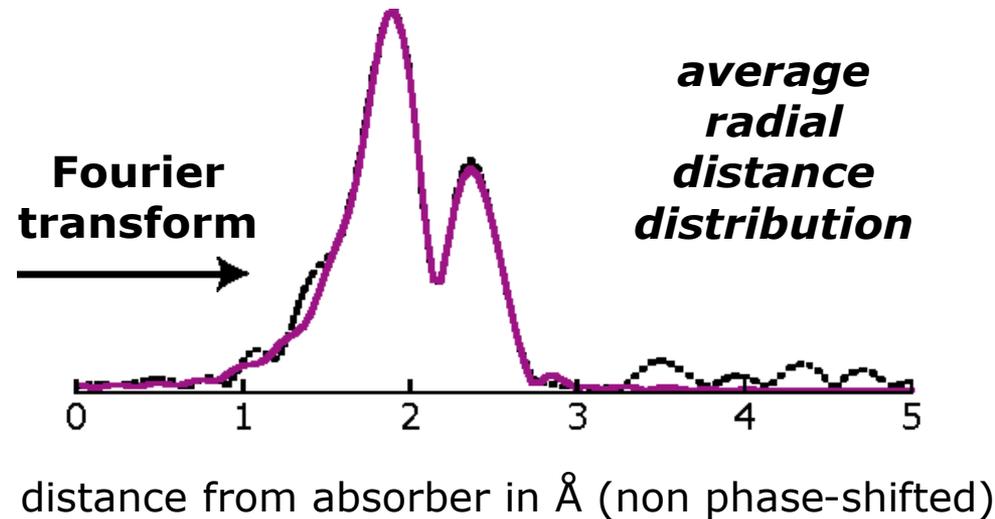
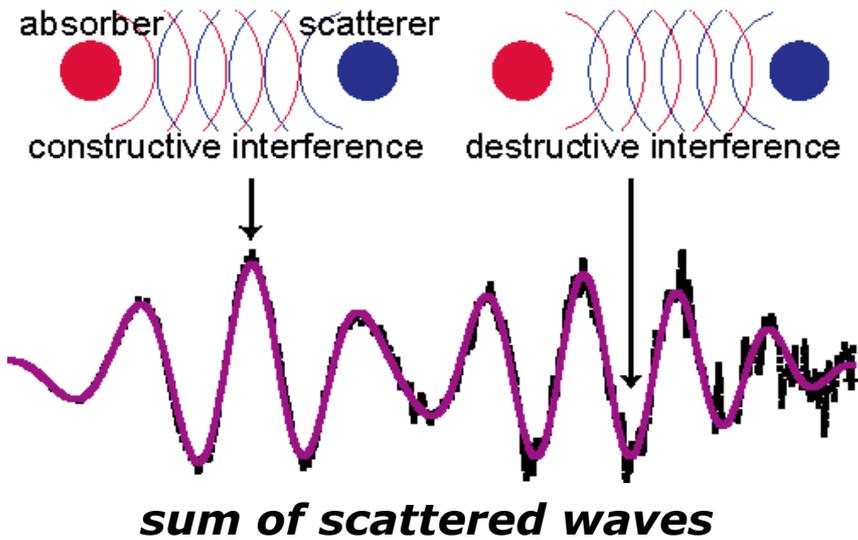
Cedervall, *et al. JACS* 133, 5626 (2011)

# X-ray Absorption Spectroscopy --- Regions

XAS is an element specific technique



# EXAFS - Extended X-ray Absorption Fine Structure

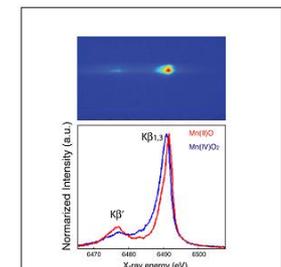
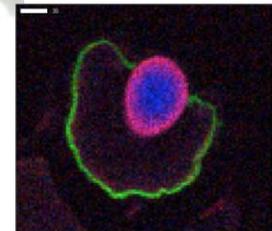
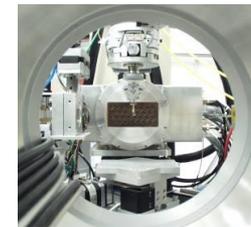
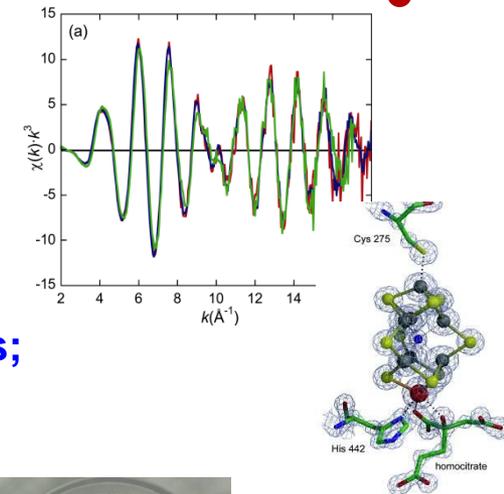


Wave Property	Information	Precision
Frequency	Inter-atomic Distance, $R$	$\pm 0.01-0.02 \text{ \AA}$
Overall Magnitude	Coordination Number, $N$	$\pm 10-20 \%$
Phase Shift & Amplitude	Atomic Number, $Z$	$> \pm 1$
Damping Effect	Structural Disorder, $\sigma^2$	$\pm 10 \%$

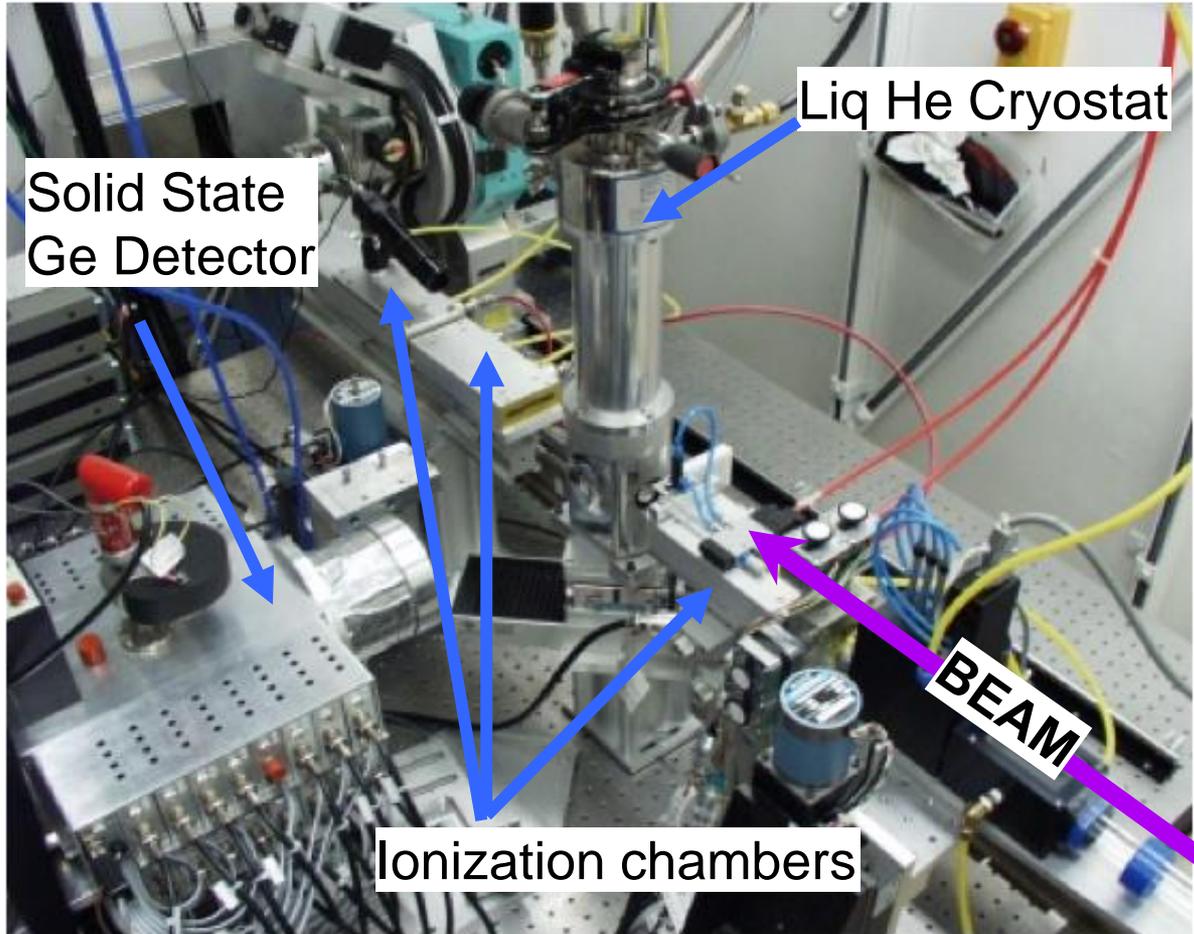
*distance resolution for scatters of equal  $Z$ ,  $\Delta R = \pi / 2\Delta k$*

# Biological X-ray Spectroscopy Approaches

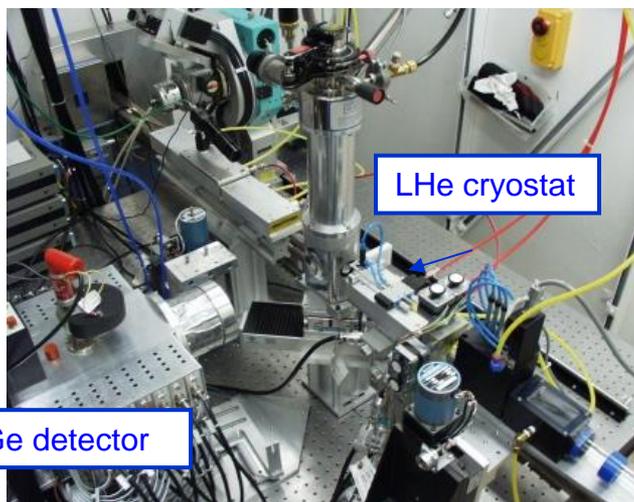
- Detailed analysis of metal centers in proteins (non-crystalline conditions)**
  - ⇒ identification of species
  - ⇒ accurate bond lengths
  - ⇒ electronic structure/covalency
  - ⇒ ligand XAS as metal center probe
- Reaction intermediates in metalloproteins (solution or crystals; trapped frozen or time-resolved)**
  - ⇒ effective oxidation states
  - ⇒ structural changes in the active site - function
- Single crystal XAS**
  - ⇒ solution vs. crystalline state of active site conformation
  - ⇒ polarization signal enhancement
- Micro- (or nano-) XAS imaging and spectro-microscopy**
  - ⇒ micron- (or nano-) level spatial resolution
  - ⇒ electronic and geometric structure
  - ⇒ live species, e.g. cells
  - ⇒ tomography
- “Advanced spectroscopy” – XES, XRS, RIXS, HERFD...**
  - ⇒ analyzer crystal based high-energy resolution fluorescence
  - ⇒ emission spectroscopy for time-resolved studies
  - ⇒ inelastic scattering approaches for expanded electronic information



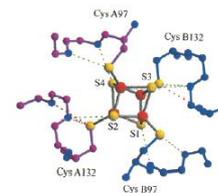
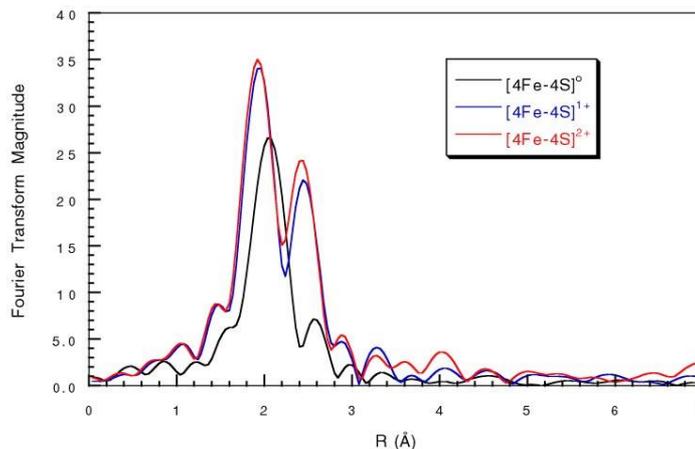
# Inside a BioXAS Experimental “Hutch”



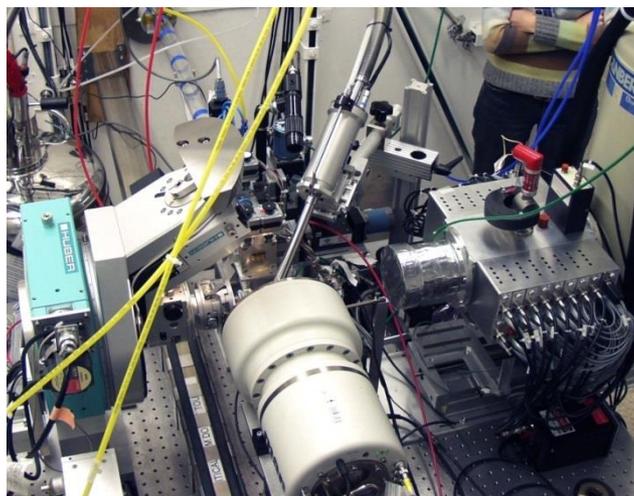
# XAS Experiment – a Few Illustrations



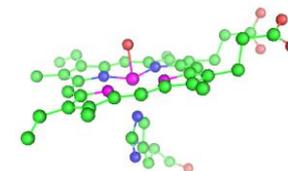
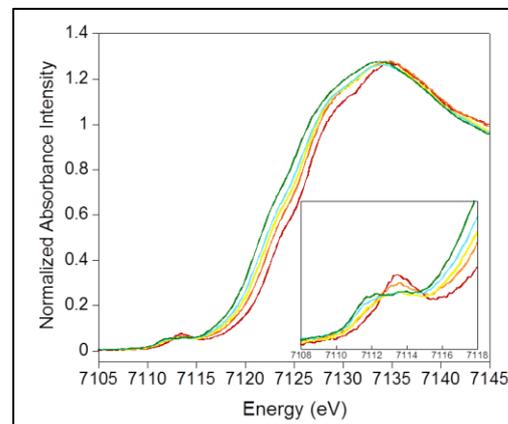
Frozen enzyme solution XAS



Structural change in 4Fe-4S site of nitrogenase Fe protein as a function of redox potential



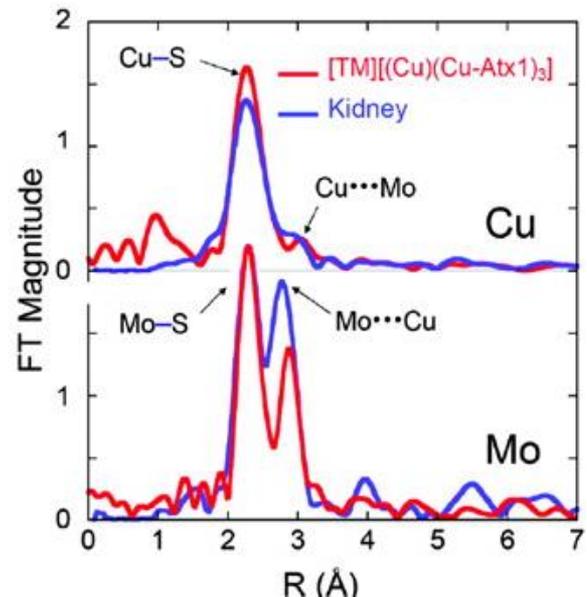
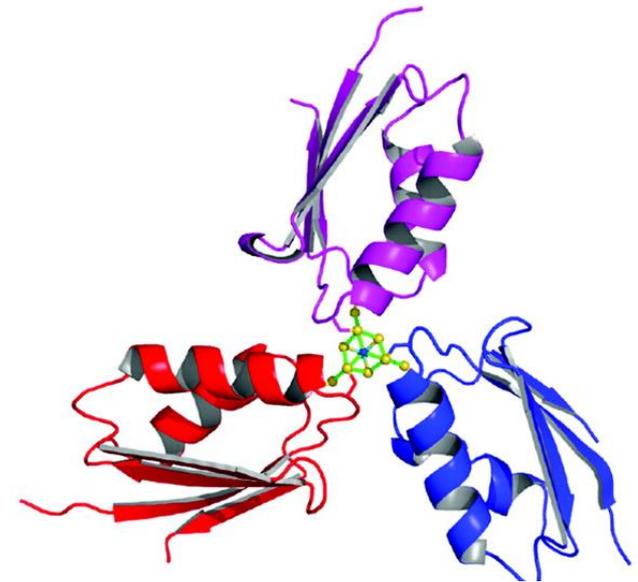
Parallel crystal XAS – MC



High-valent Fe state established for Fe in cytochrome c peroxidase crystals – and SR beam photoreduction - seen by XAS Fe K-edge change

# Potential Anti-cancer Drug Function

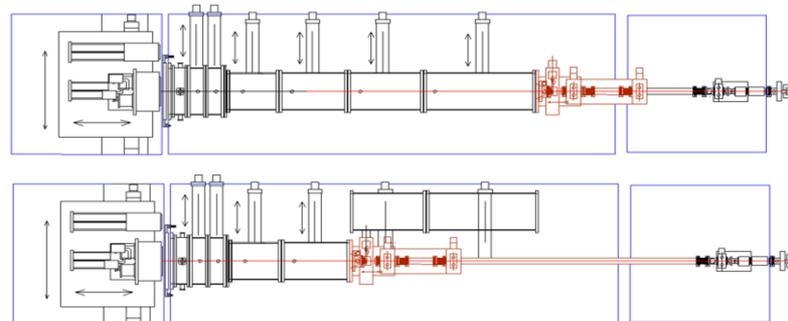
- Cells need copper function - too much copper can be toxic, such as in the case of Wilson disease
- Tetrathiomolybdate ( $\text{MoS}_4^{2-}$  or TM) is an orally active agent for the treatment of this and other disorders of copper metabolism
- The action is through a TM drug complex with the metallochaperone Atx1
- The crystal structure revealed a sulfur-bridged copper-molybdenum cluster reminiscent of those found in Mo and Fe-S proteins
- EXAFS showed that it is a  $\text{Cu}^I$ -based cluster; that it is stable in solution, and corresponds to physiological clusters isolated from TM-treated Wilson's disease animal models
- The cluster blocks the copper from being accessible to transport
- This understanding could lead to treatment of other diseases such as ALS, Parkinson's, multiple sclerosis and some cancers, since cancer cells require copper for growth



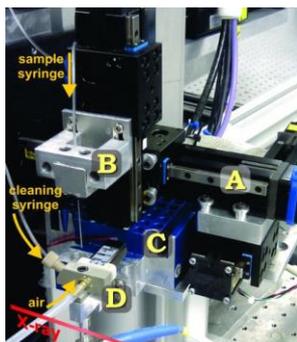
# SAXS / WAXS Experiment Approaches



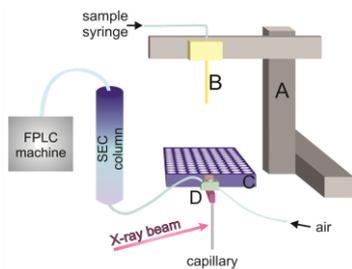
SAXS Instrument SSRL BL4-2



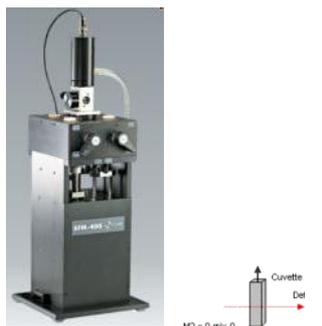
Changing camera length...



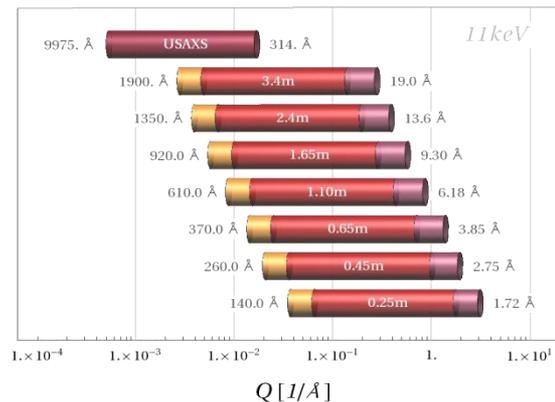
Automated sample delivery



In-situ FPLC

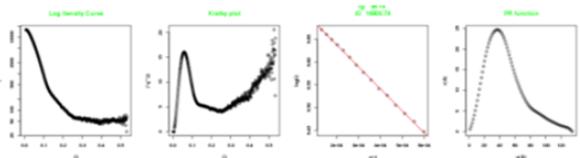


Rapid mixer (or laser) for reaction trigger – time-resolved SAXS



$Q = \frac{4\pi \sin\theta}{\lambda}$   
 $D = \frac{2\pi}{Q}$   
 $Q = 0.003/\text{Å} \dots 4.2/\text{Å}$

To match molecular size... with abilities such as



Automated data analysis

# SAXS / WAXS Covers Many “Sub-techniques”

random



partially ordered



single crystal

## Solution scattering

Shape determination (solution structure vs crystal structure)  
 Molecular interaction (interactive systems, protein crystallization)  
 Providing scattering amplitudes for EM studies

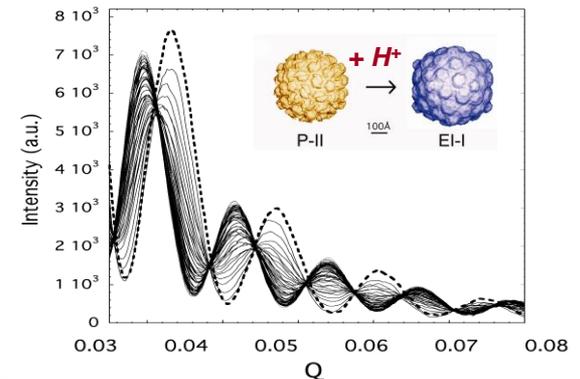
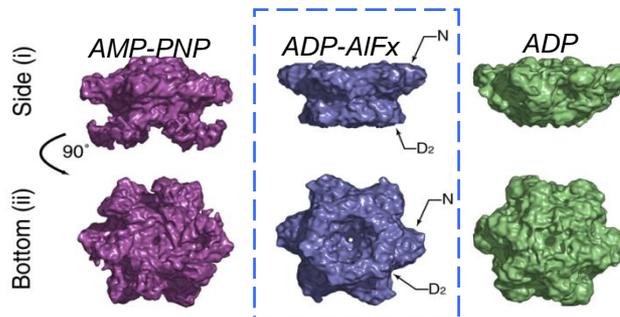
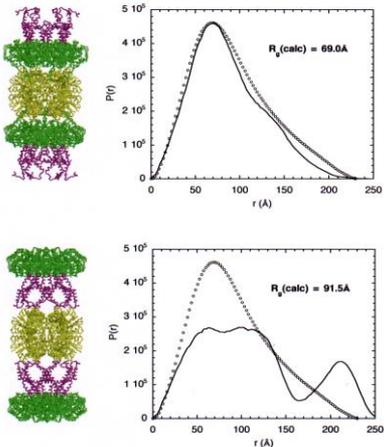
## Lipid membrane diffraction

Monolayer structures  
 Lamellar structures (phase transition)  
 Membrane protein structures (2D crystal diffraction)

## Fiber diffraction

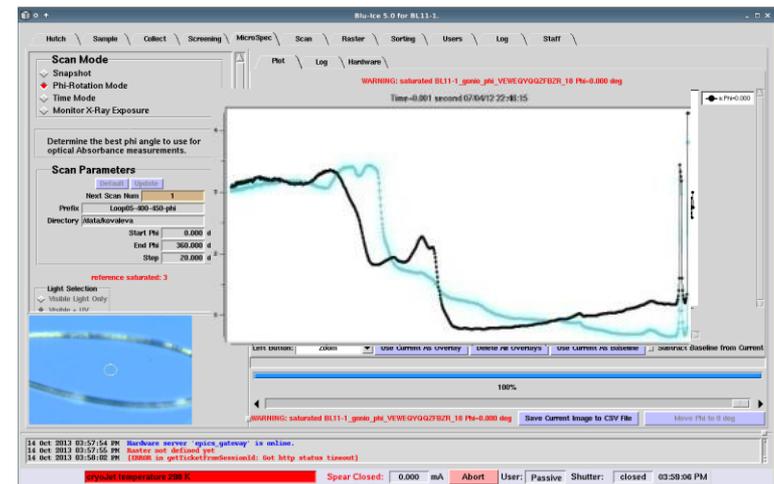
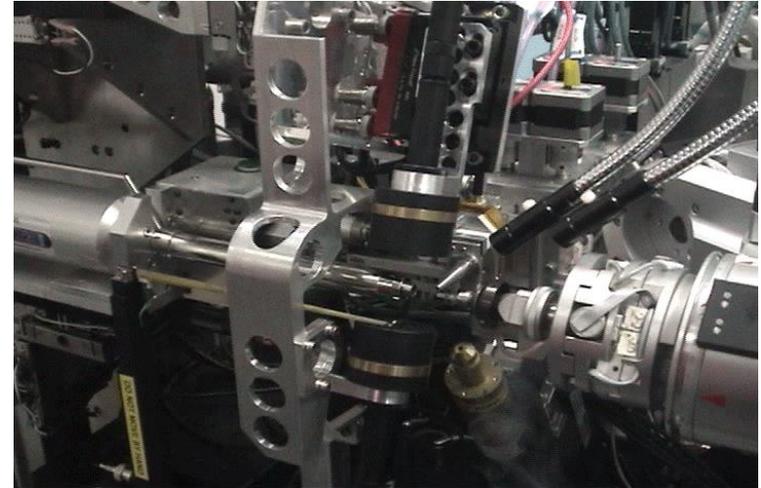
Biological fibers (amyloid/prion fibers, collagen)  
 Filamentous viruses

## Low-resolution crystallography



# So What About Other Tools – non-SR?

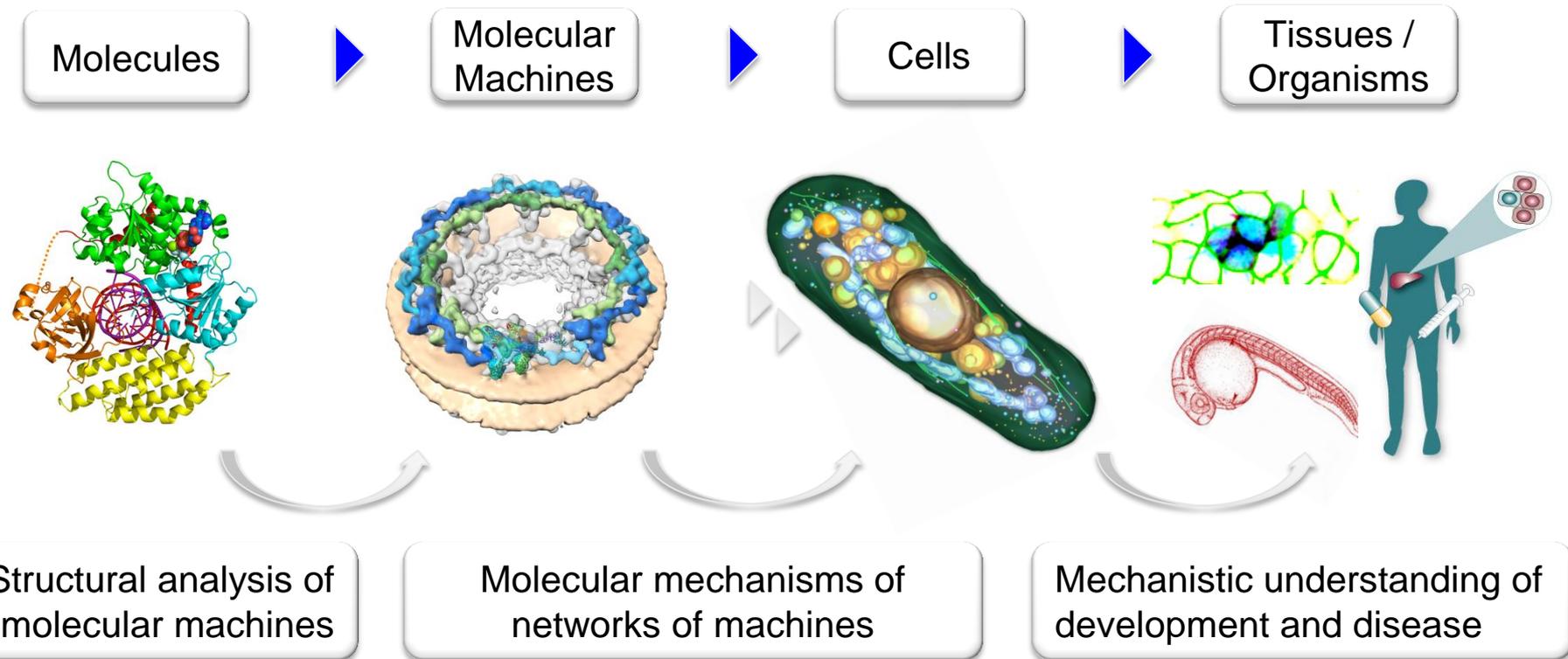
- *In-situ* UV-Vis with crystallography
- *In-situ* Raman with crystallography
- *In-situ* UV-Vis with XAS
- *In-situ* UV-Vis with SAXS
- *In-situ* DLS with SAXS
- .....



# A Frontier in Biological Science “Imaging” – from Molecules-to-Organisms

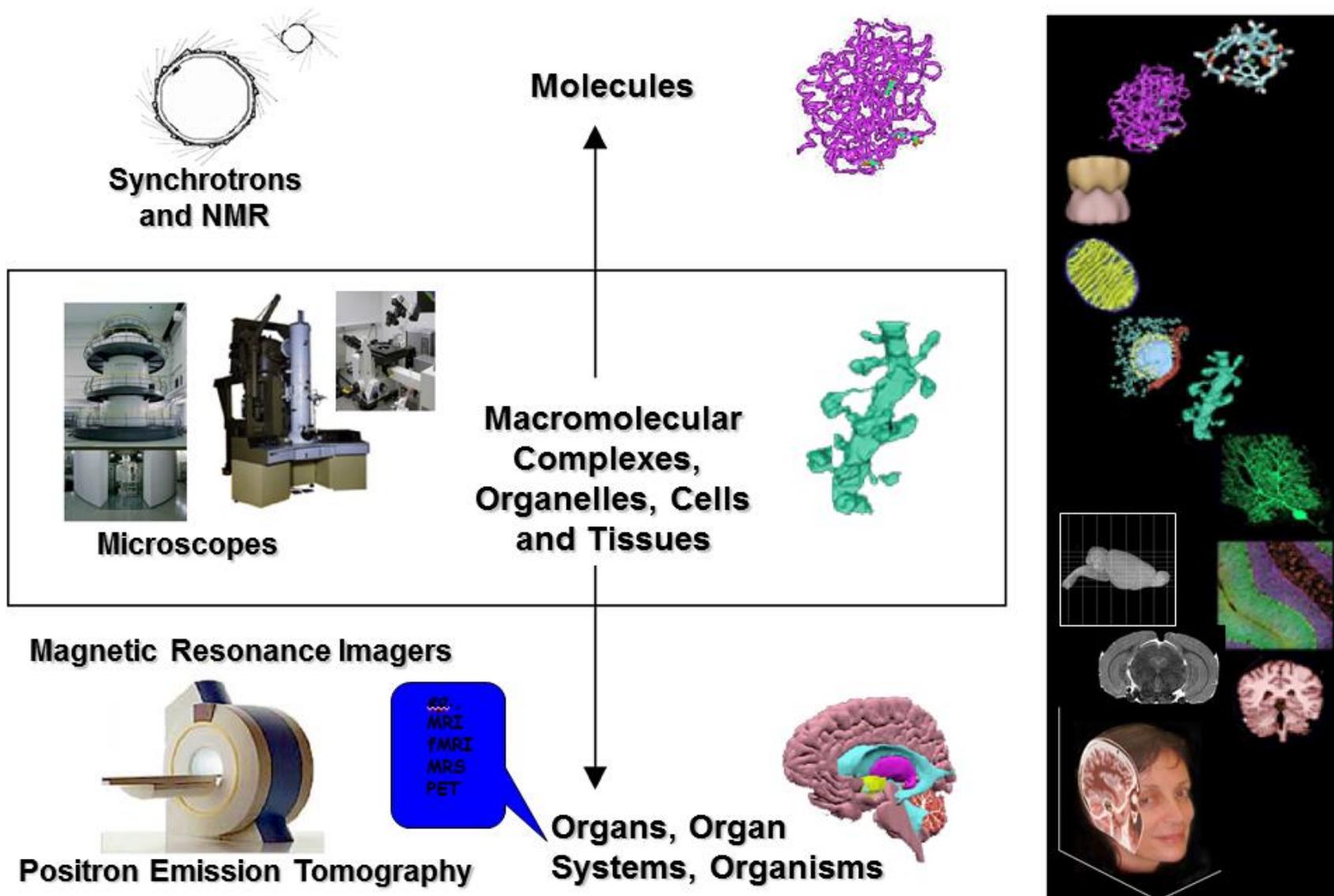


## Connecting scales with molecular mechanisms

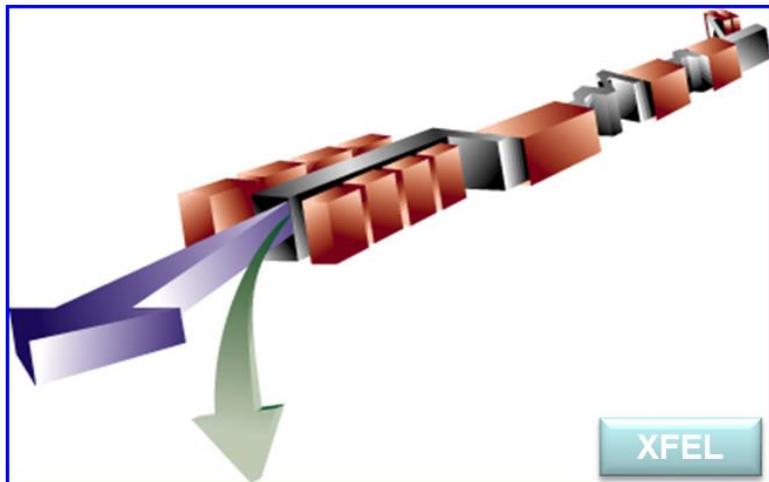


- ▶ Correlation across multiple technologies over range of space and time
- ▶ Integration of data with computational methodologies

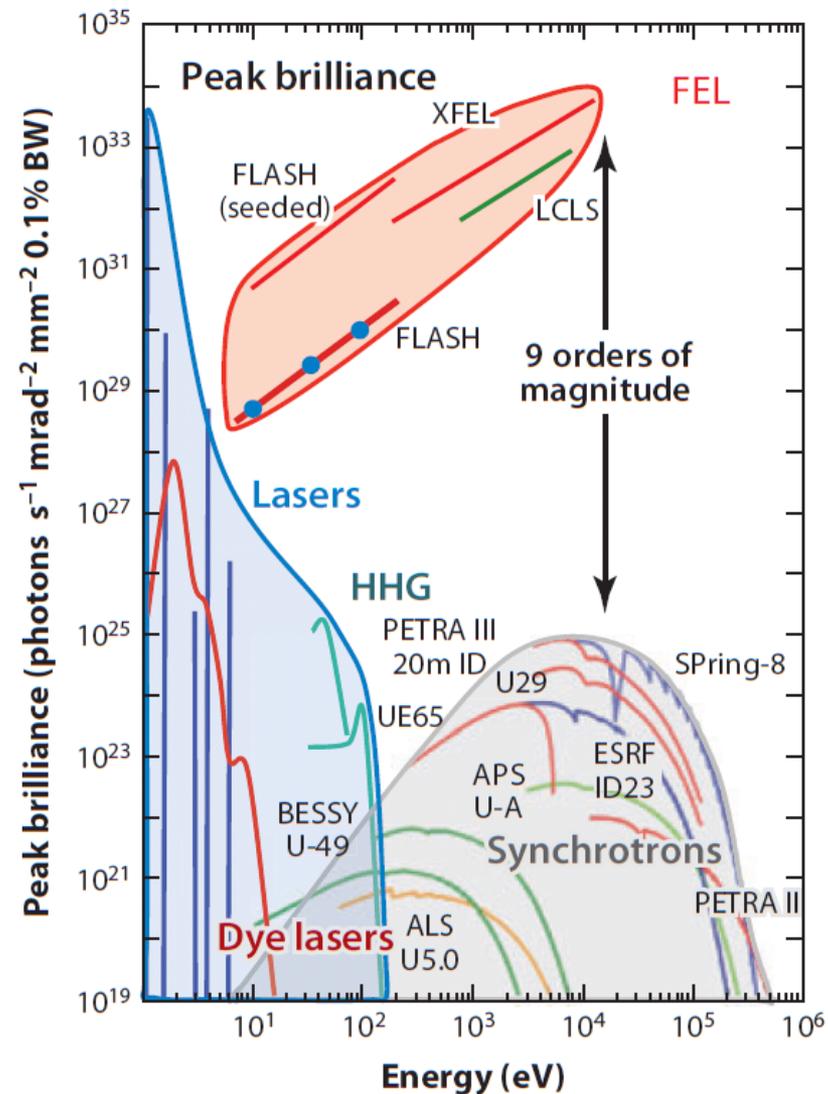
# Imaging-Based Integration over Multiple Scales – Synchrotrons, Microscopes and Non-Invasive Imagers

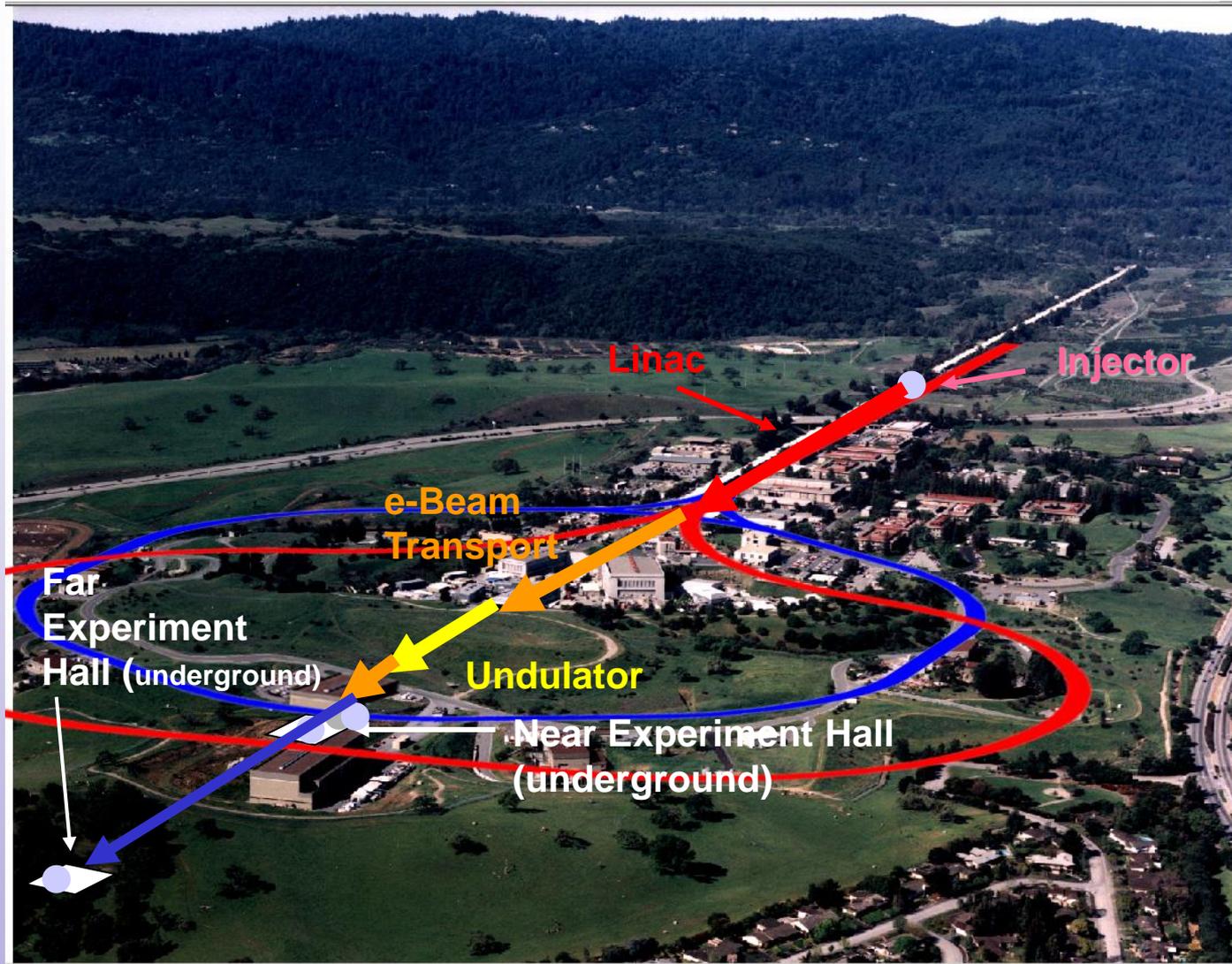


# The Next Revolution - X-ray Free Electron Lasers (2009 $\Rightarrow$ )



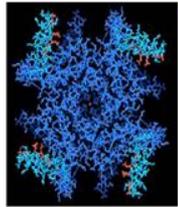
- Based on linear electron accelerator
- High average but exceedingly high peak brightness with femtosecond pulse widths
- Fully coherent
- Serves fewer beam lines and end stations (more serial)





*Complete project included LCLS construction and 6 scientific instruments*

# Synchrotron X-ray Methodologies for Research on Biological Materials – *New XFEL-Enabled Capabilities*

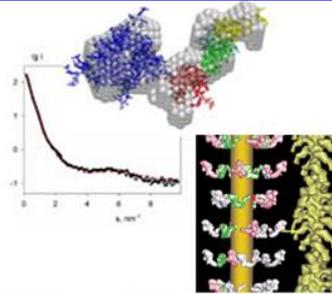


## Macromolecular Crystallography



## Time-resolved

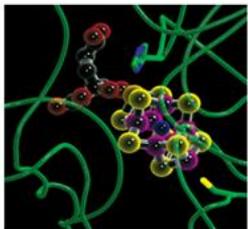
- Study of micron and sub-micron crystals
- High resolution, damage free complex structures
- Extension to imaging of non-periodic samples
- Sub ps time resolved



## Static and Time-resolved Solution X-ray Scattering and Diffraction

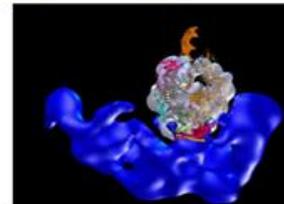
- Non-crystalline solution structure and dynamics in sub psec time domain
- Wide-angle X-ray diffraction of oriented fibrils

## X-ray Absorption and Emission Spectroscopy



- Electronic structural information in the time domain where chemical bonds are made and broken
- Correlation with PX studies on reaction intermediates

## Integrated X-ray, EM and other Methodologies (hybrid approaches)



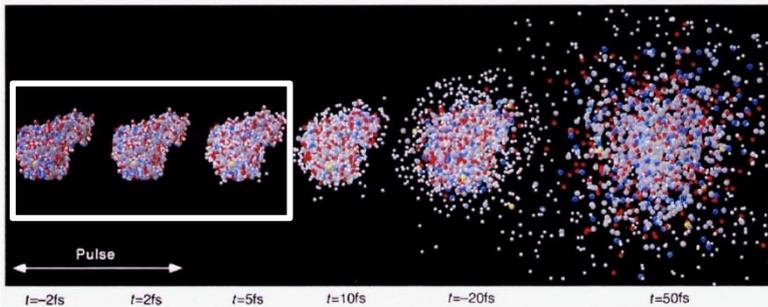
- Integration of complementary methods over a wide range of time and space
- Structural dynamics of very large complex assemblies

Note that synchrotron and XFEL x-rays are generally quite complimentary in the information that they provide – XFELs much less accessible

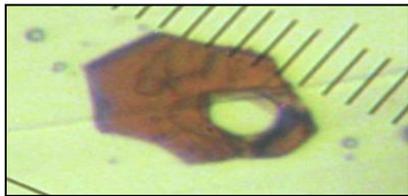
# XFEL – X-ray Properties and Unique Structural Biology Applications

X-rays are delivered in extremely short and bright pulses:

- $\sim 1 \times 10^{12}$  photons/pulse in 100 nm focus (about same as one obtains from best synchrotron sources in a second)
- X-ray pulses are each tens of fsec in duration

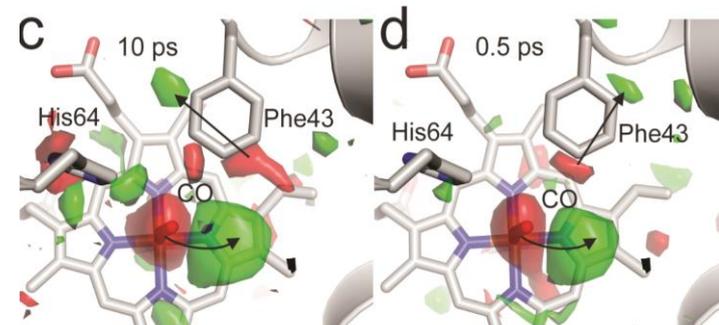


Neutze, *et al.*, Nature **406**, 752-757 (2000)



'Diffraction before destruction'

Diffraction before Destruction Concept →  
'**nano-crystallography**', '**damage-free**'  
**high resolution structures at higher resolution**



- Laser flash photolysis of CO-Myoglobin
- CO moves away within 100 fs and subsequent rearrangements can be followed

Barends *et al.* Science **350**, (2015)

Pump-probe experiments with ultrashort pulses → '**fsec structural dynamics on atomic scale**'

# Much of This Possible Only with the Support of...

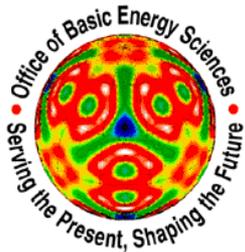
## SSRL Structural Molecular Biology Staff

Keith O. Hodgson – PI; Britt Hedman co-PI

## SMB Program Funding from



*DOE Office of Biological  
and Environmental  
Research*



*SSRL Operations is funded by DOE Office of Basic Energy Sciences*