GM-X25 Program Overview

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Photon Division

X6A SAC Meeting

February 10th, 2012

Office of Science

U.S. DEPARTMENT OF ENERGY

BROOKHAVEN NATIONAL LABORATORY

GM-X25 program

- Access for X6A users to undulator beamline X25
- Increase productivity
- Small beam (down 20um x 20um)

Outline

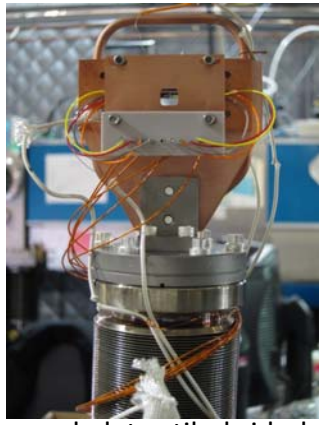
- Ongoing developments at beamline X25
- GM-X25 program statistics
- GM-X25 scientific results
- Integrated Structural Biology and SAXS

Undulator beamline X25

Collaborative effort with PXRR (A. Héroux), MX-ID management team

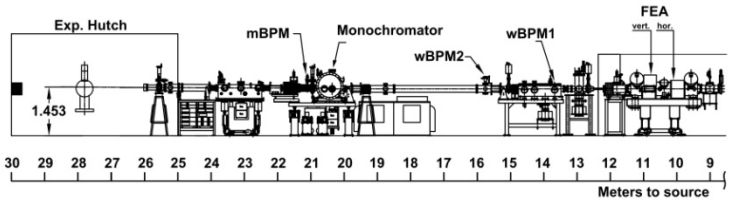
A second wBPM

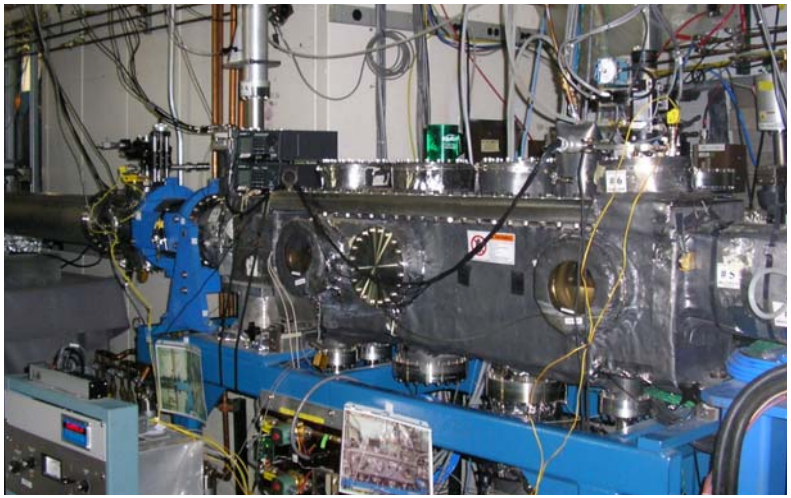
Transmission Mode Diamond White Beam Position Monitor at NSLS.
Erik M. Muller*, John Smedley, Jen Bohon, Xi Yang, Mengjia Gaowei, John Skinner, Gianluigi De Geronimo, Michael Sullivan, Marc Allaire, Jeffrey W. Keister, Lonny Berman and Annie Heroux.
J. Synchrotron Rad. (accepted)

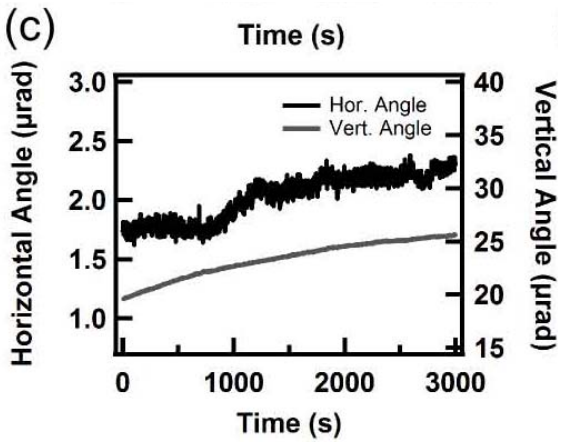


Two diamond plates tiled side-by-side
Pt-coated 4-channel current amplifier
→ Further commercialization with Sydor

Positional and angular measurements with two wBPM

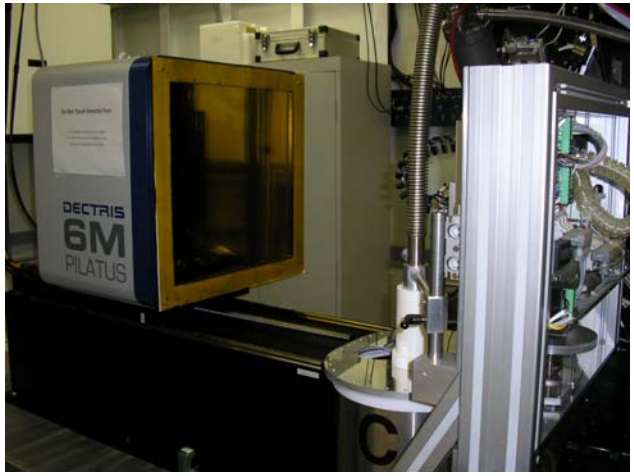


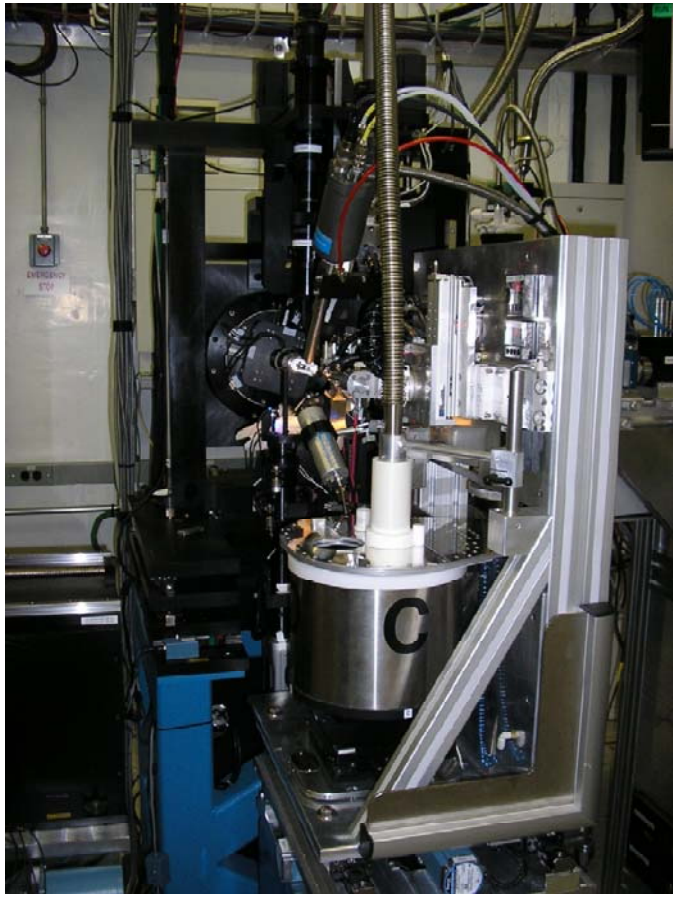


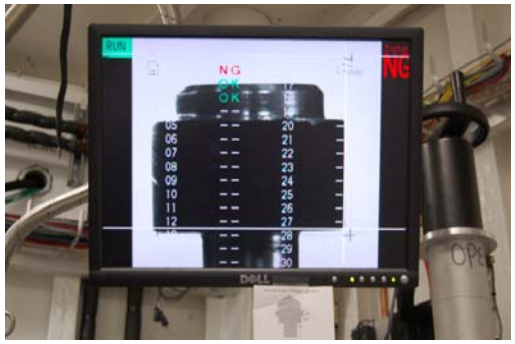


Beamline User Interlock

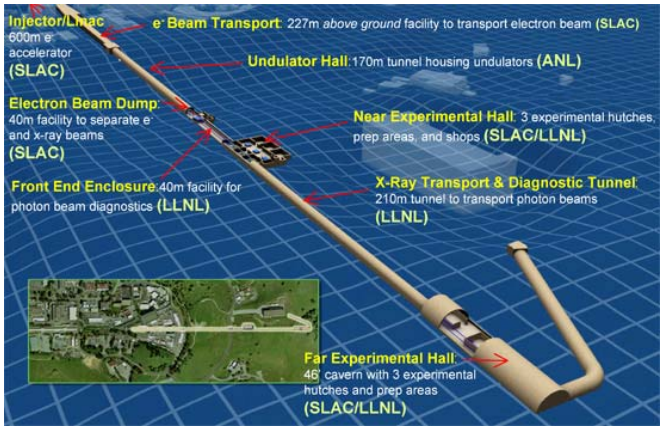
In the hutch



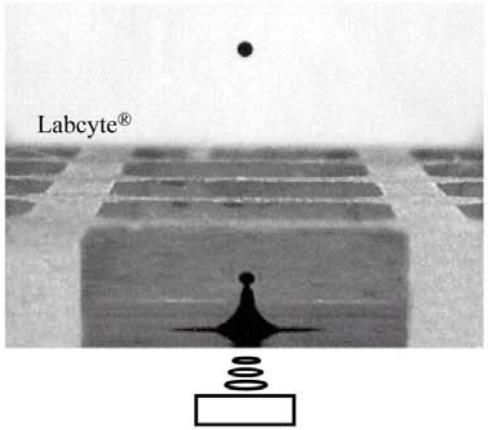




Sound wave to move crystals

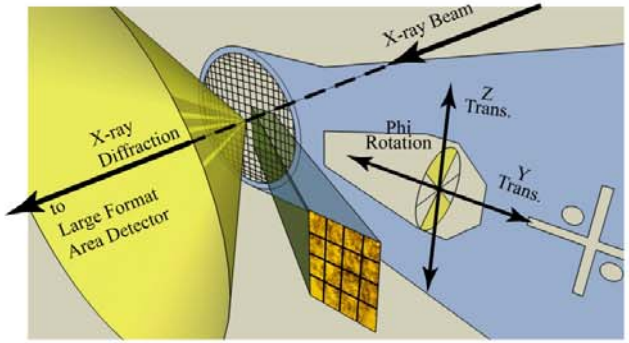


Acoustic Drop Ejection

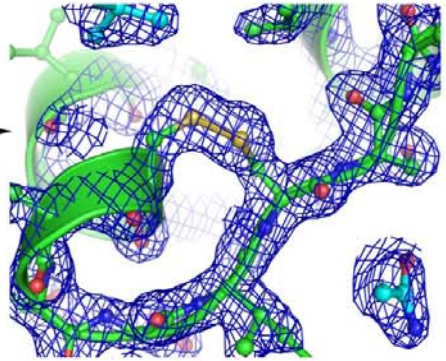


ADE Vol	~ Droplet Diameter	Max.Crystal in Sphere	~ Terminal Velocity
2.5 nL	170 µm	95 µm^3	50 cm/s
1.0 nL	124 µm	70 µm^3	25 cm/s
0.1 nL	60 µm	35 µm^3	7.5 cm/s
0.01 nL	27 µm	15 µm^3	1 cm/s

Raster-Scan Data Collection Strategy

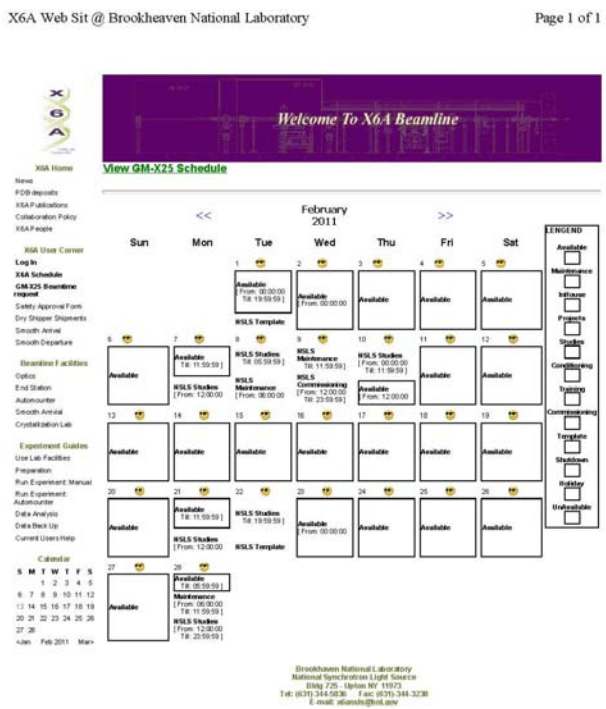


Crystal Structures



X-ray diffraction to ~ 2 Å resolution from crystals (~10µm x 10µm x 10µm). The SAD phased electron density maps for the Zn^{2+} insulin hexamer.

GM-X25 Access Mechanism



GM-X25 beamtime request

- > 7 days beamtime requested from the X6A website (min 4 hrs)
- < 7 days request sent directly by email (try for >2 hours available)

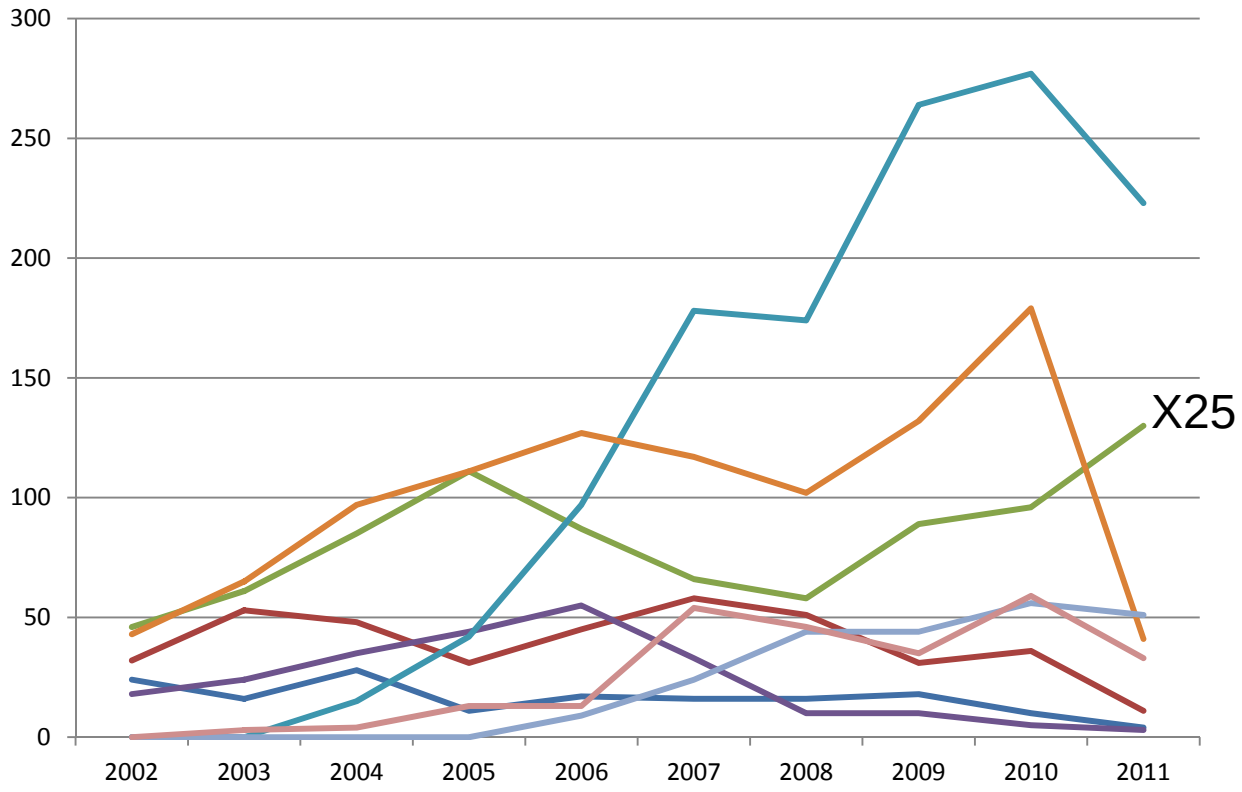
→ all requests were honored

User's quote about the combined usage of X25 after X6A
… it is like driving a Ferrari after having done 24 h in a Ford Focus ...

GM-X25 usage statistics in 2011

- 13 different groups (8 in 2010)
- 40 visits (21 in 2010)
- Total beamtime: 19.1 days (10.4 in 2010)
- Used 2 to 24 hours per visit (same)
- 134919 images collected (66616 in 2010)
- 481 datasets (278 in 2010)
- 1225 crystals screened (664 in 2010)

Growth in PDB deposits at X25

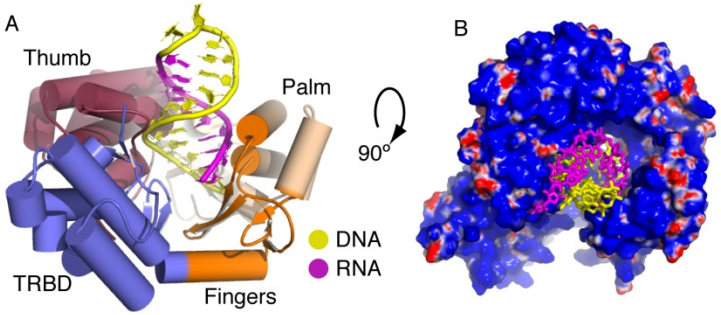


GM-X25 : 2011 Publications

1. **Visualizing Active-Site Dynamics in Single Crystals of HePTP: Opening of the WPD Loop Involves Coordinated Movement of the E Loop.** D Critton, L Tautz, R Page (2011) *J. Mol. Biol.*, **405**, 619-629.
2. **Role of Human DNA Polymerase κ in Extension Opposite from a cis–syn Thymine Dimer.** R Vasquez-Del Carpio, T Silverstein, S Lone, R Johnson, L Prakash, S Prakash, A Aggarwal (2011) *J. Mol. Biol.*, **408**, 252 - 261.
3. **tructural Basis For Antigenic Peptide Precursor Processing by the Endoplasmic Reticulum Aminopeptidase ERAP1.** T Nguyen, S Chang, I Evnouchidou, I York, C Zikos, K Rock, A Goldberg, E Stratikos, L Stern (2011) *Nat. Struct. Mol. Biol.*, **18**, 604-605.
4. **Structure of the ATP Synthase Catalytic Complex (F1) from Escherichia coli in an Autoinhibited conformation.** G Cingolani, T Duncan (2011) *Nat. Struct. Mol. Biol.*, **18**, 701-707.
5. **Three-dimensional Structure of a Viral Genome-delivery Portal Vertex.** A Olia, P Prevelige, Jr., J Johnson, G Cingolani (2011) *Nat. Struct. Mol. Biol.*, **18**, 597-603.
6. **Ligand-gated Diffusion Across the Bacterial Outer Membrane.** B Lepore, M Indic, H Pham, E Hearn, D Patel, B van den Berg (2011) *Proc Natl Acad Sci USA*, **108**, 10121-10126
7. **Mechanism for Selectivity-inactivation Coupling in KcsA Potassium Channels.** W Cheng, J McCoy, A Thompson, C Nichols, C Nimigean (2011) *Proc Natl Acad Sci USA*, **108**, 5272-5277.
8. **Crystal Structure of Escherichia coli CusC, the Outer Membrane Component of a Heavy Metal Efflux Pump.** R Kulathila, R Kulathila, M Indic, B van den Berg (2011) *PLoS One* **6**, e15610.
9. **Reductions of Baylis-Hillman Adducts.** A Walton, C Conerly, Y Pompeu, B Sullivan, J Stewart (2011) *ACS Catal.*, **1**, 989-993.

GM-X25 : The award goes to …

Telomerase Structure Paves the Way for New Cancer Therapies

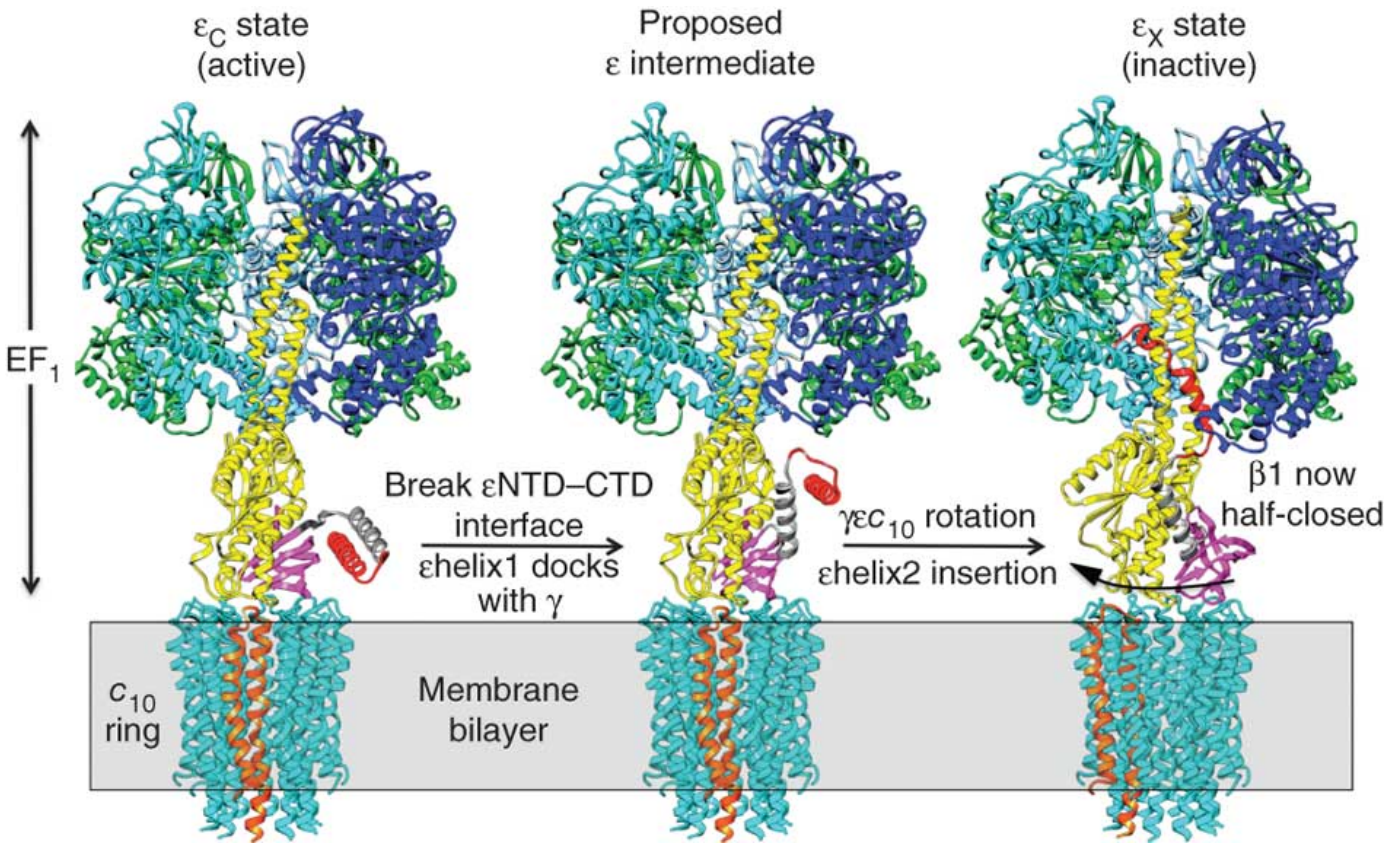


A) Structure of *tc*TERT in complex with its putative RNA template (magenta) and telomeric DNA (yellow); TERT domains are shown in color. B) TERT surface charge representation, showing the RNA-DNA hybrid (stick) docked in the highly positively charged (blue color) cavity of the ring.

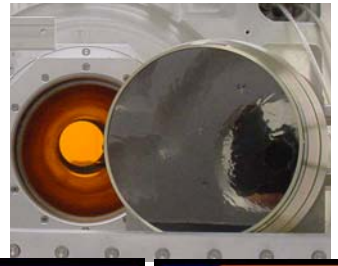
2012 award to Emmanuel Skordalakes
Margaret C. Etter Early Career Award
American Crystallographic Association

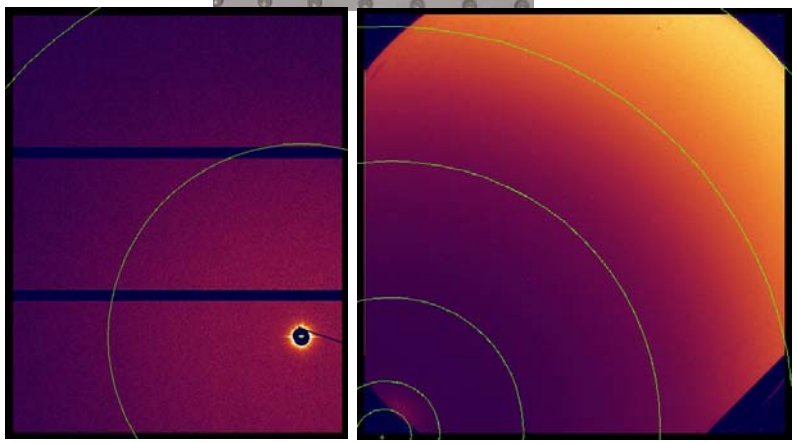
GM-X25 : ATP synthase

G. Cingolani, Thomas Jefferson University

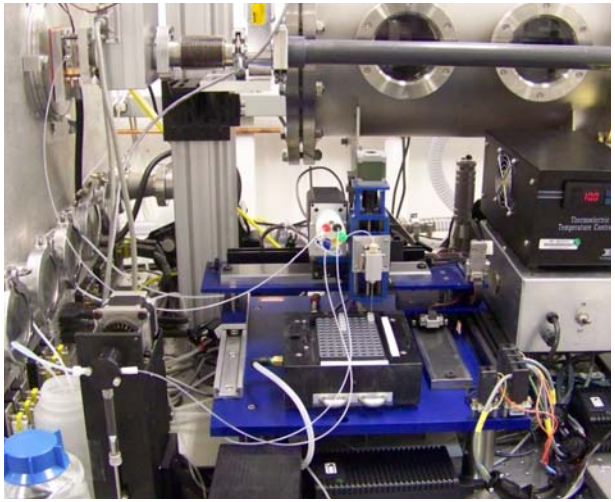


Solution X-ray Scattering at NSLS





- 10% of X9 Beamtime
- Rapid Access mechanism
  <12 hours shift
- A training program



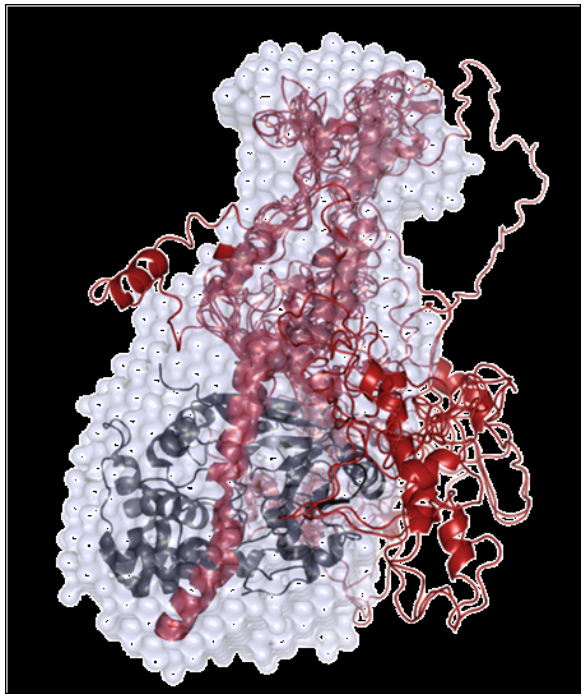
- Over 150 users
  MX
  NMR (new users)
  General SAXS
- ~ 30 publications

Integrated Structural Biology

Regulation of ser/thr Protein Phosphatase

W. Peti and R. Page (Brown University)

- PP1 is the best-characterized serine/threonine phosphatase, in charge of ~1/3 of all dephosphorylation reactions in higher eukaryotes. Free PP1 has no intrinsic specificity for substrates. Rather, it is tightly regulated by its interaction with >200 known targeting proteins4, proteins that 1) localize PP1 to distinct regions of the cell and 2) modulate its substrate specificity.
- Most of these regulators are intrinsically disordered proteins.
- Combined NMR spectroscopy, x-ray crystallography and critically SAXS is necessary to understand this class of proteins.
- Developed an ensemble approach to determine structures of protein:protein complexes, when one binding partner stays exceedingly flexible even after binding.
- They used NMR spectroscopy, combined with MD calculations and SAXS to evaluate large multi-component signaling complexes.



The PP1:Inhibitor-2 complex structure. Overlay of the SAXS envelope (surface representation, gray) with the PP1:I-2 ensemble models. PP1 is shown as a cartoon representation in black and I-2 is shown as a cartoon representation in red. PP1:I-2 ensemble models were created using a combined NMR spectroscopy and x-ray crystallography approach. The SAXS envelope was essential to prove the correctness of the model.