X-ray Footprinting at NSLSII

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What is done in Beamline X28C

— **X-ray mediated •OH radical Footprinting: Structure and dynamics of macromolecular complexes in solution.**

— **~ 40% premier publication each year: High Quality Research.**

— **Support user from US and foreign universities – collaboration and service project.**

— **Core research programs to develop radiolysis methods, detection and analysis of various biological systems.**

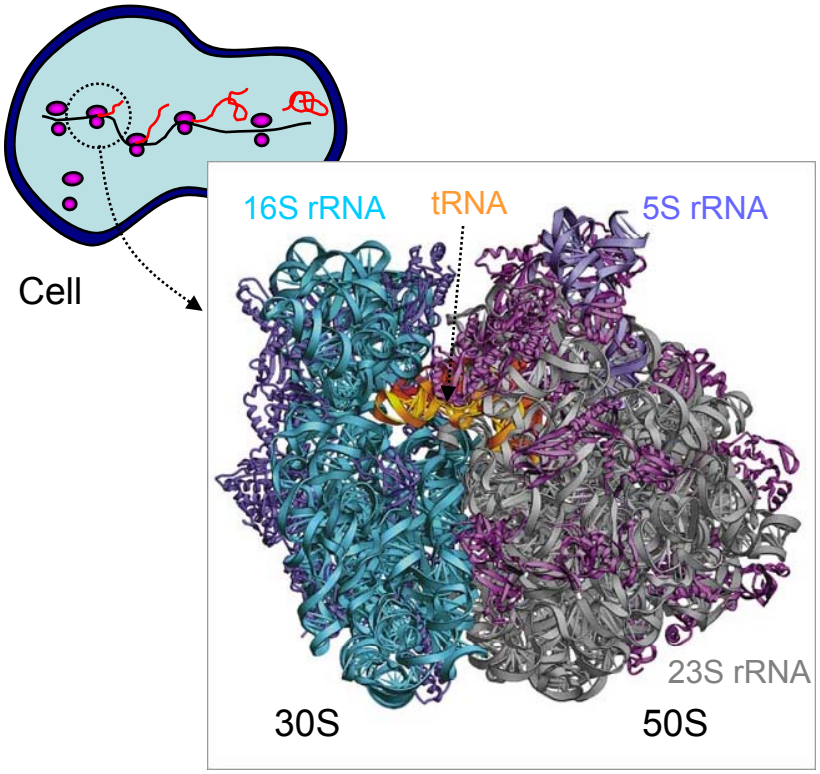
— **Core research programs to develop beam line components, mixing device and automation.**

— **Importance of Footprinting technique availability at NSLSII**

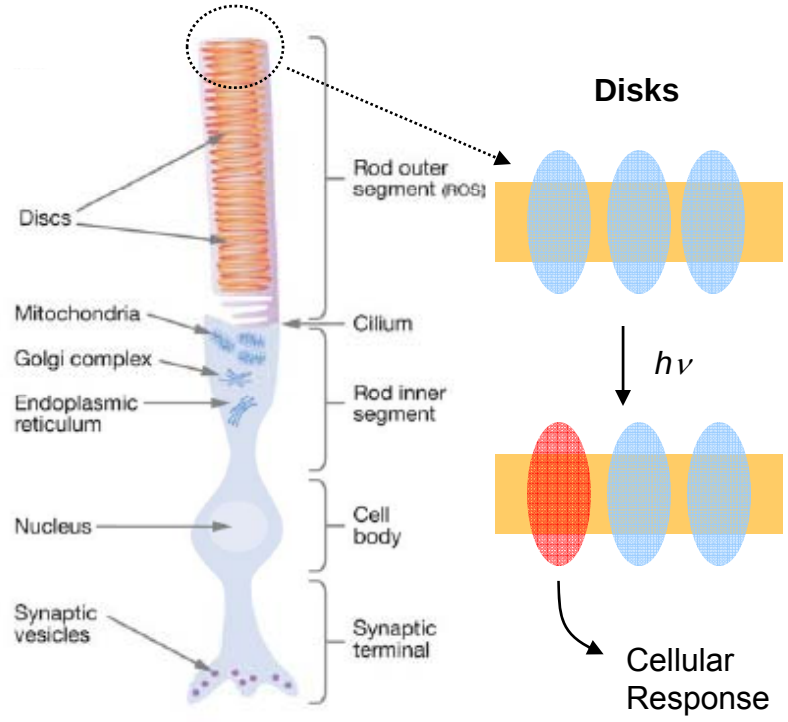
Scientific Needs and Current Limitations - 1

Macromolecular Interactions *In Vivo* and Sub-cellular Components

- Ribosome Assembly in Cell
  Johns Hopkins University, MD



- Rhodopsin Photoactivation in ROS
  Case Western Reserve University,OH

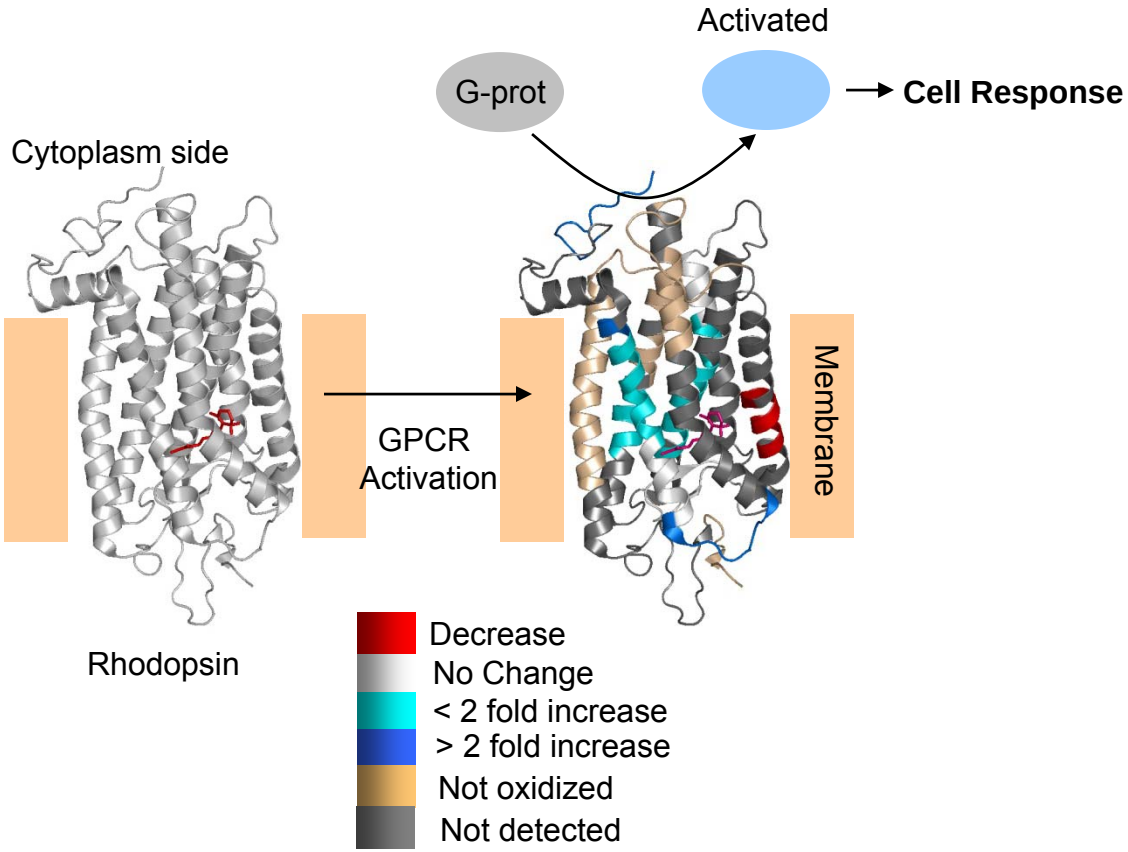


Increase in the flux density of X-ray allows μs to ms exposure times.
Shorter exposure results low perturbation in the cell and large assemblies.

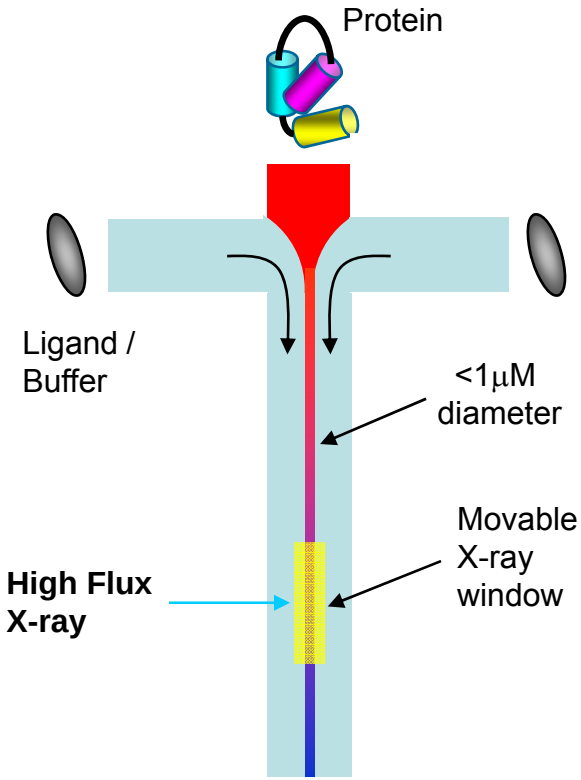
Scientific Needs and Current Limitations - 2

Membrane Protein Dynamics

- **G-protein Coupled Receptor**
  Case Western Reserve University



Micro-fabricated Flow Cell Mixer

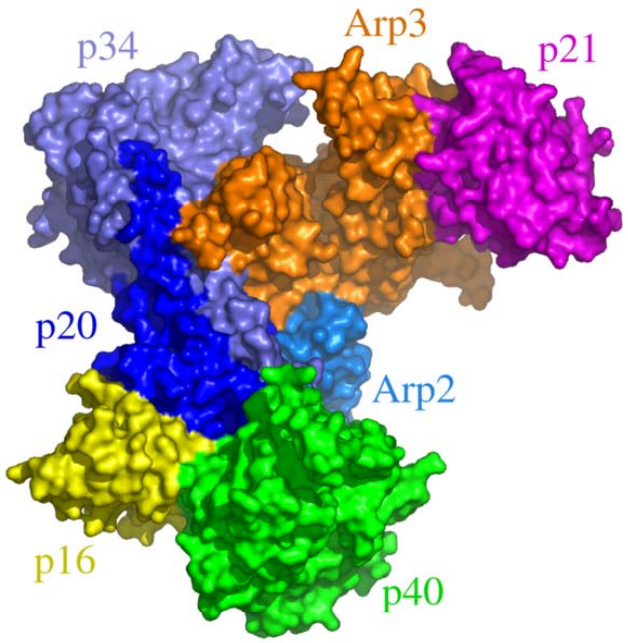


Increase in flux density and smaller beam size will allow μs exposure.
Ultra-fast kinetic studies can be developed on the biological time scale.

Scientific Needs and Current Limitation - 3

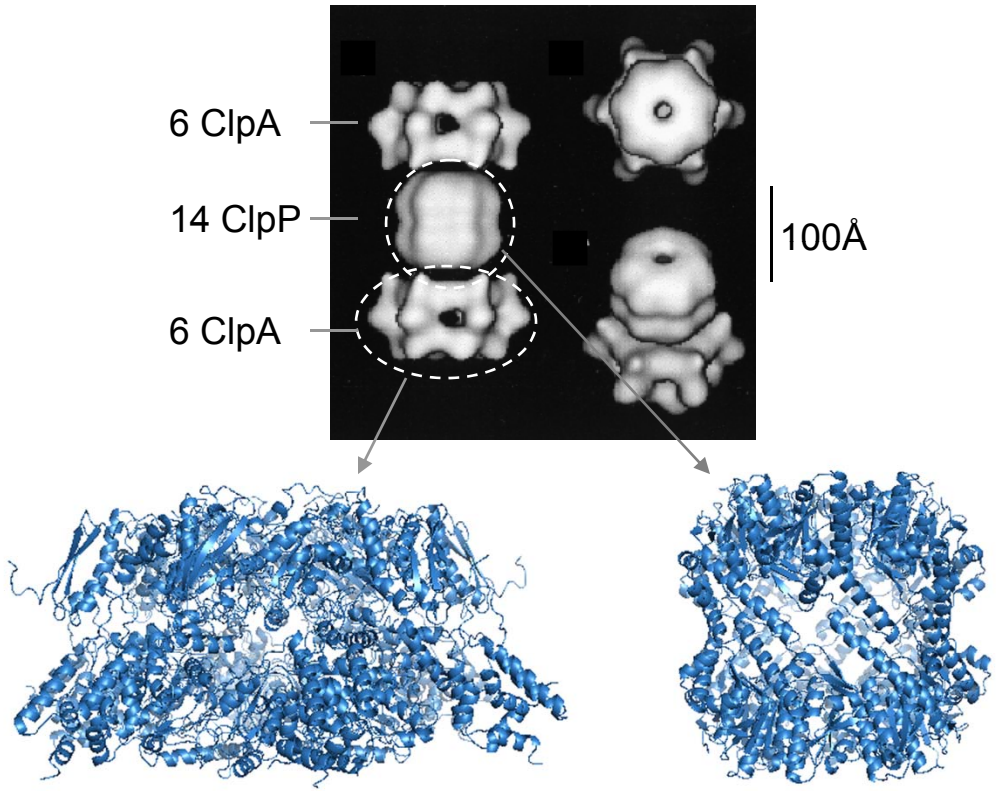
Macromolecular Assemblies

- Arp 2/3 Complex, >300kDa
  Case Western Reserve University



Kiselar et al. PNAS (2007)

- ClpAP Protease, >1300kDa
  Case Western and MIT



Increase in flux density of X-ray will allow shorter exposure. Shorter exposure results first order dose and high S/N - High Quality Data

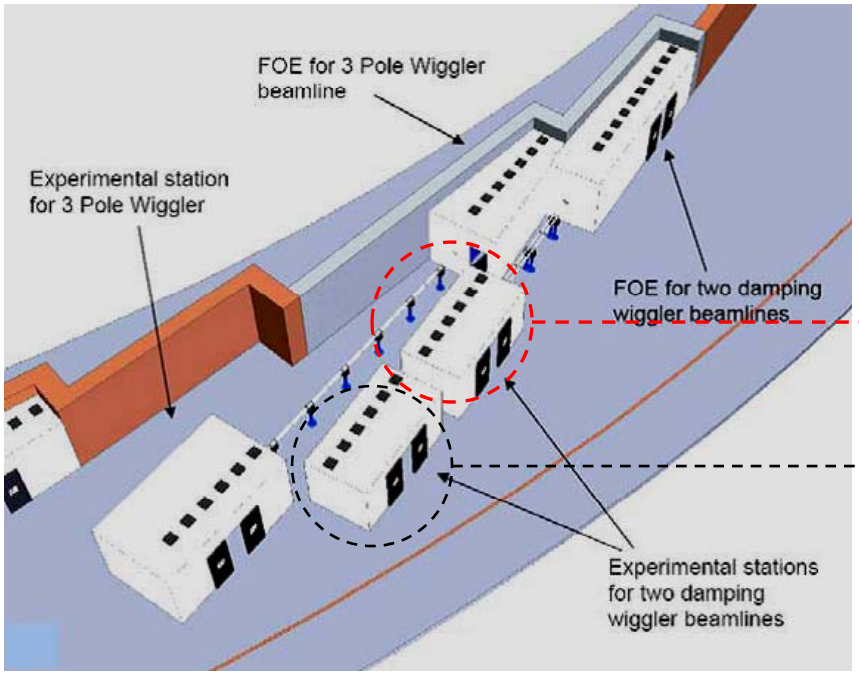
New NSLS II beamline for X-ray Footprinting

- Insertion device suitable for X-ray Footprinting: **Damping Wiggler (DW)**

- **DW** will provide high flux with a broad energy range (<10eV - ~100keV).

- Current thinking: either one 7m long device or two canted 3.5m devices (see the conceptual layout below)

Conceptual layout of the Life Science sector for X-ray Footprinting, EXAFS, SAXS



Footprinting beamline in DW or canted DW

Canted DW for EXAFS or SAXS

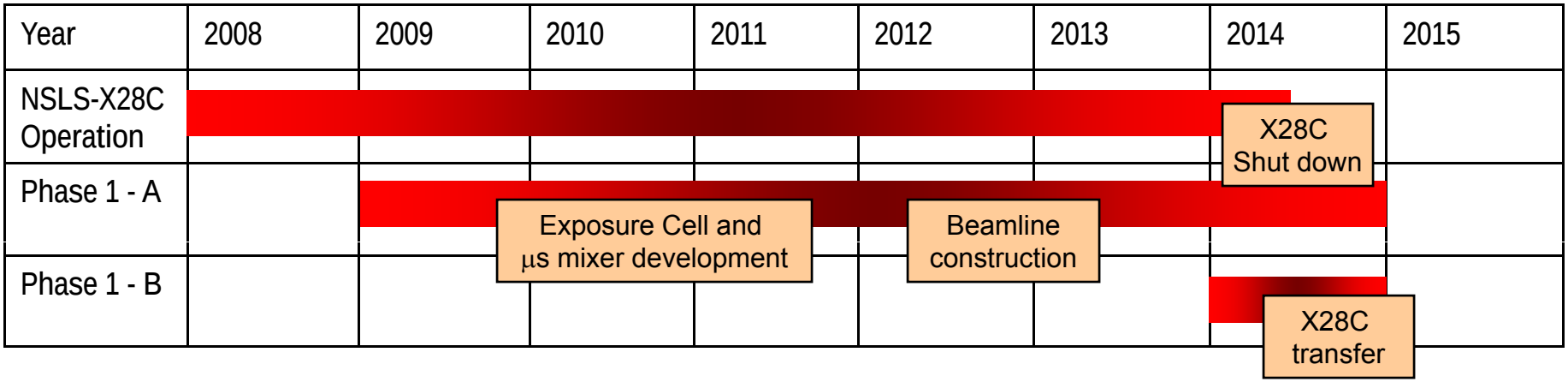
Phased Construction

Phase 1- A

- Construction of **DW beamline**.
- Incorporation of **vertical collimating mirror** within the ring tunnel, upstream of the ring wall.
- Construction of **front optic enclosure** for future upgrades.
- Construction of experimental **end station** and sample exposure set-ups.
  - Development of **sample exposure cells for NSLSII**.
  - Development of **ultra fast mixing device** for time resolved studies.

Phase 1 - B

- Transfer of existing beamline end-station components.



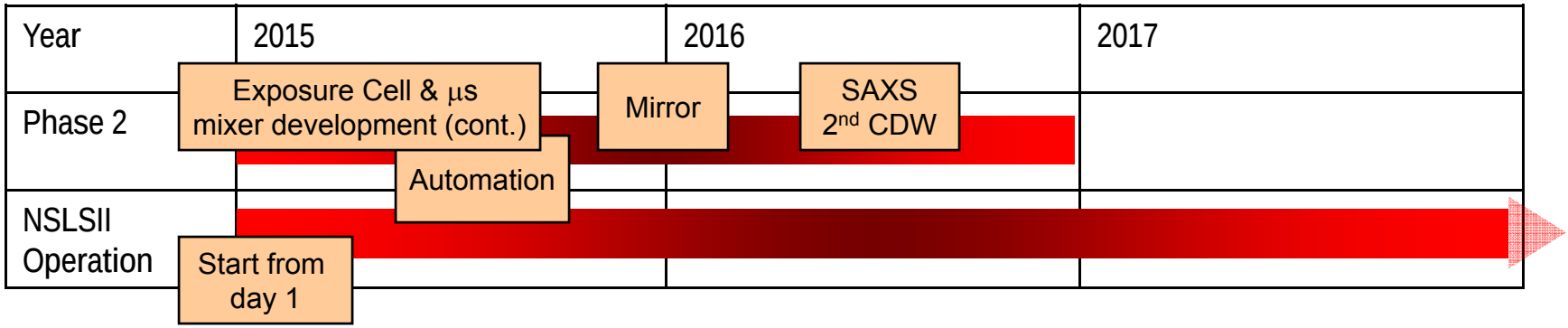
Phase II – Beamline Development and Upgrades

Phase 2 - A

- Incorporate horizontal **focusing mirror** in FOE.
- Continued development of **sample exposure cell** for the focused beam and **ultra fast mixing device** for time resolved studies.
- Precise control of beam size and shape, sample **positioning** and **alignment.**
- **Automation** of sample handling and exposure (including live cell samples)

Phase 2 - B

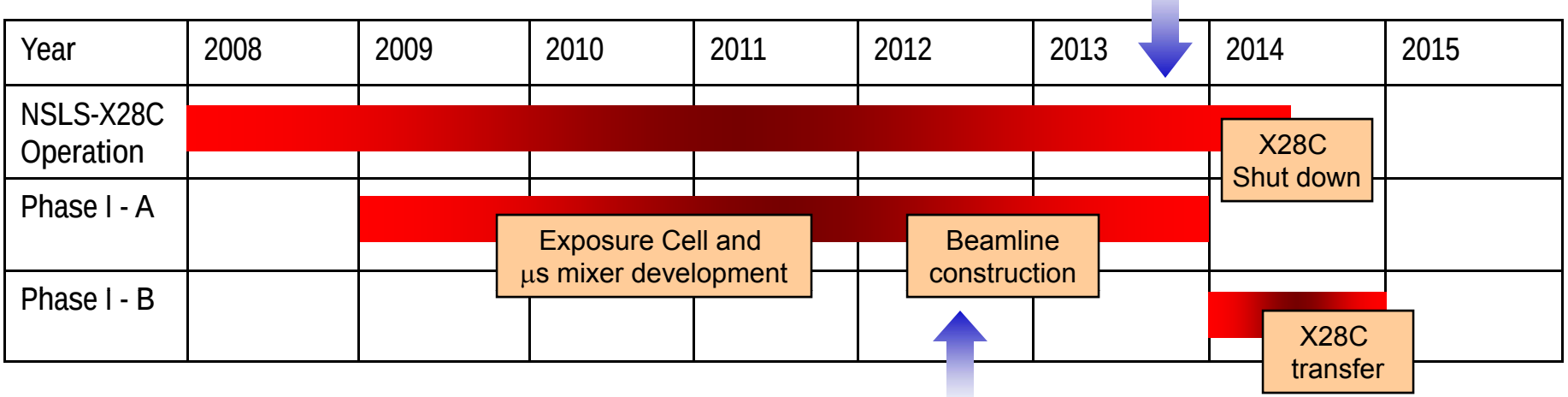
- Incorporation of **SAXS or EXAFS** on the second canted DW beamline.
- Development of joint **facility for biological SAXS** and **Footprinting**.
- Incorporation of other techniques compatible with footprinting set-up.



Funding

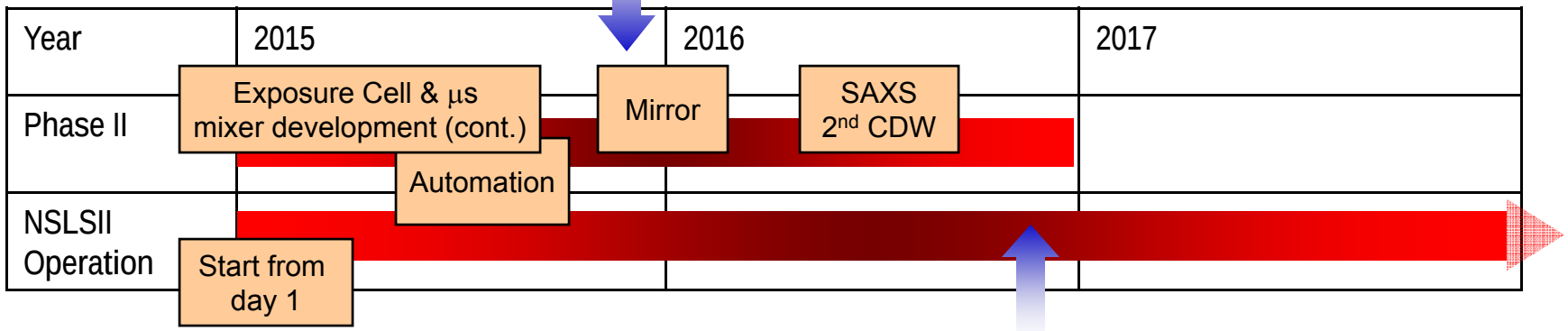
NIH-NIBIB current funding ends, renewal begins

Phase I



Supplemental Funding for Phase I - A and II

Phase II

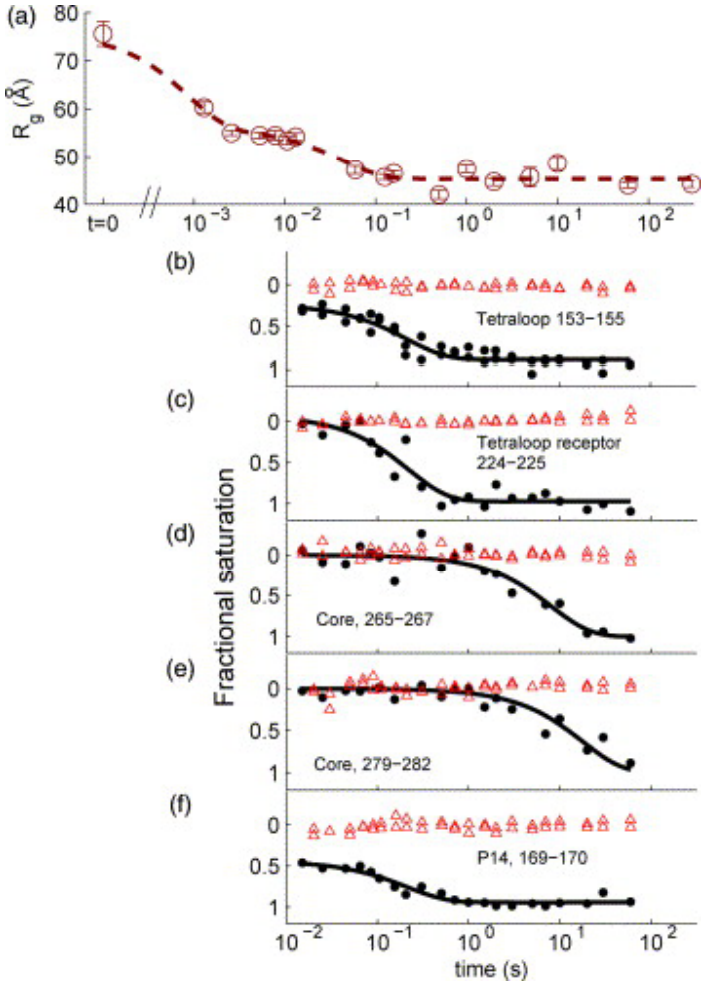


Renewed NIH-NIBIB Funding

SAXS and Footprinting : Concordant measurement of Global & Local Structure

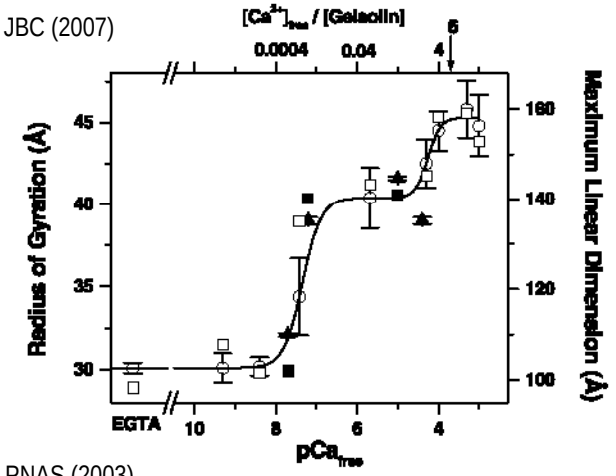
Tetrahymena **Ribozyme**

Kwok et al. JMB (2006)

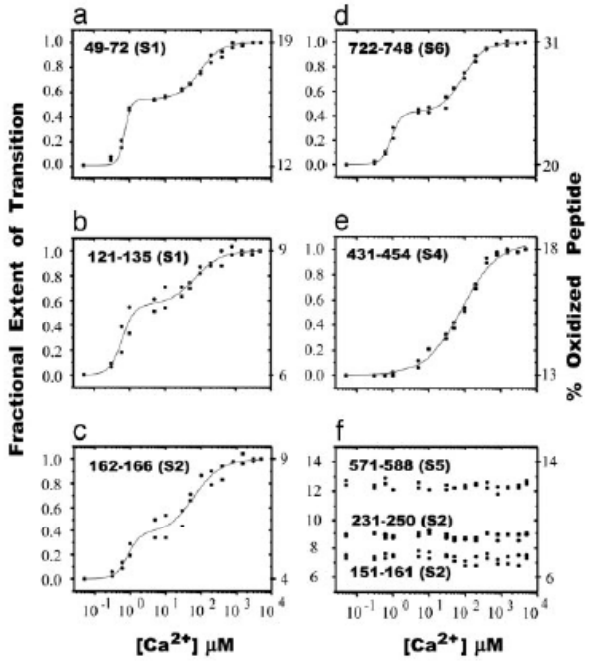


Gelsolin activation by Ca^{2+}

Ashish et al. JBC (2007)



Kiselar et al. PNAS (2003)



Footprinting and SAXS are complementary techniques

Proposal for a Joint SAXS and Footprinting Facility

— **Several footprinting users use SAXS on the same biomolecular system (> 50% in past five years)**

— **A joint facility where user can determine both global and local structural information could boost user demand significantly.**

— **Footprinting requires much less sample concentration than SAXS, so a consecutive/simultaneous data collection facility will be useful.**

— **The second CDW beamline can be built for biological SAXS (or the footprinting users need collaboration with another NSLSII SAXS facility).**

— **Efficient utilization of sample preparation time and beamtime usage.**

Summary

- **Significant need for a footprinting beamline at NSLSII**

- **Footprinting is a flux driven experiment, thus the DW is the appropriate source**

- **Construction timeline for the development of beamline and transfer.**

- **Footprinting is a technique well suited to complement other techniques (SAXS, MX) and should be included in a biological sector at NSLSII**