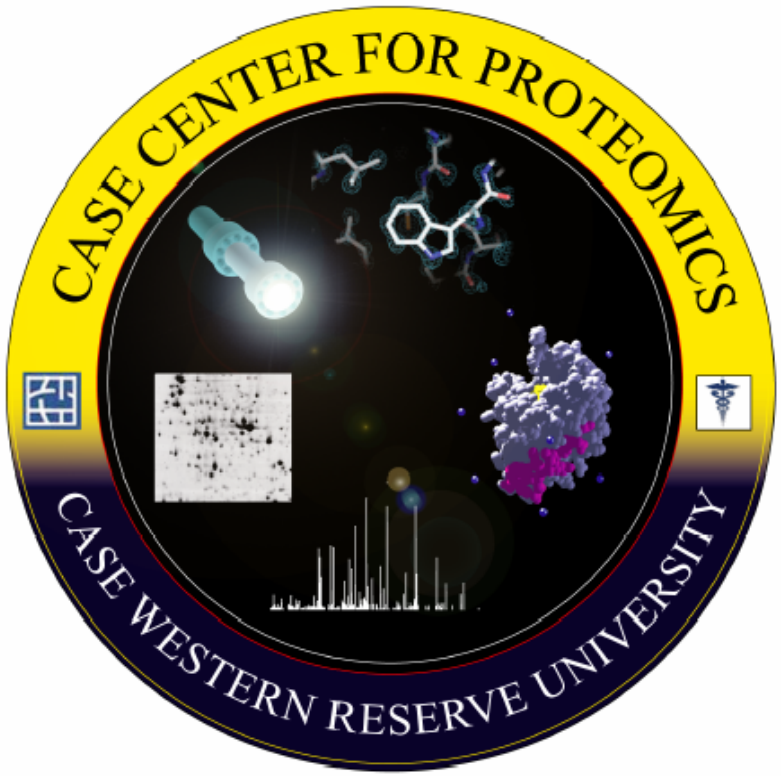
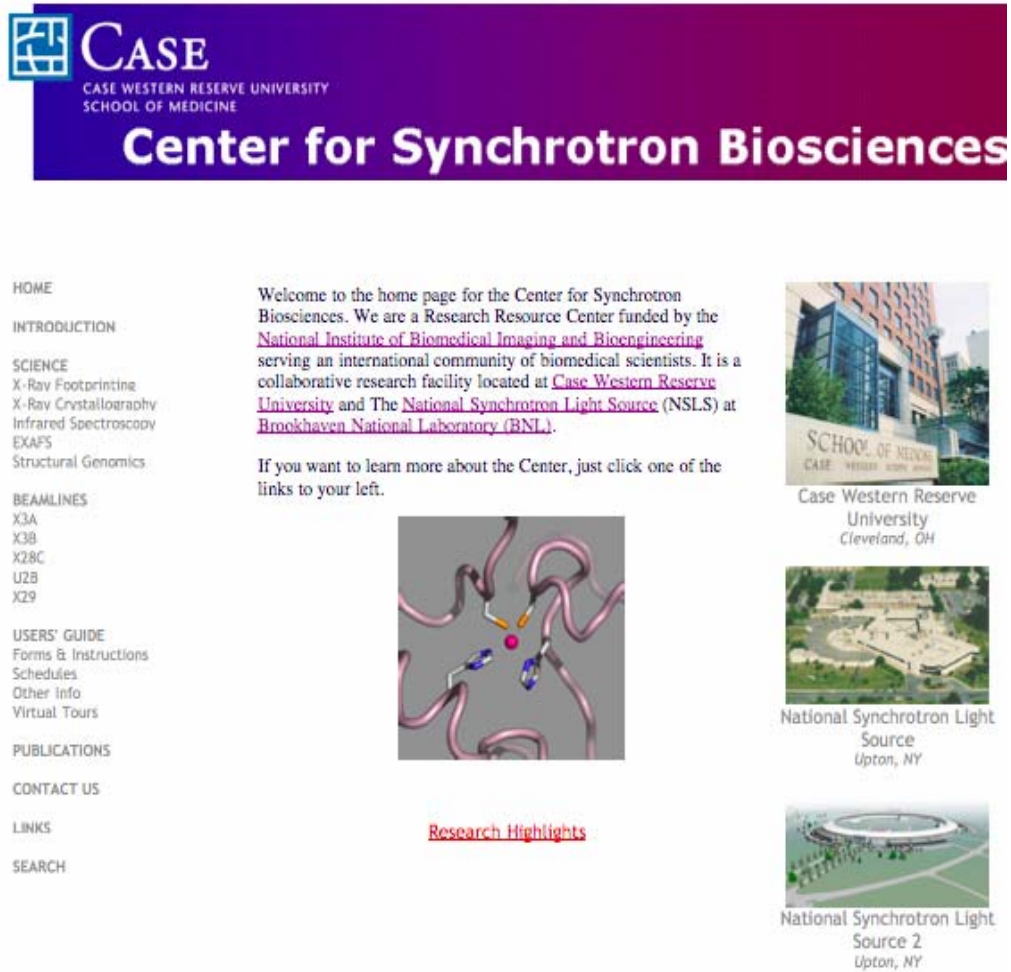
X-ray Spectroscopy and X-ray footprinting programs: Center for Synchrotron Biosciences at NSLS-I & II



Mark Chance
NSLS-II
Life Sciences Breakout
July 18, 2007

CSB Synchrotron Programs



5 beamlines for: XTAL, XAS, FP, IR

>25% NSLS Users

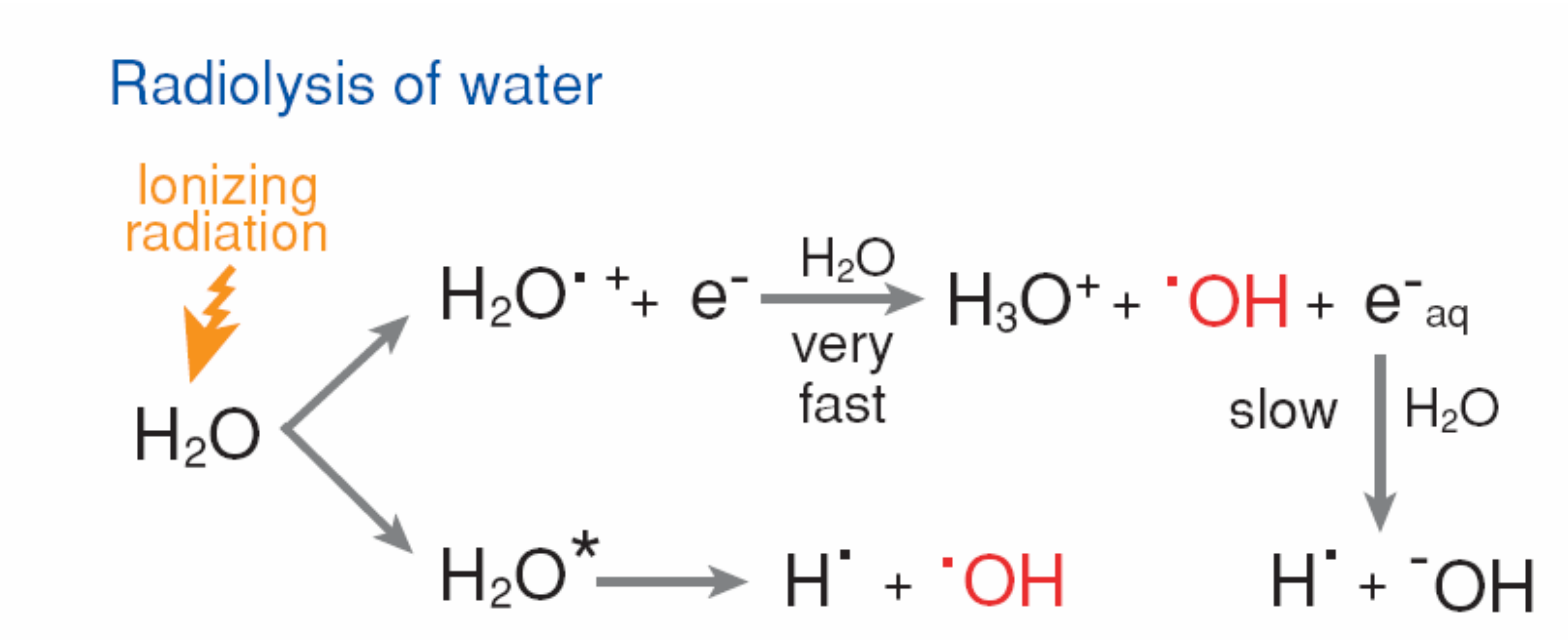
>60% Life Sciences users

150 publications last year

>580 publications last 5 years

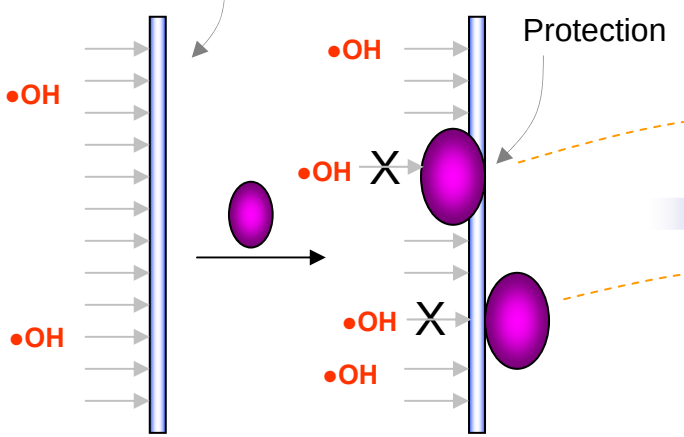
Funded by National Institute for Biomedical Imaging and Bioengineering (NIBIB)

Chemistry of Hydroxyl Radical Production for X-ray Footprinting: Flux-Density Driven Experiment



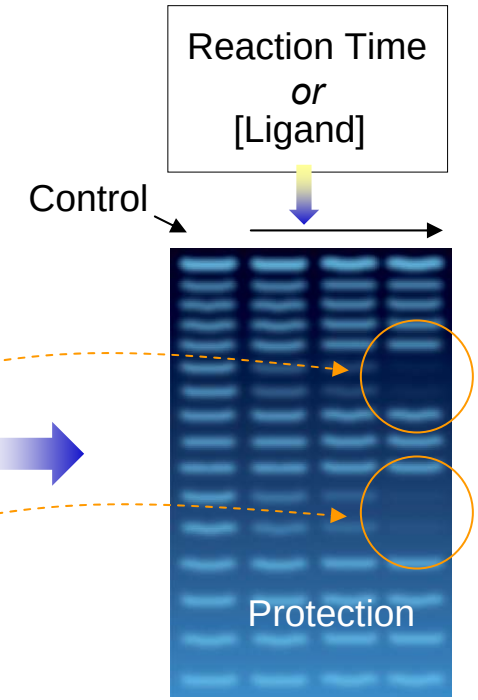
Critical Energy of Beamine and Photon Flux Important: Focused White Beam

X-ray Nucleic Acid Footprinting – The Technique



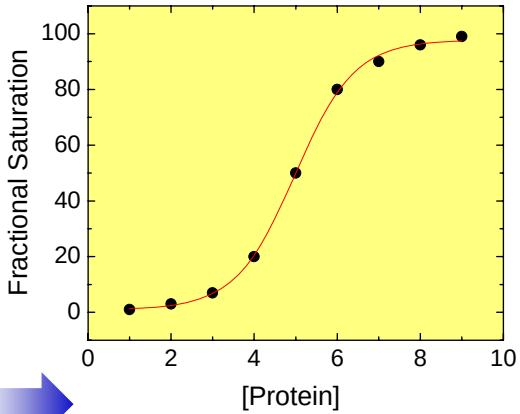
***ds*DNA**
(Control)

***ds*DNA + Ligand**



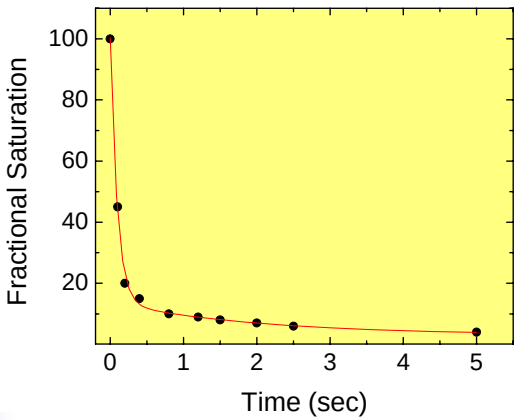
Gel Electrophoresis

Binding Isotherm



Site Specific K_d

Kinetic progress curve

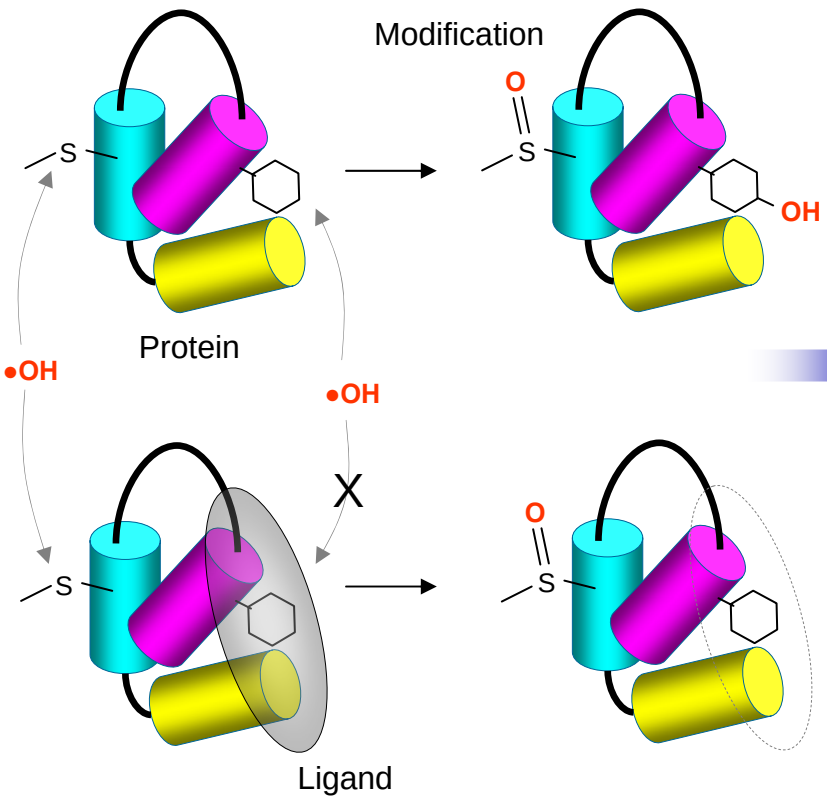


Site Specific Rate Constants, k_{obs}

Binding / Folding Mechanism

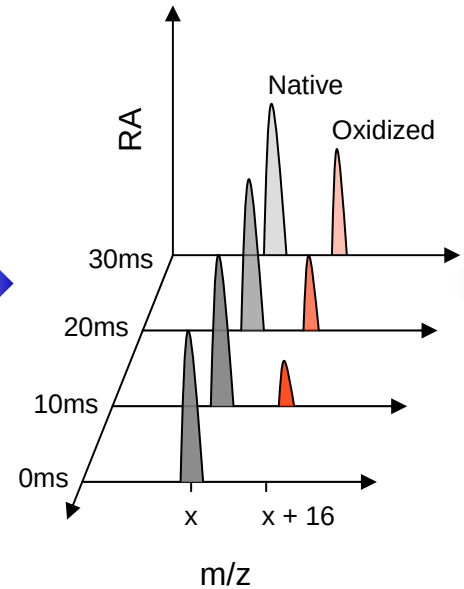
X-ray Protein Footprinting – The Technique

$H_2O \xrightarrow{\text{X-ray}} \bullet OH$

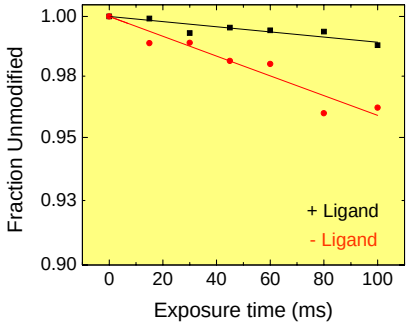


X-ray radiolysis

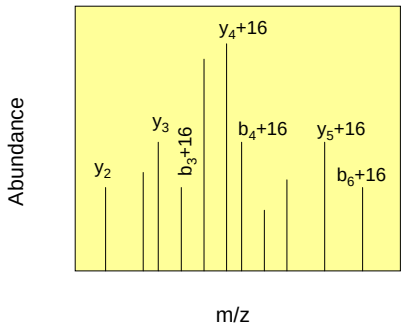
MS analysis



Dose Response



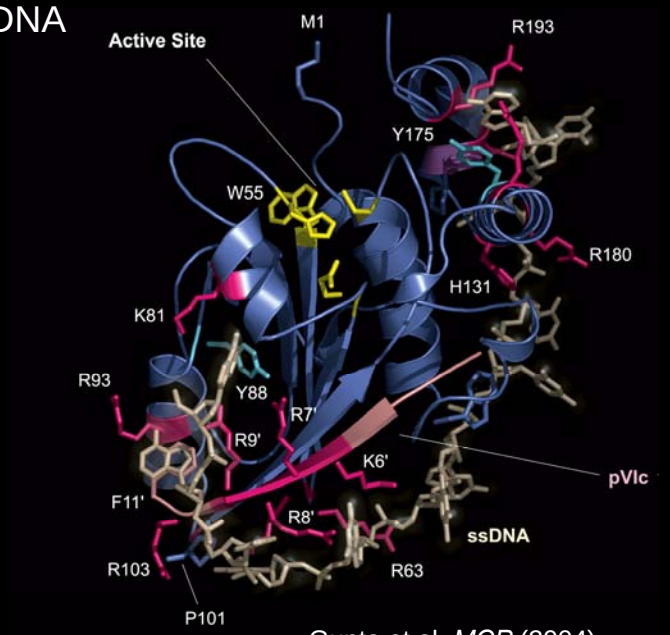
MS/MS



Structural Modeling

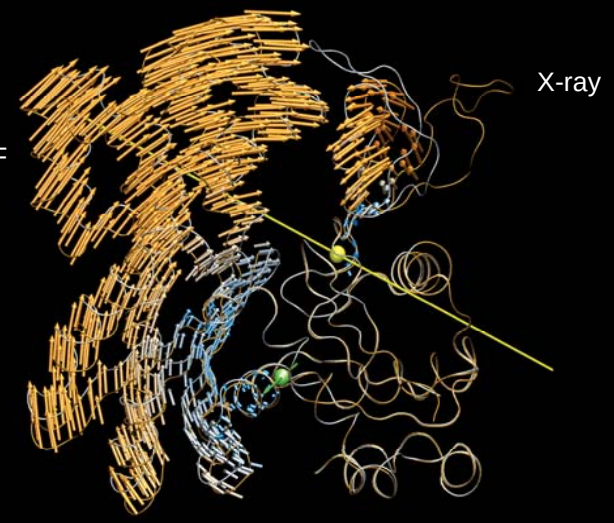
X-ray Footprinting – Protein Structure Modeling - & Future Scope

AVP-DNA



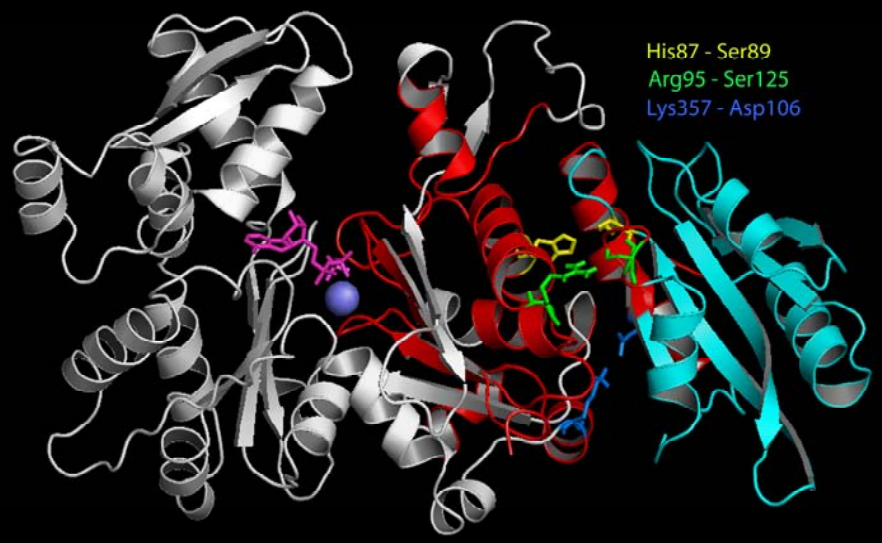
Gupta et al. *MCP* (2004)

Closure of nucleotide cleft in G-actin



Takamoto et al. *Structure* (2007)

G-Actin-Cofilin complex



Kamal et al. *PNAS* (2007)

Protein Targets

↓

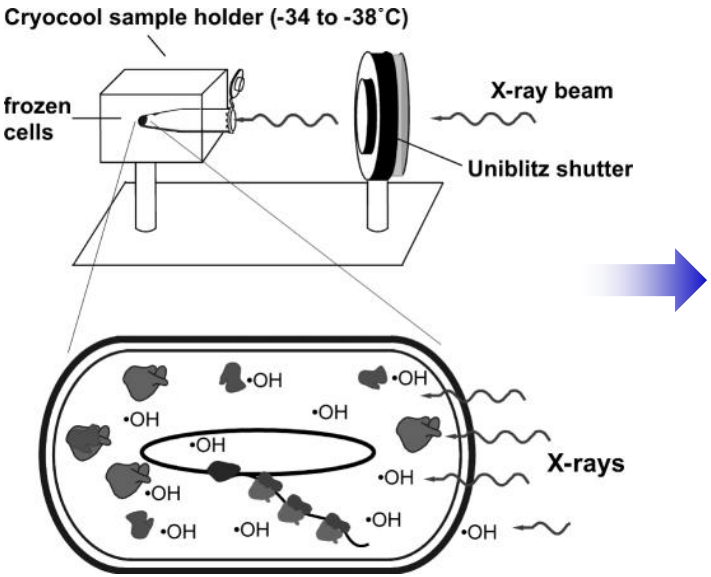
Protection Mapping by SF

↓

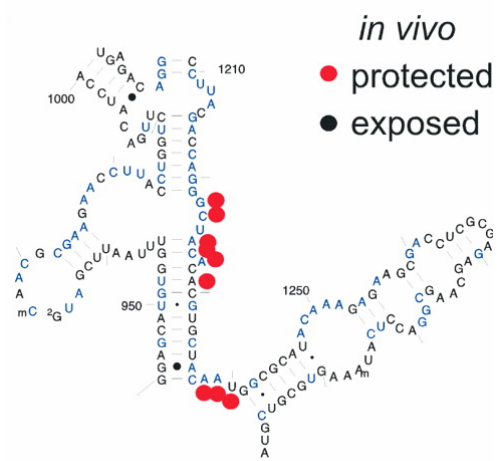
Structural Modeling

X-ray Footprinting – *In vivo* Macromolecular Interactions - & Future Scope

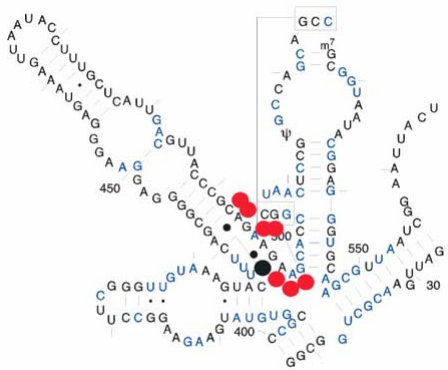
The first *in vivo* X-ray footprinting



E. Coli was grown to log phase and frozen into pellets.



In vivo footprints of 16S rRNA.

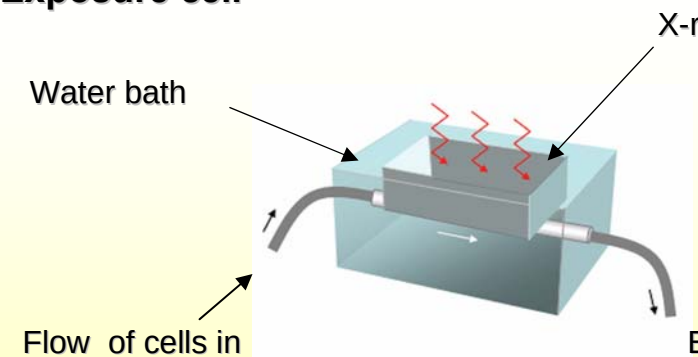


Adilakshmi et.al. *NAR*. (2006).

Systems of Interest

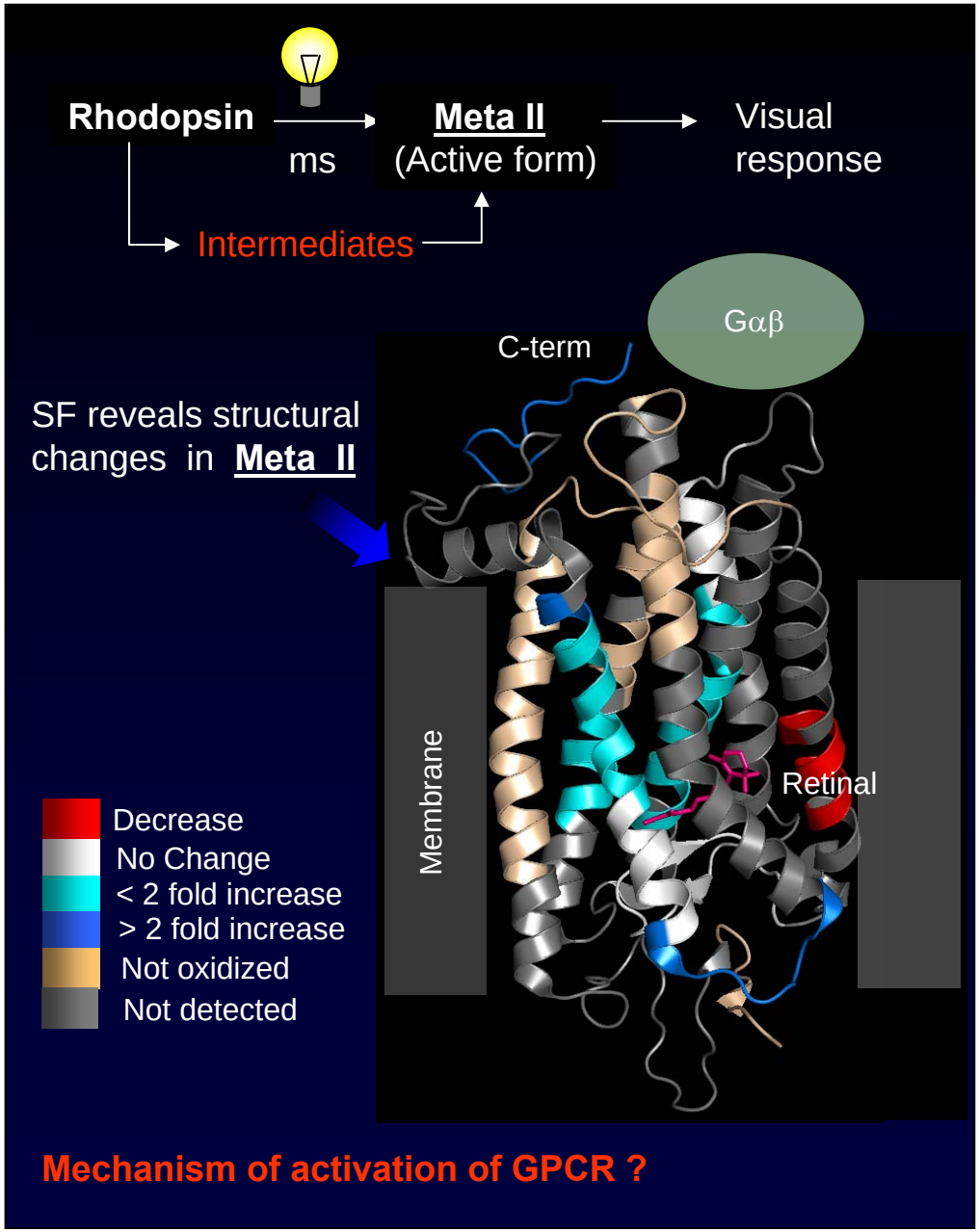
- **Ribosomal Assembly**
- **Gene expression by extra-cellular stimuli.**
- **Ligand binding in cell surface receptors.**

Exposure cell

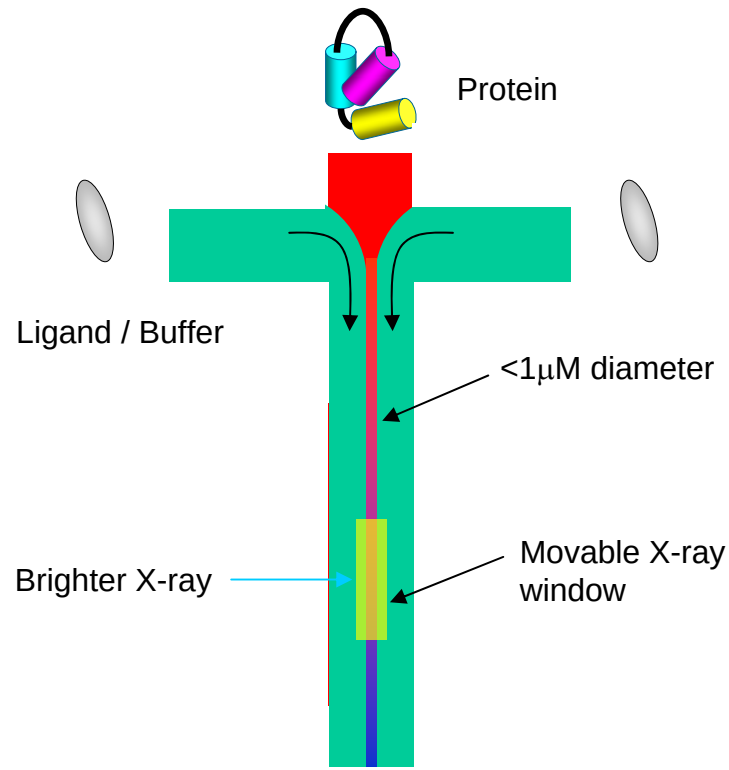


High brightness of NSLS II ▶ shorter exposure time

X-ray Footprinting – Kinetics of Macromolecular Interactions – & Future Scope



Micro-fabricated Flow Cell Mixer



Development of ultra-fast mixer with X-ray exposure cell.
The high brightness of NSLS II will revolutionize mixer experiment.

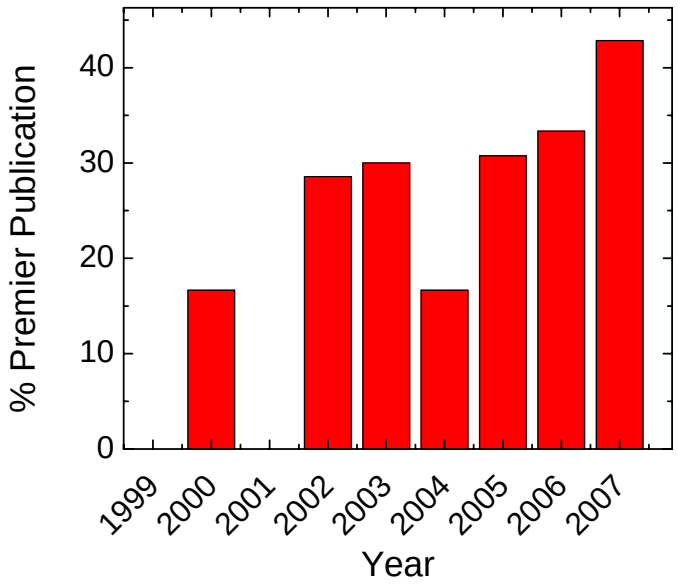
X-ray Protein Footprinting – The Beamline X28C

User access: **Core** and **Collaborative** → **Service** → **General User**

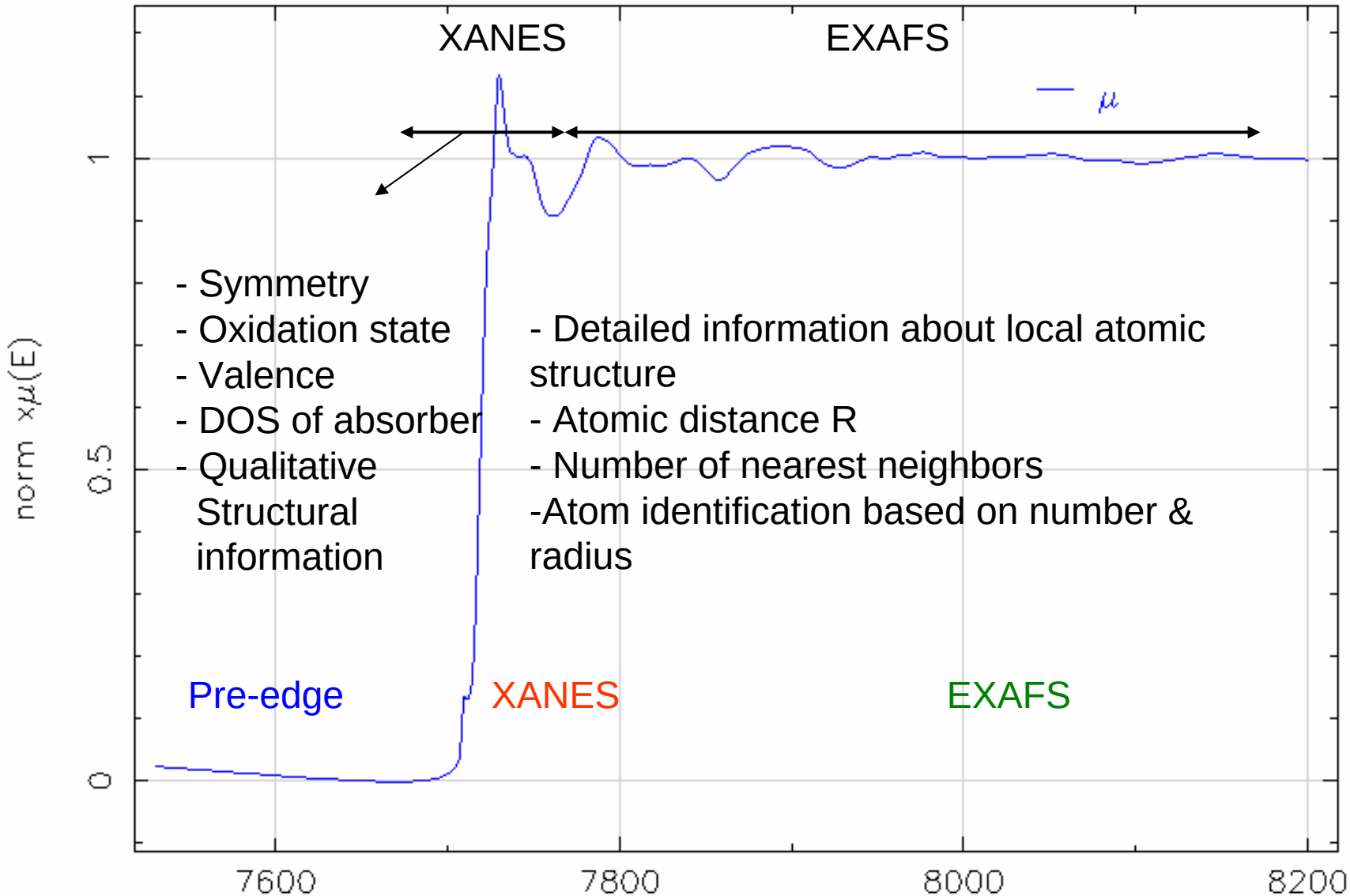
Research Institutions (2003-2007) :

1. **Case Western Research University** **(PRT)** *(GPCR & In vivo)*
2. **Albert Einstein College of Medicine** *(In vivo)*
3. University of Virginia
4. University of Georgia
5. University of Texas, Austin
6. Sloan-Kettering Institute for Cancer Research
7. Columbia University
8. **Johns Hopkins University** (*In vivo*)
9. Brookhaven National Laboratory
10. CNLRS France
11. ETH Labs, Switzerland
12. LNLS, Brazil
13. **MIT** (*Proteosome Assembly and Function*)
14. University of Berkeley
15. University of California San Francisco
16. **University of Oxford** (*Gated K^+ Channel*)

Premier Publications:



Biological XANES and EXAFS at X3B



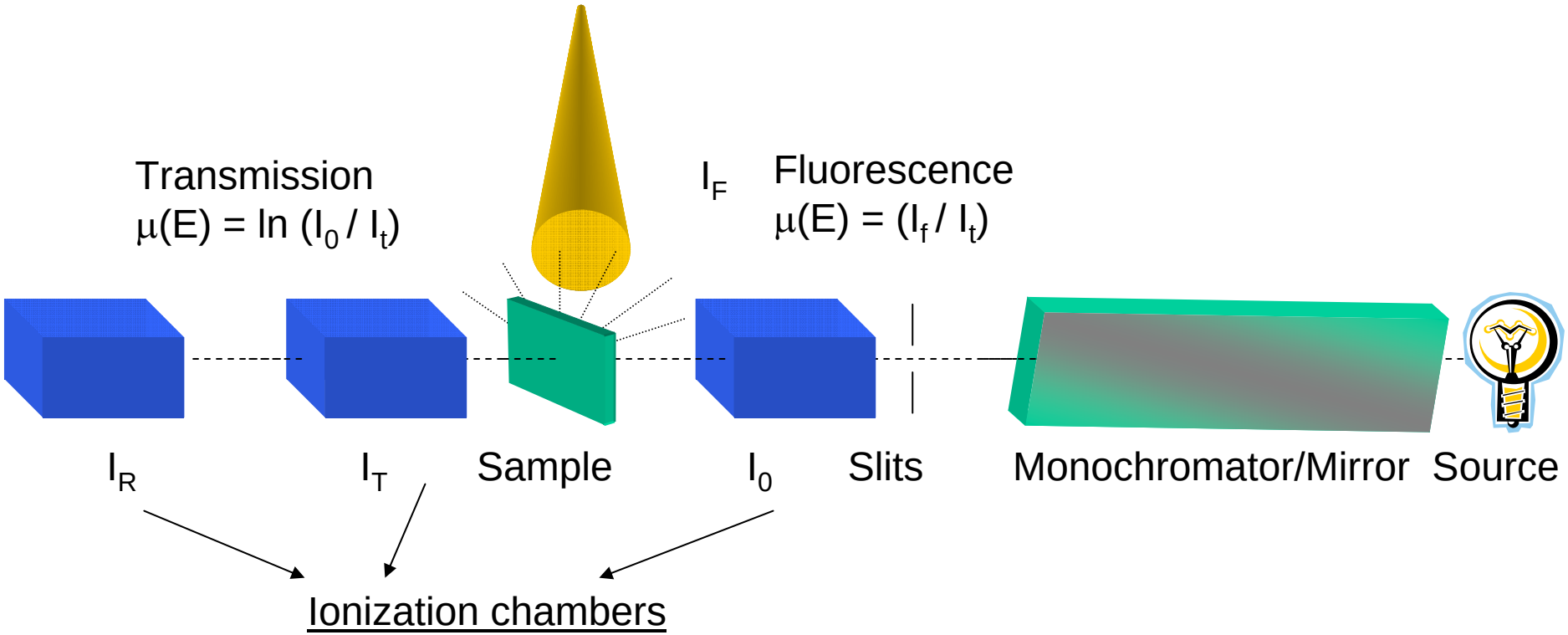
Analyzed by simulations

Analyzed by curve fitting

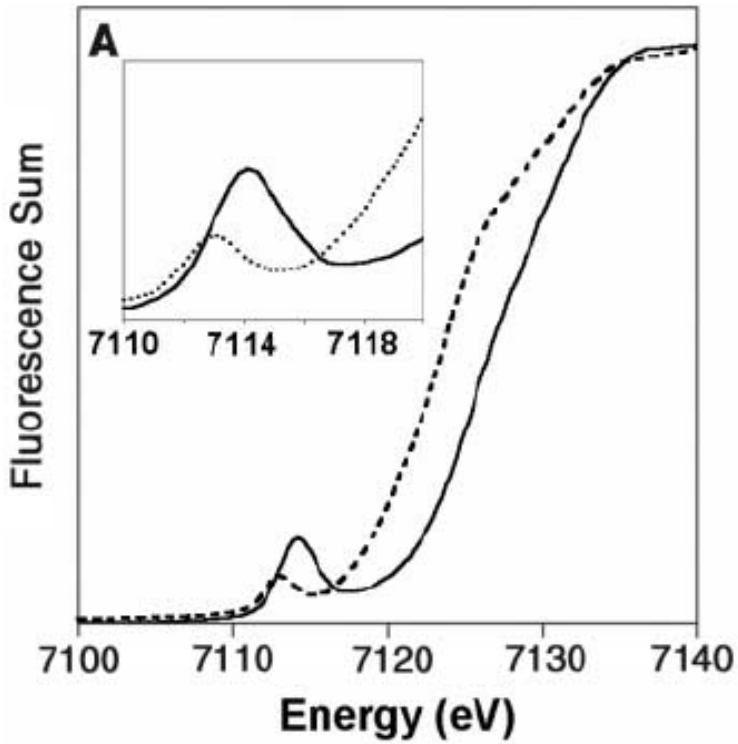
Schematics of XAS Experiment

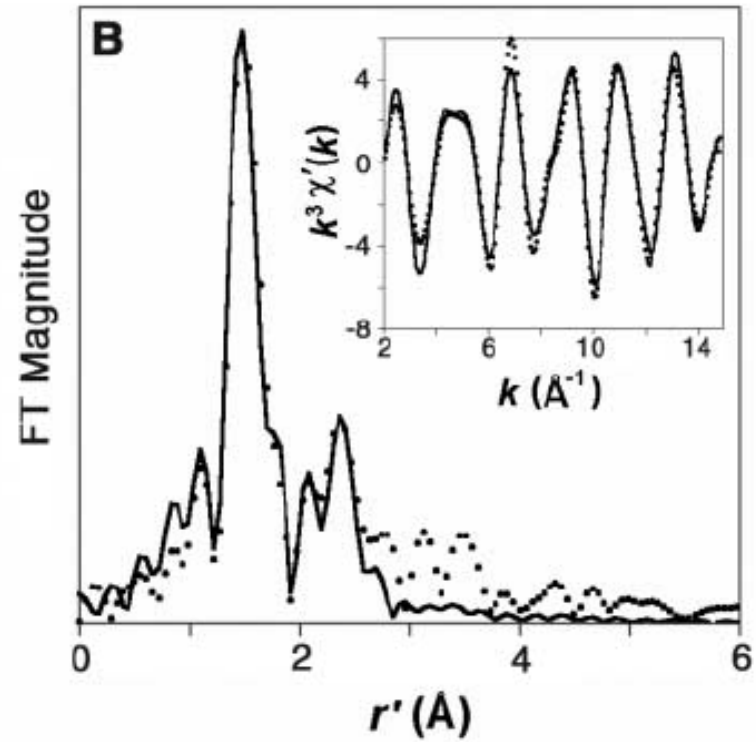
Main Components include (1) x-ray source, (2) the beamline optics, (3) the sample, (4) the detectors, and (5) data acquisition system

Multi element Ge-detector: Limiting Factor



Chemical and Spectroscopic Evidence for an Fe^{V}- Oxo Complex



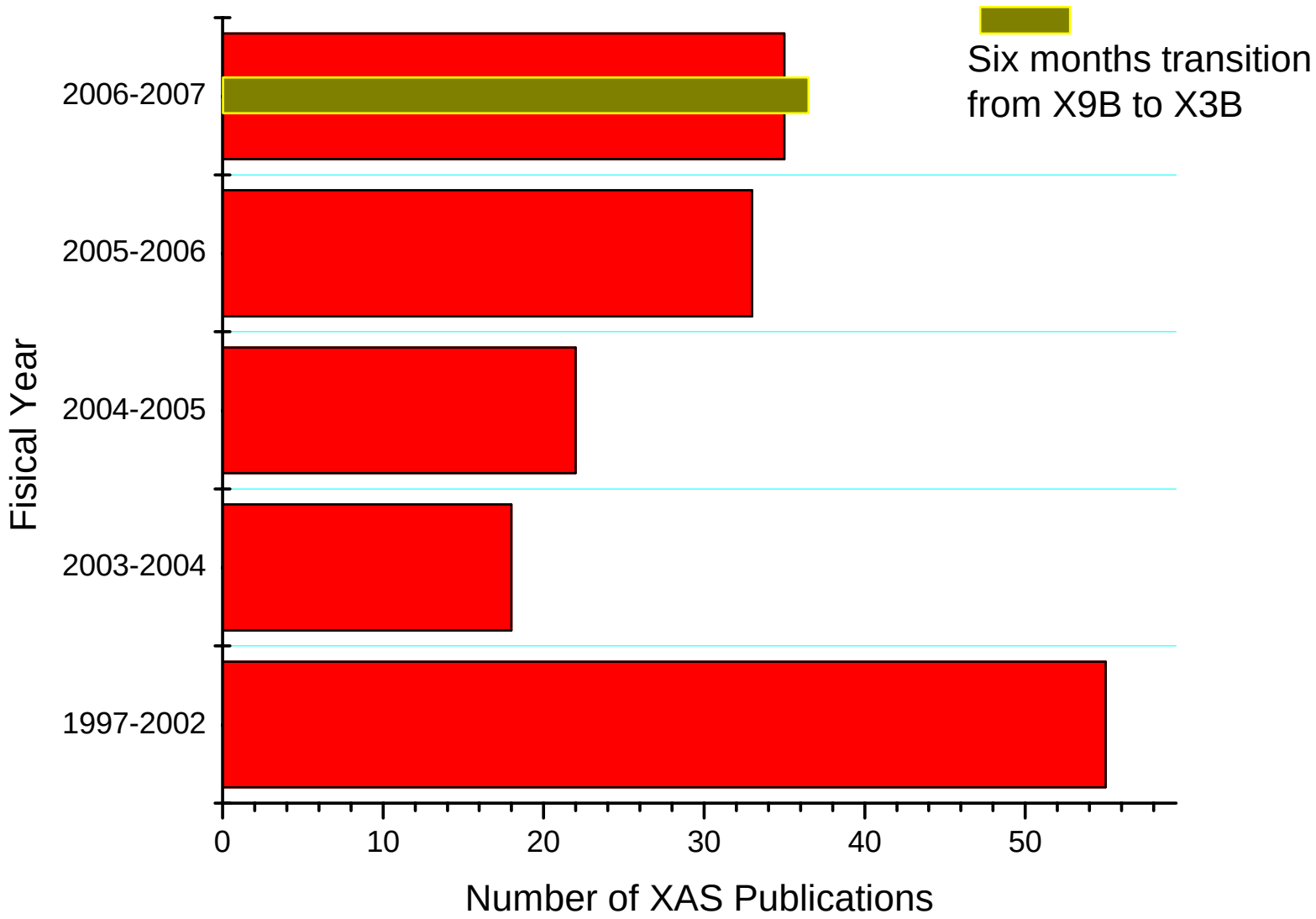


X-ray absorption near-edge spectra (Fe K edge) of Oxo species (solid line) and 1 (dashed line). The inset shows the magnified 1s→ 3d transition features.

Fourier transforms of the Fe K edge EXAFS data ($k^3c(k)$) (dots) and the fit (solid line) for 3 with 0.7 O scatterer at 1.58 Å, four N/O scatterers at 1.87 Å, and five C scatterers at 2.82 Å.

Oliveira et al Science; 315 (835) 2007

X9B/X3B-Recent Publication Count



NSLS-II Beamline Plans

- **Footprinting beamline on a Damping Wiggler part consistent with "strawman" plan-**
  #1 ranked international facility
  Will Allow microsecond dynamics of dilute *in vitro* systems and millisecond *in vivo* experiments
- **State-of-the-art EXAFS beamline dedicated to the field of biosciences will be requested on a Damping Wiggler** with additional capacity using a 3 pole wiggler as a later effort
- Extension of XAS measurements into microsecond time resolved realm; ultra-dilute systems, needs detector development
- Enhanced user support and facility-user collaboration model important features of new facility

Thanks to:

NSLS-I &II
Steve Dierker
Chi-Chang Kao
Lisa Miller
BNL
Bob Sweet & Staff

CSB Staff
Mike Sullinav
Sayan Gupta
Wuxian Shi
Sandeep Rekhi
Don Able
John Toomey
Jen Bohon
Ruijita

Biomedical Technology Program of
National Institute of Biomedical Imaging and Bioengineering

IMAT Program of National Cancer Institute

National Institute of General Medical Sciences

NSLS, DOE