Since 1982 X9B (now X3B) has been a national and international resource for X-ray Absorption Spectroscopy (XAS). X9B was moved to X3B in summer 2006 and is now fully operational.

X3B User Community:
- Biological Science, >70%
- Materials Science, ~20%
- Environmental Science, ~10%

Research Institutions:
- BNL, Energy Science Division
- Case Western Reserve University
New York Structural Genomics Research Consortium
- Northwestern University
- University of British Columbia,
- University of New Mexico,
- University of Massachusetts,
- University of Minnesota,
- Wayne State University
- Weizmann Institute of Science
- University of Nevada at Las Vegas
- Chemistry at Brookhaven National Lab
What is X-ray Absorption Spectroscopy?
There are three channels of interaction of x-ray and matter:
- elastic scattering (x-ray diffraction);
- inelastic scattering (Compton, Raman, etc);
- x-ray absorption (XAS)

XAS can be divided into two regions:
**XANES**: X-ray Absorption Near-Edge Spectroscopy
**EXAFS**: Extended X-ray Absorption Fine-Structure
Key attractions of XAS for Biological Research:
(1) XAS provides information on electronic and atomic structure for both crystalline and non-crystalline systems;
(2) XAS is a comprehensive atomic level structural tool sensitive to within ~ 4-5 Å of metal sites;
(3) XAS allows an order of magnitude more accurate bond length determination than that obtained by protein crystallography;
(4) XAS is an extremely fast probe (τ < 10^-14 s for Fe K-edge) that is suitable for multiple-scale time-resolved experiments; Combination of (1)&(4) makes XAS a unique technique for probing structure of reactive intermediates in solutions.
(5) Contrary to UV-VIS and EPR spectroscopy XAS is always detectable: There are no “spectroscopically quiet” metals;
(6) XAS is capable of probing dilute samples at the micromolar level;
(7) Since XAS measurements are usually done at 10-20K, biosamples are less susceptible to x-ray beam photoreduction compared to the XRD. Photoreduction at the active center is easy to monitor via XANES.
Beamline instrumentation:
Energy Range (keV): 4.5-13.5, Si(111)
Energy Resolution ($dE/E$): $\sim2\times10^{-4}$
Photon Flux (ph/s, 250mA): $\sim10^{11}$
Spot Size (mm): 30-0.5H and 1-0.3V

X3B is equipped with:
- 13-element Ge detector ($<10^5$cps per detector at the energy resolution of 400 eV)
- Closed Cycle Cryostat ($T=15-350K$);
- Metalloproteomics measurement assemblage
EXAMPLE: A Nickel Superoxide Dismutase Maquette That Reproduces the Spectroscopic and Functional Properties of the Metalloenzyme

Described herein is a nickel superoxide dismutase (NiSOD) maquette ([Ni(SODM1)]) based on the first 12 residues from the N-terminal sequence of Streptomyces coelicolor NiSOD. The apopeptide (SODM1) was prepared by standard solid-phase Fmoc peptide synthesis. SODM1 will readily coordinate NiII in a 1:1 ratio in slightly basic aqueous sodium phosphate buffer (0.1 M; pH 7.2) forming a lightly colored beige/pink solution. Unlike NiSOD, which is isolated as a 1:1 mixture of oxidized (NiIII) and reduced (NiII) forms, [Ni(SODM1)] can only be isolated in the NiII oxidation state. The UV/vis, X-ray absorption, and CD spectra of [NiII(SODM1)] correspond well with those reported for the reduced form of NiSOD. Despite the fact that [NiIII(SODM1)] is not isolable, [Ni(SODM1)] has an appropriate redox potential to act as an SOD (E1/2 = 0.70(2) V vs Ag/AgCl) and in fact will catalytically disproportionate >40 000 equiv of KO2.

Figure Oxidation of NiSOD to the NiIII oxidation state produces a structural change about the metal center; the N-terminal histidine imidazole coordinates to NiIII in the axial position

Example: Monomeric Yeast Frataxin is an Iron-Binding Protein

Friedreich's ataxia, an autosomal cardio- and neurodegenerative disorder that affects 1 in 50,000 humans, is caused by deficiency of the protein frataxin. To provide a molecular basis to better understand frataxin's function, the binding properties and metal-site structure of ferrous iron bound to monomeric yeast frataxin were characterized x-ray absorption spectroscopic studies. It has been observed that the yeast frataxin is stable as an iron-loaded monomer, and the protein can bind two ferrous iron atoms with micromolar binding affinity. Frataxin amino acids affected by the presence of iron are localized within conserved acidic patches located on the surfaces of both helix-1 and strand-1. XANES analysis indicates that under anaerobic conditions, bound metal is stable in the high-spin ferrous state. The metal-ligand coordination geometry of both metal-binding sites is consistent with six-coordinate iron-(oxygen/nitrogen) based ligand geometry, surely constructed in part from carboxylate and possibly imidazole side chains coming from residues within these conserved acidic patches on the protein. The metal-ligand nearest neighbor coordination environments in frataxin is nearly identical, independent of whether or not there are one or two metals bound. On the basis of these results, a model has been developed on the interaction of yeast frataxin with iron.

**Figure I**: XANES comparison of iron-yeast frataxin with ferrous and ferric models. Full XANES spectra for yeast frataxin with one iron (—) and two iron bound (—) with ferrous ammonium sulfate (----) and ferric ammonium sulfate (…..). Inset depicts expansion of background-subtracted 1s→3d region of XANES spectra of all samples.

**Figure II**: EXAFS and Fourier transforms of iron-loaded yeast frataxin XAS data. EXAFS spectra in black for yeast frataxin with one iron bound (A) and two iron bound (C) along with the corresponding Fourier transforms (B and D, respectively).

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