

**Structure and Reactivity of a Superoxide Reductase Model Complex Probed in Solution by XAFS.**

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Beamline(s): X18B

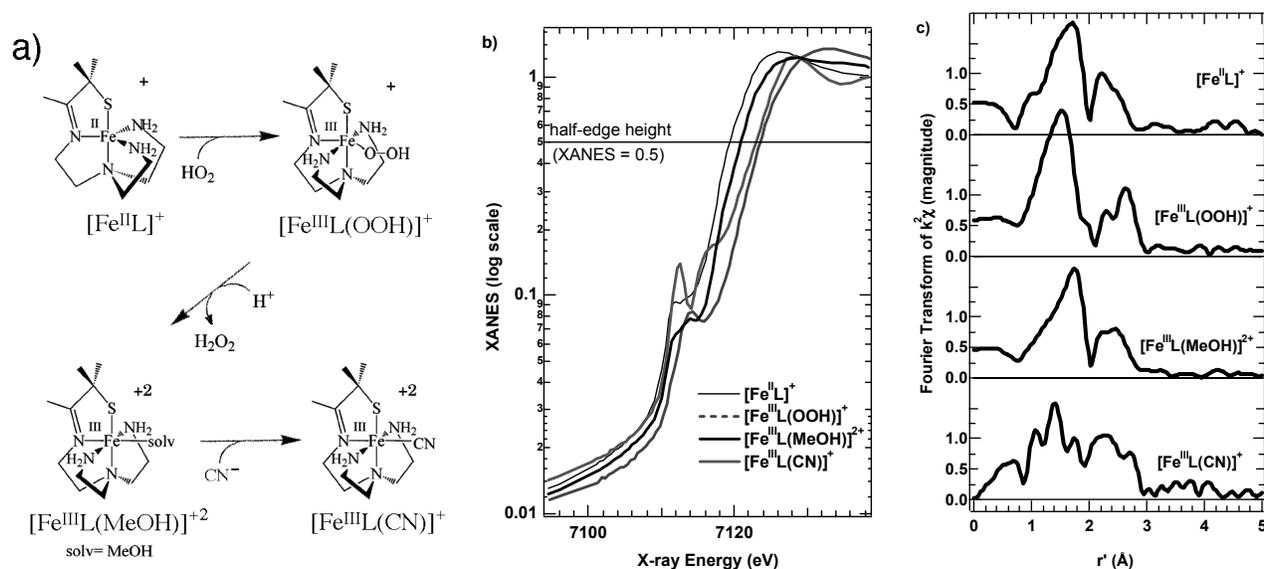
**Introduction:** Superoxide reductases (SORs) are recently-discovered metalloenzymes that degrade toxic superoxide by reducing it to hydrogen peroxide. During catalysis, the iron in the catalytic site cycles between the  $\text{Fe}^{\text{II}}$  and  $\text{Fe}^{\text{III}}$  states. The superoxide is proposed to bind to enzyme's  $\text{Fe}^{\text{II}}$ , followed by inner sphere electron transfer to produce an  $\text{Fe}^{\text{III}}(\text{OOH})$  intermediate.[1] A model ligand that shares SOR's 4 N + 1 S ligand atom set is shown in figure 1a along with some of its reactivity, including a reaction with superoxide. We have used XAFS spectroscopy to characterize the iron coordination in these complexes in solution and in the solid state.

**Methods and Materials:** 5 mg of  $[\text{FeL}(\text{CN})][\text{PF}_6]$  was ground with 100 mg of BN and packed into a 1.3 mm deep slot in aluminum sample holders. The other samples were prepared as frozen solutions in dry  $\text{O}_2$ -free methanol, with a THF solution of  $\text{KO}_2$  used to introduce the superoxide. The  $[\text{FeL}(\text{OOH})]^+$  solution was frozen within 30 seconds of mixing at  $-20^\circ\text{C}$ . 1 mil 3M Kaptan tape was used to hold the sample in the slot. XAFS data was collected in both transmission and fluorescence modes at beam line X18B. Data analysis and graphing employed "RCSEXAFSPackage" written for *Igor Pro* software and general methods that have been described previously.[2]

**Results:** The XANES spectra show that the edge energy (most conveniently approximated as the energy at which the XANES = 0.5) shifts to higher energy on conversion of the  $\text{Fe}^{\text{II}}$  starting complex to the  $\text{Fe}^{\text{III}}$  state. The edge energy is reduced somewhat in the  $[\text{FeL}(\text{MeOH})]^{2+}$  complex relative to the  $\text{CN}^-$  and  $\text{OOH}^-$  complexes; this may be associated with a spin state change (the  $\text{CN}^-$  and  $\text{OOH}^-$  complexes are low spin, while the MeOH solvate is intermediate spin ( $S=3/2$ ) based on other measurements.[2] The EXAFS spectra also show changes which were analyzed in terms of bond length changes (associated with oxidation and spin state differences) as well as second-sphere scattering from the ligand atoms. In particular, the enhanced amplitude in the FT near 2.5 Å in the  $\text{OOH}^-$  and  $\text{CN}^-$  complexes can be analyzed by including second-sphere scattering from the oxygen or nitrogen atom of these ancillary ligands; in the  $\text{CN}^-$  case, we have used multiple scattering theory in fitting the EXAFS.

**Conclusions:** XANES and EXAFS spectroscopy carried out at the NSLS shows changes in iron coordination in superoxide reductase model complexes. Some of this work has recently been published. [2]

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**References:**[1] Rusnak, F. *J. Biol. Inorg. Chem.* **2002**, 7, 646-646.[2] Shearer, J.; Scarrow, R. C.; Kovacs, J. A. *J. Am. Chem. Soc.* **2002**, 124, 11709-11717

**Figure:** (a) Reaction scheme for formation of SOR model complexes discussed in this abstract. (b) Comparison of XANES spectra of complexes. (c) Comparison of Fourier Transforms of EXAFS ( $k^2\chi$ ) for the complexes.