RESONANT X-RAY ABSORPTION BY ^{57}FE: A SITE-SELECTIVE PROBE OF PROTEIN STRUCTURE AND ELASTICITY

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Iron-containing proteins are indispensable to the life, regulation, aging, and death of the cell. Cellular metabolism depends on enzymes containing Fe and other metals and contributes to global biogeochemical cycles that exchange atmospheric pools of oxygen and nitrogen with biologically useable forms of these elements.

Measurements of resonant nuclear absorption by ^{57}Fe at synchrotron facilities selectively reveal vibrational motion of individual iron atoms within proteins containing thousands of other atoms. Quantitative comparison of the resulting information on vibrational amplitudes and directions, as well as frequencies, with quantum chemical predictions identifies vibrations of all Fe-ligand bonds and also provides a detailed test of the predicted electronic structure. I will present recent applications to enzymes that contain iron within a planar heme group.

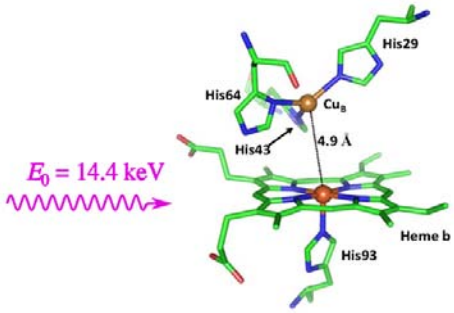

Figure 1. Mutational redesign of myoglobin introduces a second nonheme metal binding site near the heme, yielding engineered proteins Cu_BMb and Fe_BMb that mimic the catalytic sites of heme-copper oxidase and nitric oxide reductase enzymes, respectively.

One question of current interest is how a nearby nonheme metal site controls the reaction of the heme with nitric oxide (NO). NO inhibits the heme-copper site that consumes oxygen in cellular respiration, but is metabolized to produce the greenhouse gas nitrous oxide (N_2O) in closely related bacterial enzymes with iron in the nonheme site. Protein engineering mimics the catalytic site (Fig. 1) and enables selective metal replacement at either heme or nonheme site, opening the door to detailed spectroscopic investigation of reaction intermediates that are too unstable to characterize in the natural enzyme. In addition, recent results demonstrate that vibrational dynamics of heme and nonheme irons can be probed independently (Fig. 2).

Finally, averaged force constants, derived directly from the experimentally determined vibrational density of states (VDOS), quantify important aspects of structure and elasticity even when individual vibrational modes cannot be resolved and identified. Fe-ligand vibrations make the primary contribution to the *stiffness*, an effective force constant which measures the average strength of nearest neighbor interactions, while low frequency oscillations of the protein determine the *resilience*, a distinct force constant that probes the elasticity of the iron environment.

Table I: The stiffness and resilience, averaged force constants derived from vibrational measurements, quantitatively measure distinct elastic properties of heme and nonheme sites.

	force constant (pN/pm)	
	stiffness	**resilience**
Mb(II)	189 ± 20	21.2 ± 1.3
$Fe(II)_B$*Mb(II)	182 ± 9	20.3
*$Fe(II)_B$Mb(II)	155.± 6	20.1

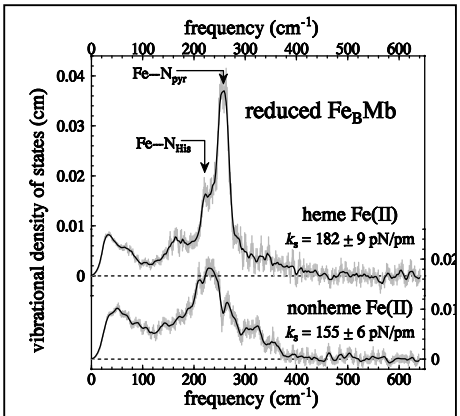

Figure 2. Selective incorporation of ^{57}Fe enables independent measurement of vibrational dynamics of iron at both heme and nonheme sites.