

Observation of Fe–H/D Modes by Nuclear Resonant Vibrational Spectroscopy

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Metal–H bonding is important in chemistry, catalysis, and the mechanisms of hydrogenase (H₂ase)¹ and nitrogenase (N₂ase).² With notable exceptions,^{3,4} H atoms are difficult to see in protein crystal structures or by EXAFS. Vibrational spectroscopy can probe M–H bonding, but in IR spectra, peptide bands often obscure M–H stretches, and these enzymes have been “black holes” for resonance Raman work. Another approach is “nuclear resonance vibrational spectroscopy” (NRVS), also known as “nuclear resonant inelastic X-ray scattering” (NRIXS).^{5,6} Here we present NRIXS data on Fe–S₄ and FeH(D)₆ model systems. We interpret the spectra and evaluate prospects for studying more complex samples.

NRIXS combines nuclear excitation and vibrational motion.⁶ The effect was predicted early on,^{7,8} but ultracentrifuge radioisotope experiments were difficult.⁹ Later, synchrotron radiation (SR) work demonstrated feasibility.^{10,11} Due to the spectral brightness of SR sources,¹² and X-ray optics progress, NRVS can now be done with high-resolution monochromators.¹³ An exciting aspect is that it provides an isotope selective vibrational spectrum—only modes with movement of the resonant nucleus couple to the excitation. With a randomly oriented sample, in the low-temperature limit, the “recoil fraction” ϕ that goes into a $n_\alpha \rightarrow n_\alpha + 1$ transition is given by: $\phi = (e^2/3)(\nu_R/\nu_\alpha)(n_\alpha + 1)f$.¹⁴ The term e^2 is the fraction of kinetic energy associated with nuclear displacement in mode α , ν_R is the nuclear recoil energy, ν_α the vibrational energy, f the recoilless fraction, and n_α is the mean occupation number of mode α at temperature T .¹⁴

Fe–H stretches have high frequencies¹⁵ and little Fe motion—both factors will reduce NRIXS intensity. To assess whether Fe–H modes could be observed in H₂ase and N₂ase, we studied two simpler systems: tetrahedral FeS₄ in *Pyrococcus furiosus* (Pf) rubredoxin (Rd), and FeH(D)₆ in [FeH(D)₆][MgBr(THF)₂]₄. The met-Rd NRIXS has bands near 61, 154, and 364 cm⁻¹ (Figure 1). Under *T_d* symmetry, there are two T₂ normal modes with Fe motion: ν_3 (mostly Fe–S stretch) and ν_4 (mostly Fe–S bend).¹⁶ In *Desulfovibrio gigas* (Dg) Rd Raman spectra, the degeneracy of ν_3 is lifted, and three bands are observed at an average value of 362 cm⁻¹.¹⁷ We assign our 364 cm⁻¹ NRIXS band to similar modes—partially resolved at 8 cm⁻¹ resolution. In the Raman data, the totally symmetric mode ν_1 is very strong; it is essentially invisible in the NRIXS spectrum, as expected for a mode that has no Fe motion in *T_d* symmetry (Figure 1).

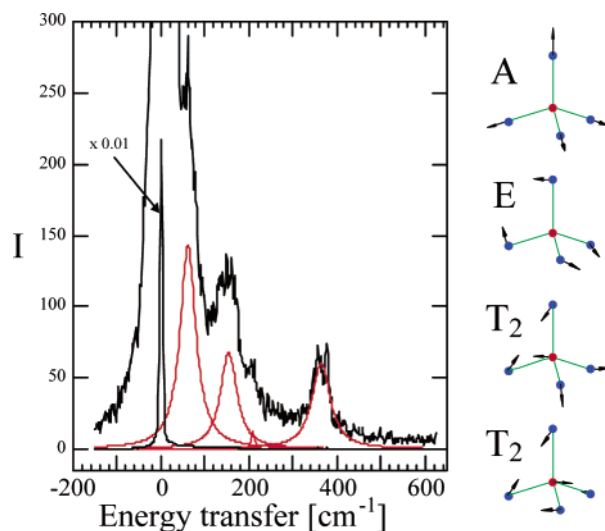


Figure 1. (Left) NRIXS data (black curve) and fits (red curve) for ⁵⁷Fe met-Rd. Ordinate scale is counts/90 s. (Inset) FeS₄ site in Pf Rd. (Right) Diagrams of tetrahedral normal modes.

The lower frequency bands involve mostly bending motion. In *T_d* symmetry, there is a pure bend, the doubly degenerate E mode (ν_2), as well as a mixed T₂ ν_4 mode. For *D_g* Rd, a band at 126 cm⁻¹ was assigned to ν_2 , and a feature at 150 cm⁻¹ to ν_4 .¹⁸ In strict *T_d* symmetry, there is no Fe motion in ν_2 , hence no intensity for this mode in our experiment. For ν_4 , although it is considered a S–Fe–S bend mode, Fe–S stretch character is not forbidden. A more detailed analysis found that S–Fe–S bending motions mix with Fe–S–C bends, and that the band at 150 cm⁻¹ has about 40% Fe–S–C bend character.¹⁷ This may explain the intensity in this region, but a more detailed analysis requires better statistics and calculations using the Pf Rd coordinates.³

The last Rd feature we consider is the band near 60 cm⁻¹. In heme proteins and models, bands between 40 and 80 cm⁻¹ are ascribed to heme doming modes.¹⁹ The Pf Rd crystal structure reveals an Fe site enclosed in a loop at the far end of the protein (Figure 1).³ The NMR structure of Zn-substituted *Dg* Rd suggests that Fe can oscillate with an amplitude of ~0.2 Å,²⁰ and we interpret the 60 cm⁻¹ mode as a collective motion of Fe and some or all cysteine ligands.

The FeH(D)₆ samples have very different spectra (Figure 2). In *O_h* symmetry, only T_{1u} modes, ν_3 and ν_4 , have central atom motion.¹⁶ The ν_4 mode is mostly bending and hence occurs at lower energy than the primarily stretching ν_3 ; we thus assign the FeD₆

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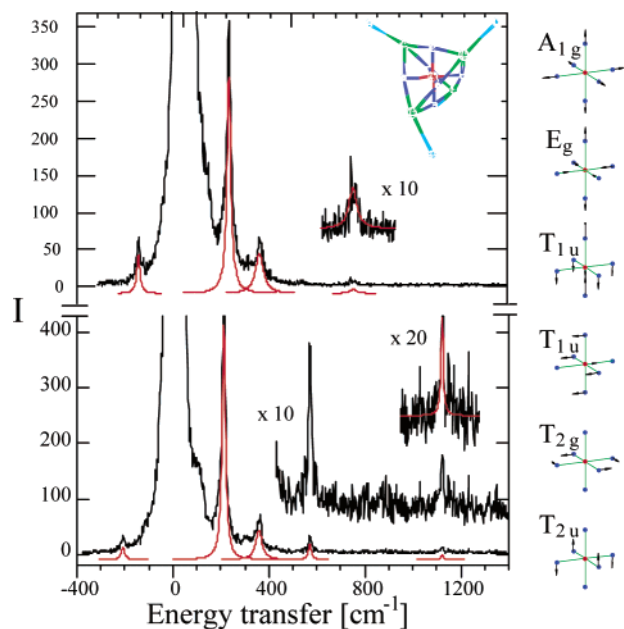


Figure 2. (Left) NRIXS for (top) FeH_6 and (bottom) FeD_6 complexes. Ordinate scales: counts/40 s. (Inset) $[\text{FeH}_6][\text{MgBr}(\text{THF})_2]_4$ structure (THF removed for clarity). (Right) Octahedral normal modes.

resonance at 571 cm^{-1} to ν_{4D} . A $\sim 787\text{ cm}^{-1}$ FeH_6 band is at $1.38\nu_{4D}$, the isotope shift is close to the $\sim\sqrt{2}$ limit for H/D substitution; we assign this as ν_{4H} . For FeD_6 , there is a feature at 1122 cm^{-1} that we ascribe to ν_{3D} : the Fe–H(D) stretch; IR bands are seen at 1138 and 1107 cm^{-1} .²¹ There could also be some contribution from an overtone of ν_{4D} . Better statistics are needed to observe ν_{3H} (seen at 1514 and 1569 cm^{-1} in the IR).²¹

The $\text{FeH}(\text{D})_6$ complexes also exhibit bands at $\sim 357 \pm 2\text{ cm}^{-1}$ and $\sim 212\text{ cm}^{-1}$. These bands show virtually no isotope effect. A plausible assignment is thus to a collective motion of the $[\text{FeH}(\text{D})_6]^{4-}$ anion. In the solid state, the $[\text{FeH}(\text{D})_6]^{4-}$ octahedron is surrounded by four $[\text{MgBr}(\text{THF})_2]^+$ ions on alternate faces (Figure 2).^{22,23} If both complex ions are considered as point masses, then the structure has T_d symmetry. In this picture, the central $[\text{FeH}(\text{D})_6]^{4-}$ anion will behave akin to Fe in Rd. As before, the T_2 vibrations will be NRIXS allowed, and we assign the 212 and 357 cm^{-1} bands to ν_4 and ν_3 respectively.

We now return to the original question—can this technique be extended to Fe in H_2ase and N_2ase ? The Rd results show that Fe–S vibrations in more complex enzymes will be readily observed but will not interfere in the Fe–H(D) bend regions. Given the difficulty in observing Fe–H stretches in $[\text{FeH}_6][\text{MgBr}(\text{THF})_2]_4$, prospects for seeing Fe–H stretches in proteins are not at first encouraging. However, the Br in this sample (K-edge at 13.5 keV) reduced the penetration length for 14.4 keV photons ~ 200 -fold vs a protein sample. We observed a count rate in the Fe–D bend region of $\sim 1\text{ s}^{-1}$, and comparable rates would be seen for proteins with ~ 1750

ppm or $\sim 35\text{ mM}$ Fe. Observation of Fe–D bonds at the mM level would require several days. Better insertion devices, optics, and detectors are needed to make this a routine experiment. The continuing evolution of SR sources and optics could yield orders of magnitude better sensitivity soon.^{24,25}

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Supporting Information Available: Procedures for ^{57}Fe -met-Rd and $[\text{FeH}(\text{D})_6][\text{MgBr}(\text{THF})_2]_4$ preps and NRIXS measurements (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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