

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

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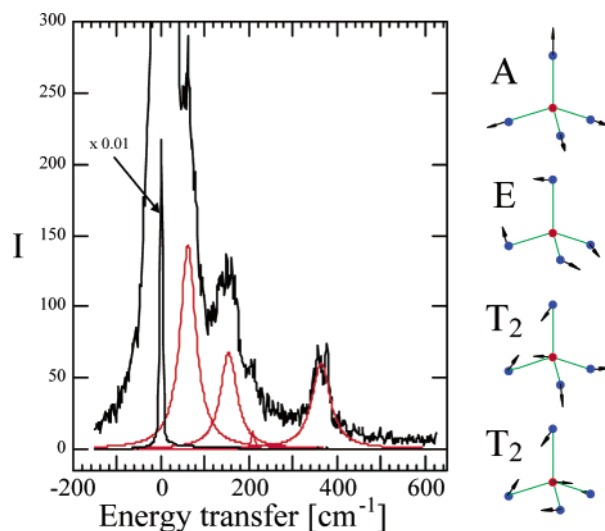
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Metal–H bonding is important in chemistry, catalysis, and the mechanisms of hydrogenase (H<sub>2</sub>ase)<sup>1</sup> and nitrogenase (N<sub>2</sub>ase).<sup>2</sup> With notable exceptions,<sup>3,4</sup> H atoms are difficult to see in protein crystal 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).<sup>5,6</sup> Here we present NRIXS data on Fe–S<sub>4</sub> and FeH(D)<sub>6</sub> model systems. We interpret the spectra and evaluate prospects for studying more complex samples.

NRIXS combines nuclear excitation and vibrational motion.<sup>6</sup> The effect was predicted early on,<sup>7,8</sup> but ultracentrifuge radioisotope experiments were difficult.<sup>9</sup> Later, synchrotron radiation (SR) work demonstrated feasibility.<sup>10,11</sup> Due to the spectral brightness of SR sources,<sup>12</sup> and X-ray optics progress, NRVS can now be done with high-resolution monochromators.<sup>13</sup> 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”  $\phi$  that goes into a  $n_\alpha \rightarrow n_\alpha + 1$  transition is given by:  $\phi = (e^2/3)(\nu_R/\nu_\alpha)(n_\alpha + 1)f$ .<sup>14</sup> The term  $e^2$  is the fraction of kinetic energy associated with nuclear displacement in mode  $\alpha$ ,  $\nu_R$  is the nuclear recoil energy,  $\nu_\alpha$  the vibrational energy,  $f$  the recoilless fraction, and  $n_\alpha$  is the mean occupation number of mode  $\alpha$  at temperature  $T$ .<sup>14</sup>

Fe–H stretches have high frequencies<sup>15</sup> and little Fe motion—both factors will reduce NRIXS intensity. To assess whether Fe–H modes could be observed in H<sub>2</sub>ase and N<sub>2</sub>ase, we studied two simpler systems: tetrahedral FeS<sub>4</sub> in *Pyrococcus furiosus* (Pf) rubredoxin (Rd), and FeH(D)<sub>6</sub> in [FeH(D)<sub>6</sub>][MgBr(THF)<sub>2</sub>]<sub>4</sub>. The met-Rd NRIXS has bands near 61, 154, and 364 cm<sup>-1</sup> (Figure 1). Under *T<sub>d</sub>* symmetry, there are two T<sub>2</sub> normal modes with Fe motion:  $\nu_3$  (mostly Fe–S stretch) and  $\nu_4$  (mostly Fe–S bend).<sup>16</sup> In *Desulfovibrio gigas* (Dg) Rd Raman spectra, the degeneracy of  $\nu_3$  is lifted, and three bands are observed at an average value of 362 cm<sup>-1</sup>.<sup>17</sup> We assign our 364 cm<sup>-1</sup> NRIXS band to similar modes—partially resolved at 8 cm<sup>-1</sup> resolution. In the Raman data, the totally symmetric mode  $\nu_1$  is very strong; it is essentially invisible in the NRIXS spectrum, as expected for a mode that has no Fe motion in *T<sub>d</sub>* symmetry (Figure 1).



**Figure 1.** (Left) NRIXS data (black curve) and fits (red curve) for <sup>57</sup>Fe met-Rd. Ordinate scale is counts/90 s. (Inset) FeS<sub>4</sub> site in Pf Rd. (Right) Diagrams of tetrahedral normal modes.

The lower frequency bands involve mostly bending motion. In *T<sub>d</sub>* symmetry, there is a pure bend, the doubly degenerate E mode ( $\nu_2$ ), as well as a mixed T<sub>2</sub>  $\nu_4$  mode. For *D<sub>g</sub>* Rd, a band at 126 cm<sup>-1</sup> was assigned to  $\nu_2$ , and a feature at 150 cm<sup>-1</sup> to  $\nu_4$ .<sup>18</sup> In strict *T<sub>d</sub>* symmetry, there is no Fe motion in  $\nu_2$ , hence no intensity for this mode in our experiment. For  $\nu_4$ , 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<sup>-1</sup> has about 40% Fe–S–C bend character.<sup>17</sup> This may explain the intensity in this region, but a more detailed analysis requires better statistics and calculations using the Pf Rd coordinates.<sup>3</sup>

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

The FeH(D)<sub>6</sub> samples have very different spectra (Figure 2). In *O<sub>h</sub>* symmetry, only T<sub>1u</sub> modes,  $\nu_3$  and  $\nu_4$ , have central atom motion.<sup>16</sup> The  $\nu_4$  mode is mostly bending and hence occurs at lower energy than the primarily stretching  $\nu_3$ ; we thus assign the FeD<sub>6</sub>

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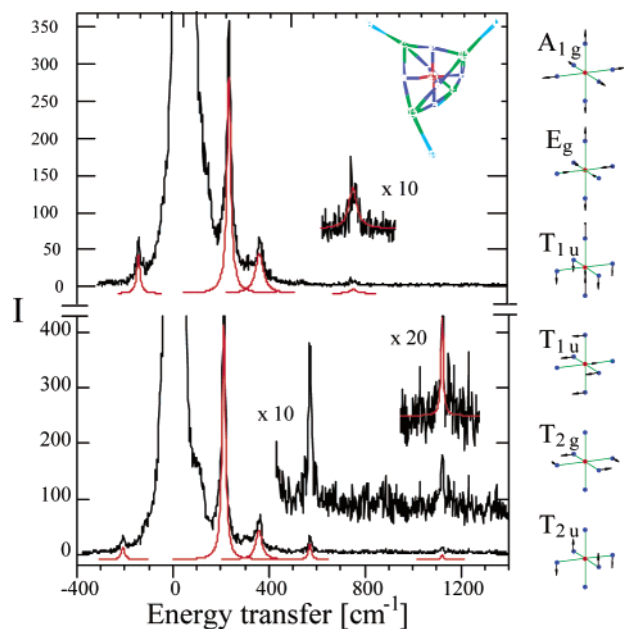
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**Figure 2.** (Left) NRIXS for (top)  $\text{FeH}_6$  and (bottom)  $\text{FeD}_6$  complexes. Ordinate scales: counts/40 s. (Inset)  $[\text{FeH}_6][\text{MgBr}(\text{THF})_2]_4$  structure (THF removed for clarity). (Right) Octahedral normal modes.

resonance at  $571\text{ cm}^{-1}$  to  $\nu_{4D}$ . A  $\sim 787\text{ cm}^{-1}$   $\text{FeH}_6$  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  $\nu_{4H}$ . For  $\text{FeD}_6$ , there is a feature at  $1122\text{ cm}^{-1}$  that we ascribe to  $\nu_{3D}$ : the Fe–H(D) stretch; IR bands are seen at  $1138$  and  $1107\text{ cm}^{-1}$ .<sup>21</sup> There could also be some contribution from an overtone of  $\nu_{4D}$ . Better statistics are needed to observe  $\nu_{3H}$  (seen at  $1514$  and  $1569\text{ cm}^{-1}$  in the IR).<sup>21</sup>

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).<sup>22,23</sup> 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  $357\text{ cm}^{-1}$  bands to  $\nu_4$  and  $\nu_3$  respectively.

We now return to the original question—can this technique be extended to Fe in  $\text{H}_2\text{ase}$  and  $\text{N}_2\text{ase}$ ? 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  $13.5\text{ keV}$ ) reduced the penetration length for  $14.4\text{ keV}$  photons  $\sim 200$ -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  $\sim 1750$

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.<sup>24,25</sup>

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**Supporting Information Available:** Procedures for  $^{57}\text{Fe}$ -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>.

## References

- (1) Cammack, R.; Frey, M.; Robson, R., Eds. *Hydrogen as a Fuel: Learning from Nature*; Taylor & Francis: New York, 2001.
- (2) Christiansen, J.; Dean, D. R.; Seefeldt, L. C. *Annu. Rev. Plant Phys. Plant Mol. Biol.* **2001**, *52*, 273–295.
- (3) Bau, R.; Rees, D. C.; Kurtz, D. M., Jr.; Scott, R. A.; Huang, H.; Adams, M. W. W.; Eidsness, M. K. *J. Biol. Inorg. Chem.* **1998**, *3*, 484–493.
- (4) Wilson, K. R.; Tobin, J. G.; Ankudinov, A. L.; Rehr, J. J.; Saykally, R. J. *Phys. Rev. Lett.* **2000**, *85*, 4289–4292.
- (5) Achterhold, K.; Sturhahn, W.; Alp, E. E.; Parak, F. G. *Hyperfine Interact.* **2002**, *141–142*, 3–12.
- (6) Gerdau, E., de Waard, H., Eds. *Nuclear Resonant Scattering of Synchrotron Radiation; Hyperfine Interactions/Kluwer Academic*: New York, 1999; Vol. 123–124.
- (7) Visscher, W. M. *Ann. Phys.* **1960**, *9*, 194–210.
- (8) Singwi, K. S.; Sjölander, A. *Phys. Rev.* **1960**, *120*, 1093–1102.
- (9) Endres, G.; Stronhkendl, F.; Langhoff, H.; E., G. *Z. Phys. B* **1981**, *44*, 253–257.
- (10) Seto, M.; Yoda, Y.; Kikuta, S.; Zhang, X. W.; Ando, M. **1995**, *74*, 3828–3831.
- (11) Sturhahn, W.; Toellner, T. S.; Alp, E. E.; Zhang, X.; Ando, M.; Yoda, Y.; Kikuta, S.; Seto, M.; Kimball, C. W.; Dabrowski, B. *Phys. Rev. Lett.* **1995**, *74*, 3832–3835.
- (12) Kunz, C. J. *Phys.: Condens. Matter* **2001**, *13*, 7499–7510.
- (13) Toellner, T. *Hyperfine Interact.* **2000**, *125*, 3–28.
- (14) Sage, J. T.; Paxson, C.; Wyllie, G. R. A.; Sturhahn, W.; Durbin, S. M.; Champion, P. M.; Alp, E. E.; Scheidt, W. R. *J. Phys.: Condens. Matter* **2001**, *13*, 7707–7722.
- (15) Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochmann, M. *Advanced Inorganic Chemistry*; Wiley-Interscience: New York, 1999.
- (16) Herzberg, G. *Molecular Spectra and Molecular Structure*; Van Nostrand Reinhold: New York, 1945; Vol. II.
- (17) Czernuszewicz, R. S.; Kilpatrick, L. K.; Koch, S. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1994**, *116*, 1134–1141.
- (18) Long, T. V.; Loehr, T. M.; Allkins, J. R.; Lovenberg, J. *J. Am. Chem. Soc.* **1971**, *93*.
- (19) Rai, B. K.; Durbin, S. M.; Prohofskey, E. W.; Sage, J. T.; Wyllie, G. R. A.; Scheidt, W. R.; Sturhahn, W.; Alp, E. E. *Biophys. J.* **2002**, *82*, 2951–2963.
- (20) Lamosa, P.; Brennan, L.; Vis, H.; Turner, D. L.; Santos, H. *Extremophiles* **2001**, *5*, 303–311.
- (21) Linn, D. E., Jr.; Skidd, G. M.; Tippmann, E. M. *Inorg. Chim. Acta* **1999**, *291*, 142–147.
- (22) Bau, R.; Ho, D.; Gibbons, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 4960–4962.
- (23) Bau, R.; Chiang, M. Y.; Ho, D. M.; Gibbons, S. G.; Enge, T. J.; Koetzle, T. F. *Inorg. Chem.* **1984**, *23*, 2383–2829.
- (24) Cho, A. *Science* **2002**, *296*, 1008–1010.
- (25) Levi, B. G. *Phys. Today* **2002**, *55*, 23–25.

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