Nuclear Resonance Vibrational Spectroscopy (NRVS) of Hydrogen and Oxygen Activation by Biological Systems

The enzymes nitrogenase (N_2ase), hydrogenase (H_2ase), methane monooxygenase (MMO), and cytochrome P-450 catalyze environmentally or medically critical reactions [1]. These proteins all contain Fe in their active sites. Although crystal structures are available for resting forms (Fig. 1), many key intermediates can only be observed by spectroscopy. The goal of our program is to use nuclear resonant vibrational spectroscopy (NRVS) to characterize the structures of these intermediates. This information will help to better define catalytic mechanisms, and it may eventually prove useful for the development of synthetic small molecule 'mimics' that can catalyze the same reactions.

NRVS is a relatively new technique that involves scanning an extremely monochromatic X-ray beam through a nuclear resonance [4-6]. Apart from the familiar 'zero phonon' Mössbauer resonance, there are additional transitions that correspond to nuclear excitation in combination with the excitation (Stokes) or de-excitation (anti-Stokes) of vibrational modes. For ^{57}Fe, the nuclear resonance is at 14.412 keV, and vibrational linewidths are less than 1 meV. The experiments therefore require a strong, tunable source with a resolution on the order of 10^{-7}. Only a third-generation synchrotron radiation source such as SPring-8 can provide sufficient spectral brightness to make such measurements feasible. Experiments were carried out at beamline **BL09XU**.

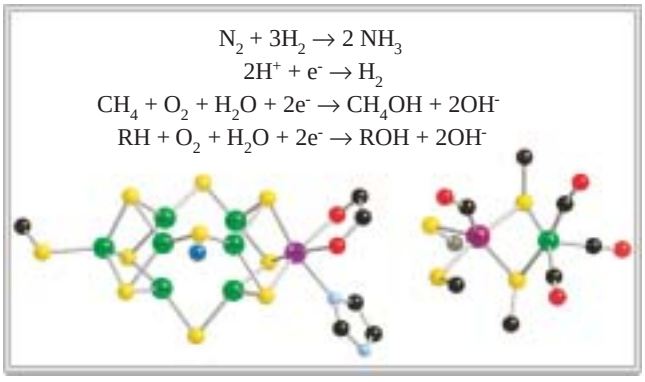

Fig. 1. Active site crystal structures for two key enzymes in this project. Left: high-resolution crystal structure of *Azotobacter vinelandii* N_2ase M center (PDB 1M1N) [2]. Sulfur atoms are drawn in yellow, oxygen in red, and carbon in black. Right: high-resolution crystal structure for a CO-inhibited form of *Desulfovibrio vulgaris* Miyazaki F H_2ase [NiFe] center (PDB 1UBJ) [3]. One or two of the CO ligands to Fe may well be CN^- instead. In the active forms of the enzyme, CO is not bound to Ni, and there are proposals that a hydride ion bridges the Ni and Fe ions.

Since metalloprotein samples are dilute, only a small fraction of the incident beam is absorbed in the NRVS process. The measurement technique exploits the relatively long lifetime of the nuclear excitation, along with the pulsed nature of the synchrotron source, by electronically gating the Fe Kα emission that occurs following internal conversion in between synchrotron pulses (Fig. 2).

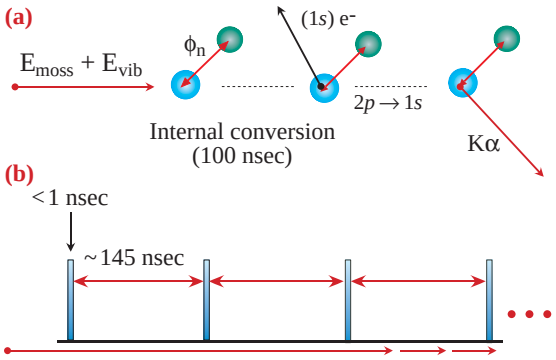

Fig. 2. **(a)** Chemist's view of the NRVS process. A photon excites a nucleus in the system to an excited state (E_{moss}), along with promoting the system to a higher vibrational level (E_{vib}). The nucleus relaxes (primarily) by internal conversion (emission of a core electron) followed by Kα fluorescence. **(b)** The NRVS detection scheme relies on gating the detector only during an interval between prompt synchrotron pulses.

In principle, clusters such as the FeMo-cofactor and the [NiFe] cluster have dozens of discrete normal modes that require hundreds of parameters for the definition of vibrational frequencies. One approach to simplifying the problem depends on model compounds and Urey-Bradley force fields to derive a set of transferable parameters for the Fe-S clusters. In this 'aufbau' approach, one starts with mononuclear tetrahedral compounds, and then builds up to binuclear, trinuclear, and more complex structures. As seen in Fig. 3, the spectra of Fe-S clusters divide approximately into high-frequency modes with a strong Fe-S stretching motion, and a wide range of lower frequency bands. Low-frequency modes can be further subdivided into simple three-atom bends, more complex ruffling and twisting motions of the entire cluster, and lattice modes.

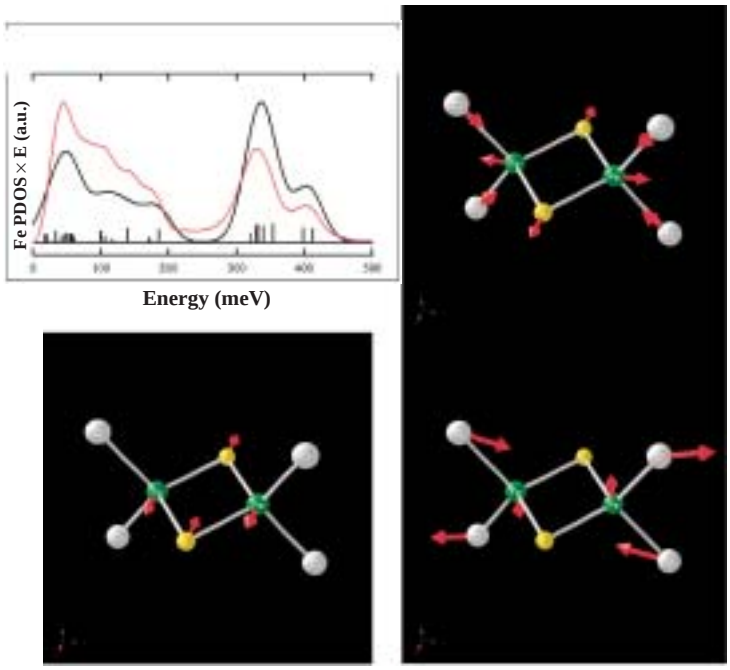

Fig. 3. Top left: spectrum (—) and simulation (— and sticks) for $(NEt_4)[Fe_2S_2Cl_4]$. Top right: the totally symmetric A_{1g} mode. Bottom left: the highest frequency B_{3u} mode, which combines with the A_{1g} mode to produce the > 400 cm^{-1} peak. Bottom right: the B_{3g} mode that contributes to the low frequency intensity near 100 cm^{-1}. The cluster of features around 50 cm^{-1} has been assigned to the lattice vibrations of the entire cluster. The normal modes in all figures were calculated using the 'VIBRATZ' analysis program from Shape Software (http://www. shapesoftware.com/).

The N_2 ase spectrum is significantly more structured than the $[Fe_2S_2Cl4]^{2-}$ spectrum (Fig. 4), partly because of the large number of Fe in the FeMo-cofactor, but also because of the presence of another eight Fe in the so-called 'P cluster' (not shown). Despite this complexity, the normal modes of FeMo-co are gradually becoming untangled (Fig. 4). The richness of these spectra, combined with relatively straightforward analysis procedures and the potential for isotopic substitution, suggest that NRVS will become a powerful tool for the characterization of metals in enzymes.

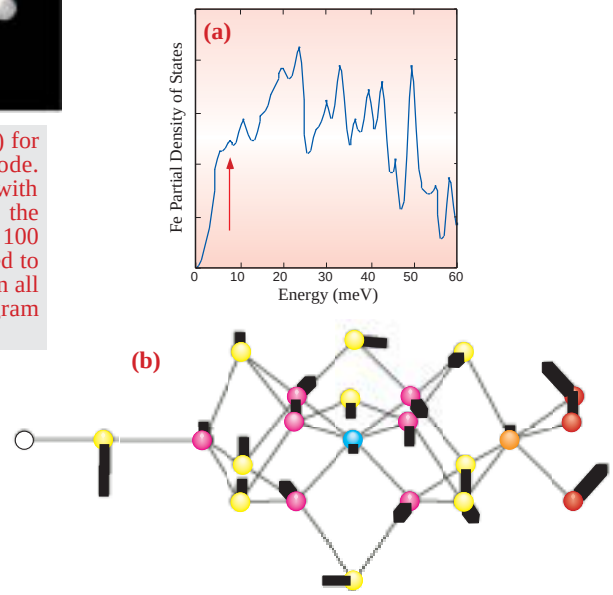

Fig. 4. **(a)** NRVS spectrum for the nitrogenase enzyme. **(b)** the twisting normal mode for the FeMo-cofactor cluster, predicted to occur around 10 meV in the NRVS spectrum. The cluster was simplified by making all Mo (orange) ligands oxygen atoms (red). Fe atoms are purple and Sulfurs are yellow.

References

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