Nuclear Spectroscopy of Nitrogenase and Hydrogenase

In nature, the reduction of dinitrogen to ammonia ($N_2 \rightarrow 2NH_3$), and the reversible oxidation of H_2 to protons and electrons ($2H^+ + 2e^- \rightleftarrows H_2$), rely on two critical enzymes – respectively nitrogenase (N_2ase), and hydrogenase (H_2ase). N_2 fixation is the key step in the nitrogen cycle, and the biological process is responsible for about half of the protein available for human consumption. The other half is produced using natural gas in fertilizer factories by the Haber-Bosch process – an activity that corresponds to ~2% of world energy production. The H_2 used in the synthetic process is derived from natural gas, and the production of ammonia currently consumes about 5% of global natural gas consumption. H_2 processing is crucial for the metabolism of many anaerobic organisms, and knowledge about the mechanism of H_2 evolution may prove critical for a future 'H_2 economy'. Bacteria are able to catalyze the reversible oxidation of H_2 using cheap and abundant Fe, while the best synthetic catalysts rely on precious Pt group metals. In summary, a better understanding of N_2ases and H_2ases may have an impact on our use of fossil fuels and our ability to transition to a more sustainable energy economy.

In nature, at least three different forms of each enzyme have evolved – what they have in common is the use of Fe at the active site, either with or without the assistance of a second metal (Mo, V, Ni). As illustrated in Fig. 1, X-ray crystallography has provided detailed atomic level structures for the electron transfer chains and active sites of these enzymes. However, so far it has been difficult to capture key enzyme intermediates in the crystalline state, and important questions remain about both structure and mechanism. For this reason, we have turned to a spectroscopic technique – Nuclear Resonance Vibrational Spectroscopy (NRVS).

The NRVS experiment involves scanning an extremely monochromatic X-ray beam through a nuclear resonance. Apart from the 'zero phonon' (recoil-free) Mössbauer resonance, there are additional transitions that correspond to nuclear excitation plus excitation or de-excitation of vibrational modes. A time-gated APD detector allows separation of these nuclear events from the almost instantaneous scattering and X-ray fluorescence (Fig. 2). NRVS offers a less restrictive selection rule than infrared or resonance Raman spectroscopy – the only requirement for intensity is motion of the resonant nucleus (in our case ^{57}Fe) along the direction of the incident X-ray beam in a given normal mode. The bottom line is that the intensity for a particular normal mode is proportional to the fraction of kinetic energy due to ^{57}Fe in that mode. The benefit of NRVS for characterization of Fe in N_2ase and N_2ase should be clear – we only observe normal modes associated with motion of the Fe atoms.

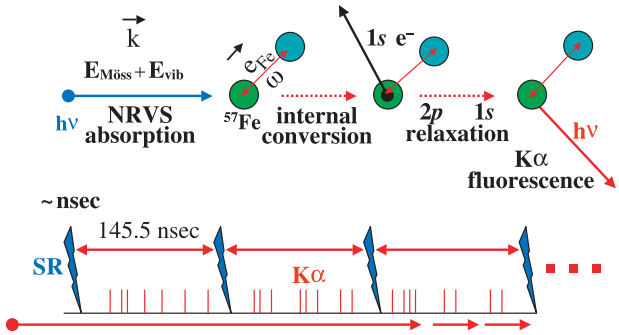

Fig. 2. Schematic illustration of the NRVS measurement. Left to right: monochromatic photons from SPring-8 beamline BL09XU excite an ^{57}Fe nucleus and a vibration with frequency ω. The excited ^{57}Fe nucleus relaxes by internal conversion with a lifetime of ~100 ns by expelling a 1*s* electron. The subsequent Kα radiation is measured between synchrotron pulses (bottom) by an avalanche photodiode detector.

The partial vibrational density of states (PVDOS) that can be measured by NRVS covers a frequency range from ~10 cm^{-1} to nearly 1000 cm^{-1}, as illustrated in Fig. 3 for the electron transfer protein rubredoxin [1,2], the FeMo cofactor of *A. vinelandii* N_2ase [3], and the mononuclear H_2ase from *M. marburgensis* called Hmd [4]. A wide variety of normal modes occur over these two orders of magnitude. At the low end are modes that involve large regions of polypeptide chain, as shown in Fig. 4, while at

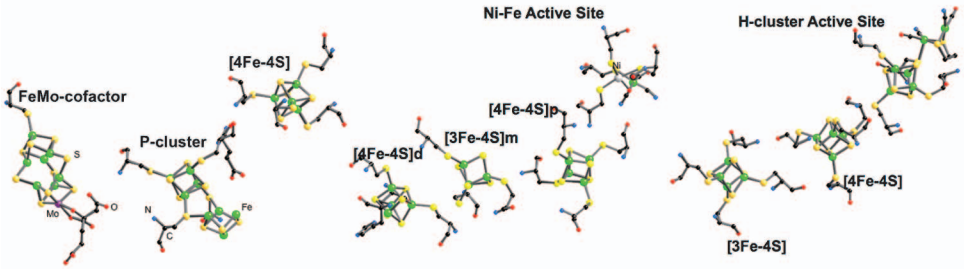

Fig. 1. The electron transfer chains and active sites for (left to right): *A. vinelandii* Mo N_2ase, *D. vulgaris* [NiFe] H_2ase, and *C. pasteurianum* [FeFe] H_2ase.

the high end are the stiffest modes (such as Fe-CO bends) or those involving the lightest atoms, such as Fe-H and Fe-D stretches (not shown). In between are Fe-S stretching modes and cluster breathing modes that involve S-Fe-S and Fe-S-Fe bending.

We have used normal mode calculations, based on empirical force fields as well as based on DFT calculations, to interpret Fe metalloprotein NRVS. For example, by combining the CHARMM force field for peptide motion with our own empirical Fe-S force constants, we have modeled the entire spectrum for rubredoxin [2]. The lowest frequency mode is illustrated in Fig. 4. Also shown are the totally symmetric 'breathing' mode at 200 cm^{-1} for the FeMo cofactor [3], and the highest frequency mode, which is mostly Fe-C-O bending in nature, for the mononuclear Hmd H_2ase [4].

What have we learned from NRVS so far, and what does this technique promise for the future? With N_2ase, we have seen how the cluster dynamics are dominated by the presence of interstitial atom 'X'. For Hmd H_2ase, we have seen the importance of CO ligands in the Fe dynamics and obtained new data regarding the cysteine and tentative water ligands. Longer term, NRVS could allow characterization of bound substrates such as N_2 and H-, which is critical for understanding the catalytic mechanisms. But, if NRVS is to achieve its full potential, we will eventually need another order of magnitude in flux, as well as better monochromators and detectors. With such improvements, NRVS will become an extraordinarily powerful probe of Fe enzyme biochemistry.

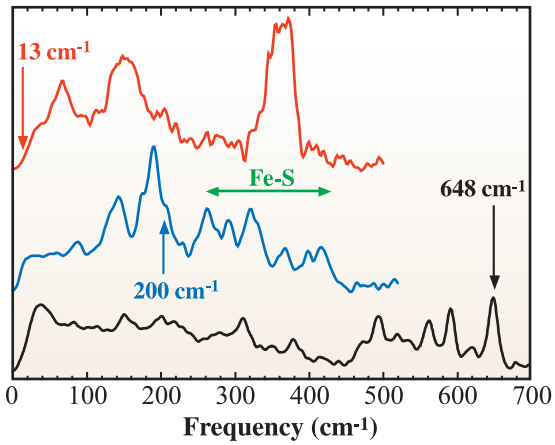

Fig. 3. Representative ^{57}Fe metalloprotein NRVS spectra. Top to bottom: **(a)** rubredoxin, **(b)** Hmd H_2ase, and **(c)** the isolated FeMo cofactor from N_2ase.

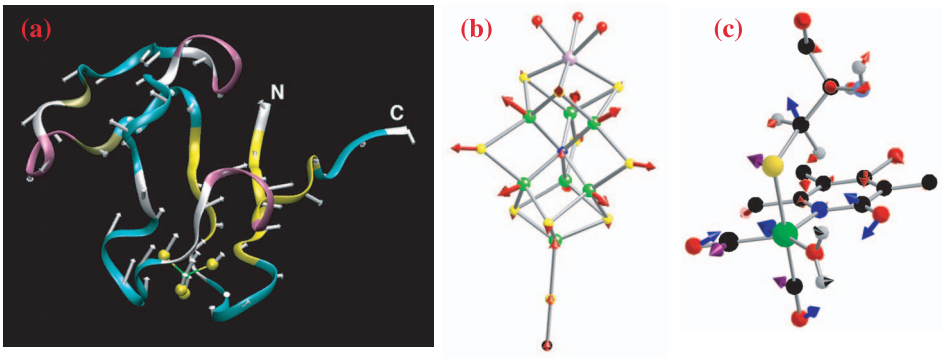

Fig. 4. Atomic motion in representative ^{57}Fe metalloprotein normal modes. Color code: S – yellow, Fe - green, C – black, O – red, and N – blue. Left to right: **(a)** the lowest frequency mode at 13 cm^{-1} for rubredoxin, **(b)** the totally symmetric mode at 200 cm^{-1} for the N_2ase FeMo cofactor, and **(c)** highest frequency mode at 648 cm^{-1} for the mononuclear Fe site in Hmd H_2ase. In the latter case, the motion of Fe and CO groups has been modified for clarity.

Stephen P. Cramer [a,b,*], Yuming Xiao [b] and Yisong Guo [b]

[a] Physical Biosciences Division, Lawrence Berkeley National Lab., USA
[b] Department of Applied Science, UC Davis, USA

*E-mail: spjcramer@ucdavis.edu

References

[1] Y. Xiao *et al.*: J. Am. Chem. Soc. **127** (2005) 14596.
[2] *M.-L. Tan, A.R. Bizzarri, Y. Xiao, S. Cannistraro, T. Ichiye, C. Manzoni, G. Cerullo, M.W.W. Adams, J.F.E. Jenney, S.P. Cramer: J. Inorg. Biochem.* ***101*** *(2006) 375.*
[3] *Y. Xiao, K. Fischer, M.C. Smith, W. Newton, D.A. Case, S.J. George, H. Wang, W. Sturhahn, E.E. Alp, J. Zhao, Y. Yoda, S.P. Cramer: J. Am. Chem. Soc.* ***128*** *(2006) 7608.*
[4] *Y. Guo, H. Wang, Y. Xiao, S. Vogt, R.K. Thauer, S. Shima, T. Rauchfuss, P. Volkers, E. Alp, W. Sturhahn, Y. Yoda, S.P. Cramer: J. Am. Chem. Soc.* ***129*** *(2007) - in preparation.*