A dynamic view of [NiFe] hydrogenase by means of nuclear resonance vibrational spectroscopy

Hydrogenases (H_2ases) are enzymes that catalyze both the production and consumption of molecular hydrogen [1,2]. They play an important role in biology, allowing organisms to efficiently use protons as electron acceptors and evolving H_2, and also allowing capture of H_2 in their role as 'uptake H_2ases' [3]. They are also of interest for potential applications in a hydrogen economy, either used directly or serving as inspirational targets for biomimetic inorganic catalysts [4]. A detailed knowledge of the catalytic mechanisms of H_2ases is thus an important goal for biology, chemistry, and clean energy technology.

In the [NiFe] family of H_2ases, catalysis takes place at a Ni-Fe dimetallic center (Fig. 1). In this unique active site, Fe is linked to Ni by a pair of cysteine thiolate ligands. Depending on the redox poise or degree of aerobic deactivation, the Fe and Ni centers may also be bridged by hydride, oxo/hydroxo, and possibly hydroperoxo ligands. The Fe site also carries one CO and a pair of CN^- ligands. [NiFe] H_2ases also possess additional Fe-S clusters that relay electrons to and from the active site.

Although crystallography has revealed many essential features about the NiFe active site, there are additional details that are more easily addressed by spectroscopy. For example, infrared spectroscopic studies have revealed more than ten different chemical species, using the CO and CN^- stretching bands in the 1800–2100 cm^{-1} region. Only a few forms of the enzyme can be crystallized as homogeneous chemical species, and even then, it is hard to see all of the details by crystallography with atomic resolution. The alternate species often differ by the bridge between Ni and Fe, which is often hard to see clearly in the crystallographic data. In the oxidized, inactive forms Ni-A and Ni-B, it is proposed that Ni and Fe are bridged by O^{2-}, OH^- or even OOH^- species, while the reduced and active forms 'Ni-C' and 'Ni-R' are both thought to possess a hydride (H^-) bridge. However, the critical hydride bridge is not visible in the crystal structures. In the current study [5], we employed a relatively new technique called Nuclear Resonance Vibrational Spectroscopy (NRVS), which is only sensitive to motion of ^{57}Fe, to identify H_2ase Fe-CN and Fe-CO modes in the 400–600 cm^{-1} region. Such studies, combined with measurement of model compounds and Density Functional Theory (DFT) calculations will help characterize the geometry of the bridges to iron and the nature of hydrogen activation.

NRVS has evolved into a powerful technique for probing the dynamics of Fe in metalloproteins. The measurement employs scanning a highly monochromatic (meV) X-ray beam through a nuclear resonance. Unlike Mössbauer spectroscopy, which detects 'recoil-free' nuclear transitions, NRVS detects the inelastic events that involve creation or destruction of phonons. The resulting spectrum is similar to a conventional IR or Raman spectrum, but with different selection rules. The NRVS intensity for a given normal mode is related to the amount of motion of the resonant nucleus (in this case ^{57}Fe) in that mode. One can therefore observe only the ^{57}Fe-based vibrations for a protein sample involving thousands of other atoms. The measurements were conducted at beamline **BL09XU**.

As seen in Fig. 2, the NRVS of H_2ase is dominated by contributions from the Fe-S clusters in the electron transport chain. The structure around 150 cm^{-1} derives from cluster breathing and bending modes, while the features between 200 and 400 cm^{-1} are mostly Fe-S stretching modes. Fortunately, the Fe-S cluster NRVS dies out rapidly above 400 cm^{-1}, and at

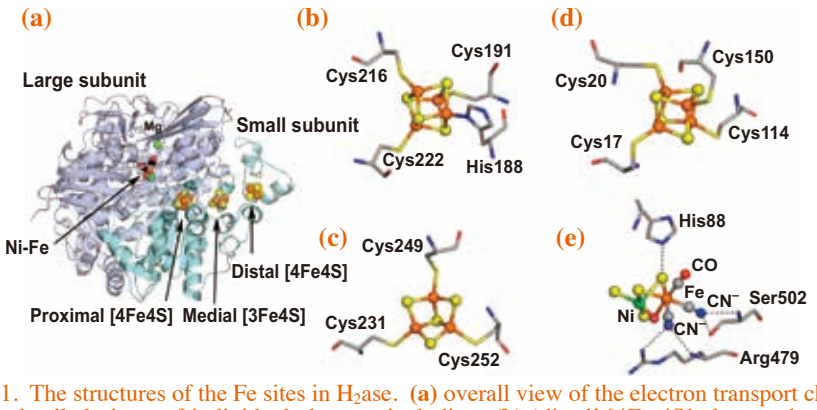

Fig. 1. The structures of the Fe sites in H_2ase. **(a)** overall view of the electron transport chain. **(b-e)** detailed views of individual clusters, including: **(b)** 'distal' [4Fe-4S] cluster, showing Cys_3His ligation, **(c)** 'medial' [3Fe-4S] cluster, **(d)** 'proximal' [4Fe-4S], and **(e)** Ni-Fe active site. The atoms are Fe (brown), Ni (green), S (yellow), C (gray), N (blue) and O (red).

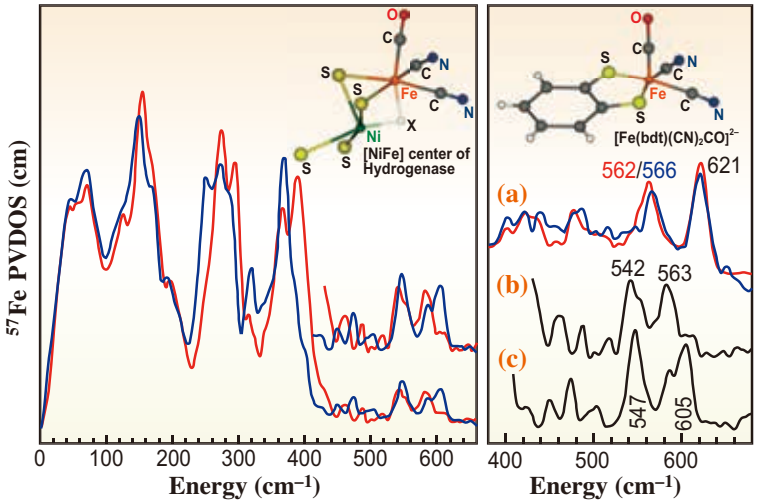

Fig. 2. NRVS-derived PVDOS for Fe sites in H_2ase. Left: overall spectra for oxidized (—) *vs* reduced (—) H_2ase. Right: close-ups for Fe-CO/CN region. Top to bottom: **(a)** $(NEt_4)_2[^{57}Fe(bdt)(CN)_2(CO)]$ (—) and $(NEt_4)_2[^{57}Fe(tdt)(CN)_2(CO)]$ (—), **(b)** oxidized H_2ase, and **(c)** reduced H_2ase.

still higher frequencies it is still possible to distinguish Fe-CN and Fe-CO stretching and bending modes from the active site Fe. Upon reduction of the protein, we were able to see modest upshifts in the Fe-CO stretch that correlated with shifts seen in the mid-IR CO stretching bands.

Going forward, a truly exciting prospect of this work is the potential for observation of Fe hydride/deuteride stretching and bending modes. In recent work on a Ni-H/D-Fe model compound (Fig. 3), we found that weak Fe-H stretches could be seen past 1500 cm^{-1}, while much stronger Fe-H-Ni bending modes were visible near 800 cm^{-1}. Finding the position of these bands in H_2ase will provide valuable information about the nature of hydrogen activation at the active site. The continuing improvement in the emittance of the SPring-8 storage ring, the brightness available from improved undulators, and the stability of high-resolution X-ray monochromators have all contributed to make this work possible.

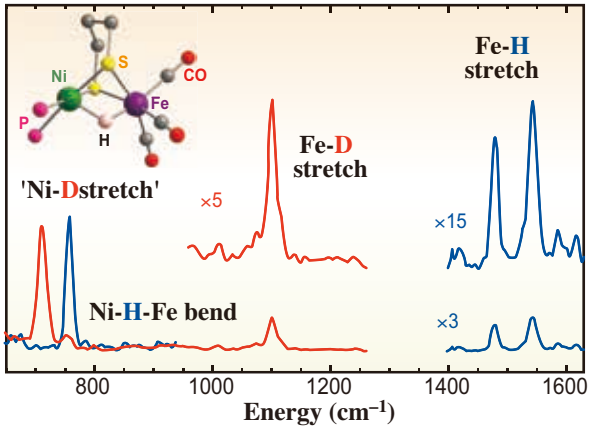

Fig. 3. Recent NRVS data on $[(CO)_3{}^{57}Fe(pdt)H/DNi(dppe)]BF_4$ (blue for H and red for D) - a Ni-Fe model for NiFe H_2ase. The model structure is as shown in the insert.

H. Wang[a,c], H. Ogata[b], W. Lubitz[b,*] and S. P. Cramer[a,c,*]

[a] Department of Chemistry, University of California, USA
[b] Max Planck Institute for Chemical Energy Conversion, Germany
[c] Physical Biosciences Division, Lawrence Berkeley National Laboratory, USA

*Email: wolfgang.lubitz@cec.mpg.de, spjcramer@ucdavis.edu

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