

## 2D-IR Spectroscopy Reveals Structural and Dynamical Details of [NiFe] Hydrogenases

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Utilising active-site CO/CN<sup>-</sup> ligands as vibrational reporter groups (A), we have studied hydrogen-transforming enzymes, so-called hydrogenases, by pump-probe and two-dimensional (2D) infrared (IR) techniques. 2D-IR spectroscopy verified unusual vibrational features of a unique [NiFe] hydrogenase (B) and allowed insights into the constrained structure of its oxygen-protected state (doi:10.1021/jacs.2c06400). Moreover, detailed information on CO bond properties of multiple redox-structural states of the active site were obtained.

The data indicate that the CO bond dissociation energy does not follow the trend of the CO-stretch fundamental frequency (C) but is significantly affected by the shape of the bond potential.

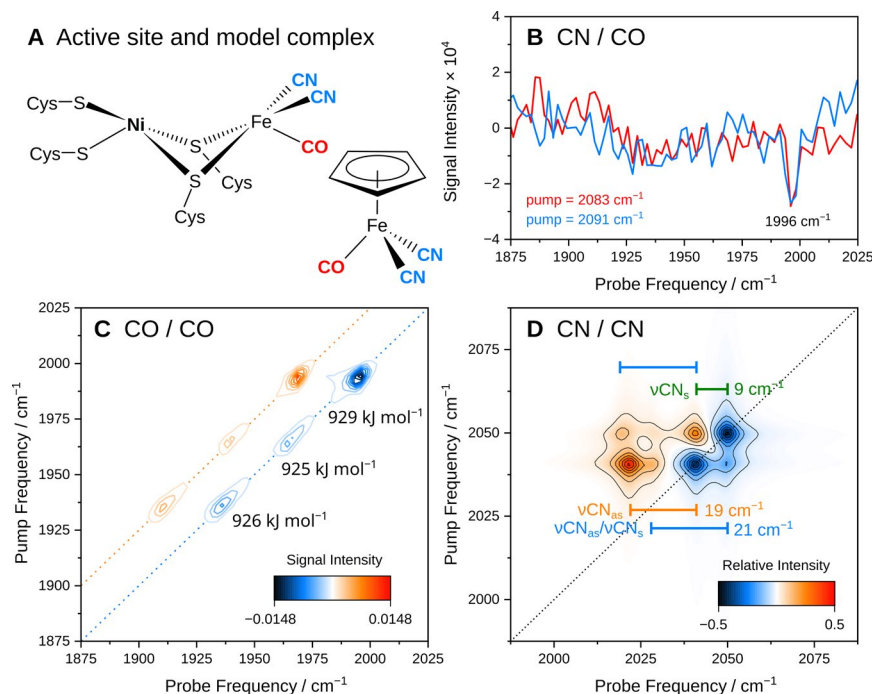
This finding was supported by a study on another hydrogenase (doi:10.1039/D2CP04188J), which additionally revealed differences between the protein-embedded active site and a bioinspired model complex in different solvents (A).

This work also resolved a complex signal pattern related to the CN<sup>-</sup> ligands, which could be explained and structurally interpreted by quantum chemical calculations (D)(doi:10.3390/catal12090988).

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(A) Structures of the active site of [NiFe] hydrogenase and a biomimetic model complex. Diatomic ligands used as IR reporter groups are highlighted. (B) Pump slice through the 2D-IR spectrum, revealing coupling between CN stretch modes and a high-frequency CO stretch mode, confirming that the latter reflects an active-site ligand. (C) CO stretch signals of three redox-structural states of the [NiFe] active site, resolved by 2D-IR spectroscopy. Indicated bond dissociation energies do not correlate with CO stretch fundamental frequencies. (D) Simulated 2D-IR spectrum reproducing the experimental CN-stretch signature by including resonant interactions in the underlying calculations.

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