# Photodynamics of Asymmetric Di-Iron-Cyano Hydrogenases Examined by Time-Resolved Mid-Infrared Spectroscopy

Amber Meyers,<sup>1</sup> Edwin J. Heilweil<sup>\*2</sup> and Christopher J. Stromberg<sup>\*1</sup>

<sup>1</sup>Department of Chemistry and Physics, Hood College, Frederick, MD 21701-8524, USA <sup>2</sup>Nanoscale Device Characterization Division, Physical Measurement Laboratory, National Institute of Standards and Technology (NIST), Gaithersburg, MD 20899, USA

### Abstract

Two anionic asymmetric Fe-Fe Hydrogenase model compounds containing a single CN and five CO ligands:  $[Et_4N][Fe_2(\mu-S_2C_3H_6)(CO)_5(CN)_1]$  and  $[Et_4N][Fe_2(\mu-S_2C_2H_4)(CO)_5(CN)_1]$  dissolved in room temperature acetonitrile are examined. The molecular asymmetry affects the redox potentials of the central iron atoms thus changing the photophysics and possible catalytic properties of the compounds. Femtosecond ultraviolet excitation with mid-infrared probe spectroscopy of the model compounds was employed to better understand the ultrafast dynamics of the enzyme active site. Continuous ultraviolet lamp excitation with Fourier Transform infrared (FTIR) spectroscopy was also used to explore stable product formation on the second timescale. For both model compounds, two timescales are observed; a 20 ps to 30 ps decay and formation of a long-lived photoproduct. The picosecond decay is assigned to vibrational cooling and rotational dynamics while the residual spectra remains for up to 300 ps suggesting formation of new photoproducts. Static FTIR spectroscopy yielded a different stable photoproduct than observed on the ultrafast timescale. Density functional theory (DFT) calculations simulated photoproducts for CO-loss and CN-loss isomers and the resulting photoproduct spectra suggest the picosecond transients arise from a complex mixture of isomerization after CO-loss while dimerization and formation of a CN-containing Fe-CO-Fe bridged species are also considered. \*Corresponding authors: stromberg@hood.edu and edwin.heilweil@nist.gov

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## Introduction

[FeFe]-hydrogenases are natural bacterial enzymes that catalyze the production of hydrogen gas. These enzymes, which contain two iron atoms in their active site, are of great interest because of the increasing need to develop less expensive substitutes for platinum commonly used as a catalyst for hydrogen generation.<sup>1-9</sup> Symmetric model compounds containing all CO ligands or mixed CO/CN and CO/PMe<sub>3</sub> ligands on the irons have been previously studied.<sup>8</sup> However, asymmetric model compounds which more closely mimic the natural species have not been the focus of photochemical studies and are examined in this work.

Hydrogen is a potential replacement for fossil fuels. Currently, hydrogen gas is catalytically generated from methane and fossil fuels prompting industry to strive to find alternative means for hydrogen generation. For hydrogen to be a useful replacement fuel, it must be generated more efficiently. In nature, hydrogen gas is produced by bacteria in a slightly acidic water environment and the microorganisms utilize hydrogenase enzymes to catalyze the reduction of protons to hydrogen gas.<sup>1–9</sup> Three types of hydrogenases exist in nature: the two most common being [NiFe]- and [FeFe]-hydrogenases. The [NiFe]-hydrogenases generally catalyze the oxidation of hydrogen gas into protons, whereas [FeFe]-hydrogenases generally catalyze the reduction of protons to hydrogen gas.<sup>3,4,6,9,10</sup> The reduction of hydrogen ions into hydrogen gas is an essential step in producing hydrogen gas from water.

The active site of [FeFe]-hydrogenases contains a di-iron core with two bridging sulfur ligands and multiple pendant carboxyl (CO) and cyano (CN) ligands.<sup>8,11–13</sup> The general

molecular structure of the native form [FeFe]-hydrogenase in one possible oxidation state is shown in Figure 1.<sup>13</sup> Many studies of small model compounds resembling the [FeFe]hydrogenase active site have been completed, and although these synthesized model compounds resemble the active site in structure, they are typically very poor catalysts on their own.<sup>10,14–23</sup> One of the simplest of these model compounds contains all CO ligands and is based on Fe<sub>2</sub>( $\mu$ -S<sub>2</sub>C<sub>3</sub>H<sub>6</sub>)(CO)<sub>6</sub>.<sup>7</sup> Related model compounds are synthesized by making substitutions to the pendant CO ligands or by altering the carbon bridge between the two sulfurs of this species.<sup>15,23,24</sup> Different pendant ligands alter the electronic properties at the Fe-Fe core and influence the catalytic properties of the molecule.<sup>10</sup>



**Figure 1.** Structure of the asymmetric active site of the native [FeFe]-hydrogenase.<sup>3,7,25–27</sup> Atomic color codes are Fe (purple), S (yellow), C (grey), O(red), N(blue) and H(white).

Other model compounds have been designed to use light to drive hydrogen gas production. One approach has been to bind the hydrogenase models to elements of Photosystem



**Figure 2.** Structure of the predominant (a) axial isomer of  $[Fe_2(\mu-S_2C_3H_6)(CO)_5(CN)_1]^{1-}$  (1) and (b) axial isomer of  $[Fe_2(\mu-S_2C_2H_4)(CO)_5(CN)_1]^{1-}$  (2). Atomic color codes are Fe (purple), S (yellow), C (grey), O(red), N(blue) and H(white).

L<sup>13,28,29</sup> while another approach binds a photosensitizer directly to the hydrogenase model system.<sup>30–41</sup> In the latter approach, light absorbed by the photosensitizer drives the redox reactions for hydrogen production, similar to the mechanism of Photosystem II.<sup>33</sup> However, on their own the photodynamics of the [FeFe]-hydrogenase active site (and similar model compounds), especially on the ultrafast timescale, are not well understood. Symmetric model compounds containing all CO, mixed CO and CN, and mixed CO and PMe<sub>3</sub> ligands have been studied by us using ultraviolet (UV) or visible excitation with ultrafast infrared (IR) probe spectroscopy.<sup>42–46</sup> However, asymmetric model compounds containing only one CN ligand, for example, have been synthesized but not yet investigated using time-resolved infrared (TRIR) spectroscopy. These species are the focus of this study.

In this work, the photodynamics of two asymmetric model compounds were examined at room temperature in acetonitrile solution:  $[Et_4N][Fe_2(\mu-S_2C_3H_6)(CO)_5(CN)_1]$  and  $[Et_4N][Fe_2(\mu-S_2C_2H_4)(CO)_5(CN)_1]$ . The room temperature ground state anions ( $[Fe_2(\mu-S_2C_3H_6)(CO)_5(CN)_1]^{1-}$  (1) and  $[Fe_2(\mu-S_2C_2H_4)(CO)_5(CN)_1]^{1-}$  (2)) exist in different isomeric forms, and the dominant isomeric structures of the studied anions are shown in Figure 2. We will show that these models exhibit a fast (20 ps to 30 ps) decay followed by a long-lived photoproduct offset. As was found for symmetric models containing two CN ligands,<sup>44-46</sup> these mono-substituted anions did not exhibit an initial (ca. 150 ps) decay, as previously observed in all CO or mixed CO/PMe\_3 compounds, implying that the symmetry and inclusion of CN ligands alters the catalytic

capabilities of these models.<sup>43,44,47–50</sup> Several Density Function Theory (DFT) simulated spectra for CO-loss isomers also suggest the possibility of forming a bridging Fe-CO-Fe species, as found in the native structure. Formation of a bridging Fe-CO-Fe species could also affect the catalytic properties of the molecule and is believed to be an important intermediate during hydrogen generation.<sup>8</sup>

### Methods

**Synthesis.** Syntheses of **1** (as  $[N(C_2H_5)_4][Fe_2(\mu-S_2C_3H_6)(CO)_5(CN)_1]$ ) and **2** (as  $[N(C_2H_5)_4][Fe_2(\mu-S_2C_2H_4)(CO)_5(CN)_1]$ ) were conducted by a previously published method.<sup>15</sup> The synthesis of **1** is summarized as follows: previously synthesized<sup>15</sup> Fe<sub>2</sub>( $\mu$ -S<sub>2</sub>C<sub>3</sub>H<sub>6</sub>)(CO)<sub>6</sub> was combined with trimethylamine N-oxide (ONMe<sub>3</sub>·2H<sub>2</sub>O) and acetonitrile (MeCN) under nitrogen atmosphere. The solution was cooled to 233 K (-40 °C). One equivalent of Et<sub>4</sub>NCN was combined with MeCN under nitrogen atmosphere and added to the cold flask. The reaction mixture warmed to room temperature for 2 h then the solution evaporated to dryness under vacuum. The remaining dark red oil was extracted into tetrahydrofuran and filtered. The volume was reduced under vacuum and the product was precipitated upon washing with hexanes and evaporated to dryness. Purity of the product was checked using FTIR. Synthesis of **2** is similar to **1** above, except the initial reaction is started using the ethyl-bridged reagent Fe<sub>2</sub>( $\mu$ -S<sub>2</sub>C<sub>2</sub>H<sub>4</sub>)(CO)<sub>6</sub>.

**Time Resolved UV-Pump/IR-Probe Laser Spectroscopy (TRIR).** A 40 fs Ti:sapphire oscillator tuned to 800 nm seeded a home-built linear cavity Ti:sapphire regenerative amplifier.<sup>42</sup> The regenerative amplifier produced 800 nm, 80 fs, 700 µJ pulses at a frequency of 1 kHz. These pulses were used to pump a Spectra-Physics OPA-800F optical parametric amplifier (OPA).<sup>51</sup> The OPA produced signal and reference pulses, which were difference frequency mixed in a

AgGaS crystal resulting in 120 fs, ~1  $\mu$ J probe pulses in the mid-IR centered around 2000 cm<sup>-1</sup> (CO- and CN-stretching region) with a bandwidth of ca. 230 cm<sup>-1</sup> full-width at half-maximum (FWHM). Residual 800 nm light from the OPA was either doubled (400 nm) or tripled (267 nm) to produce sample photoexcitation (pump) pulses. These excitation wavelengths line up relatively well with molecular absorptions, as shown in Figure 3. The transition at 350 nm is assumed to be a metal-to-ligand charge transition, based on work with a similar Fe<sub>2</sub>( $\mu$ -S<sub>2</sub>)(CO)<sub>6</sub> compound.<sup>52</sup> All beam paths after the OPA were purged with dry and CO<sub>2</sub>-free air to reduce gas-phase spectral interference in the CO-stretching region.



Figure 3. UV-Vis spectra of 1, along with  $Fe_2(\mu-S_2C_3H_6)(CO)_6$  (3),  $Fe_2(\mu-S_2C_3H_6)(CO)_4(PMe_3)_2$  (4), and  $[Fe_2(\mu-S_2C_3H_6)(CO)_4(CN)_2]^{2-}$  (5).

The pump and probe beams were overlapped, focused by a 10 cm focal length  $CaF_2$  lens and transmitted through a flow cell with 2 mm liquid pathlength and  $CaF_2$  windows. Samples of **1** and **2** were prepared in anhydrous MeCN at ca.  $10^{-3}$  mol/dm<sup>3</sup> concentration to achieve optical densities (OD) of approximately 0.8 for IR CO-stretching mode peak absorptions. The timing of the probe pulses relative to the pump pulses was adjusted using a computer-controlled optical delay stage with a maximum delay time of 300 ps. The IR probe beam traversed a 50% beam splitter so that a second IR beam is directed through the sample cell separated from the pump beam and used as a reference to normalize the IR probe signal. The pump beam was chopped at 500 Hz to allow iterative collection of excited versus unexcited sample transmission.

The transmitted IR signal and reference beams were collimated by a second 10 cm CaF<sub>2</sub> lens, passed through a scanning grating monochromator with 4 cm<sup>-1</sup> FWHM resolution and imaged onto a matched pair of Mercury-Cadmium-Telluride (MCT) detectors. The detector pre-amplified outputs for each laser pulse were sampled by boxcar averagers and analog-to-digital converters. Collected signals were processed by home-built software to extract the averaged pump-induced differential optical density ( $\Delta$ OD) at each wavelength as the monochromator was scanned across the spectrum.

Two types of ultrafast time-resolved IR (TRIR) scans were conducted. First, entire spectral difference scans were obtained at selected time-delays. Two separate runs for each delay time were averaged to obtain improved signal-to-noise (approximately  $\pm 0.002$  OD baseline noise; Type B k=1 analysis). From these spectra, wavelengths of importance were strategically chosen. Kinetic scans were then obtained at the selected wavelengths with pump-probe delay time scanned from -35 ps to 285 ps with a resolution of approximately 2 ps. Higher resolution scans were also taken around t=0 with delay time ranging from -8 ps to 25 ps with resolution of approximately 0.2 ps.

**Minute Delay Time FTIR Spectroscopy.** Samples were loaded into a static 1.0 mm pathlength IR cell fitted with CaF<sub>2</sub> windows and placed in a dry-air and carbon dioxide purged Nicolet Magna 550 FTIR spectrometer.<sup>51</sup> CO-stretching mode IR peak absorption was kept to <1 OD. Each sample was exposed to UV light from an unfiltered broad-band mercury pen lamp (max

output 254 nm, Oriel Corp.) for 30 s intervals and IR absorption spectra were taken at the beginning and after each interval of UV exposure. Difference OD spectra were calculated for each interval of UV exposure by subtracting absorption spectra exposed to UV by spectra that had not been exposed to UV. This analysis permits identification of starting molecule loss (- $\Delta$ OD) and formation of new photoproduct species (+ $\Delta$ OD) from new IR absorption features. Equivalent UV-Vis spectra were obtained using a Perkin-Elmer Lambda 2 spectrometer.

**DFT Calculations.** IR spectra for isomeric and transient species were calculated using Gaussian 16W.<sup>53,54</sup> Gas-phase calculations were performed using the BP86 functional<sup>55–57</sup> with the TZVP basis set,<sup>58,59</sup> which has performed well for related model compound studies.<sup>42–46</sup> While absolute frequency agreement between DFT results and experiments is generally found to be poor, relative frequency shifts and amplitudes adequately agree to make favorable product and intermediate structural assignments.<sup>30-34</sup> Calculated infrared "stick" spectra were convolved with a 15 cm<sup>-1</sup> FWHM Lorentzian function to mimic features broadened by the room temperature MeCN solvent. Calculations were performed for each isomer of 1 and 2 and for all possible COand CN-loss photoproduct fragments that could result from UV excitation. CO- and CN-loss photoproduct fragments were also run with an acetonitrile solvent molecule added to the vacant site to see the results of potential solvent coordination. The possible ground state and CO-loss isomers for 1 and 2 are shown in Figures 3 and 4, respectively. Detailed DFT calculation results including relative energies ( $\Delta G$ ), dipole moments, CO- and CN-stretching mode assignments, frequencies and intensities may be found in the Supplemental Information (SI) Tables S1 through S11.



Figure 4. Ground state and CO-loss isomeric structures and identifier labels for 1.



Figure 5. Ground state and CO-loss isomeric structures with identifier labels for 2.

### **Results and Discussion**

#### **Steady State Infrared Spectra**

Species **1** exists as four possible ground state isomers where **1-1** and **1-2** (as well as **1-3** and **1-4**) differ only by the direction the propyl bridge atoms. For these species, as with others previously studied,<sup>42,44,46</sup> the bridge flip isomeric forms do not significantly affect room temperature vibrational spectra. **2** can exist as two possible isomers as the species is ethylbridged and does not possess a bridge flip (numbered **2-1** and **2-3**, for consistency with **1**).

The FTIR ground state spectrum for **1** (Figure 6(a)), displays distinct CO-stretching band absorptions at 1914 cm<sup>-1</sup>, 1976 cm<sup>-1</sup>, and 2030 cm<sup>-1</sup>. There is also a broad feature centered around 1950 cm<sup>-1</sup> resulting from of at least two overlapping CO-stretching absorptions. The isolated weaker CN-stretching band is located at 2092 cm<sup>-1</sup>. IR spectra for each isomer of **1** were calculated using DFT methods with results from these calculations shown in Figure 6(b). Note that the spectra of **1-1** and **1-2** are nearly identical, while the spectra of **1-3** and **1-4** are indistinguishable. There is a slight shift in the frequency scale of the calculated spectra (no frequency scaling was applied), which causes the calculated spectra to be slightly more compressed than the experimental spectra.



**Figure 6.** (a) Experimental FTIR spectrum of **1** in room temperature acetonitrile, (b) Simulated IR spectra for each ground state isomer of **1** based on DFT calculations, (c) comparison of simulated IR spectra assuming a Boltzmann distribution of isomers (brown) and a mixture of 70% of isomer **1-3** and 30% isomer **1-1** (orange).

By comparing simulated FTIR spectra of the possible isomers (via DFT calculations) with the spectra of the synthesized sample, the isomers and their relative amounts present in the sample can be determined. None of the simulated spectra on their own match the experimental spectra exactly, which indicates a mixture of CN-containing isomers was initially synthesized. If only isomers **1-3** and/or **1-4** were present in solution, only one distinct weaker band at a lower frequency from the strongest band would be observed instead of the broad overlapping bands in the experimental spectrum. If only isomers **1-1** and/or **1-2** were present in solution, the experimental spectra would not show the strongest band near 1976 cm<sup>-1</sup>. Since the experimental spectrum cannot be reproduced by the simulated spectra of a single isomer alone, a possible mixture of isomers was considered. The strong band in the experimental spectrum at 1976 cm<sup>-1</sup>

solution. The broad band to lower wavenumber of the strong band in the experimental spectrum is likely composed of at least two overlapping bands. Since it is not a distinct peak, which would be the case if only isomers **1-3** and/or **1-4** were present, isomers **1-1** and/or **1-2** must also be present and so a mixture of these isomers was simulated, as shown in Figure 6(c). We conclude there is approximately a 70:30 abundance ratio of isomers **1-3** and/or **1-4** to isomers **1-1** and/or **1-2**. The ratio of isomers may be closer to 60:40 or 80:20, but isomers **1-3** and/or **1-4** clearly dominate. As was found for related CN-containing species,<sup>42</sup> isomers **1-3** and **1-4** are the most polar isomers and are likely stabilized by being in acetonitrile solution. This potentially explains why they are present in higher abundance than isomers **1-1** and **1-2** (which are less polar) despite being the lowest energy isomers in pure gas phase calculations (see SI Table S2).

#### Time Resolved UV-Pump/IR-Probe Laser Spectroscopy

**1 in MeCN.** Ultrafast TRIR was used to collect scans at several pump-probe time delays with 400 nm excitation and the resultant difference spectral data is shown in Figure 7. Additional data was collected for **1** using both 266 nm and 400 nm pump wavelengths (see Figure 8), which generated qualitatively very similar difference spectra. Any apparent differences are due to a small calibration error that distorted the data in Figure 8 (the error was corrected when the data in Figure 7 was collected, but 266 nm pump data was not collected at that time). The analysis below is based on the data shown in Figure 7, although the same conclusions result from a detailed analysis of the data in Figure 8. Parent species absorptive loss (bleaches) are shown below the baseline and new product absorptions occur above the zero-difference baseline. Bleaches occur for **1** at 1930 cm<sup>-1</sup>, 1979 cm<sup>-1</sup>, and 2036 cm<sup>-1</sup>. These bleaches generally correspond well with FTIR ground state absorptions, except for ca. 1956 cm<sup>-1</sup> where there is an absorption in the FTIR spectra, but very little signal in the TRIR spectra. At this spectral

position, there is likely a new absorption that overlaps the bleach expected there, resulting in cancellation of the bleach. New absorptions are observed for photoexcited **1** at 1963 cm<sup>-1</sup>, and 2038 cm<sup>-1</sup> out to 300 ps delay time. A weak new absorption feature may also be present around 1900 cm<sup>-1</sup> (more clearly seen in Figure 8).



**Figure 7.** Time-resolved UV and visible pump, IR probe difference spectra for **1** in MeCN (293 K) at indicated time delays after excitation with (a) 400 nm, and (b) ground-state FT-IR spectrum of **1** in MeCN solution at 293 K. The solid horizontal line in (a) is the  $\Delta OD = 0$  baseline.



**Figure 8.** Time-resolved UV and visible pump, IR probe difference spectra for **1** in MeCN (293 K) at indicated time delays after excitation with (a) 266 nm and (b) 400 nm, and (c) ground-state FTIR spectrum of **1** in MeCN solution at 293 K. Solid horizontal lines in (a) and (b) are the  $\Delta OD = 0$  baseline.

The difference spectra scans were examined to select wavenumbers associated with bleach and new absorption features to perform dynamical measurements. Time-delay scans were taken at these fixed wavenumbers with high and low time-step densities in order to better understand the dynamics of these bleaches and absorptions. The raw long-time delay scans for **1** at the noted wavenumber positions are shown in Figure 9(a). For all cases, an instrument-limited risetime at delay time t=0 is observed followed by a rapid intensity decrease and flat long-time

offset. Two different timescales are extracted from the time-delay scans for this species using a simple single exponential fit with offset model. To avoid the rise time and instrument-dependent coherence effects, fits started at 1 ps after t = 0. These fits are complicated by new absorptions that nearly entirely cancel out the bleaches. The fits for two dynamical scans are shown for **1** in Figures 8(b) and 8(c). The observed time-dependence of seven different wavelengths were averaged to give a rapid  $28 \pm 11$  ps decay, followed by the long-lived offset. All fits used in this analysis are shown in Figure S1.



**Figure 9.** Time-dependent scans for **1** in MeCN (293 K) using 400 nm excitation at (a) multiple selected spectral wavenumbers, (b) 2038 cm<sup>-1</sup>, and (c) 1943 cm<sup>-1</sup>. Single-exponential with long-time offset fits to these dynamics are also shown (red).

The 20 ps to 30 ps initial decay lifetime likely arises from vibrational cooling of photoproducts resulting from relaxation of the excited state.<sup>47,49,50</sup> This behavior is especially noticeable at ~1950 cm<sup>-1</sup> and ~2021 cm<sup>-1</sup>, where the short-time spectra are broad and shifted to lower wavenumbers, indicative of anharmonic overtone absorption of coupled hot bands to CO-stretching vibrations. After approximately 20 ps to 30 ps, the product species return to their ground vibrational states and the intermediate spectra "relax" to the long-time spectrum, which is narrower and shifted to higher wavenumbers. Evidence of vibrational cooling has been observed in previously examined related molecules<sup>42,43</sup>, but not as distinctly as in **1** (and **2**, see below). In addition to vibrational relaxation, there is also rotational dynamics potentially contributing to the early-time dynamics, as observed in other similar model compounds.<sup>60–62</sup> The long-lived spectrum at ~300 ps delay suggests that one or more new photoproducts are formed that may be stable with ns or longer lifespans.

Somewhat surprisingly, no ~150 ps decay is seen in this data, despite attempts to fit the data to a bi-exponential decay. TRIR work on all-CO and mixed CO/PMe<sub>3</sub> models has shown a ~150 ps decay that was assigned to the decay of a long-lived electronic excited state.<sup>43,44,47–49</sup> The exception to this were the di-cyano model species which only showed a ~20 ps decay followed by a long offset, similar to the mono-cyano species studied here.<sup>42</sup>

Interestingly, all compounds that exhibit a 150 ps decay component have a weak UV-Vis absorption band around 450 nm (see Figure 3). In these spectra, both cyano-functionalized species **1** and **5** ( $[Fe_2(\mu-S_2C_3H_6)(CO)_4(CN)_2]^{2-}$ ) do not show a distinct absorption band at 450 nm (although a long tail is present in this area that could obscure a very weak band), and their time-dependence are also missing the 150 ps decay component.  $Fe_2(\mu-S_2C_3H_6)(CO)_6$  (**3**) and  $Fe_2(\mu-S_2C_3H_6)(CO)_4(PMe_3)_2$  (**4**) both have the weak 450 nm absorption and produce the 150 ps decay

lifetime. Based on earlier work on Fe<sub>2</sub> ( $\mu$ -S)<sub>2</sub>(CO)<sub>6</sub>, this band around 450 nm seems to be related to a transition between the Fe-Fe  $\sigma$ -bonding HOMO and an antibonding LUMO orbital,<sup>52</sup> although why this transition is absent in the cyano-functionalized species is unclear. Detailed TD-DFT calculations for all of the involved species are planned to help clarify the role of the 450 nm absorption band.

The missing 150 ps decay component may also explain why the spectra using a 266 nm pump wavelength are so similar to that with a 400 nm pump (Figure 8). In most other model compounds studied thus far at multiple pump wavelengths, the amount of signal from the 150 ps component varies depending on the pump wavelength used, causing differences in the spectra using different pump wavelengths at short times.<sup>42–45</sup> This variation is not seen in this study, as there is no 150 ps component.

In order to better locate new absorption features for the long-time delay photoproduct(s) without overlapping bleaching features, the ground state IR spectrum for **1** was added to the longest time-delayed spectrum obtained (at 286 ps, dark blue in Figure 7), as shown in Figure 10. In order to account for the difference in intensities between the TRIR spectrum and the FTIR spectrum, a multiplying normalization factor was applied to the product spectrum. This multiplicative factor was approximated and adjusted in order to minimize the negative absorption at ~1928 cm<sup>-1</sup>. However, it was not possible to fully minimize both negative absorptions at ~1928 cm<sup>-1</sup> and ~2000 cm<sup>-1</sup>. These negatively absorbing features in the product spectrum are likely due to broadening in the TRIR spectrum that is not observed in the FTIR spectrum. Using this analysis, the 1916 cm<sup>-1</sup> absorption is found to shift to the red (lower wavenumber) from the corresponding absorption at 2033 cm<sup>-1</sup> shifts very slightly to the blue (higher wavenumber).



**Figure 10.** Long time-delay product spectrum (gray) extrapolated from the 286 ps time delayed TRIR and ground state FTIR spectra for **1** in MeCN at 293 K.

In order to identify potential product species' structures, the possibility of isomerization upon excitation or relaxation to the ground state was considered since these processes were previously observed in related di-iron model compounds.<sup>42,44,47</sup> In this case, the possibility of isomerization alone can be ruled out by comparing the observed shifts to the expected shifts that would occur due to isomerization. For example, assuming there is approximately a 70:30 ground state isomer ratio as previously described, all of **1-1** and/or **1-2** could potentially isomerize to **1-3** and/or **1-4** upon excitation. If this were the case, the absorption at 1903 cm<sup>-1</sup> would shift to the red while the absorptions at 1961 cm<sup>-1</sup> and 2033 cm<sup>-1</sup> would be expected to shift considerably to the blue (Figure 6(b)). Since the observed shift is to the red at 1961 cm<sup>-1</sup>, the possibility of **1-1** and/or **1-2** isomerizing completely to **1-3** and/or **1-4** cannot fully explain the observed spectrum. Using a similar analysis, the possibility of **1-3** and/or **1-4** isomerizing to **1-1** and/or **1-2** also cannot, by itself, explain the observed spectrum. In that case, a blue shift at 1900 cm<sup>-1</sup> and red shifts at 1961 cm<sup>-1</sup> and 2033 cm<sup>-1</sup> would be expected, which is not observed experimentally.

Another possibility may be loss of the CN ligand upon UV excitation. To address this possibility, DFT calculations were performed to simulate the spectra of all possible CN-loss isomers, shown for **1** in Figure 11(a) (with no coordinated acetonitrile) and Figure 11(f) with a coordinated acetonitrile (solvent) molecule. In both coordinated and uncoordinated cases, CN-loss would result in a significant blue shift of all CO-stretching bands, which is not observed in the product spectrum. Furthermore, the CN-stretch band at 2094 cm<sup>-1</sup> persists with nearly identical starting intensity (see Fig. 9) suggesting little if any CN is photolyzed. The possibility of CN ligand loss is thus entirely ruled out.

A third possibility is loss of a CO ligand upon UV excitation, which is observed in many metal-carbonyl species. DFT calculations were performed to simulate all possible CO-loss isomers, shown for **1** in Figure 10(b-e) with no solvent coordination and Figure 10(g-j) with solvent coordination. Several CO-loss isomers match the observed shifts to the red at 1900 cm<sup>-1</sup> and 1961 cm<sup>-1</sup>. However, not a single calculated spectrum for any CO-loss isomers results in a blue shift at 2033 cm<sup>-1</sup> (relative to the calculated ground state spectrum) as is observed experimentally. The loss of a CO,  $\pi$ -acceptor ligand is expected to cause the remaining CO stretches to weaken and shift to lower wavenumbers, as is seen in the DFT-calculated CO-loss spectra. Thus, the loss of a CO ligand cannot, by itself, result in the product spectra.



**Figure 11.** Calculated DFT spectra for all CN- and CO-loss photoproduct isomers of **1**: (a) CN-loss isomers and (b-e) CO-loss isomers originating from possible ground state isomeric structures. Isomers that differ only by the direction the propyl bridge typically have nearly

identical spectra and are indistinguishable. (f-j) are the same as (a-e) but with an acetonitrile molecule coordinated at the CO-loss site.

Since isomerization and ligand loss can each explain portions of the observed spectrum but not all of it, the observed shifts likely arise from a complex mixture of isomerization and COloss. For example, the only possibility that results in the observed product blue shift at 2033 cm<sup>-1</sup> is isomerization of **1-1/1-2** to **1-3/1-4**. This isomerization, combined with partial CO-loss products nearly matches the red shifts at 1900 cm<sup>-1</sup> and 1961 cm<sup>-1</sup>, as well at the blue shift at 2033 cm<sup>-1</sup>. Other, more complicated explanations are possible, such as the formation of a bridging Fe-CO-Fe species, a change in the coordination of one or both of the sulfur atoms, or dimerization, but these would be difficult to model or test with any certainty.

A transient CO-loss photoproduct could possibly generate a more stable bridging Fe-CO-Fe species. Specifically, the DFT calculated isomer structures **1-2.3**, **1-2.5**, and **1-4.1** all show evidence of a bridging or semi-bridging CO bond in the uncoordinated cases (see Figures 10(c) and 10(e)) with lowest frequency CO-stretch absorptions near 1800 cm<sup>-1</sup>. Somewhat surprisingly, adding an acetonitrile molecule in the vacant site still results in semi-bridging CO in isomers **1-2.3** and **1-2.5**, however the **1-4.1** isomer is no longer bridging under these conditions. Unfortunately, we did not acquire TRIR spectral data with sufficient signal-to-noise in the bridging CO-stretching region (typically 1700 cm<sup>-1</sup> to 1800 cm<sup>-1</sup>) permitting us to conclude whether or not a bridging CO product is generated. However, we can conclude that a bridging CO isomer alone does not explain the product spectrum. If a bridging CO species were formed, the entire spectrum of the product would be shifted significantly to the red, which is not experimentally observed. Additionally, DFT calculations suggest the bridging CO isomers have smaller dipoles than other CO-loss isomers in that same isomer "family" (see SI Tables S4 and S6). The use of acetonitrile, a very polar solvent, makes it unlikely that bridging CO isomers

with smaller dipoles would be formed over other isomers with larger dipoles. Further TRIR investigations are required to confirm these conclusions.

**2** in MeCN. Ultrafast TRIR was also used to collect spectral scans at different time delays using 400 nm excitation of **2** (See Supplemental Information Figures S2 to S7 for corresponding figures above for **1**). Similar results obtained for **1** were also observed for **2**. Time delay scans were taken for **2** (Figure S3(a)) and the fits for seven of these scans are shown in Figure S3(b-h). Two relaxation timescales were again observed for **2**; a  $36 \pm 23$  ps decay, followed by a long-lived offset. The larger value and large standard deviation for this time constant compared to that for **1** is primarily due to a single wavelength (1899 cm<sup>-1</sup>) with an anomalously long time constant. As was performed for **1** in order to better identify the locations of new absorptions, the long-time TRIR spectra for **2** was added to the FTIR ground-state spectrum for **2** (Figure S4) and the same analysis of comparing shifts performed for **1** above was applied. Product spectra were again compared to DFT simulated spectra for different possible isomeric structures (see Figure S5) and similar conclusions for CN and CO-loss, dimerization and bridging CO photoproducts drawn as for **1** discussed previously.

One interesting difference was observed in the DFT calculations for 2 compared to 1. When uncoordinated with acetonitrile, 2 showed semi-bridging CO-loss isomers in two cases (2-1.3 and 2-1.5), similar to 1. However, 2 also yielded a CN-bridging isomer in one case (2-3.2). When coordinated with acetonitrile, however, the bridging configurations were no longer the lowest energy for any of the CO-loss isomers of 2.

## Minute Time-Delay FTIR Spectroscopy:

Minute timescale FTIR difference spectra for **1** are shown in Figure 12, while very similar spectra for **2** may be found in Figure S6, respectively. As described earlier, the longest time spectra for each species was added to its corresponding ground-state FTIR spectrum so that the locations of new absorptions would be easier to identify, as shown in Figures 13 and S7. For both **1** and **2**, new absorptions are observed at 1905 cm<sup>-1</sup>, 1941 cm<sup>-1</sup>, 1978 cm<sup>-1</sup>, and 2086 cm<sup>-1</sup>. A distinct red shift is observed for all CO-stretching bands. The weaker CN-stretch absorption at 2086 cm<sup>-1</sup>, which was barely distinguishable from noise in the TRIR data, remains in the spectrum but is observed to clearly shift to the red. These shifts were compared to those observed in the TRIR data. The directions of the shifts at corresponding wavelengths between transient FTIR and TRIR data do not match. Of particular note is the strong red shift of the 2030 cm<sup>-1</sup> band, which shifted very slightly to the blue in the TRIR data. This implies that different photoproducts are formed on the second timescale than on the picosecond timescale.

The large red shift (especially of the band at 2030 cm<sup>-1</sup>) implies that the new photoproduct is not the result of isomerization (compare the individual isomer spectra in Figure 6(b)). The spectra also do not at all match the calculated spectra of the CN-loss isomers shown in Figure 11(a) and (f), which would result in a strong blue shift for all of the CO stretches.

The significant red-shift of the CO and CN absorptions would be consistent with the formation of stable CO-loss photoproducts. Comparing the product spectrum in Figure 13 with the calculated CO-loss spectra in Figure 11(b-e) and (g-h), several of the CO-loss isomers would be consistent with the observed product spectra, especially those from isomers **1-1** and **1-2**. The product bands in the minute timescale spectra are also narrower and better resolved than those in the TRIR data, which might imply the presence of fewer CO-loss isomers in the mixture. This

possibility would be consistent with initially higher-energy CO-loss isomers being formed that relax to lower-energy isomers on a longer (> ns) timescale.

The formation of stable CO-loss isomers on the minute timescale is surprising, because CO is expected to recombine within microseconds at millimolar concentrations of the starting compound. The coordinating nature of the MeCN solvent may help to stabilize these long-lived CO-loss photoproducts. Production of a stable bridging Fe-CO-Fe photoproduct was considered, but a detailed analysis of the 1600-1800 cm<sup>-1</sup> region reveals no evidence of a bridging CO-stretching mode.

The most likely explanation for the difference between the photoproducts generated on the picosecond and minute timescales is slow relaxation to the most stable CO-loss isomers. Higher-energy CO-loss isomers would eventually isomerize to a more limited set of isomers, greatly simplifying the spectrum. Any non-equilibrium isomerization of 5-CO (non-CO-loss) isomers would revert to the equilibrium ratio for MeCN solvent on this timescale, removing them from the spectrum, as well. For comparison to these FTIR results, equivalent electronic UV-Vis minute time-delayed and difference spectra may be found in Figure S8.



**Figure 12.** Long time UV-FTIR difference spectra for **1** at the indicated total UV-lamp exposure times.



**Figure 13.** Long time product spectrum extrapolated from 180 s UV exposure and FTIR spectrum for **1**.

#### Conclusions

The photodynamics of two asymmetric cyano-containing Fe-Fe hydrogenase model compounds were studied. **1** was excited with 266 nm and 400 nm wavelengths, while **2** was excited using only 400 nm excitation. Picosecond photo-dynamics were examined using picosecond TRIR spectroscopy, while stable product formation was investigated using UV-lamp exposure with FTIR and UV-Vis difference spectroscopies on the minute timescale.

Ultrafast transient dynamics of bleaches and absorptions occur on two timescales. An initial 20 ps to 30 ps decay followed by a long-lived offset is observed for both **1** and **2**. The rapid 20 ps to 30 ps decay is likely the result of vibrational cooling and rotational dynamics after a much faster electronic state reorganization. As was found earlier for symmetric models containing two CN and other ligands, these mono CN-substituted molecules did not exhibit an initial (ca. 150 ps) decay previously observed in all CO or mixed CO/PMe<sub>3</sub> compounds, implying that the symmetry and inclusion of anionic CN ligands significantly participates in the photodynamics and possible function of these models.<sup>43,44,47–50</sup> The long-lived (>150 ps) offset spectra and dynamics also suggests that one or more stable and soluble products are formed on the picosecond to minute timescale.

TRIR spectra were compared to DFT calculated spectra of different isomeric structures for each anion. This comparison indicated that the TRIR product spectra were not the result of isomerization, CO-loss, or CN-loss alone. Rather, the product spectra likely arise from a complex combination of CO-loss and isomerization, which would be nearly impossible to simulate. As is present in the native enzyme, low energy bridging Fe-CO-Fe species may form

in low yield after CO-loss, as indicated from DFT calculations, but more detailed TRIR spectral measurements are required to confirm this possibility. Identification of these products via further DFT modelling and NMR spectroscopy will be further explored.

On the minute timescale, no evidence for CN loss or isomerism occurs and initial groundstate isomerism likely equilibrates by this time. Only CO-loss photoproducts were observed and possibly stabilized by coordinating MeCN solvent. No evidence of a bridging CO ligand was spectroscopically detected on this timescale.

# **AUTHOR INFORMATION**

The authors declare no competing financial interests.

**Supplemental Information:** Detailed DFT calculation results for ground state and all isomeric structures for **1** and **2** including relative energies ( $\Delta$ G), dipole moments, CO- and CN-stretching mode assignments, frequencies and intensities, with analogous picosecond transient spectra and time-resolved decays with minute timescale FTIR and UV-Vis spectra for **2** may be found in the Supplemental Information (SI).

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# References

- (1) Adams, M. W. W. The Structure and Mechanism of Iron-Hydrogenases. *Biochim Biophys Acta. Bioenergetics* **1990**, *1020* (2), 115–145.
- Adams, M. W. W.; Stiefel, E. I. BIOCHEMISTRY: Biological Hydrogen Production: Not So Elementary. *Science* 1998, 282 (5395), 1842–1843. https://doi.org/10.1126/science.282.5395.1842.
- (3) Adams, M. W.; Stiefel, E. L. Organometalic Iron: The Key to Biological Hydrogen Metabolism. *Chem. Biol.* **2000**, *4*, 214–220.
- (4) Bennett, B.; Lemon, B. J.; Peters, J. W. Reversible Cabon Monoxide Binding and Inhibition at the Active Site of the Fe-Only Hydrogenase. *Biochemistry* **2000**, *39*, 7455–7460.
- Hambourger, M.; Gervaldo, M.; Svedruzic, D.; King, P. W.; Gust, D.; Ghirardi, M.; Moore, A. L.;
  Moore, T. A. [FeFe]-Hydrogenase-Catalyzed H2 Production in a Photoelectrochemical Biofuel Cell.
  J. Am. Chem. Soc. 2008, 130 (6), 2015–2022. https://doi.org/10.1021/ja077691k.
- (6) Nicolet, Y.; Lemon, B. J.; Fontecilla-Camps, J. C.; Peters, J. W. A Novel FeS Cluster in Fe-Only Hydrogenases. *Trends Biochem. Sci.* **2000**, *25* (3), 138–142.
- Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. X-Ray Crystal Structure of the Fe-Only Hydrogenase (CpI) from Clostridium Pasteurianum to 1.8 Angstrom Resolution. *Science* 1998, 282 (5395), 1853–1858. https://doi.org/10.1126/science.282.5395.1853.
- Tard, C.; Pickett, C. J. Structural and Functional Analogues of the Active Sites of the [Fe]-, [NiFe]-, and [FeFe]-Hydrogenases. *Chem. Rev.* 2009, 109 (6), 2245–2274. https://doi.org/10.1021/cr800542q.
- (9) Wang, X.-B.; Niu, S.; Yang, X.; Ibrahim, S. K.; Pickett, C. J.; Ichiye, T.; Wang, L.-S. Probing the Intrinsic Electronic Structure of the Cubane [4Fe-4S] Cluster, Nature's Favorite Cluster for Electron Transfer and Storage. J. Am. Chem. Soc. 2003, 125, 14072–14081.
- (10) Gloaguen, F.; Lawrence, J. D.; Rauchfuss, T. B. Biomimetic Hydrogen Evolution Catalyzed by an Iron Carbonyl Thiolate. *J. Am. Chem. Soc.* **2001**, *123*, 9476–9477.
- (11) Lubitz, W.; Ogata, H.; Rüdiger, O.; Reijerse, E. Hydrogenases. *Chemical Reviews* 2014, 114 (8), 4081–4148. https://doi.org/10.1021/cr4005814.
- Siegbahn, P. E. M.; Tye, J. W.; Hall, M. B. Computational Studies of [NiFe] and [FeFe]
  Hydrogenases. *Chem. Rev.* 2007, *107* (10), 4414–4435. https://doi.org/10.1021/cr050185y.
- (13) Parkin, A. Understanding and Harnessing Hydrogenases, Biological Dihydrogen Catalysts; Metal Ions in Life Sciences; Springer, Dordrecht, 2014; Vol. 14, pp 99–124.
- (14) Daraosheh, A. Q.; Harb, M. K.; Windhager, J.; Görls, H.; El-khateeb, M.; Weigand, W. Substitution Reactions at [FeFe] Hydrogenase Models Containing [2Fe3S] Assembly by Phosphine or Phosphite Ligands. Organometallics 2009, 28 (21), 6275–6280. https://doi.org/10.1021/om9005752.
- (15) Gloaguen, F.; Lawrence, J. D.; Schmidt, M.; Wilson, S. R.; Rauchfuss, T. B. Synthetic and Structural Studies on [Fe2(SR)2(CN)x(CO)6-x]x- as Active Site Models for Fe-Only Hydrogenases. J. Am. Chem. Soc. 2001, 123, 12518–12527.
- (16) Harb, M. K.; Apfel, U.-P.; Kuì<sup>^</sup>bel, J.; Goì<sup>^</sup>rls, H.; Felton, G. A. N.; Sakamoto, T.; Evans, D. H.; Glass, R. S.; Lichtenberger, D. L.; El-khateeb, M.; Weigand, W. Preparation and Characterization of Homologous Diiron Dithiolato, Diselenato, and Ditellurato Complexes: [FeFe]-Hydrogenase Models. *Organometallics* 2009, *28* (23), 6666–6675. https://doi.org/10.1021/om900675q.
- (17) Harb, M. K.; Niksch, T.; Windhager, J.; Görls, H.; Holze, R.; Lockett, L. T.; Okumura, N.; Evans, D. H.; Glass, R. S.; Lichtenberger, D. L.; El-khateeb, M.; Weigand, W. Synthesis and Characterization of Diiron Diselenolato Complexes Including Iron Hydrogenase Models. *Organometallics* 2009, *28* (4), 1039–1048. https://doi.org/10.1021/om800748p.
- (18) Lawrence, J. D.; Li, H.; Rauchfuss, T. B. Beyond Fe-Only Hydrogenases: N-Functionalized 2-Aza-1,3-Dithiolates Fe2[(SCH2)2NR](CO)x (x = 5,6). *Chem. Commun.* **2001**, No. 16, 1482–1483.

- (19) Le Cloirec, A.; Best, S. P.; Borg, S.; Davies, S. C.; Evans, D. J.; Jughes, D. L.; Pickett, C. J. A Di-Iron Dithiolate Possessing Structural Elements of the Carboyl/Cyande Sub-Site of the H-Centre of Fe-Only Hydrogenase. *Chem. Commun.* **1999**, 2285–2286.
- (20) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. Coordination Sphere Flexibility of Active-Site Models for Fe-Only Hydrogenase: Studies in Intra- and Intermolecular Diatomic Ligand Exchange. *J. Am. Chem. Soc.* **2001**, *123*, 3268–3278.
- (21) Song, L.-C.; Gao, W.; Feng, C.-P.; Wang, D.-F.; Hu, Q.-M. Investigations on Synthesis, Structure, and Properties of New Butterfly [2Fe2Se] Cluster Complexes Relevant to Active Sites of Some Hydrogenases. *Organometallics* **2009**, *28* (20), 6121–6130. https://doi.org/10.1021/om900572t.
- (22) Tye, J. W.; Darensbourg, M. Y.; Hall, M. B. De Novo Design of Synthetic Di-Iron(I) Complexes as Structural Models of the Reduced Form of Iron-Iron Hydrogenase. *Inorg. Chem.* 2006, 45, 1552– 1559.
- (23) Zhao, X.; Georgakaki, I. P.; Miller, M. L.; Yarbrough, J. C.; Darensbourg, M. Y. H/D Exchange Reactions in Dinuclear Iron Thiolates as Activity Assay Models of Fe–H2ase. *J. Am. Chem. Soc.* 2001, *123* (39), 9710–9711. https://doi.org/10.1021/ja0167046.
- (24) Zhao, X.; Georgakaki, I. P.; Miller, M. L.; Mejia-Rodriguez, R.; Chiang, C.-Y.; Darensbourg, M. Y. Catalysis of H2/D2 Scrambling and Other H/D Exchange Processes by [Fe]-Hydrogenase Model Complexes. *Inorg. Chem.* 2002, *41*, 3917–3928.
- (25) Nicolet, Y.; Piras, C.; Legrand, P.; Hatchikian, C. E.; Fontecilla-Camps, J. C. Desulfovibrio Desulfuricans Iron Hydrogenase: The Structure Shows Unusual Coordination to an Active Site Fe Binuclear Center. *Structure* 1999, 7 (1), 13–23.
- (26) Darensbourg, M. Y.; Lyon, E. J.; Zhao, X.; Georgakaki, I. P. The Organometallic Active Site of [Fe]Hydrogenase: Models and Entatic States. *Proc. Natl. Acad. Sci. U.S.A.* 2003, 100 (7), 3683– 3688. https://doi.org/10.1073/pnas.0536955100.
- (27) Popescu, C. V.; Eckard, M. Electronic Structure of the H Cluster in [Fe]-Hydrogenases. J. Am. Chem. Soc. **1999**, *121*, 7877–7884.
- Ihara, M.; Nishihara, H.; Yoon, K.-S.; Lenz, O.; Friedrich, B.; Nakamoto, H.; Kojima, K.; Honma, D.; Kamachi, T.; Okura, I. Light-Driven Hydrogen Production by a Hybrid Complex of a [NiFe]-Hydrogenase and the Cyanobacterial Photosystem I. *Photochemistry and Photobiology* 2006, 82 (3), 676–682. https://doi.org/10.1562/2006-01-16-RA-778.
- (29) Lubner, C. E.; Grimme, R.; Bryant, D. A.; Golbeck, J. H. Wiring Photosystem I for Direct Solar Hydrogen Production. *Biochemistry* **2010**, *49* (3), 404–414. https://doi.org/10.1021/bi901704v.
- (30) Fukuzumi, S.; Lee, Y.-M.; Nam, W. Thermal and Photocatalytic Production of Hydrogen with Earth-Abundant Metal Complexes. *Coordination Chemistry Reviews* **2018**, *355*, 54–73. https://doi.org/10.1016/j.ccr.2017.07.014.
- (31) Hongguang, C.; Mei, W.; Duan, L.; Licheng, S. Preparation, Characterization and Electrochemistry of an Iron-Only Hydrogenase Active Site Model Covalently Linked to a Ruthenium Tris(Bipyridine) Photosensitizer. *Journal of Coordination Chemistry* **2008**, *61* (12), 1856–1861. https://doi.org/10.1080/00958970701767002.
- (32) Sun, L.; Åkermark, B.; Ott, S. Iron Hydrogenase Active Site Mimics in Supramolecular Systems Aiming for Light-Driven Hydrogen Production. *Coord. Chem. Rev.* **2005**, *249* (15–16), 1653–1663. https://doi.org/10.1016/j.ccr.2005.01.013.
- (33) Wolpher, H.; Borgstrom, M.; Hammarstrom, L.; Bergquist, J.; Sundstrom, V.; Styring, S.; Sun, L.; Akermark, B. Synthesis and Properties of an Iron Hydrogenase Active Site Model Linked to a Ruthenium Tris-Bipyridine Photosensitizer. *Inorg. Chem. Commun.* **2003**, *6* (8), 989–991.
- (34) Ott, S.; Kritikos, M.; Åkermark, B.; Sun, L. Synthesis and Structure of a Biomimetic Model of the Iron Hydrogenase Active Site Covalently Linked to a Ruthenium Photosensitizer. *Angew. Chem. Int. Ed.* **2003**, *42*, 3285–3288.

- (35) Song, L. C.; Tang, M. Y.; Mei, S. Z.; Huang, J. H.; Hu, Q. M. The Active Site Model for Iron-Only Hydrogenases Coordinatively Bonded to a Metalloporphyrin Photosensitizer. *Organometallics* 2007, 26 (7), 1575–1577.
- (36) Song, L.-C.; Wang, L.-X.; Tang, M.-Y.; Li, C.-G.; Song, H.-B.; Hu, Q.-M. Synthesis, Structure, and Photoinduced Catalysis of [FeFe]-Hydrogenase Active Site Models Covalently Linked to a Porphyrin or Metalloporphyrin Moiety. *Organometallics* 2009, 28 (13), 3834–3841. https://doi.org/10.1021/om900141x.
- Ott, S.; Borgstrom, M.; Kritikos, M.; Lomoth, R.; Bergquist, J.; Åkermark, B.; Hammarstrom, L.;
  Sun, L. Model of the Iron Hydrogenase Active Site Covalently Linked to a Ruthenium
  Photosensitizer: Synthesis and Photophysical Properties. *Inorg. Chem.* 2004, 43 (15), 4683–4692.
- (38) Gao, S.; Huang, S.; Duan, Q.; Hou, J.; Jiang, D.; Liang, Q.; Zhao, J. Iron–Iron Hydrogenase Active Subunit Covalently Linking to Organic Chromophore for Light-Driven Hydrogen Evolution. *Int. J. Hydrogen Energy* March 7, 39 (20), 10434–10444. https://doi.org/10.1016/j.ijhydene.2014.05.003.
- (39) Gao, W.; Liu, J.; Jiang, W.; Wang, M.; Weng, L.; Åkermark, B.; Sun, L. An Azadithiolate Bridged Fe2S2 Complex as Active Site Model of FeFe-Hydrogenase Covalently Linked to a Re(CO)3(Bpy)(Py) Photosensitizer Aiming for Light-Driven Hydrogen Production. C. R. Chim. 2008, 11 (8), 915–921.
- (40) Ekström, J.; Abrahamsson, M.; Olson, C.; Bergquist, J.; Kaynak, F. B.; Eriksson, L.; Sun, L.; Becker, H.-C.; Åkermark, B.; Hammarström, L.; Ott, S. Bio-Inspired, Side-on Attachment of a Ruthenium Photosensitizer to an Iron Hydrogenase Active Site Model. *Dalton Trans.* 2006, 4599–4606.
- Magnuson, A.; Anderlund, M.; Johansson, O.; Lindblad, P.; Lomoth, R.; Polivka, T.; Ott, S.; Stensjö, K.; Styring, S.; Sundström, V.; Hammarström, L. Biomimetic and Microbial Approaches to Solar Fuel Generation. Acc. Chem. Res. 2009, 42 (12), 1899–1909. https://doi.org/10.1021/ar900127h.
- (42) Stromberg, C. J.; Heilweil, E. J. Ultrafast Photodynamics of Cyano-Functionalized [FeFe]
  Hydrogenase Model Compounds. *The Journal Of Physical Chemistry. A* 2018, *122* (16), 4023–4030. https://doi.org/10.1021/acs.jpca.8b00661.
- Bingaman, J. L.; Kohnhorst, C. L.; Van Meter, G. A.; McElroy, B. A.; Rakowski, E. A.; Caplins, B. W.;
  Gutowski, T. A.; Stromberg, C. J.; Webster, C. E.; Heilweil, E. J. Time-Resolved Vibrational
  Spectroscopy of [FeFe]-Hydrogenase Model Compounds. *J. Phys. Chem. A* 2012, *116* (27), 7261–7271. https://doi.org/10.1021/jp2121774.
- Johnson, M.; Thuman, J.; Letterman, R. G.; Stromberg, C. J.; Webster, C. E.; Heilweil, E. J. Time-Resolved Infrared Studies of a Trimethylphosphine Model Derivative of [FeFe]-Hydrogenase. J. Phys. Chem. B 2013, 117 (49), 15792–15803. https://doi.org/10.1021/jp4067873.
- (45) Meyer, R. L.; Zhandosova, A. D.; Biser, T. M.; Heilweil, E. J.; Stromberg, C. J. Photochemical Dynamics of a Trimethyl-Phosphine Derivatized [FeFe]-Hydrogenase Model Compound. *Chemical Physics* 2018, *512*, 135–145. https://doi.org/10.1016/j.chemphys.2017.12.014.
- (46) Thornley, W.; Wirick, S. A.; Riedel-Topper, M.; DeYonker, N. J.; Bitterwolf, T. E.; Stromberg, C. J.; Heilweil, E. J. Photodynamics of [FeFe]-Hydrogenase Model Compounds with Bidentate Heterocyclic Ligands. *J. Phys. Chem. B* 2019, *123* (33), 7137–7148. https://doi.org/10.1021/acs.jpcb.9b04675.
- (47) Kania, R.; Frederix, P. W. J. M.; Wright, J. A.; Ulijn, R. V.; Pickett, C. J.; Hunt, N. T. Solution-Phase Photochemistry of a [FeFe]Hydrogenase Model Compound: Evidence of Photoinduced Isomerisation. J. Chem. Phys. 2012, 136 (4), 044521–044529.
- (48) Kaziannis, S.; Santabarbara, S.; Wright, J. A.; Greetham, G. M.; Towrie, M.; Parker, A. W.; Pickett, C. J.; Hunt, N. T. Femtosecond to Microsecond Photochemistry of a [FeFe]Hydrogenase Enzyme Model Compound. *J. Phys. Chem. B* 2010, *114* (46), 15370–15379. https://doi.org/10.1021/jp107618n.

- (49) Caplins, B. W.; Lomont, J. P.; Nguyen, S. C.; Harris, C. B. Vibrational Cooling Dynamics of a [FeFe]-Hydrogenase Mimic Probed by Time-Resolved Infrared Spectroscopy. *The Journal of Physical Chemistry A* 2014, *118* (49), 11529–11540. https://doi.org/10.1021/jp510517z.
- (50) Frederix, P. W. J. M.; Adamczyk, K.; Wright, J. A.; Tuttle, T.; Ulijn, R. V.; Pickett, C. J.; Hunt, N. T. Investigation of the Ultrafast Dynamics Occurring during Unsensitized Photocatalytic H2 Evolution by an [FeFe]-Hydrogenase Subsite Analogue. *Organometallics* **2014**, *33* (20), 5888– 5896. https://doi.org/10.1021/om500521w.
- (51) Certain Commercial Equipment, Instruments, or Materials Are Identified in This Paper to Foster Understanding. Such Identification Does Not Imply Recommendation or Endorsement by the National Institute of Standards and Technology, nor Does It Imply That the Materials or Equipment Identified Are Necessarily the Best Available for the Purpose.
- (52) Silaghi-Dumitrescu, I.; Bitterwolf, T. E.; King, R. B. Butterfly Diradical Intermediates in Photochemical Reactions of Fe2(CO)6(μ-S2). J. Am. Chem. Soc. 2006, 128 (16), 5342–5343. https://doi.org/10.1021/ja061272q.
- (53) Parr, R. G.; Yang, W. *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.
- (54) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.;
  Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; et al. *Gaussian 16 Rev. B.01*; Wallingford, CT, 2016.
- (55) Becke, A. D. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A* **1988**, *38* (6), 3098–3100.
- (56) Perdew, J. P. Erratum: Density-Functional Approximation for the Correlation Energy of the Inhomogeneous Electron Gas. *Phys. Rev. B* **1986**, *34* (10), 7406–7406.
- (57) Perdew, J. P. Density-Functional Approximation for the Correlation Energy of the Inhomogeneous Electron Gas. *Phys. Rev. B* **1986**, *33* (12), 8822–8824.
- (58) Schafer, A.; Huber, C.; Ahlrichs, R. Fully Optimized Contracted Gaussian Basis Sets of Triple Zeta Valence Quality for Atoms Li to Kr. *J. Chem. Phys.* **1994**, *100* (8), 5829–5835.
- (59) Schafer, A.; Horn, H.; Ahlrichs, R. Fully Optimized Contracted Gaussian Basis Sets for Atoms Li to Kr. *J. Chem. Phys.* **1992**, *97* (4), 2571–2577.
- (60) Ridley, A. R.; Stewart, A. I.; Adamczyk, K.; Ghosh, H. N.; Kerkeni, B.; Guo, Z. X.; Nibbering, E. T. J.; Pickett, C. J.; Hunt, N. T. Multiple-Timescale Photoreactivity of a Model Compound Related to the Active Site of [FeFe]-Hydrogenase. *Inorg. Chem.* 2008, 47 (17), 7453–7455. https://doi.org/10.1021/ic800568k.
- (61) Stewart, A. I.; Clark, I. P.; Towrie, M.; Ibrahim, S. K.; Parker, A. W.; Pickett, C. J.; Hunt, N. T. Structure and Vibrational Dynamics of Model Compounds of the [FeFe]-Hydrogenase Enzyme System via Ultrafast Two-Dimensional Infrared Spectroscopy. J. Phys. Chem. B 2008, 112 (32), 10023–10032. https://doi.org/10.1021/jp803338d.
- (62) Stewart, A. I.; Wright, J. A.; Greetham, G. M.; Kaziannis, S.; Santabarbara, S.; Towrie, M.; Parker, A. W.; Pickett, C. J.; Hunt, N. T. Determination of the Photolysis Products of [FeFe]Hydrogenase Enzyme Model Systems Using Ultrafast Multidimensional Infrared Spectroscopy. *Inorg. Chem.* 2010, 49 (20), 9563–9573. https://doi.org/10.1021/ic101289s.

TOC Graphic:

