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Probing the effect of the solution environment on the vibrational dynamics of an enzyme model system with ultrafast 2D-IR spectroscopy


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Ultrafast 2D-IR spectroscopy has been applied to study the structure and vibrational dynamics of (µ-C(CH3)(CH2S)2(CH2S(CH2)2Ph)Fe2(CO)5, an organometallic model of the active site of the FeFe[hydrogenase] enzyme. 2D-IR spectra have been obtained in solvents ranging from non-polar to polar and protic. The influence of the solvent bath on vibrational relaxation, including rapid intramolecular population transfer, has been characterized. In addition, the temporal dependence of the 2D-IR lineshape has been used to extract information relating to hydrogen bond-mediated spectral diffusion via the frequency–frequency correlation function. Comparisons with previous 2D-IR studies of hydrogenase model systems offer insights into the dependence of the rate of population transfer upon vibrational mode separation and solvent environment, with important implications for the composition and reactivity of the active site of the enzyme.

Introduction

Ultrafast 2D-IR spectroscopy began its development some 10 years ago and has since shown itself to be a powerful probe of both chemical and biological systems. The methodology is closely related to that of multidimensional NMR methods and is based upon a sequence of ultrashort mid-infrared laser pulses. The main advantage of 2D-IR spectroscopy arises from an inherent high time resolution that is sensitive to processes many orders of magnitude faster than can be observed using NMR methods. This arises from the short free-induction decay times and lifetimes of vibrational transitions along with advances in laser technology that enable generation of mid-IR pulses with durations of the order of 100 fs. As the technique has developed, so has the range of topics that 2D-IR has been used to explore, including vibrational dynamics, solvent–solute interactions, the investigation of rates at which populations at chemical equilibrium interconvert (chemical exchange), protein dynamics and non-equilibrium systems via transient 2D-IR methods. This list is not exhaustive and these applications have been the subject of recent review articles.

Of most relevance to the present study is the ability of ultrafast 2D-IR spectroscopy to extract information from the 2D lineshape relating to the nature of solute–solvent interactions alongside vibrational relaxation dynamics. In the former case, the 2D-IR lineshape provides a measurement of the inhomogeneous
broadening of a vibrational transition, which is observed as an elongation of the lineshape along the diagonal of the 2D-IR spectrum. \textsuperscript{11,27–229} Furthermore, in the event that the timescale of the processes responsible for the inhomogeneous broadening is comparable with the delay between excitation and detection events in the experiment, spectral diffusion leads to a transition from diagonally elongated to circular line profiles as the molecule is able to sample all possible environments within the timescale of the experiment. \textsuperscript{11,27} It has been shown that it is possible to obtain from this lineshape evolution the frequency–frequency, or solvation, correlation function (FFCF or SCF). \textsuperscript{11,27,29} This provides a measurement of the frequency and amplitude of the motions within the solvent bath that lead to inhomogeneous broadening of the absorption. Several approaches have been used to extract the FFCF from 2D-IR lineshapes including evaluation of the ellipticity parameter, \textsuperscript{11} the centre line slope method\textsuperscript{27} and the angle of the nodal plane between the positive and negative contributions to a 2D-IR lineshape. \textsuperscript{11,29} These methods have been used to gain insight into the solvation dynamics of a range of samples including HOD in D\textsubscript{2}O,\textsuperscript{30} NaBr solutions\textsuperscript{31} and small peptides,\textsuperscript{32} as well as larger biological systems such as transmembrane proteins.\textsuperscript{43} Furthermore, the method has been employed to elucidate the ultrafast backbone fluctuations of neuroglobin via the FFCF.\textsuperscript{21}

Here, we report the application of 2D-IR spectroscopy to study the vibrational dynamics and solvent–solute interactions of a model compound of the active subsite of the hydrogenase enzyme system. The hydrogenases consist of three phylogenetically distinct families of metalloprotein-based enzymes; the NiFe[H], FeFe[H] and cluster-free Fe[H] hydrogenases. Their function is to catalyse the reversible activation of molecular hydrogen according to the reaction $\text{H}_2 \rightleftharpoons 2\text{H}^+ + 2\text{e}^-$. In the case of NiFe[H] and FeFe[H], the active subsite consists of a dimetallic cluster (either Ni–Fe or Fe–Fe) bridged by two sulfur atoms. In addition, the metal atoms are coordinated by carbonyl and cyanide ligands. Interest in these species is driven by the view that understanding the chemistry involved will lead to new electrocatalytic systems for hydrogen production or uptake. Such technology will result in new materials allowing the replacement of the expensive platinum metal catalysts used in fuel cells with those made from the more abundant iron and nickel. While the Ni-Fe[H] enzymes have been extensively studied,\textsuperscript{34–37} it is the FeFe[H] system that has been the focus of considerably more synthetic model studies resulting from the structural similarity of the active site to complexes of the form ($\mu$-SRS)Fe\textsubscript{2}(CO)\textsubscript{6}.\textsuperscript{38–41} The advantage of the latter lies in the ability to study analogues of the active site of these enzymes without the complication of the surrounding protein. Furthermore, it has been shown that such synthetic analogues are capable of catalysing the reduction of protons, a phenomenon that has been extensively explored using compounds which share a common topology with the active site.\textsuperscript{38}

Relevant to this study is the fact that the active site of the FeFe[H] enzyme has been shown to be devoid of solvating water molecules.\textsuperscript{32} This implies that the protein scaffold surrounding the active site plays a key role in dissipating excess vibrational energy during the reaction mechanism. The active site itself is connected to the protein backbone by just one cysteine residue that is coordinated to one of the Fe centres in the Fe\textsubscript{2}S\textsubscript{2} active site cluster. The remaining links with the protein backbone are believed to be through H-bonding and electrostatic interactions.\textsuperscript{43}

In order to understand this situation more fully and to determine the behaviour of active site models in solution we have applied 2D-IR methods to the study of a model compound in solution. In a previous publication, the solution phase structure and vibrational dynamics of ($\mu$-S(CH\textsubscript{2})\textsubscript{3}S)Fe\textsubscript{2}(CO)\textsubscript{6} in polar and non-polar solvents were determined.\textsuperscript{7} It was observed that the carbonyl stretching modes underwent rapid vibrational population transfer and equilibration prior to relaxation to the ground state. This process was observed to occur at an increased rate in polar solvents. Here we extend this study to the model compound ($\mu$-C(CH\textsubscript{3})(CH\textsubscript{2}S)\textsubscript{2}(CH\textsubscript{2}S(CH\textsubscript{2})\textsubscript{2}Ph)Fe\textsubscript{2}(CO)\textsubscript{5}, 1 (see Fig. 1). This model provides a closer approximation to the active site for two reasons: firstly, the Fe\textsubscript{2}S\textsubscript{2} cluster...
features a third sulfur ligand, mimicking the enzyme cysteine linkage and, secondly, the CO stretching modes of this species display a greater frequency separation, more in keeping with the enzyme active site. The latter is of particular interest because it is widely believed that rapid population transfer occurs through interactions with the solvent low frequency density of states, as such a close relationship between the peak in the density of states and the population transfer rate might be expected to exist. By using ultrafast 2D-IR spectroscopy to study in three solvents, non-polar heptane, polar acetonitrile and protic 1,7-heptanediol, we aim to further determine the role played by the solvent in the vibrational relaxation of FeFe[H] model species. We will examine the vibrational population relaxation pathways and exploit the ability of 2D-IR to extract information relating to the FFCF from the 2D lineshape to gain insights into the hydrogen bonding dynamics of . It is anticipated that these studies will extend previous work and further lay the groundwork for future studies of the enzyme active site, where comparisons of the vibrational relaxation of the active site with a range of model–solvent combinations will yield insights into the properties of the protein pocket.

Experimental

Ultrafast 2D-IR spectra were obtained using the well-established quasi-frequency-domain double resonance approach. The overall design of the spectrometer including a description of the pulse-shaping device used to produce the tunable narrow bandwidth pump pulses has been discussed previously. Briefly, mid-infrared (IR) laser pulses (ca. 150 fs duration; 5 μJ pulse energy) were produced following difference frequency generation of the signal and idler outputs of a white light-seeded optical parametric amplifier (OPA). These pulses produced a usable bandwidth of around 200 cm⁻¹ centred near 2000 cm⁻¹, resonant with the carbonyl-stretching vibrational modes of the model compound . The OPA was pumped using one third of the 3 mJ per pulse output of a laser system comprising an 80 MHz repetition rate Ti:sapphire oscillator seeding a regenerative amplifier. The pulses produced by this combination were of the order of 35 fs duration at a central wavelength of 800 nm and a 1 kHz repetition rate.

The mid-IR laser pulses were split using a beamsplitter in the approximate intensity ratio 90 : 10. The more intense beam was routed through the pulse shaping device to produce narrowband pump pulses of 5–10 cm⁻¹ bandwidth before being temporally and spatially overlapped with the probe pulse at the sample. The probe was subsequently dispersed and detected using a spectrometer and 64-element HgCdTe array detector. Modulation of the pump–pulse train at half the laser repetition rate enabled acquisition of the pump on–pump off difference spectra which constitute a single slice through the 2D-IR spectrum. The latter is produced by scanning the pump frequency for a fixed pump–probe delay time. Laser intensity

![Molecular structure of the model compound I studied herein. Grey spheres represent C; white, H; yellow, S; red, O; maroon, Fe.](image)
fluctuations were accounted for by normalisation of the signals to the output of a single element HgCdTe detector onto which a small fraction of the probe beam was directed.

2D-IR spectra were recorded using the magic angle relationship for the relative pump and probe polarization directions to yield vibrational relaxation dynamics free from the effects of molecular rotation. Spectra were also recorded using parallel and perpendicular polarization geometries and a linear combination of the responses used to recover the magic angle relationship. Comparisons of the two datasets showed excellent agreement between the two approaches.

In the case of heptane and acetonitrile solutions of 1, samples were held between two CaF$_2$ windows separated by $\sim 630$ $\mu$m using a PTFE spacer. It was not necessary to flow the sample, though the air-sensitive nature of the metal carbonyl-derivative samples required that the solutions in each solvent be produced in an inert-atmosphere environment following degassing of the solvents. The concentration of the solutions was selected to give a peak optical density in the carbonyl stretching region of the mid-IR of around 0.4; sufficiently high to yield good signal-to-noise ratios but not high enough to result in concentration-related 2D-IR lineshape distortions. In the case of 1,7-heptanediol solutions, a broad overtone absorption from the solvent necessitated a reduced pathlength of 250 $\mu$m, this combined with the lower solubility of 1 in heptanediol meant that the peak OD was limited to around 0.1. In general, this did not adversely affect the recovered signal-to-noise ratios. The model compound 1 was produced using established methods$^{38,45}$ and all solvents were obtained from Sigma-Aldrich Ltd and used without further purification. Samples were filtered using 0.2 $\mu$m filters in order to reduce experimental noise arising from scattered pump radiation.

**Results and discussion**

2D-IR spectroscopy

Ultrafast 2D-IR spectra of solutions of 1 in heptane, acetonitrile and 1,7-heptanediol in the carbonyl stretching region of the mid-IR are shown in Fig. 2(a)–(c) alongside the respective FTIR spectra. The FTIR spectra are similar in each of the three solvents with two intense absorptions near 2050 and 1990 cm$^{-1}$, allowing for small solvatochromic shifts, and a weaker transition near 1940 cm$^{-1}$. In the case of heptane solution, the narrow linewidths associated with this solvent lead to the observation of a second weaker band near 1970 cm$^{-1}$, this mode is also present near 1960 cm$^{-1}$ in the FTIR spectra of the acetonitrile and heptanediol solutions, though as a shoulder.

**Fig. 2** FTIR and 2D-IR spectra of 1 in heptane (a), acetonitrile (b) and 1,7-heptanediol (c). 2D-IR spectra were recorded with a pump–probe delay time of 2 ps. All spectra are plotted using a colour scheme ranging from red (negative values) to blue (positive values).
to the most intense band near 1990 cm\(^{-1}\). Molecule 1 possesses \(C_1\) symmetry and as such a fifth carbonyl stretching mode would be anticipated. To date, a complete assignment of the carbonyl stretching modes of this species is not available but preliminary density functional theory calculations (not reported here) suggest that it may be of low intensity and located close to the 1990 cm\(^{-1}\) band. There is some evidence in support of this in the FTIR spectrum of the heptane solution (Fig. 2(a)) where a small shoulder is observed between the 1990 cm\(^{-1}\) mode and the weak mode near 1970 cm\(^{-1}\), this is however inconclusive. For the present study, it is intended to focus upon the 2D-IR spectroscopy and dynamics of the three most intense CO stretching transitions near 2050, 1990 and 1940 cm\(^{-1}\). These bands will henceforth be referred to using these wavenumber designations to prevent ambiguity brought about by reference to individual solvatochromic transition frequencies. It is interesting to note the variation in FWHM for the individual transitions, heptane giving rise to a value of \(\sim 5\) cm\(^{-1}\) while the more polar acetonitrile and the protic heptanediol lead to a FWHM value of \(\sim 10\) cm\(^{-1}\). The similar values for the polar and protic solvents are particularly interesting in light of the somewhat different major mechanisms of solvent–solute interaction, namely dipole–dipole interaction and hydrogen bonding, respectively. The effect of this on the vibrational relaxation dynamics of 1 will be discussed subsequently.

The 2D-IR spectra shown in Fig. 2 were recorded with a pump–probe delay time of 2 ps and are largely as would be expected from previous work on similar compounds. In heptane (Fig. 2(a)) the three main transitions near 2050, 1990 and 1940 cm\(^{-1}\) are present as bleaches (negative-going peaks) on the diagonal of the spectrum as a result of depletion of the ground state of each of these modes by the pump radiation and stimulated emission from the \(v = 1\) state. Each diagonal bleach is accompanied by a red-shifted transient absorption arising from the population of the \(v = 0\) mode. The separation of these two lines gives an indication of the anharmonic frequency shift between the \(v = 0–1\) and \(v = 1–2\) transitions of the molecule; of the order of 15 cm\(^{-1}\) for each mode. In addition to the diagonal transitions, each of the three main diagonal peaks is linked to the other two by off-diagonal peaks indicating coupling or population transfer between the modes, these are shown \(\textit{via}\) dashed lines in Fig. 2. That the off-diagonal peaks are of appreciable amplitude close to zero pump–probe time delay and the amplitudes of the off-diagonal peaks are generally observed to become stronger relative to the diagonal peaks with increased pump–probe time delays suggests a contribution from each of these phenomena. This would also be consistent with previous observations on organometallic species.\(^7\)\(^{14}\)\(^{46}\) In heptane, off diagonal peaks linking the second weak mode near 1970 cm\(^{-1}\) to the three main diagonal peaks are also observed. This occurs even though the diagonal peak itself is weak and arises from the fact that the off-diagonal peak intensities result from a contribution from each of the two diagonal mode intensities; as such, the more intense diagonal mode intensities lead to the off-diagonal peaks becoming more clearly visible than the diagonal peak at 1970 cm\(^{-1}\). Once again, the off-diagonal peaks constitute negative–positive pairs of peaks attributable to radiation–matter interaction pathways involving the \(v = 0–1\) and \(v = 1–2\) vibrational transitions. Peaks arising from transitions involving the combination band modes would also be expected, though these are unresolvable from the \(v = 1–2\) peaks.

The patterns described above are repeated for both acetonitrile (Fig. 2(b)) and heptanediol (Fig. 2(c)), though the broader absorptions lead to less well-resolved spectra, particularly in the region of the 1970 cm\(^{-1}\) transition. In the case of heptanediol, only the more intense peaks near 2050 and 1990 cm\(^{-1}\) are shown, the lower solubility leading to poorer signal-to-noise ratios in the 2D-IR spectra near the 1940 cm\(^{-1}\) band, these peaks are however clearly observed in cross-section allowing extraction of vibrational relaxation dynamics (see below).

It is interesting to compare the lineshapes obtained from the 2D-IR spectra of 1 in each of the three solvents. In heptane, the lines are virtually circular, excepting...
a small amount of broadening along the pump frequency axis direction arising from
the finite bandwidth of the narrow-band pump pulse. This suggests that the line-
shapes are either largely homogeneously broadened or that any inhomogeneous
contributions arise from processes that are fast on the timescale of the experiment.
The same is true in acetonitrile, though the pump-axis distortion is exacerbated by
the somewhat broader transitions. The observation of such circular lineshapes, indi-
cating homogeneously broadened or motionally-narrowed lineshapes, is consistent
with previous studies of hydrogenase model compounds in heptane and acetonitrile.\textsuperscript{7}
The relative line broadening observed for acetonitrile solutions was attributed to
processes that are rapid on the experimental timescale.\textsuperscript{7}

In contrast, the diagonal peaks of the heptanediol solution at this short pump–
probe time delay are noticeably elongated along the diagonal of the spectrum.
This effect has been observed previously in solutions featuring hydrogen-bonding
solvents and is indicative of inhomogeneous broadening of the lineshape.\textsuperscript{11,27–29}
This effect has been discussed in detail elsewhere, but, briefly, if spectral diffusion,
the rate at which the solute samples the range of possible environments created by
ultrafast fluctuations of the solvent bath, is slow with respect to the pump–probe
delay time then the excitation and detection frequencies will be similar and hence
result in a diagonal elongation of the peak. As the pump–probe delay time increases,
spectral diffusion leads to an evolution of the lineshape towards a circular profile as
the detection frequency becomes less likely to equal the excitation frequency. The
timescale for this process provides a measurement of the FF CF.\textsuperscript{11,27–29}

In order to gain more insight into this process for \textit{I} in heptanediol, 2D-IR spectra
of the most intense band near 1990 cm\textsuperscript{-1} were recorded as a function of pump–probe
time delay from 2–70 ps. Example spectra are shown in Fig. 3. The evolution of the
lineshape can be clearly observed as a function of pump–probe delay time. In order
to quantify this and to extract information pertaining to the FF CF, we have
employed two previously established approaches, the centre line slope (CLS)\textsuperscript{27} and
nodal line slope (NLS) methods.\textsuperscript{11,29} The former has been shown to produce an
accurate measurement of the FF CF, including contributions from homogeneous

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Evolution of the 2D-IR lineshape of the 1990 cm\textsuperscript{-1} mode of \textit{I} in heptanediol as a func-
tion of pump–probe delay time. The solid lines indicate the results of applying the CLS method
for determining the FF CF (see text).\textsuperscript{27}}
\end{figure}
or motionally-narrowed contributions, while the latter has been applied to double resonance 2D-IR measurements and shown to reflect the degree of inhomogeneous broadening of the transition. A third method, using the ellipticity of the 2D-IR lineshape has also been applied to determine the FFCF, however this method was not applied here as the convolution of the 2D-IR lineshape with the pump-beam bandwidth profile in the double resonance methodology leads to a distortion of the lineshape that would diminish the accuracy of a measurement that relies upon the relationship between diagonal and antidiagonal linewidths of the transition. Very briefly, the CLS method determines the (pump, probe) coordinates of the maximum signal of the diagonal $v = 0\rightarrow 1$ peak for each pump frequency, leading to a line consisting of these points in frequency space. The slope of this line is then determined and the inverse taken such that a perfectly inhomogeneously broadened transition, with a CLS slope along the diagonal, will return a CLS parameter of 1, while a circular profile, with vertical CLS axis, will yield a value of 0. The temporal decay of the CLS parameter is a measure of the FFCF. The NLS method takes a similar approach but plots the position of the point where the two halves ($v = 0\rightarrow 1$ and $v = 1\rightarrow 2$) of the 2D-IR diagonal peakshape lead to a zero value. The angle of this slope is treated in a similar way so that CLS and NLS results should be comparable.

The results of the CLS and NLS analyses are shown in Fig. 4. It is clear that the results obtained are in very good agreement and can be well-represented by a biexponential decay function with timescales of 3.6 and 70 ps (CLS) and 3.2 and 76 ps (NLS). It is also notable from Fig. 4 that extrapolating the CLS and NLS data to zero pump–probe time delay will yield a value of less than one. It is noted that the double resonance approach to 2D-IR places certain limitations on the time resolution of the data obtained as a result of narrowing the bandwidth of the pump pulse. In addition, effects caused by overlap of the pump and probe pulses can lead to lineshape distortions at times shorter than 1.5 ps, the effective time resolution of the spectrometer used here, hence the need to extrapolate this data to zero rather than measure it directly.

The implications of this data are that the solvent–solute interactions of 1 in heptanediol feature motionally-narrowed or homogeneous components that are beyond the time resolution of the spectrometer, leading to the initial CLS/NLS parameter of less than unity, alongside a component with a temporal dependence of around 3–4 ps and a slower contribution of around 70 ps. Comparison of the CLS data with other hydrogen bonding solutions show a large variation in the trends observed. Studies of the FFCF of HOD in D$_2$O reveal a very

![Fig. 4](image_url) Results of CLS (black squares) and NLS (red triangles) analysis of the 2D-IR lineshape evolution with pump–probe delay time for 1 in heptanediol solution. Solid lines show results of fitting to a biexponential function.
fast component of around 50 fs followed by an oscillation with a period of around 200 fs and a longer decay of 1.4 ps,\textsuperscript{13,47,48} while studies of aqueous salt solutions show biexponential FFCF decay profiles obtained via the CLS approach with sub-picosecond and few-picosecond components, respectively.\textsuperscript{31} Furthermore, measurements of the 2D-IR lineshape evolution of the amide I band of N-methylacetamide in aqueous solution showed that the inhomogeneous broadening contribution to the lineshape persisted beyond 4 ps.\textsuperscript{28} Despite the variation, these are all somewhat different to the temporal dependence of the CLS parameter measured here. It should be noted that the heptanediol solution of I is unlikely to show close agreement with aqueous solutions because of the high viscosity of 1,7-heptanediol. The absolute viscosity of heptanediol is not currently available but it has been measured as being in excess of 20 mPa s at 298 K, the value being limited by the range of the viscometer. Furthermore, the viscosity of 1,2-pentanediol and 1,2-hexanediol are reported to be 39.16 and 42.25 mPa s respectively at 303 K.\textsuperscript{49} This indicates that the viscosity of heptanediol is significantly higher than the aqueous solutions discussed previously and is likely to have a bearing on the timescales mentioned. This solvent is perhaps more likely to resemble the dynamics of the protein pocket where hydrogen bonding interactions occur with the large, slowly moving protein scaffold rather than with the aqueous solvent.

The lower time resolution of the double resonance 2D-IR approach means that any dynamics occurring on timescales faster than around 1.5 ps will appear to be motionally narrowed and it is possible that the faster processes observed previously in aqueous solutions are still beyond the scope of this experiment. Possible origins of this effect include rapid hydrogen bond fluctuations, vibrations and librational motion of the heptanediol molecule. H-bond fluctuations have been attributed to the 50 fs decay in the FFCF of HOD, while H-bond vibrations might be expected on sub ps timescales, similar to those responsible for oscillatory features in the FFCF observed in HOD containing systems.\textsuperscript{13,47,48} The dynamics on the few-picosecond timescale in the FFCF of aqueous solutions have been previously assigned to spectral diffusion linked to hydrogen bond rearrangement.\textsuperscript{31} Despite the increased viscosity of the sample considered here, it would seem reasonable to conclude that this is likely to be responsible for the 3–4 ps dynamics observed; in a viscous solvent where rotational and diffusive motion is slow, hydrogen bond rearrangements within the first solvation shell would be expected to be somewhat decoupled from the macroscopic viscosity. The origin of the longer timescale processes would thus seem likely to arise from slower motions related to molecular rotation or diffusive rearrangements involving disruption of the first hydration shell of the solute. Considering that the molecular rotation time measured for a similar hydrogenase model compound in heptane was of the order of 17 ps and the viscosity of heptane is around 0.387 mPa s at 298 K,\textsuperscript{50} the value for I in heptanediol would be expected to be significantly in excess of 100 ps following the Debye–Stokes–Einstein relation, though for substances of such high viscosity such a simple approach is unlikely to be exact. This would however be consistent with the slower contribution to the FFCF observed in these experiments, though given the high viscosity it may be overly simplistic to attribute this timescale to straightforward molecular rotation and diffusion when reorientational processes involving the disruption of the first hydration shell may be more appropriate. Slow and static FFCF contributions have been previously observed in large protein samples and attributed to slow structural motions of the protein backbone,\textsuperscript{21} supporting possible comparisons between heptanediol solution and the role of the protein scaffold in FeFe[H].

It is interesting to note that the spectral diffusion effects observed in the diagonal lineshapes are not repeated for the off-diagonal peaks, which show a little elongation along the diagonal at very short pump–probe delay times but this is lost rapidly, within 10 ps. This suggests that the solvent processes giving rise to the two peaks are not strongly correlated for the different modes.
Vibrational dynamics

Vibrational relaxation dynamics for 1 in each of the three solvents were extracted by recording slices through the 2D-IR spectra at a range of pump–probe time delays with pump frequencies corresponding to the three most intense absorptions near 2050, 1990 and 1940 cm\(^{-1}\), respectively. The temporal decay of the amplitudes of the diagonal and cross peaks were then extracted and fitted to exponential functions. The function chosen was either a single exponential decay, bi-exponential decay or an exponential rise plus decay function depending on the nature of the data. Example data for pumping the 1990 cm\(^{-1}\) mode of 1 in heptane are shown in Fig. 5 alongside a representative slice through the 2D-IR spectrum to show the experimental S/N ratios obtained. A summary of the fitted data is given in Table 1 for data recovered when pumping the two most intense transitions at 2050 and 1990 cm\(^{-1}\). The data recovered for the weaker transition at 1940 cm\(^{-1}\) followed the same general patterns but is not included due to the variability of the data arising from poorer S/N levels. It is stressed that, for clarity of discussion, the three main modes of 1 in each solvent are consistently referred to as 2050, 1990 and 1940 cm\(^{-1}\).

Fig. 5  (a) Example slice through the 2D-IR spectrum of 1 in heptane solution, recorded with a pump frequency of 1991 cm\(^{-1}\) and a 2 ps pump–probe time delay. Also shown (b–f) are temporal dependences of the diagonal and off-diagonal peak amplitudes. Red lines indicate fits to the data using exponential functions (see text). The legends indicate the transition to which the dynamics belong, through reference to the labels in (a).
1940 cm\(^{-1}\) rather than the actual frequencies, which are given in the footnotes to Table 1. Similarly, the transient absorption corresponding to the \(v = 1-2\) transition of each of these modes is labeled using the \(v = 0-1\) frequency.

Taking the dynamics associated with pumping the 2050 cm\(^{-1}\) mode (Table 1), the temporal dynamics of the diagonal peak amplitudes in each solvent for both for \(v = 0–1\) and 1–2 transitions can be well-represented by biexponential decay functions with a short (few-picosecond) decay time and a longer (\(\approx 50–100\) ps) decay timescale. The off-diagonal modes are well represented by a function featuring a few-picosecond rise time followed by a tens of picoseconds decay. The one exception to this is the off-diagonal \(v = 0–1\) transition of the 1990 cm\(^{-1}\) mode in heptane, which displays single-exponential dynamics. In general, the rise times observed in heptane solution are of the order of 10 ps, those in acetonitrile are between 1 and 4 ps, though it is noted that the lower end of this range is approaching the limits of the temporal resolution achievable here, while those in heptanediol are intermediate at around 5 ps. The decay times also show a solvent dependence, averaging 85 ps in heptane, 78 ps in acetonitrile and 59 ps in heptanediol. There is some scatter evident in the data though this is generally attributable to poorer signal-to-noise levels at longer decay times. It is also noteworthy that a slight increase in the observed rise times occurs on moving from the 1990 cm\(^{-1}\) off-diagonal peak pair to the 1940 cm\(^{-1}\) off-diagonal peaks.

Similar patterns are observed for pumping the 1990 cm\(^{-1}\) mode (Fig. 5(b)–(f)). In this case the biexponential decays of the diagonal modes are observed to show a slightly faster initial decay parameter and, in the case of the 0–1 transition in heptane and acetonitrile, the data is better represented by a single exponential function with a decay time of the order of 80 ps; evidence for a fast component, close to the experimental time resolution, was observed (Fig. 5(c)) but was not reliably recovered using the fitting procedure. It is noted that a non-resonant contribution to the data from the sample cell windows is expected near time zero though

<table>
<thead>
<tr>
<th>2D-IR peak freq label/cm(^{-1})</th>
<th>Heptane (\tau_1/ps)</th>
<th>AcCN (\tau_2/ps)</th>
<th>Heptanediol (\tau_2/ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[2050]</strong></td>
<td>(v = i-j)</td>
<td>(\tau_1/ps)</td>
<td>(\tau_2/ps)</td>
</tr>
<tr>
<td>Diagonal [2050] (0–1)</td>
<td>5</td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td>Diagonal [2050] (1–2)</td>
<td>7</td>
<td>116</td>
<td>3</td>
</tr>
<tr>
<td>Off diag [1990] (0–1)</td>
<td>90</td>
<td>1</td>
<td>79</td>
</tr>
<tr>
<td>Off diag [1990] (1–2)</td>
<td>10</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>Off diag [1940] (0–1)</td>
<td>10</td>
<td>100(^a)</td>
<td>1(^a)</td>
</tr>
<tr>
<td>Off diag [1940] (1–2)</td>
<td>19(^a)</td>
<td>54(^a)</td>
<td>4(^a)</td>
</tr>
</tbody>
</table>

| **[1990]**                     | \(v = i-j\) | \(\tau_1/ps\) | \(\tau_2/ps\) | \(\tau_1/ps\) | \(\tau_2/ps\) |
| Diagonal [1990] (0–1)          | 10            | 77             | 1              | 82             | 1              | 57             |
| Diagonal [1990] (1–2)          | 13            | 97             | 1              | 85             | 1              | 56             |
| Off diag [1990] (0–1)          | 92            | 76             | 4              | 53             |
| Off diag [1990] (1–2)          | 1             | 91             | 2              | 81             | 5              | 54             |
| Off diag [1940] (0–1)          | 10            | 99             | 2              | 82             | 52             |
| Off diag [1940] (1–2)          | 14            | 61             | 1              | 76             | 3\(^a\)       | 54\(^a\)       |

\(?a\) Inferior S/N ratio. \(b\) Exact line positions [2050]: 2054 cm\(^{-1}\) (heptane); 2049 cm\(^{-1}\) (acetonitrile); 2046 cm\(^{-1}\) (heptanediol). [1990]: 1991 cm\(^{-1}\) (heptane); 1984 cm\(^{-1}\) (acetonitrile); 1983 cm\(^{-1}\) (heptanediol). [1940]: 1945 cm\(^{-1}\) (heptane); 1928 cm\(^{-1}\) (acetonitrile); 1933 cm\(^{-1}\) (heptanediol).
comparisons to the negative time rising edge of the data clearly showed the difference between signal decays associated with relaxation dynamics and instrument cross-correlation effects. The off-diagonal peaks again showed rise-time dynamics when pumping the 1990 cm\(^{-1}\) transition. Once again in heptane these were of the order of 10 ps, in acetonitrile this value reduced to 1–2 ps, consistent with the values obtained in heptanediol. The overall long-term decay timescales of the peaks showed excellent agreement for both pump frequencies, the averages for the 1990 cm\(^{-1}\) band being 86 ps in heptane, 80 ps in acetonitrile and 54 ps in heptanediol.

It is interesting to note that the rise times observed for the off-diagonal peaks when pumping the 1990 cm\(^{-1}\) mode are generally somewhat shorter than those observed when pumping the 2050 cm\(^{-1}\) mode. This effect is particularly apparent for acetonitrile and heptanediol solutions.

The data obtained when pumping the 1940 cm\(^{-1}\) mode follows the same general trends in that biexponential decays are observed for the diagonal peaks and rise times are observed for the off-diagonal modes. Once again, it was observed that the rise times for the off-diagonal modes showed a slight general increase with the separation frequency of the diagonal and off-diagonal peaks and the slow decay time was observed to be faster in heptanediol than in heptane and acetonitrile.

The data described is consistent with a model in which the pumped transition undergoes vibrational relaxation through a combination of rapid intramolecular vibrational redistribution (IVR) to close-lying carbonyl stretching modes on sub-10 ps timescales followed by vibrational relaxation on 50–80 ps timescales depending upon the solvent. This is not dissimilar to the results obtained for another FeFe[H] model compound (µ-S(CH\(_2\)_3)S)Fe\(_2\)(CO)\(_6\) (2), which showed 5 and 125 ps relaxation timescales in heptane solution.\(^7\) The heptane data for 1 is comparable, though it is noteworthy that, while the IVR processes occur at comparable or slightly slower rates, the overall relaxation is faster. One possible explanation for the latter effect may arise from the pendant phenyl group on 1, which will increase the number of low frequency vibrations of 1 in comparison to 2 and also may be expected to lead to more favourable interactions with the alkane solvent both of which will facilitate vibrational relaxation. Comparisons of relative IVR rates are considered below.

It is interesting to note that the relaxation for species 2 in acetonitrile showed only single exponential dynamics, which were assigned as arising from IVR processes beyond the time resolution of the spectrometer used. In this case, fast IVR processes are observed, indicating either slightly slower dynamics or superior time resolution. In the case of a polar solvent, the pendant phenyl group may be expected to reduce solvent interaction somewhat, though the overall slow relaxation time is little changed in 1 compared to 2.

The patterns in the data upon changing the solvent are also of interest: moving from non-polar to polar solvents increases the rate of IVR markedly as may be expected if IVR is solvent mediated due to stronger interactions between solute and solvent,\(^7,44\) though this has little effect on the slow vibrational relaxation rate. Moving to a protic solvent however leads to a substantial increase in the overall vibrational relaxation rate at the expense of a slower IVR rate. This implies that the excess energy is dissipated via intramolecular pathways into low frequency vibrational modes and intermolecularly into solvent modes much more efficiently than in the polar or non-polar case. This situation which may be beneficial for enzyme reaction dynamics as these are expected to be modulated by hydrogen-bonding interactions. It is noted that the presence of a solvent overtone band in heptanediol near the carbonyl stretching modes may facilitate vibrational relaxation into solvent vibrational modes. A similar, though weaker, band is present in heptane solutions but stronger interactions with this mode in heptanediol are a possibility. Acetonitrile also possesses a close-lying vibrational transition at ~2250 cm\(^{-1}\), though this is perhaps too distant to play a key role in vibrational relaxation. As such, no simple relationship between solvent vibrational modes and vibrational relaxation times would seem to exist. Another possibility, given the relative insensitivity of the
vibrational relaxation time to the identity of the solvent, is that the intramolecular vibrational modes of the solute play a key role in this process.

It is instructive to consider the overall patterns in IVR rate in relation to the low frequency density of states (DoS) of the solvent systems, which are expected to act as a bath for the IVR energy transfer process. Overall the IVR rate was observed to be faster in acetonitrile and heptanediol than heptane and displayed a slight correlation with the frequency separation of the modes of 1 in each of the three solvents studied here. It is also apparent that the IVR rate is slower for 1 versus 2 in heptane and acetonitrile.

Considering that the average carbonyl mode separation is around 30 cm\(^{-1}\) in 2 and around 50 cm\(^{-1}\) in 1, these are largely as may be expected if the DoS displays a peak at very low frequencies (below 30 cm\(^{-1}\)), with fewer modes at higher frequencies restricting IVR-facilitating interactions with the solute. However, Optical Kerr Effect (OKE) studies suggest that acetonitrile and heptanediol will display a broader distribution in the DoS (FWHM \(\approx 150\) cm\(^{-1}\)) with a higher peak frequency (\(\sim 50\) cm\(^{-1}\)) than heptane (\(\sim 100\) and 30 cm\(^{-1}\), respectively) due to stronger intermolecular interactions. Qualitatively, this would tend to suggest that IVR between modes in 2 would be enhanced in heptane relative to acetonitrile and heptanediol, while IVR between modes with separations of 50 cm\(^{-1}\) would tend to be preferred over those with 30 cm\(^{-1}\) separations in polar and protic solvents, which is not observed. It is noted however, that the spectra obtained from OKE studies lacks the factor of \(\omega\) (vibration frequency) required to transfer from the Raman spectrum to the genuine DoS, which will shift the peak in the DoS to a still higher wave-number. OKE data is also skewed by the relative changes in polarisability attributable to the near continuum of individual modes that constitute the DoS and as such it is not trivial to obtain information on the absolute peak in the DoS. Similar complications arise from applying neutron scattering measurements to gain information on the DoS. The implications from the data recovered here are that the peak may be expected to lie below 30 cm\(^{-1}\) while polar and protic solvents facilitate IVR as a result of stronger interactions between solvent and solute modes.

These results are of particular interest for comparisons with the active site of the enzyme itself in which the presence of a mixture of carbonyl and cyano ligands leads to even greater mode separation than observed in 1. The indications arising from the data presented herein imply that the IVR rate between ligand stretching modes of the active site may be expected to be slower than observed for 1, with overall vibrational relaxation into modes of the protein scaffold mediated by hydrogen bonding interactions.

**Conclusions**

In conclusion, the vibrational relaxation and solvent–solute interaction dynamics of a model compound of the active site of the FeFe[H] enzyme have been studied in a range of solvents. Extraction of data relating to the FFCF from the temporal evolution of the 2D-IR lineshape has revealed dynamics on three timescales; a sub-picosecond motionally-narrowed contribution, a 3–4 ps contribution that has been attributed to hydrogen bond reorganisation and a slow, 70 ps, component relating to solvent rotational and diffusive motions. Vibrational relaxation has been shown to occur through rapid IVR between carbonyl stretching vibrational modes followed by slow relaxation to the ground state. The solvent dependence of these processes is attributed to the different low frequency density of states and interaction mechanisms of these solvents.

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References