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C–D Modes as structural reporters via dual-frequency 2DIR spectroscopy

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Abstract

Implementation of weak C–D stretching modes as structural reporters via dual-frequency 2DIR spectroscopy is demonstrated. It is experimentally shown that interactions among CD and C≡N modes can provide useful structural constraints. The dephasing times, diagonal and off-diagonal anharmonicities for the CD and CN modes in acetonitrile-d_3 are measured. These experiments open an avenue of using deuteration of carbon atoms as a universal labeling strategy for structural measurements via 2DIR spectroscopy.

1. Introduction

An open challenge in chemistry and biology is to develop methods of measuring dynamics of structural changes of molecules in solution on a wide range of time scales. Two-dimensional infrared spectroscopy (2DIR) has recently emerged as a powerful tool for determining structures of molecules in solution at ambient temperatures [1–3] able to acquire snapshots of structural distributions in a picosecond time window and having a potential of measuring structural dynamics [4–6]. Being analogous to 2D correlation methods of NMR, 2DIR provides convenient ways of measuring structural constraints, such as distances between the modes (coupling strengths), angles between the transition moments and energy transfer rates. Most 2DIR experiments reported were analogous to homonuclear 2D NMR [1,2,7]. To measure atom-sensitive structural constraints in such experiments using CO modes in proteins, one or several CO modes have to be decoupled from the rest of the CO modes. Incomplete, but shown to be sufficient, decoupling can be obtained by $^{13}C{=}^{18}O$ isotope substitution [8]. The presence of unlabeled CO modes with, similar frequencies as that of the label, reduces the dynamic range for the cross-peak measurements. A novel, dual-frequency 2DIR method analogous to heteronuclear 2D NMR, has recently been implemented [9,10]. The method allows measuring interactions of vibrational modes with frequencies in different spectral regions without contributions from diagonal peaks, that leads to an accurate determination of structural constraints [9,10]. To obtain atom-sensitive structural constraints it is important to use localized vibrational modes that are not abundant in the studied molecule and/or the solvent and can therefore serve as vibrational labels. A C–D stretching mode is an attractive candidate in this respective. It is often localized and has a frequency in a water transparency region. On the other hand, deuteration is non-invasive and simple type of labeling that offers ultimate labeling flexibility. While the C–D label is very attractive, it has a very weak transition dipole that amounts only at ca. 2–3% from that of the C=O mode. C–D modes were used in linear IR spectroscopy as structural probes [11,12], but their usage was limited because of the small transition dipole.

In this Letter, we demonstrate implementation of C–D stretching modes as structural probes via dual-frequency 2DIR. Interactions between C–D modes and another mode having frequency in the water transparency region, the C≡N mode, were studied. Because the interaction between

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two weak modes, such as CN and CD, is expected to be weak, the dual-frequency 2DIR spectroscopy is the method of choice, as it allows elimination, partially or completely, of the diagonal peaks and delivers cross peaks in a true background-free manner.

2. Experimental details

2.1. Dual-frequency 2D IR

Details of the dual-frequency 2DIR setup (Fig. 1c) were recently reported [10]. Briefly, the Ti:Sapphire oscillator/regenerative amplifier laser system (Coherent Inc.) producing pulses at 804 nm, 44 fs duration and 0.9 mJ energy were used to pump two in-house built optical parametric amplifiers (OPA) which generated signal-idler pulse pairs. Frequency-difference pulses were generated from each signal-idler pair in 2 mm thick $AgGaS_2$ crystals providing independently tunable mid-IR pulses with ca. 90 fs duration and 2.5 μJ energy at ca. 5 μm wavelength. Each mid-IR beam was split into two equal parts: three pulses were focused onto the sample and the fourth was used as a local oscillator (LO), Fig. 2. A third-order signal generated in the sample was picked at the phase matching direction $(-k_1 + k_2 + k_3)$, mixed with the LO, delayed by time delay t, and detected by an MCT detector (Fig. 2). The time delays between the pulses (Fig. 2b-I), τ, T, and t, were controlled by linear-motor-driven translation stages. The two-dimensional (τ, t) spectra were recorded using the step of 6.8 fs with ca. 270 and 350 points in τ and t directions, respectively. Two-dimensional (ω_τ, ω_t) spectra were generated via double Fourier transformation. Because the absolute phases of the IR pulses were not known, the phases in both the rephasing and non-rephasing spectra had to be corrected. An accurate way to tune the phase is to match the pump-probe spectra with an ω_t-projection of the real part of the 2D spectrum [13]. However, because the cross-peak signals in the dual-frequency pump/probe measurements were too small to measure, the phases of the spectra were adjusted aiming for the ratio of the negative

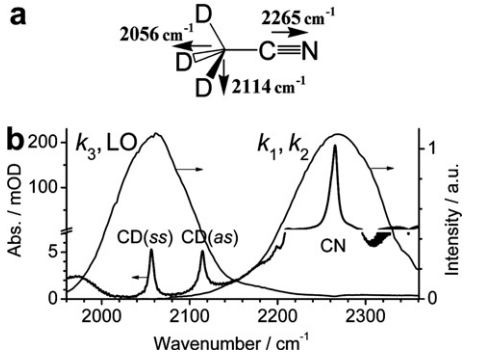

Fig. 1. (a) Structure of acetonitrile-d_3 with transition dipoles of the CD and CN modes shown with arrows. (b) Linear spectrum of acetonitrile-d_3 and the spectra of mid-IR pulses k_1, k_2, k_3, and LO used for the experiment shown in Fig. 3a.

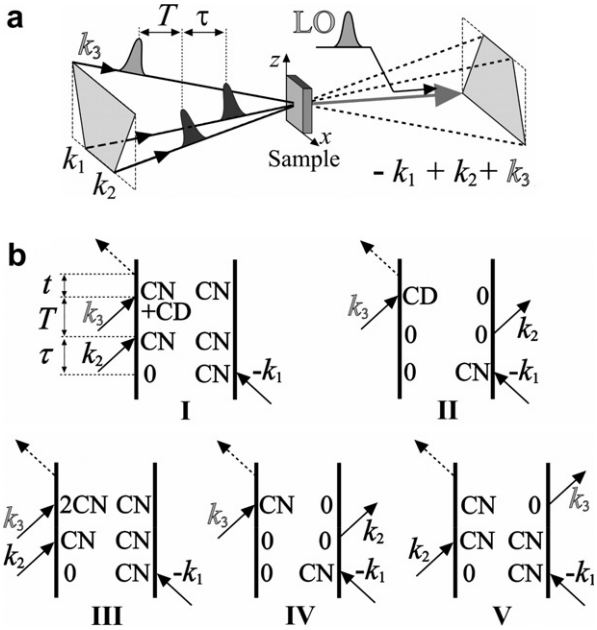

Fig. 2. (a) Geometry of the mid-IR pulses interacting with the sample in the dual-frequency 2DIR measurements. (b) Feynman diagrams describing the cross peaks (I and II) and diagonal peaks (III–V) in rephasing experiments. The notation CD stands for both CD_{ss} and CD_{as} modes.

to positive peak amplitudes in a peak pair at 1.05. If the dynamics in the combination band level is the same as that in the singly excited state, the negative and positive features in a peak pair would have the same absolute amplitudes. The 1.05 value, although arbitrary, reflects the lifetime shortening in the combination state with respect to the singly excited-state. In addition, a phase correction, linearly varying with frequency, was applied for simultaneous phase restoration for the cross and diagonal peaks. The necessity of such phasing is dictated by the choice of τ and t scanning ranges, which excluded the regions with of $\tau = 0$ and $t = 0$. Note that modeling of all 2DIR spectra was performed using the same scanning ranges to those used in the respective experiment.

To avoid an overwhelming contribution from the CN diagonal peaks, the spectra of the pulses were tuned so that the k_1 and k_2 pulses matched the absorption spectrum of the CN mode at 2265 cm^{-1} while the k_3 and LO pulses matched the CD modes at 2056 and 2114 cm^{-1}, Fig. 1b. The phase-match geometry of the beams interacting with the sample is shown in Fig. 2a. Rephasing and non-rephasing experiments were performed by interchanging the order of the first and second pulses (Fig. 2b). Polarization of the beams was controlled by half-wave plate / wire-grid polarizer pairs.

2.2. Sample preparation and linear spectrum

For spectral measurements the samples were held in a 200 μm path length cell with CaF_2 windows. The concentration of the sample, acetonitrile-d_3 in chloroform

(Fig. 1a), was ca. 0.45 M. All experiments were performed at room temperature.

2.3. Spectral modeling

The lineshapes of both CD and CN transitions in acetonitrile-d_3,vide infra are dominated by homogeneous broadening. Spectral diffusion therefore does not play significant role and can be neglected. The Bloch approximation, which has been successfully applied to describe dephasing of vibrational modes, is used for spectral modeling. Using the Bloch approximation, a time-domain third-order signal, that describes the cross peaks at (ω_2, ω_1) and ($\omega_2 - \Delta_{12}$, ω_1) in the 2D IR spectra, is given by

$$S_{\mathrm{R/NR}}(\tau, T, t) = B\mu_1^2\mu_2^2 e^{\pm i\omega_1\tau} e^{-i\omega_2 t}[e^{i\Delta_{12}t - t/T_1^{(1)}} - 1] \times e^{-\gamma_1\tau - \gamma_2 t - \sigma_1^2\tau^2/2 - \sigma_2^2 t^2/2} e^{\pm f\sigma_1\sigma_2 t\tau}, \quad (1)$$

where the upper and lower signs correspond to the rephasing and non-rephasing experiments, respectively, Δ_{12} is the off-diagonal anharmonicity, $T_1^{(1)}$ is the population relaxation time of the ω_1 mode, and $\gamma_{1,2}$, $\sigma_{1,2}$, and $\mu_{1,2}$ are the total dephasing time, inhomogeneous width, and transition dipole of the fundamental transitions with ω_1 and ω_2 frequencies, respectively. The distributions of ω_1 and ω_2 frequencies was assumed to be static with the correlation coefficient f given by $\langle\delta\omega_1\delta\omega_2\rangle/\sigma_1\sigma_2$, where $\delta\omega_{1,2}$ are the frequency deviations from the respective mean frequencies. The diagonal signals where also modeled with Eq. 1, taking $\omega_2 = \omega_1$, $\mu_2 = \sqrt{2}\mu_1$, $f = 1$ [1,9], and $\Delta_{12} = \Delta_{11}$, where Δ_{11} is the value of the diagonal anharmonicity. The factor B stands for ensemble averaged tensor element dependent of polarizations of IR pulses and angles between the vibrational modes. For (*zzzz*) and (*zzxx*) polarization conditions, isotropic stationary distribution of molecules these factors are given by $1 + \frac{4}{5}P_2(\cos\theta)$ and $1 - \frac{2}{5}P_2(\cos\theta)$, respectively, where P_2 in the second Legendre polynomials and θ is the angle between transition dipoles of ω_1 and ω_2 modes [14,15]. Because the laser pulses used are much shorter than the fastest characteristic time in the system, no convolution with the pulse profiles was performed.

3. Results and discussion

As a first step in using CD modes as reporters, interactions among CD and C≡N modes were measured for acetonitrile-d_3 in chloroform. There are three CD stretching modes in the CD_3 group, one symmetric stretch (*ss*) at 2056.3 cm^{-1} and two degenerate antisymmetric stretching modes (as) at 2114.4 cm^{-1} (Fig. 1b), all localized on the four atoms. The transition moment direction of the symmetric stretch is parallel to the CC bond while the transition dipoles for the two asymmetric stretch modes are at 90° to the CC bond. As a result, CD_3 modes constitute naturally a perfect angular probe as several planar angles can be measured from interactions of the CD modes with another mode, characterizing the mutual group orientation in the three-dimensional space. The absorption bands of CD_{ss} and *as* modes in acetonitrile-d_3 have extinction coefficients of ca. 1.3 and 0.6 $M^{-1}\,cm^{-1}$, respectively, while the CN transition at 2265 cm^{-1} is much stronger with the extinction coefficient of ca. 46 $M^{-1}\,cm^{-1}$ (Fig. 1b).

Fig. 3a shows the non-rephasing spectrum measured with the polarizations of all four pulses along the z direction, (*zzzz*) polarization conditions. Three pairs of peaks can be seen: two CD/CN cross-peak pairs at ca. (2056, 2265 cm^{-1}) and (2114, 2265 cm^{-1}) and the CN diagonal peak pair at ca. (2265, 2265 cm^{-1}), where the (CD_{ss}, CN) cross peaks are dominant. The presence of the (CD, CN) cross peaks indicates interactions among respective modes, while the diagonal peaks reflect properties of the CN mode potential and the CN mode dynamics [16]. Interestingly, the (CD_{as}, CN) cross peaks are ca. 3 times weaker than the (CD_{ss}, CN) cross peaks. Several factors determine the relative amplitudes of the cross peaks: the values of respective off-diagonal anharmonicities, $\Delta_{\mathrm{CN/CDss}}$ and $\Delta_{\mathrm{CN/CDas}}$, electric field amplitude of the k_3 and LO beams at 2056 and 2114 cm^{-1} frequencies, the linewidths of the transitions, the values of the transition dipoles of the CD modes, and the mutual orientation of the CN and CD transition dipoles. The ratio of the cross-peak amplitudes provides a good estimate of the ratio of the off-diagonal anharmonicities, $\Delta_{\mathrm{CN/CD}_{ss}}/\Delta_{\mathrm{CN/CD}_{as}}$.

The (CD_{as}, CN) cross peaks measured in (*zzzz*) polarizations are much weaker than the (CD_{ss}, CN) peaks and their shapes are substantially perturbed by the presence of the strong peaks nearby. Performing 2DIR measurements with (*zzxx*) polarizations allowed obtaining the spectrum where the (CD_{as}, CN) cross-peak pair is dominant (Fig. 3c). While the light intensity of the k_3 and LO pulses at the CD_{as} mode frequency in these experiments was ca. 1.7 times larger than that at the CD_{ss} mode, effecting the cross-peak amplitudes, the angles between the transition moments, $\theta_{\mathrm{CN/CD}_{ss}} = 0°$ and $\theta_{\mathrm{CN/CD}_{as}} = 90°$, played the main role in determining these amplitudes (via B factor). Because the amplitude of the (CD_{as}, CN) cross peaks in the (*zzxx*) spectrum is 7.8-fold larger than that of the (CD_{ss}, CN) cross peaks (Fig. 3c), it is clearly possible to measure selectively the two cross-peak pairs and avoid their interference. The negative cross peak at (2165, 2265 cm^{-1}) is persistent through a series of experiments with (*zzxx*) pulse polarizations and has unclear origin.

The off-diagonal anharmonicities, $\Delta_{\mathrm{CN/CD}_{ss}}$ and $\Delta_{\mathrm{CN/CD}_{as}}$, and the peak broadening parameters were obtained via the global modeling of the 2D spectra and the linear absorption spectrum. Two features of the 2DIR spectra were the most crucial in the fitting: the ratio of the amplitudes of the cross peaks and the exact ω_t peak frequencies of the cross and diagonal peaks. Based on molecular geometry and our own anisotropy measurements, the angles, θ, between the CN/CDss and CN/CD as transition moments were taken to be 0 and 90°. The ratio of the two cross-peak amplitudes measured with (*zzzz*) polarizations, $A_{(\mathrm{CD}_{ss},\mathrm{CN})}/A_{(\mathrm{CD}_{as},\mathrm{CN})} = 3.0$, was

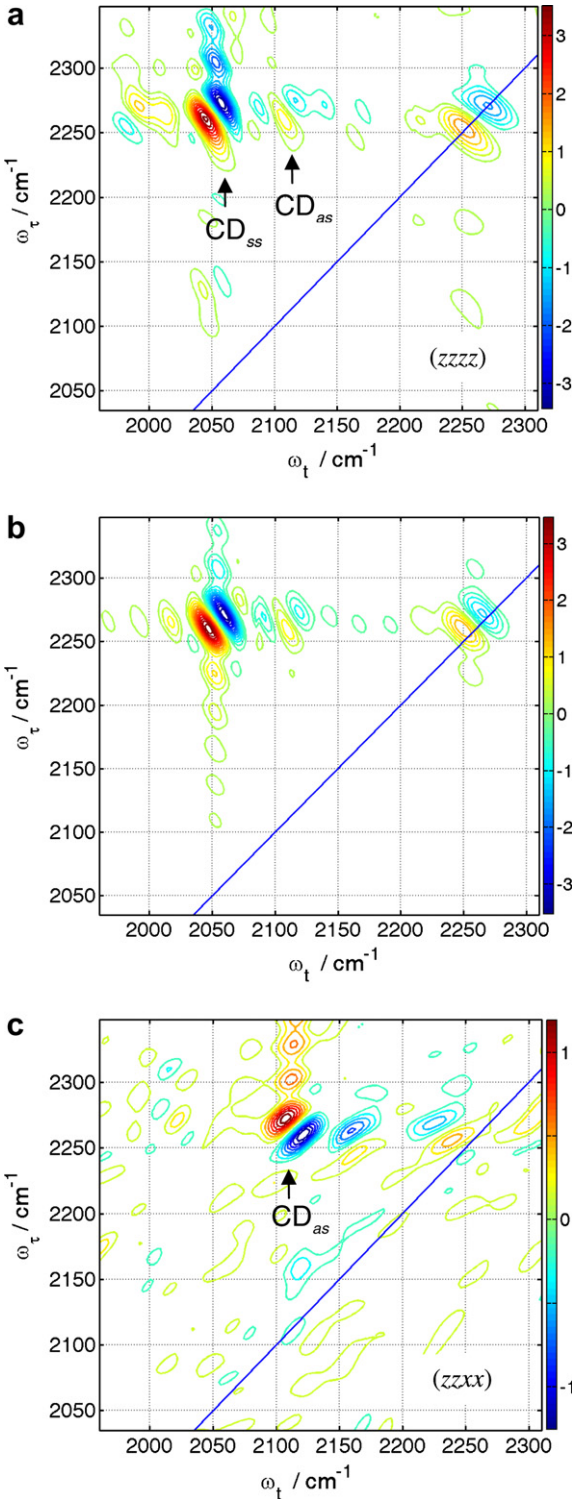

Fig. 3. (a) Non-rephasing 2DIR spectrum measured at $T = 600$ fs with (*zzzz*) polarizations of the pulses; (b) The modeling of the experiment shown in graph (a) with the parameters given in the text; (c) Rephasing 2DIR spectrum measured at $T = 300$ fs with (*zzxx*) polarizations of the pulses.

targeted in the fit, (Fig. 3a) giving the ratio of the off-diagonal anharmonicities at $\Delta_{CN/CD_{ss}}/\Delta_{CN/CD_{as}} = 0.45 \pm 0.05$. The exact ω_t frequencies of the cross and diagonal peaks allowed determination of the absolute values of the anharmonicities, which were determined to be $\Delta_{CN/CD_{ss}} = 2.5 \pm 1.5\ cm^{-1}$ and $\Delta_{CN/CD_{as}} = 5.5 \pm 3\ cm^{-1}$. Interestingly the CN mode interacts with the CD_{as} modes stronger than it does with the CD_{ss} mode, despite the orthogonal orientation of their transition moments. This indicates that mechanical coupling is dominant in interactions of the CN and CD modes [17]. Contribution of the electric coupling can be estimated assuming a point dipole–dipole interaction. The dipole interaction energy was calculated at ca. 1.0 cm^{-1} [16]. The value of the off-diagonal anharmonicity calculated from this interaction energy is only ca. $2 \times 10^{-3}\ cm^{-1}$, which is much smaller than the experimentally observed anharmonicity and supports strongly the conclusion that mechanical coupling is the dominant coupling mechanism.

The properties of the CD_{ss} and CD_{as} modes, such as diagonal anharmonicities, dephasing times and lifetimes, were determined from simultaneous modeling of the diagonal CD peaks in rephasing (Fig. 4) and non-rephasing 2DIR spectra with the linear spectrum. Based on the diagonal peak lineshape and amplitudes of the diagonal peaks in rephasing and non-rephasing spectra, that differ by less than 5%, the CD modes appear to be almost entirely homogeneously broadened in moderately polar solvent such as chloroform with the total dephasing times of ca. 1.6 and 1.4 ps for the *ss* and *as* modes, respectively. Small inhomogeneity of the CD modes, $\sigma \leqslant 1\ cm^{-1}$, did not allow determination of the correlation coefficient $f_{CD/CN}$ with confidence. The diagonal anharmonicities for CD_{ss} and CD_{as} were measured at 29 ± 2 and $24 \pm 2\ cm^{-1}$, respectively. Note that these values are smaller than the value of 57 cm^{-1} obtained for the CD mode in formamide-D, presumably due to a delocalization effect in CD_3 and smaller bond energy in formamide [18]. The diagonal anharmonicity of the CN mode was measured at $16.5 \pm 1.5\ cm^{-1}$.

The CD modes, while very weak, have been previously used as structural reporters in linear IR spectroscopy

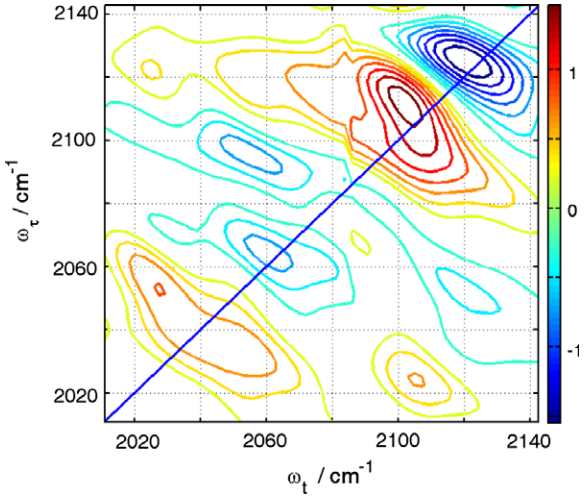

Fig. 4. CD-diagonal non-rephasing 2D IR spectrum of acetonitrile-d_3.

[11,12]. In this work we have demonstrated that pairwise interaction among CD and CN modes can readily be measured via dual-frequency 2DIR. While the acetonitrile-d_3 molecule used here is very simple and rigid, these proof-of-principle experiments demonstrate usefulness of the pair of modes, CD and CN, as structural labels. Because the CD and CN modes are localized on the respective CD_3 and CN groups, the cross peaks caused by their pairwise interactions in molecules can be used to obtain structural constraints, such as distances and angles between transition dipoles. The recovery of the intermode distance is straightforward if electric coupling is dominant [16]. In case the mechanical mode coupling is significant, as in the case of CD/CN interaction in acetonitrile-d_3, quantum mechanical calculations are needed to link the off-diagonal anharmonicity value to the distance between the groups. The linewidths of the CD and CN modes in non-polar solvents are very narrow, ca. 6–6.5 cm^{-1}, and the frequencies fall into the water transparency region, which makes these mode pairs potentially powerful structural reporters.

4. Summary

In summary, we have demonstrated that interactions among the CD and CN modes can be measured via dual-frequency 2DIR spectroscopy. Despite the small transition dipole of the CD modes nonlinear 2DIR methods allowed discrimination of narrow CD bands from background absorption as the 2DIR spectra are only sensitive to pairwise interactions. The measured dephasing times and anharmonicities of the CD and CN modes could be useful for further experiments. While the current sensitivity of the setup built in our laboratory is not sufficient to measure interactions of CD modes in proteins, a 50-fold sensitivity increase will allow working with proteins at millimolar concentrations. Such advances would require more stable laser sources; current RMS fluctuations of mid-IR-pulse intensity are ca. 0.2%. Because the CN and CD groups in acetonitrile are only ca. 2 Å apart a steep decrease of the off-diagonal anharmonicity is expected at larger distances. However, the relaxation-assisted 2DIR method reported recently, can be implemented to help observing interactions of modes separated by larger distances [19]. We believe that these experiments open an avenue of using deuteration of carbon atoms as a universal labeling strategy for structure measurements via 2DIR.

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Appendix A. Supplementary data

Supplementary data associated with this Letter can be found, in the online version, at doi:10.1016/j.cplett.2007.02.020.

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