Two-dimensional electronic spectroscopy of bacteriochlorophyll a with synchronized dual mode-locked lasers

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How atoms and electrons in a molecule move during a chemical reaction and how rapidly energy is transferred to or from the surroundings can be studied with flashes of laser light. However, despite prolonged efforts to develop various coherent spectroscopic techniques, the lack of an all-encompassing method capable of both femtosecond time resolution and nanosecond relaxation measurement has hampered various applications of studying correlated electron dynamics and vibrational coherences in functional materials and biological systems. Here, we demonstrate that two broadband (>300 nm) synchronized mode-locked lasers enable two-dimensional electronic spectroscopy (2DES) study of chromophores such as bacteriochlorophyll a in condensed phases to measure both high-resolution coherent vibrational spectrum and nanosecond electronic relaxation. We thus anticipate that the dual mode-locked laser-based 2DES developed and demonstrated here would be of use for unveiling the correlation between the quantum coherence and exciton dynamics in light-harvesting protein complexes and semiconducting materials.
Our understanding of natural light-harvesting systems involving excitonic energy transfers has been revolutionized by the application of femtosecond nonlinear optical spectroscopy. Delocalized quantum excited states created by photoexcitation of and electronic couplings between light-absorbing chromophores relax through a myriad of pathways, such as exciton migration, excitation localization induced by the thermal fluctuation of coupled bath degrees of freedom, and long-distance Förster energy transfer. A series of events involving electron and proton transfer reactions subsequently occur in photosynthetic systems. One of the most crucial techniques for studying such complex photoinduced reaction dynamics of generic self-assembled multichromophoric systems is two-dimensional electronic spectroscopy (2DES), which is capable of providing the time correlation between the initial and final states by mapping their nonlinear optical response onto 2D excitation- and detection-frequency space.

The 2DES requires multiple femtosecond optical pulses to interrogate molecular systems, such as light-harvesting protein complexes, optical chromophores in solutions, semiconducting materials, metallic nanoparticles, and chiral aggregates. It provides information on not only ultrafast energy transfer kinetics but also dephasing time scales of electronic and/or vibrational coherences of coupled chromophore systems like photosynthetic proteins. Recently, the 2DES of the Fenna–Matthews–Olson complex revealed an important role of vibronic couplings in the ultrafast energy transfer process on the one-exciton state manifold. It turns out that the vibronically excited vibrational modes play a crucial role in enhancing the pigment-to-pigment energy transfer rates. In addition, the excited-state dynamics and relaxations of chlorophyll molecules that are critical pigments in photosynthetic complexes are of fundamental importance.

In 2DES experiments, the time evolution of excited states initiated by a pair of pump pulses generating temporally interfered electric fields is monitored by using another time-delayed probe and local oscillator pulses. The waiting time that is the time delay between the pump and probe pulses is usually scanned by mechanically changing the optical path lengths of the excitation and detection pulses. It was shown that the Tω-scanning speed can be dramatically enhanced by utilizing two repetition rate-stabilized mode-locked lasers (MLs), where the technique is called asynchronous optical sampling (ASOPS). In ASOPS, each ML generates a train of optical pulses separated by an equal time interval. When the repetition rates of the two MLs are slightly detuned from each other by ∆f, the optical time delay (Tω) between a pair of pulses from the two MLs increases linearly in laboratory time (t). The corresponding time delay increment (∆Tω) is determined by the repetition rate and the detuning factor ∆f as

\[
\Delta T_\omega = \frac{1}{f_i} - \frac{1}{f_i + \Delta f} \approx \frac{\Delta f}{f_i} = f_o \frac{1}{f_i},
\]

Here, f_o is the down-conversion factor defined by ∆f/f_i. Although the automatic time-delay scanning scheme called ASOPS was proposed and demonstrated in 1987 (ref. 26), the application of ASOPS to coherent multidimensional spectroscopy has not been achieved, even though the ASOPS has been employed in the development of dual-frequency comb spectroscopic techniques. Recently, synchronized MLs without photodetector array nor mechanical time-delay devices have been used to perform Raman microspectroscopy, dual-frequency comb 2DES, and time- and frequency-resolved transient absorption and refraction spectroscopy.

Here, we experimentally demonstrate that synchronized mode-locked laser-based 2DES (SM-2DES) without a long mechanical delay line for the Tω-scan enables ultrafast time-resolved studies of sub-picosecond electronic and vibrational dephasings, picosecond solvation dynamics, and nanosecond population relaxation without sacrificing its femtosecond time-resolvability and detection sensitivity. We show that the data acquisition time for the measurement of the Tω-dependent 2DES signals is substantially shortened in comparison to the conventional 2DES technique. We, here, present the SM-2DES experimental results for bacteriochlorophyll a (BChl) 1-propanol solution to demonstrate the feasibility and capability of a novel coherent 2D spectroscopic technique. It should be noted that the ASOPS-based femtosecond time-resolved spectroscopy with an interferometric detection of the nonlinear signal, including SM-2DES, requires tightly repetition-rate-stabilized MLs to achieve a sub-fs timing jitter. The fast and efficient data acquisition rate of SM-2DES enabled us to study ultrafast solvation dynamics, vibrational coherences of vibronically coupled modes, and non-Condor effects on the coherent vibrational spectra extracted from the SM-2DES signals.

**Results**

**Synchronized mode-locked laser-based 2DES**

The experimental setup for SM-2DES is illustrated in Fig. 1a (see Supplementary Note 1 for detailed optical layout). SM-2DES in a boxcar geometry requires four temporally and spatially separated pulses that are referred to as their wave vectors k_A, k_B, k_C, and k_LO, which represents the local oscillator used to interferometrically detect the generated four-wave mixing signal electric field. The repetition rates of ML1 and ML2 are slightly different from each other by ∆f (Fig. 1a), where the repetition rate (f) of ML2 is 80 MHz, and that of ML1 is 80 MHz + ∆f. ∆f was chosen as 38.4 Hz and 3.2 kHz to set ∆Tω ≈ 6 fs and 500 fs, respectively, according to Eq. (1). First, two pump pulses with wave vectors k_A and k_B, respectively, that are used to excite chromophores in solutions are produced by ML1, and the time delay between the two pulses, denoted as τ1, is experimentally controlled using a high-precision translational stage. This spatial interference fringe generated by the two pump fields propagating in the two different directions determined by the wave vectors of k_A and k_B acts like a grating. Then, the probe pulse propagating along the direction of k_C arrives at the sample after a waiting time Tω, and then the probe beam is scattered due to the third-order field–matter interaction. Such a three-pulse scattering process was initially studied by Weiner and Ippen. The Tω-dependent relaxation of the transient grating diffracted in the direction of k_LO results from the decay of the population of excited-state molecules in a dissipative medium. The scattered signal is under interferometric detection with an added LO pulse (with wave vector k_LO) from ML2. Here, the delay time between the probe and LO pulses, denoted as τ2, is scanned using another high-precision translational stage.

Figure 2a shows a representative set of SM-2DES raw data of the IR125/ethanol solution. When the pump pulse with wave vector k_A precedes the k_B pulse, the photon echo response dominates the detected 2DES signal for an optically
heterogeneous system. The opposite time-ordered field-matter interactions in the order of $\mathbf{k}_B \rightarrow \mathbf{k}_A \rightarrow \mathbf{k}_C$ pulses generate the non-rephasing 2DES signal. At a fixed $\tau_1$, a continuous $\tau_2$-scan is performed while ASOPS with two trains of $\mathbf{k}_B$ and $\mathbf{k}_C$ pulses from the two MLs is used to scan the waiting time ($T_w$) that is related to laboratory time $t$ by $T_w = f_{\text{LO}} \Delta t$. $\Delta t$ is the time elapsed after the optical trigger signal generated when a pair of pulses from the two MLs temporally overlap. The most prominent interference pattern of the SM-2DES signal at $\tau_1 = 0$ shown in Fig. 2b arises from the interference between the local oscillator electric field and the 2DES electric field. However, the interferogram in Fig. 2b depends on $T_w$ too, even though it is not clearly visible in the interferogram plotted in Fig. 2b. One can retrieve the $T_w$-dependent 2DES signals by rearranging the raw data. The $T_w$-dependent feature of the SM-2DES signal can be clearly seen in the zoomed-in plot in Fig. 2c. Each decay curve (Fig. 2d) at fixed $\tau_1$ and $\tau_2$ corresponds to the measurement (or waiting) time-dependent interference signal, i.e., $I(\tau_2, \Delta t, \tau_1) = I(\tau_2, T_w/f_{\text{LO}}, \tau_1)$, which shows how the vibrational quantum beats in the infrared frequency domain can be measured with a photodetector in the RF domain by employing an ASOPS-based down-conversion measurement scheme. The experimental data in Fig. 2a is, therefore, a 3D time-domain interferometric signal $I(\tau_2, \Delta t, \tau_1)$ that depends on $\tau_1$, $\Delta t (= T_w/f_{\text{LO}})$, and $\tau_2$.

To establish the relationship between the measured time-domain interferogram in Fig. 1b and the nonlinear response function $S^{(3)}(t_3, t_2, t_1)$ of optical chromophores in condensed phases, we employ time-dependent perturbation theory, use optical frequency comb field expressions, and invoke an impulsive pulse approximation. One can find that the experimentally measured signal $I(\tau_2, \Delta t, \tau_1)$ that results from the interference between the LO and 2DES signal electric fields, $E_{\text{LO}}$ and $E_{\text{signal}}$, respectively, is given by (see Supplementary Note 2 for detailed theoretical derivation and ref. 40):

$$I(\tau_2, \Delta t, \tau_1) \propto \text{Re} \left[ E_{\text{LO}}(t) \times E_{\text{signal}}(t) \right] \propto \text{Re}[R_{\text{echo}}(\tau_2, T_w, \tau_1)],$$  

(2)

where $R_{\text{echo}}(\tau_2, T_w, \tau_1)$ is the time-domain nonlinear response function associated with photon-echo spectroscopy. Equation (2) suggests that the all-time-resolved SM-2DES interferogram is simply proportional to the real part of the photon-echo response function. As manifested in Eq. (2), the time scale of femtosecond and nanosecond molecular processes during $T_w$ is converted to...
that of laboratory time $\Delta t$ on the nanosecond to millisecond time scales by the inverse of the down-conversion factor, i.e., $1/f_{\text{r}} = 2.5 \times 10^3$ and $2.08 \times 10^6$ for $\Delta f_r = 3.2$ kHz and 38.4 Hz, respectively, with $f_r = 80$ MHz in the present work.

Fourier-transforming the 3D interferometric signal, $I(T_\omega, \Delta t, \tau_1)$, with respect to $\tau_2$ and $\tau_3$, we were able to obtain the 2DES spectra with respect to the excitation angular frequency ($\omega_1$) and detection angular frequency ($\omega_2$). A notable advantage of SM-2DES compared to the conventional 2DES is that both the amplitude and phase of the 2DES data can be analytically normalized and corrected, respectively, by using the interferometric pump-probe signals measured with the same instrument (see Methods and Supplementary Note 3). Here, the phase stability between the probe and LO pulses is maintained by an additional reference interferometer (see Methods). In practice, it should be noted that the present phase correction and spectral amplitude calibration methods are useful when the complex (real and imaginary) pump-probe data are available. However, the spectral shapes of the pump pulses are unavoidably imprinted in the measured SM-2DES spectra. Since the automatic $T_\omega$-scan was performed up to 3.0 ns with a time interval of 6 fs or 500 fs, when the repetition rate detuning factor is 38.4 Hz or 3.2 kHz, respectively, the sheer size and information density of 2DES data (many thousands to millions of 2DES spectra) are extraordinarily large, which attests to the capability of our SM-2DES technique.

**SM-2DES of bacteriochlorophyll a.** The spectral bandwidth of our Ti:Sapphire oscillators is broad enough to cover both the $Q_y$-absorption and -emission bands of the BCHla in propanol as well as the high-lying vibronic bands (Fig. 3a). Three representative transitions between the ground ($S_0$) and $Q_y$ states, which are the 0–0 $Q_y$-absorption ($Q_{y,0}$) at 387 THz, 0–1 $Q_y$-absorption ($Q_{y,1}$) at 420 THz, and 0–0 $Q_y$-emission at 375 THz, are marked by dashed lines. The $Q_{y,0}$-emission peak is Stokes-shifted from the $Q_{y,0}$-absorption peak due to free energy minimization processes such as solvation. The time resolution of our SM-2DES is 12.5 fs (see Method and Supplementary Note 1) so that one can study the wave packet oscillations of vibronically coupled modes with frequencies up to 2670 cm$^{-1}$. During the time required for a full $T_\omega$-scan, which is $1/\Delta f_r$, the number of $T_\omega$-points in the dynamic range of the $T_\omega$-resolved measurements from 0 to 12.5 ns is $2.08 \times 10^6$ when $\Delta f_r = 38.4$ kHz and $f_r = 80$ MHz and $2.5 \times 10^4$ when $\Delta f_r = 3.2$ kHz and $f_r = 80$ MHz. However, the conventional 2DES with a mechanical delay line for the $T_\omega$-scan is, in practice, not useful to collect such a large number of $T_\omega$-dependent 2DES signals. Due to the high data acquisition rate of the SM-2DES compared to the conventional 2DES methods using a single ML and a mechanical delay line for scanning the waiting time, the frequency resolution of the coherent vibrational spectrum extracted from the time-resolved 2DES signals can be substantially enhanced. Furthermore, the large number of data equally spaced over a wide range of waiting time enables to perform boxcar averaging, which can, in turn, enhance the signal-to-noise ratio of the 2DES spectrum even at a long waiting time at which the amplitude of the signal is expected to be small.

In Fig. 3b, the six representative 2DES spectra at waiting times of 50 fs, 500 fs, 5 ps, 50 ps, 500 ps, and 3.0 ns are shown. At a short waiting time ($T_\omega = 50$ fs), both ground-state bleach (GSB) and stimulated emission (SE) positively contribute to the absorptive 2DES signal. In general, the coherent electronic artefact significantly contributes to the 2DES signal at zero waiting time when the three pulses that interact with the sample overlap in time$^{35}$. The diagonal peak at (387 THz, 387 THz) and the cross-peak at (420 THz, 387 THz) originating from both the GSB and SE terms become vertically elongated along the detection frequency due to rapid intramolecular vibrational redistribution and solvation dynamics, which causes the detection frequency of the SE contribution to shift down to 375 THz in less than 5 ps$^{37}$. The two GSB contributions denoted as GSB$_0$ at (387 THz, 387 THz) and GSB$_1$ at (420 THz, 387 THz) result from the GSBs produced at $Q_{y,1}$ by the $Q_{y,0}$- and $Q_{y,1}$-excitations, respectively. The $Q_{y,1}$ diagonal peak at (420 THz, 420 THz) is not clearly visible in Fig. 3b because its amplitude is much smaller than the GSB$_0$ diagonal peak, denoted as GSB$_0$, by a factor of $\sim$0.06. Also, there should be another GSB peak at (387 THz, 420 THz), which results from the GSB of $Q_{y,1}$ state when the pump induced a $Q_{y,0}$ transition. The amplitude of the cross-peak at (387 THz, 420 THz) could be comparable to that of GSB$_1$. However, the GSB$_1$ peak is found to be approximately four times stronger than that of the cross-peak at (387 THz, 420 THz) (Supplementary Fig. 2c). Such a reduction in the amplitude of the cross-peak at (387 THz, 420 THz) is caused by the negative contribution from an excited-state absorption$^{25}$. The two SE contributions denoted as SE$_0$ at (387 THz, 375 THz) and SE$_1$ at (420 THz, 375 THz) result from the Stokes-shifted excited states $Q_{y,1}$ and $Q_{y,2}$, respectively. The GSB$_1$ and SE$_1$ peaks in Fig. 3b are frequency-resolved from the GSB$_0$ and SE$_0$ peaks (Fig. 3a), even though both the absorption and emission spectra have broad lineshapes.
Picosecond relaxation and solvation dynamics. The time profiles of the 2DES signals at the four peak positions (GSB₀, SE₀, GSB₁, and SE₁) over the broad range (six decades) of waiting times are plotted in Fig. 3c and d. The amplitudes of the slow time profiles from 200 ps to 3 ns are matched with each other so that one can directly compare the early parts of the four decaying signals with $T_w$ (Fig. 3d). At short waiting times (<100 ps), the decay profiles of SE₀ and GSB₀ that are associated with the $Q_{y,0}$ excitation differ from those of the SE₁ and GSB₁ signals, respectively (Fig. 3c and d). Recently, Moca et al. carried out a series of 2DES experiments for chlorophyll $a$ in a few different solvents$^{42}$, and they found that the change of the 2DES spectra at short waiting times is dictated by the solvation dynamics. If the dipole moments of the ground and excited states differ from each other, the surrounding solvent molecules would rearrange their orientations and move longitudinally from the excited state molecule to stabilize the newly created charge distributions of the solute molecule. Due to the ultrafast solvation dynamics, the SE peak that overlaps with the corresponding GSB peak at $T_w = 0$ undergoes a frequency red-shift along the detection frequency axis$^{37}$, which makes the diagonal peak vertically broadened and elongated along the detection frequency axis. Therefore, the amplitude of the GSB₀ (SE₀) peak at (387 THz, 375 THz) shows a short-time decaying (rising) feature. Therefore, our observation that the short time changes of the SE₀ and GSB₀ signals are notably large suggests that the charge distribution of the $Q_{y,0}$ state differs from that of the ground state, $S₀$. This result is consistent with Moca et al.’s experimental observation$^{42}$. To confirm this interpretation, we carried out density functional theory (DFT) and time-dependent DFT calculations of the ground ($S₀$) and excited ($Q_{y}$)
states of BCHla, respectively. Indeed, these ab initio calculation results show that the dipole moments of these two states are distinctively different from each other (Supplementary Fig. 4). However, still, it is not entirely clear why the signals at SE₁ and GSB₁ generated by the transition to the $Q_{υ,1}$ state induced by the pump field–matter interaction do not relax with $T_{w}$ for ~100 ps. One possible explanation is that the charge distribution of the $Q_{υ,1}$ state is similar to that of the ground state, which makes the solvation dynamics contributions to the decays of the SE₂ and GSB₂ signals small.

**Femtosecond dynamics and vibrational coherences.** Using the short-time 2DES signals at four different points in a given 2DES spectrum, one can obtain the spectra of vibronically excited modes, which will be referred to as coherent vibrational spectrum (CVS) (Fig. 3e). We found four strongly vibronically coupled modes with frequencies of 190, 564, 728, and 1156 cm$^{-1}$. Here, the frequency resolution is estimated to be 8 cm$^{-1}$ by considering the scan range of the waiting time ($0 \leq T_{w} \leq 5.0$ ps) and the width (2.5 ps) of the apodization window. It should be emphasized that the SM-2DES technique does not need additional data acquisition (2.5 ps) of the apodization window. It should be emphasized that the SM-2DES technique does not need additional data acquisition time for a longer waiting time scanning to achieve an improved frequency resolution for the spectral analysis of vibrational quantum beats.

The vibrational mode at 728 cm$^{-1}$ is found to be the most strongly vibronically coupled to the $Q_{υ}$ transition, which indicates that the displacement between the corresponding vibrational potential energy surfaces of the $S_0$ and $Q_{υ}$ states is large. The vibronic coupling between the $Q_{υ}$ and the 1156 cm$^{-1}$ mode is responsible for the $Q_{υ,13}$ band because the frequency difference between the $Q_{υ,0}$ and $Q_{υ,1}$ bands in the absorption spectrum is close to 1156 cm$^{-1}$. The amplitudes of the other two modes at 190 and 564 cm$^{-1}$ depend on the excitation and detection frequencies. Although the information density of the three-dimensional 2DES data obtained with wide dynamic range detection frequencies. Although the information density of the three-dimensional 2DES data obtained with wide dynamic range 2DES measurements is very high, it is still difficult to thoroughly analyse the vibrational coherence spectra in Fig. 3e. Below, we shall present principal components analysis results and compare the DFT results for the ground and excited states of BCHla.

**Nanosecond dynamics.** In Fig. 3f, we present the decay-constant distributions of the 2DES signals at the four peak positions, GSB₀, SE₀, GSB₁, and SE₁, which are the weighted histograms of the decay constants from the tri-exponential fitting analyses of the waiting-time-dependent signals. The amplitude of each exponential component is used as the weighting factor. From the total absorptive 2DES signals (Supplementary Fig. 5), we found that the lifetime of the $Q_{υ}$ state is about 2.1 ns, which is consistent with the fluorescence lifetime of BCHla in 1-propanol. The sub-nanosecond components could be associated with the rotational diffusion of BCHla because such a component was not observed in the magic-angle transient absorption study. Although the nanosecond dynamics of the BCHla solution is mainly determined by the rotational diffusion and population relaxation, the SM-2DES with nanosecond scanning capability could be useful for studying systems requiring both 2DES and nanosecond measurements in the future.

**Principal components analysis.** Vibrational coherence analysis at a single frequency point in the 2D map could cause a misinterpretation of the 2DES signal, even though the relative amplitudes and linewidths of vibronically coupled modes found in the CVS obtained by Fourier-transforming the 2DES signal with respect to $T_{w}$ provide crucial information on their vibrational coupling strengths and decoherences. Therefore, it would be useful to analyse both the coherent vibrational spectra and the 2D coherence maps to extract substantially detailed information on the nature and origin of coherent oscillations found in the spec-trally congested $T_{w}$-resolved 2DES spectra. Here, we carried out a principal component analysis to identify the major 2D spectral patterns of the oscillating components in the 2DES signal and draw correlation between the vibronically coupled modes found in the CVS and the 2D spectra of major principal components. The entire three dimensional ($N_{υ,1} \times N_{w} \times N_{w}$) 2DES data, where $N_{υ,1}$, $N_{w}$, and $N_{w}$ are the numbers of data points of excitation frequency, detection frequency, and waiting time, respectively, are then represented with two 2D electronic spectra and two coherent vibrational spectra, which are the two principal components denoted as PC₁ and PC₂ in Fig. 4. All the other principal components are found to have negligibly small singular values.

Because the peak frequencies of the 2D coherence maps of the PC₁ and PC₂ overlap with the absorption and emission spectra of BCHla, respectively, the majority of PC₁ can be identified with combination of GSB₀ and SE₁ and that of the PC₂ with SE₂ (see Supplementary Fig. 6 for detailed descriptions of these peak assignments and for the corresponding PC spectra extracted from the non-rephasing 2DES signals). There are six strong peaks at 190, 337, 564, 728, 901, and 1156 cm$^{-1}$ in the coherent vibrational spectra associated with the two principal components, PC₁ and PC₂, of the 2DES data (Fig. 4b). The relative amplitudes of the three peaks at 337, 564, and 901 cm$^{-1}$ in the CVS of the PC₂ are found to be much weaker than those in the CVS of the PC₁. Such detection-frequency dependences of the three peaks are notable when comparing the single-frequency point CVS at the maximum positions of the 2D coherence maps of the PC₁ and PC₂ (Fig. 4c). Consistent with the present PCA results on the vibronically coupled modes, we found that the amplitudes of the 2D coherence maps at 337, 564, and 910 cm$^{-1}$ (Supplementary Fig. 10) at (387 THz, 375 THz) are comparatively small. This indicates that the vibronic contributions from these modes to the SE₀ are negligible. The similarity and difference between the CVS of PC₁ and PC₂ suggest interesting information about the vibronic coupling strengths and their non-Condon effects, as will be discussed below.

**DFT calculation of vibronic coupling strength.** To assign the vibronically excited vibrational modes of BCHla, we carried out two different DFT calculations to estimate the vibronic coupling strengths. The first approach, scheme 1, is to calculate the coordinate displacements of all the normal modes using the normal mode projection method, which is based on direct comparisons of the optimized structures of the $S_0$ and $Q_{υ}$ states (see the black bar spectrum in Fig. 4d). The second approach, scheme 2, is based on the numerical calculation of the gradient of vibrational potential energy surface of the $Q_{υ}$ state. Once the slope of the vibrational potential energy curve at the equilibrium position of the ground state is obtained, one can estimate the vibrational coordinate displacement with invoking the harmonic approximation (red bar spectrum in Fig. 4d) (see Supplementary Note 6 for more details). Although these two approaches are not perfect, they still provide useful information about which modes are strongly coupled to the electronic $Q_{υ}$ transition. From the DFT calculations, we could assign the six notable peaks in Fig. 4b at 190, 337, 564, 728, 901, and 1156 cm$^{-1}$ (see Supplementary Fig. 11 for their vibrational eigenvectors). The peak frequencies of the observed vibrational modes of BCHla are consistent with the previous experimental results obtained with hole-burning spectroscopy and 2DES studies.

**Non-Condon effects and symmetry.** The electronic structures of the HOMO and LUMO are shown in Fig. 5a and b. The
The amplitudes of the three vibrational modes at 337, 564, and 901 cm$^{-1}$ and the 2DES signals at (390 THz, 390 THz) and (390 THz, 375 THz) are discussed in the theoretical and 2DES studies$^{48-50}$. In general, non-Condon effects refer to the dependence of the TDM on the vibrational coordinates. Such a breakdown of Condon approximation is already manifest in the broken mirror symmetry between the steady-state absorption and emission spectra in Fig. 3a$^{31}$. However, the linear spectra do not provide information about which molecular vibrations have strong Herzberg-Teller vibronic couplings. For those non-Condon modes, the TDM at the equilibrium position ($Q^0$) in the electronically excited state could differ from that at the equilibrium position ($Q^0$) in the ground state. In the 2DES experiments, such a non-Condon effect can be studied by examining the detection frequency-dependence of the CVS. As can be seen in Fig. 4b and c, the vibronic coupling strengths of the 337, 564, and 901 cm$^{-1}$ modes in the CVS associated with the SE$_0$ term are negligible, whereas they are strong in the CVS associated with the GSB$_0$ band. These disparities in the vibronic coupling strengths can be considered as experimental evidence of the non-Condon effects from these $D_{4h}$-to-$C_s$ symmetry-breaking modes. Here, it should be mentioned that the CVS of the GSB and SE contributions are similar to each other in the case of a dye (IR125) solution (Supplementary Fig. 7). Therefore, the difference between the two CVS in Fig. 4b and c is a characteristic feature of BChla. To understand how such a difference in the coherent vibrational spectra is related to the breakdown of the Condon approximation, let us consider the TDM associated with the electronic transition of BChla by probe beam interaction with BChla. The TDM determining the SE$_0$ signal should be evaluated at $Q^0$, whereas that associated with the GSB$_0$ is the TDM at $Q^0$ (Fig. 5e). In the present work, we performed all parallel polarization 2DES measurements. However, it would be useful to carry out polarization-controlled 2DES spectroscopic studies, e.g., 2DES anisotropy measurements$^{19}$, to extract more information about the relative directions of the absorptive and emissive TDMs ($\mu_{eg}(Q^0)$ and $\mu_{eg}(Q^0)$) in Fig. 5e) in the future. Our experimental results suggest that the vibronic TDMs are notably vibrational coordinate-dependent for the three (337, 564, and 901 cm$^{-1}$) modes, which cannot be revealed by linear spectroscopic studies of BChla. Noting that the molecular distortions along these three vibration coordinates ($Q_{a,b}$ involve a $D_{4h}$-to-$C_s$ symmetry breaking, we anticipate that a further investigation about how such symmetry-breaking modes could affect the vibronic couplings and excitation transfer processes in photosynthetic reaction centres and light-harvesting complexes would be interesting.

**Discussion**

In the present article, we showed that, as proof-of-principle experiments, direct time-domain measurement of the photon-echo response function of optical chromophores in solution is feasible using the SM-2DES technique. This measurement enables information on the correlation between two time-separated electronic coherences and femtosecond-resolved molecular relaxation occurring on femtosecond to nanosecond time scales to be obtained with a single-point radio frequency detector and without a metre-long mechanical delay line. This work is an experimental demonstration of SM-2DES for studying the excited-state dynamics of a photosynthetic pigment (BChla) in solution. The efficient 2DES measurement of BChla solution within wide time and frequency windows by SM-2DES enabled observation and analysis of characteristic excited-state vibrational coherences of BChla. The experimental results on BChla can be of use to understand the nature of the photosynthetic energy transfer processes. We anticipate that SM-2DES will be useful for investigating molecular structure changes of chemically reactive systems due to its femtosecond time-resolvability and wide dynamic range measurement capability. In addition, the use of
Such a non-Condon effect is also manifest in the broken mirror symmetry between the steady-state absorption and emission spectra in Fig. 3a. The thick red arrow represents the $Q_\text{r}$ transition dipole moment ($\mu_{Qr}$). Blue and yellow balls represent nitrogen and magnesium atoms, while the grey balls do carbon atoms. x- and y-axes are defined from two orthogonal N-Mg-N lines. Thin red arrows emphasize the nuclear motions of N atoms in the BChla centre. The key eigenvector elements of the $D_{abh}\rightarrow D_{abh}$ (c) and $D_{abh}\rightarrow C_1$ (d) symmetry-breaking vibrational modes at the BChla centre are shown here. The full vibrational eigenvectors of these six modes are presented in Supplementary Fig. 11. The red arrows in c and d show the directions (eigenvector elements) of nitrogen atoms. e Schematic diagram describing a non-Condon effect on the reduced vibrational amplitudes of the three symmetry-breaking modes found in the CVS from the 2DES signal at (390 THz, 375 THz). Due to the destructive interference between the Franck-Condon factor and the Herzberg-Teller vibronic coupling, the oscillator strength associated with the $Q_{y'0}\rightarrow S_2$ transition at the equilibrium coordinate $Q^0_{y0}$ in the excited state is smaller than that at the equilibrium coordinate $Q^0_{y0}$ in the ground state. Such a non-Condon effect is also manifest in the broken mirror symmetry between the steady-state absorption and emission spectra in Fig. 3a.

Methods

Synchronized mode-locked laser-based 2DES. Two Ti:Sapphire lasers (Rainbow 2, Femtolasers) producing a train of pulses with a duration time of 7 fs were used for the SM-2DES setup. The repetition frequencies of the MLs were phase-locked to a reference radio frequency (RF) signal from an 8-channel frequency synthesizer (Holzworth, HS9008A), the time-base of which is linked phase-coherently to a GPS-disciplined RB atomic clock. The repetition frequencies of ML1 and ML2 were adjusted to be 80 MHz + $\Delta f_r$ and 80 MHz, respectively. The waiting time $T_w$ was automatically scanned due to the difference in the repetition frequencies, $\Delta f_r$, between the two MLs, which is the key aspect of the ASOPS technique. The sampling rate was synchronized to the repetition rate of ML2 (80 MHz) to record every pulse-to-pulse intensity difference of the signal. We set $\Delta f_r$ to be either 38.4 Hz or 3.2 kHz, which corresponds to $\Delta T_w$ values of 6 fs and 500 fs, respectively, for the SM-2DES measurements. Fractions of ML1 and ML2 are non-collinearly focused at a nonlinear crystal to generate a sum-frequency-generation field, which is utilized as an optical trigger for the automatic $T_w$-scan. The time jitter of the $T_w$-scan is determined by the pulse shape of the optical trigger and it was <1 fs. Due to the fast automatic scanning of $T_w$, $T_2$ can be scanned continuously by using a mechanical delay stage. The time interval for the $T_2$ step-scan was controlled to be $\Delta T_2 = 1.868$ fs, which corresponds to a translational stage step of 0.14 μm for each continuous $T_2$-scan. The time resolution of our SM-2DES was 12.5 fs, which was estimated by measuring the cross-correlation between ML1 and ML2 at the sample position (see Supplementary Note 1 for details).

IR125 and BChla were purchased from Exciton and ChemCruz, respectively. The two molecules were used without further purification. IR125 (BChla) was dissolved in ethanol (1-propanol), and the light absorbance of the solution was estimated by measuring the cross-correlation between ML1 and ML2 at the sample position (see Supplementary Note 1 for details). We checked that there was no additional signal from the sample degration.

Phase correction and amplitude calibration. Small portions of the local oscillator fields from ML1 before and after the $T_2$-scanning stage were taken using two beam splitters and were used to construct a Mach–Zehnder interferometer. This process provides the reference interferograms that are synchronized with the SM-2DES data collections. The reference interferograms are used to compensate for the phase fluctuation error in the detection frequency and the nonorthogonality between $T_w$ and $T_2$-scans.

In interferometric SM-PP spectroscopy, the probe field itself provides a phase reference automatically, and the spectral amplitudes of the measured PP spectrum can be simply normalized by using the pulse spectrum. On the other hand, SM-2DES signals cannot be normalized, and their electric field phases cannot be easily corrected. However, the phase and amplitude of SM-2DES can be analytically calibrated and measured, respectively, by using interferometrically measured SM-PP spectra with exactly the same instrument (see Supplementary Note 3). The calibration factor is the ratio of the integrated SM-2DES spectrum over the excitation frequency to the interferometrically measured SM-PP spectrum. Unlike the conventional approach using the projection theorem, the present calibration and phase correction scheme
works well because the complex (both real and imaginary) SM-PP signals can be obtained and used as both amplitude and phase references.

Data availability
The data that support the findings of this study are available within the paper and its supplementary information files. Raw data are available from the corresponding author on a reasonable request.

Received: 13 July 2020; Accepted: 28 October 2020; Published online: 27 November 2020

References
1. Brixner, T. et al. Two-dimensional spectroscopy of electronic couplings in photosynthesis. *Nature* **434**, 625–628 (2005).
2. Collini, E. et al. Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. *Nature* **463**, 644 (2010).
3. Jang, S. J. & Mennucci, B. Delocalized excitons in natural light-harvesting antennas. *Rev. Mod. Phys.* **90**, 035003 (2018).
4. Blankenship, R. E. *Molecular Mechanisms of Photosynthesis* (John Wiley & Sons, 2014).
5. van Amerongen, H., van Grondelle, R. & Valkunas, L. Evidence for wavelike energy transfer through quantum coherence in monomeric bacteriochlorophyll a monomers in solution. *J. Phys. Chem. B* **120**, 10542–10556 (2005).
6. Tretiak, S. & Mukamel, S. Density matrix analysis and simulation of electronic excitations in conjugated and aggregated molecules. *Chem. Rev.** **102**, 3171–3212 (2002).
7. Cho, M. Coherent two-dimensional optical spectroscopy. *Chem. Rev.** **108**, 1331–1418 (2008).
8. Schöles, G. D. et al. Using coherence to enhance function in chemical and biophysical systems. *Nature* **543**, 647 (2017).
9. Engel, G. S. et al. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. *Nature* **446**, 782 (2007).
10. Steige, K. & Brixner, T. Two-dimensional spectroscopy of electronically coupled excitons in photosynthetic complexes. *Nature* **543**, 355–365 (2017).
11. Hybl, J. D., Albrecht, A. W., Gallagher Faeder, S. M. & Jonas, D. M. Two-dimensional optical spectroscopy of bacteriochlorophyll a monomers in solution. *J. Phys. Chem. Lett.** **9**, 3053–3057 (2018).
12. Mooney, G. et al. Coherent coupling of excitons and triads in a photosynthetic reaction center. *J. Phys. Chem. Lett.** **9**, 3070–3074 (2018).
13. Lietard, A., Hsieh, C.-S., Rhee, H. & Cho, M. Electron heating and thermal relaxation of gold nanorods revealed by two-dimensional electronic spectroscopy. *J. Am. Chem. Soc.** **133**, 1866–1871 (2019).
14. Novoderezhkin, V. I., Romero, E. & van Grondelle, R. How exciton-vibrational coherences control charge separation in the photosystem II re...
