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Identification and characterization of diverse coherences in the Fenna–Matthews–Olson complex

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The idea that excitonic (electronic) coherences are of fundamental importance to natural photosynthesis gained popularity when slowly dephasing quantum beats (QBs) were observed in the two-dimensional electronic spectra of the Fenna–Matthews–Olson (FMO) complex at 77 K. These were assigned to superpositions of excitonic states, a controversial interpretation, as the strong chromophore–environment interactions in the complex suggest fast dephasing. Although it has been pointed out that vibrational motion produces similar spectral signatures, a concrete assignment of these oscillatory signals to distinct physical processes is still lacking. Here we revisit the coherence dynamics of the FMO complex using polarization-controlled two-dimensional electronic spectroscopy, supported by theoretical modelling. We show that the long-lived QBs are exclusively vibrational in origin, whereas the dephasing of the electronic coherences is completed within 240 fs even at 77 K. We further find that specific vibrational coherences are produced via vibronically coupled excited states. The presence of such states suggests that vibronic coupling is relevant for photosynthetic energy transfer.

Through billions of years of evolution, nature has found a solution for the efficient harvesting of sunlight in the form of densely packed pigments embedded in protein environments[1,2]. In aiming to understand the functionality of these complexes, particular attention has been paid to the Fenna–Matthews–Olson (FMO) complex[3], a small protein homo-trimer situated between the chlorosome antennae and the photosynthetic reaction centre (RC) of green sulfur bacteria.[4,5] This historic interest in the FMO complex has been due to the early resolution of its crystal structure[6,7], its relative structural simplicity (Fig. 1a) and its high water solubility. Together, these properties make the complex both experimentally accessible and simple enough to allow for detailed theoretical work. The assumption has thus been that it could serve as an exemplar system to unravel the mechanisms that underlie photosynthetic light harvesting. Decades of experimental and theoretical studies have thus resulted in detailed descriptions of the excitonic structure and energy-transfer dynamics[8–13], and recent two-dimensional electronic spectroscopy (2DES)[14,15] studies have enabled direct tracking of the energy flow in both isolated[9,16] and in situ[5] complexes.

The prevailing model for energy transfer in weakly or intermediately coupled systems such as the FMO complex is based on incoherent excitation 'hopping'[17], with models based on this picture being highly successful in explaining energy transfer in a wide variety of photosynthetic complexes. In 2007, a strongly contrasting picture received significant attention when long-lived quantum beats (QBs) were reported in the 2DES signals of the FMO complex at 77 K (refs [18,19]) and attributed to coherent superpositions of excitonic states. Excitonic coherence had already been identified in 1997[20], but the dephasing time was then estimated to be less than ~180 fs even at 19 K. Although this suggested a very limited timespan of excitonic superpositions, in particular at physiological temperatures, subsequent observations of similar QBs in the 2DES signals from other photosynthetic complexes were interpreted to imply that such coherence dynamics could be crucial to photosynthetic function[21–23].

Since its proposition, this coherent excitonic interpretation has been highly controversial, as the broad homogeneous spectral lines of light-harvesting complexes[24] suggest strong coupling of electronic states to the environment and, as a consequence, fast dephasing. To overcome this apparent contradiction, correlated site energy fluctuations for the protein-bound pigments were proposed[18,25]. Subsequent simulations failed, however, to identify any such 'protection' of coherences[26,27].

Recently, a mutagenesis approach made it possible to investigate the dynamics of FMO complexes with substantially different energy-level structure[28]. Contrary to expectation, given by an excitonic coherence interpretation, the observed long-lived QBs appeared to have a negligible dependence on the exciton energies. Moreover, theoretical studies indicated that the observed spectroscopic signals could be explained by vibronic coupling of excited states. Several excitonic energy gaps in the FMO complex are found at around 150–240 cm^{-1}, a range that contains a number of weakly Franck–Condon active ring-deforming vibrational modes of the bacteriochlorophyll a (BChl) molecules[29–31]. By explicitly incorporating such modes into a vibronic exciton model, it was shown that long-lived coherences of a mixed vibronic character could be produced in the excited state[32]. Later it was demonstrated that ground-state vibrations also can produce signals similar to those observed in the FMO complex[33] when excited via vibronically coupled transitions. A subsequent study that incorporated the entire FMO subunit concluded that ground-state coherences, in fact, dominate the 2DES signal.[34]

In this study we apply extensive analysis of the QBs in data obtained from two distinct sequences of polarized pulses to

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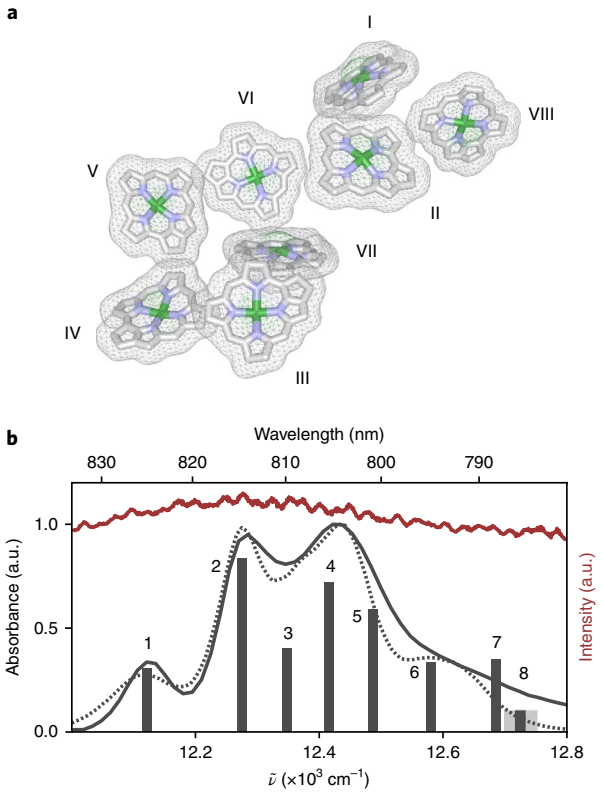

Fig. 1 | Structure and absorption of the FMO complex . a, Structural arrangement of the bacteriochlorophyll *a* molecules in the FMO complex (from published data[6]) with site numbering according to Fenna. **b**, Experimental (solid line) and calculated (broken line) absorption spectra of the FMO complex at 77 K. Experimentally determined[16] exciton energies (vertical bars) and laser spectrum (red) used in 2DES experiments are also shown. The eighth excitonic state (in the shaded area) was not included in the modelling. a.u., arbitrary units.

characterize coherences in the FMO complex at 77 K. We clearly distinguish short-lived excitonic coherences and long-lived vibrational coherences both in the ground and excited states.

Results

Structure and absorption of the FMO complex. The initial crystallographic work on the FMO complex found the protein subunits to contain well-defined structures of seven[3] (later amended to eight[6]) BChl pigments (Fig. 1a). We identified the spectroscopic signatures of the eighth BChl in a preceding FMO study[16], and we presume that the isolated FMO complexes investigated here also contain eight BChl molecules. The previously extracted exciton energies and 77 K absorption spectrum of the FMO complex isolated from the green sulfur bacteria *Chlorobium tepidum* are shown in Fig. 1b.

Coherence signals in polarization-controlled 2DES. The 2DES technique[15] and our specific implementation[35] have been detailed previously. The recorded data set appears as a sequence of two-dimensional (2D) maps in which the complex emitted field, $E^{(3)}(\tilde{\nu}_1,t_2,\tilde{\nu}_3)$, is displayed as a function of excitation and detection energies (proportional to the wavenumbers $\tilde{\nu}_1$ and $\tilde{\nu}_3$, respectively) and evolves with the population time t_2. QBs may appear along t_2, the excitation/detection energy dependence of which can be conveniently identified by a Fourier transform over population time. We refer to the resulting maps as $\tilde{\nu}_2$ oscillation maps.

2DES data sets contain the entire third-order response of the system, and the resulting information density may lead to problematic spectral congestion in multichromophore systems. Polarization techniques can be used to alleviate such congestion, because the measured signals are dependent on both the relative angles between transition dipole moments and the relative polarization angles of the incident laser pulses. These approaches are particularly powerful in fully noncollinear 2DES geometries, where one can control the polarizations of all incident pulses and the detected signal.

Most reported studies on the FMO complex[18,19] and other photosynthetic complexes[21,23,36,37] have, nevertheless, exclusively employed a series of parallel-polarized pulses, denoted here as 'all-parallel' (AP) or ⟨0°,0°,0°,0°⟩. Although this sequence typically yields the strongest signal, it preferentially generates signals that originate from interactions between parallel dipoles. As a consequence, coherence dynamics in these experiments are dominated by intramolecular vibrational motion.

To suppress such intramolecular signals and allow the signatures of coherences across multiple pigments to emerge, we applied a sequence of two perpendicularly polarized pulse pairs, denoted here as 'double-crossed' (DC) or ⟨45°,−45°,90°,0°⟩ (Fig. 2a). First applied in 2D vibrational spectroscopy[38,39] and later in 2DES[22,40], it suppresses both all non-coherence signals (for example, population dynamics) and coherence signals that involve interactions with pairwise parallel transition dipoles. Thus, signals from localized vibrational modes are suppressed, whereas signals from, for example, intermolecular electronic coherence remain.

In coupled multichromophore systems, certain linear combinations of vibrational modes can also contribute to the DC signal[33] (the details are published elsewhere[41]). This occurs when a coherence is generated through transitions to (vibronically) mixed excited states. As detailed by Tiwari et al.[33], this results in the electronic character of the excited states taking on a vibrational coordinate dependence, which effectively causes the transition polarization to oscillate with the vibrational frequency. Vibronically coupled states are thus revealed through the presence of vibrational contributions in the DC signal.

To provide support for the experimental assignment of the coherence signals, we simulated the time evolution of the FMO subunit using a vibronic exciton model to calculate a polarization-resolved 2D spectrum at each population time step. The model (details are given in Supplementary Section 1 and published elsewhere[34]) explicitly includes a Raman-active vibrational mode for each BChl, which was parametrized with a Huang–Rhys factor of 0.02 and a wavenumber of 160 cm^{-1}. The weakly coupled eighth BChl and the weak interactions between BChls on different trimeric units were neglected, and the calculations were thus based on the seven-site electronic Hamiltonian obtained in previous studies[8]. The calculated absorption spectra of FMO are shown in Fig. 1b.

2D spectra of FMO. To characterize the coherence dynamics in the FMO complex with a high wavenumber resolution, polarization-controlled 2DES experiments were performed at 77 K, scanning the population time t_2 to 1.8 ps. The dramatic difference in the 2D spectral structure between the pulse-polarization sequences is clear from inspection of the spectra in Fig. 2b,c, in which the real (absorptive) part of representative (t_2 = 40 fs) rephasing 2D spectra are shown (the total 2D spectra are shown in Supplementary Fig. 1).

The AP spectra (Fig. 2b) are dominated by diagonal peaks associated with features in the absorption spectrum (Fig. 1b), whereas the patterns of off-diagonal features reveal correlations between the transitions. The excitonic structure and relaxation pathways that emerge from analysis of the time evolution of the 2D spectrum are detailed elsewhere[16]. In the DC spectra (Fig. 2c), signals from population dynamics are suppressed, and the remaining signals are 'running waves' across the 2D map of alternating negative and positive features. The time evolution also differs radically; whereas population dynamics dominate the AP spectra, with only weak QBs