Coherent wavepackets in the Fenna-Matthews-Olson complex are robust to excitonic-structure perturbations caused by mutagenesis

Margherita Maiuri[1], Evgeny E. Ostroumov[1], Rafael G. Saer[2,3], Robert E. Blankenship[2,3,4], Gregory D. Scholes[1]*

[1]Department of Chemistry, Princeton University, Washington Road, Princeton, New Jersey 08544, United States.

[2]Department of Biology, Washington University in St. Louis, St. Louis, MO 63130, United States.

[3]Photosynthetic Antenna Research Center, Washington University in St. Louis, St. Louis, MO 63130, United States.

[4]Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130, United States.

*Correspondence to: gscholes@princeton.edu

Femtosecond pulsed excitation of light-harvesting complexes creates oscillatory features in their response. This phenomenon has inspired a large body of work aimed at uncovering the origin of the coherent beatings and possible implications for function, and is still questioned today. Here we exploit site-directed mutagenesis to change the excitonic level structure in Fenna–Matthews–Olson (FMO) complexes and compare the coherences using broad-band pump-probe spectroscopy. Our experiments detect two oscillation frequencies with dephasing on a picosecond time scale—both at 77K and room temperature. By studying these coherences with selective excitation pump-probe experiments, where pump excitation is in resonance only with the lowest excitonic state, we show that the key contributions to these oscillations stem from ground-state vibrational wavepackets. These experiments explicitly show that the coherences—though in the ground electronic state—can be probed at the absorption resonances of other bacteriochlorophyll molecules because of delocalization of the electronic excitation over several chromophores.

Introduction

Progress in the field of ultrafast laser spectroscopy has enabled researchers to examine more incisively the properties of various multi-chromophoric systems. The observation of coherent oscillations has enthused discussions of the roles that quantum phenomena may play and how the function arising from coherence might be probed [1]. Towards this end it is important to elucidate precisely how ultrafast spectroscopy triggers and detects coherence.

Coherent phenomena have been observed in several studies of various photosynthetic antenna [2-6] by coherent femtosecond spectroscopy. A landmark study in this field was performed on the Fenna-Matthews-Olson (FMO) complex— a photosynthetic protein of green sulfur bacteria [4,5]. The main function of the FMO complex is the mediation of energy transfer from the chlorosome antenna to the reaction center of green sulfur bacteria [7]. The FMO complex has a trimeric structure where each subunit contains eight bacteriochlorophyll-*a* (BChl-*a*) pigments [8]. Seven of these are strongly bound within the protein scaffold and are interacting with each other, owing to their arrangement within the protein scaffold [6]. By exploiting two-dimensional electronic spectroscopy (2DES) long-lived quantum beatings were observed, and initially assigned to superpositions of excitonic states formed from electronically coupled BChls [4]. The persistence of coherences up to a few picoseconds at cryogenic temperatures—beyond the typical optical dephasing time of electronic states (~200 fs [9])—and their signature during the first 300 fs even at close to physiological temperatures [5], motivated researchers to work out how the detection of these coherences changes the prevailing models for energy transfer in light-harvesting complexes. However, despite a decade of extensive studies, the question of coherence in photosynthesis is still not entirely resolved [10,11].

To understand the origin and the implications of the reported coherent oscillations, it is necessary to consider the interaction between the nuclear motion and the electronic coordinates of coupled light-harvesting chromophores and how this interaction manifests itself in spectroscopic data [12]. Theoretical models attempted to explain the long-lasting oscillatory features in FMO and other photosynthetic complexes in terms of interchromophore vibronic coupling producing quantum mechanically mixed electronic and vibrational levels [9,13-21]. It was suggested that these beats are generated even if the Huang Rhys factors of the vibrations involved are very small [22,23].

The current consensus is that the coherent beats, when produced by broad-band photoexcitation, are associated with excited electronic states—wavepackets formed by superposition of vibronic levels (formed by mixture of electronic and vibrational levels). Vibronic coupling was proposed as a mechanism to preserve the striking excited state coherence at 77K for such an unusually long time (longer than 1ps [9,12]). Based on the same vibronic coupling model, others have posited that the coherent oscillations are wavepackets comprising a superposition of ground state vibrational levels [13], where vibronic coupling in the excited state manifold acts as an amplifier of the ground state coherences [9,13]. It is apparent that to explicitly determine the origin of the long-lived coherent oscillations, and therefore what information they convey, theoretical models need additional experimental information, not yet available in the literature.

Here we report a pump-probe investigation of a series of FMO complexes, where site-directed mutagenesis enabled us to perturb the transition energy of selected BChl molecules, thereby changing the electronic interaction and thus the excitonic distribution of the FMO complex [24]. This approach allowed us to probe the contributions of specific excitons to the

coherent beatings. Through a systematic comparison of the series of mutated FMO complexes measured under two excitation conditions and at two temperatures, we show that the strongest oscillations detected in our experiment are insensitive to mutagenesis, having similar frequencies and dephasing times in all of the pump-probe data. We also show that in the selective pump-probe experiments the oscillations are due to the ground state vibrational wavepackets. Additionally, the selective pump-probe experiments reveal significant interchromophore coupling, which is observed throughout the absorption spectrum of the FMO complexes.

Results

The arrangement of the BChls in the FMO complex is depicted in Fig. 1a, which shows the crystal structure of the FMO subunit and highlights the perturbed BChls. The cryogenic absorption spectrum of the FMO wild type (Fig. 1b) reveals three distinct Q_y bands with peaks at 805 nm, 813 nm, and 823 nm. As has been shown previously, the spectral positions of these bands are primarily determined by the charged amino acids and ligands, which surround and interact with the BChls [25]. Additionally, after photoexcitation BChls interact with each other by electronic coupling, which results in the delocalization of excitonic states among different BChl molecules.

Of particular interest is the band at 823 nm, which is assigned to the lowest excitonic state, mainly localized on BChl 3 [25]. Figure 1a highlights the two BChls (BChl 6 and BChl 3) that have been perturbed by introducing two phenylalanines at the target position and the corresponding spectra of the mutated FMO complexes (W184F and Y16F) are reported in Fig. 1d [24]. The absorption spectrum of the W184F mutant (mutation at BChl 6) reveals major changes at the Q_y transitions in the high energy side [24]. The absorption changes associated with the Y16F

(mutation at BChl 3) include a dramatic decrease in the amplitude at 823 nm, confirming that the BChl 3 is the main pigment responsible for the last step of the energy funnel process within the FMO complex [24,25]. Circular dichroism spectroscopy and excitonic simulations suggested that the energy of the lowest energy absorption band of FMO is highly sensitive to the interaction between the C3 acetyl group of BChl 3 and its surrounding protein environment, causing a blue shift of the BChl 3 absorption from 823 nm to 794 nm [24].

Figures 1d–f show the results of the broad-band pump-probe experiments performed at 77K on the three samples (FMO wild type, W184F and Y16F complexes). The $\Delta T/T$ maps point out different features, with the main positive photo-bleaching bands following the changes observed in the linear absorption spectra, e.g. a lack of the 823 nm band for Y16F. Investigation of the photo-induced dynamics in these three proteins can inform us on how the energy transfer is modified by the mutations. However, the population dynamics study is beyond the scope of this work, rather we focus on the coherent oscillations superimposed on the pump-probe decay traces in order to uncover the couplings and coherences in the FMO complex.

To investigate coherent oscillations, the exponential decay contribution has been removed from the pump-probe maps and the residual oscillatory traces have been analyzed both in time and frequency domains. Figs. 2a–c report residual oscillation traces extracted from the low temperature data of the wild type, W184F and Y16F FMO complexes, each at three selected probe wavelengths. We observe distinguishable beating signals lasting more than 2 ps in all the traces, which were fit by two damped cosine functions (black solid lines in Figure 2). The

Fourier transformed power spectra of both the experimental and simulated data are shown in Figs. 2d–f. The two observed oscillation frequencies (157 cm^{-1} and 194 cm^{-1}) are very similar across the spectrum for all the samples; the calculated standard deviations are ± 2 cm^{-1} and ± 3 cm^{-1}, respectively.

The broad-band pump-probe experiments were repeated at room temperature for all the samples (Supplementary Fig. 3) and the residuals oscillations, as well as the corresponding Fourier spectra, are displayed in Figs. 2g–n. The room temperature wave packets show features similar to those obtained from the 77K data: they oscillate with frequencies of 160 ± 3 cm^{-1} and 199 ± 4 cm^{-1}, and dephase on a ~2 ps timescale.

To further examine the coherent wavepackets we performed another series of pump-probe experiments where the excitation spectrum was narrowed and tuned to the red edge of the FMO absorption spectrum (see Supplementary Fig. 2). Fig. 3 summarizes this set of experiments in the same fashion as in Fig. 2. Upon excitation of the red shoulder of the FMO absorption spectrum, we detect coherent oscillations at all probe wavelengths, even on the blue edge of the probe spectrum where no direct pump excitation takes place. The oscillation frequencies of 159 ± 3 cm^{-1} and 197 ± 5 cm^{-1} agree with those of the broad-band experiment within the experimental error. These are the two strongest modes and are consistently observed in all mutants, at all excitation conditions and at all temperatures. These modes have similar frequencies to the oscillations reported in previous 2DES studies where the relevance of coherence in light harvesting has been discussed [4,5,26,27]. There may be other weak oscillations present in our data, however we focus on the two most significant features.

Discussion

The recent creation of a series of FMO complexes with mutations at specific BChl sites offers a unique way to verify experimentally the assignment of coherent oscillations to excitonic manifold wavepackets. Indeed, since the excitonic structure of the mutated FMO complexes is significantly altered (which is evident from the steady-state spectra, Fig. 1), variations in the beating frequencies corresponding to electronic coherence are expected, which would reflect changes in the energies of the exciton states. In particular, in the W184F FMO complex, BChl 6 band is shifted from 800 nm to 815 nm, mainly affecting excitons 2, 3 and 7 of the wild type FMO [24,28]. In the Y16F FMO complex the BChl 3 band is shifted from 823 nm to 811 nm, which significantly disturbs excitons 1, 2 and 4 [24,28]. However, no changes in the beating frequencies are observed in our data (Fig. 2) among these FMO mutants as compared to wild type. Even in the Y16F mutant the frequencies remain unaffected, whereas the stationary and transient spectra change drastically with the disappearance of exciton 1 (Fig. 1). Therefore, the observed wavepackets seem to be independent of the superposition of excitonic levels in the excited state. These comparisons of mutants with different excitonic splitting clearly show that these oscillations are not electronic coherences. Our work yields clear experimental evidence for a vibrational origin of these oscillatory features in the FMO complex detected by means of ultrafast spectroscopy.

An important previous 2DES study[5] reported a strong temperature dependence of dephasing of coherent beats. Thanks to the high signal-to-noise level achieved in our experiment

and the appropriate signal averaging (100 acquisitions), our data show that coherent beats in pump-probe measurements at room temperature have the same spectral and temporal features as at 77K. These long-lasting oscillations point to vibrational wavepackets as the origin of the coherence. Indeed, it is typical for vibrational wavepackets to persist on the timescale of several picoseconds, since the dephasing of vibrational coherences comes mainly from intramolecular vibrational relaxation, (by energy redistribution among other vibrational modes)[29,30].

The third indication of the vibrational nature of the coherent beats comes from steady-state spectroscopy. Vibrational modes in the FMO complex have been measured by fluorescence line narrowing (FLN) [23,22] and were assigned to intra-molecular BChl *a* vibrations. Similar vibrational modes were observed in isolated BChl *a* by hole burning [31]. The ~160 cm^{-1} and ~195 cm^{-1} frequencies observed in our experiments are comparable with the frequencies reported in these other studies.

Taken together, our observations indicate that vibrational modes are the origin of the coherent beats. Broad-band pump pulses tend to produce ground and excited state vibrational coherences [32,33]. The broad-band family of ultrafast experiments (both 2DES and pump-probe) in principle can discriminate ground versus excited state vibrational coherences [34,35]. However, the FMO spectrum consists of multiple chromophores and it is too congested to allow such distinction by using the analysis of the phase and the nodal structure in the Fourier domain [36,37]. Nevertheless, we find here that selective-pump excitation experiments are very revealing, since they allow only specific pathways to contribute to the measured signal.

In the second series of pump-probe experiment (Fig. 3) we selectively study the wavepackets evolving on the ground electronic state. Let us analyze in more detail the pump-probe data of wild type FMO complexes, measured at 77K using the pump pulse tuned to the far-

red edge of the absorption spectrum (see Fig. 4a). Three photo-bleaching signals with identical kinetics are observed, including those at wavelengths which are far to the blue from the excitation region (Supplementary Fig. 4). These multiple, distinct photo-bleaching bands correspond to at least three coupled BChl molecules, which evidently share a common ground state, i.e. the lowest excitonic wavefunction is delocalized over several BChl *a* sites. Multiple photo-bleaching features are also observed in the two FMO mutants (Figs. 4b and c), following their corresponding absorption spectra. Common ground states have been observed previously as cross-peaks in 2D maps at time t=0 [6,7,28], and pronounced delocalization was previously discussed [25] and reported in a recent 2DES experimental study [28]. The intensity of the bleaching signal of each of these BChls depends on the degree of delocalization. Furthermore, considering that the energy gaps between these bleach bands span over ca. 300 cm^{-1}, we conclude that the coupling among the BChls should involve both vibrational and electronic levels of the excited state manifold, and is therefore vibronic in its nature. It is important to note, that although in the large body of theoretical work, the full structure of the FMO complex is taken into account[12,38-40], in some studies the vibronic coherence has been treated using a dimer model[13,19,20]. Our observation that at least three molecules are coupled and contributing to the signal, points out that one might need to go beyond the dimer model system in order to more accurately describe the coherent spectroscopy of chromophores in the FMO proteins.

The pump-probe maps in Figs. 4a-c clearly show that the pattern of oscillating signals is imposed on the ground state bleaching features. We rationalize the coherent data (reported in Fig. 3a and b) in terms of four-wave-mixing pathways [41,42]. The excitation spectrum of the pump pulse is tuned to the very red edge of the FMO absorption (Supplementary Fig. 2), and therefore only the lowest level of the excited state manifold is photo-excited. Under these excitation

conditions the coherences cannot be created in the excited state. Therefore, the coherent oscillations are produced via a ground state bleach pathway, and they are observed in our data across all the absorption spectrum of the FMO complexes, due to a shared ground state. The schematic representation of the ground state bleach wave-mixing pathways is shown in Fig. 4d (for the details of the four-wave-mixing pathways see SI). The non-linear interaction of the pump pulse with the sample creates a wavepacket that oscillates on the potential energy surface of the common ground state, (see the first two black arrows in Fig. 4b). The frequency of that oscillation is equal to the energy of the corresponding ground state vibrational mode. The second pulse, which arrives after delay, probes the optical response consisting of a coherent oscillatory trend imposed at each probe wavelength (indicated by three pairs of colored arrows) onto the photo-bleaching signals. Clearly, the oscillatory feature can be observed at any wavelength present in the spectrum of the probe pulse. However, those wavelengths of the probe pulse, which correspond to transitions with stronger Franck-Condon factors, are amplified.

We note that in addition to the ground state, some excited state coherences can be present in the broad-band experiments. However, as it is clear from our results, the coherent oscillations in selective excitation experiments and in broad-band experiments closely resemble each other with an exclusively ground state contribution in the selective pump-probe experiments case. Therefore, the excited state coherent contribution to the four-wave-mixing signals (both in pump-probe and 2DES data) if present, is difficult to disentangle from the ground state coherences.

Conclusion

By comparing a series of mutant FMO complexes and comparing broad-band and selective excitation conditions, we have shown that coherent wavepackets in the transient absorption spectra of FMO complexes comprise ground-state vibrations, which are observed in our data via coherently delocalized exciton states. Since broad-band pump-probe and 2D electronic spectroscopies involve the same four wave-mixing signals, our results imply that the coherent oscillations we detected comprise at least part of those observed in 2DES experiments. We find that the wavepackets evolving on the ground electronic state are robust to environmental fluctuations (they dephase in approximately 2 ps at both 77K and room temperature) and insensitive to changes in excitonic structure (induced by site-directed mutagenesis). Our observations contribute to the discussion about the roles that vibronic coupling and coherent exciton delocalization play in photosynthetic light harvesting.

Methods

Preparation of FMO Complexes.

Preparation of FMO wild type and FMO mutants isolated from *Chlorobaculum tepidum* has been reported elsewhere [43]. The perturbation of the excited state manifold by site-directed mutagenesis of the FMO complexes has been recently studied in detail by Saer et al. [24]. All samples were made in a buffer of 20 mM tris/HCl (pH 8.0) and mixed 50:50 vol / vol in glycerol. The optical density was approximately ~0.25 - 0.3 at the maximum absorption peak measured in a 0.5 mm fused silica cell (Starna) at room temperature. A Janis cryostat was used to cool the sample to 77 K.

Ultrafast Spectroscopy.

The time-resolved measurements were performed using a regeneratively amplified Ti:sapphire laser system (Libra, Coherent Inc.) delivering ~90 fs pulses at ~800 nm with an average power of ~4.0 W at 10 kHz. The amplified laser pulses pumped a custom-built two-stage optical parametric amplifier (OPA). The generated pulses spanning 700–950 nm were compressed by a prism-pair compressor, and split using a beam-splitter into pump (<10 nJ) and probe (<100 pJ) pulses. Typical pulse durations (FWHM) were ~12 fs (for details see Supplementary Fig. 1). All measurements were performed with pump and probe parallel polarizations. The pump beam spot size was estimated to be 60 μm. In two-color pump-probe experiments (selective pump excitation in the text) the pump pulse spectrum was tuned to be resonant with the red edge of the sample absorption by using a long-pass filter. The pulse duration after passing through the filter was <30 fs (Supplementary Fig. 2).

Each measurement is the average of 100 acquisitions. The values of the extracted frequencies (with 95% confidence interval) shown in Figs. 2 and 3 are calculated using the Matlab Fit-Tool. The slow-varying decay is subtracted from the pump-probe maps by fitting the data with a two-exponential function. It is important to note that the amplitudes of the resulting oscillations (Figs. 2 and 3) are low (<0.005% of the total amplitude at 77K for the broadband experiments) and therefore these oscillations can be observed only when very high signal-to-noise ratio (S/N) is achieved (S/N ~ 100).

Data availability

The data supporting the findings of this study are available upon request from the corresponding author (including data presented in the main text and in the Supplementary Information

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Author Contributions. R.E.B and G.D.S. conceived the research. E.E.O. designed the ultrafast spectrometer. M.M. and E.E.O. performed the pump-probe experiments. M.M. analysed the experimental data. M.M., E.E.O. and R.S. discussed the experimental data. R.S. prepared the mutated FMO complexes. M.M., E.E.O and G.D.S. wrote the paper. All the authors commented on the manuscript.

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Figure captions

Fig. 1. Crystal structure, stationary and transient absorption spectra of WT and mutated FMO complexes. a, Crystal structure of WT FMO monomer (PDB ID: 3ENI) showing positions and relative distances of W184 and Y16 side chains to the C3 acetyl groups of BChls 6 and 3, respectively. **b**, Steady state spectra at 77K of wild type FMO. **c**, Steady state spectra at 77K of W184F and Y16F FMO mutants. **d**-**f**, Broad-band pump-probe maps at 77K for FMO Wild Type, W184 F and Y16F respectively. The data are plotted as $\Delta T/T$ functions of probe wavelength and delay. All the stationary spectra have been normalized to their respective maxima and the light blue shaded areas correspond to the laser pulses used in the broad-band pump-probe experiments.

Fig. 2. Coherent oscillations and corresponding Fourier spectra obtained from broad-band pump-probe data of various FMO complexes. The residual oscillations were obtained after removing a two-exponential decay component from the pump-probe data. The data are reported for the three indicated probe wavelengths at 77K (**a, b, c**) and room temperature (**g, h, i**) and the Fourier power spectra were calculated at 77K (**d, e, f**) and room temperature (**l, m, n**), for the wild type, W184F and Y16F FMO complexes, respectively. The oscillation traces are fit to the product of two cosine functions and two exponential decays (solid black lines in each figure). The corresponding Fourier spectra are plotted on top of the experimental data. The fits are obtained with a 95% confidence interval and fit standard error <0.002 at 77K and <0.009 at room temperature.

Fig. 3. Coherent oscillations and corresponding Fourier spectra obtained from selective pump-probe data of various FMO complexes. The residual oscillations were obtained after removing a two-exponential decay component from the pump-probe data. The data are reported

for the three indicated probe wavelengths at 77K (**a, b, c**) and room temperature (**g, h, i**) and the Fourier power spectra were calculated at 77K (**d, e, f**) and room temperature (**l, m, n**), for the wild type, W184F and Y16F FMO complexes, respectively. The oscillation traces are fit to the product of two cosine functions and two exponential decays (solid black lines in each figure). The corresponding Fourier spectra are plotted on top of the experimental data. The fits are obtained with a 95% confidence interval and fit standard error <0.004 at 77K and <0.006 at room temperature.

Fig. 4. Selective pump-probe data of wild type FMO complex. a, Selective pump-probe map of the wild type FMO complex at 77K. The black arrow in panel **a** indicates the wavelength of pump excitation. Solid lines specify the probe traces reported in Supplementary Fig 4**. b, c,** Selective pump-probe map of the W184F and Y16F FMO mutant complexes at 77K. **d**. Corresponding four-wave mixing diagrams of the bleach signal the representative probe wavelengths.