Light-harvesting in photosynthesis is determined by the excitonic interactions in disordered antennae and the coupling of collective electronic excitations to fast nuclear motions, producing efficient energy transfer with a complicated interplay between exciton and vibrational coherences. In this chapter we apply 2DES to an excitonically coupled bacteriochlorophyll dimer, the B820 subunit of the light harvesting complex 1 (LH1-RC) of R. rubrum G9. Fourier analysis of the measured kinetics and modeling of the spectral responses in a complete basis of electronic and vibrational states allow us to distinguish between pure vibrational, mixed exciton-vibrational (vibronic), and predominantly exciton coherences. Although the B820 dimer is a model system, the approach presented here represents a basis for further analyses of more complicated systems, providing a tool for studying the interplay between electronic and vibrational coherences in disordered photosynthetic antennae and reaction centres.

Parts of this chapter have been published as: M. Ferretti, V. I. Novoderezhkin, E. Romero, R. Augulis, A. Pandit, D. Zigmantas and R. van Grondelle, Physical Chemistry Chemical Physics 16, 9930 (2014) [1]
3. The coherences in the B820 bacteriochlorophyll dimer revealed by two-dimensional electronic spectroscopy

3.1. Introduction

The success of natural photosynthesis is based on two ultrafast processes: excitation energy transfer after light absorption by the photosynthetic light harvesting antenna followed by transmembrane charge separation in the photosynthetic reaction centre [2–4]. Both processes occur with a quantum efficiency approaching unity in spite of the highly disordered nature of these biological systems. In recent years, the role of quantum mechanics, disorder and coherence [5–8] has been proposed to explain the high energy conversion efficiency of the primary steps of photosynthesis.

Two-dimensional electronic spectroscopy (2DES) is a tool to study the presence and the role of quantum coherences in biological complexes [9–11]. In this technique three spectrally broad and ultra-short laser pulses are used to set and detect the coherent superposition of quantum states in the complex. The three pulses are time delayed, and fast Fourier transformation (FFT) with respect to the coherence time \( \tau \) (the time between the first and the second pulse) and with respect to the rephasing time \( t \) (the time between the third pulse and the emitted signal) results in 2D spectra which correlate the absorbed frequency \( \omega_\tau \) with the emitted frequency \( \omega_t \) for a fixed population time \( T \) (the time between the second and the third pulse). The population time dynamics is related to the evolution of quantum coherence between quantum states and to energy transfer. The coherence appears as peak amplitude oscillations during \( T \), whereas energy transfer appears as non-oscillating crosspeaks. Although originally off-diagonal amplitude oscillations were associated with exciton coherence [11] and diagonal amplitude oscillations with vibrational coherences, recently, electronic-vibrational (vibronic) models, with both diagonal and off-diagonal contributions to the oscillations, have been proposed [12–16]. Within the proposed mechanism, vibronic coherences appear when the exciton splitting energy is resonant with vibrational modes, and this resonance is potentially able to sustain, regenerate, or even (re-)create coherences between electronic states during the time scale of energy and electron transfer [12, 13, 15, 17–19]. However, due to the complexity of the exciton manifold of most of the photosynthetic complexes, the nature of the observed coherences has not been unambiguously determined yet.

The B820 pigment–protein complex is a good candidate for testing the technique and the theoretical models describing coherence in biology due to its simple exciton manifold. The B820 subunit consists of a protein-bound bacteriochlorophyll (BChl) dimer which is the basic building block of the light-harvesting 1 (LH1) pigment–protein complex from purple bacteria [20–22]. This pigment–protein subunit consists of a heterodimer of one \( \alpha \) and one \( \beta \) polypeptide which non-covalently binds the two BChl pigments [23]. The B820 dimer exciton manifold is described by four exciton states: one ground state, two one-exciton states absorbing at 795 nm and 820 nm, and one double exciton state (Fig. 3.1). The exciton state at 795 nm is almost dark and it appears as a shoulder in the steady state absorption spectrum whereas the exciton state at 820 nm is super-radiant [23] (Fig. 3.1). The exciton split-
3.1. **Introduction**

The energy of the B820 dimer (400–500 cm\(^{-1}\)) is comparable to the frequencies of the most dominant intramolecular BChl vibrational modes (as observed in a transient absorption experiment on mutants of the bacterial reaction center\(^{[24]}\)). Moreover, the absorption intensity of the higher energy exciton component is of the same order of magnitude as the intensities of the vibrational satellites in the blue wing of the absorption in this complex. Thus, the B820 is an excellent model system to investigate the interplay between exciton and vibrational coherences. The energetic disorder in B820, caused by slow protein motions (with respect to the ultrafast dynamics probed by 2DES), is large enough\(^{[25–27]}\) to create a spread of the apparent exciton splitting from 400 to 800 cm\(^{-1}\), which can become resonant with the most intense vibrational modes.

**Figure 3.1:** Top left frame: expected pigment–protein arrangement of the B820 subunit adapted from the crystal structure of the reaction center light-harvesting complex 1 of *Rhodopseudomonas palustris*\(^{[24]}\). Top right frame: the B820 exciton energy level scheme without vibrational coupling. Bottom frame: in red, the B820 room temperature steady-state absorption spectrum and in black, the laser spectral profile.

Here, we have measured the 2D spectra of B820 at room temperature and studied the population time dynamics, in order to understand the nature of the coherences. A Fourier transformation with respect to the population time \(T\) is applied in order to select only those spectral features oscillating with particular frequencies during the population time. The amplitude oscillations reflect quantum coherences within the system which can have exciton, vibrational or vibronic origins. We have
applied a model based on a two-pigment Hamiltonian in the complete diabatic basis of electronic and vibrational states to calculate the linear and non-linear (2D) spectral responses. The model enables us to explore the interplay between exciton and vibrational coherences for a single realization of the disorder, and to reproduce the experimentally obtained disorder-averaged 2D frequency maps. We find the simultaneous presence of exciton and vibrational coherence and estimate the degree of exciton-vibrational mixing.

3.2. EXPERIMENTAL RESULTS

3.2.1. 2D ELECTRONIC SPECTRA

The 2D real rephasing spectra, for \( T \) equal to 20, 100, 220 and 500 fs, are shown in Fig. 3.2. The signal along the diagonal is dominated by a positive band at 815 nm which corresponds to the contributions of ground state bleach (GSB) and stimulated emission (SE) from the 820 nm super-radiant state. The almost-dark state at 795 nm is undistinguishable in the 2D spectra in agreement with its low amplitude in the steady state absorption spectrum shown in Fig. 3.1. The off-diagonal peaks are both negative and correspond to the intrinsic lineshape of the rephrasing 2D spectrum and to the excited state absorption (ESA).

![Figure 3.2: 2D real rephasing spectra at population time (T) equal to 20, 100, 220 and 500 fs at room temperature.](image)

The (superposition) states created by the three laser pulses and their rephasing can be described by double-sided Feynman diagrams. These diagrams allow us to assign certain processes to specific locations \((\lambda_\tau, \lambda_t)\) in the 2D spectra. With the Feynman diagrams (SUPPLEMENTARY) it is possible to show that the peak at (830,795)
3.2. **Experimental results**

nm corresponds to the excitation from the one-exciton 820 nm state to the double-exciton \( f \) state (where both monomers are excited), whereas the peak at (804,845) nm corresponds to an excitation from the 795 nm state to the \( f \) state (see SUPPLEMENTARY for 2D total real spectrum).

### 3.2.2. 2D Traces and Quantum Beats

The 2D traces (Fig. 3.3, top-left frame), the real rephasing amplitude as a function of population time \( T \), are analyzed in order to study the dynamic evolution of the spectra. The traces show a bi-exponential decay modulated by oscillations. The origin of the multi-exponential decay is due to several relaxation processes, which occur on different time scales, e.g. excitonic relaxation in hundreds of femtoseconds and vibrational relaxation in a few picoseconds [28]. The presence of amplitude oscillations, the so called quantum beats, is a signature of quantum coherence.

![figure](image)

**Figure 3.3:** Top-left frame: population time \( (T) \) dynamics of representative 2D real rephasing peaks. Top-right frame: quantum beats obtained after subtraction of the bi-exponential decay from the traces shown in the top frame. Some of the traces have been vertically translated for better visualization. Bottom frame: Power spectral density of the quantum beats indicating the different oscillation frequencies of the system. For a list of the most prominent frequencies see Table 3.1

The traces are fitted with a bi-exponential decay which is then subtracted from the original traces. The resulting residues, which are the quantum beats, are shown in Fig. 3.3 (top-right frame). A fast Fourier transformation (FFT) is applied to the
residues in order to evaluate the oscillation frequencies contained in the quantum beats. The power spectral density (PSD) of the FFT is shown in Fig. 3.3 bottom, and the corresponding frequencies are reported in Table 3.1. For the interpretation of the frequencies and the nature of the coherences see the modelling of 2D frequency maps section below.

### 3.2.3. 2D REAL REPHASING FREQUENCY MAPS

The analysis of the 2D traces presented in the previous section is applied to the whole 2D spectrum, i.e., to all the different $(\lambda_T, \lambda_I)$ combinations. The result is represented by 2D frequency maps, each of them corresponding to different $\omega_T$ frequencies, instead of being associated with different time $T$ (as is the case for the 2D spectra). These maps show only the state super-positions which oscillate with a certain frequency $\omega_T$ during $T$. Four maps corresponding to $\omega_T$ equal to 345, 416, 546 and 735 cm$^{-1}$ are shown in Fig. 3.4. Note that the $\omega_T$ resolution is about 90 cm$^{-1}$.

The low frequency map at 345 cm$^{-1}$ shows an intense diagonal peak at 820 nm and two weaker off-diagonal peaks at (830, 810) nm and at (810, 830) nm. In the maps corresponding to frequencies near the exciton splitting (400–550 cm$^{-1}$) the below-diagonal peaks at (800, 830) nm becomes dominant. At higher frequencies, such as 735 cm$^{-1}$, the amplitudes of both diagonal and off-diagonal peaks are comparable, and the off-diagonal peaks show complicated structures. Even though the 735 cm$^{-1}$ frequency exceeds the expected exciton splitting energy, exciton coherence can still appear in realizations of the disorder with significant asymmetry between the two BChl molecules, for instance, realizations with an energy difference of 200 cm$^{-1}$ between the two site energies. In this way the disorder can produce a mixed exciton-vibrational or predominantly exciton coherence at any frequency. To understand the origin of the coherences, we model the third-order response of the system in the basis of electron-vibrational eigenstates, taking into account the disorder in the site energies and the coupling of single vibrational modes to the exciton manifold of the system.

### 3.3. MODELLING

#### 3.3.1. EXCITON-VIBRATIONAL HAMILTONIAN

To study the interplay of electronic and vibrational coherences in the B820 dimer we use a model containing two sites (diabatic states) coupled to a single vibrational mode. The system (exciton-vibrational) Hamiltonian in the site (diabatic) representation is:
3.3. Modelling

\[
H_{\text{ex-vib}} = H_g + H_e + H_f
\]

\[
H_g = |g\rangle \left[ \Omega \sum_{s=x,y} \left( \frac{1}{2} \left( \Delta^s_g \right)^2 + \left( \beta^s_1 \beta_s + \frac{1}{2} \right) - \frac{1}{\sqrt{2}} \Delta^s_g \left( \beta_s + \beta^s_1 \right) \right) \right] |g\rangle
\]

\[
H_e = \sum_{n=1,2} |n\rangle \left[ \omega_{n0} + \Omega \sum_{s=x,y} \left( \frac{1}{2} \left( \Delta^s_n \right)^2 + \left( \beta^s_1 \beta_s + \frac{1}{2} \right) - \frac{1}{\sqrt{2}} \Delta^s_n \left( \beta_s + \beta^s_1 \right) \right) \right] |n\rangle
\]

\[
H_f = |f\rangle \left[ \omega_{10} + \omega_{20} + \Omega \sum_{s=x,y} \left( \frac{1}{2} \left( \Delta^s_f \right)^2 + \left( \beta^s_1 \beta_s + \frac{1}{2} \right) - \frac{1}{\sqrt{2}} \Delta^s_f \left( \beta_s + \beta^s_1 \right) \right) \right] |f\rangle
\]

where \( H_g \), \( H_e \) and \( H_f \) represent the Hamiltonian for none, one or both monomers excited, respectively.

The basic states are given by a direct product of the electronic wavefunctions (ground \( |g\rangle \), one-exciton \( |n\rangle \), two-exciton \( |f\rangle \)) and vibrational wavefunctions \( |a_s\rangle \) depending on the effective nuclear coordinates \( s = x, y \). Note that \( x \) and \( y \) do not represent spatial coordinates, but the displacement in two independent directions. The basis wavefunctions \( |a_s\rangle \) are unshifted, i.e., have zero displacement along the \( x \)- and \( y \)-coordinates. The creation and annihilation phonon operators \( \beta^s_1 \) and \( \beta_s \) work on this unshifted basis. The displacements of the electronic surfaces along the \( s \)-coordinates \( (\Delta^s_g, \Delta^s_n, \Delta^s_f) \) are accounted for by the shifting operators \( \Omega \Delta^s \left( \beta_s + \beta^s_1 \right) / \sqrt{2} \), where \( \Omega \) is the vibrational frequency. Excitation of the diabatic state \( n \) corresponds to the \( g \rightarrow n \) transition with the electronic transition dipole \( d_n \) and zero-phonon transition energy \( \omega_{n0} \). The interaction between the diabatic states is given by the energy \( M_{12} \) that is supposed to be independent of the vibrational coordinates. The two-exciton manifold consists of a single state corresponding to excitation of the two sites, i.e., \(|f| = |1, 2\rangle\). Diagonalization of the Hamiltonian gives the exciton-vibrational (vibronic) eigenstates:

\[
H_g \Phi^g = \Phi^g E^g; \quad |c\rangle > \sum_a C^g_{ac} |g, a\rangle; \quad E^g_{cc'} = \delta_{cc'} \omega_c
\]

\[
H_e \Phi^e = \Phi^e E^e; \quad |b\rangle > \sum_{n,a} C^e_{n,ab} |n, a\rangle; \quad E^e_{bb'} = \delta_{bb'} \omega_b
\]

\[
H_f \Phi^f = \Phi^f E^f; \quad |r\rangle > \sum_a C^f_{ar} |f, a\rangle; \quad E^f_{rr'} = \delta_{rr'} \omega_r
\]

where \(|a\rangle = |a_x, a_y\rangle\) is the product of the vibrational wavefunctions corresponding to the \( x \)- and \( y \)-coordinates; \(|g, a\rangle, |n, a\rangle, \) and \(|f, a\rangle\) denote a product of the electronic and vibrational wavefunctions. The transition dipoles between the ground, one- and two-exciton vibronic manifolds (denoted as \( c, b \), and \( r \), respectively) are:
The coherences in the B820 bacteriochlorophyll dimer revealed by
two-dimensional electronic spectroscopy

\[ d_{bc} = \sum_{n,a} C_{n,ab}^e d_n C_{ac}^g \]
\[ d_{rb} = \sum_{n\neq m} C_{ar}^f \left( d_m C_{n,ab}^e d_n C_{m,ab}^e \right). \]

Note that a similar approach has been previously employed to model electron transfer coupled to a coherent vibrational motion along two nuclear coordinates in the bacterial reaction centre [29], and recently the same Hamiltonian was used to explore the structure of the electronic and vibrational coherences in the 2D spectral responses of a molecular dimer [16, 30]. In the present modelling we use displacements \( \Delta x_g, \Delta y_g = \Delta/2-1, -1 \) for the ground state \(|g>\); \( \Delta x_1, \Delta y_1 = \Delta/21, -1 \) and \( \Delta x_2, \Delta y_2 = \Delta/2-1, 1 \) for the excited states \(|1>\) and \(|2>\); and \( \Delta x_f, \Delta y_f = \Delta/21, 1 \) for the two-exciton state \(|f>=\ |1,2>\). Note that the relative displacements for the \(|1> \rightarrow |1,2>\) and \(|2> \rightarrow |1,2>\) transitions are the same as for the \(|g> \rightarrow |2>\) and \(|g> \rightarrow |1>\) transitions, respectively. The minima of the potential surfaces of the diabatic states 1 and 2 are displaced (with respect to each other) along the \( x-y \) direction. Therefore, the nuclear motion along this direction is affected by the mixing between the diabatic states. Note that the dynamics along the \( x-y \) direction corresponds to anticorrelated nuclear motion within the states 1 and 2.

3.3.2. EXCITON-VIBRATIONAL STRUCTURE OF THE ABSORPTION SPECTRUM

We consider a symmetric dimer with equal unperturbed site energies \( \omega_{10} = \omega_{20} \). The static disorder is modeled by uncorrelated shifts of the site energies randomly taken from a Gaussian distribution with full width at half maximum (FWHM) of \( \sigma = 650 \) cm\(^{-1}\). The interaction energy \( M_{12} \) was varied from -250 to -300 cm\(^{-1}\). These values are close to the parameters determined from a quantitative fit of the B820 non-linear transient absorption (pump–probe) spectra [24]. The angle between the two transition dipoles \( \alpha \) was varied from 0\(^{\circ}\) to 30\(^{\circ}\). First, we calculate the absorption spectra of one specific realization of the disorder with the site energies \( \omega_{10} = \delta \omega/2 \) and \( \omega_{20} = \delta \omega/2 \), where \( \delta \omega = 400 \) cm\(^{-1}\). The stick absorption spectra of this specific realization coupled to three different vibrational frequencies are shown in Fig. 3.5. The calculation is restricted to the low-temperature limit, when transitions occur only from the lowest vibrational level of the ground state. As a result, the absorption components can be characterized by a single index \( b \) corresponding to the number of one-exciton vibronic state (the vibronic states are numbered in increasing order of their energies).

The \( \omega = 300 \) cm\(^{-1}\) top frame in Fig. 3.5 illustrates the situation when the vibrational frequency that couples to the excitonic manifold is lower than the exciton splitting (2\( M_{12} = 500 \) cm\(^{-1}\)). In this case the spectrum consists of an intense zero-phonon line (\( b = 1 \)) of the lowest exciton component accompanied by a vibrational
3.3. Modelling

**Figure 3.4:** B820 2D frequency maps at $\omega_T = 345, 416, 546$, and $735$ cm$^{-1}$; experimental (left frame) and calculated (right frame) maps. The calculation is performed at room temperature, averaging over disorder ($\sigma = 650$ cm$^{-1}$), with $\alpha = 251$ cm$^{-1}$ and $M_{12} = -270$ cm$^{-1}$. The excited states are coupled to a single vibrational mode, taken from a specific manifold as discussed in the text. In the calculated maps the energy is counted from the unperturbed zero-phonon energies $\omega_{10} = \omega_{20}$, which correspond to the electronic excitation of a BChl monomer from the lowest vibrational level of the ground state to the lowest vibrational level of the excited state (without disorder).

satellite (containing two almost degenerate $b = 2, 3$ levels). The $b = 3$ level has a symmetric wavefunction (see insets in Fig. 3.5) with the maxima oriented along the $x + y$
3. The Coherences in the B820 Bacteriochlorophyll Dimer Revealed by Two-Dimensional Electronic Spectroscopy

**Figure 3.5:** Top frame: calculated absorption spectra of a dimer with $\delta \omega = 400 \text{ cm}^{-1}$ and $\Omega = 300 \text{ cm}^{-1}$ (top), 450 cm$^{-1}$ (middle), and 750 cm$^{-1}$ (bottom). All the spectra are normalized to unity. Blue curves correspond to parallel dipole moments ($\alpha = 0^\circ$), whereas red curves show additional components (or increase in amplitudes) appearing at $\alpha = 30^\circ$. The parameters of the model are: $M_{12} = -250 \text{ cm}^{-1}$ (both for $\alpha = 0^\circ$ and $30^\circ$), low temperature limit (only the lowest vibrational level of the ground state is populated), equal displacement $\delta = 0.7$ for all the vibrational frequencies, and equal line-width of 10 cm$^{-1}$ (at HWe) for all the absorption components. The energies on the abscissa are counted from the unperturbed $\omega_1 = \omega_2$ value. The insets show the shape of the wavefunctions (plotted as contour lines in the $x,y$ plane) for the exciton-vibrational levels corresponding to the most intense components of the absorption spectra, i.e., $b=1,2,3,7$ ($\Omega = 300 \text{ cm}^{-1}$), $b=1-5$ ($\Omega = 450 \text{ cm}^{-1}$), and $b=1-6$ ($\Omega = 750 \text{ cm}^{-1}$). Bottom frame: the vibronic level scheme normalized to the corresponding vibrational frequency $\Omega$. The zero phonon line of the lower exciton component (i.e., level 1) is shown in green; the first vibrational satellites of the lower exciton component (levels 2 and 3) are shown in red; the levels of the higher exciton component are shown in blue. Dashed lines refer to anticorrelated wavefunctions, whereas continuous lines to correlated ones. The pure vibrational levels (level 3) are all at the same energy (after normalization to $\Omega$). The higher the frequency $\Omega$, the larger the shift of the level 2 from the pure vibrational level 3. When $\Omega$ exceeds the exciton splitting ($\Omega = 750 \text{ cm}^{-1}$) the level 2 becomes predominantly excitonic (shown in blue in the $\Omega = 750 \text{ cm}^{-1}$ level diagram).

direction. This direction (indicated in the inset of Fig. 3.5) corresponds to a correlated nuclear motion in the sites ($n = 1$ and 2), i.e., motion along the $x = y$ line. The $b = 3$ level is shifted from the lowest transition exactly by $\Omega$ and the dipole strength is independent of the frequency $\Omega$ and the mutual dipole orientation $\alpha$, differently from the other vibronic states. On the other hand, the second ($b = 2$) level corresponds to the ‘anticorrelated’ direction $x - y$, that is affected by the mixing of the two sites. This direction (indicated in the inset of Fig. 3.5) corresponds to a motion along the $x = -y$ line. In the disordered case ($\delta \omega = 400 \text{ cm}^{-1}$ in our example), the $b$
2 wavefunctions are asymmetric reflecting exciton mixing between the sites with different energies. In addition, the mixing changes the shape of the potentials in the \( x\text{–}y \) direction and produces vibronic levels with smaller splitting as compared to the original frequency \( \Omega \). At low frequencies, \( \Omega \ll 2M_{12} \), the \( b = 2 \) and \( b = 3 \) levels have the same intensity and almost the same energy. Increasing the vibrational frequency \( \Omega \) results in an increasingly bigger splitting between the \( b = 2 \) and \( b = 3 \) levels and increasingly larger dipole strength for the \( b = 2 \) level due to exciton mixing. Further to the blue from the \( b = 2, 3 \) pair we find the lowest vibronic state of the higher exciton component (\( b = 7 \)) that is relatively weak (due to the small angle between the dipoles). Disorder increases the splitting between the main vibronic levels of the two exciton components (\( b = 1 \) and \( b = 7 \)) and makes the higher component allowed even for \( \alpha = 0^\circ \).

The \( \Omega = 450 \text{ cm}^{-1} \) middle frame in Fig. 3.5 shows the situation when the vibrational frequency approaches the exciton splitting. In this case the anticorrelated component of the first vibrational satellite (\( b = 2 \)) becomes more intense and shifted from the correlated one (\( b = 3 \)), and contains some degree of exciton mixing (depending on the angle between the dipoles as can be seen from the small, but distinguishable difference between the red and the blue curves). The upper exciton component is split into two sublevels (\( b = 4 \) and \( 5 \)). The value of the splitting as well as the intensities of these levels increases with the disorder value \( \delta \omega \). Consequently, in this case the stick absorption spectrum consists of one pure vibrational level (correlated vibrational component \( b = 3 \)), one exciton-vibrational level (anticorrelated vibrational component \( b = 2 \)) and two predominantly exciton components (\( b = 4, 5 \)).

The lower frame in Fig. 3.5 corresponds to \( \Omega = 750 \text{ cm}^{-1} \). This vibrational frequency exceeds the exciton splitting, and the order of the upper exciton and vibrational components is opposite to the low-frequency case. Close to the main zero-phonon line (\( b = 1 \)) we find a single and predominantly exciton level corresponding to the upper exciton component. In fact this is the anticorrelated \( b = 2 \) component, but strongly transformed and red-shifted due to significant exciton contribution. Other predominantly exciton components (\( b = 4 \) and \( b = 5, 6 \)) are similar to the \( b = 4 \) and \( 5 \) levels in the \( \Omega = 450 \text{ cm}^{-1} \) case, but they are less intense and more split. In this case the absorption spectrum can be treated as consisting of the lower exciton component (with the \( b = 1 \) origin and \( b = 3 \) vibrational satellite) and a higher exciton component (with the \( b = 2 \) origin and \( b = 5,6 \) satellites, where \( b = 5 \) is the anticorrelated and \( b = 6 \) is the correlated component as can be seen from their wavefunctions). Disorder increases the splitting between these states.

### 3.3.3. EXCITON AND VIBRATIONAL COHERENCES

In order to illustrate the interplay of exciton and vibrational coherences in the 2D spectra we will show the positions of the main components oscillating at a certain frequency in the 2D frequency maps. The calculation is restricted to the low temper-
The coherences in the B820 bacteriochlorophyll dimer revealed by two-dimensional electronic spectroscopy

ature limit and to coherent dynamics (i.e., relaxation between exciton-vibrational components is not included). We calculate the intensities of the rephasing stimulated emission (SE) components oscillating with the frequencies $\omega_{b'b'} = \omega_{b'} - \omega_{b'}$ on the $(\omega_r, \omega_t)$ plane, where $\omega_r = \omega_{b'0}$ is the excitation frequency (corresponding to transition from the lowest $c = 0$ level of the ground state to the exciton-vibrational level $b'$ of the excited-state manifold) and $\omega_t = \omega_{bc}$ is the emitting frequency (emission from the excited state $b'$ to the state $c$ of the ground state). The coherence between the $b$ and $b'$ states can have purely vibrational, mixed exciton-vibrational, or purely exciton origin. The degree of exciton mixing $P_{coh}$ present in the $b-b'$ coherence can be defined as:

$$P_{coh}(b, b') = \left| \sum_a C_{1,ab}^{e} C_{2,ab'}^{e} \right| + \left| \sum_a C_{2,ab}^{e} C_{1,ab'}^{e} \right|$$

where the maximal value $P_{coh} = 1$ corresponds to a pure exciton mixing with complete delocalization over the two sites. For a purely vibrational coherence without any exciton mixing $P_{coh}$ is equal to zero. In Fig. 3.6 we consider three cases with $\Omega = 300$, 450, and 750 cm$^{-1}$ (with the same parameters as in the examples shown in Fig. 3.5).

In the $\Omega = 300$ cm$^{-1}$ case (Fig. 3.6, top frame), the coherence between the $b = 1$ and $b = 3$ levels $(1,3)$ produces four intense SE peaks oscillating with the frequency $\Omega = 300$ cm$^{-1}$. The structure of this quadruplet is exactly the same as for the SE of a single electronic transition coupled to one vibrational mode. The $(1,2)$ coherence produces a very similar quadruplet, but with a slightly different oscillation frequency, i.e., 278 cm$^{-1}$. The $(1–3)$ and $(1–2)$ coherences correspond to coherent nuclear motion along correlated $x+y$ and anticorrelated $x−y$ coordinates, respectively (as can be seen from the shapes of the wavefunctions shown in Fig. 3.5). The degree of exciton mixing is $P_{coh}(1,3) = 0$ and $P_{coh}(1,2) = 0.17$, corresponding to a pure vibrational and a mixed exciton-vibrational coherence, respectively. The $(1–7)$ coherence yields two intense excitonic peaks with $P_{coh}(1,7) = 0.88$ and with symmetric positions with respect to the diagonal in the 2D frequency map. The oscillation frequency in this case is 680 cm$^{-1}$ (which is significantly larger than the exciton splitting, due to the inclusion of the energy difference $\delta \omega = 400$ cm$^{-1}$) that accounts for one realization of the site energy disorder). This oscillation frequency will of course be different in other realizations of the disorder. There is also coherence between the $b = 2$ and 7 levels with $P_{coh}(2,7) = 0.34$ and 402 cm$^{-1}$ oscillation frequency. This duplet is not purely excitonic, has a smaller amplitude, and its position is shifted along the antidiagonal direction (i.e., one peak is near the diagonal and the other is below the diagonal). Both $(1–7)$ and $(2–7)$ coherences correspond to a motion along the $x−y$ coordinate (Fig. 3.5).

At $\Omega = 450$ cm$^{-1}$ we obtain two vibrational quadruplets $1–3$ and $1–2$ (now with a larger difference in oscillation frequencies as compared to the $\Omega = 300$ cm$^{-1}$ case), two symmetric exciton duplets $1–4$ and $1–5$ (oscillating at 670 and 837 cm$^{-1}$), and
one exciton duplet 2–5 with antidiagonal shift (oscillating at 447 cm\(^{-1}\)). The \(P_{\text{coh}}(1,2)\) value for the anticorrelated vibration has increased indicating larger exciton mixing, whereas the \(P_{\text{coh}}(1,4)\) value for the most intense exciton pair is reduced. Thus, the exciton and vibrational degrees of freedom are more strongly mixed when the vibrational frequency is near the excitonic splitting.

At \(\Omega = 750\) cm\(^{-1}\) the anticorrelated vibrational quadruplet 1–2 becomes more ex-
citonic than vibrational with $P_{\text{coh}}(1,2) = 0.6$. Its contribution to the signal oscillates with a frequency of 505 cm$^{-1}$, which is close to the exciton splitting (and far from the original 750 cm$^{-1}$ vibration). In addition, this quadruplet becomes more intense than the purely vibrational quadruplet 1–3. Moreover, the upper pair of the 1–2 peaks is much more intense than the lower pair, so the anticorrelated quadruplet appears to be more similar to a typical exciton duplet (symmetric with respect to the diagonal). There are minor peaks with a lower degree of exciton mixing, i.e., the exciton quadruplet 1–5 ($P_{\text{coh}}(1,5) = 0.47$) and the exciton duplet 2–5 ($P_{\text{coh}}(2,5) = 0.40$), oscillating with high frequencies (1214 and 709 cm$^{-1}$). There are also two minor purely vibrational quadruplets (due to $b = 2–3$ and 1–6 coherences) oscillating at frequencies 245 and 1255 cm$^{-1}$ corresponding (approximately) to $\Omega - 2M_{12}$ and $\Omega + 2M_{12}$.

We conclude that coupling to just a single vibration produces a manifold of coherent peaks of different physical nature (purely vibrational, exciton-vibrational, and predominantly excitonic) oscillating with different frequencies, depending on the disorder-induced shifts of the electronic transition energies[30, 31]. Thus, the 2D frequency map for any particular frequency may contain contributions from all (or at least many) vibrational modes coupled to the electronic transitions in a dimer. As a rough approximation we can collect all these contributions by calculating the 2D spectra of a dimer coupled to a single vibrational mode, and repeat this for a sequence of different vibrational frequencies. In such an approximation we neglect the effects produced by a combined action of many modes. However such a model allows the reproduction of the main features observed in 2D frequency maps, at frequencies that are below, near, and above the exciton resonance in the B820 dimer.

### 3.3.4. Modeling of 2D Frequency Maps

Now we switch to the modelling of the B820 experimental 2D frequency maps, obtained after Fourier transformation of the measured 2D spectra with respect to the population time. Fig. 3.4 shows the 2D maps at 345, 416, 546, and 735 cm$^{-1}$ oscillation frequencies together with the calculated maps. The calculated data include all the components, i.e., SE, ground-state bleaching (GSB) and excited-state absorption (ESA) averaged over disorder and over all possible orientations of the complex. Initial ground-state populations correspond to room temperature. In the excited state we restrict to coherent dynamics, i.e., we do not consider the relaxation dynamics between different vibronic states. Without relaxation we cannot calculate the homogeneous broadening, so the line shapes are represented by some phenomenological line shapes. We consider the coupling to a single vibration, but its frequency is taken from the manifold of vibrational modes with frequencies $\Omega = 195.345.416.546.735.970.1100.1360.1600$ cm$^{-1}$, and displacements $\Delta = 0.51.0.86.0.43.0.46.0.83.0.63.0.67.0.66.0.69$. Note that in the single-mode model, the vibrational frequency should correspond to the oscillation frequency, i.e., to reproduce the 2D map, for example at 345 cm$^{-1}$, we need to include the 345 cm$^{-1}$ vibration. Shifting of the vibrational
frequency by a few cm\(^{-1}\) from 345 cm\(^{-1}\) will result in some sizeable changes in the 2D map for the 345 cm\(^{-1}\) oscillation frequency. The displacements of vibrational modes listed above correspond approximately to the shape of the MD/QC spectral density for the B850 \(a/\beta\) dimer\(^{32}\), but we have increased the coupling for the 735, 970, and 1360 cm\(^{-1}\) modes according to the Fourier amplitudes of the measured 2D kinetics. Note also that intense peaks near 735, 970, and 1360 cm\(^{-1}\) are present in the measured spectral density for other complexes, for instance, for LHCII (see for a comparison of these spectral densities our modelling of bacterial LH2\(^{33}\)). After calculating the 2D spectra with contributions from all these frequencies (calculated sequentially and added) we perform a Fourier transformation of the resulting coherent dynamics and plot the 2D frequency maps for the 345, 416, 546, and 735 cm\(^{-1}\) oscillation frequencies. Thus, the calculated 2D frequency maps are compared to the measured ones in Fig. 3.4. Notice that oscillations at any particular frequency, for example at 416 cm\(^{-1}\), contain not only contributions from the original 416 cm\(^{-1}\) vibrational mode but also contributions from mixed exciton-vibrational or pure exciton coherences produced by coupling to other modes (and oscillating at frequencies near 416 cm\(^{-1}\) within a window of about 10 nm corresponding to phenomenological decay of the coherences that is supposed to be in the 0.5–1 ps range in our modeling). Thus, coupling to 970, 1100, 1360, and 1600 cm\(^{-1}\) modes produces a lot of predominantly exciton coherences in the 400–550 cm\(^{-1}\) region. Oscillations at 345 cm\(^{-1}\) (that is lower than the exciton splitting) are determined mostly by vibrational coherences from the 345 cm\(^{-1}\) mode and minor contributions from higher frequency modes. The structure of these minor contributions is illustrated by the low-frequency 245 cm\(^{-1}\) vibrational quadruplet produced by the coupling of the exciton manifold to the 750 cm\(^{-1}\) vibrational mode as shown in the bottom frame of Fig. 3.6.

At low frequencies (345 cm\(^{-1}\)) the most intense peak is the diagonal vibrational one. At the frequencies near the exciton splitting (416 and 545 cm\(^{-1}\)) there are several components with significant exciton-type mixing that give symmetric off-diagonal peaks and some peaks shifted below the diagonal. Due to exciton contributions the diagonal peak becomes less intense, whereas the peak below the diagonal becomes dominant (due to specific excitonic peaks shifted below the diagonal and also due to the cancelation of the excitonic SE and ESA components above the diagonal). At 735 cm\(^{-1}\) there is still a significant contribution from the exciton peaks appearing in realizations with significant asymmetry of the two molecules (thus increasing the apparent splitting between exciton components up to 735 cm\(^{-1}\)). The average degree of exciton mixing \(P_{\text{coh}}\) is maximal for the off-diagonal peaks of the 2D frequency maps shown in Fig. 3.4, \(i.e., \langle P_{\text{coh}} \rangle = 0.21, 0.55, 0.63, \text{ and } 0.41 \) for the off-diagonal peaks of the 345, 416, 546, and 735 cm\(^{-1}\) maps, respectively. For lower (195 cm\(^{-1}\)) and higher (970 cm\(^{-1}\)) frequencies the maximal \(P_{\text{coh}}\) value drops to 0.07 and 0.10, respectively. Thus, the relative increase of the amplitude of off-diagonal peaks, with respect to the
diagonal peak, is a general feature reflecting the presence of exciton-type coherence. On the other hand, the amplitudes of the diagonal and off-diagonal peaks in the 2D frequency maps are very sensitive to the parameters of the model, in particular to the line shapes. We have found that predominantly vibrational coherence can produce 2D maps where the diagonal peak is the most intense or maps dominated by a peak below the diagonal, i.e., appearing very similar to the exciton-type peaks. Therefore, the only possibility to fully distinguish between exciton and vibrational coherences is to perform a consistent fit of all the 2D kinetics or 2D Fourier-transformation maps at various frequencies. Such an analysis as performed here for the B820 complex allows us to reveal the simultaneous presence of different types of coherences, i.e., pure vibrational, mixed exciton-vibrational coherence, and predominantly exciton coherence.

3.4. DISCUSSION

2DES is a powerful tool to study both the static and the dynamic properties of the exciton manifold in photosynthetic pigment–protein complexes. Static features such as the spectral distribution of the excited state absorption band can be studied from the population time spectra as shown in Fig. 3.2. In fact 2DES, differently from transient absorption, is a selective technique, meaning that the excited state absorption (ESA) contribution to the signal is not necessarily superimposed on the ground state bleaching or on the stimulated emission contributions. However the 2D rephasing spectra also contain contributions from the intrinsic lineshape, therefore for the study of such features it is better to consider the total real spectra (see SUPPLEMENTARY). The spectral distribution of ESA can be extrapolated from the spectra without any deconvolution, for example the (820,790) nm peak corresponds to ESA from the super-radiant state. This negative peak is centered at 790 nm with a broadening of about 40 nm (in the real total spectrum). This result is in full agreement with the spectral deconvolution of the transient absorption data[34].

The dynamics can be extrapolated from the T dependency of the traces and from the frequency maps. In the traces, the fact that the oscillations last for more than 600 fs is a signature of vibrational or vibronic coherences. In fact, pure electronic coherences should de-phase in a few hundred femtoseconds[28, 30]. The frequencies in the power spectral density reported in Table 3.1 were previously found in transient absorption[35] and in 3 Photon Echo Peak Shift[36] experiments. These frequencies, which are between 100 and 750 cm⁻¹, reflect pure vibrational and mixed exciton-vibrational coherences. In the latter case the exciton interaction between the two sites does not create a significant mixing of the electronic states, but changes the shape of the excited-state potential surface, thus reducing the vibronic frequency. The exciton coherence contribution becomes significant for the oscillating peaks corresponding to frequencies near the exciton splitting value (400–550 cm⁻¹).

Significant exciton coherences are also present at higher frequencies, i.e., up to
700–800 cm\(^{-1}\), being determined by realizations of the disorder with a large energy gap between the two pigments (which increases the apparent value of the exciton splitting). Thus, in the region of 400–800 cm\(^{-1}\) the exciton splitting can become resonant with the coherent vibrational motion (depending on the realization of the disorder), producing a non-trivial interplay between the vibrational and exciton degrees of freedom (accompanied by specific spectral signatures which appear in the linear absorption and in the 2D frequency maps).

Our study of the B820 subunit demonstrates that coherence transfer occurring in a system with quasi-static conformational disorder and with coupling of exciton states to a manifold of vibrations, generally produces a rich dynamic picture with a variety of coherences varying from purely vibrational to mixed and to predominantly exciton coherences. Note that in the calculated frequency maps, the maximum averaged degree of exciton mixing \(\langle P_{\text{coh}} \rangle\) is 0.63, meaning that the long lasting oscillations are never due to pure exciton coherences. Assignment of these types of coherences is a challenging problem. We show that the frequency analysis and its representation in 2D frequency maps, in combination with modelling, provide a unique tool which gives new insights into this puzzle. Note that in this model the exciton manifold is coupled to different single vibrational modes, and the exciton-vibrational relaxation is neglected. However this relaxation most likely occurs on the timescale of the measurements and probably it will play a role in the dynamics of the coherences. In order to take into account the exciton-vibrational relaxation, the interaction with all the vibrational modes should be included in the model. This would allow the calculation of the homogeneous broadening and the corresponding decay rate of the coherences.

### 3.5. Conclusions

The Fourier analysis of the 2D spectra in combination with modeling based on a two-pigment Hamiltonian in the basis of electronic and vibrational states is applied to study the nature of quantum coherences in the protein-bound BCHl dimer B820, the basic unit of the bacterial light-harvesting complex LH1. We observe the simultaneous presence of different types of coherence, including pure vibrational, mixed exciton-vibrational and predominantly exciton coherences. This causes the complicated 2D-echo responses with long-lived oscillatory components over a wide range of frequencies (with the most intense components between 100 and 750 cm\(^{-1}\)). In conclusion, we present the specific 2D frequency map features which are characteristic of pure vibrational, mixed exciton-vibrational and predominantly exciton coherences.

Differently from previous work we apply a Fourier analysis in combination with modelling which allows us to understand clearly the nature of quantum coherences in the B820 dimer. The understanding of these features is an essential tool aimed at assisting in the assignment of the nature and therefore the role and interplay of
3. The coherences in the B820 bacteriochlorophyll dimer revealed by two-dimensional electronic spectroscopy

electronic and vibrational coherences in other photosynthetic complexes.

3.6. MATERIALS AND METHODS

SAMPLE
The B820 dimer is prepared from the carotenoidless strain R. rubrum G9 as described elsewhere[22]. Samples are in buffer containing 50 mM phosphate buffer pH 7.8, 1.3% β-OG, and 20% (v/v) glycerol.

SPECTROSCOPY
2D electronic spectra are recorded at room temperature with a passively stabilized diffractive optics based set-up, which was described previously in detail[37]. Double modulation lock-in detection is used in order to reduce the noise and to enhance the sensitivity[37]. The coherence time (τ) is scanned from -70 fs to 120 fs, with a step of 1 fs. The population time (T) is scanned from 0 to 100 fs, with a 10 fs step, and from 100 fs to 700 fs with a 20 fs step, in addition, spectra at longer T (1 ps, 5 ps and 10 ps) are recorded. The laser system (PHAROS, Light Conversion) repetition rate is set to 1 kHz. The laser pulses are generated using a home-built non-collinear optical parametric amplifier (NOPA). Each pulse is centered at 820 nm with a full width half maximum (FWHM) of 90 nm in order to excite the QY bands of the dimer, as is shown in Fig. 3.1. The pulse duration is 16 fs, the energy per pulse is 10 nJ and the spot diameter on the sample is 100 μm.

3.7. ACKNOWLEDGEMENTS
M. Ferretti, E. Romero and R. van Grondelle were supported by the Royal Dutch Academy of Sciences (KNAW), the VU University Amsterdam, the TOP grant (700.58.305) from the Foundation of Chemical Sciences part of NWO and the advanced investigator grant (267333, PHOTPROT) from the European Research Council. M. Ferretti, E. Romero and R. van Grondelle thank the support from the EU FP7 project PAPETS (GA 323901). V. I. Novoderezhkin was supported by the Russian Foundation for Basic Research (Grant No. 12-04-01085). R. Augulis and D. Zigmantas acknowledge the support from the Swedish Research Council, The Knut and Alice Wallenberg Foundation, Wenner-Gren Foundations and Crafoord Foundation.
### 3.8. Appendix: Beating Frequencies

<table>
<thead>
<tr>
<th>$\Omega_T$ (cm$^{-1}$)</th>
<th>TA</th>
<th>3PEPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td></td>
<td></td>
</tr>
<tr>
<td>340</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>440</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>553</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>745</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Table 3.1:** Oscillation frequencies of the 2D real rephasing spectra obtained by Fourier analysis of residues in Fig. 2. Almost all of them were previously measured by transient absorption (TA)\([35]\) or by 3 pulse echo peak shift (3PEPS)\([36]\).
REFERENCES


