On the Mixed Upper Exciton State of the Special Pair in Bacterial Reaction Centers

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ABSTRACT: Excitonic interactions between two closely separated bacteriochlorophyll *a* molecules (BChls) in the special pair of the reaction center (RC) of purple bacteria determine the positions and relative oscillator strengths of its two excitonic components. While the absorption of the lower excitonic band is well defined, the position and the intensity of the upper excitonic band (P_{Y+}) are still under debate. Recent 77 K two-dimensional electronic spectroscopy data on *Rba. capsulatus* suggested that the P_{Y+} component absorbs at ~840 nm i.e. at a significantly lower energy than previously suggested. In the present work we argue that the P_{Y+} state is mixed with the excited states of the accessory BChls (B^*/P_{Y+}) leading to excitons contributing to the 785-825 nm spectral region which is consistent with previously published data. This conclusion is based on hole-burning/linear dichroism data and modeling studies of the excitonic structure of the RC using non-Markovian reduced density matrix approach.

INTRODUCTION

The reaction centers from purple bacteria (bRCs), whose first structure was already reported about 30 years ago,[1,2] are still of great interest and widely studied model systems for probing excitonic structure and charge separation.[3–9] The bRC pigments include a strongly coupled "special pair" of bacteriochlorophyll *a* (BChl), i.e. P_A/P_B, collectively called the *P*-band, as well as two additional accessory BChls (B_A and B_B) and two bacteriopheophytins (BPheos) (H_A and H_B), labeled for their respective location on the *A*- and *B*-branches. Despite decades of studies characterizing the photoexcitation dynamics, many questions remain open; for example, neither the excitonic structure and the coupling of various excitons with charge transfer (CT) states nor the role of bath fluctuations are well understood. Due to the presence of strong coupling within the P_A/P_B dimer, the excited state is split into states that are approximately symmetric (+) and antisymmetric (–) linear combinations of $P_A{}^*P_B$ and $P_AP_B{}^*$ denoted as the P_{Y+} and P_{Y-} exciton states of the *P*-band (asterisk indicates an excited pigment). The cofactor arrangement and absorption spectra in a very broad energy range for both *Rba. sphaeroides* and *Rba. capsulatus* (including band assignments) are given in Figures S1 and S2 in the Supporting Information (SI). In this work we focus on the energetic position and oscillator strength of the upper exciton level (P_{Y+}) whose position and oscillator strength are not well established even though its energy has significant implications regarding the nature of the very fast and efficient excitation energy transfer (EET) in photosynthetic bRCs.[10–15] For example, if the energy of the P_{Y+} state is between the *P* and *B* bands[6] or on the low-energy side of the *B*-band,[14,16] the EET from the excited *B** band to the lower excitonic state of the special pair, P_{Y-}, would occur by a two-step mechanism, with P_{Y+} serving as the kinetic intermediate.[6,11,16] In contrast, if P_{Y+} lies at much higher energies, i.e. somewhere between B_A and $H_{A/B}$ bands as calculated in refs 5,12, then P_{Y+} could be the key

acceptor state involved in fast energy transfer from *H* to *P*. However, as we argue below, it is likely that P_{Y+} lies within the *B*-band, and due to *strong mixing* with the B_A/B_B chromophores[17] it is highly delocalized and its direct detection is difficult.

Over the years many researchers have addressed this problem and suggested that the P_{Y+} energy level in bRCs (recently termed P_+*)[6] must contribute to the absorption near 12120 – 12340 cm^{-1} (810-825 nm), although detailed assignments due to its delocalization have proved somewhat problematic,[11,14,16,18] as other experiments on *Rba. sphaeroides* suggested the location of the P_{Y+} band between 12820-12225 cm^{-1} (780-818 nm).[12,19–21] Our previous modeling studies suggested that the large bleach at low temperatures near 810-818 nm is largely due to blue electrochromic shifts of the B_A/B_B cofactors and straightforward assignment of the P_{Y+} state in this region is difficult,[22] most likely due to mixing with other transitions. Nevertheless, up to very recently, a consensus was emerging that low temperature P_{Y+} band lies near 810-815 nm in agreement with linear dichroism (LD),[16] hole-burning (HB)[14] and pump-probe data.[23] The average lifetime of the P_{Y+} exciton (that was never revealed as a "pure" component) based on various theoretical and low temperature experimental data should be on the order of ~50-100 fs.[11,23] For example, Arnett et al. argued that the energy transfer time from P_{Y+} to P_{Y-} in *Rba. sphaeroides* is about 65 fs,[11] but the latter experiment was performed at room temperature and, as a result, the respective energies of these two states in their assignment were shifted to 11494 and 12121 cm^{-1} (870 and 825 nm), respectively, and are not directly comparable with low-temperature data. That is, the correspondence between the low- and room-temperature excitonic states of the *P*-band is not resolved as yet. However, Voss et al.[23] using multicolor transient absorption spectroscopy and differently shaped excitation pulses of 30 fs (both at room-temperature and 15 K) showed that the kinetics of downhill energy transfer are virtually temperature independent. Thus, the kinetics are

likely similar, but the spectral positions may differ. The latter work also showed that upon excitation of P_{Y+} with 820 nm pulses (T = 15 K), internal conversion takes place in about 50-100 fs.[23] These authors also suggested that P_{Y+} strongly contributes to the 800 nm band, or, alternatively, that under these excitation conditions B^* and P_{Y+} states are coherently excited.

Very recently, to solve this conundrum, the Ogilvie group,[6] based on 77 K 2D electronic spectroscopy (2DES) data obtained for the W(M250)V mutant of the *Rba. capsulatus* that lacks the primary acceptor ubiquinone, suggested that the hidden upper exciton state of the special pair lies near 11900 cm^{-1} (~840 nm), i.e. at significantly lower energy than previously suggested.[10,14–16,24] Namely, these authors argued that using 2DES they directly resolved the P_+^* state of the special pair (P_{Y+} in our nomenclature used in this work), which based on their kinetics data rapidly decayed to the lower energy exciton state, P_-^* (= P_{Y-}) in 24 fs.[6] Comparison of the above-mentioned energies of the P_{Y-} and P_{Y+} states is complicated, as the energy of the P_{Y-} band is strongly temperature dependent, i.e. the P_{Y-} band of *Rba. sphaeroides* shifts from 11560 cm^{-1} (865 nm) at room temperature[25] to about 11300 cm^{-1} (885 nm) at 77K[26] and 11144 cm^{-1} (897.3 nm) at 4 K,[12,27] due to the significant charge-transfer character of the P_{Y-} band as a result of mixing with the CT state(s).[28] Unfortunately, the temperature dependence of the special pair (SP) BChls excitonic splitting (P_{Y+} minus P_{Y-}) due to thermodynamic thermal expansion is unknown. In particular, there is no information on the temperature shift of the upper excitonic band P_{Y+}, although it is believed to be weakly coupled with CT states. For example, previously, to account for the large temperature shift of the P_{Y-} band, the excitonic coupling of the special pair BChls was varied with temperature from 618 cm^{-1} at 15 K to 400 cm^{-1} at 298 K.[15] On the other hand, Renger[28] described the temperature dependent blue shift of the *P*-band by considering coupling of the optical excitation with an intermolecular CT state coupled non-linearly with the vibrational bath. The

latter work, however, used a simplified model of a special pair dimer where only the lowest energy transition P_{Y-} was considered.

As mentioned above, recent 77 K 2DES experiment in *Rb. capsulatus* demonstrated the presence of a weak and short lived P_{Y+} state near 11900 cm^{-1} (840 nm) assigned to the upper exciton band.[6] Very recently Konar et al.[4] suggested that their two-color 2D electronic spectroscopy data (see Figure 2B in ref 4) also support this assignment. Although, in our opinion the origin of the short-lived (24 fs) state near 840 nm is not clear, we question its assignment to the P_{Y+} state since there is a large collection of literature data that quite convincingly demonstrated that the P_{Y+} component in WT bRCs is likely mixed and contributes at low temperatures to the 810-820 nm region and that its position is weakly temperature-dependent. Moreover, the blue shift of the B_B state in the D_{LL} mutant of *Rhodobacter capsulatus* (which lacks an electron transfer acceptor H_A), led to a decrease of its overlap with P_{Y+}, which in turn led to the appearance of the larger negative LD signal at 813 nm, believed to be largely contributed by P_{Y+}[29] (vide infra). Besides, we argue below that a $P \rightarrow P_{Y+}$ transition near 840 nm cannot carry 19% of the oscillator strength of $P \rightarrow P_{Y-}$ as suggested in ref 6. In addition, earlier room temperature experiments, via polarization characteristics suggested that the intensity ratio of P_{Y-} to P_{Y+} is smaller (i.e. approximately 1 to 0.1, i.e. about 10% of the oscillator strength was assigned to the P_{Y+} level).[11] In this assignment, the P_{Y+} band was also placed between the P and B bands,[11] though its energy was significantly blue-shifted in comparison with the energy suggested in ref 6. Stark experiments also suggested that the intensity of the P_{Y+} component is significantly weaker than that of the P_{Y-} band. Nevertheless, the literature data offer no consensus, and the key challenge remains to provide more information on the mixed upper exciton state of the special pair (and its oscillator strength) at low

temperatures. This is attempted below via an analysis (including modeling) of low temperature absorption/nonresonant transient (photochemical) HB spectra and literature[16] linear dichroism (LD) data. We anticipate that our data shown below will shed more light on the elusive P_{Y+} level.

EXPERIMENTAL AND THEORETICAL METHODS

Experimental methodology. Details about the experimental setup were described elsewhere.[30] Briefly, absorption and hole-burned (HB) spectra were measured by a Bruker HR125 Fourier transform spectrometer. For all absorption and nonresonant HB spectra a spectral resolution of 4 cm^{-1} was used. The laser source (λ_B = 488.0 nm) for nonresonant HB spectra was produced by a Coherent Innova 200 argon ion laser. Laser power was set by a continuously adjustable neutral density filter. Experiments were performed at 4 K inside an Oxford Instruments Optistat CF2 cryostat. Sample temperature was read and controlled with a Mercury iTC temperature controller. The glass-forming solution was 55:45 (v/v) glycerol:ethylene glycol. Samples were diluted 40:60 (v/v) with a buffer:glass solution.

Excitonic calculations. Our modeling method is based on excitonic calculation using Frenkel Hamiltonian and non-Markovian reduced density matrix approach.[31–36] Briefly, site energy disorder is taken into account via Monte Carlo simulations. We adopted spectral density, site-distribution function (SDF) and zero phonon line widths as well as Huang-Rhys factor values for special pair pigments P_A and P_B from the ref 37 and adjusted corresponding values to take into account the effect of excitonic delocalization on electron-phonon coupling parameters and inhomogeneous broadening.[34] Many relevant parameters including pigment-pigment and pigment-CT coupling constants, lineshape function and spectral densities for monomeric BChls, BPheos

and CT states are taken from refs 5 and 28. For details of our theoretical approach see the Supporting Information.

RESULTS AND DISCUSSION

Transient HB spectra. We demonstrate first that if the P_{Y+} band appears in the 820-840 nm spectral range, its oscillator strength must be significantly smaller (in both *Rba. capsulatus* and *Rba. sphaeroides*) than that suggested in ref 6. Figure 1 shows absorption (curve a) and transient $P^+Q_A^-$ HB spectra (curve b) for the W(M250)V mutant of *Rba. capsulatus*, i.e. the same mutant as that studied by the above mentioned 2DES experiments.[6] (Both spectra were measured at T = 4 K). Special care was taken to obtain perfect glasses for low temperature HB experiments to minimize scattered light that often affects the baseline of many low temperature absorption spectra obtained for bRCs. Curve b (with a hole depth at *P*-band of 20%) is obtained at burning frequency (ν_B) of 20492 cm^{-1} (λ_B = 488.0 nm) with a laser intensity of 300 mW. Curve b′ is the inverted and scaled curve b to fit the shape of the P_{Y-} band, clearly demonstrating that the entire P_{Y-} state is bleached. Importantly, no clear bleach associated with the P_{Y+} state is observed near 820-845 nm. Assume for the moment that the assignment of the P_{Y+} state near 820-845 nm with an oscillator strength of ~19% relative to P_{Y-} (as suggested in ref 6 at 77 K) also holds for 4 K. Then its contribution in this energy range (even if we allow for about 10 nm blue spectral shift due to a different species or lower temperature) should carry about 19% of the P_{Y-} oscillator strength and, more importantly, the corresponding bleach should be observed in our transient nonresonant HB spectra. Figure 1 shows that this is not the case; that is, the optical density in between the *P* and *B* bands is very weak and there is no clear band in the 820-845 nm spectral range. Besides,

previous[5,12] and current modeling studies for *Rba. sphaeroides* (vide infra) show that the above region is largely contributed to by BChl vibrations.[5,12]

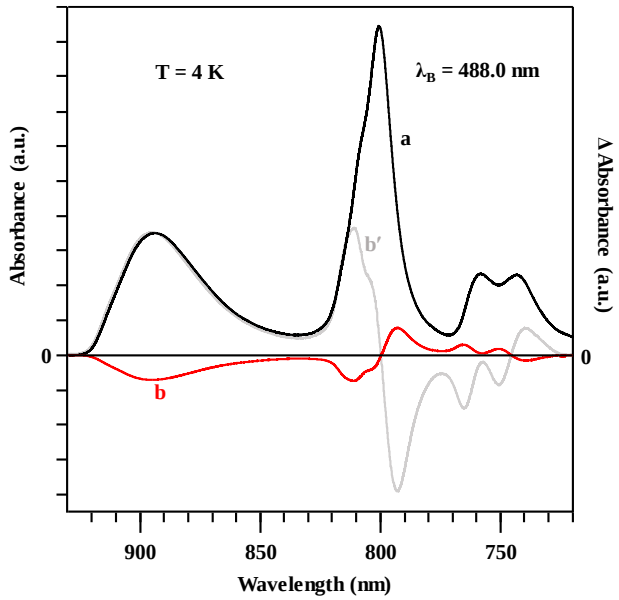

Figure 1. Spectra a and b are the absorption and transient HB $P^+Q_A^-$ spectra, respectively, obtained at 4 K for the W(M250)V mutant of the *Rba. capsulatus*. Curve b (with a hole depth at the *P*-band of 20%) is obtained using λ_B = 488.0 nm (ν_B = 20492 cm^{-1}) with laser intensity of 300 mW. Curve b′ (gray) is the inverted and scaled curve b to fit the shape of the *P*-band.

We note also that it is widely believed that this mutant (with valine in position M250 instead of tryptophan, which is located near Q_A) leads to a Q_A-less bRC. However, as shown in Figure 1, we observe a relatively deep (~20%) transient $P^+Q_A^-$ hole, which clearly suggests that in our sample (which is from the same batch as that studied in ref 6) not all Q_A cofactors were removed. Assuming that electron transfer on the *B*-side in this mutant is only ~2-3%[7] and the spectrum is very different from the spectrum expected for ^3P,[38,39] the observed bleach must be

largely assigned to $P^+Q_A^-$ formation. This could explain why the data reported in ref 6 are quite noisy, as not all forward processes $P^+H_A^- \rightarrow P^+Q_A^-$ are blocked and a relatively large fraction of *P* spends a significant time in the photooxidized state unable to be excited and perform charge separation. Discussion of resonant (persistent/transient) HB spectra obtained for the W(M250)V mutant is beyond the scope of this work and will be published elsewhere.

Since there is no obvious bleach that could be assigned to P_{Y+} state near 820-845 nm in Figures 1, we suggest that either the P_{Y+} state does not contribute in this spectral range or its oscillator strength *is negligibly small*. Thus, it is likely, as suggested in the literature, that P_{Y+} in *Rba. sphaeroides* contributes somewhere in the 800-820 nm spectral region and is strongly mixed with B_B/B_A chromophores.[17] We will return to this issue in the modeling part of this work described and discussed briefly below.

Because the X-ray structure of the RC of *Rba. capsulatus* is not available, modeling studies are performed using the X-ray structure of the RC of *Rba. sphaeroides*[40] which are believed to be very similar (see SI for details). However, before we discuss the results of our modeling studies for *Rba. sphaeroides*, Figure 2 shows the corresponding absorption (curve a) and transient $P^+Q_A^-$ HB spectra (curve b) measured at T = 4 K. Curve b (with a hole depth at P_{Y-} band of 30%) is obtained at burning frequency (ν_B) of 20492 cm^{-1} (λ_B = 488.0 nm). As expected, also in this case there is no clear bleach of the P_{Y+} band (near 825-845 nm), and such a state (even if present) could not carry ~19% of oscillator strength (in comparison to P_{Y-}); vide supra.

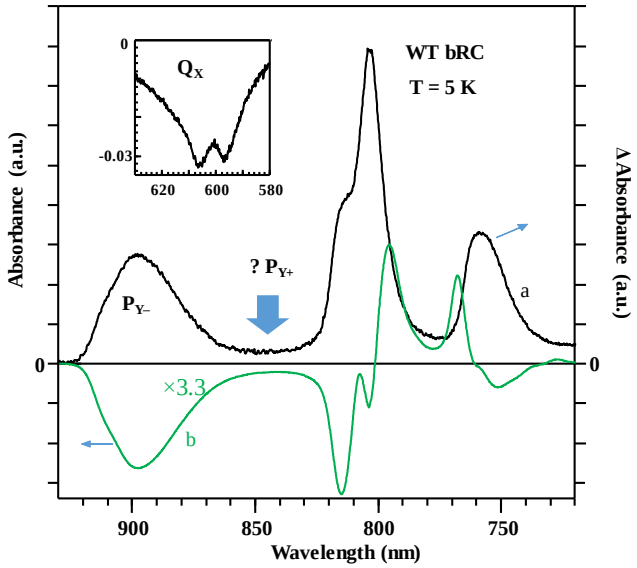

Figure 2. Spectra a and b are the absorption[12] and transient HB $P^+Q_A^-$ spectra, respectively, obtained for wild type of *Rba. sphaeroides* at 4 K (spectral resolution of 4 cm^{-1}). Curve b was obtained for the long lived (~100 ms) bottleneck $P^+Q_A^-$ state using burning wavelength of λ_B = 488 nm (ν_B = 20492 cm^{-1}) with a laser intensity of 150 mW. The fractional hole depth at the *P*-band is about 30%. The inset shows the corresponding bleach in the Q_x-region.

Special pair dimer calculations. The conclusions regarding the P_{Y+} state reached above (based on the experimental low temperature absorption and transient HB spectra) are consistent with our simplified excitonic calculations obtained only for the SP dimer P_A/P_B of *Rba. sphaeroides* using the non-Markovian reduced density matrix approach.[31–36] In this case we focused on the SP lower- and higher-energy excitonic states, and the shapes of the corresponding LD and HB spectra. This is illustrated in Figure S3 in the SI. Here, for brevity we only note that the lower- and higher-exciton states (i.e. P_{Y-} and P_{Y+} states, respectively) should be observed (bleached) for a broad range of the special pair coupling matrix elements, i.e. 350-650 cm^{-1}.[5,12,41,42] That is, if the P_{Y+} state indeed lies in the vicinity of 820-845 nm, the P_{Y+} bleach should be clearly

observed in experimental HB spectra (i.e. in spectra b shown in Figures 1 and 2), though its intensity may change depending on the extent of mixing with other cofactors not included in these simple models. Since no clear bleach is observed in our experimental transient HB spectra discussed above, we find it unlikely that the upper energy exciton level (P_{Y+}) lies near 820-845 nm. (Note that in our transient holes bleaches as small as 0.1% can be easily distinguished; data not shown). Besides, $V_{PA/PB}$ of about 300 cm^{-1} (estimated for the P_{Y+} lying near 840 nm) seems to be too small, as the well-accepted value for *Rba. sphaeroides* at low temperatures is about 600-700 cm^{-1}.[5,12,41] Even larger $V_{PA/PB}$ (~910-1030 cm^{-1})[43,44] were suggested for *Rba. viridis*. Although the center-to-center distance between P_A and P_B is almost the same in the crystal structures for the two species, $V_{PA/PB}$ is larger in *Rba. viridis* because the two BChls overlap in ring I.[42]

Excitonic calculations for all chromophores of the RC including CT states. To further support our conclusion, we consider below all six chromophores of the RC of *Rba. sphaeroides* and two dark CT states: $P_A^+P_B^-$ (CT1) and $P_A^-P_B^+$ (CT2),[44] with CT1 state having a lower energy as reported in ref.[45] Results of our excitonic calculations are illustrated in the Figure 3. That is, the left panel of this figure shows our best simultaneous fits (solid lines) to the low temperature absorption, LD, and transient HB spectra (filled spectra) for $V_{PA/PB}$ = 625 cm^{-1}. Despite inclusion of two CT-states, which borrow intensity from *P** band,[44,46] we had to scale transition dipole moments of P_A and P_B molecules, as often done in the literature.[5,12,47] The origin of decrease in transition dipole moment for *P*-band in Q_y region might be also a result of intensity borrowing by other BChl transitions (Q_x, B_x, B_y). The best fits are obtained when the value of the transition dipole moments was decreased by a factor of 1.25 when normalized to the transition dipole moment of the B_A/B_B pigments (Table S1 in the SI). The Hamiltonian and the parameters used to obtain best

description of all three spectra shown in the left panel of Figure 3 are given in Table S1 and S2. Here, the key question is whether or not it is possible to fit the above mentioned three experimental spectra, including the *P*-band intensity, without scaling the transition dipole moments of P_A and P_B. Concerning the fits, the answer is yes, as shown in Figure S4 of the SI. However, to obtain reasonable fits $V_{PA/PB}$ must be small (about 325 cm^{-1}) and the difference between site energies of P_A and P_B must be very large (i.e. $\Delta E = P_A - P_B = 1130\ cm^{-1}$). For such parameters the contribution from CT1 and CT2 states to P_{Y-} band is zero, as illustrated in Tables S3 and S6 in the SI. Moreover, the delocalization of the lowest-energy exciton would be only 1.2. We find these parameters to be unphysical. For more detail see Tables S1, S2, S3, S5 and S6 in the SI.

Many $V_{PA/PB}$ values and site energies of P_A and P_B were tested but the best fits, as mentioned above, are obtained for $V_{PA/PB}$ of 625 cm^{-1} (see left panel of Figure 3; solid lines). Note that our Hamiltonian (Table S2 in the SI) provides a very good fit not only to the absorption spectrum, but also to the LD and transient nonresonant HB spectra. Moreover, in our best fit all four BChl molecules have similar site energies and $\Delta E = P_A - P_B = 315\ cm^{-1}$, which is closer to theoretically predicted value of ~500 cm^{-1} for the same bRC.[45,48] We emphasize that the theoretical fit of the LD spectrum of *Rba. sphaeroides* at T < 10 K is reported here for the first time. Interestingly, the LD spectrum is well described, including the tiny feature near 12240 cm^{-1} as indicated by the upward solid arrow in the left middle frame in Figure 3. The calculated LD signal shows two excitonic bands assigned to the SP; i.e. the lower-energy band (P_{Y-}) near 897 nm, with the upper (mixed) excitonic band located near 12280 cm^{-1} (~814.3 nm). This small dip in LD signal was assigned to the P_{Y+} state.[16] The experimental data shown in the right panel of Figure 3 are the same (filled curves) as those in the left panel, and are shown for easy comparison with best fits (solid

lines) obtained for $V_{PA/PB}$ = 300 cm^{-1} as suggested by the 2DES data reported in ref 6. In this case the agreement with experimental data is poor, implying again that it is unlikely the P_{Y+} state (not mixed with B*) lies between the P and B bands with an oscillator strength of ~19% (relative to P_{Y-}).

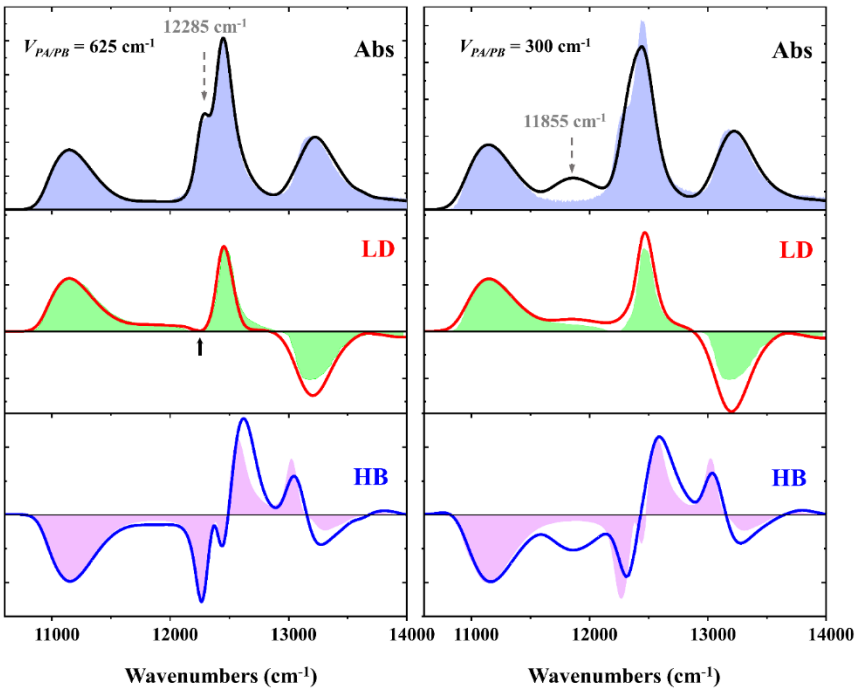

Figure 3. Experimental (filled curves) and corresponding simulated absorption (black curves), LD (red curves) and transient HB (blue curves) calculated for P_A/P_B coupling strength equal to 625 cm^{-1} (left panel) and 300 cm^{-1} (right panel). Solid arrow in the middle left frame shows a dip in LD at ~12240 cm^{-1}. Grey dashed arrows show maxima of an upper excitonic component of the special pair (P_{Y+}) (right frame) or the position of the largest contribution of P_A and P_B to mixed excitons 2-4 (left frame; see also pigment contributions in frame C of Figure 4 and text below for details).

Frames A and E of Figure 4 show the excitonic states of simulated absorption/LD spectra from Figure 3, obtained for coupling matrix elements $V_{PA/PB}$ = 625 cm^{-1}. The same type of data is shown in frames B and F of Figure 4 for simulated spectra from the right panel of Figure 3 with a smaller coupling matrix element (i.e. 300 cm^{-1}). Corresponding pigment contributions to the simulated absorption and LD spectra from Figure 3 with $V_{PA/PB}$ of 625 cm^{-1} (left panels) and 300 cm^{-1} (right panels) are shown in frames C, G and D, H of Figure 4, respectively. The delocalization length (L_M) for exciton 1-6 in frames A and B of Figure 4 are 2.1, 3.0, 2.1, 3.6, 1.1, 1.0 and 1.8, 1.8, 1.3, 1.4, 1.1, 1.0 respectively (see also Tables S5 and S7). Note that the upper exciton state of the special pair (for $V_{PA/PB}$ = 625 cm^{-1}) is delocalized as it is strongly mixed with the excited states of accessory BChls (*B**), though it contributes mostly to the low-energy side of the *B*-band, as often indicated in the literature.[10,15,17] That is, P_A and P_B also contribute to the 2nd, 3rd, and 4th exciton states. This is clearly visible in frame C of Figure 4, which illustrates the contributions from all pigments and CT1 state. The latter contributes 7% to the first exciton state (*M*=1) and 5% to *M* = 2 and 4 states in the absorption spectrum. The contribution of higher-energy CT2 is negligible in the region of interest and, therefore, is not shown (for details see Table S5 in the SI). Note that CT states do not significantly affect P_{Y+} band; however, as expected for an excitonically coupled system, P_{Y+} also borrows intensity from *B** (compare with calculations for the *P*-dimer shown in Figure S3 in the SI). Note that, neglecting mixing with B_A/B_B, the P_{Y+} state would lie near 12410 cm^{-1} (~806 nm). Since the P_{Y+} component is mixed with *B**, its major contribution is more clearly revealed in the calculated LD spectra; i.e. this state lies near 12285 cm^{-1} (814 nm) as shown in the exciton states in frame E of Figure 4 (contributed mostly by bands 2 and 3). The latter value agrees with the largest contribution of P_A and P_B to the absorption at the same energy (see the top left frame in Figure 3 and frame C of Figure 4). Data shown in frame E of Figure 4, with a

positive LD signal for exciton 3, illustrates why the upper excitonic component in the experimental LD spectrum is so weak. That is, the weak feature indicated by a solid arrow below the experimental LD spectrum in the middle left frame of Figure 3 does not reflect the entire P_{Y+} state. Pigment contributions to the LD spectra for different coupling matrix elements discussed in this work are shown in frames G and H of Figure 4.

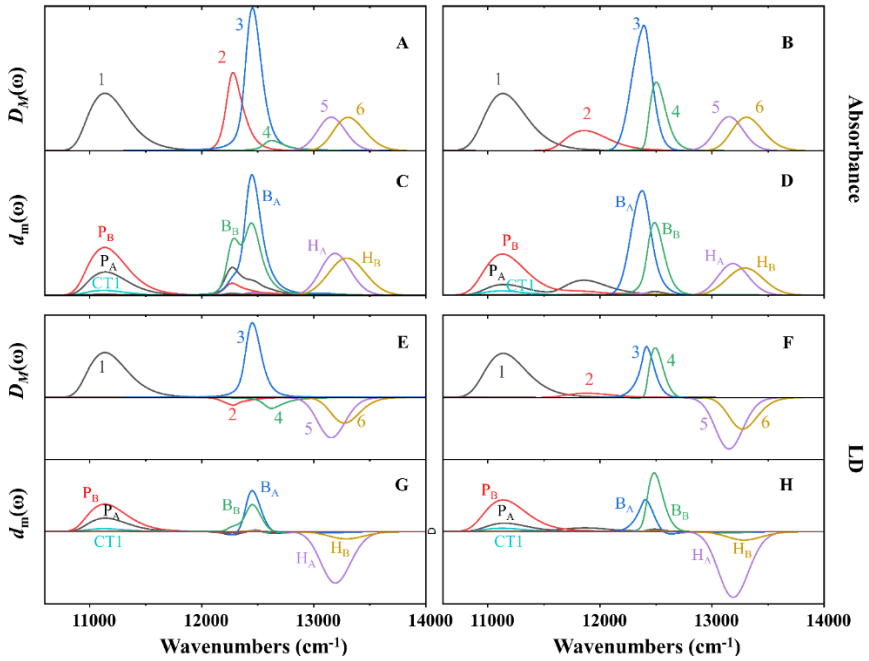

Figure 4. Frames A, B, E and F show six excitonic states ($D_M(\omega)$) of simulated absorption/LD spectra, obtained for coupling matrix elements $V_{PA/PB}$ of 625 cm^{-1} (left panels) and 300 cm^{-1} (right panels), respectively. Corresponding pigment and CT1 contributions ($d_m(\omega)$) to the simulated absorption and LD spectra from Figure 3 with $V_{PA/PB}$ of 625 cm^{-1} (left panels) and 300 cm^{-1} (right panels) are shown in frames C, G and D, H, respectively. The delocalization length (L_M) for exciton 1-6 of absorptions in frames A and B are 2.1, 3.0, 2.1, 3.6, 1.1, 1.0 and 1.8, 1.8, 1.3, 1.4, 1.1, 1.0 respectively (see Tables S5 and S7).

The excitonic states and pigment contributions to simulated absorption and LD spectra for $V_{PA/PB}$ = 300 cm^{-1} (right panels of Figure 3) are given in the right panels of Figure 4. In this case, the dashed arrows in the right frames of Figure 3 indicate a nearly "pure" P_{Y+} state (band 2 in frames B and F of Figure 4 with 87% contribution from P_A/P_B and a maximum near 843 nm). That is, in this case the P_{Y+} state very weakly mixes with B_A or B_B as summarized in Table S7. We do not discuss these data in detail as visual inspection of data in the right frames of Figure 3 (poor fits), as well as the contribution numbers ($|c_{mM}|^2$) of pigments *m* to excitonic states *M* reported in Table S7 in the SI suggest that $V_{PA/PB}$ must be much larger than 300 cm^{-1}, as argued above. Summarizing, our Hamiltonian reveals that the P_{Y+} state is strongly mixed with B_A and B_B, contributing mostly to the 2nd, 3rd, and 4th excitons that are largely delocalized (see Table S5) with contribution from P_A/P_B of 29%/12%, 6%/1%, and 30%/13% respectively. Thus, it is not surprising that historically it has been difficult to directly identify the P_{Y+} level explicitly.

Thus, the key question is whether the results of our modeling study are in agreement with earlier experimental findings. Indeed, earlier low temperature LD studies of Breton et al.[16] and HB studies by the Small group[14,49] obtained for *Rba. sphaeroides*, as mentioned above, are consistent with data shown in Figures 1 and 2, and left panels in Figure 3 and 4. Mikhailyuk et al.[10] also concluded (via analysis of absorption spectra of *Rba. sphaeroides* by higher order derivative spectroscopy) that the P_{Y+} band must have a maximum near 810 nm. The Scheer and Meech groups also found P_{Y+} near 805-810 nm (at 8 K) in refs 17,20,21, while Gorokhov[50] placed P_{Y+} near 809 nm. Importantly, Small's group showed[51,52] that there was a large degree of correlation between the P_{Y-} state and a low-energy shoulder (LES) near 811 nm (using a different host matrix) of the *B*-band to which the P_{Y+} state contributes via mixing with B_A and B_B chromophores, in good

agreement with our calculations (see also Table S2). That is, only one feature of the Q_y-region of the transient HB spectra lying higher in energy than P_{Y-} showed observable burning wavelength (λ_B) dependence when resonantly excited across the *P*-band.[51] λ_B-dependence was most pronounced for PVOH host films because of the additional ~120 cm^{-1} of inhomogeneous broadening in comparison to the glass host used in our laboratory. The LES shoulder, in our glassy matrix observed near 813 nm (12300 cm^{-1}) is mostly contributed by exciton 2 and 4 to which P_A/P_B contributes 29%/12% and 30%/13%, respectively. Neglecting delocalization, i.e. mixing with other states, P_{Y+} would carry a broad bandwidth (about 400 cm^{-1}) comparable to that of the P_{Y-} state. Only due to mixing (i.e. delocalization) the response from the special pair upper exciton appears narrower. Thus, our results are consistent with many papers published previously, and we do not think that the significantly red-shifted (and short lived (24 fs) state should be assigned to the state P_{Y+}.

CONCLUSIONS

To summarize, experimental HB spectra suggest that if the P_{Y+} upper exciton level in *Rba. sphaeroides* or *Rba. capsulatus* indeed lies near 820-845 nm,[6] its oscillator strength would have to be extremely small as no P_{Y+} related bleach is observed in that region in our transient HB spectra. Moreover, extremely weak oscillator strength of the P_{Y+} state in the 820-845 nm spectral region is not supported by our modeling studies and/or literature experimental polarization characteristics, where a typically estimated value for P_{Y+} oscillator strength is about 0.1,[11] 0.12,[14] or 0.17[10] relative to P_{Y-}. Based on our modeling, we conclude that P_{Y+} state is strongly mixed with the excited states of the accessory BChls (B^*/P_{Y+}) leading to narrowed excitons 2-4, which contribute to the 785-825 nm spectral region. This assignment is consistent with multiple literature findings[10,11,14–

[17,20,23,50] which clearly suggest that the P_{Y+} state is located within the *B*-band (mostly on the low-energy side of this band with a major contribution near 814 nm; see frames A and C in Figure 4) and serves as an energetic intermediate that facilitates effective excitation energy transfer to the acceptor *P*. Haran et al. also argued that agreement between theory and experiment is achieved if the P_{Y+} state is strongly mixed with the excited states of accessory BChls.[17] The lifetime of the mixed B^*/P_{Y+} states (for excitons 3 and 4) gives values of ~70 fs, which is in good agreement with experimental data.[11,23] However, the key question remains what is the origin of the short-lived (24 fs) state observed near 11900 cm^{-1} by 77 K 2DES electronic spectroscopy and assigned to P_{Y+}. We suggest tentatively that the 24 fs lifetime might correspond to a short-lived unidentified CT state in the excited state of the *P*-band. Interestingly, a similar (very short) lifetime of 29 fs was recently observed at room temperature 2DES data obtained for a similar variant of the bRC of *Rba. sphaeroides* also lacking the primary acceptor ubiquinone.[3] In this case, however, the first species associated spectrum was assigned to P^* at the Franck-Condon point, and the very short 29 fs lifetime was assigned to $P^* \rightarrow P_A^+P_B^-$. This room temperature data, however, did not reveal the P_{Y+} state in total absorptive 2D spectra, and, as a result this state has not been discussed. We anticipate that our data, as well as the very recent 2DES results reported in refs 3,6 will guide future experimental efforts and more advanced theoretical calculations to provide more insight into the mixed upper excitonic component of the special pair in these widely studied bRCs.

ASSOCIATED CONTENT:

The Supporting Information (SI) contains: 1) Cofactor arrangement and labeled absorption spectra; 2) Arrangement of cofactors in the vicinity of the mutated side chain Trp M250 in *Rba. capsulatus*; 3) Description of methodologies used; 4) Modeling of optical spectra for the special pair P_A/P_B

dimer using different sets of parameters; 5) Magnitudes of dipole moments of the pigments used in calculations; 6) Simulated absorption, LD and HB spectra (for all cofactors) without scaling the dipole strength of BChls; 7) Hamiltonians; 8) Contribution numbers ($|c_{mM}|^2$) of pigments *m* to excitonic states *M* for the simulated spectra with different sets of parameters.

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Notes

The authors declare no competing financial interests.

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