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Energy transfer dynamics of isolated B800–850 and B800–820 pigment-protein complexes of *Rhodobacter sphaeroides* and *Rhodopseudomonas acidophila*

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We have compared the energy-transfer dynamics of different B800–850 and B800–820 isolated pigment-protein complexes. Picosecond absorption spectroscopy under annihilation-free conditions has been used to measure the energy-transfer rate from BChl 800 to BChl 850/820 at 296 and 77 K. It is found that at room temperature the BChl 800 → BChl 850/820 transfer is very fast (< 1 ps in all studied complexes). At 77 K in the type I B800–850 complex of *Rhodobacter sphaeroides* and *Rhodopseudomonas acidophila* the BChl 800 to BChl 850 transfer is about 1–2 ps, whereas in the other complexes the decay of the BChl 800 excited state occurs within less than 1 ps, also at 77 K. The observed subtle differences between the different complexes give insight into the fine details of energy transfer. The excited state of BChl 850/820 has a long lifetime (≈ 1 ns), but measurements of induced anisotropy reveal very fast energy transfer between more or less identical BChl 850 molecules.

Introduction

The photosynthetic light-harvesting antenna of purple bacteria is a highly organized arrangement of pigment-protein complexes allowing very efficient energy transfer from the periphery of the antenna to the reaction center [1]. The number of different pigment-protein complexes forming the antenna varies from one bacterial species to another. Thus, *Rhodospirillum rubrum* is an example of a species having only one major pigment B880, and an additional minor component B896. *Rhodobacter sphaeroides* has two major complexes B800–850 and B875 as well as the minor B896. *Rhodopseudomonas acidophila* is a representative of the group of bacteria containing three major pigment-protein complexes, B800–820, B800–850, and B875. In all cases the pigment-protein complexes are believed to be arranged in order of decreasing energy towards the reaction center.

The B800–850 light-harvesting complex of *Rb. sphaeroides* was the first membrane-bound photosynthetic antenna complex obtained in purified form [2]. Since then, a similar complex has been isolated from several species of purple bacteria, and extensively studied with a number of spectroscopic techniques. Two spectroscopically slightly different B800–850 complexes, termed type I and type II, respectively, have been identified [3,4]. The type I complex is represented by the complex isolated from *Rb. sphaeroides* and *Rps. acidophila* (strain 7750) and it is characterized by an ap-

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proximately 1:1.5 intensity ratio of the 800 and 850 nm absorption bands. The type II complex has been isolated from *Rps. acidophila* (strain 7050) [5] and is characterized by a near 1:1 absorbance ratio of the two absorption bands.

Based on the results of the spectroscopic studies a model of the minimum functional unit of B800–850 was suggested by Kramer et al. [6]. In this model four BChl 850, two BChl 800 and three carotenoid molecules occupy a basic unit which is composed of two pairs of an α,β polypeptide dimer. Other models involving the same or other aggregation states of α,β dimers have also been suggested by other authors. For instance, such basic units of α,β dimers were proposed to form structures of cyclic hexamers of (B800–850) or dodecamers of (B875/880) which surround the reaction center and direct the excitation energy into the trap [7,8].

When isolated and solubilized by conventional detergents such as lauryldimethylamine *N*-oxide (LDAO), Triton, or lithium dodecyl sulfate (LDS) the basic units aggregate into a highly ordered structure and in some cases several hundred chromophores may be coupled through energy transfer. For instance, the B800–850 complex isolated from *Rb. sphaeroides* has a variable degree of aggregation, sensitive to the relative detergent/protein concentration, which may vary from about 30 BChl 850 molecules in LDS to hundreds of BChl 850's in LDAO [9]. This aggregation could at least in principle be compared with the aggregation of complexes existing in the photosynthetic membrane. However, little is known about the relation between the aggregation of these complexes in the intact membrane and in the detergent-solubilized state.

In order to understand the function of the light-harvesting antenna it is necessary to examine the energy transfer dynamics both in whole membrane preparations, containing the complete photosynthetic apparatus, and in individual pigment-protein complexes. In this paper we report the results of picosecond absorption recovery measurements at room temperature and 77 K on the B800–850 complex of *Rb. sphaeroides* and *Rps. acidophila* and the B800–820 complex of *Rps. acidophila*. The results show that all studied complexes have quite similar overall energy transfer dynamics and that this dynamics is in general agreement with previous observations made for both chromatophores [10,11] and the isolated complex [12]. However, the present study of several complexes allows us to identify a number of subtle differences between the different complexes, which yields additional insight into the fine details of energy transfer of these light-harvesting complexes.

Materials and Methods

The energy transfer dynamics of the following complexes was studied: the type I B800–850 complex of *Rb. sphaeroides* and *Rps. acidophila* (strain 7750), B800–850 type II of *Rps. acidophila* (strain 7050), and the B800–820 complex of *Rps. acidophila* (strain 7050). Procedures for their preparation can be found in Refs. 2 and 5.

Wavelengths and temperatures at which the energy transfer kinetics of these complexes were studied are summarized in Table I. Thus, the B800–850 type I complex of *Rps. acidophila* was studied by exciting and probing both in the 800 and 850 nm absorption bands, at room temperature as well as at 77 K. For the other three complexes meaningful kinetics could not be obtained at 77 K when exciting and probing in the 850/820 nm band, due to the accumulation of

TABLE I

SUMMARY OF STUDIED SYSTEMS AND EXPERIMENTAL CONDITIONS

r.t. = room temperature; X means studied in this work.

complex	Wavelength and temperature			
	r.t. 800 nm band	77 K 800 nm band	r.t. 850/820 nm band	77 K 850/820 nm band
B800–850 [a] *Rb. sphaeroides*	×	×	×	–
B800–850 type I *Rps. acidophila*	×	×	×	×
B800–850 type II *Rps. acidophila*	×	×	×	–
B800–820 *Rps. acidophila*	×	×	×	–

[a] See also Ref. 12.

long-lived photoproducts which efficiently quenched the excited state of the final emitter BChl 850/820, and thereby shortened the lifetime drastically. This effect has previously also been observed for the B800–850 complex of *Rb. sphaeroides* at room temperature [12]. The observation by Rafferty et al. [13] that intense continuous illumination of *Rb. sphaeroides* R26 chromatophores bleaches the 850 nm absorption band is probably a manifestation of the same process; it was attributed to the photooxidative loss of BChl 850 antenna chlorophyll.

Measurements of isotropic and anisotropic absorption recovery kinetics were performed as previously described, by using a high repetition rate (800 kHz) synchronously pumped and cavity-dumped dye laser system, operating in the near-infrared wavelength region (700–910 nm) [10]. For the room temperature measurements the concentrated detergent-solubilized sample was diluted with plain buffer solution (Tris) to yield an absorbance of about 0.3/mm. A high flow speed of the sample was mimicked by using a rotating cell [10], which renewed the irradiated sample volume for every excitation pulse. The low-temperature measurements were performed with a stationary sample in a liquid-N_2 cryostat. The measured decay curves were analyzed as a sum of exponentials, and the associated lifetimes and amplitudes were obtained by using a non-linear least-square fitting program. In cases where very short (< 20 ps) decay components were observed a deconvolution procedure was used to obtain the decay parameters [11]. In this way lifetimes equal to or longer than 1 ps could be determined accurately, although 10 ps long laser pulses were used. This fact is demonstrated by the measured recovery kinetics of a cyanine dye molecule in Fig. 1. The decay time of this molecule was determined to be 5 ps by direct measurement using 300 fs pulses at 575 nm. When using 20 ps excitation and probing pulses the deconvolution procedure yields a time constant of 4 ps, which is quite close to the actually measured one. This result shows that time constants larger than approx. 1 ps can be obtained with this method.

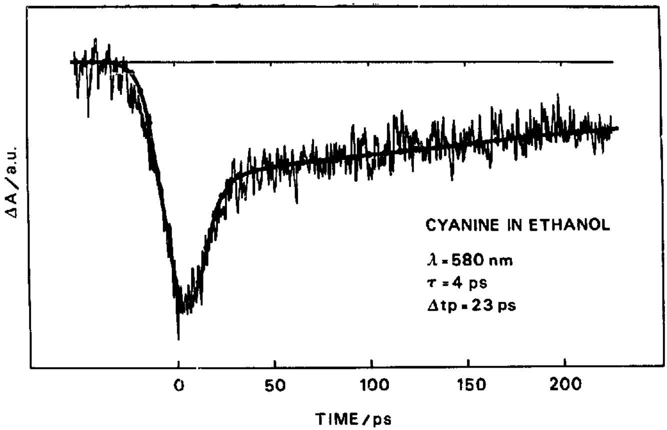

Fig. 1. Absorption recovery kinetics of 1,1′-diethyl-4,4′-cyanine at 580 nm measured with a 23 ps long pulse. The solid line is a theoretical fit including deconvolution with a Gaussian pulse having the same width at half maximum as the measured pulse autocorrelation function.

Results and Discussion

All studied complexes have the same general energy transfer pattern. However, from the observed differences it is possible to divide them into two different classes. The type I B800–850 complexes appear to form one category distinct from the B800–820 and B800–850 type II complexes. The similarities and differences between the two groups will be illustrated in the following discussion.

The general decay pattern common to all complexes is illustrated by the isotropic recovery curves of Fig. 2 for the B800–B850 type I complex of *Rps. acidophila* and the B800–820 complex. Excitation at 77 K and probing at about 800 nm results in an initial bleaching which decays very quickly into an absorption which in turn decays on a much slower timescale. At room temperature the initial bleaching has a much lower intensity, in fact it is only clearly distinguishable in the decay curve of the B800–850 type I complex (Fig. 2A). Similar decays were earlier also observed at the same wavelength in chromatophore preparations [11], and the very fast decay was then attributed to BChl 800 → BChl 850 energy transfer. Since this kind of decay is present in all examined complexes this assignment now appears to be well documented. We assign the much slower nanosecond decay to the quenching of the excited state of the final emitter BChl 850/820. Similar nanosecond lifetimes have previously been observed in the

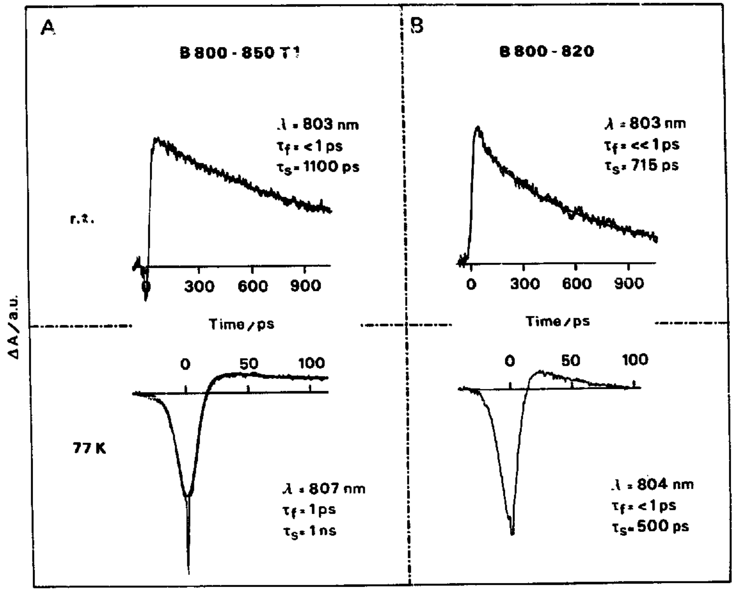

Fig. 2. Isotropic absorption recovery kinetics around 800 nm, measured at 77 K and room temperature. (A) B800–850 type I of *Rps. acidophila*. (B) B800–820 of *Rps. acidophila*.

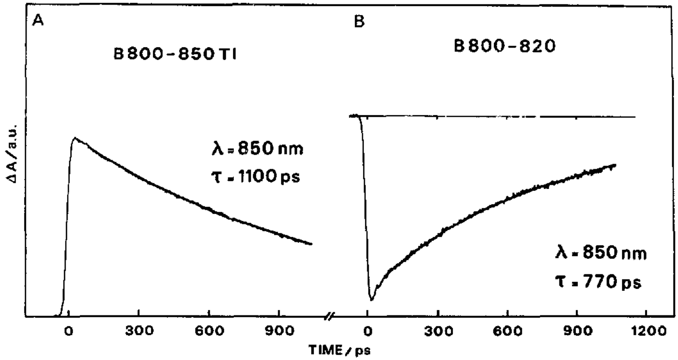

Fig. 3. Isotropic kinetics at 850 nm measured at room temperature for (A) B800–B850 type I; (B) B800–820.

energy transfer kinetics of a number of different isolated pigment-protein complexes of purple bacteria [12,14,15]. Consequently, such nanosecond lifetimes appear to be typical of the quenching of BChl excited states of purple bacteria antenna complexes. The slow decay of the BChl 850/820 excited state is much more pronounced in the decay curves measured within the absorption band of BChl 850/820, where the decay is observed as an intense excited state absorption or a strong bleaching (see Fig. 3).

A detailed analysis of the decay curves of Fig. 2, including deconvolution with the pulse autocorrelation function, reveals a clear difference in the initial fast time constant between the two groups of complexes; the BChl 800 → BChl 850 transfer time at 77 K in the type I complex is clearly resolvable and amounts to $\tau_f = 2 \pm 1$ ps, whereas the corresponding time constant of the B800–820 complex is too short to be resolved. The latter is estimated to be in the range 0.5–1 ps at 77 K. Previous studies of energy transfer dynamics in chromatophores of *Rb. sphaeroides* [10,11] suggested that the BChl 800 → BChl 850 transfer rate is temperature-dependent. This is also clearly demonstrated by comparing the decay curves of Fig. 2 obtained at room temperature and 77 K. At room temperature (r.t.) the initial decay is too fast to be resolved by the deconvolution technique. However, we can use the amplitude of the fast (bleaching) component to estimate the lifetime of the BChl 800 excited state. Thus we obtain the following relation with tentative time constants indicated.

$$\underset{1\text{–}2\ \text{ps}}{\text{B800–850 TI(77 K)}} > \underset{0.5\text{–}1\ \text{ps}}{\text{B800–820 (77 K)}} > \underset{\approx 0.5\ \text{ps}}{\text{B800–850 TI (r.t.)}}$$

$$> \underset{< 0.5\ \text{ps}}{\text{B800–820 (r.t.)}}$$

In an attempt to explain the difference in energy transfer rate between the B800–850 type I and the B800–820 complexes we calculated the spectral overlap integral between the fluorescence of BChl 800 and the absorption of BChl 850/820. The result of this calculation suggests that the overlap, and thus the rate of Förster energy transfer, is at least a factor of three higher in the B800–820 complex as compared to the B800–850 complex. Consequently, this could explain the observed differences in energy transfer rates.

Similarly, the observed temperature dependence of the BChl 800 → BChl 850/820 transfer rate can be attributed to a temperature-induced change in the spectral overlap.

Petrich, J.W., Breton, J. and Martin, J.L. (private communication) also measured the rate of BChl 800 → BChl 850 energy transfer in the isolated B800–850 complex of *Rb. sphaeroides* by exciting BChl 800 with very short 150 fs pulses ($\lambda = 797$ nm), and probing the rise of the BChl 850 excited state at 855 nm. It was concluded that the transfer occurs within less than 100 fs. This result appears to be in contradiction to our results. However, it is important to note that the femtosecond experiments were performed with very high pulse intensity which caused extensive excitation annihilation to occur. Thus, instead of its normal approx. 1 ns lifetime the BChl 850* was seen to decay with an approximate time constant of 2.4 ps. Such an extensive quenching of the excited state will most likely result in an apparent shortening of the risetime of the BChl 850 excited state. The situation is even further complicated by the fact that some BChl 850 is also directly excited at 797 nm. Thus, the rise of the absorption signal due to BChl 850* will have a contribution from an instantaneous rise in addition to the contribution from the BChl 800 → BChl 850 transfer. To resolve the true energy transfer time (expected to be 0.5–1 ps) from such a mixed signal is probably a difficult task.

Upon excitation and probing within the BChl 850/820 absorption band a single long lifetime due to the quenching of the BChl 850/820 excited state is observed. As is seen from Tables II–IV the B800–820 and the type II B800–850 complexes appear to have a somewhat shorter lifetime (τ_s = 700 ps) than the type I B800–850 complexes (τ_s = 1100 ps). It was mentioned above that some of the complexes are exposed to efficient quenching of the BChl 850/820 excited state (see Table I) under experimental conditions favoring accumulation of long-lived photoproducts. It appears that the complexes that are most sensitive to this quenching also have the shortest decay time of the final emitter, in the absence of quenching. Both these observations could be the result of a structural difference in the part of the polypeptide chain where the BChl 850/820 chromophores are situated. A more open structure in this part of the protein would make the BChl chromophores more accessible to external influence which could affect the excited state lifetime in the described manner.

TABLE III

ISOTROPIC LIFETIMES AND LIMITING ANISOTROPY FOR THE B800–820 COMPLEX OF *RPS. ACIDOPHILA* STRAIN 7050

The standard deviations of the nanosecond lifetimes are 10% of the measured value.

Temp.	Wavelength (nm)	τ_f (ps)	τ_s (ps)	$r(0)$	$r(\infty)$
296 K	803	< 0.5	715	0.05	0.05
	808	< 0.5	610	0.04	0.04
	820	–	800	0.07	0.05
	827	< 1	630	0.08	0.08
	850		770	0.11	0.08
77 K	804	< 1	–	0.39	≈ 0

For the type I B800–850 complex of *Rps. acidophila* the lifetime of the BChl 850 excited

TABLE II

ISOTROPIC LIFETIMES AND LIMITING VALUES OF ANISOTROPY FOR B800–850 TYPE I FROM *RPS. ACIDOPHILA* STRAIN 7750

The standard deviations of the nanosecond lifetimes are 10% of the measured value.

Temp.	Wavelength (nm)	τ_f (ps)	τ_s (ps)	$r(0)$	$r(\infty)$
296 K	795	< 1	1195	0.05	0.05
	803	< 1	1110	0.05	0.05
	850	–	1100	0.08	0.07
	870	–	1090	0.11	0.11
	880	–	1050	0.10	0.10
77 K	807	2 ± 1	1000	0.28	< 0.05
	880	–	1250	0.11	0.11

TABLE IV

ISOTROPIC LIFETIMES AND LIMITING ANISOTROPY FOR THE B800–850 TYPE II COMPLEX OF *RPS. ACIDOPHILA* STRAIN 7050

The standard deviations of the nanosecond lifetimes are 10% of the measured value.

Temp.	Wavelength (nm)	τ_f (ps)	τ_s (ps)	$r(0)$	$r(\infty)$
296 K	803	< 0.5	870	0.05	0.05
	808	< 0.5	863	0.06	0.05
	820	–	610	0.07	0.07
	831	–	830	0.07	0.07
	870	–	733	0.11	0.11
77 K	804	< 1	–	0.37	≈ 0
	807	< 1	–	0.41	≈ 0

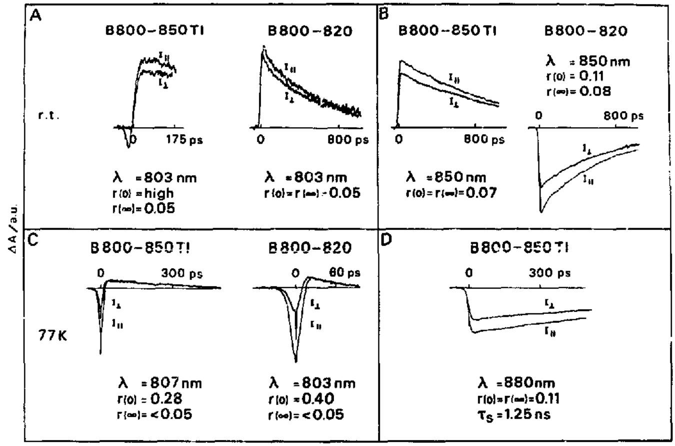

Fig. 4. Absorption anisotropy of the B800–850 type I and B800–820 complexes. (A) 800 nm and room temperature (r.t.); (B) 850 nm and room temperature; (C) 800 nm and 77 K; (D) 850 nm and 77 K.

state was also measured at 77 K (Fig. 4D), and found to be only slightly longer (20%) as compared to the room temperature value.

The time dependence of the induced absorption anisotropy is most easily followed at those wavelengths where a high intensity of the long-lived decay is observed, i.e. in this case in the BChl 850/820 band. Here we have a time window of more than 1 ns to monitor the time evolution of the anisotropy of the BChl 850/820 excitations. For BChl 800 the situation is more difficult, due to the very short excited state lifetime. Only at 77 K are the decay times long enough to allow quantitative measurements of the anisotropy. However, even here the results are limited to an initial, $r(0)$ and final, $r(\infty)$, value of the anisotropy. No reliable time dependence could be obtained at these wavelengths due to the limited time resolution.

The results summarized in Fig. 4 show that the decay of anisotropy in the studied complexes follows the same general behavior as previously observed in chromatophores [10,11] and in the B800–850 complex of *Rb. sphaeroides* at room temperature [12]. This means that excitation and probing in the BChl 800 band is associated with a relatively high anisotropy, $r(0) > 0.2$ at 77 K, whereas excitation and probing in the BChl 850/820 band yields very low anisotropy, $r(0) = r(\infty) < 0.1$. There is seen to be a clear difference in the initial anisotropy between the B800–850 type I and the B800–820 complexes as measured at 800 nm and 77 K (see Fig. 4C). While the maximum anisotropy for the B800–850 type I complex is about 0.25 it comes very close to the theoretical maximum of 0.4 for the B800–820 and B800–850 type II complexes. The lower-than-maximum value of $r(0)$ in the type I complex indicates that depolarizing BChl 800 ↔ BChl 800 energy transfer proceeds on a timescale similar to that of BChl 800 → BChl 850 transfer [11]. If we assume that the BChl 800's of the B800–820 complex are organized in a similar way as those in the B800–850 complex, and that BChl 800 ↔ BChl 800 transfer has about the same rate in both complexes, then the observed difference in anisotropy around 800 nm suggests a higher rate of BChl 800 → BChl 820/850 transfer in the B800–820 and B800–850 type II complexes as compared to the type I complex. This conclusion is in accordance with the results obtained from the isotropic measurements (Fig. 2).

At long times a low value of the anisotropy is observed at 800 nm, $r(\infty) < 0.05$ (Fig. 4). This suggests an efficient depolarization during the BChl 800 → BChl 850/820 and BChl 850/820 ↔ BChl 850/820 transfer steps.

The anisotropy measured within the BChl 850/820 band is independent of time (within the time resolution), $r(0) = r(\infty) = 0.08 \pm 0.02$, and temperature. These results suggest that there is a fast initial energy transfer between adjacent BChl 850/820 molecules, presumably within one minimum unit, resulting in efficient depolarization of the excited states. The fact that $r(\infty) = 0.08$ suggests that the Q_y transition dipole moments of the BChl 850/820 molecules are oriented roughly in one plane, or several parallel planes. The same conclusion was earlier drawn from fluorescence and picosecond absorption depolarization measurements [6,10].

Finally we turn to a peculiar observation made for the B800–820 complex in a narrow wavelength interval around the isosbestic point of absorbance changes due to BChl 820 ($\lambda = 827$ nm). In this wavelength range, where the amplitude of the absorbance changes due to BChl 820 is very low, a pulse limited decay of an initial bleaching is also observed. This type of decay is strongly reminiscent of similar observations previously made for BChl 850 of *Rb. sphaeroides* [10] and BChl 880 of *Rs. rubrum* [11]. Since this kind of kinetics has now been observed for all types of major antenna pigments of purple bacteria it may be suggested that very rapid decays around the isosbestic points of BChl antenna pigments is a general property that reflects some fundamental process common to all pigment-protein complexes.

We have previously suggested two possibilities: (i) spectral inhomogeneity of the BChl absorption band; (ii) spectral shifts due to exciton effects [11]. Since the effect appears in exactly the same way (same relative wavelength position and same kind of signal) in all complexes, it seems likely that the reason is more fundamental than spectral inhomogeneity, which would be expected to show some variation between different complexes. We therefore suggest that the effect arises as a result of breaking exciton interaction after excitation. If the absorption band of the pigment that is excited corresponds to exciton-coupled BChl molecules, the initial excited state is expected to be a collective excitation. The lifetime of such a state is probably very short (< 1 ps) [16,17], and would be expected to decay into a localized excited state, where the energy is localized on a single BChl molecule. Exciton-coupled BChl molecules of light harvesting pigment-protein complexes seem generally to have a red-shifted absorption spectrum relative the spectrum of the non-interacting molecules. A transfer from coupled to less coupled chromophores, induced by exciton localization, is thus expected to be associated with a spectral blue-shift. The very rapid kinetics around the isosbestic wavelengths described above are all consistent with this picture.

Acknowledgements

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