Angewandte Chemie International Edition

DOI: 10.1002/anie.201003771

**Photosynthetic Mechanisms**

# Ultrafast Energy-Transfer Pathway in a Purple-Bacterial Photosynthetic Core Antenna, as Revealed by Femtosecond Time-Resolved Spectroscopy**

*Daisuke Kosumi,* Satoshi Maruta, Tomoko Horibe, Ritsuko Fujii, Mitsuru Sugisaki, Richard J. Cogdell, and Hideki Hashimoto**

The photosynthetic apparatus of purple bacteria is designed to absorb light energy, which is used to power transmembrane charge separation.[1,2] In the primary stages of light harvesting, carotenoids (Car) bound to light-harvesting antenna complexes absorb sunlight in blue and green regions of the spectrum and transfer this excitation energy to nearby bacteriochlorophyll (Bchl). This process involves singlet–singlet excitation-energy transfer (EET) from Car to Bchl. Interestingly, even though it is energetically possible for Bchl to transfer excitation energy to Car, reverse singlet–singlet EET from Bchl to Car is not considered to be an active pathway (Figure 1).

In the $S_0$ ground state, Car displays $A_g^-$ symmetry if its linear polyene backbone has $C_{2h}$ point-group symmetry. Since the lowest singlet excited state, $S_1$ ($2\,^1A_g^-$), is one-photon optically forbidden, the $S_2$ ($1\,^1B_u^+$) state is the lowest one-photon optically allowed state.[3] Bchl has two distinct absorption bands due to $\pi\rightarrow\pi^*$ transitions in the visible–infrared region; the bands are designated as the $Q_x$ and $Q_y$ bands.[4] Ultrafast spectroscopic measurements of Car in solution have revealed that the internal conversion (IC) from $S_2$ to $S_1$ and $S_1$ to $S_0$ occurs in approximately 100 fs and a few picoseconds, respectively.[2,5–7] Steady-state fluorescence excitation and femtosecond kinetic measurements have been used to estimate EET efficiency in photosynthetic systems, with reported values ranging from 30 to nearly 100 %.[2] In purple-bacterial light-harvesting complexes, ultrafast spectroscopic measurements have shown that singlet–singlet EET involves the pathways $S_2\rightarrow Q_x$ and $S_1\rightarrow Q_y$.[2] Singlet–singlet EET from $Q_y$ of chlorophyll (Chl) to $S_1$ of Car was postulated by Frank et al. to explain nonphotochemical quenching of the Chl $Q_y$ excited state in higher plants.[8] This EET process has been observed in light-harvesting complex II of higher plants[9,10] and in an artificial light-harvesting dyad often used as a model for studies of energy dissipation in oxygenic photosynthesis.[11] Herein, we report the discovery of a novel EET pathway involving singlet–singlet EET from Bchl $Q_x$ to Car $S_1$ following excitation of the $Q_x$ band of Bchl. This energy-transfer pathway was clearly visible in steady-state fluorescence excitation spectra, but only in the presence of Car. We also discuss possible roles of this new pathway in *Rhodospirillum (Rsp.) rubrum* S1 purple bacteria.

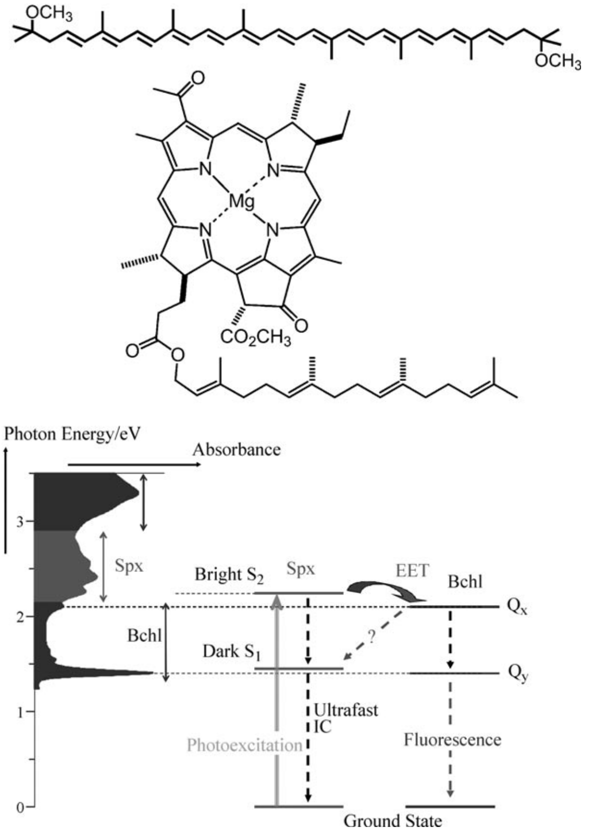

***Figure 1.*** Steady-state absorption and energy diagram of EET processes in the core complexes from *Rsp. rubrum* S1. The chemical structures of spirilloxanthin and bacteriochlorophyll *a* are also shown.

The bacterial photosynthetic core-antenna complexes from *Rsp. rubrum* S1 contain the carotenoid spirilloxanthin (Spx), which has the longest naturally occurring conjugated

---

[*] Dr. D. Kosumi, S. Maruta, T. Horibe, Dr. R. Fujii, Prof. M. Sugisaki, Prof. H. Hashimoto
CREST/JST and Department of Physics
Graduate School of Science, Osaka City University
3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585 (Japan)
E-mail: kosumi@sci.osaka-cu.ac.jp
hassy@sci.osaka-cu.ac.jp

Prof. R. J. Cogdell
Glasgow Biomedical Research Centre, University of Glasgow
126 University Place, G12 8QQ Scotland (UK)

[**] D.K. and S.M. contributed equally to this study. This research was supported in part by a Grant-in-Aid for JSPS Fellows (No. 20002429 to D.K.). H.H. thanks the Nissan Science Foundation and the HFSP for financial support. R.J.C. thanks the EPSRC, the HFSP, and the DOE for financial support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201003771.

polyene chain, with 13 double bonds (Figure 1). In these bacterial light-harvesting complexes, the reported efficiency of $S_2 \rightarrow Q_x$ EET is approximately 30 %, whereas that of $S_1 \rightarrow Q_y$ EET is 0 %.[12–14] We recorded steady-state absorption spectra of the chromatophores of *Rsp. rubrum* S1, the corresponding carotenoidless mutant, *Rsp. rubrum* G9, and Spx (Figure 2 A).

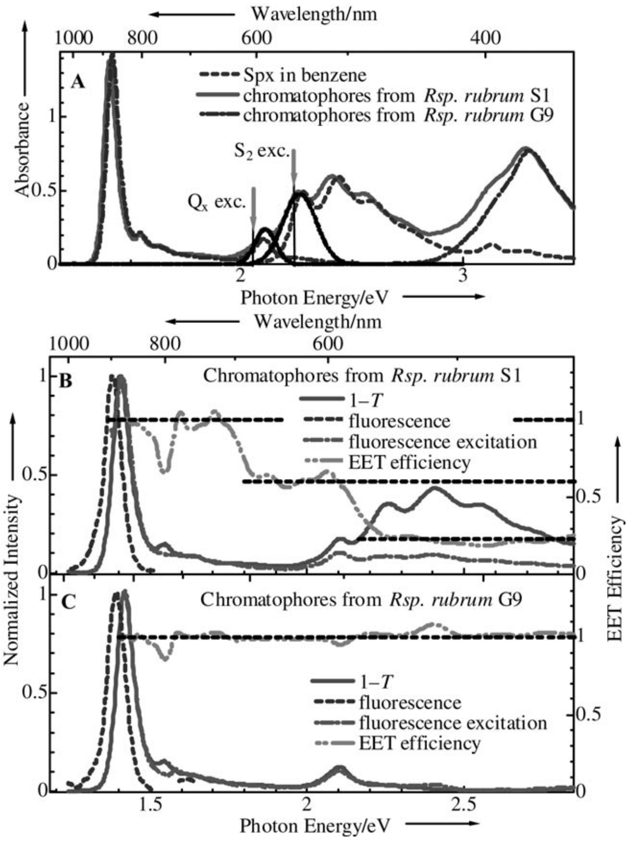

***Figure 2.*** A) Steady-state absorption spectra of chromatophores from *Rsp. rubrum* S1 and G9 and spirilloxanthin (Spx) in benzene. Dashed-and-double-dotted lines are the best fits of the $Q_x$ band and the 0–0 transition of $S_2$ with a Gaussian function. B,C) Fluorescence emission and excitation, 1−*T*, and EET-efficiency spectra of chromatophores from *Rsp. rubrum* S1 and G9, respectively.

A comparison of the absorption spectra for *Rsp. rubrum* S1 and G9 revealed that the peak of the Bchl $Q_y$ band of S1 was red-shifted approximately 10 meV in the presence of Spx. The only other difference between these spectra was observed in the visible region, in which the absorption bands due to Spx were absent in the case of G9.

We also measured the fluorescence emission, excitation, and $1-T$ (*T*, transmittance) spectra of *Rsp. rubrum* S1 and G9 membranes (Figures 2 B,C). The fluorescence excitation and $1-T$ spectra were normalized at the $Q_y$ band of Bchl. The EET-efficiency spectrum, which corresponds to the wavelength dependence of the ratio between $1-T$ and the fluorescence excitation spectra, was also plotted. The visible dip at 1.55 eV in the EET spectra was due to the reaction centers. Notably, three distinct plateaus were observed in the EET-efficiency spectrum of *Rsp. rubrum* S1 in the regions in which absorbance by $S_2$ of Spx (above 2.24 eV) and the $Q_x$ (1.84–2.10 eV) and $Q_y$ bands (below 1.75 eV) of Bchl occurs. The overall Spx→Bchl EET efficiency, which was obtained by averaging the efficiency over the entire region of the spectrum in which Spx absorbs, was found to be 22 %. Surprisingly, the average efficiency in the region of the Bchl $Q_x$ band was not unity (60 %). In the case of *Rsp. rubrum* G9 membranes, an EET efficiency of 100 % was observed when the Bchl $Q_x$ band was excited. A comparison of the EET efficiency in these two cases upon excitation of the Bchl $Q_x$ band clearly showed that in the presence of Spx, the efficiency of IC to the $Q_y$ band was decreased from 100 to 60 %. In other words, Spx provides an alternative pathway for energy transfer from the excited $Q_x$ state that competes with IC to the $Q_y$ state. This interesting phenomenon appears to have been overlooked in previous determinations of the EET efficiency of Spx to Bchl in *Rsp. rubrum* S1, as the $1-T$ and fluorescence excitation spectra at the Bchl $Q_x$ band were routinely normalized.

We next investigated excited states and energy-transfer dynamics by femtosecond pump–probe spectroscopy. The Spx→Bchl EET processes of *Rsp. rubrum* S1 after excitation into $S_2$ of Spx have been reported previously[12–14] (see also the Supporting Information); therefore, we focused our attention on the dynamics of reverse Bchl→Spx EET after excitation into the $Q_x$ band of Bchl. We recorded a set of photoinduced absorption spectra of the chromatophores of *Rsp. rubrum* S1 and G9 after excitation into the $Q_x$ band of Bchl at 2.07 eV (Figure 3 A). In the transient absorption spectra of *Rsp. rubrum* G9, a large dip clearly observed around 2.1 eV is assignable to the bleaching of the $Q_x$ band and a positive absorbance change due to transient absorption from $Q_y$ to a higher-lying excited state, following IC from the $Q_x$ band. The photoinduced absorption spectrum obtained for chromatophores from *Rsp. rubrum* S1 at 0.6 ps after excitation into the $Q_x$ band shows several transient signals identical to those observed for *Rsp. rubrum* G9. The differences between the spectra take the form of transient signals that appear to correspond to bleaching of the ground state of Spx above 2.2 eV and absorption from its excited states at 2.00 and 2.15 eV. Although the energies of $S_2$ of Spx and $Q_x$ of Bchl are quite similar, the absorbance of $S_2$ at 2.07 eV is estimated to be ten times lower than that of $Q_x$, as revealed by the decomposition of the steady-state absorption spectrum. As the excitation energy of 2.07 eV is found in the plateau of the $Q_x$ region in the *Rsp. rubrum* S1 EET spectrum, which contains a minimal contribution of $S_2$ of Spx (see the Supporting Information), direct excitation to $S_2$ of Spx at 2.07 eV is essentially negligible. It has been reported that ultrafast band shifts (electrochromic shifts) of Cars bound to nearby Bchls often occur upon excitation of the $Q_y$ band of Bchl owing to changes in the local electric fields of Cars.[15] The same effect may be occurring in this case upon excitation of the $Q_x$ band. However, the spectral structure of the absorbance change above 2.25 eV shown in Figure 3 A for *Rsp. rubrum* S1 exactly mirrors the steady-state absorption, which is not consistent with a band shift resulting from an electrochromic effect.

To investigate the role of Spx in the observed changes, we examined the differences between *Rsp. rubrum* S1 and G9 in

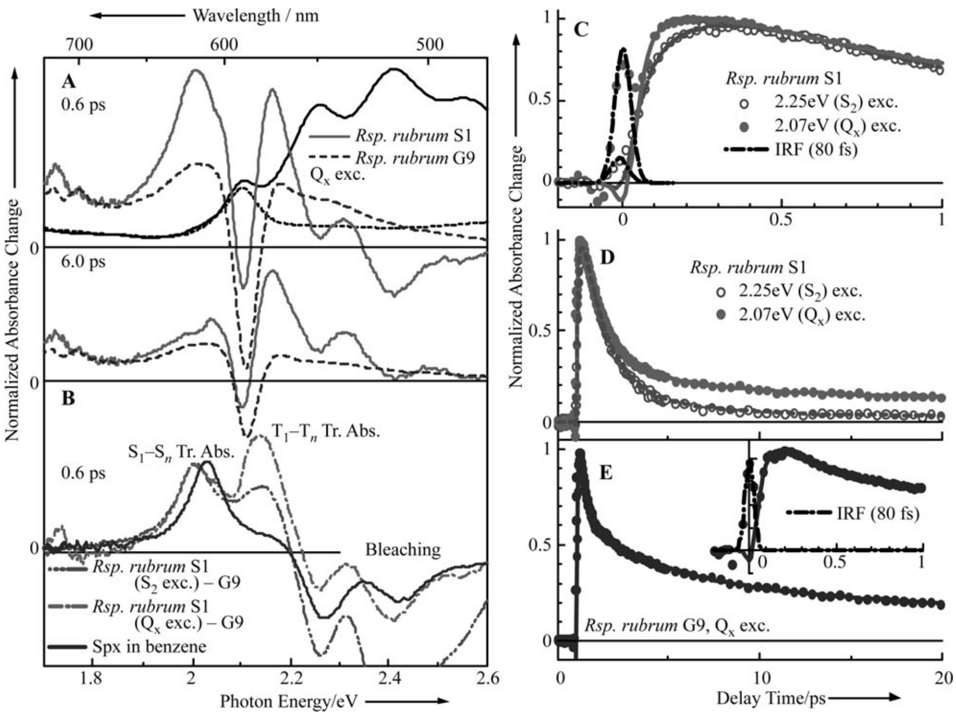

*Figure 3.* A) Photoinduced absorption spectra of *Rsp. rubrum* S1 and G9 chromatophores after excitation into the $Q_x$ band of Bchl at 2.07 eV. The steady-state absorption spectra are also plotted (thinner solid and dashed lines). B) Difference absorbance spectra between *Rsp. rubrum* S1 and G9. The photoinduced absorption spectrum of spirilloxanthin (Spx) in benzene upon excitation of $S_2$ is also plotted. C,D) Kinetics of the absorbance change of *Rsp. rubrum* S1 chromatophores after excitation to $S_2$ and into the $Q_x$ band with short and long time delays, respectively. The probe energy was selected as 2.00 eV to correspond to the peak of the $S_1 \rightarrow S_n$ transient absorption. The solid and dashed lines are best-fit curves. E) Kinetics of the absorbance change of *Rsp. rubrum* G9 chromatophores at 2.00 eV after excitation into the $Q_x$ band. The inset is a plot with a short time delay. Tr. Abs. = transient absorption.

the excited states. The difference absorbance spectra between *Rsp. rubrum* S1 and G9 after excitation into the $Q_x$ bands and between *Rsp. rubrum* S1 after excitation to $S_2$ and *Rsp rubrum* G9 after excitation into the $Q_x$ band are shown in Figure 3B. The transient absorption spectra of *Rsp. rubrum* S1 and G9 were normalized at 1.85 eV, since changes caused by $S_1$ of Spx in benzene are quite small below 1.9 eV (Figure 3B). The difference spectra for *Rsp. rubrum* S1 after excitation to $S_2$ and into the $Q_x$ band display essentially identical spectral futures, but the amplitudes of each component differ. Furthermore, the difference spectra include the bleaching signal above 2.25 eV, the $S_1 \rightarrow S_n$ transient absorption at 2.00 eV, and the $T_1 \rightarrow T_n$ transient absorption at 2.15 eV.[12,14] Even though Spx is not excited directly, the appearance of the $S_1 \rightarrow S_n$ transient absorption and $S_0$ bleaching signal strongly suggests that an energy-transfer pathway from Bchl to Spx is active after excitation into the $Q_x$ band.

Figure 3C,D shows the kinetic traces of the $S_1 \rightarrow S_n$ transient absorption of *Rsp. rubrum* S1 after excitation to $S_2$ and into the $Q_x$ band. The kinetic traces of absorbance change for *Rsp. rubrum* G9 after excitation into the $Q_x$ band are shown in Figure 3E. The solid and dashed lines in Figure 3C–E are best-fit curves for the rise and decay phases convoluted with the instrument-response function (IRF) with the assumption of a Gaussian temporal profile. An oscillating signal was observed around 0 ps after excitation into the $Q_x$ band and was present throughout the visible spectral region. Such signals have been assigned previously to coherent nonlinear optical effects.[16,17] The kinetic traces of the $S_1 \rightarrow S_n$ transient absorption of *Rsp. rubrum* S1 after excitation to $S_2$ and into the $Q_x$ band contain an identical decay component (lifetime of $1.4 \pm 0.1$ ps), as well as an additional decay component due to the transient absorption from $Q_y$ of Bchl that remains at longer delays. The rise of the $S_1 \rightarrow S_n$ transient absorption after excitation into the $Q_x$ band is slightly faster than that after the excitation of Spx to $S_2$, even though the initial dynamics of the $S_1 \rightarrow S_n$ transient absorption after excitation into the $Q_x$ band have interference from the initial coherent oscillatory signals. If the reasonable assumption is made that the signal around $t = 0$ is a result of these coherent effects, the temporal response of the absorbance changes is limited by the IRF.[17] Therefore, we performed fitting analysis by including the coherent signal to determine the precise rise time. The rise time of the $S_1 \rightarrow S_n$ transient absorption was determined to be $40 \pm 10$ fs following excitation via $S_2$, and $30 \pm 10$ fs after excitation into the $Q_x$ band.

The extremely fast appearance of transient absorbance by the $S_1$ population after excitation into the $Q_x$ band indicates ultrafast EET from Bchl to Spx. A likely donor state for Bchl→Spx EET upon excitation of the $Q_x$ band of Bchl is the $Q_x$ band, because the $S_1$ energy of Spx in solution has been reported to be 1.48 eV,[18] a value that is slightly higher than the $Q_y$ energy of 1.40 eV. Moreover, if $Q_y$ is assumed to be the donor for the EET to $S_1$ of Spx, it is expected that at least part of the transient absorption of $Q_y$ would be rapidly quenched with a time constant of 30 fs. However, such rapid quenching does not appear to occur, as the broad transient absorption due to the excited $Q_y$ state does not disappear at longer delays. From our analyses of the formation dynamics of the excited $Q_y$ state after excitation into the $Q_x$ band, the rate of this reaction in chromatophores from *Rsp. rubrum* G9 was determined to be $50 \pm 10$ fs (Figure 3E, inset). On the basis of the $Q_x$ lifetime of 50 fs, the efficiency and rate of the $Q_x \rightarrow S_1$ EET were calculated to be 40% and 75 fs$^{-1}$, respectively. An efficiency of 40% for the $Q_x \rightarrow S_1$ EET is in good agreement with the value determined by the fluorescence excitation measurements.

In this study, through fluorescence excitation measurements and determination of the kinetics of transient absorption upon the excitation of Spx and Bchl, we discovered reverse singlet–singlet EET from Bchl to Car in photosynthetic core-antenna complexes from *Rsp. rubrum* S1. The EET processes revealed in this study are described in Figure 4. In this EET pathway, after transfer of the energy absorbed by $S_2$ of Spx to the $Q_x$ state of Bchl (43 % efficiency), a large amount of this excitation energy (40 %) is returned to Spx by the reverse singlet–singlet EET reaction. These results indicate that Spx acts as an efficient quencher of the Bchl $Q_x$ excited state in the photosynthetic core antenna of this purple bacterium. Although the novel reverse EET reaction identified in this study has interesting parallels in nonphotochemical quenching observed in higher plants, the prevalence of this pathway in nature is not yet clear. If this EET channel is indeed widespread, it may be necessary to reevaluate many of the reported values of the efficiency of EET from Car to Bchl, and even those for EET from carotenoids to chlorophyll.

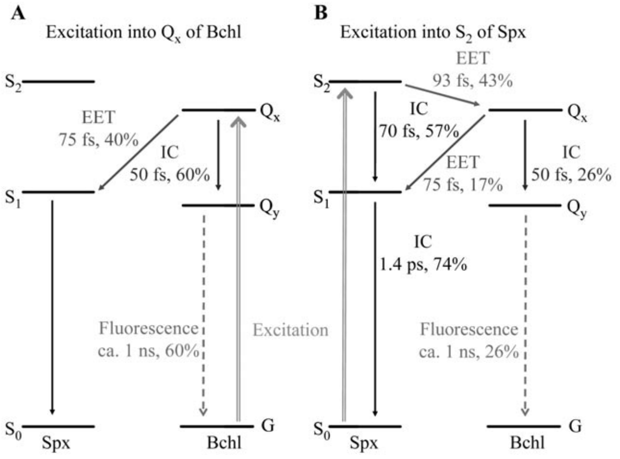

***Figure 4.*** Active EET pathways in chromatophores from *Rsp. rubrum* S1 after excitation A) into the $Q_x$ band of Bchl and B) to the $S_2$ state of Spx. The $S_2$ and $S_1$ lifetimes of Spx in solution were determined independently (see the Supporting Information).

Received: June 21, 2010
Revised: September 28, 2010

**Keywords:** bacteriochlorophyll · carotenoids · energy transfer · femtochemistry · photosynthesis

[1] H. A. Frank, R. J. Cogdell, *Photochem. Photobiol.* **1996**, *63*, 257 – 264.
[2] T. Polívka, V. Sundström, *Chem. Rev.* **2004**, *104*, 2021 – 2072.
[3] B. S. Hudson, B. E. Kohler, K. Schulten in *Excited States*, *Vol. 6* (Ed.: E. C. Lim), Academic Press, New York, **1982**, p. 95.
[4] J. D. Petke, G. M. Maggiora, L. L. Shipman, R. E. Christoffersen, *Photochem. Photobiol.* **1980**, *32*, 399 – 414.
[5] D. Kosumi, M. Fujiwara, R. Fujii, R. J. Cogdell, H. Hashimoto, M. Yoshizawa, *J. Chem. Phys.* **2009**, *130*, 214506.
[6] T. Buckup, J. Savolainen, W. Wohlleben, J. L. Herek, H. Hashimoto, R. R. B. Correia, M. Motzkus, *J. Chem. Phys.* **2006**, *125*, 194505.
[7] D. Polli, G. Cerullo, G. Lanzani, S. De Silvestri, K. Yanagi, H. Hashimoto, R. J. Cogdell, *Phys. Rev. Lett.* **2004**, *93*, 163002.
[8] H. Frank, A. Cua, V. Chynwat, A. Young, D. Gosztola, M. Wasielewski, *Photosynth. Res.* **1994**, *41*, 389 – 395.
[9] A. V. Ruban, R. Berera, C. Ilioaia, I. H. M. van Stokkum, J. T. M. Kennis, A. A. Pascal, H. van Amerongen, B. Robert, P. Horton, R. van Grondelle, *Nature* **2007**, *450*, 575 – 578.
[10] Y.-Z. Ma, N. E. Holt, X.-P. Li, K. K. Niyogi, G. R. Fleming, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4377 – 4382.
[11] R. Berera, C. Herrero, I. H. M. van Stokkum, M. Vengris, G. Kodis, R. E. Palacios, H. van Amerongen, R. van Grondelle, D. Gust, T. A. Moore, A. L. Moore, J. T. M. Kennis, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5343 – 5348.
[12] C. C. Gradinaru, J. T. M. Kennis, E. Papagiannakis, I. H. M. van Stokkum, R. J. Cogdell, G. R. Fleming, R. A. Niederman, R. van Grondelle, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2364 – 2369.
[13] E. Papagiannakis, I. H. M. van Stokkum, R. van Grondelle, R. A. Niederman, D. Zigmantas, V. Sundström, T. Polívka, *J. Phys. Chem. B* **2003**, *107*, 11216 – 11223.
[14] F. S. Rondonuwu, Y. Watanabe, R. Fujii, Y. Koyama, *Chem. Phys. Lett.* **2003**, *376*, 292 – 301.
[15] J. L. Herek, M. Wendling, Z. He, T. Polívka, G. Garcia-Asua, R. J. Cogdell, C. N. Hunter, R. van Grondelle, V. Sundström, T. Pullerits, *J. Phys. Chem. B* **2004**, *108*, 10398 – 10403.
[16] D. Kosumi, M. Komukai, H. Hashimoto, M. Yoshizawa, *Phys. Rev. Lett.* **2005**, *95*, 213601.
[17] D. Kosumi, M. Fujiwara, H. Hashimoto, M. Yoshizawa, *J. Phys. Soc. Jpn.* **2009**, *78*, 104715.
[18] R. Fujii, T. Ishikawa, Y. Koyama, M. Taguchi, Y. Isobe, H. Nagae, Y. Watanabe, *J. Phys. Chem. A* **2001**, *105*, 5348 – 5355.