



# Initial photo-induced dynamics of the photoactive yellow protein chromophore in solution

Delmar S. Larsen <sup>a,\*</sup>, Mikas Vengris <sup>a</sup>, Ivo H.M. van Stokkum <sup>a</sup>,  
Michael A. van der Horst <sup>b</sup>, Robert A. Cordfunke <sup>b</sup>, Klaas J. Hellingwerf <sup>b</sup>,  
Rienk van Grondelle <sup>a</sup>

<sup>a</sup> Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

<sup>b</sup> Department of Microbiology, Swammerdam Institute for Life Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WS Amsterdam, The Netherlands

Received 1 October 2002; in final form 21 December 2002

## Abstract

The initial photoinduced dynamics of thiomethyl *p*-coumaric acid (TMpCA) in solution has been studied with dispersed time-resolved pump–probe spectroscopy with a ~100 fs instrument response and extending over a wavelength range of ~300 to ~600 nm. TMpCA is a model chromophore for the intrinsic chromophore found in photoactive yellow protein (PYP). Stimulated emission from the chromophore is quenched on a timescale similar to chromophore within the PYP protein. A product state absorption is also observed and is formed earlier than the relaxation of the excited state and that of an observed transient intermediate.

© 2003 Elsevier Science B.V. All rights reserved.

## 1. Introduction

The photoactive yellow protein (PYP), isolated from the purple phototropic eubacterium *Ectothiorhodospira halophila*, is an ideal experimental system for investigating how the dynamical response of a protein results in biological function. PYP is a small, 125 residue, water-soluble protein, presumed responsible for the initial step of a pathway that results in the negative phototaxis of

the bacterium to blue light [1]. Upon excitation, the photoactive chromophore within PYP, thiol-ester linked anionic *p*-coumaric acid (pCA), undergoes a *trans/cis* isomerization around its double bond [2–4]. This conformational change then initiates a complex photocycle that includes multiple proton transfer steps and a partial unfolding of the protein [4–6], which exposes part of the hydrophobic protein core to the aqueous solvent environment. The partially unfolded PYP then refolds to assume its native state. The resulting photocycle proceeds on timescales that encompasses over twelve orders of magnitude from picoseconds to seconds [7–10].

\* Corresponding author. Fax: +31-20-444-7999.

E-mail address: [dslarsen@nat.vu.nl](mailto:dslarsen@nat.vu.nl) (D.S. Larsen).

When studying the dynamics of the pCA chromophore embedded within the PYP protein, a simple question arises: is the observed PYP dynamics controlled by the inherent dynamics of the intrinsic pCA chromophore, or is the observed PYP dynamics the result of the motions of the protein environment on the embedded chromophore? This ‘chicken or the egg’ query can only be properly addressed by first studying the inherent isomerization dynamics of the chromophore without the protein environment interfering with the studied photodynamics. In this Letter, we present a dispersed pump–probe study of the initial photo-induced dynamics of a model PYP chromophore, thiomethyl *p*-coumaric acid (TMpCA), in alkaline solution (i.e., without the influence of the PYP protein).

In the PYP protein, the pCA chromophore is covalently linked to the unique cysteine of the apoprotein and, therefore, in the model TMpCA compound a methyl group replaces and mimics this protein linkage (Fig. 1) [11]. This only minimally affects the conjugated electronic system of the chromophore, but of course the tuning effect of the surrounding protein [12] is lacking in TMpCA. As with the pCA chromophore in PYP, upon

excitation, the TMpCA chromophore is presumed to isomerize from a *trans* isomer to the *cis* form (Fig. 1). This reaction was studied in alkaline buffer with a pH of 10.5 in order to simulate the conditions found for the chromophore in the native protein. In both, the phenolic moiety of the chromophore is deprotonated [3,13]. The data presented here are discussed without the aid of numerical analysis and hence without introducing a specific reaction model. A multi-wavelength global analysis along with a detailed comparison between the TMpCA and PYP dynamics will be presented elsewhere.

## 2. Experimental

The experimental apparatus used has been described in detail elsewhere, [14] but is briefly presented here with some key modifications. An amplified Ti:sapphire laser system (Coherent and BMI Alpha 1000) operating at 1 kHz produces 400  $\mu$ J pulses at 790 nm with a duration of  $\sim 60$  fs (FWHM). Part of this 790 nm light was used to generate a white light continuum used for the probe light by focusing the 790 nm beam into a 2 mm thick CaF<sub>2</sub> plate. To prevent burning of the CaF<sub>2</sub> plate, the plate was slowly ( $\sim 1$  cm/s) translated perpendicular to the focused beam. The CaF<sub>2</sub> plate generates a white light probe continuum that extends to 300 nm, which is needed for studying the isomerization reaction of TMpCA. A second portion of the 790 nm light was frequency-doubled in a 0.5 mm BBO crystal producing 395 nm light and used as an excitation source. The intensity of the pump light at the sample was maintained at 100–150 nJ/pulse with neutral density filters. The duration of the pump pulses at the sample is estimated at sub-100 fs resulting in an instrument response function of  $\sim 120$  fs (FWHM). The sample was prepared with an optical density of  $\sim 5$  (per cm) and was flowed through a 1-mm path length quartz optical flow cell with a peristaltic pump.

To remove effects from reorientational motion of the absorbing molecules, the polarization of the pump beam was set to magic angle (54.7°) with respect to the probe polarization with a Berek

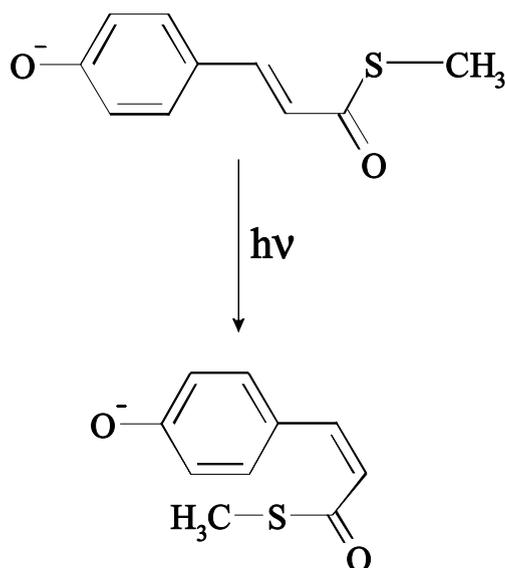


Fig. 1. Scheme of the isomerization reaction of the *trans* isomer of TMpCA into the *cis* isomer in buffer at pH 10.5.

compensator (New Focus 5540) [15]. Time delay between the pump and the probe was achieved by varying the distance traversed by the probe beam with a motorized mechanical stage. The pump and probe beam were focused in the sample (spot size  $\sim 300 \mu\text{m}$ ) with a 10 cm focal length quartz lens at a crossing angle of  $6^\circ$ . After the sample, the probe light was dispersed onto a double-diode array detector with an imaging spectrograph (Chromex 250IS). The diode signals were then digitized and collected with an attached computer for further analysis [16]. Dispersion-corrected data is constructed by measuring the cross-phase modulation signals of pure water [17], calculating the dispersion of the white light probe pulse, and then applying the determined wavelength-dependent time shift to the raw data. The timing accuracy in the dispersion corrected data is less than 50 fs.

TMpCA was synthesized from *p*-coumaric acid (using the 1,1-carbonyldiimidazole derivative of the chromophore) and sodiumthiomethoxide (Acros Organics). Equimolar amounts were mixed and allowed to react overnight at room temperature. The compound was purified with a silicagel column by washing with two column volumes of petroleum ether, and elution with a 1:1 ethylacetate/petroleum ether mixture. The identity and purity of the synthesized TMpCA was confirmed with mass spectrometry and NMR analysis. The samples were buffered with 10 mM of CAPS buffer to a pH of 10.5.

### 3. Results and discussion

The absorption spectrum of the *trans* TMpCA chromophore in buffer at pH 10.5, shown in Fig. 2, peaks at 385 nm and extends out to 450 nm. Overlaying the *trans* spectrum is the reconstructed spectrum of the photo-product, determined by subtracting the *trans* absorption spectrum from the 5 ns transient spectrum until a smooth, completely positive absorption band was generated. The absorption spectrum of this TMpCA photo-product is both narrower and more blue-shifted ( $\sim 350 \text{ nm}$ ) than the *trans* spectrum, which is similar to the photoproduct isomer of the related pCA chromophore at alkaline pH [18].

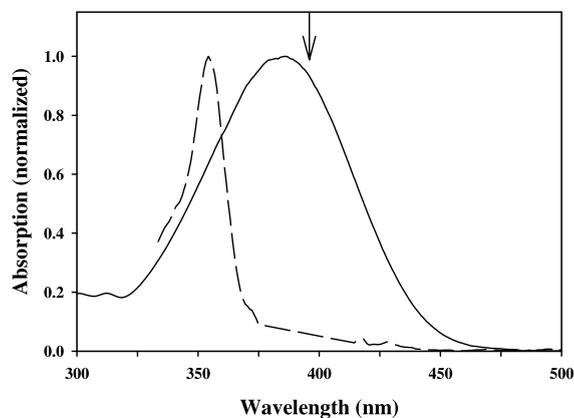


Fig. 2. Normalized absorption spectrum of TMpCA (*trans*) at pH 10.5 (solid line) and reconstructed photoproduct absorption spectrum (dashed line). The arrow indicates the excitation wavelength used in this study (395 nm).

A contour plot of the dispersed pump–probe signal is shown in Fig. 3, with selected wavelength and time traces and spectra presented in Fig. 4. A narrow wavelength region ( $\sim 15 \text{ nm}$ ) around the excitation wavelength ( $\sim 395 \text{ nm}$ ), which is obscured by scattered pump light, is not shown. Three characteristic spectral regions are identified in the data: (1) a  $>480 \text{ nm}$  region, (2) a  $400\text{--}480 \text{ nm}$  region, and (3) a  $<400 \text{ nm}$  UV region. The data in each of these three regions exhibit dynamics with differing origins.

#### 3.1. Region I: 480–560 nm

The signals in Region I consist mainly of a broad stimulated emission band (SE). The short time resolution of these measurements allows two distinguishable dynamical processes to be clearly observed: a red-shifting and a quenching of the SE band. The red-shifting of the SE occurs on a sub-picosecond timescale and is ascribed to the aqueous solvation of the excited chromophore. This shift is approximately  $\sim 50 \text{ nm}$  (see also Fig. 4c) and is similar to the spectral shift that is also observed for a locked derivative of TMpCA chromophore [19]. The solvent dynamics extracted from this time-resolved Stokes shift also coincides with similar results from other aqueous solvation studies [20,21].

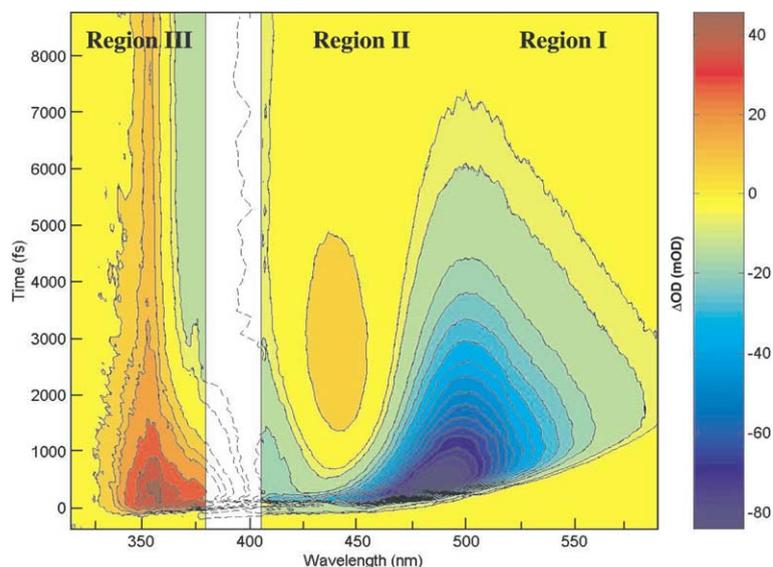


Fig. 3. Dispersion-corrected transient absorption spectra of TMpCA in buffer at pH 10.5 observed during the first 10 ps. The dashed lines in the scatter region (385–405 nm) are contours of interpolated data (linear) between the data on both sides.

On a slower picosecond time scale, the SE is observed to decay. In contrast to the dynamical shift of the SE band, the origin of this quenching is in doubt. It is tempting to interpret this quenching of the stimulated emission as a sign of the isomerization reaction and thus as a useful probe for investigating its reaction dynamics.

### 3.2. Region II: 405–480 nm

As the trace at 450 nm highlights (Fig. 4), Region II starts out initially negative, but after a picosecond it evolves into a positive band that peaks around 3 ps and the decays back to zero. Various processes may contribute to this positive band, including a rising excited state absorption (ESA) due to the probing of a different part of the potential energy surface as the reaction evolves, or perhaps a ground state absorption (GSA) from a vibrationally hot ground electronic state. Recent measurements suggest that this band originates from a GSA and not from an ESA and its assignment will be discussed elsewhere [19]. Although the growth of a positive band at 450 nm, on the blue side of the SE band, certainly contributes to the observed red-shifting of the SE in Region I, the clear rise on the red side of the SE

band highlights the inherent shifting of the SE, due to solvation.

### 3.3. Region III: 330–380 nm

A complex set of dynamics is observed in the third region. This spectral window is an important feature of our data since a product state absorption (PSA) is clearly observed as a positive ridge around 355 nm. Since no wavelength or temporal changes in the transient spectra were observed from 10 ps to the 5 ns time range of our experimental setup (data not shown), this 355 nm band is tentatively ascribed to PSA of the isomerized TMpCA photoproduct (Fig. 4c). As Fig. 4a shows, this PSA band most likely forms within 3 ps and then maintains a constant level. Unfortunately, the rise of this PSA band is obscured by a larger contribution from a decaying ESA band (with an instrument-limited rise), resulting in the overall appearance of a decaying band for times longer than 1 ps. In addition to the positive PSA, a negative signal is observed near the excitation wavelength (395 nm, see Fig. 3), which is attributed to the ground state bleach (GSB) band, because it coincides with the absorption spectrum of *trans* TMpCA (Fig. 2). However, the scatter from the

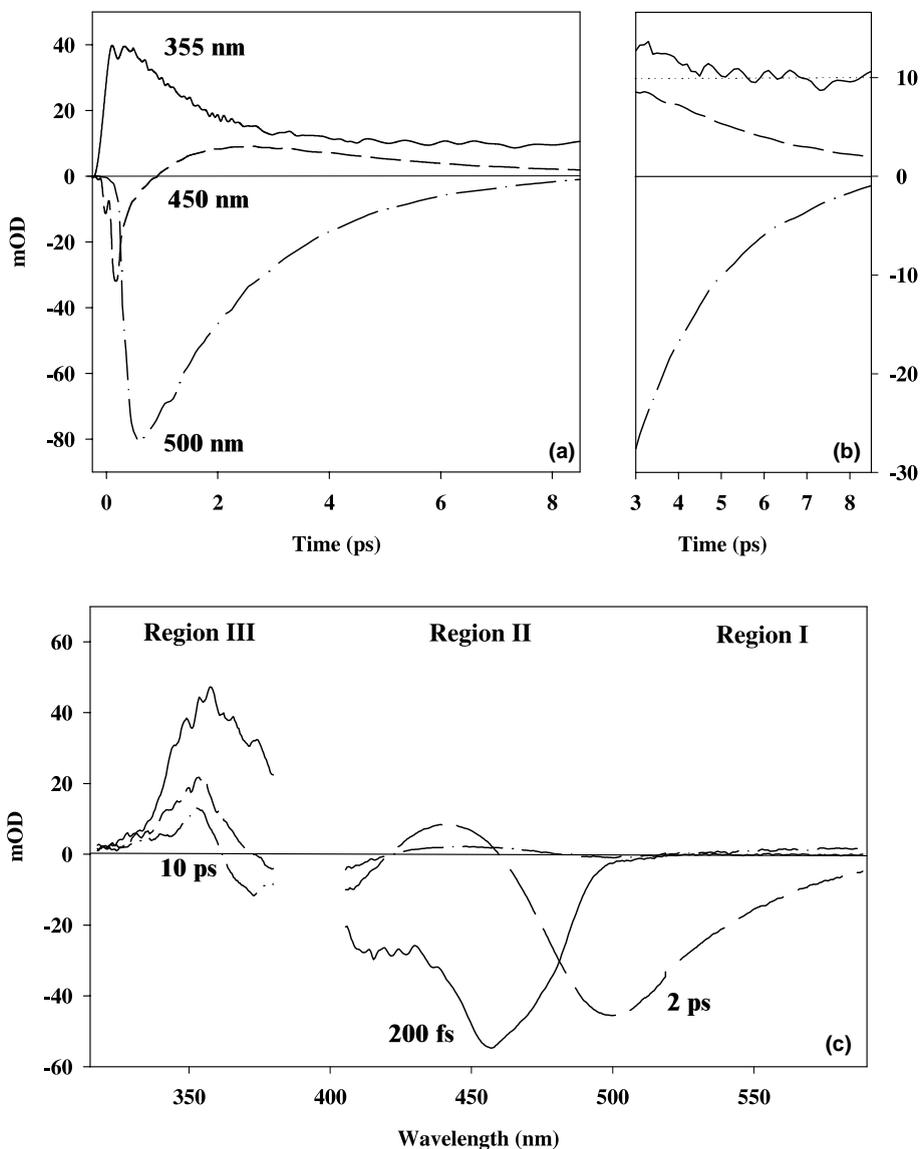


Fig. 4. (a) Dispersion corrected time traces at 355 nm (solid line), 440 nm (dashed line) and 500 nm (dashed-dot line). (b) Emphasized slower timescales of the same dynamics shown in panel A (also same demarcation as panel A). Note: the time and absorption axes here are different from panel A. The light dashed line was placed at the time-independent value for the product state absorption. (c) Transient spectra (dispersion corrected) measured at 200 fs (solid line), 2 ps (dashed line), and 8 ps (dashed-dot line).

pump pulse around 395 nm obscures the observation of the complete GSB.

#### 3.4. Comparing regions

The three time traces in Fig. 4a, highlight the photo-induced reaction dynamics of TMpCA.

Comparing the time traces measured at 355 and at 450 nm, one can see a strong correlation between the decay of the ESA in the UV, (which obscures the growth of the PSA) and the rise of the intermediate absorption band. In contrast, no appreciable correlation exists between the observed decay of the ESA with the SE decay at 500 nm,

strongly suggesting that the ESA band does not directly reflect the excited state population that is responsible for the stimulated emission.

Despite the obscuring ESA that complicates the direct observation of the isomerized product, a maximum isomerization time can be extracted from the data since no clear rise is observed in the PSA (355 nm) absorption band even after 3 ps. Therefore, the PSA rises faster than the decay of the SE band at 500 and 450 nm (Fig. 4b). Comparing the magnitude of the three bands at a specific time emphasizes this aspect. At 4 ps, the SE and the 450 nm band have decayed to  $\sim 25\%$ , and  $\sim 80\%$  of their respective initial values; however, the PSA band at 355 nm has already reached its final value and does not change in amplitude for the duration of the remainder of the experiment ( $\sim 5$  ns). Thus the PSA dynamics does not appear to be directly coupled to the dynamics of the stimulated emission decay.

Though quite unlikely, two alternative interpretations may be proposed. First, this effect could be explained by an experimental error in accounting for the dispersion of the white light. However, this would require a many-picosecond error, a value much greater than our estimated error in timing ( $\sim 50$  fs). Alternatively, an odd coincidental circumstance may occur where a decaying ESA from a transient intermediate exactly compensates for the rising product state absorption, resulting in a constant apparent value of the PSA. However, since the full PSA spectrum does not evolve in time (after  $\sim 4$  ps) this would require the ESA to have the *exact* spectrum and extinction of the product state, which is also highly improbable. Therefore, the data supports the conclusion that a branching occurs in the photocycle dynamics.

The observation that the product state evolves on a different (and faster) time scale than the quenching of the stimulated emission, and the decaying of the observed intermediate at 450 nm suggests that neither is solely responsible for forming the observed PSA. This subsequently requires adopting a non-sequential model to describe the photoinitiated dynamics of the TMpCA chromophore. Indeed, a parallel model has been suggested for describing the dynamics of PYP that involves two pathways for the generation of a

specific photocycle intermediate [22–24]. This conclusion is backed by recent multi-pulse ultrafast experiments on TMpCA and PYP [19].

#### 4. Conclusion

The ultrafast photoinduced dynamics of TMpCA, a model compound for the PYP chromophore has been studied in alkaline buffer, with several dynamical processes observed. Upon excitation, a strong stimulated emission band is observed that red-shifts on a sub-picosecond timescale (attributed to solvation dynamics) and is then quenched on a longer timescale ( $\sim 2$  ps). Within the first picosecond, an intermediate, transient band, centered at 450 nm grows in and then decays on a  $\sim 3$  ps timescale, which is slightly longer than the decay time of the stimulated emission quenching. In addition to these dynamics, a product state absorption is directly observed; though its rise time is obscured by an interfering excited state absorption.

At first glance, the decay timescale of the stimulated emission and of the intermediate appear connected to the isomerization reaction. However, a direct comparison of the dynamics of representative time traces shows that the dynamics of neither appear correlated with the product state absorption. The origin of the quenching of the stimulated emission and its relation to the isomerization reaction is not known, and a more detailed study using different chromophores and solvents is in progress.

#### Acknowledgements

This research was supported by the Netherlands Organization for Scientific Research (NWO) via the Dutch Foundation for Earth and Life Sciences (ALW). D.S.L. is grateful to the Human Frontier Science Program Organization for providing financial support with a long-term fellowship.

#### References

- [1] W.W. Sprenger, W.D. Hoff, J.P. Armitage, K.J. Hellingwerf, *J. Bacteriol.* 175 (1993) 3096.

- [2] W.D. Hoff, P. Dux, K. Hard, B. Devreese, I.M. Nugteren-Roodzant, W. Crielaard, R. Boelens, R. Kaptein, J. van Beumen, K.J. Hellingwerf, *Biochemistry* 33 (1994) 13959.
- [3] M. Baca, G.E. Borgstahl, M. Boissinot, P.M. Burke, D.R. Williams, K.A. Slater, E.D. Getzoff, *Biochemistry* 33 (1994) 14369.
- [4] U.K. Genick, G.E. Borgstahl, K. Ng, Z. Ren, C. Pradervand, P.M. Burke, V. Srajer, T.Y. Teng, W. Schildkamp, D.E. McRee, K. Moffat, E.D. Getzoff, *Science* 275 (1997) 1471.
- [5] B. Perman, V. Srajer, Z. Ren, T. Teng, C. Pradervand, T. Ursby, D. Bourgeois, F. Schotte, M. Wulff, R. Kort, K. Hellingwerf, K. Moffat, *Science* 279 (1998) 1946.
- [6] G. Rubinstenn, G.W. Vuister, F.A. Mulder, P.E. Dux, R. Boelens, K.J. Hellingwerf, R. Kaptein, *Nat. Struct. Biol.* 5 (1998) 568.
- [7] A. Baltuška, I.H.M. van Stokkum, A. Kroon, R. Mons-houwer, K.J. Hellingwerf, R. van Grondelle, *Chem. Phys. Lett.* 270 (1997) 263.
- [8] T. Gensch, C.C. Gradinaru, I.H.M. van Stokkum, J. Hendricks, K. Hellingwerf, R. van Grondelle, *Chem. Phys. Lett.* 356 (2002) 347.
- [9] H. Chosrowjan, N. Mataga, N. Nakashima, I. Yasushi, F. Tokunaga, *Chem. Phys. Lett.* 270 (1997) 267.
- [10] Z. Ren, B. Perman, V. Srajer, T.-V. Teng, C. Pradervand, D. Bourgeois, F. Schotte, T. Ursby, R. Kort, M. Wulff, K. Moffat, *Biochemistry* 40 (2001) 13788.
- [11] G.E. Borgstahl, D.R. Williams, E.D. Getzoff, *Biochemistry* 34 (1995) 6278.
- [12] M. Yoda, H. Houjou, Y. Inoue, M. Sakurai, *J. Phys. Chem. B* 105 (2001) 9887.
- [13] T.E. Meyer, M.A. Cusanovich, G. Tollin, *Arch. Biochem. Biophys.* 306 (1993) 515.
- [14] C.C. Gradinaru, I.H.M. van Stokkum, A.A. Pascal, R. van Grondelle, H. van Amerongen, *J. Phys. Chem. B* 104 (2000) 9330.
- [15] G.R. Fleming, *Chemical Applications of Ultrafast Spectroscopy*, Oxford University Press, New York, 1986.
- [16] W.D. Hoff, I.H.M. van Stokkum, H.J. van Ramesdonk, M.E. van Brederode, A.M. Brouwer, J.C. Fitch, T.E. Meyer, R. van Grondelle, K.J. Hellingwerf, *Biophys. J.* 67 (1994) 1691.
- [17] K. Ekvall, P. van der Meulen, C. Dhollande, L.E. Berg, S. Pommeret, R. Naskrecki, J.C. Mialocq, *J. Appl. Phys.* 87 (2000) 2340.
- [18] P. Changenet-Barret, P. Plaza, M.M. Martin, *Chem. Phys. Lett.* 336 (2001) 439.
- [19] D.S. Larsen, M. Vengris, I.H.M. van Stokkum, M. van der Horst, R. Cordfunke, K. Hellingwerf, R. van Grondelle, in preparation.
- [20] M.J. Lang, X.J. Jordanides, X. Song, G.R. Fleming, *J. Chem. Phys.* 110 (1999) 5884.
- [21] R. Jimenez, G.R. Fleming, P.V. Kumar, M. Maroncelli, *Nature* 369 (1994) 471.
- [22] Y. Imamoto, M. Kataoka, F. Tokunaga, *Biochemistry* 35 (1996) 14047.
- [23] Y. Imamoto, Y. Shirahige, F. Tokunaga, T. Kinoshita, K. Yoshihara, M. Kataoka, *Biochemistry* 40 (2001) 8997.
- [24] M. Unno, M. Kumauchi, J. Sasaki, F. Tokunaga, S. Yamauchi, *Biochemistry* 41 (2002) 5668.