Supplementary Material (ESI) for PCCP


# Electronic Supplementary Information

# Primary photodynamics of a biomimetic model of Photoactive Yellow Protein (PYP)

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## Contents



## 1. Structural characterization of dp-CD-PYP1 complexes

This section briefly summarizes the structural characterization of dp-CD-PYP1 by 1D and 2D 1H NMR spectroscopy. A full report, as well as the details of the synthesis of the molecule, has been submitted for publication elsewhere.[1]

Figure S1 displays the 2D ROESY NMR spectrum of dp-CD-PYP1 in deuterated borate buffer at pH=10.1. The spectrum shows strong cross peaks between the CD cavity protons (H3, H5 and/or H6 protons) and the chromophore aromatic protons (Hc-f protons) and weaker ones with one of the chromophore vinylic protons (Hb proton), giving evidence for the inclusion of the chromophore phenolate group and the adjacent vinylic CH group, inside the CD-cavity in basic aqueous solution.

The nature of chromophore-CD complexes (self-inclusion complexes vs. intermolecular complexes) has been accessed by measuring 1H NMR spectra of solutions with different concentrations of dp-CD-PYP1 (from 1 to 10 mM). The absence of any shift of the peaks

associated to the chromophore phenolate and vinylic protons upon decreasing the concentration of dp-CD-PYP1 shows the formation of intramolecular complexes, as represented on Scheme 3 (main text).

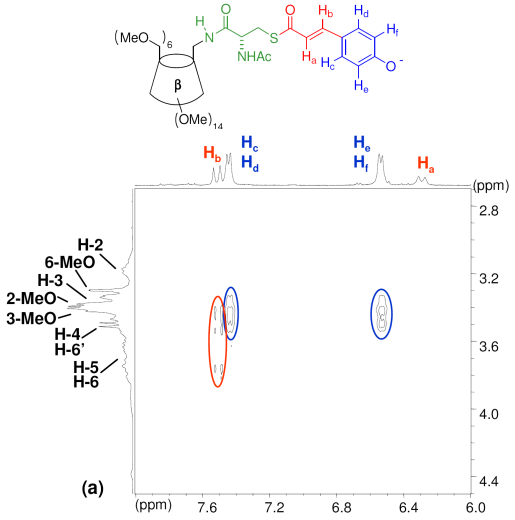


**Fig. S1.** Partial contour plot of the 2D ROESY NMR spectrum (400 MHz, T=25°C, mixing time: 200 ms) of 10 mM dp-CD-PYP1 in deuterated borate buffer at pH=10.1.

We finally conclude to the formation of strong self inclusion complexes of dp-CD-PYP1 in basic aqueous solution.

## 2. Steady-state photolysis of dp-CD-PYP1

Figure S2 (up) illustrates the evolution of the differential steady-state absorption spectra of dp-CD-PYP1, in basic aqueous solution, upon continuous irradiation at 370 nm. In contrast with $pCT^{-}$, irradiation of dp-CD-PYP1 leads to the formation of a stable *cis*-isomer absorbing in the red edge of the initial *trans* conformation with a maximum around 420 nm.[1] The absorption coefficient spectrum of the produced *cis* isomer is compared to that of the *trans* form in Figure S2 (bottom).

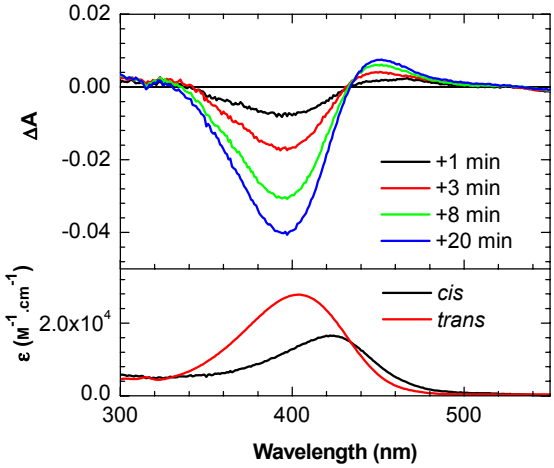


**Fig. S2:** (up) Evolution of the differential absorbance of dp-CD-PYP1 in 50 mM CAPS buffer solution at pH 10.1, upon continuous irradiation at 370 nm. (down) Absorption spectrum of the *cis* isomer extracted from the differential spectra shown above together with the absorption spectrum of the *trans* dp-CD-PYP1.

## 3. Transient spectra of pCT⁻

Although the transient absorption spectra of pCT⁻ have been already described in details in previous publications,[2-4] we report here new measurements on pCT⁻, in CAPS buffer at pH 10.1, with improved signal-to-noise ratio and time resolution (by factors of 5 and 10, respectively). These are the same experimental conditions as those used for dp-CD-PYP1.

Figure S3 shows the evolution of the transient absorption spectra of pCT⁻ for pump-probe delays ranging from 150 fs to 1.3 ns (The corresponding normalized steady-state absorption and fluorescence spectra are recalled in the lower frame).

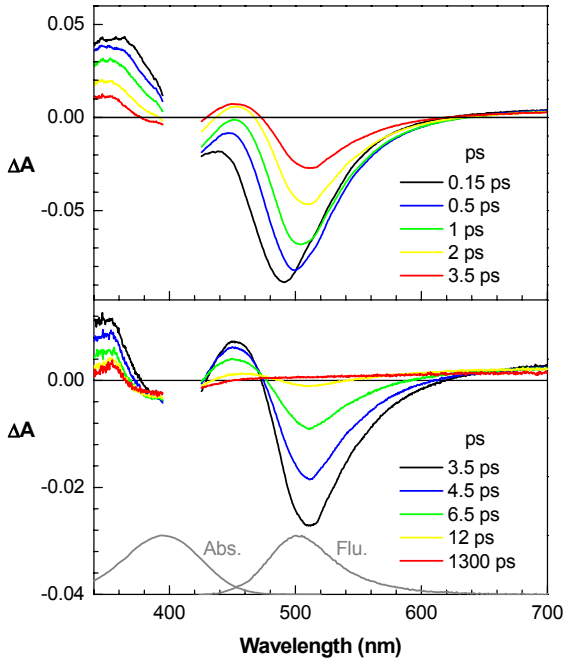


**Fig. S3:** Transient absorption spectra of pCT⁻ in 50 mM CAPS buffer solution at pH 10.1, for various pump-probe delays after 50-fs excitation at 405 nm (the scattered pump light has been masked). The corresponding normalized steady-state absorption and fluorescence spectra are recalled in the lower frame.

At short times, the ΔA spectra of pCT⁻ exhibit three intense bands associated to ESA at 350 nm, GSB around 400 nm and SE band at 490 nm. Above 550 nm, one also notes the presence of the characteristic broad and long-lived band of solvated electrons produced by biphotonic ionization of pCT⁻ phenolate moiety.[5] Since global analysis of the ΔA spectra was unsuccessful (structured residues), ΔA kinetics were independently fitted at different wavelengths, with a sum of two or three exponential functions and a plateau. At time delays up to 3.5 ps, the ESA and the SE bands decay, while a small transient absorption band located around 450 nm rises, in 1.2 ± 0.1 ps. In the meantime the ESA and the SE bands undergo a rapid dynamical solvatochromic shift to the blue and red respectively. In contrast with CD-PYP1, the 450-nm absorption band of pCT⁻ rapidly disappears in 3.5± 0.5 ps. The spectral region between 500 and 570 nm, dominated by the SE, exhibits an exponential decay of 2.7 ± 0.1 ps. In the sub-nanosecond regime, only a small residual bleaching signal and the characteristic absorption bands of the radical–solvated electron pairs are observed.

Figure S4 displays the effect of the excitation energy on the ΔA spectrum, at 1 ps and 1 ns time delays, at different wavelengths. In contrast to dp-CD-PYP1 (main text Figure 2), the figure shows that, in all cases, the ΔA signal measured at 1 ns exhibits a non-linear dependence, (with log-log slopes of about 1.9 ± 0.3) confirming that they are solely due to the two-photon process.

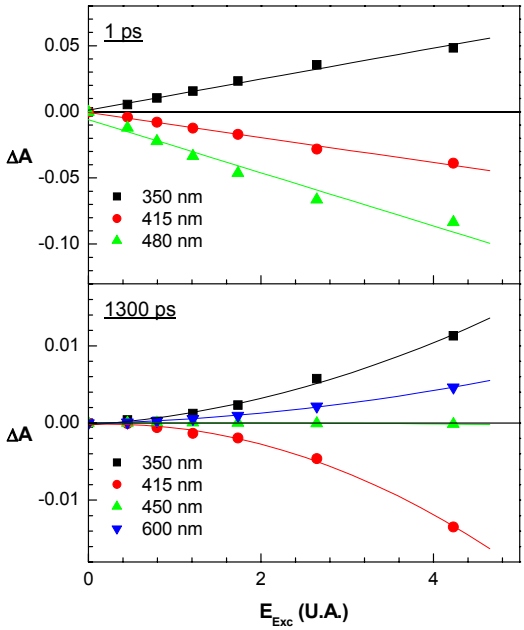


**Fig. S4:** Effect of the excitation energy ($E_{exc}$ expressed in arbitrary units) on different spectral regions of the ΔA spectra of $pCT^-$ in 50 mM CAPS buffer solution at pH 10.1, for pump-probe delays of 1 ps and 1300 ps, after excitation at 405 nm.

## 4. Estimation of the quantum yield of photoisomerization of dp-CD-PYP1

In order to estimate the quantum yield of photoisomerization of dp-CD-PYP1, the first step consists in determining the initial concentration of excited state produced by one-photon absorption. The second step consists in calculating the concentration of *cis* isomer in the nanosecond regime. Since the radical-solvated electron pairs produced by two-photon ionization, also contribute to the persistent signal observed in the nanosecond regime (see Figure 2 in the main text), we performed a global fit of the ΔA signal between 425 and 700 nm, a spectral region where the absorption of the radical is negligible (see section 5 below).

### *4.1. Initial concentration of 'one-photon' excited state*

In the picosecond regime, the ΔA signals of dp-CD-PYP1 display a linear dependence with the excitation energy between 350 and 480 nm (in spectral regions respectively dominate by ESA and SE contributions; see main text Figure 3: log-log slopes of about 1.00 ± 0.03), showing that the one-photon process is dominant. The energy used to excite dp-CD-PYP1 at 405 nm was about 530 nJ per pulse ($1.08\times10^{12}$ photons), focused on a surface of 0.035 $mm^2$. The sample was held in a 1-mm thick cell and had an absorbance of 0.58 at the excitation wavelength. In these conditions, about 74% of the incident photons were absorbed in the excited volume, i.e. the concentration of absorbed photon in the excited volume was about 38 µM.

Immediatly after the femtosecond excitation, at a pump-probe delay of 150 fs, we estimated that the concentration of solvated electrons due two-photon ionization from the ΔA signal (= 0.0025) at 700 nm (Figure 2, main text), where only solvated electron contributes. By using the well-known absorption coefficient of solvated electrons in water ($\varepsilon_{e^-} = 22\,545\ M^{-1}\,cm^{-1}$ at 700 nm),[6,7] we found a value of about 1.1 µM. The corresponding concentration of absorbed photons leading to solvated electrons is thus

about 2.2 µM, under the assumption that the electron recombination or scavenging processes are rather limited at this very short time scale.[8] Thus, about 6% of the absorbed photons are involved in the two-photon ionization process. The remaining absorbed photons lead to the one-photon process that eventually produces the *cis* isomer. We finally deduce that the initial concentration (in the excited volume) of dp-CD-PYP1 in the excited state produced by one-photon absorption is 35.8 µM.

### *4.2. Concentration of cis isomer in the nanosecond regime*

The concentrations of *cis* isomers and radical-solvated electron pairs contributing to the transient spectrum of dp-CD-PYP1 at 1 ns (see Figure 3 in the main text) have been quantified by fitting the ΔA signal between 425 and 700 nm, a spectral region where the absorption of the radical is negligible (see section 5), with the following equation:

$$\Delta A = l \{[\varepsilon_{cis} - \varepsilon_{trans}]\, c_{cis} + [f \times \varepsilon_{elec} - \varepsilon_{trans}]\, c_{rad}\}$$

where $\varepsilon_{cis}$ and $\varepsilon_{trans}$ are the absorption coefficients of the *cis* and *trans* dp-CD-PYP1 isomers represented on Figure S2 (section 2); $\varepsilon_{elec}$ is the absorption coefficient of the solvated electron taken from the literature;[6,7] $c_{cis}$ and $c_{rad}$ are the concentrations of the *cis* isomers and radicals, respectively. Finally, f is a parameter taking into account a possible scavenging reaction of the solvated electrons, as observed for $pCT^-$ (see section 5) that potentially makes the concentration of solvated electron lower than that of the radical ($c_{elec} = f \times c_{rad}$, with $f < 1$).

Figure S5 displays the ΔA spectrum of dp-CD-PYP1 and the corresponding best fit obtained for $c_{cis} = 1.58 \pm 0.07$ µM, $c_{rad} = 0.58 \pm 0.03$ µM and $f = 1.05 \pm 0.06$ (no scavenging of the solvated electrons occurs in this case).

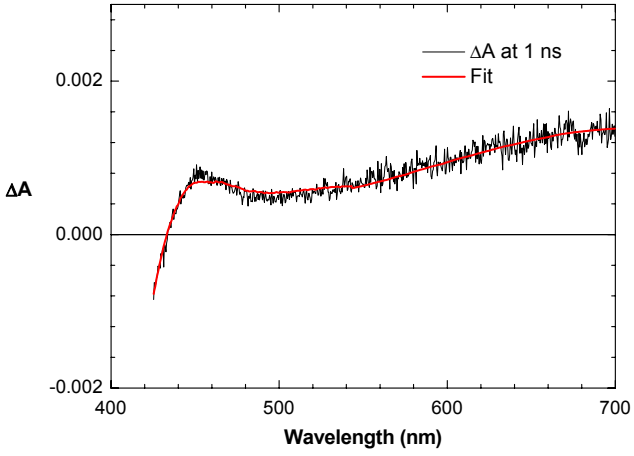


**Fig. S5:** Transient spectrum of dp-CD-PYP1 in 50 mM CAPS buffer solution at pH 10.1, 1ns after 50-fs excitation at 405 nm and the corresponding best fit obtained with concentrations of 0.58 ± 0.03 µM of radicals (see text) and 1.58 ± 0.07 µM of *cis* isomers.

### *4.3. Quantum yield of isomerization*

We finally estimated the quantum yield of isomerization as the ratio of the concentration of the *cis* isomer formed over the initial concentration of excited state produced by one-photon. We found $\phi_{isom} = 0.04 \pm 0.01$ (standard error).

## 5. Absorption spectrum of the pCT$^{\bullet}$ radical

This section is dedicated to extracting from the transient absorption spectra of pCT$^{-}$ the spectrum of the pCT$^{\bullet}$ radical. It will then be assumed that the absorption spectrum of the dp-CD-PYP1 radical is very similar to this one. The particular interest of pCT$^{-}$ is that it does not give rise to any photoisomerization product. The signal observed in the nanosecond regime is thus here solely due to the contributions of solvated electrons and pCT$^{\bullet}$ radicals, produced by two-photon absorption.

We estimated that the concentration of solvated electron by taking the ΔA signal of pCT$^{-}$ at 700 nm (i.e. in a spectral region where only the solvated electron absorption contributes) and using the well-known absorption coefficient spectrum of solvated electron in water ($\varepsilon_{elec}$ = 22 545 M$^{-1}$ cm$^{-1}$ at 700 nm).[6,7] At a delay of 200 fs after excitation, this concentration is found to be about 1.8 µM. At 1.3 ns, it is however reduced 0.7 µM. This latter value is significantly lower than the concentration of the pCT$^{\bullet}$ radical, namely 1.0 µM, as calculated from the persistent bleaching signal ($\varepsilon_{pCT^-}$ = 27 820 M$^{-1}$ cm$^{-1}$ at 395 nm) observed at 1.3 ns. It is known that, in the presence of high solute concentrations, solvated electrons can be scavenged at a rate which depends on the nature of the solute. As matter of a fact, at pH 10.1, a fast scavenging reaction with the hydronium cations can be excluded ($k = 2.3\times10^{10}$ M$^{-1}$ s$^{-1}$).[8] The exact nature of the reaction involved in the present experiment is not yet clear but it might involve a very efficient reaction between solvated electrons and the CAPS buffer.

Figure S6 displays the absorption spectrum of the pCT$^{\bullet}$ radical, and compares it to that of pCT$^{-}$. It has been calculated by subtracting the contributions of ground-state bleaching and solvated electron absorption from the ΔA spectrum of pCT$^{-}$ at 1.3 ns.

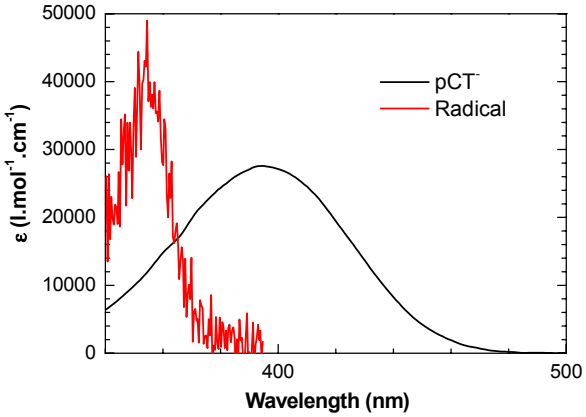


**Fig. S6** Absorption spectra of pCT$^{-}$ and pCT$^{\bullet}$ radical, in 50 mM CAPS buffer solution at pH 10.1. The absorption spectrum of pCT$^{\bullet}$ has been obtained by subtracting from the transient spectra of pCT$^{-}$ at 1.3 ns (see Figure S2) the contributions of ground-state bleaching and solvated electron absorption.

## References

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