Supporting Information

Structural effects on the ultrafast photoisomerization of Photoactive Yellow Protein. Transient absorption spectroscopy of two point mutants.

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Contents

1. Target Analysis

A global analysis was first performed by singular value decomposition (SVD).[1] The minimal number of retained singular values was chosen so as to get essentially noise-like residuals after reconstruction of the differential spectra and difference with the original data. Global fits of the kinetic vectors were performed with a sum of four exponentials and a step function, convoluted with a Gaussian representing the experimental response function. The FWHM of the Gaussian was found to be about 1.2 ± 0.1 ps. The results are presented as Decay Associated Differential Spectra (DADS).

A target analysis[2] of the kinetic traces at 18 significant wavelengths was then carried out on SVD-reconstructed kinetics. The analytical model was constructed as follows. A set of differential equations representing the (spontaneous) temporal evolution of the concentrations of each transient ($c_i(t)$) was first constructed and solved. In general form it reads:

$$\frac{d}{dt} c_i(t) = -k_i \; c_i(t) + \sum_{j \neq i} \phi_{ji} \; k_j \; c_j(t) \qquad (1)$$

with k_i the total decay rate of state i and ϕ_{ji} the branching ratio (or reaction yield) of the state-j-to-state-i reaction. The kinetic traces were then globally fitted (Levenberg-Marquardt algorithm) to the following expression:

$$\Delta A(\lambda, t) = \left(\sum_i [\varepsilon_i(\lambda) - \varepsilon_g(\lambda)] \, x_i(t) \right) x_{exc} \; c_{tot} \; \ell \qquad (2)$$

where ℓ is the optical path length, c_{tot} is the total sample concentration and x_{exc} is the fraction of molecules being initially excited by the pump pulse. The fraction of initial excited concentration ($c_{exc} = c_{tot} \, x_{exc}$) found in state i at time t is noted $x_i(t)$ ($x_i(t) = c_i(t)/c_{exc}$). $\varepsilon_i(\lambda)$ are unknown generalized extinction coefficients (including transient absorption and stimulated emission) attached to each transient and $\varepsilon_g(\lambda)$ is the known ground-state absorption coefficient. The fit led to a set of rate constants, branching ratios and Species Associated Spectra (SAS, i.e. $\varepsilon_i(\lambda)$ spectra). SAS were directly adjusted by imposing spectral constraints on them, aimed at preventing them from displaying ground-state absorption features (i.e. $-\varepsilon_g(\lambda)$ contributions) or at canceling any trace of stimulated emission. The detail

of these constraints is described below. The fraction x_{exc} of excited molecules was treated as an adjustable parameter.

Using the kinetic model of Scheme 2, we performed a global fit of 18 ΔA kinetic traces ranging from 360 nm and 530 nm, for WT and its three mutants. In order to limit the number of solutions and obtain more reliable results, the following constraints were applied to the fits:

(i) The three different excited-state populations were assumed to have identical absorption and SE spectra. The amplitude of the SE signal was fixed during the fits. It was estimated from the steady-state absorption and fluorescence spectra of WT using Peterson[3] and Strickler-Berg expressions.[4] The molar extinction coefficients of the three mutants were taken from Imamoto et al.[5] The oscillator strength of the S_0-S_1 transition being the same for all compounds,[5] the radiative lifetime of the three mutants was assumed to be the same as that of WT.

(ii) Since femtosecond time-resolved fluorescence measurements did not exhibit any delayed fluorescence,[6-16] transients X, I_0 and I_1 were supposed to be non-emissive, hence not to display any stimulated emission. The corresponding SAS (species-associated spectra) were consequently constrained to have positive values. This condition also helps guaranteeing that no bleaching contribution is present, as generally expected for any SAS.

(iii) The SAS of X was assumed to have negligible values for wavelengths above 510 nm since the contribution of any absorption from this state is found to be negligible in this spectral region in DADS1.

(iv) As far as calculating the SAS of I_1 is concerned, two possibilities were considered. Given the presence of GSB in DADS5, one may suppose the absorption spectrum of I_1 is negligible in this spectral region (below 420 nm). As a result, the reaction from I_0 to I_1 is found to be only partial ($\phi_{I_0 \to I_1} < 1$). Alternatively one may allow I_1 to absorb light in this spectral region (above 420 nm) but, in order to achieve unique solution, one must impose an additional constraint. Fixing $\phi_{I_0 \to I_1}$ to 1 was chosen as it seems quite improbable that I_0, which is a ground-state protein-unrelaxed full

cis configuration, thermally isomerizes back to *trans* in the sub-nanosecond regime. In the present work, we chose the second alternative, although it was not retained in a previous target analysis of WT transient spectra.[2] As matter of fact, the quality of our fits was improved and the spectrum of I_1 was found comparable to the ones previously reported by van Brederode et al.[17] and Joshi et al.[18]

(v) Since the contribution of GSB in DADS5 is smaller than that of DADS4 (see Figure 2), the above constraint ($\phi_{I_0 \rightarrow I_1} = 1$) could only be fulfilled if the absorption of I_0 was imposed to be negligible below 450 nm.

2. Photoionization of the PYP chromophore

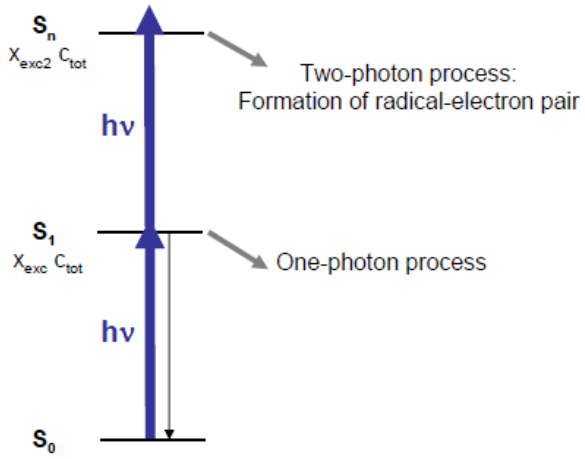
Previous pump-probe spectroscopy studies carried out on the isolated PYP chromophore in water[19-21] and on WT[22] showed the formation of radical-electron pairs (rep) due to two-photon ionization of the chromophore. Although the behavior of radical-electron pairs in proteins is not fully understood, they were found to be long-lived species on the scale of few nanoseconds.[22] The absorption of the radical occurs between 340 nm and 400 nm with a maximum centered around 370 nm.[22] The absorption of the associated electrons is qualitatively similar to that of the "solvated electron", with a broad characteristic band covering the whole visible spectral region above 450 nm.[22] As stated by Larsen et al.,[20] the actual behavior of ejected electron is poorly understood in proteins. If the electron does not escape from the chromophore-binding pocket and gets solvated by water, it might be stabilized by nearby amino-acid residues.

In order to include a possible photoionization process in our present target analysis, an additional pathway was added to the kinetic model of Scheme 2, as follows:

$$\Delta A(\lambda, t) = \left[x_{exc2} \, [\varepsilon_{rep}(\lambda) - \varepsilon_g(\lambda)] + x_{exc} \left(\sum_i [\varepsilon_i(\lambda) - \varepsilon_g(\lambda)] \, x_i(t) \right) \right] c_{tot} \, \ell \qquad (3)$$

A schematic representation of the modified kinetic model is displayed on Scheme S1. In this modified model, a fraction x_{exc2} of the total concentration is supposed to be two-photon ionized. The

radical-electron pair is treated as one species, the spectrum of which ($\varepsilon_{rep}(\lambda)$) displays the signature of the radical below 440 nm and the absorption of the associated electron above 440 nm.



Scheme S1. Modified kinetic model of Scheme 2 taking into account a possible two-photon ionization process from the excited-state. c_{tot} is the total sample concentration, x_{exc} is the fraction of molecules initially excited by the pump pulse leading to one photon process and x_{exc2} represents the fraction of the excited population involved in a two-photon ionization process.

Due to the low extinction coefficient of the ejected electron (about 4 times smaller than that of the radical)[22] and its long lifetime, we do not expect to separate its contribution from that of the longest-lived intermediate I_1, which absorbs in the same spectral region. In the spectral region above 440 nm, $\varepsilon_{rep}(\lambda)$ was thus assumed to be negligible for all compounds. Below 440 nm, in the spectral region of the absorption of the radical, $\varepsilon_{rep}(\lambda)$ was left free. In order to reduce the number of parameters and ensure unique convergence, the population leading to ionization was adjusted in such a way as to guarantee that the radical absorption coefficient extracted from the target analysis is comparable to that previously reported by Larsen et al., for WT.[22] For the mutants, the radical absorption coefficient of which is unknown, the contribution of the population leading to ionization was roughly estimated from the part of the signal that exhibits a quadratic dependence with excitation energy.

3. Additional fitting results

Figures S1 and S2 display the results of target analyses performed on our previously published[23] transient absorption spectra of WT and R52Q, respectively. The kinetic model of Scheme 2 allows a good description of the spectra in all cases.

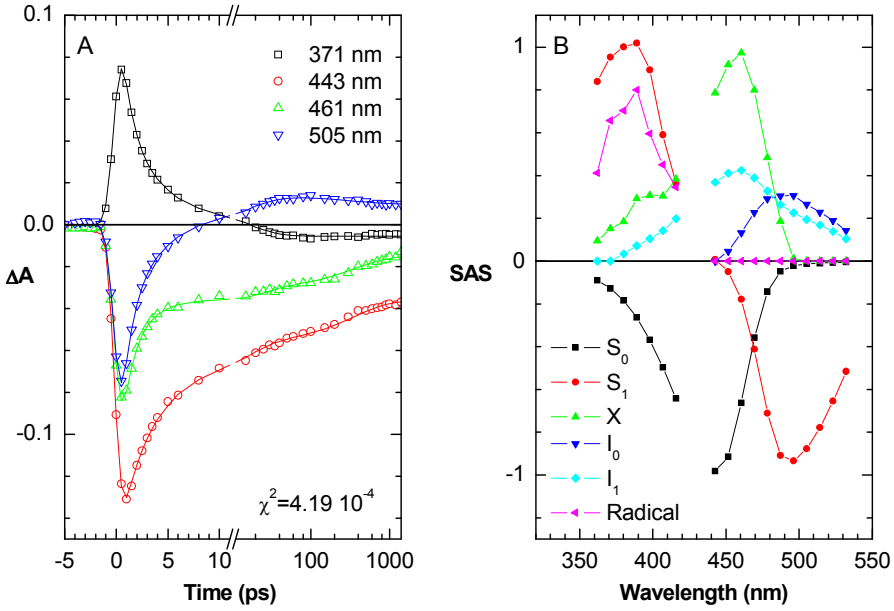


Figure S1. Left: ΔA(t) kinetics at selected wavelengths of mutant WT, in 10 mM Tris-HCl buffer solution at pH 8.1, after excitation at 428 nm. The fits corresponding to the target analysis with the model of Scheme 2 are represented by the solid lines. Right: SAS of species S_1, X, I_0,I_1 and radical, obtained by target analysis of the differential absorption spectra of WT. The initial S_0 absorption spectrum is recalled for comparison.

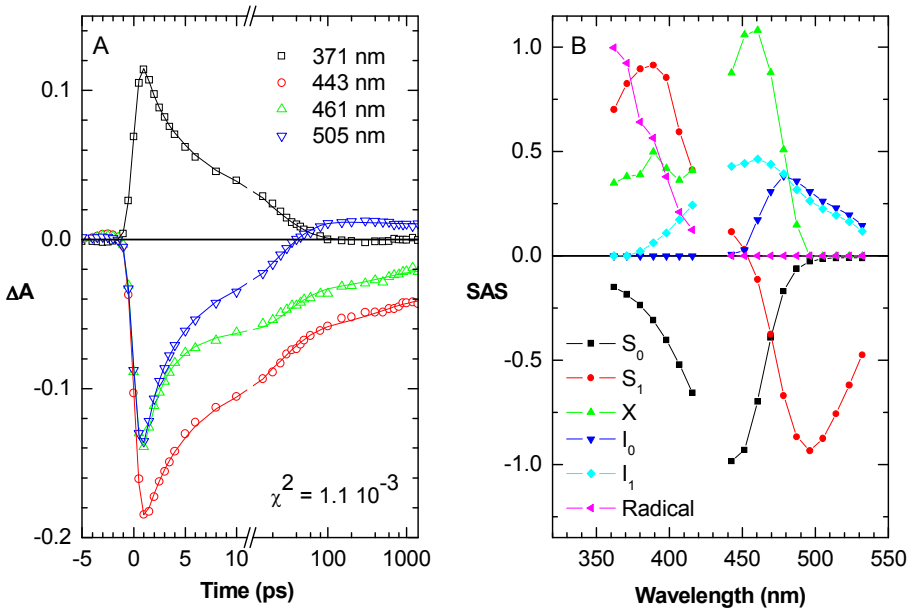


Figure S2. Left: ΔA(t) kinetics at selected wavelengths of mutant R52Q, in 10 mM Tris-HCl buffer solution at pH 8.1, after excitation at 428 nm. The fits corresponding to the target analysis with the model of Scheme 2 are represented by the solid lines. Right: SAS of species S_1, X, I_0,I_1 and radical, obtained by target analysis of the differential absorption spectra of R52Q. The initial S_0 absorption spectrum is recalled for comparison.

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