Supplementary Material: How light-induced charge transfer accelerates the receptor-state recovery of photoactive yellow protein from its signaling state

L. Premvardhan,* M.A. van der Horst ,† K.J. Hellingwerf ,†,* R. van Grondelle*

* Department of Biophysics and Physics of Complex Systems, Faculty of Sciences, Vrije Universiteit, de Boelelaan 1081, 1081 HV Amsterdam, The Netherlands; † Laboratory for Microbiology, Swammerdam Institute for Life Sciences, Universiteit van Amsterdam, BioCentrum, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

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lp2f@few.vu.nl OR premvard@gmail.com

Materials and Methods

Synthesis M100A PYP was produced and isolated as described by Kort *et al.* (Kort and others 1996) and Hendriks *et al.*(Hendriks and others 2002) as a hexa-histidine tagged apo-protein in *Escherichia coli*. The M100A mutation was introduced using the QuickChange kit (Stratagene) and with pHISP as a template. The sequences for the mutagenic primers of the M100A mutated protein were 5' CGATGTTCGAATACACCTTCGATTACCAAG CGACGCCCACGAAGG 3' and 5' CCTTCGTG GGCGTCGCTTGGTAATCGAAGGTGTATTCGAA CATCG 3'. The mutations were confirmed using nucleotide sequence analysis. Protein samples were used without removal of their hexa-histidine-containing N-terminal tag in 10 mM Tris/HCl, pH8.

Experimental Procedure: The procedure outlined in Ref (Premvardhan and others 2003) is applied here to prepare the sample cell and to measure the Stark spectra of M100A. Included below are the additional steps taken to trap the pB intermediate at low temperature.

The sample cell, containing the M100A solution in 66 % (w/v) of glycerol, is illuminated at 450 nm with light from a Xe lamp for 5 – 10 minutes, at room temperature, and then immersed in liquid nitrogen. Room temperature absorption spectra, taken immediately after illumination, invariably resulted in regeneration of at least 50 % of pG because of the time it take to acquire the absorption spectra. Therefore, the relative amount of pB trapped at low temperature depends on the time delay between illumination at room temperature and immersion of the sample in liquid nitrogen. Shorter delays generate absorption spectra such as I, where hardly any pG is re-generated, else type II spectra are acquired where a noticeable amount of pG is present (Figure 1a).

Theory of Stark effect. Although the analytical approach has been described extensively in the literature, a brief description is included below to better the results presented in the manuscript. Essentially, the field-induced change in the absorption spectrum (Stark effect), $A(\tilde{\nu})$, of an ensemble of molecules, arises from the difference in the intensity of light transmitted through the sample in the presence and absence of an external field. The change in absorbance ($\Delta A(\tilde{\nu})$), averaged over all orientations, is analyzed using the Liptay expression (Liptay 1974):

$$-\Delta A(\tilde{\nu}) = \vec{F}_{eff}^{\,2}\left[\begin{array}{l} a_\chi A(\tilde{\nu}) + \dfrac{b_\chi \tilde{\nu}}{15h}\left\{\dfrac{\partial}{\partial\tilde{\nu}}\left(\dfrac{A(\tilde{\nu})}{\tilde{\nu}}\right)\right\} \\ + \dfrac{c_\chi \tilde{\nu}}{30h^2}\left\{\dfrac{\partial^2}{\partial\tilde{\nu}^2}\left(\dfrac{A(\tilde{\nu})}{\tilde{\nu}}\right)\right\} \end{array}\right] \quad \text{Eq. 1}$$

$\Delta A(\tilde{\nu})$, expressed as a function of the energy in wavenumbers, $\tilde{\nu}$, is proportional to the square of the

effective field, $\vec{F}_{eff}$, at the site of the solute and to the weighted sum of: the (field-free) absorption lineshape, the first, and second derivatives of $A(\tilde{\nu})$. The effective field, $\vec{F}_{eff}$, arises from the enhancement of the external field due to the polarization of the solvent, such that $\vec{F}_{eff} = f_c \times \vec{F}_{ext}$, where $\vec{F}_{ext}$ is the external applied field and , f_c, the cavity-field factor.

When, χ, the angle between the applied AC electric-field vector and the electric-field vector of the polarized light, is set at the magic angle (54.7°) the expressions for the co-efficients of the derivatives are:

$$a_{54.7} = \frac{1}{30|\vec{m}|^2}\sum_{ij}\left[10A_{ij}^2\right] + \frac{1}{15|\vec{m}|^2}\sum_{ij}\left[10B_{ijj}\right] \quad \text{Eq. 2}$$

$$b_{54.7} = \frac{1}{|\vec{m}|^2}\sum_{ij}\left[10m_i A_{ij}\Delta\mu_j\right] + \frac{15}{2}\overline{\Delta\alpha}_{el} \quad \text{Eq. 3}$$

$$c_{54.7} = 5|\Delta\vec{\mu}|^2 \quad \text{Eq. 4}$$

The co-efficient a_χ (Eq. 2), usually small, is related to the electric-field induced change in the transition moment, $\vec{m}$: $\vec{m}(\vec{F}_{eff}) = \vec{m} + \underline{A}\cdot\vec{F}_{eff} + \vec{F}_{eff}\cdot\underline{\underline{B}}\cdot\vec{F}_{eff}$, where $\underline{A}$ and $\underline{\underline{B}}$ are the transition-moment polarizability and hyperpolarizability tensors, respectively. The first-derivative coefficient, $b_{54.7}$, is proportional to the second-rank tensor, $\underline{\underline{\Delta\alpha}}_{el}$, the difference electronic polarizability. In Eq. 3, $b_{54.7}$ is expressed in terms of the scalar quantity $\overline{\Delta\alpha}_{el}$, the average change in electronic polarisability between the ground and excited states. An accurate expression for $b_{54.7}$ includes the additional cross term comprising the tensor element of $\underline{A}$. The latter term is often neglected to be able to obtain $\overline{\Delta\alpha}_{el}$ directly from $b_{54.7}$. This assumption may however be erroneous, particularly if $|\Delta\vec{\mu}|$ is large. If A_{ij} is not negligible, then the value of $\overline{\Delta\alpha}_{el}$ reported as such actually includes the component equal to $2/15|\vec{m}|^2\sum_{ij}\left[10m_i A_{ij}\Delta\mu_j\right]$.

The co-efficient of the second derivative at $\chi = 54.7°$ is directly proportional to the square of the change in static dipole moment, $|\Delta\vec{\mu}|$ (Eq. 4). Note, however, that only the *magnitude*, and not the sign, of $\Delta\mu$ can be determined from the Stark signal, because the Liptay analysis follows from the assumption that the molecules in the sample are frozen in an isotropic orientation, in the presence and absence of the field.

Fitting the Stark spectra The absorption spectrum of M100A, composed of overlapping bands of pG and pB, is deconvolved into a sum of gaussians using a simplex algorithm to find the global minimum. The transitions underlying the absorption spectrum, either electronic or vibronic, are presumed to be modeled by these gaussians. For instance, the absorption of pG is modeled as a sum of gaussians, where each gaussian corresponds either to a vibronic transition or a geometric isomer. Fitting the Stark spectrum to separate bands, each composed of one or more gaussians, yields a better fit and also yields the electronic properties for the individual transitions.

In M100A, when pB is the pre-dominant species (see spectrum I in Figure 1a), the Stark spectrum can be directly fit to the derivatives of the smoothed absorption spectrum in the region of pB absorbance. In the case where pG and pB absorbances are comparable (spectrum II), the absorption is deconvolved into two bands: between 20,000 – 25000 cm^{-1} and 25500 – 32000 cm^{-1}, to model pG and pB, respectively. A more detailed explanation of the fitting procedure can be obtained in Refs (Premvardhan and Peteanu 1999) and (Reimers and Hush 1991). The coefficients of the derivatives, a_χ, b_χ and c_χ, are extracted by means of a linear least-squares (LLSQ) fit of the electro-absorption signal to the sum of $A(\tilde{\nu})$, and the first and second derivatives of $A(\tilde{\nu})$ of each band. All reported electrostatic properties are the average of at least three independent measurements and are uncorrected by the cavity field factor f_c. Strictly speaking, the units for $\overline{\Delta\alpha}_{el}$ and $|\Delta\vec{\mu}|$ are $Å^3/f_c^2$ and Debye/f_c, respectively. Note that f_c could enhance the externally applied field by 10-20 %.

REFERENCES

(1) Hendriks J, Gensch T, Hviid L, van der Horst MA, Hellingwerf KJ, van Thor JJ. 2002. Transient exposure of hydrophobic surface in the Photoactive Yellow Protein Monitored with Nile Red. Biophys. J. 82:1632-43.
(2) Kort R, Hoff WD, van West M, Kroon AR, Hoffer SM, Vlieg KH, Crielaard W, van Beeuman JJ, Hellingwerf KJ. 1996. EMBO J. 15(13):3209-18.
(3) Liptay W. 1974. Dipole moments and polarizabilities of molecules in excited states. In: Lim EC, editor. Excited States. New York: Academic Press. p 129-229.
(4) Premvardhan LL, Peteanu LA. 1999. Dipolar Properties of and Temperature Effects on the Electronic States of 3-Hydroxyflavone (3HF) Determined using Stark-Effect Spectroscopy and Compared to Electronic Structure Calculations. J. Phys. Chem. A 103:7506-7514.
(5) Premvardhan LL, van der Horst MA, Hellingwerf KJ, van Grondelle R. 2003. Stark spectroscopy on Photoactive Yellow Protein, E46Q and a non- isomerising derivative, probes photo-induced charge motion. Biophys. J. 84: 3226-3239.
(6) Reimers JR, Hush NS. 1991. Electronic Properties of Transition-Metal Complexes Determined from Electroabsorption (Stark) Spectroscopy. 2. Mononuclear Complexes of Ruthenium(II). J. Phys. Chem. 95:9773-9781.