

Supplemental information to: Structural evolution of the chromophore in the primary stages of trans/cis isomerization in photoactive yellow protein

Karsten Heyne,^{†,*} Omar F. Mohammed,[†] Anwar Usman,[†] Jens Dreyer,[†]
Erik T.J. Nibbering,^{†,*} and Michael A. Cusanovich^{†,§*}

Max Born Institut für Nichtlineare Optik und Kurzzeitspektroskopie, Max-Born-Strasse 2A, D-12489 Berlin, Germany, and Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, Arizona 85721, USA

Received: March 3, 2005; In Final Form: November 1, 2005

1. Full listing of Reference 29

Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; G. Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98 (Revision A.2)*; Gaussian, Inc.: Pittsburgh PA., 1998.

2. Previous work on vibrational band assignments

Until now three different approaches have been followed in the elucidation of the nature of the vibrational bands of PYP observed in low-temperature trapped intermediates and in transient states of solution phase PYP. Chromophore model systems enable identification of marker modes of functional groups, such as phenolate ring vibrations or the C=O stretching mode.¹⁻⁴ PYP mutants where specific molecular units have been changed reveal the distinct contributions of these units to the vibrational spectra. For instance, in the E46Q PYP mutant Glu46 has been exchanged by Gln, and with this mutant it has been possible to identify the C=O stretching mode of the COOH-group of Glu46.^{5,6} Isotope substitution provides direct insight into the involvement of motions of specific atoms in the normal modes underlying the vibrational transitions. H/D exchange at the C₂ and C₄ positions, for instance, affect vibrational modes to which the phenolate group contributes. H/D exchange at C₈ reveals the vibrations with motions of the ethylenic group, and ¹²C-¹³C exchange at C₉ affects modes with C=O stretching involvement. The latter approach of isotope substitution has been pursued in an extensive study of low-temperature trapped intermediates.^{3,7} The determination of marker modes in these studies have provided

conclusive evidence on structural changes that accompany the transition of the P-state to the intermediate I₁ and I₂ states and the low-temperature trapped intermediates PYP_B, PYP_H, PYP_L and PYP_M.

3. Dark P state chromophore geometry

For the electronic ground dark state P, where the chromophore is in the trans configuration, we focus our analysis on the spectral range from 1800 to 1660 cm⁻¹ and 1450 to 970 cm⁻¹ of the fingerprint region, where amide I and amide II mode contributions are negligible. We record thirteen bleached bands with a magnitude large enough to determine the dichroic ratio (Table S1). In the spectral range from 1450 to 970 cm⁻¹ four bands strongly support the chromophore geometry with the C=O tilted out-of-plane (3PYP structure, Fig. 4b). These four bands are the hydrogen out-of-plane (H-oop) HC₇=C₈H wagging mode at 986 cm⁻¹, the C₈-C₉^{trans} stretching mode at 1060 cm⁻¹, the δCH(9a) phenolate vibration (also indicated as Y9a) at 1166 cm⁻¹ and the C=C-C(-S)=O stretching vibration at 1300 cm⁻¹.^{1-3,7}

Using the 3PYP/1NWZ geometry the calculated frequency $\nu^c_{\gamma\text{HCCH}} = 996 \text{ cm}^{-1}$ and angle $\Theta^c_{\gamma\text{HCCH}} = 25^\circ$ of the H-oop mode correspond much better to the experimental values of $\nu^e = 986 \text{ cm}^{-1}$ and $\Theta^e = 31 \pm 5^\circ$ than the values of $\nu^c_{\gamma\text{HCCH}} = 952 \text{ cm}^{-1}$ and $\Theta^c_{\gamma\text{HCCH}} = 82^\circ$ calculated with the 3PHY structure.

Furthermore, the C₈-C₉^{trans} stretching vibration at $\nu^c_{\text{vC8C9}} = 1060 \text{ cm}^{-1}$, assigned as a trans geometry marker band³, has an angle of $\Theta^c_{\text{vC8C9}} = 23 \pm 5^\circ$. This is in better agreement with the values for the 3PYP/1NWZ structure ($\nu^c_{\text{vC8C9}} = 1117 \text{ cm}^{-1}$ and $\Theta^c_{\text{vC8C9}} = 18^\circ$) than for the planar 3PHY geometry (calculated values of $\nu^c_{\text{vC6C7}} = 1141 \text{ cm}^{-1}$ and $\Theta^c_{\text{vC6C7}} = 5^\circ$ or $\nu^c_{\text{vC8C9}} = 939 \text{ cm}^{-1}$ and $\Theta^c_{\text{vC8C9}} = 18^\circ$).

The marker band for a deprotonated chromophore at 1166 cm⁻¹ has been assigned to the δCH(9a) (or Y9a) vibration.² This band also exhibits different vibrational character for the two reported trans structures. While the calculations for the 3PYP/1NWZ structure supports the assignment with a frequency of $\nu^c_{\delta\text{CH}(9a)} = 1161 \text{ cm}^{-1}$, the calculations for the 3PHY structure result in a frequency of $\nu^c_{\delta\text{CH}(9a)} = 1092 \text{ cm}^{-1}$ and a vibrational character with additional contributions of in-plane hydrogen HC₇=C₈H bending. In addition to that, the low-temperature study of

Imamoto *et al.* reveals that H/D exchange at the chromophore positions C₂ and C₄ leads to a significant frequency down shift of more than 90 cm⁻¹ (no absorption band between 1170 and 1060 cm⁻¹).⁷ H/D exchange at these positions down shifts the calculated δCH(9a) frequency position for the 3PYP/1NWZ geometry by -92 cm⁻¹ (Table S2). In contrast, for the planar 3PHY geometry H/D exchange leads to a significantly minor frequency down shift of -64 cm⁻¹ due to the in-plane hydrogen HC₇=C₈H bending contribution (Table S3).

Finally, around 1300 cm⁻¹ a C-C(-S)=O stretching vibration is observed¹. We measure an angle of $\Theta^{\circ}_{\nu_{\text{CCSO}}} = 29 \pm 3^{\circ}$, whereas we calculate values of $\Theta^{\circ}_{\nu_{\text{C8C9}}} = 9^{\circ}$ with the 3PHY structure. For the 3PYP/1NWZ geometry three vibrations are located around 1300 cm⁻¹ with angles of 13, 18 and 59 degrees. This implies that our experimentally determined angle may be the resulting average value of these overlapping bands.

For one single band we found an apparent better correspondence for the 3PHY planar geometry. This band, located at 1445 cm⁻¹, has a measured angle $\Theta^{\circ} = 42 \pm 10^{\circ}$. In the case of the 3PHY geometry two vibrational transitions with angles of 50 and 12 degrees contribute to the signal. In contrast, the value calculated with the 3PYP/1NWZ geometry is $\Theta^{\circ}_{\text{phe}} = 24^{\circ}$. We feel that this contrasting behaviour may be caused by interference of the bleach signals with positive absorbance bands of the I₀ state.

4. Orientation of the COOD-group of Glu46 in the P, P* and I₀ states

In the early stages of isomerization of PYP from the P to the I₀ state geometric changes are primarily due to chromophore rearrangements. Only the carboxyl side group of Glu46 exhibits a marked response upon electronic excitation. Analysis of the direction of the νC=O transition dipole moment of the COOD group of Glu46 provides insight into the response of the protein surroundings to chromophore rearrangements. The direction of the C=O vibrational transition moment is similar in the P and P* states indicating that the COOD side group of Glu46 does not change its orientation upon electronic excitation. Instead, optical excitation of PYP leads to a weakening of the hydrogen bond between Glu46 and the phenolate of the chromophore, presumably due to a net charge flow from the phenolate to the (C₉=O)-S region of the chromophore. Moreover, when P* relaxes to the first product state I₀, no transient response of Glu46 is observed for either polarization directions of the mid-IR probe pulse. From this we conclude that the hydrogen bond between Glu46 and the phenolate of the chromophore recovers its strength while retaining the orientation of the COOD-group. As a result we can conclude that in the primary stages of the PYP photoinduced geometry changes the chromophore isomerization reaction does not involve a significant relocation of the oxygen side of the phenolate group that is directly linked to Glu46.

Because additional calculations including Glu46 show that deviation of the νC=O transition dipole moment to the C=O double bond direction is less than 20°, we directly calculate the angle between the

electronic transition dipole moment of the chromophore and the C=O double bond direction of Glu46 to be $\Theta^{\circ}_{\text{Glu46}} = 61 \pm 20^{\circ}$ for the planar 3PHY geometry. For the slightly tilted chromophore geometries 3PYP and 1NWZ we calculate $\Theta^{\circ}_{\text{Glu46}} = 35 \pm 20^{\circ}$ and $\Theta^{\circ}_{\text{Glu46}} = 72 \pm 20^{\circ}$, respectively. The measured angle of $\Theta^{\circ}_{\text{Glu46}} = 84 \pm 6^{\circ}$ has the best correspondence with the 1NWZ structure. From that we conclude that although the 3PYP structure, obtained with X-ray diffraction on crystalline PYP, is a better representation of the chromophore geometry of PYP in solution, we expect that the flexible amino acids in the protein binding pocket may be more sufficiently described with the 3PHY structure, that has been obtained with nuclear magnetic resonance on solution phase PYP. However, the more recent refined 1NWZ structure obtained with X-ray diffraction on crystalline PYP turns out to describe most appropriately both the tilted chromophore trans geometry and the nearly perpendicular orientation of the C=O group of Glu46. Regrettably a more refined PYP_B structure has not been reported so far, and thus it remains a future task to test further the structural similarities between the transient I₀ and PYP_B states.

5. First intermediate I₀ state chromophore geometry

For the first intermediate I₀ state we observe five chromophore bands in the difference spectrum, but only three are located in the spectral range not potentially affected by amide I and amide II absorptions.

The C₈-C₉^{cis} marker band for the cis chromophore geometry³, experimentally observed at $\nu^{\circ}_{\nu_{\text{C8C9}}} = 1000$ cm⁻¹ is calculated to absorb at $\nu^{\circ}_{\nu_{\text{C8C9}}} = 972$ cm⁻¹ in the case of the low-temperature trapped cis chromophore 3PYP geometry (Fig. 4e). A better correspondence with experiment results in the case of the chromophore with relaxed planar geometry with $\nu^{\circ}_{\nu_{\text{C8C9}}} = 983$ cm⁻¹ (Fig. 4d).

The vibrational band at 1495 cm⁻¹, with measured angle $\Theta^{\circ} = 35 \pm 4^{\circ}$ (Table S1), is probably caused by several modes. For the 3PYP cis geometry we calculate two corresponding vibrations around this frequency position with angles of 18 and 4 degrees (Table S4). Here, also the relaxed cis chromophore geometry has a better correspondence with the experimental signals as three vibrations with calculated angles of 30, 10 and 26 degrees can contribute (Table S4).

Strikingly better results are obtained in the calculations for the vibrational band experimentally observed at $\nu^{\circ}_{\nu_{\text{CO}}} = 1669$ cm⁻¹ with an angle of $\Theta^{\circ}_{\nu_{\text{CO}}} = 69 \pm 10^{\circ}$. This vibration has been assigned to the C=O stretching vibration in I₁ by Unno *et al.*⁴ With the relaxed planar chromophore geometry we calculate a vibration with dominant C=O stretching character located at $\nu^{\circ}_{\nu_{\text{CO}}} = 1696$ cm⁻¹ with an angle $\Theta^{\circ}_{\nu_{\text{CO}}} = 76^{\circ}$, in good agreement with the experimental values. In contrast, for the 3PYP cis chromophore geometry two bands with frequencies of 1639 and 1787 cm⁻¹ and angles of 5 and 16° have a vibrational mode character with little C=O stretching involvement (Table S4). From this latter feature we conclude that only the relaxed planar cis chromophore geometry, as presented in Fig. 4d, is responsible for the measured vibrational frequencies and angles in the I₀ state.

TABLE S1 : Frequency Positions ν^e and Angles Θ^e Obtained from the Experimental Absorbance Difference Spectra of PYP.

Bleach signals (electronic ground P state structure)		Transient absorbance signals (I_0 product state structure)	
Θ^e	ν^e	Assignment	Assignment
84 ± 6	1727	$\nu\text{C}=\text{O}$ of Glu46;	$\nu\text{C}=\text{O}$ of Glu46 (electronic excited state);
58 ± 6	1647	amide I + $\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g)$	$\nu\text{C}=\text{O} + \delta\text{HC}=\text{CH}(A_1) + \nu\text{PhO}(8a) + \delta\text{CH}(9a)$;
57 ± 5	1614	amide I + $\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g) + \nu\text{C}=\text{O}$	$\nu\text{C}=\text{O} + \delta\text{HC}=\text{CH}(A_1) + \nu\text{PhO}(8a) + \delta\text{CH}(9a)$;
39 ± 3	1564	$\nu\text{PhO}(8b) + \delta\text{CH}(3)$; $\nu\text{PhO}(14) + \delta\text{CH}(18a)$;	$\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C} + \delta\text{HC}=\text{CH}(A_1)$
45 ± 2	1511	$\nu\text{PhO}(19a) + \delta\text{CH}(18a) + \nu\text{C}=\text{O}$; $\nu\text{C}=\text{O} + \nu\text{C}_8-\text{C}_9 + \delta\text{CH}(18a)$	$\nu\text{C}-\text{O} + \nu\text{C}=\text{O} + \nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C} + \delta(=\text{C}_7\text{H})$
42 ± 10	1445	$\nu\text{PhO}(18b) + \delta\text{HC}=\text{CH}(B_u)$	$\nu\text{PhO}(19b) + \delta\text{CH}(18b) + \nu\text{C}-\text{O}$
29 ± 3	1302	$\delta\text{CH}(3) + \delta\text{HC}=\text{CH}(A_g)$; $\delta\text{CH}(3) + \delta\text{HC}=\text{CH}(B_u)$;	$\nu\text{C}_8-\text{C}_9 + \delta\text{HC}=\text{CH}(B_1) + \delta\text{CH}(18a)$;
14 ± 14	1242	$\nu\text{C}-\text{O} + \delta\text{HC}=\text{CH}(A_g) + \delta\text{CH}(3)$	
16 ± 16	1227	$\delta\text{HC}=\text{CH}(B_u) + \delta\text{CH}(\text{PhO}) + \nu\text{C}_6-\text{C}_7-\text{C}_8$	
23 ± 9	1166	protein	
23 ± 5	1060	$\delta\text{CH}(9a)$;	
42 ± 7	1045	$\nu\text{C}_8-\text{C}_9 + \delta(=\text{C}_7\text{H})$;	
31 ± 5	986	$\delta\text{CH}(18b)$	
		$\gamma\text{HC}=\text{CH}(A_u)$;	
		$\gamma\text{HC}_8=\text{C}_9\text{H}$ (oop) ^d	

a : ν : stretching vibration; δ : in-plane vibration; γ : out-of-plane vibration.

b : Ref. 7.

c : Refs. 1,2,8.

d : Refs. 3,9.

e : Ref. 10.

TABLE S2: Frequency Positions ν^c and Angles Θ^c of the Electronic Ground P State Calculated with B3LYP/6-31+G(d,p) (Frequency Scaling Factor 0.96) using the 3PYP Structure Reported in the Crystalline PYP X-Ray Diffraction Study^{11,12}.

<i>trans</i> chromophore structure ^a			2,4-d ^b			
No	Θ^c	ν^c	Assignment	No	ν^c	Assignment
76	22	1690	$\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g)$	76	1687	$\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g)$
75	6	1648	$\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g) + \nu\text{C}=\text{O}$	75	1643	$\nu\text{PhO}(8a) + \delta\text{CH}(9a) + \nu\text{C}=\text{C}(A_g) + \nu\text{C}=\text{O}$
73	87	1599	$\nu\text{PhO}(8b) + \delta\text{CH}(3)$	73	1586	$\nu\text{PhO}(8b) + \delta\text{CH}(3)$
72	18	1539	$\nu\text{PhO}(14) + \delta\text{CH}(18a)$	72	1531	$\nu\text{PhO}(14) + \delta\text{CH}(18b)$
71	13	1520	$\nu\text{C}=\text{O} + \nu\text{C}_8-\text{C}_9 + \delta\text{CH}(18a)$	71	1514	$\nu\text{C}=\text{O} + \nu\text{C}_8-\text{C}_9$
70	25	1509	$\nu\text{PhO}(19a) + \delta\text{CH}(18a) + \nu\text{C}=\text{O}$	70	1483	$\nu\text{PhO}(19a) + \delta\text{CH}(18a)$
65	24	1407	$\nu\text{PhO}(18b) + \delta\text{HC}=\text{CH}(B_u)$	65	1396	$\nu\text{PhO}(19b) + \delta\text{CH}(18b) + \delta\text{CH}=\text{CH}(B_u)$
62	32	1356	$\delta\text{HC}=\text{CH}(B_u) + \delta\text{CH}(18a) + \nu\text{C}_6-\text{C}_7$	62	1345	$\delta\text{HC}=\text{CH}(B_u) + \nu\text{C}_6-\text{C}_7 + \delta\text{C}_1\text{H}(\text{PhO})$
59	59	1308	$\delta\text{CH}(3) + \delta\text{HC}=\text{CH}(A_g)$	59	1305	$\delta\text{HC}=\text{CH}(A_g)$
58	18	1292	$\delta\text{CH}(3) + \delta\text{HC}=\text{CH}(B_u)$	58	1289	$\delta\text{C}_{1,5}\text{H}(\text{PhO}) + \delta(=\text{C}_7\text{H})$
57	13	1290	$\nu\text{C}-\text{O} + \delta\text{HC}=\text{CH}(A_g) + \delta\text{CH}(3)$	55	1233	$\delta\text{CH}(3)$
54	4	1228	$\delta\text{HC}=\text{CH}(B_u) + \delta\text{CH}(\text{PhO}) + \nu\text{C}_6-\text{C}_7-\text{C}_8$	54	1215	$\delta\text{HC}=\text{CH}(B_u) + \nu\text{C}_6-\text{C}_7 + \delta\text{C}_{1,5}\text{H}(\text{PhO})$
53	11	1161	$\delta\text{CH}(9a)$	50	1117	$\nu\text{C}_8-\text{C}_9 + \delta=\text{C}_8\text{H} + \delta\text{C}_5\text{H}(\text{PhO})$
49	18	1117	$\nu\text{C}_8-\text{C}_9 + \delta(=\text{C}_7\text{H})$	49	1069	$\delta\text{CH}(9a)$
48	59	1111	$\delta\text{CH}(18b)$	48	1068	$\nu\text{PhO}(19a) + \delta\text{CH}(9a) + \text{Cys69}$
45	63	1003	$\nu\text{PhO}(19a) + \delta\text{CH}(18a)$	46	995	$\gamma\text{HC}=\text{CH}(A_u)$
44	25	996	$\gamma\text{HC}=\text{CH}(A_u)$	44	940	$\gamma\text{PhO}(\text{C}_4\text{H}+\text{C}_5\text{H})$
43	22	976	$\gamma\text{PhO}(\text{C}_4\text{H}+\text{C}_5\text{H})$	43	916	$\gamma\text{PhO}(\text{C}_1\text{H}+\text{C}_2\text{H})$
41	23	953	$\gamma\text{HC}=\text{CH}(A_u) + \gamma\text{PhO}(\text{C}_1\text{H}+\text{C}_2\text{H}+\text{C}_3\text{H})$			

a: The C-H and N-H distances have been optimised.

b: Column refers to case of H/D isotope substitution on C₂ and C₄.

Table S3. Frequency positions ν^c and angles Θ^c of the electronic ground P state calculated with B3LYP/6-31+G(d,p) (frequency scaling factor 0.96) using the 3PHY structure reported in the solution phase PYP NMR study¹³.

No		Θ^c	ν^c	<i>trans</i> chromophore structure		2,4-d ^a	
				Assignment	No	ν^c	Assignment
83	35	1793	$\nu\text{C}=\text{C}(\text{A}_g)+\nu\text{C}=\text{O}+\nu\text{C}=\text{O}+\delta\text{HC}=\text{CH}(\text{A}_g)$		83	1792	$\nu\text{C}=\text{C}(\text{A}_g)+\nu\text{C}=\text{O}+\nu\text{C}=\text{O}+\delta\text{HC}=\text{CH}(\text{A}_g)$
82	10	1759	$\nu\text{C}-\text{O}+\nu\text{C}=\text{O}+\nu\text{C}=\text{C}(\text{A}_g)+\text{Cys69}$		82	1757	$\nu\text{C}-\text{O}+\nu\text{C}=\text{O}+\nu\text{C}=\text{C}(\text{A}_g)$
80	22	1617	$\nu\text{C}=\text{O}+\nu\text{C}=\text{C}(\text{A}_g)$		80	1651	$\nu\text{C}=\text{O}+\nu\text{C}=\text{C}(\text{A}_g)$
79	36	1554	$\nu\text{PhO}(8b)+\delta\text{CH}(3)+\delta\text{HC}=\text{CH}(\text{B}_u)$		79	1543	$\nu\text{PhO}(8b)+\delta\text{CH}(3)+\delta\text{HC}=\text{CH}(\text{B}_u)$
78	6	1502	$\nu\text{PhO}(8a)+\delta\text{CH}(18a)+\nu\text{C}_6-\text{C}_7$		78	1495	$\nu\text{PhO}(8a)+\delta\text{CH}(9a)+\nu\text{C}_6-\text{C}_7$
73	12	1405	$\nu\text{PhO}(19a)+\delta\text{CH}(18a)$		72	1379	$\nu\text{PhO}(19b)+\delta\text{CH}(18b)$
71	50	1383	$\nu\text{PhO}(19b)+\delta\text{CH}(18b)$		70	1364	$\nu\text{PhO}(19a)+\delta\text{CH}(18a)+\delta\text{HC}=\text{CH}(\text{A}_g)$
68	11	1348	$\nu\text{PhO}(19b)+\delta\text{CH}(18b)+\delta\text{HC}=\text{CH}(\text{B}_u)$		67	1332	$\nu\text{PhO}(19b)+\delta\text{CH}(18b)+\delta\text{HC}=\text{CH}(\text{B}_u)$
66	9	1300	$\delta\text{HC}=\text{CH}(\text{A}_g)$		66	1299	$\delta\text{HC}=\text{CH}(\text{A}_g)$
64	17	1233	$\delta\text{CH}(3)+\delta\text{HC}=\text{CH}(\text{B}_u)$		63	1223	$\delta\text{HC}=\text{CH}(\text{B}_u)$
62	86	1222	$\delta\text{CH}(3)+\delta\text{HC}=\text{CH}(\text{B}_u)$		60	1161	$\delta\text{CH}(3)+\delta\text{HC}=\text{CH}(\text{B}_u)$
59	5	1141	$\nu\text{C}_6-\text{C}_7+\delta\text{CH}(9a)+\delta\text{HC}=\text{CH}(\text{B}_u)$		58	1117	$\nu\text{C}_6-\text{C}_7+\delta\text{CH}(9a)+\delta\text{HC}=\text{CH}(\text{B}_u)$
55	11	1092	$\delta\text{CH}(9a)+\delta\text{HC}=\text{CH}(\text{B}_u)$		53	1028	$\delta\text{CH}(9a)$
53	47	1043	$\delta\text{CH}(18b)+\delta\text{HC}=\text{CH}(\text{B}_u)$		50	951	$\gamma\text{HC}=\text{CH}(\text{A}_u)$
50	50	971	$\nu\text{PhO}(12)+\delta\text{CH}(18a)$		49	939	$\nu\text{C}_8-\text{C}_9+\delta(=\text{C}_8\text{H})$
48	82	952	$\gamma\text{HC}=\text{CH}(\text{A}_u)$				
47	18	939	$\nu\text{C}_8-\text{C}_9+\delta(=\text{C}_8\text{H})$				

 a: Column refers to case of H/D isotope substitution on C₂ and C₄.

TABLE S4: Frequency Positions ν^c and Angles Θ^c of the I_0 Product State Calculated with B3LYP/6-31+G(d,p) (Frequency Scaling Factor 0.96) using the 3PYP Structure Reported for PYP_B in the Crystalline PYP X-Ray Diffraction Study¹¹ (Right Column) and the Planar Chromophore Geometry after Optimization of the C7=C8-(C9=O)-S Moiety (Left Column).

I_0 product state with optimised cis geometry ^a			PYP _B structure ^a		
No	ν^c	Θ^c	No	ν^c	Θ^c
76	1696	76	76	1787	16
					Assignment
					$\nu\text{C}=\text{O} + \delta\text{HC}=\text{CH}(\text{A}_1) + \nu\text{PhO}(\text{8a}) + \delta\text{CH}(\text{9a})$
75	1632	65	75	1639	5
					$\nu\text{PhO}(\text{8a}) + \delta\text{CH}(\text{9a}) + \nu\text{C}=\text{C} + \delta\text{HC}=\text{CH}(\text{A}_1)$
74	1574	6	74	1587	46
					$\nu\text{C}-\text{O} + \nu\text{C}=\text{O} + \nu\text{PhO}(\text{8a}) + \delta\text{CH}(\text{9a}) + \nu\text{C}=\text{C} + \delta(=\text{C}_7\text{H})$
72	1526	7	72	1531	4
					$\nu\text{C}-\text{O} + \delta\text{CH}(\text{18a}) + \nu\text{C}=\text{C} + \delta\text{HC}=\text{CH}(\text{A}_1)$
71	1510	26	71	1508	18
					$\nu\text{PhO}(\text{19b}) + \delta\text{CH}(\text{18b}) + \nu\text{C}-\text{O}$
70	1462	10	67	1407	15
					$\delta\text{HC}=\text{CH}(\text{B}_1) + \nu\text{PhO}(\text{8b}) + \delta\text{CH}(\text{3})$
69	1457	30	66	1398	20
					$\nu\text{PhO}(\text{8b}) + \delta\text{CH}(\text{3}) + \delta\text{HC}=\text{CH}(\text{B}_1)$
64	1404	10	64	1386	38
					$\nu\text{PhO}(\text{19a}) + \delta\text{CH}(\text{18a}) + \delta\text{HC}=\text{CH}(\text{B}_1) + \text{Cys69}$
62	1361	17	62	1328	86
					$\nu\text{PhO}(\text{14}) + \delta\text{CH}(\text{3}) + \delta\text{HC}=\text{CH}(\text{A}_1)$
59	1277	10	60	1298	78
					$\delta\text{HC}=\text{CH}(\text{A}_1) + \delta\text{CH}(\text{3})$
56	1248	53	55	1214	23
					$\nu\text{PhO}(\text{14}) + \delta\text{CH}(\text{3}) + \delta\text{HC}=\text{CH}(\text{A}_1)$
55	1216	77	54	1183	50
					$\nu\text{C}_6-\text{C}_7 + \delta\text{CH}(\text{18a}) + \delta\text{HC}=\text{CH}(\text{B}_1)$
53	1168	11	52	1172	7
					$\delta\text{CH}(\text{9a})$
49	1106	46	49	1109	40
					$\delta\text{CH}(\text{18b})$
48	1042	52	47	1061	56
					$\gamma\text{PhO}(\text{C}_4\text{H} + \text{C}_5\text{H})$
45	983	12	46	1004	79
					$\nu\text{C}_8-\text{C}_9 + \delta\text{HC}=\text{CH}(\text{B}_1) + \delta\text{CH}(\text{18a})$
44	971	14	44	972	13
					$\gamma\text{HC}=\text{CH} + \gamma\text{PhO}(\text{C}_1\text{H} + \text{C}_2\text{H})$
43	959	17	43	954	18
					$\nu\text{PhO}(\text{12}) + \delta\text{CH}(\text{18a})$
41	943	15	43	954	18
					$\gamma\text{HC}_7-\text{C}_8\text{H} + \gamma\text{PhO}(\text{C}_1\text{H} + \text{C}_2\text{H})$

a: The C-H and N-H distances have been optimised.

References

- (1) Kim, M.; Mathies, R. A.; Hoff, W. D.; Hellingwerf, K. *J. Biochemistry* **1995**, *34*, 12669-12672.
- (2) Zhou, Y.; Ujj, L.; Meyer, T. E.; Cusanovich, M. A.; Atkinson, G. H. *J. Phys. Chem. A* **2001**, *105*, 5719-5726.
- (3) Unno, M.; Kumauchi, M.; Sasaki, J.; Tokunaga, F.; Yamauchi, S. *J. Phys. Chem. B* **2003**, *107*, 2837-2845.
- (4) Unno, M.; Kumauchi, M.; Hamada, N.; Tokunaga, F.; Yamauchi, S. *J. Biol. Chem.* **2004**, *279*, 23855-23858.
- (5) Imamoto, Y.; Mihara, K.; Hisatomi, O.; Kataoka, M.; Tokunaga, F.; Bojkova, N.; Yoshihara, K. *J. Biol. Chem.* **1997**, *272*, 12905-12908.
- (6) Brudler, R.; Rammelsberg, R.; Woo, T. T.; Getzoff, E. D.; Gerwert, K. *Nat. Struct. Biol.* **2001**, *8*, 265-270.
- (7) Imamoto, Y.; Shirahige, Y.; Tokunaga, F.; Kinoshita, T.; Yoshihara, K.; Kataoka, M. *Biochemistry* **2001**, *40*, 8997-9004.
- (8) van Thor, J. J.; Pierik, A. J.; Nugteren-Roodzant, I.; Xie, A. H.; Hellingwerf, K. *J. Biochemistry* **1998**, *37*, 16915-16921.
- (9) Unno, M.; Kumauchi, M.; Sasaki, J.; Tokunaga, F.; Yamauchi, S. *Biochemistry* **2002**, *41*, 5668-5674.
- (10) Groot, M. L.; van Wilderen, L. J. G. W.; Larsen, D. S.; van der Horst, M. A.; van Stokkum, I. H. M.; Hellingwerf, K. J.; van Grondelle, R. *Biochemistry* **2003**, *42*, 10054-10059.
- (11) Genick, U. K.; Soltis, S. M.; Kuhn, P.; Canestrelli, I. L.; Getzoff, E. D. *Nature* **1998**, *392*, 206-209.
- (12) Getzoff, E. D.; Gutwin, K. N.; Genick, U. K. *Nat. Struct. Biol.* **2003**, *10*, 663-668.
- (13) Düx, P.; Rubinstenn, G.; Vuister, G. W.; Boelens, R.; Mulder, F. A. A.; Hard, K.; Hoff, W. D.; Kroon, A. R.; Crielaard, W.; Hellingwerf, K. J.; Kaptein, R. *Biochemistry* **1998**, *37*, 12689-12699.