Supporting Information for

Vibrational Assignment of the 4-Hydroxycinnamyl Chromophore in Photoactive Yellow Protein

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Table 1S. Calculated Optimized Geometries of Deprotonated *trans*-4-Hydroxycinnamyl Chromophore of PYP_{dark}.

	model 1	model 2	model 3	PYP_{dark} (Xtal)[a]
		Bond Length (Å)		
O1–C1	1.2517	1.2884	1.2959	1.315
C1–C2	1.4622	1.4368	1.4339	1.403
C2–C3	1.3677	1.3751	1.3751	1.360
C3–C4	1.4319	1.4220	1.4220	1.382
C4–C5	1.4305	1.4202	1.4204	1.402
C5–C6	1.3691	1.3771	1.3769	1.364
C6–C1	1.4584	1.4319	1.4295	1.389
C4–C7	1.4146	1.4300	1.4283	1.426
C7–C8	1.3827	1.3690	1.3709	1.337
C8–C9	1.4277	1.4432	1.4380	1.419
C9–O2	1.2266	1.2308	1.2339	1.250
C9–S	1.8829	1.8328	1.8368	1.773
C2–H	1.0877	1.0864	1.0860	
C3–H	1.0887	1.0873	1.0870	
C5–H	1.0906	1.0890	1.0885	
C6–H	1.0875	1.0863	1.0858	
C7–H	1.0906	1.0901	1.0898	
C8–H	1.0857	1.0857	1.0852	
O1…O (Y42)		2.7054	2.6188	2.4848
O1…O (E46)		2.5871	2.6119	2.5777

O2…N (C69)		3.1083	2.8914	2.7173
		Bond Angle (deg)		
O1–C1–C2	122.65	121.75	121.35	119.09
C1–C2–C3	122.63	121.51	121.28	120.00
C2–C3–C4	122.13	121.84	121.75	122.54
C3–C4–C5	116.16	116.71	116.86	116.81
C4–C5–C6	122.69	122.28	122.22	121.82
C3–C4–C7	124.08	123.93	123.87	122.88
C4–C7–C8	129.67	129.26	129.03	120.19
C7–C8–C9	120.88	120.53	120.50	121.59
C8–C9–O2	128.99	126.93	126.97	124.78
O2–C9–S	119.43	120.21	120.38	121.83
C9–S–C10	99.07	99.47	100.66	102.86
C9–O2…N (C69)		115.63	103.41	107.13
		Dihedral Angle (deg)		
O1–C1–C2–C3	180.00	-179.89	179.85	-178.34
O1–C1–C6–C5	-180.00	179.99	-179.87	177.81
C1–C2–C3–C4	0.00	-0.06	0.05	-0.38
C2–C3–C4–C7	180.00	-179.68	179.89	-175.65
C3–C4–C7–C8	-180.00	-0.87	0.28	-2.54
C5–C4–C7–C8	180.00	179.42	-179.99	-178.39
C4–C7–C8–C9	180.00	179.42	-179.40	165.10
C7–C8–C9–O2	0.01	0.12	1.79	-11.47
C7–C8–C9–S	180.00	-179.97	-178.29	173.11
H–C7–C8–H	-180.00	179.50	179.92	
H–C8–C9–O2	-180.00	-179.91	-177.81	
O2–C9–S–C10	0.00	1.50	9.43	1.71
C8–C9–O2..N (C69)		-119.49	-144.94	-138.45

[a] Getzoff et al. (2003).

Table 2S. Observed and Calculated Vibrational Frequency (cm^{-1}) of PYP_{dark} and Its Models[a]

ν_{obs}[b]	ρ_{obs}[c]	ν_{cal}[d] (model 1)	ρ_{cal}[e]	ν_{cal}[d] (model 2)	ρ_{cal}[e]	ν_{cal}[d] (model 3)	ρ_{cal}[e]	Assignment[f]
1631 (–2, –33, –4)	0.33	1671 (–36, –2)	0.32	1646 (–1, -33, –2)	0.27	1636 (–2, –31, –3)	0.29	ν_{11} νC9=O2
1600 (0, –2, –21)	nd	1623 (–2, –42)	0.28	1606 (0, 0, –33)	0.17	1607 (–1, 1, –36)	0.68	ν_{12} νCC(8a), νC7=C8
1556 (–4, –5, –33)	0.33	1572 (–1, –49)	0.65	1548 (0, –8, –29)	0.36	1548 (–5, –7, –25)	0.36	ν_{13} νC7=C8, νCC(19a)
1495 (+2, –2, –34)	0.33	1538 (–3, –10)	0.58	1509 (–4, –1, –37)	0.36	1507 (–7, 0, –47)	0.35	ν_{14} νCC(19a), νC7=C8
1470 (+2, 0, –30)	0.33	1441 (0, –49)	0.25	1493 (–1, 0, –50)	0.37	1498 (–4, 0, –52)	0.38	ν_{15} νCC+δCH(8b)
1438 (+1, –1, –38)	0.40	1490 (0, –51)	0.75	1447 (0, 0, –45)	0.45	1447 (0, 0, –45)	0.44	ν_{17} νCC+δCH(19b)
1343 (*0, 0, nd*)	nd	1406 (–1, –29)	0.55	1373 (+2, –1, –29)	0.71	1361 (0, –1, –30)	0.57	ν_{22} νC1–O1(7a')
1325 (*0, 0, nd*)	nd	1325 (-3, -10)	0.53	1314 (+1, -1, -8)	0.42	1323 (0, –3, –13)	0.68	ν_{18} δC7H=C8H(B_u)
1305 (+5, –2, nd)	0.29	1297 (0, -21)	0.73	1296 (0, -1, -17)	0.34	1299 (+1, –1, –19)	0.35	ν_{20} νCC+δCH(14), δC7H=C8H(A_g)
1288 (–1, 0, –5)	0.29	1253 (0, -3)	0.27	1271 (0, 0, -7)	0.34	1271 (+2, 0, –7)	0.35	ν_{23} δC7H=C8H(A_g)
1283 (–1, 0, –14)	0.29	1215 (0, 0, -47)	0.75	1255 (-1, 0, -28)	0.60	1266 (–2, 0, –31)	0.47	ν_{19} νCC+δCH(3)
nd		1194 (0, -14)	0.68	1197 (0, 0, -14)	0.41	1200 (0, 0, –14)	0.39	ν_{24} νC4–C7(7a), δC7H=C8H(B_u)
1163 (0, 0, -7)	0.40	1135 (0, -4)	0.49	1146 (-1, 0, -5)	0.20	1150 (–1, 0, –6)	0.25	ν_{25} δCH(9a)
nd	nd	1067 (-2, -6)	0.20	1084 (0, 0, -13)	0.75	1091 (0, 0, –14)	0.31	ν_{27} δCH(15)
1054[g] (–, –13, –3)	0.33	1054 (-9, -13)	0.18	1052 (0, -13, -4)	0.54	1055 (+1, –12, –3)	0.57	ν_{29} νC8–C9
999 (0, 0, –28)	nd	946 (0, -27)	0.26	966 (0, 0, -27)	0.21	969 (0, 0, –27)	0.14	ν_{28} νCC+δCCC(18a)
983 (+1, -1, +1)	0.35	967 (0 -1)	0.75	980 (0, 0, -1)	0.44	979 (0, 0, –1)	0.64	γ_2 γC7H=C8H(A_u)
961 (0, 0, -9)	nd	928 (0, -7)	0.75	928 (0, 0, -8)	0.52	931 (0, 0, –8)	0.53	γ_5 γCH(5)
943 (0, 0, -7)	nd	919 (0, -7)	0.75	918 (0, 0, -8)	0.75	926 (0, 0, –8)	0.69	γ_3 γCH(17a)
889 (–1, –4, *–6*)	0.26	853 (-6, -10)	0.13	866 (+1, -6, -9)	0.21	866 (0, –6, –10)	0.22	ν_{31} δC7C8C9, νCC(~1)
nd	nd	825 (0, -18)	0.75	824 (0, 0, -14)	0.39	830 (0, 0, –14)	0.68	γ_6 γCH(11)
847 (0, 0, -12)	0.33	786 (-1, -23)	0.10	816 (-3, -1, -21)	0.10	817 (+5, –1, –22)	0.10	ν_{32} νCC+νCO(1)
825 (-1, -1, -3)	0.33	2 x 416 (0, -11)	0.75	2 x 414 (0, 0, -11)	0.70	2 x 414 (–1, 0, –11)	0.64	2 x γCC(16a)
nd	nd	773 (0, -3)	0.75	798 (0, 0, -3)	0.70	801 (0, 0, –3)	0.75	γ_8 γC7H=C8H(B_g), γCH(10a
nd	nd	766 (0, -7)	0.75	781 (+1, 0, -7)	0.75	788 (0, 0, –7)	0.52	γ_7 γCH(10a), γC7H=C8H(B
766 (0, -7, -9)	nd	717 (-6, -6)	0.28	735 (-1, -7, -8)	0.33	733 (+7, –7, –8)	0.31	ν_{33} νCO+νCC(13), δC9=O2
733 (–1, –1, –21)	0.31	714 (0, -20)	0.75	709 (0, -1, -21)	0.75	711 (0, –1, –21)	0.72	γ_9 γCO+γCH+γCC(4), γC7H=C8H(B_g)
nd	nd	696 (-2, -2)	0.36	687 (0, -1, -1)	0.27	636 (0, –1, –1)	0.15	ν_{34} γS–C10
652 (+2, -19, -2)	nd	624 (-18, 0)	0.75	633 (0, -18, -1)	0.52	627 (+6, –16, –1)	0.35	γ_{10} γC9=O2
640 (-1, *+1*, -16)	nd	610 (0, -20)	0.75	625 (+3, 0, -19)	0.64	626 (–1, 0, –20)	0.75	ν_{35} δCCC(6b)
539 (-1, -1, -8)	0.32	515 (-1, -9)	0.61	525 (-1, -1, -9)	0.26	525 (–1, –1, –9)	0.66	ν_{36} δC4C7C8, δCCC(~6b) ring-deform.
nd	nd	485 (0, -10)	0.75	502 (0, 0, -11)	0.74	505 (0, 0, –11)	0.75	γ_{11} γCH+γCO+γCC(16b)
nd	nd	437 (-1, -6)	0.36	482 (-5, 0, -7)	0.46	493 (–7, 0, –6)	0.51	ν_{39} δCO(9b)
459 (-2, -1, -1)		464 (-1, -4)		465 (-1, -2, -2)		464 (–3, –1, –2)	0.35	ν_{37} δC8C9S, δC9SC10, δCCC(6a)
445 (-2, 0, -2)		404 (-1, -2)		427 (-4, 0, -1)		440 (–2, –1, –1)	0.17	ν_{38} δC8C9S, δC9SC10, δCCC(6a)
		416 (0, -11)		414 (0, 0, -11)		414 (–1, 0, –11)	0.64	γ_{12} γCC(16a)
376 (-1, 0, -8)		351 (0, -7)		358 (0, 0, -7)		359 (0, 0, –7)	0.71	γCO+γCC+γC4-C7(10b)
342 (-4?, 0, 0)								
		293 (0, -1)		274 (0, 0, -2)				ν_{40} δC9SC10, δC8C9S (??)
		218 (0, -4)		221 (0, 0, -3)				τCC+γCO(17b), γC7=C8

[a] This table summarizes the observed and calculated vibrational frequencies and depolarization ratio. nd = not determined. [b] Observed vibrational frequencies of PYP_{dark}. The numbers in parentheses are the isotope shifts of D_2O – na, ^{13}C=O – na, and $^{13}C_6$-ring – na, respectively. The numbers in italic are not accurately determined. [c] Observed depolarization of PYP_{dark}. [d] Calculated vibrational frequencies of model 3. The numbers in parentheses are the isotope shifts of D_2O – na, ^{13}C=O – na, and $^{13}C_6$-ring – na, respectively. [e] Calculated depolarization of model 3. [f] The observed Raman bands are assigned to the calculated in-plane (ν_i) or out-of-plane (γ_i) normal modes. Approximate descriptions of the calculated modes are also described. For ring vibrations, the corresponding vibrational modes of tyrosine are indicated. The –HC7=C8H– moiety of the chromophore can be approximately described by the point group C_{2h} for the two hydrogens, so that the local symmetry description is given for the hydrogen bending modes. [g] The observed frequency and isotope shifts for PYP_{dark} in D_2O, where the ν_{29} band is observed as a single band.

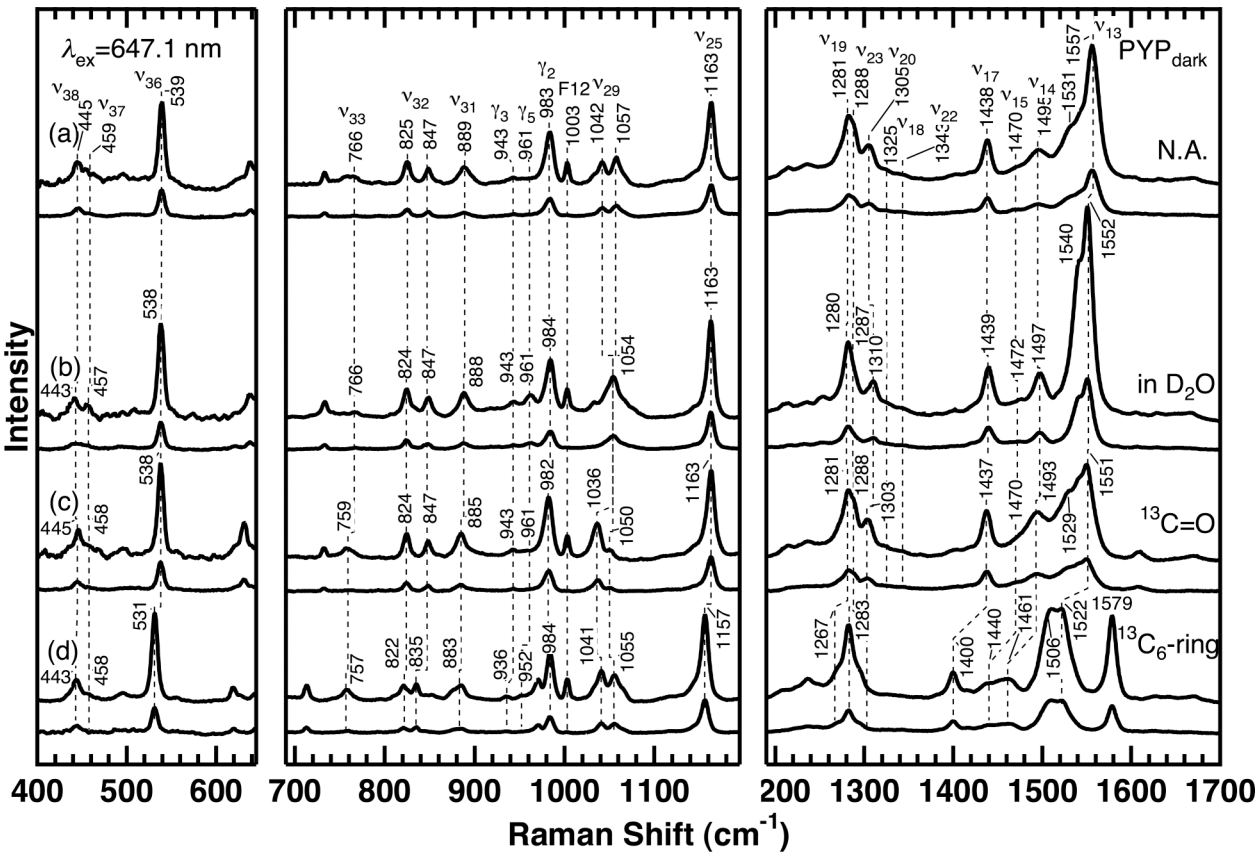

Figure 1S. Raman spectra of PYP_{dark} and its isotopomers in 10 mM Tris-HCl, pH 7.4. The spectra were obtained with 647.1 nm excitation.

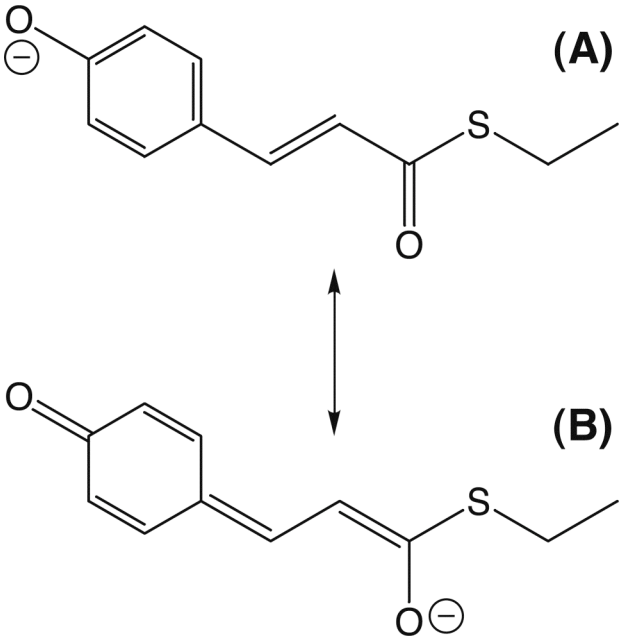

Figure 2S. Two possible resonance structures of the deprotonated *trans*-4-hydroxycinnamyl chromophore for PYP_{dark}.