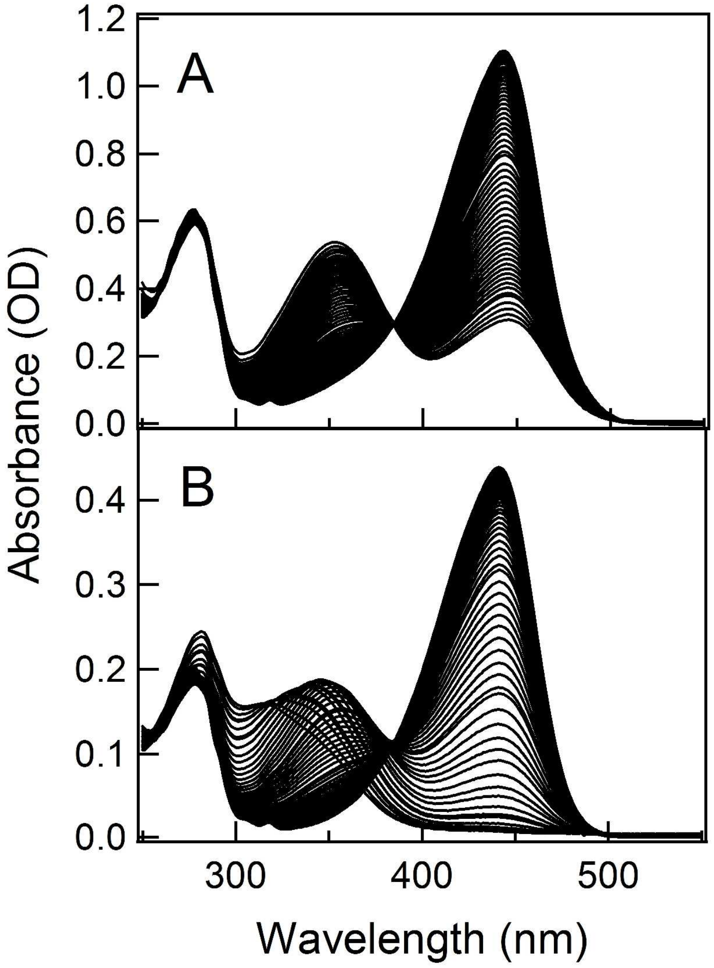
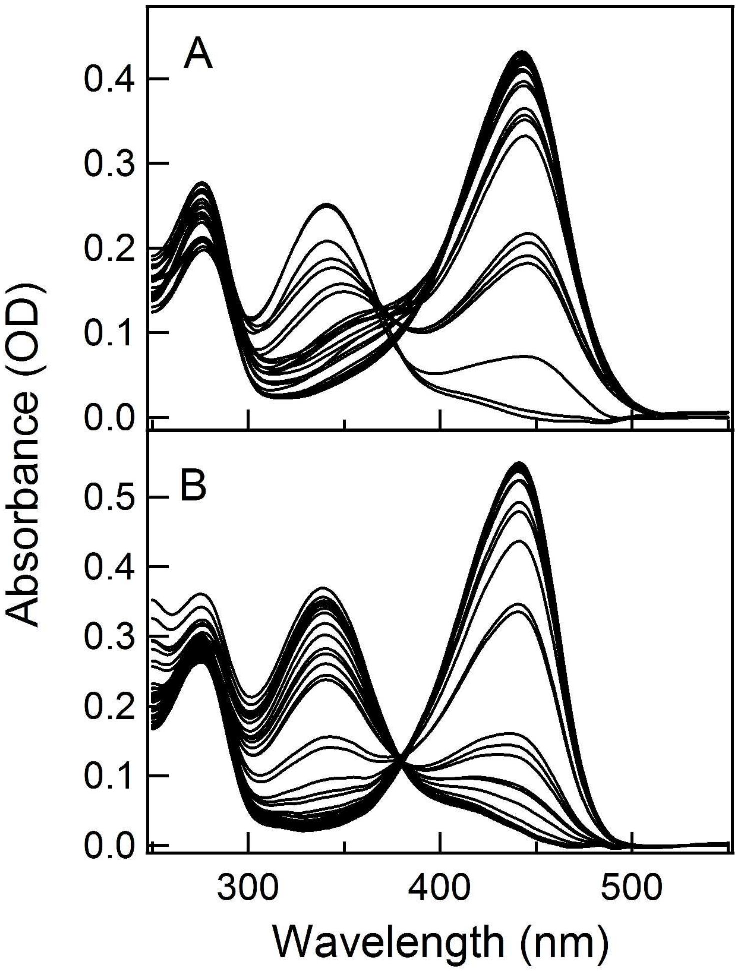
SUPPLEMENTAL MATERIALS

A Conserved Helical Capping Hydrogen Bond in PAS Domains Controls Signaling Kinetics in the Superfamily Prototype Photoactive Yellow Protein

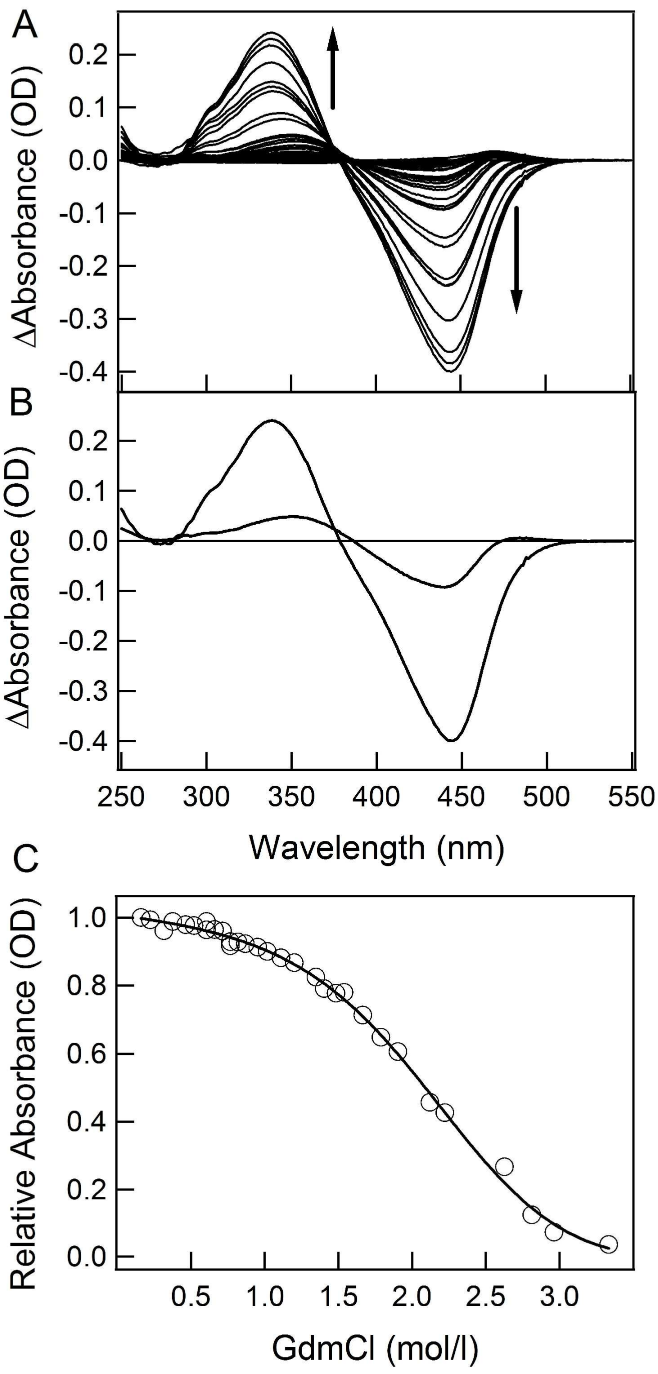
Masato Kumauchi, Sandip Kaledhonkar, Andrew Philip, James Wycoff, Miwa Hara, Yunxing Li, Aihua Xie, Wouter D. Hoff



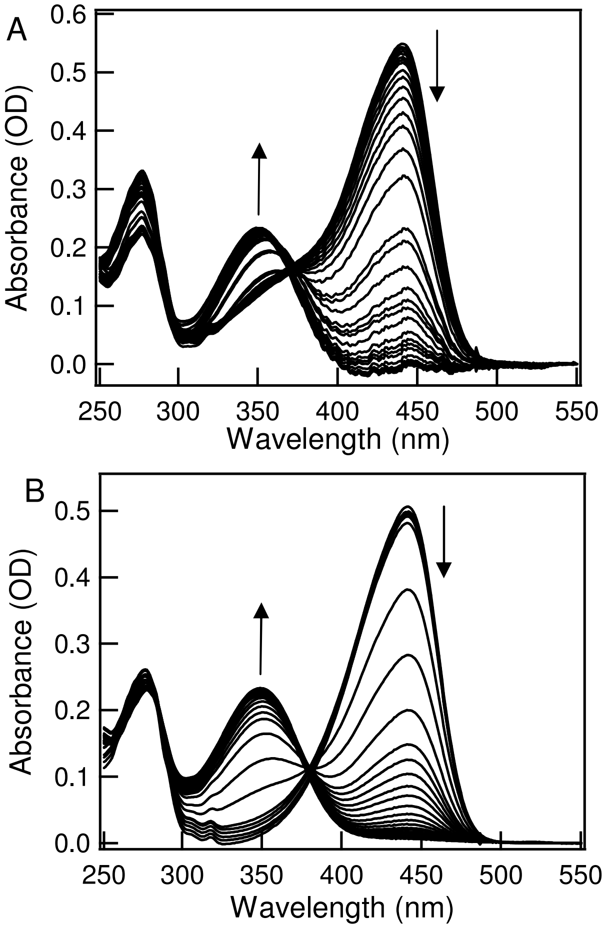
Supplemental Fig. 1. Absorbance spectra as a function of temperature for the N43A (A) and N43S (B) mutants of PYP. At lower temperatures the spectra exhibit a transition with an isosbestic point at 384 nm. The arrows indicate the direction of the spectral changes upon increasing the temperature.



Supplemental Fig. 2. Absorbance spectra as a function of Gdn-HCl concentration for the N43A (A) and N43S (B) mutants of PYP. The arrows indicate the direction of the spectral changes upon increasing the Gdn-HCl concentration.



Supplemental Fig. 3. Complex denaturant-induced unfolding in the N43A mutant of PYP. (A) Absorbance difference spectra as a function of Gdn-HCl concentration for the N43A (calculated from the data in supplemental Fig. 2A). (B) Characteristic difference spectra for the first (below 0.8 M Gdn-HCl) and second phase of the unfolding transition. (C) Dependence of the absorbance at 446 nm on Gdn-HCl. Despite the non-isosbestic nature of the transition and the spectral differences during the first and second half of the transition, the denaturant dependence of the transition as detected by the absorbance at 446 nm appears two-state.



Supplemental Fig. 4. Determination of the pK_a of the *p*CA in the N43A (A) and N43S (B) mutants of PYP by acid titration monitored with UV/vis absorbance spectroscopy. The arrows indicate the direction of the spectral changes upon lowering the pH. The pH values for the spectra of N43A PYP are 7.98, 7.68, 7.45, 7.00, 6.58, 6.48, 5.96, 5.88, 5.79, 5.66, 5.57, 5.38, 5.32, 5.16, 5.03, 4.09, 4.75, 4.42, 4.34, 4.28, 4.25, 4.10, 4.04, 4.01, 3.87, 3.81, 3.75, 3.69, 3.51, 3.38, 3.33, 3.21, 3.05. The pH values for the spectra of N43S PYP are 7.46, 7.46, 7.39, 7.00, 5.84, 4.88, 4.21, 3.50, 3.26, 3.08, 2.89, 2.82, 2.76, 2.70, 2.58, 2.48, 2.35, 2.26, 2.20, 2.15, 2.08, 2.00, 1.92, 1.87, 1.69, 1.55, 1.46. The spectral shoulder of N43A PYP near 350 nm around neutral pH is due to a small amount of photoexcitation by ambient and/or measurement light and the very slow rate of pB decay in this mutant.

Supplemental Table 1. Conservation of PYP Asn43 hydrogen bonding in PAS domains.

Protein[a] (PDB ID)	Residue	Helical cap (n + i) α-helix 3			Link to β-strand 1	
Asn		OD1	i	length (Å)(°)[b]	OD1	length (Å)(°)
Hh PYP (1NWZ)	Asn43	Glu46 NH	3	2.9 (136)	Ala30 NH	2.9 (159)
Rc PYP (1MZU)	Asn43	Glu46 NH	3	3.3 (150)	Ala30 NH	2.8 (168)
Rm FixL (1D06)	Asn163	Ala166 NH	3	2.8 (137)	Thr150 NH	2.8 (172)
Av NifL (2GJ3)	Asn51	Phe54 NH	3	2.9 (145)	Ile38 NH	3.0 (159)
Ec DOS - O_2 (1VB6)	Asn46	Ala49 NH	3	2.9 (138)	Ala33 NH	2.8 (164)
Ec DOS + O_2 (1S67)	Asn46	H_2O	-	2.6	-	
Bs YtvA (2PR5)	Asn43	Phe46 NH	3	2.9 (129)	Val27 NH	2.9 (154)
Gs Hkin2 (2R78)	Asn40	Ile43 NH	3	3.0 (128)	Ile27 NH	3.0 (159)
Hs ERG (1BYW)	Asn45	Phe48 NH	3	2.9 (145)	Phe29 NH	3.2 (158)
Hs PasK (lLL8)	Asn25	Ala28 NH	3	3.2 (147)	Ile11 NH	3.4 (142)
Vp NR(II) (3B33)	Asn30	Ala33 NH	3	3.0 (156)	Thr17 NH	2.9 (167)
Ro rha1 (3FG8)	Asn144	Cys147 NH	3	3.0 (165)	Phe131 NH	3.0 (158)
Hm HTR-l (3BWL)	Asn426	Phe429 NH	3	2.9 (156)	Ile413 NH	2.9 (119)
Hm HTR-l (3FC7)	Asn161	SeMet164NH	3	3.8 (107)	Ile148 NH	2.5 (127)
Ec TYRR (2JHE)	Asn106	Ser109 NH	3	2.8 (163)	[c]	
Ser		OG			OG	
Ss Cph1 (2VEA)	Ser46	Asn48 OD1	2	2.6 (163)	Val32 NH	3.1 (166)
Bj FixL (1DP6)	Ser169	Ala172 NH	3	3.4 (130)	Met156 NH	3.2 (173)
Ac LOV2 (1G28)	Ser947	Phe950 NH	3	3.3 (141)	Phe931 NH	3.0 (137)
Cr LOV1 (1N9L)	Ser38	Phe41 NH	3	3.2 (140)	Phe22 NH	3.1 (159)
As LOV2 (2V1A)	Ser431	Phe434 NH	3	3.1 (162)	Phe415 NH	3.3 (140)
At LOV1 (2Z6C)	Ser215	Ala216 C=O	1	3.1 (159)	Phe199 NH	2.9 (166)
NcVivid (2PD7)	Ser89	Phe92 NH	3	3.4 (148)	Leu73 NH	3.1 (166)
PF0695 (3CAX)[d]	Ser379	Arg383 NH_2	4	3.0 (122)	Val366 NH	2.8 (152)
Rp BphP3 (2OOL)	Ser59	Asn61 OD1	2	2.6 (163)	Leu45 NH	3.1 (169)
Dr Bphy (2O9C)	Ser55	Asn57 OD1	2	2.7 (160)	Leu41 NH	3.0 (157)
Bs Kina (2VLG)	Ser31	Asn33 OG	2	2.9 (168)	His18 NH	2.8 (161)
Asp		OD1			OD2	
Hs ARNT (2B02)	Asp377	Arg379 NH	2	2.9 (168)	Ile364 NH	2.8 (164)
Hs Hif2a (3F1N)	Asp258	Arg260 NH_2	2	2.0 (152)	Leu245 NH	1.9 (155)
Thr		OG1			OG1	
Dm PER (1WA9)	Thr255	Ser257 OG1	3	2.7 (102)	Cys241 NH	3.2 (140)
Gly						
Nc STHK (2P04)	Gly33	-	-		-	

[a] Hh = *Halorhodospira halophila*; Rc = *Rhodospirillum centenum*; Rm = *Rhizobium meliloti*; Av = *Azotobacter vinelandii*; Ec = *Escherichia coli*; Bs = *Bacillus subtilis*; Gs = *Geobacter sulfurreducens*; Hs = *Homo sapiens*; Bj = *Bradyrhizobium japonicum*; Ac =*Adantium capillus-veneris*; Cr = *Chlamydomonas reinhardtii*; Hm = *Haloarcula marismortui*; Vp = *Vibrio parahaemolyticus*; Ro = *Rhodococcus sp.*; At = *Arabidopsis thaliana*; As = *Avena sativa*; Rp = *Rhodopseudomonus palustris*; Dm = *Drosophila melanogaster* ; Ss = *Synechocystis sp. pcc 6803*; Nc = *Neurospora crassa*; Dr = *Deinococcus radiodurans*; Nc = *Nostoc punctiforme pcc 73102*. [b] The hydrogen bonding length and angle are indicated. [c] NH_2 – Glu91C=O (3.0 Å, 153 degrees); [d] Uncharacterized protein PF0695.