Supporting Information

Rockwell et al. 10.1073/pnas.0902370106

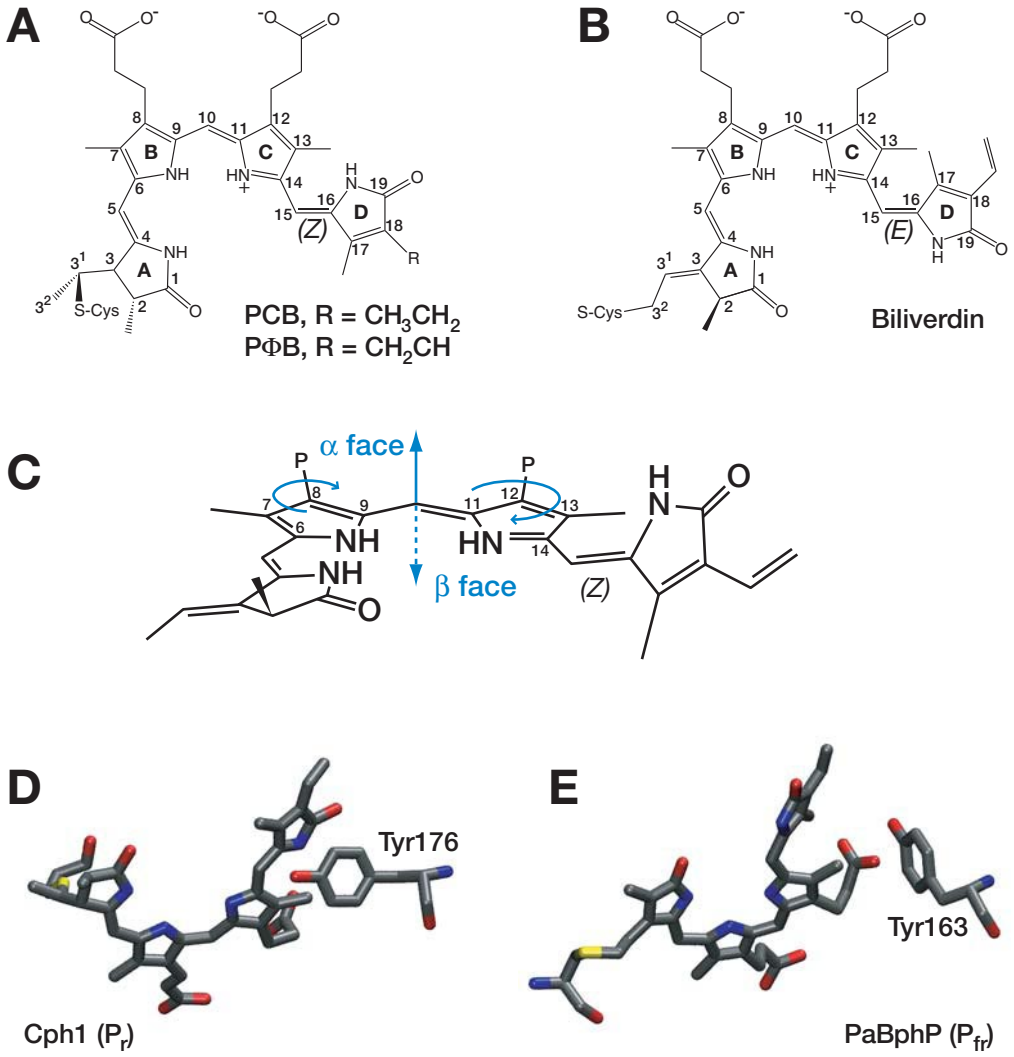


Fig. S1. Structure and nomenclature of bilin chromophores. (*A*) The structure of the phytobilins phycocyanobilin (PCB) and phytochromobilin (PΦB) is shown in the C5-*Z*,*syn* C10-*Z*,*syn* C15-*Z*,*anti* configuration of Cph1 in the P_r state. Ring names and numbering are shown, as is the *Z* configuration of the 15,16 double bond. (*B*) The structure of biliverdin IXα (BV) is shown in the C5-*Z*,*syn* C10-*Z*,*syn* C15-*E*,*anti* configuration of *Pa*BphP in the P_{fr} state. Stereochemistry at C2 is inferred from that observed in the high-resolution structure of DrBphP in the P_r state. (*C*) The convention for naming bilin facial dispositions is shown for the BV chromophore of *Dr*BphP in the P_r state. The P_r D-ring lies on the α face of the coplanar B- and C-rings. Views in *A* and *B* are thus from the α face. (*D*) The locations of PCB and Tyr-176 in the crystal structure of the Cph1 P_r state (Protein Data Bank ID code 2VEA) are shown from the β-face. (*E*) A similar view is shown for BV and Tyr-163 in the crystal structure of the PaBphP P_{fr} state (Protein Data Bank ID code 3C2W).

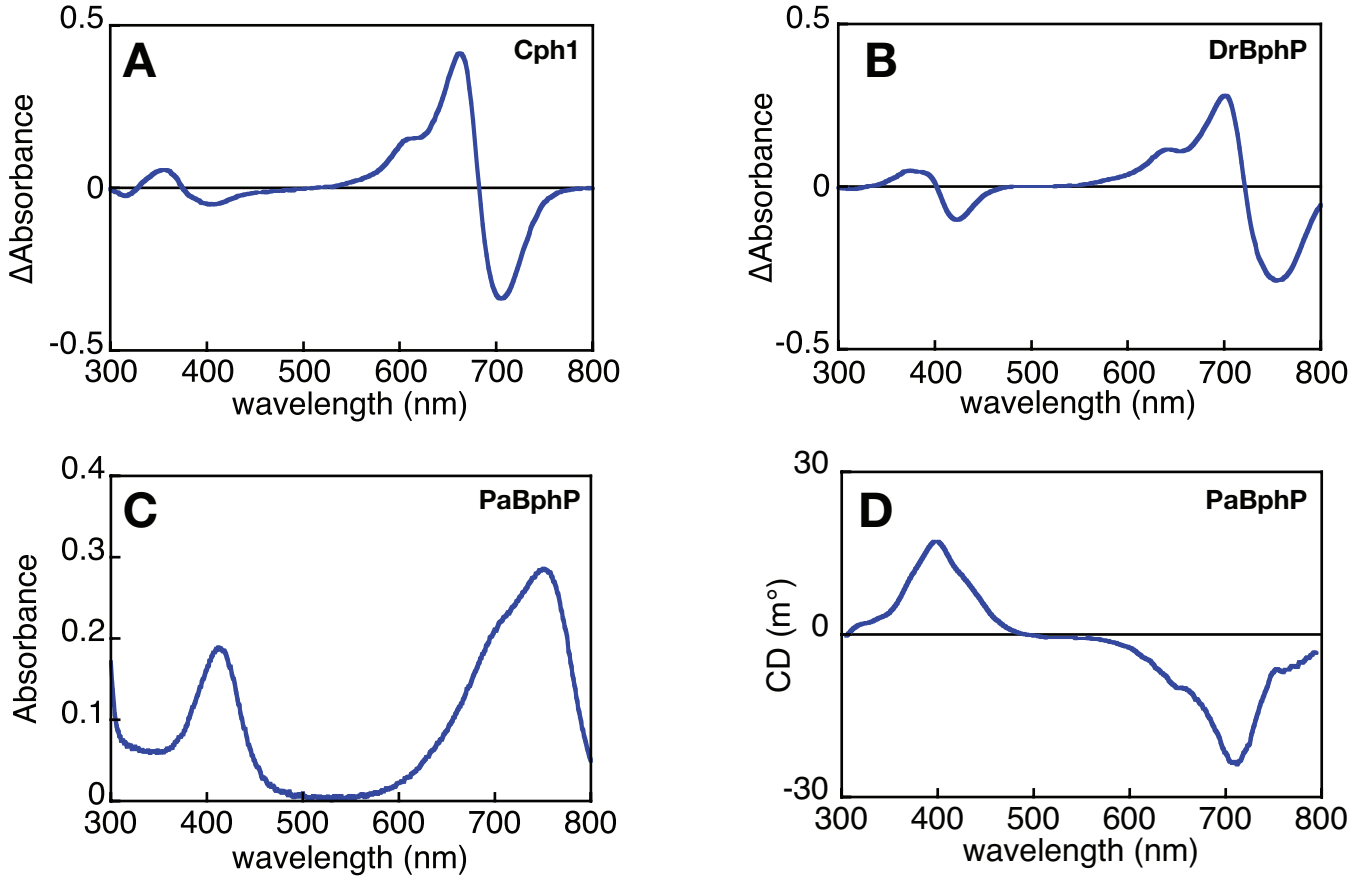


Fig. S2. Spectroscopic characterization of Cph1, *Dr*BphP, and *Pa*BphP. (*A*) The photochemical difference spectrum is shown for Cph1. (*B*) The photochemical difference spectrum is shown for DrBphP. (*C*) The absorbance spectrum of the thermally-stable P_r/P_{fr} equilibrium state of *Pa*BphP is shown. (*D*) The CD spectrum of *Pa*BphP is shown.

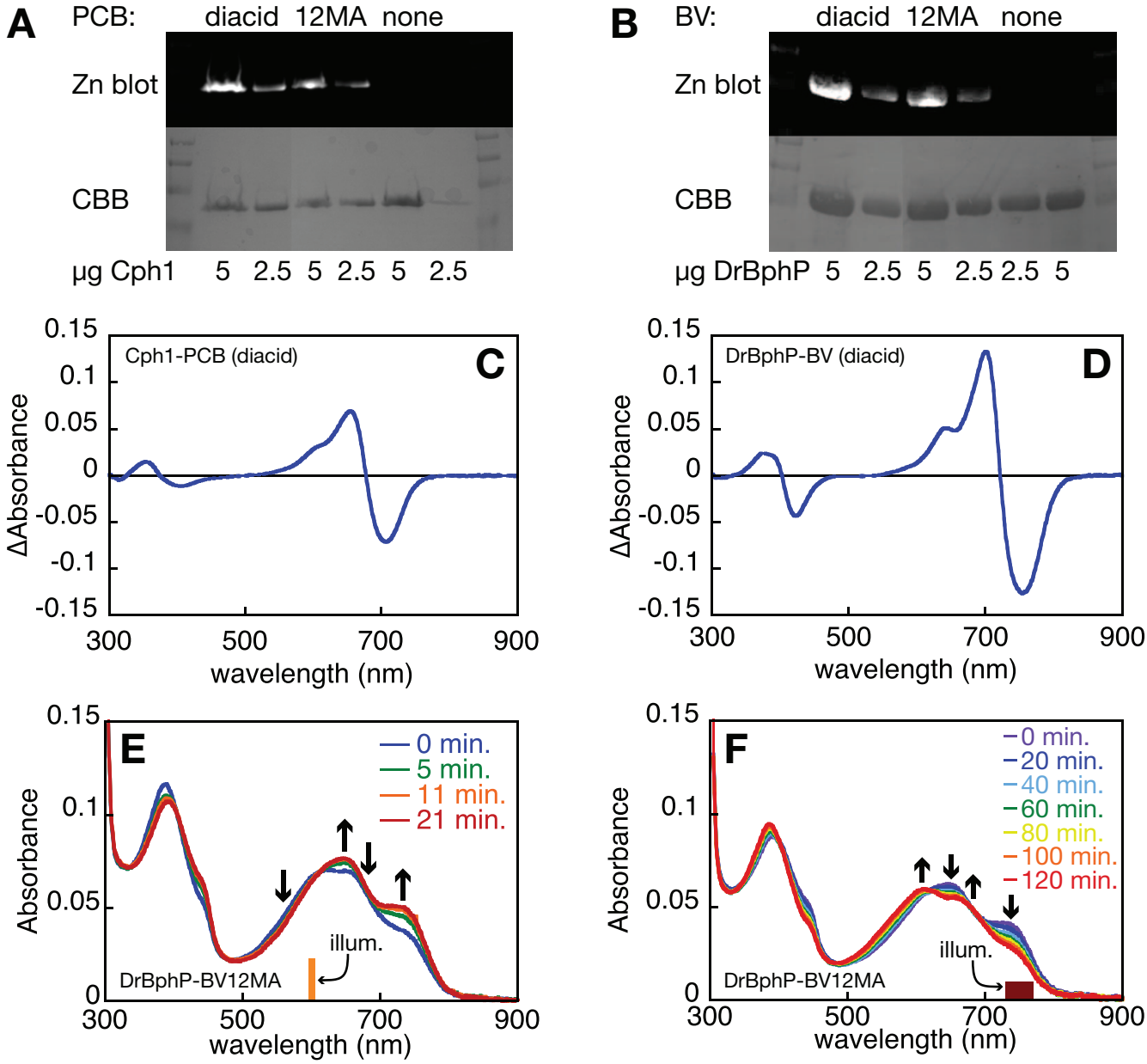
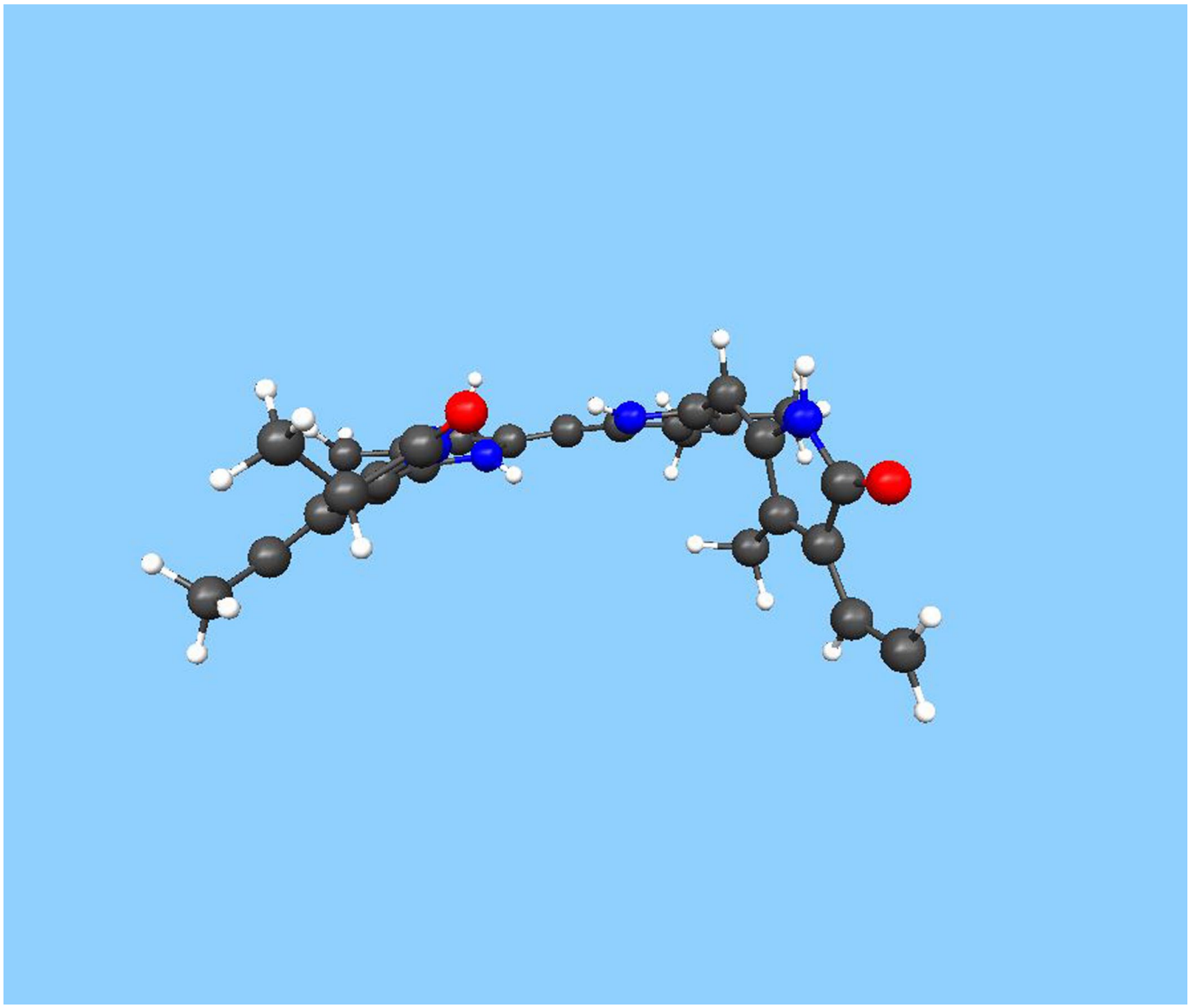
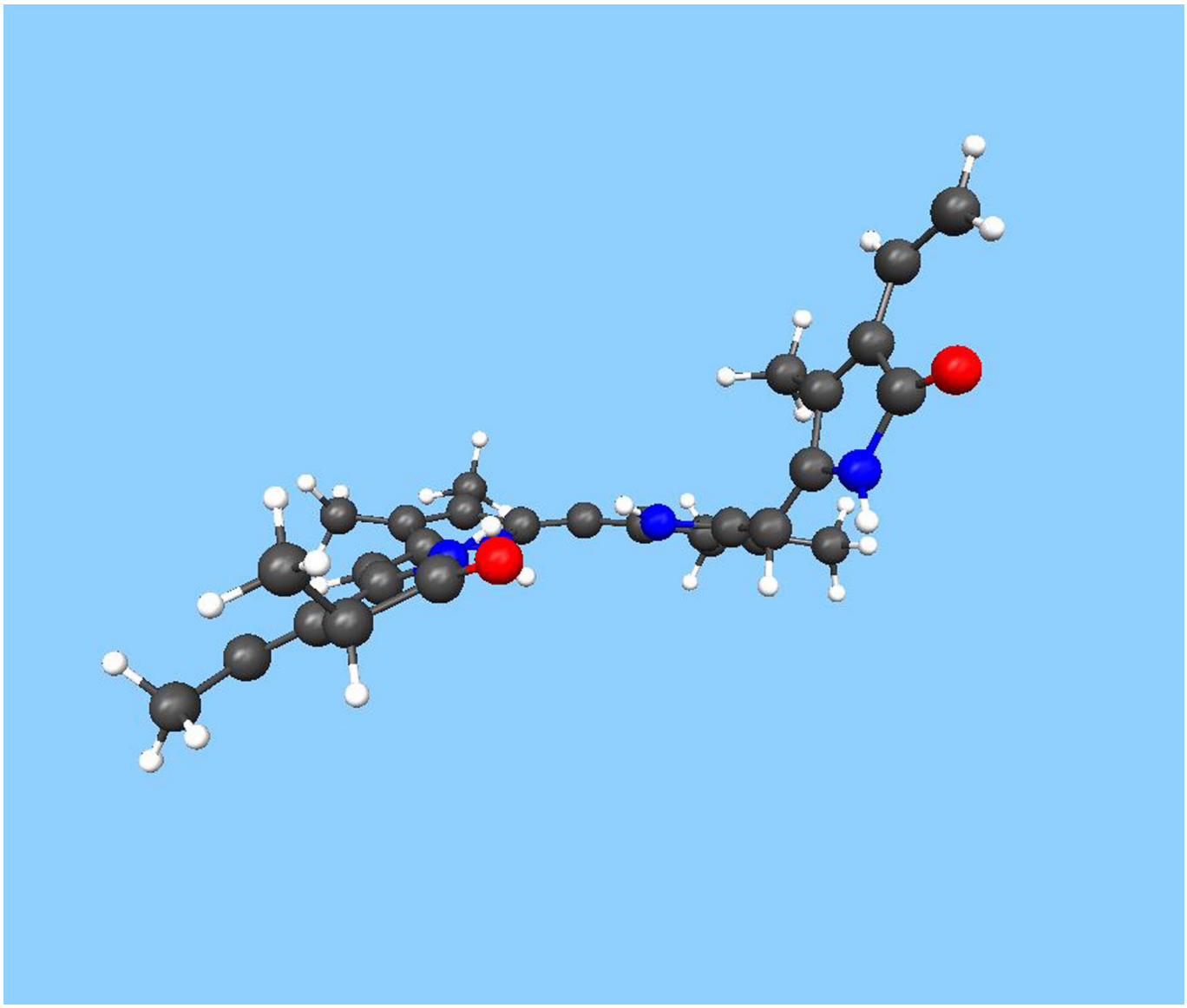


Fig. S3. Characterization of phytochromes assembled with chromophore 12-monoamides. (*A*) ApoCph1 was incubated with PCB, PCB 12-monoamide (PCB12MA), or a solvent control (DMSO) for 2 h and then subjected to overnight dialysis to remove unbound chromophore. The resulting preparations were characterized by SDS/PAGE and zinc blotting to confirm the presence of covalently-bound chromophore. (*B*) Apo*Dr*BphP was similarly incubated and analyzed. (*C*) The photochemical difference spectrum is shown for Cph1 after assembly with PCB. (*D*) The photochemical difference spectrum is shown for *Dr*BphP after assembly with BV. (*E*) The covalent adduct of *Dr*BphP with BV12MA (*Dr*BphP-BV12MA) was illuminated with 600 ± 5 nm light for the indicated times, and conversion of both dual-P_r peaks to both dual-P_{fr} peaks was observed. (*F*) The photoequilibrium mixture, generated by 600 nm illumination (*E*), was then irradiated with 750 ± 20 nm light for the indicated times to examine regeneration of dual-P_r.



Movie S1. A model compound mimicking the P_r chromophore of *Dr*BphP was manually rotated counterclockwise about the 15/16 bond to give a formally C15-*E*,*anti* geometry. The resulting geometry was then optimized and animated as described in *Materials and Methods*. Initial counterclockwise rotation results in movement of the D-ring to the bilin β-face.

Movie S1 (MOV)



Movie S2. A model compound mimicking the P_r chromophore of *Dr*BphP was manually rotated clockwise about the 15/16 bond to give a formally C15-*E*,*anti* geometry. The resulting geometry was then optimized and animated as described in *Materials and Methods*. Initial clockwise rotation results in retention of the D-ring on the bilin α-face.

Movie S2 (MOV)

Table S1. Experimental spectral parameters

Protein	Chromophore	Species	Peak wavelengths, nm	R/B
Cph1	PCB*	P_r	356, 662	3.1
		P_{fr}	406, 704	2.7
	PCB†	P_r	355, 654	0.8
		P_{fr}	406, 706	0.8
	PCB-12MA	Dual-P_r	349, 559, 633	1.0
DrBphP	BV*	P_r	396, 702	3.1
		P_{fr}	422, 754	2.6
	BV†	P_r	394, 702	1.9
		P_{fr}	421, 754	1.7
	BV12MA	Dual-P_r (dark state)	385, 598, 667	1.0
		Dual-P_r (670 nm DS)	381, 561, 683	ND
		Dual-P_r (600 nm DS)	383, 556, 684	ND
		Dual-P_r (750 nm DS)	379, 556, 685	ND
		Dual-P_r (Dk. Rev. DS)	380, 566, 685	ND
		Dual-P_{fr} (670 nm DS)	439, 649, 738	ND
		Dual-P_{fr} (600 nm DS)	438, 647, 735	ND
		Dual-P_{fr} (750 nm DS)	438, 648, 746	ND
		Dual-P_{fr} (Dk. Rev. DS)	441, 650, 739	1.2‡
PaBphP	BV	Dark equilibrium	412, 751	2.3
none	BV	Free chromophore	386, 651	0.2
	BV12MA		379, 688	0.5
	PCB		370, 629	0.4
	PCB12MA		364, 624	0.6

R:B ratio was defined as the ratio of integrated absorbance at >500 nm to that at 320–500 nm. Dk. Rev., dark reversion; DS, difference spectrum; ND, not determined.

*Measured with samples expressed and purified as holoprotein for CD spectroscopy.

†From expression and purification of apoprotein followed by in vitro assembly with diacid BV or PCB.

‡Calculated for the sample most enriched in Dual-P_{fr}.