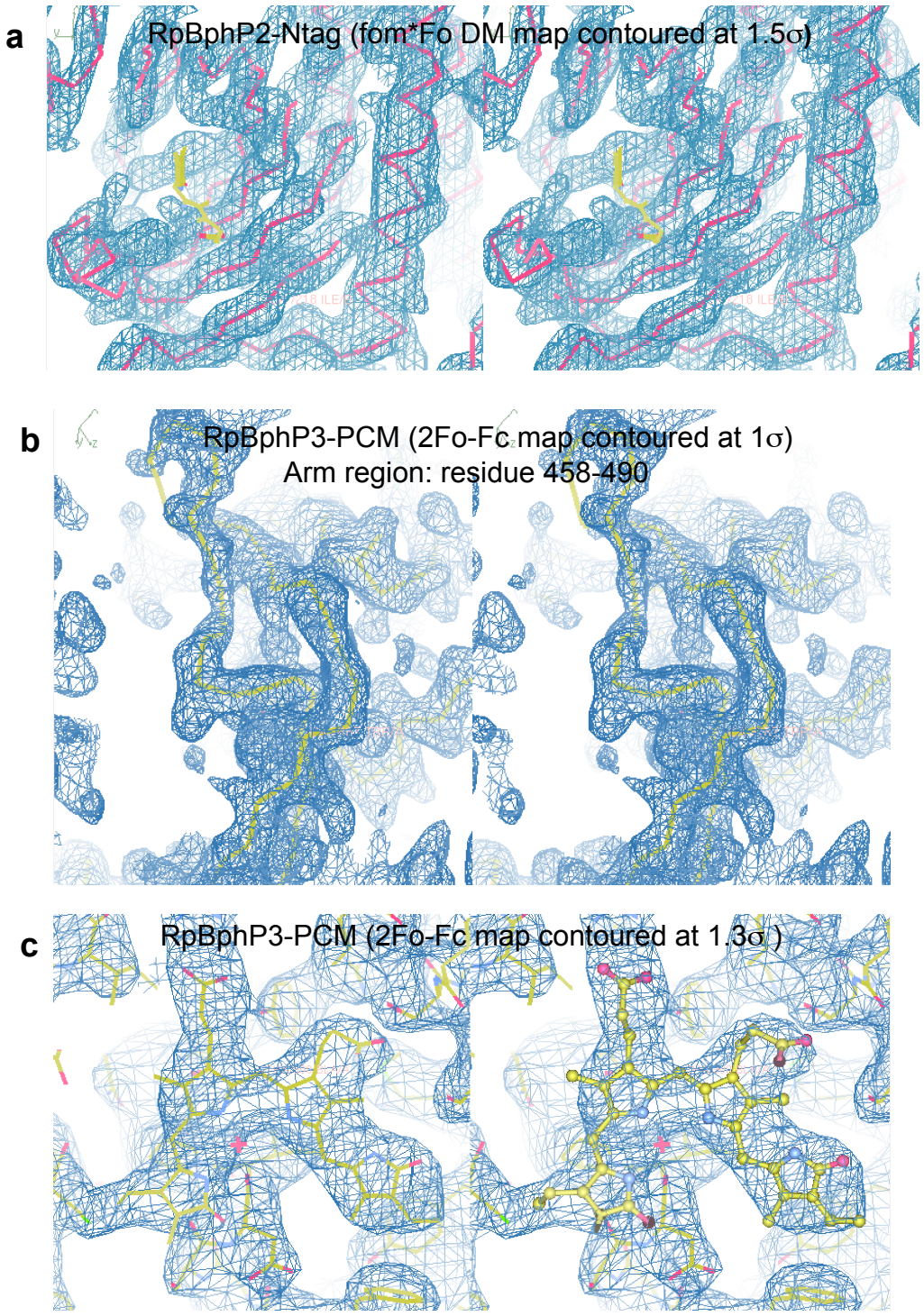


Figure S1. The electron density maps in stereo-view. a) The figure-of-merit weighted experimental SAD map of RpBphP2-Ntag following density modification (PDB ID 4S21). The chromophore is colored in yellow. b) The 2Fo-Fc map in the arm region of the subunit A of the RpBphP3-PCM structure (PDB ID 4R70); c) 2Fo-Fc map of the chromophore binding pocket in RpBphP3-PCM. The biliverdin chromophore is highlighted in ball-and-stick. Related to Figure 1 and Figure 4.

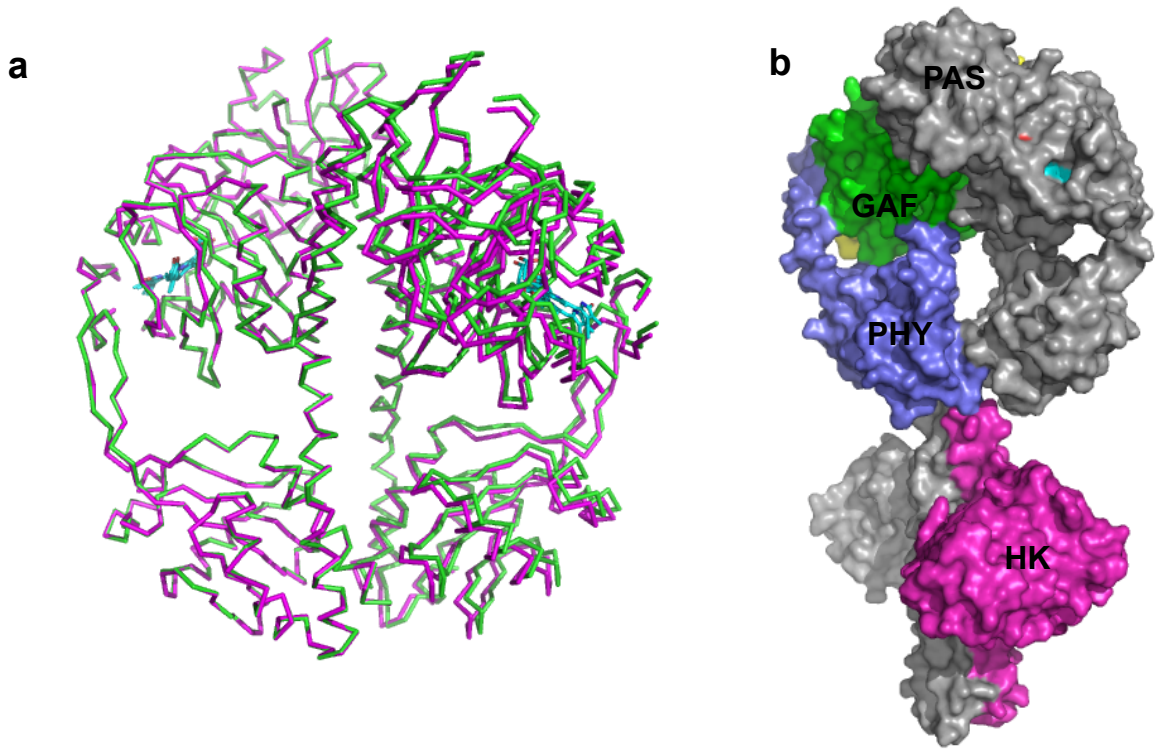


Figure S2. The dimer scaffold and full-length modeling of RpBphP2. a) Superposition of the RpBphP2-Ctag (green) and RpBphP2-Ntag (magenta) structures according to least square fitting of molecule A (monomer at left) shows a slight displacement in molecule B (monomer at right). Related to Figure 1a. b) A surface representation of the full-length RpBphP2 model in the Pr state. One RpBphP2 monomer is colored in solid grey, and the other is colored according to their individual domains (PAS, yellow; GAF, green; PHY, blue; and HK, magenta).

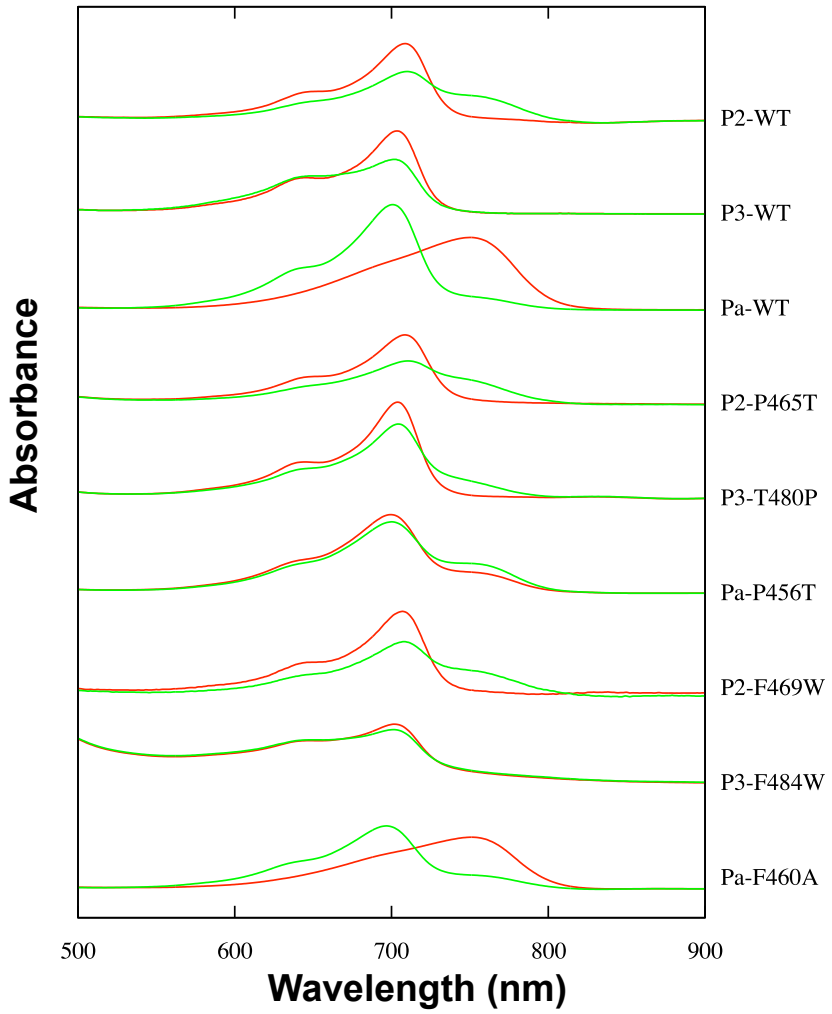
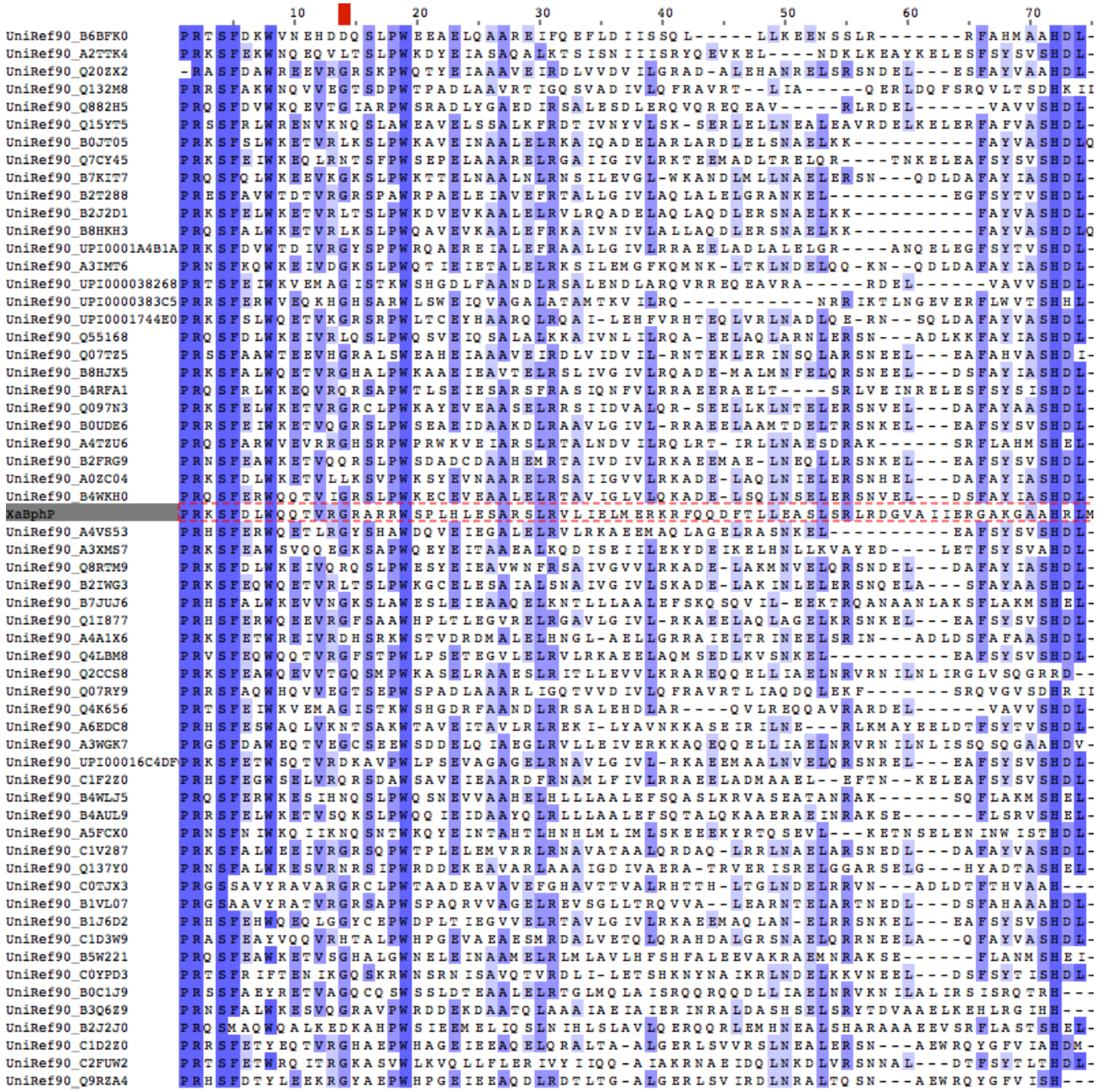


Figure S3. Absorption spectra of RpBphP2 (P2), RpBphP3 (P3) and PaBphP (Pa) wild type (WT) and mutants in the PRxSF motif. Dark-adapted spectra are colored in red, and light-induced spectra measured after 5-min illumination are shown in green curve**.** Related to Figure 4.

a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UniRef90_B3Q6Z9
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b

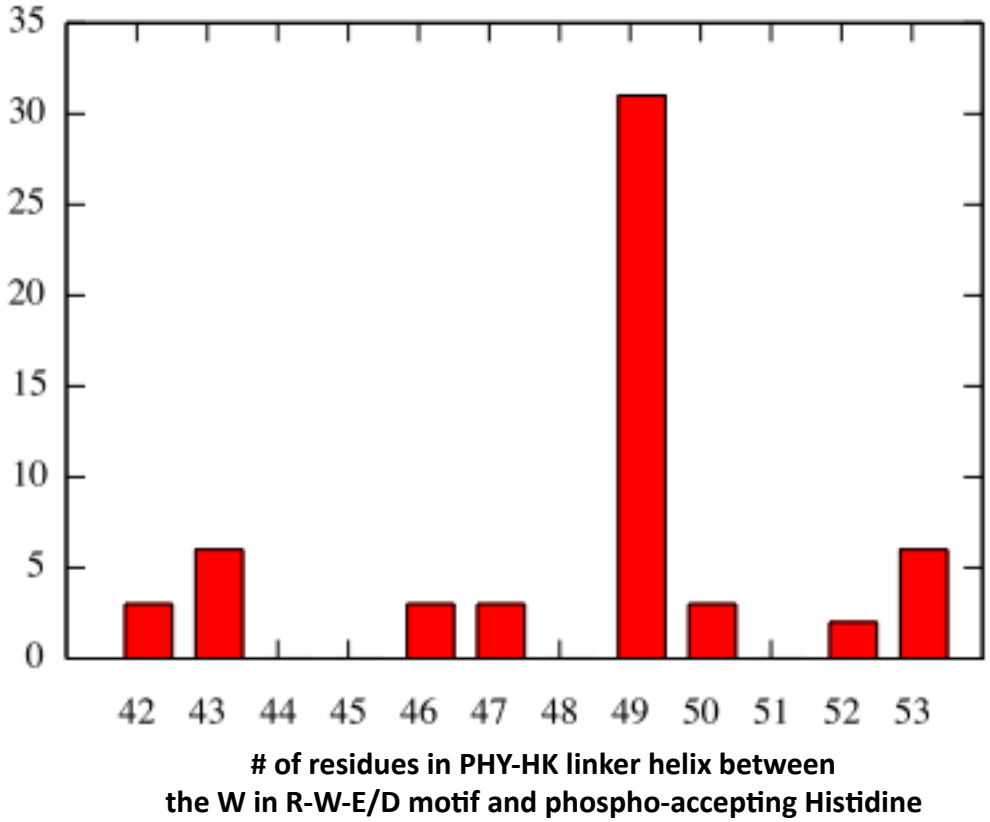

Figure S4. **The PHY-HK linker sequence.** a) Multiple sequence alignment in the range from the PRxSF motif to the phospho-accepting histidine in 60 putative BphP sequences retrieved from UniPROT. XaBphP (highlighted in grey and red dashed line) was used as query sequence (Jalview). b) Histogram of the helical length of the PHY-HK linker helix between the conserved Trp in the R-W-E/D motif and phospho-accepting histidine as measured by number of residues. Related to Figure 5.

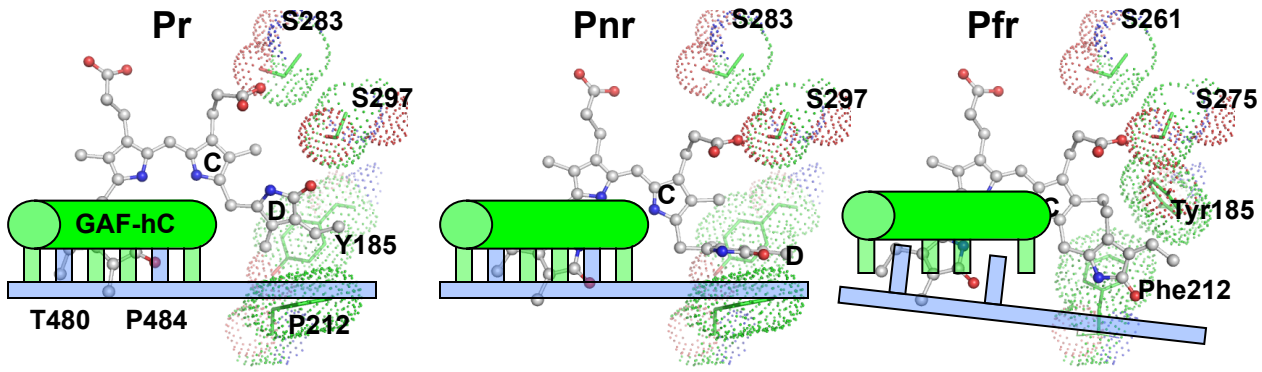

Figure S5. Cartoon representations of the Pr, Pnr and Pfr state. The RpBphP3-PCM crystal structure illustrates the Pr conformation. The PaBphP structure (PDB ID 3NHQ) illustrates Pfr. Structural analysis and mutational data in this work suggest the Pnr conformation. Compared to the Pr state, the chromophore undergoes an overall rotation relative to the protein matrix in the Pnr and Pfr states, in which the propionate group of ring C moves away from Ser283 and closer to Ser297. In the Pnr state, ring D disengages from the conjugated system of the chromophore due to steric hindrance arising from the "tongue-and-groove" interactions at the GAF-PHY interface (shown in a green cylinder representing the GAF-hC helix and a long blue bar representing the arm containing the PRxSF motif). In the Pfr state, the arm is dislodged allowing further relaxation of ring D to adopt the *ZZEssa* configuration. Related to Figure 4b.

Table S1. Inter-atomic distances at the GAF-PHY interface in RpBphP3-PCM and Cph1 (2VEA) calculated by the CCP4 contact program (related to Figure 4).

	arm of PHY	GAF	Distance (Å)
RpBphP3	Thr460-O	His211-N	2.94
	Gly262-N	His211-O	3.18
	Arg477-NE	Glu27-OE1	3.12
	Ala478-O	Thr268-CG2	3.07
	Ala478-CB	Thr268-OG1	3.15
	Thr480-OG1	Thr268-O	3.18
	Thr480-OG1	Tyr272-N	2.99
	Thr480-OG1	Tyr272-CA	2.99
	Thr480-OG1	Tyr272-CB	3.14
	Arg481-O	Asn275-OD1	2.62
	Ala482-CA	Asn275-OD1	3.14
	Ala482-C	Asn275-OD1	2.96
	Ala482-O	Asn275-OD1	2.68
Cph1	Asn449-O	His202-N	2.74
	Gly451-N	His202-O	2.90
	Tyr458-OH	Ser11-OG	2.97
	Ile467-CB	Gln14-OE1	3.19
	Ile467-CG2	Gln14-OE1	3.16

Table S2. Equivalent residues among known phytochrome structures (related to Figure 4 and Figure 5).

Structure (PDB ID)	**GAF-PHY interface**			**PRxSF motif**				**R-W-E/D motif**		
RpBphP3	Y272	D216	L207	T480	R481	S483	F484	R452	W498	E502
RpBphP2	Y258	D202	Y193	P465	R466	S468	F469	R439	W483	E487
PaBphP	Y250	D194	Y185	P456	R457	S459	F460	R428	W474	D478
Cph1	Y263	D207	Y198	P471	R472	S474	F475	R441	W489	E493
DrBphP	Y263	D207	Y198	P465	R466	S468	F469	R442	W483	E487
RpBphP1 (4GW9)	Y257	D201	Y192	P465	R466	S468	F469	R437	W483	D487
AtPhyB (4OUR)	Y361	D307	Y298	P581	R582	S584	F585	R554	W599	E603