Supporting Information

Lyophilization Reveals a Multitude of Structural Conformations in the Chromophore of a Cph2-like Phytochrome

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Figures S1–S4

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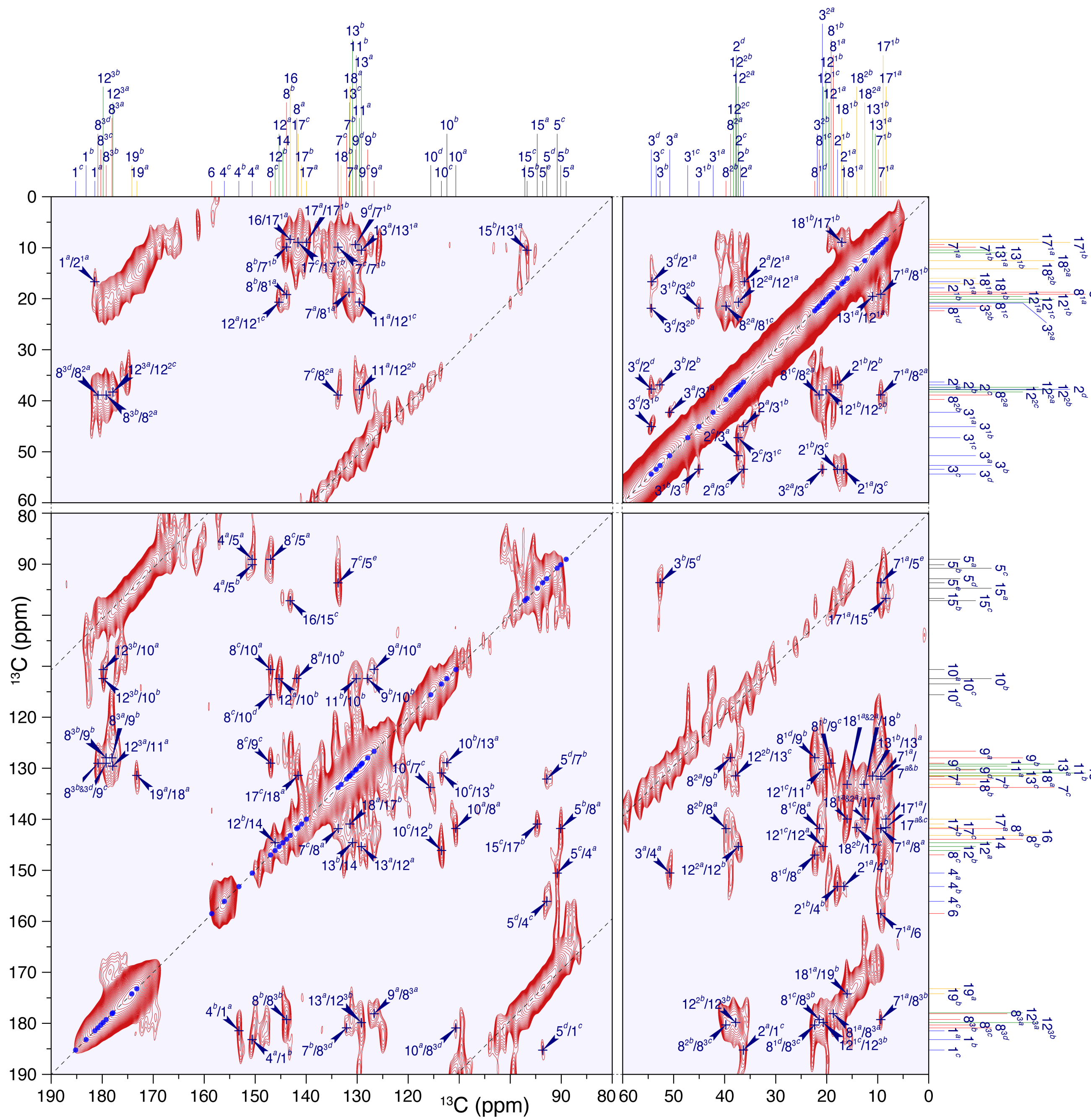

Figure S1. Enlarged contour plot of the DARR spectrum shown in Figure 2a. The DARR spectrum of lyophilized *u*-[^{13}C,^{15}N]-PCB-All2699GAF1 as Pr was acquired with a mixing time of 50 ms. The 1D traces of the 2D spectrum (along ω_1-, *right*, and ω_2-dimension, *top*) are shown as stick spectra with the assignment of ^{13}C peaks (see Figure 1d for chromophore numbering).

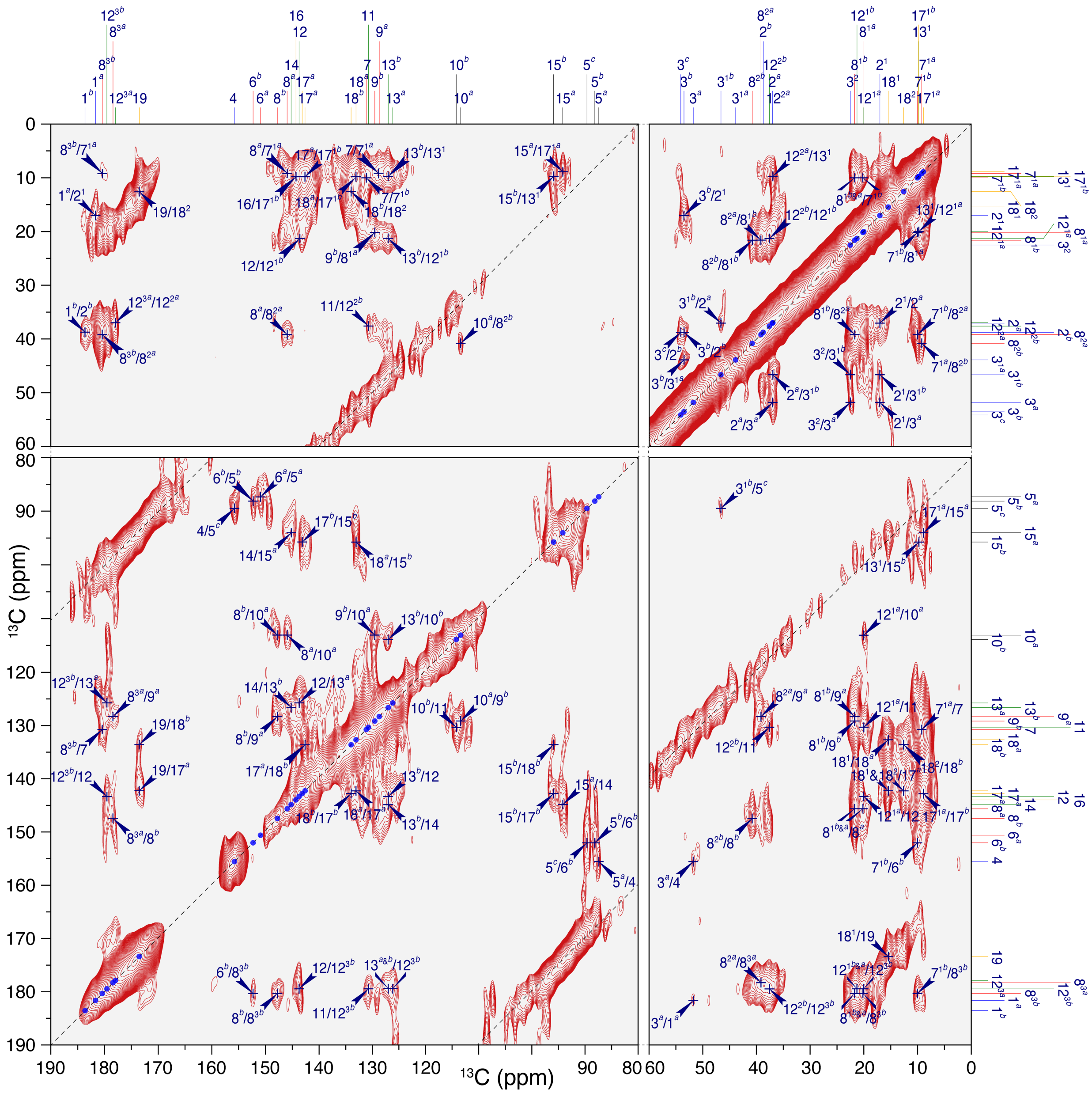

Figure S2. Enlarged contour plot of the DARR spectrum shown in Figure 2b. The DARR spectrum of lyophilized *u*-[^{13}C,^{15}N]-PCB-All2699(GAF1–PHY) as Pr was acquired with a mixing time of 50 ms. The 1D traces of the 2D spectrum (along ω_1-, *right*, and ω_2-dimension, *top*) are shown as stick spectra with the assignment of ^{13}C peaks (see Figure 1d for chromophore numbering).

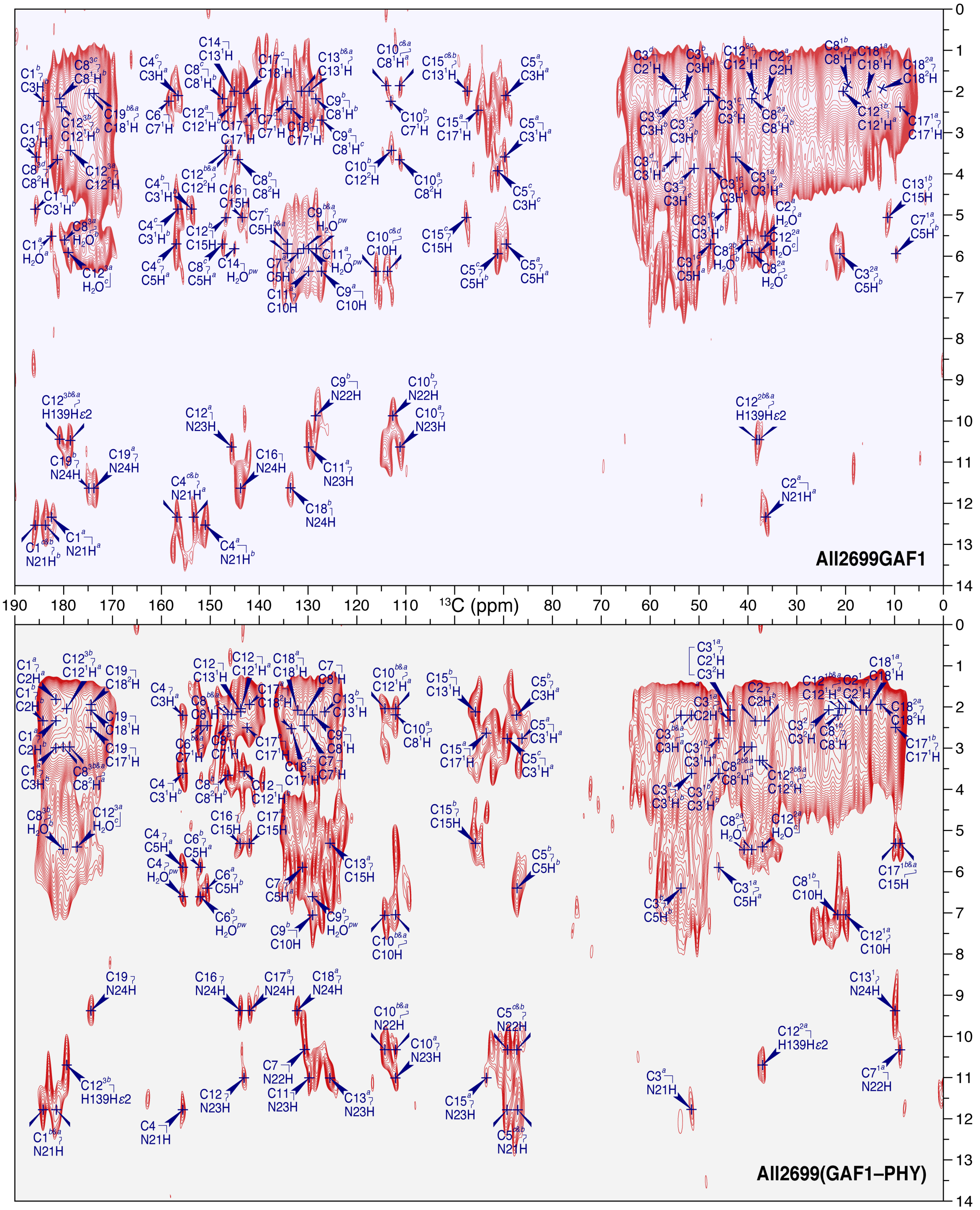

Figure S3. Full contour plots of the HETCOR spectra shown in Figure 3a,b. 2D ^{1}H–^{13}C supercycled PMLG-decoupled dipolar correlation spectra of the *u*-[^{13}C,^{15}N]-PCB chromophore in All2699GAF1 and All2699(GAF1–PHY) with a contact time of 1 ms. The complete assignments including intramolecular $^{1}H^{N21-N24}$–$^{13}C^{PCB}$ and interfacial $^{1}H^{residue/water}$–$^{13}C^{PCB}$ correlations are shown.

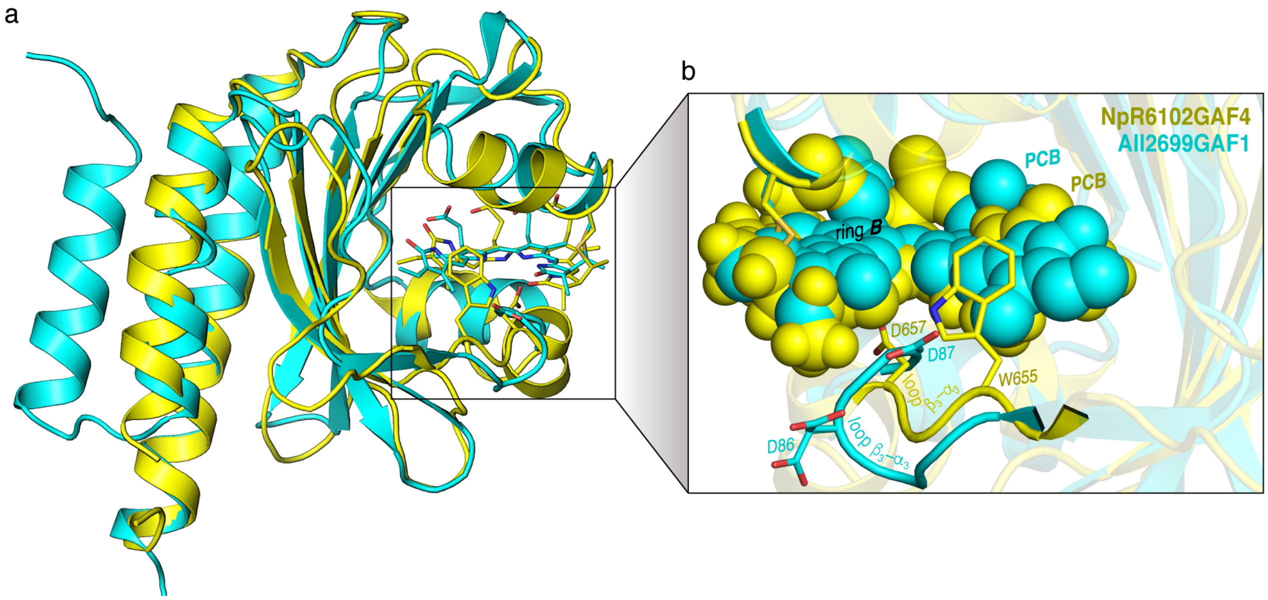

Figure S4. Structural comparison of All2699GAF1 and NpR6102GAF4 in the red-absorbing Pr state. (a) Superposition of All2699GAF1 6OZA (cyan) and NpR6102GAF4 6BHN (yellow) structures. The respective PCB chromophores are shown as sticks using the same color coding as for the ribbon diagrams. (b) Structural views of the PCB-chromophore binding pocket in the two GAF-only domains, highlighting the chromophore and the loop β_3–α_3 on the bilin β face. Local structural flexibility of this loop is given by the alternative side-chains of D86 in All2699GAF1 and W655 and D657 in NpR6102GAF4.

Table S1. ^{13}C chemical shifts of the PCB chromophore incorporated in All2699GAF1 and All2699(GAF1–PHY) in their Pr dark states as lyophilized powder. Published ^{13}C data of frozen All2699GAF1 and All2699(GAF1–PHY) solutions are listed for reference. The ^{13}C chemical shift values obtained from All2699GAF1 and All2699(GAF1–PHY) as lyophilized powder are compared with those from the frozen solution samples. The ^{13}C chemical shift differences of PCB chromophore in the two photoreceptors between the lyophilized and frozen solution states are listed and illustrated in Figure 2. The chromophore numbering is according to Figure 1d.

PCB carbons		^{13}C chemical shift (ppm)				^{13}C chemical shift difference (ppm)			
		All2699GAF1		All2699(GAF1–PHY)		$\Delta_{\text{Lyophilized state – Frozen solution}}$		$\Delta_{\text{All2699(GAF1–PHY) – All2699GAF1}}$	
		Frozen solution	Lyophilized state	Frozen solution	Lyophilized state	GAF1	GAF1–PHY	Frozen solution	Lyophilized state
ring A	**1**	181.6 (1^{a})	182.0 (1^{a})	180.8 (1^{a})	181.6 (1^{a})	+0.4	+0.8	−0.8	−0.4
		182.7 (1^{b})	183.8 (1^{b})	181.8 (1^{b})	183.6 (1^{b})	+1.1	+1.8	−0.9	−0.2
		-	185.8 (1^{c})	-	-	-	-	-	-
	2	33.4 (2^{a})	36.7 (2^{a})	-	-	+3.3	-	-	-
		35.9 (2^{b})	37.2 (2^{b})	36.5 (2^{a})	37.1 (2^{a})	+1.3	+0.6	+0.6	−0.1
		36.9 (2^{c})	37.8 (2^{c})	-	-	+0.9	-	-	-
		-	38.1 (2^{d})	37.1 (2^{b})	38.8 (2^{b})	-	+1.7	+0.2	+0.7
	2^{1}	17.1 (2^{1a})	17.0 (2^{1a})	18.0	17.0	−0.1	−1.0	+0.9	0.0
		17.3 (2^{1b})	18.2 (2^{1b})			+0.9		+0.7	−1.2
	3	51.7 (3^{a})	51.2 (3^{a})	53.1 (3^{a})	51.8 (3^{a})	−0.5	−1.3	+1.4	+0.6
		53.7 (3^{b})	53.1 (3^{b})	53.6 (3^{b})	53.5 (3^{b})	−0.6	−0.1	−0.1	+0.4
		54.5 (3^{c})	53.8 (3^{c})	-	54.1 (3^{c})	−0.7	-	-	+0.3
		-	54.8 (3^{d})	-	-	-	-	-	-
	3^{1}	42.9 (3^{1a})	42.6 (3^{1a})	46.5 (3^{1a})	43.9 (3^{1a})	−0.3	−2.6	-	+1.3
		46.7 (3^{1b})	45.4 (3^{1b})	-	-	−1.3	-	−0.2	-
		47.3 (3^{1c})	47.8 (3^{1c})	47.4 (3^{1b})	46.7 (3^{1b})	+0.5	−0.7	+0.1	−1.1
	3^{2}	20.4 (3^{2a})	21.2 (3^{2a})	20.6 (3^{2a})	22.5	+0.8	+1.9	+0.2	+1.3
		21.3 (3^{2b})	22.2 (3^{2b})	21.6 (3^{2b})		+0.9	+0.9	+0.3	+0.3
	4	154.3 (4^{a})	151.1 (4^{a})	154.5 (4^{a})	155.6	−2.3	+1.1	+0.2	+4.5
		155.7 (4^{b})	153.8 (4^{b})	155.1 (4^{b})		−1.9	+0.5	−0.6	+1.8
		158.2 (4^{c})	156.6 (4^{c})	-		−1.6	-	-	−1.0
A–B	**5**	87.3 (5^{a})	89.5 (5^{a})	87.7	87.3 (5^{a})	+2.2	−0.4	+0.4	−2.2
		88.5 (5^{b})	90.6 (5^{b})		88.1 (5^{b})	+2.1	+0.4	−0.8	−2.5
		-	91.2 (5^{c})		89.5 (5^{c})	-	+1.8	-	−1.7
		-	93.3 (5^{d})	-	-	-	-	-	-
		-	94.1 (5^{e})	-	-	-	-	-	-
ring B	**6**	150.2	159.1	149.5	150.6 (6^{a})	+8.9	+1.1	−0.7	−8.5
					152.0 (6^{b})		+2.5		−7.1
	7	126.5	132.1 (7^{a})	127.6	130.7	+5.6	+3.1	+1.1	−1.4
			132.6 (7^{b})			+6.1			−1.9
			134.2 (7^{c})			+7.7			−3.5
	7^{1}	8.8	9.7 (7^{1a})	9.2	9.2 (7^{1a})	+0.9	0.0	+0.4	−0.5
			10.2 (7^{1b})		10.0 (7^{1b})	+1.4	+0.8		−0.2
	8	146.7	142.2 (8^{1a})	-	-	−4.5	-	−0.7	-
			144.4 (8^{1b})	146.0	145.6 (8^{1a})	−2.3	−0.4		+1.2
			147.5 (8^{1c})		146.4 (8^{1b})	−1.2	+0.4		−1.1
	8^{1}	-	19.0 (8^{1a})	-	-	-	-	-	-
		19.3 (8^{1a})	19.5 (8^{1b})	20.8 (8^{1a})	20.2 (8^{1a})	+0.2	−0.6	+1.5	+0.7
		21.7 (8^{1b})	21.8 (8^{1c})	22.0 (8^{1b})	21.6 (8^{1b})	+0.1	−0.4	+0.3	−0.2
		-	22.7 (8^{1d})	-	-	-	-	-	-
	8^{2}	39.9 (8^{2a})	39.2 (8^{2a})	39.1 (8^{2a})	39.2 (8^{2a})	−0.7	+0.1	−0.8	0.0
		40.7 (8^{2b})	40.1 (8^{2b})	40.1 (8^{2b})	40.8 (8^{2b})	−0.6	+0.7	−0.6	+0.7
	8^{3}	178.1 (8^{3a})	178.6 (8^{3a})	179.4 (8^{3a})	178.3 (8^{3a})	+0.5	−1.1	-	−0.3
		179.2 (8^{3b})	179.8 (8^{3b})	179.9 (8^{3b})	180.3 (8^{3b})	+0.6	+0.4	+0.2	+0.5
		180.4 (8^{3c})	180.8 (8^{3c})	-	-	+0.4	-	−0.5	-
		-	181.4 (8^{3d})	-	-	-	-	-	-
	9	128.1	127.2 (9^{a})	-	-	−0.9	-	−0.1	-
			128.4 (9^{b})	128.0	128.3 (9^{a})	+0.3	+0.3		−0.1
			129.5 (9^{c})		129.1 (9^{b})	+1.4	+1.1		−0.4
			130.8 (9^{d})	-	-	+2.7	-		-

Continued on following page.

PCB carbons		^{13}C chemical shift (ppm)				^{13}C chemical shift difference (ppm)			
		All2699GAF1		All2699(GAF1–PHY)		$\Delta_{\text{Lyophilized state – Frozen solution}}$		$\Delta_{\text{All2699(GAF1–PHY) – All2699GAF1}}$	
		Frozen solution	Lyophilized state	Frozen solution	Lyophilized state	GAF1	GAF1–PHY	Frozen solution	Lyophilized state
B–C	**10**	111.6	111.2 (10^{a})	-	-	−0.4	-	+0.3	-
			112.9 (10^{b})	111.9	113.1 (10^{a})	+1.3	+1.2		+0.2
			114.0 (10^{c})		113.9 (10^{b})	+2.4	+2.0		−0.1
			116.1 (10^{d})	-	-	+4.5	-		-
ring C	**11**	128.5	130.1 (11^{a})	128.7	130.3	+1.6	+1.6	+0.2	+0.2
			130.7 (11^{b})			+2.2			−0.4
	12	145.8	145.8 (12^{a})	144.4	143.3	0.0	−1.1	−1.4	−2.5
			146.8 (12^{a})			+1.0			−3.5
	12^{1}	21.9	20.0 (12^{1a})	21.1	20.0 (12^{1a})	−1.9	−1.1	−0.8	0.0
			20.5 (12^{1b})		-	−1.4	-		-
			21.0 (12^{1c})		21.3 (12^{1b})	−0.9	+0.2		+0.3
	12^{2}	37.8	37.7 (12^{2a})	38.3	37.0 (12^{2a})	−0.1	−1.3	+0.5	−0.7
			38.2 (12^{2b})		37.6 (12^{2b})	+0.4	−0.7		−0.6
			38.7 (12^{2c})		-	+0.9	-		-
	12^{3}	179.5	178.6 (12^{3a})	178.1	177.9 (12^{3a})	−0.9	−0.2	−1.4	−0.7
			180.4 (12^{3b})		179.4 (12^{3b})	+0.9	+1.3		−1.0
	13	125.0	129.7 (13^{a})	126.2	125.7 (13^{a})	+4.7	−0.5	+1.2	−4.0
			131.4 (13^{b})		126.6 (13^{b})	+6.4	+0.4		−4.8
			132.0 (13^{c})	-	-	+7.0	-		-
	13^{1}	10.9	10.8 (13^{1a})	11.1	9.8	−0.1	−1.3	+0.2	−1.0
			11.4 (13^{1b})			+0.5			−1.6
	14	143.1	145.1	144.9	144.9	+2.0	0.0	+1.8	−0.2
C–D	**15**	94.2 (15^{a})	95.2 (15^{a})	94.3	94.1 (15^{a})	+1.0	−0.2	+0.1	−1.1
		94.9 (15^{b})	97.2 (15^{b})		95.8 (15^{b})	+2.3	+1.5	−0.6	−1.4
		-	97.6 (15^{c})	-	-	-	-	-	-
ring D	**16**	144.2	143.6	144.1	144.0	−0.6	−0.1	−0.1	+0.4
	17	-	140.5 (17^{a})	-	-	-	-	-	-
		141.1 (17^{a})	141.4 (17^{b})	142.2	142.2 (17^{a})	+0.3	0.0	+1.1	+0.8
		142.3 (17^{b})	142.1 (17^{c})		142.8 (17^{b})	−0.2	+0.6	−0.1	+0.7
	17^{1}	9.6	8.7 (17^{1a})	10.0	8.9 (17^{1a})	−0.9	−1.1	+0.4	+0.2
			9.3 (17^{1b})		9.8 (17^{1b})	−0.3	−0.2		+0.5
	18	133.3	131.9 (18^{a})	133.9	132.7 (18^{a})	−1.4	−1.2	+0.6	+0.8
			133.6 (18^{b})		133.6 (18^{b})	+0.3	−0.3		0.0
	18^{1}	15.3	16.4 (18^{1a})	15.9	15.5	+1.1	−0.4	+0.6	−0.9
			17.4 (18^{1b})			+2.1			−1.9
	18^{2}	12.1	12.9 (18^{2a})	12.7	12.6	+0.8	−0.1	+0.6	−0.3
			14.5 (18^{2b})			+2.4			−1.9
	19	172.2	173.8 (19^{a})	172.7	173.4	+1.6	+0.7	+0.5	−0.4
			174.8 (19^{b})			+2.6			−1.4

Table S2. ^{1}H chemical shifts of the carbon-bound protons in PCB chromophore as incorporated in All2699GAF1 and All2699(GAF1–PHY) in their Pr dark states as lyophilized powder. Published ^{1}H data of frozen All2699GAF1 and All2699(GAF1–PHY) solutions are listed for reference. The ^{1}H chemical shift values obtained from All2699GAF1 and All2699(GAF1–PHY) as lyophilized powder are compared with those from the frozen solution samples. The ^{1}H chemical shift differences of chromophore carbon-bound protons in the two photoreceptors between the lyophilized and frozen solution states are listed and illustrated in Figure 3. The chromophore numbering is according to Figure 1d.

PCB carbon-bound protons		^{1}H chemical shift (ppm)				^{1}H chemical shift difference (ppm)			
		All2699GAF1		All2699(GAF1–PHY)		$\Delta_{\text{Lyopholized state – Frozen solution}}$		$\Delta_{\text{All2699(GAF1–PHY) – All2699GAF1}}$	
		Frozen solution	Lyophilized state	Frozen solution	Lyophilized state	GAF1	GAF1–PHY	Frozen solution	Lyophilized state
ring A	2	1.9 (2^a)	2.2	-	-	+0.3	-	-	-
		2.0 (2^b)		1.8 (2^a)	1.8 (2^a)	+0.2	0.0	−0.2	−0.4
		2.5 (2^c)		2.4 (2^b)	2.3 (2^b)	−0.3	−0.1	−0.1	+0.1
	2^1	1.7	1.9	2.1	2.1	+0.2	0.0	+0.4	+0.2
	3	-	2.1 (3^a)	1.9 (3^a)	2.2 (3^a)	-	+0.3	−0.6	+0.1
		2.5 (3^a)	2.2 (3^b)	2.7 (3^b)	3.0 (3^b)	−0.3	+0.3	−0.4	+0.8
		3.1 (3^b)	3.9 (3^c)	-	-	+0.8	-	-	-
	3^1	2.9 (3^{1a})	3.7 (3^{1a})	3.3	2.8 (3^{1a})	+0.8	−0.5	+0.4	−0.9
		4.9 (3^{1b})	4.8 (3^{1b})		3.6 (3^{1b})	−0.1	+0.3	−1.6	−1.2
	3^2	1.6 (3^{2a})	1.9	1.9	2.0	+0.3	+0.1	+0.3	+0.1
		1.9 (3^{2b})				0.0		0.0	
A–B	5	6.8 (5^a)	5.7 (5^a)	5.4 (5^a)	5.9 (5^a)	−0.9	+0.5	−1.4	+0.2
		7.5 (5^b)	6.0 (5^b)	6.2 (5^b)	6.4 (5^b)	−1.5	+0.2	−1.3	+0.4
ring B	7^1	2.3	2.2	2.4	2.5	−0.1	+0.1	+0.1	+0.3
	8^1	1.6 (8^{1a})	1.7 (8^{1a})	1.7 (8^{1a})	2.2	+0.1	+0.5	+0.1	+0.5
		-	2.2 (8^{1a})	2.3 (8^{1b})		-	−0.1	-	0.0
		3.0 (8^{1b})	2.7 (8^{1c})	-	-	−0.3	-	−0.3	−0.5
	8^2	3.4	3.6	2.8 (8^{2a})	3.0 (8^{2a})	+0.2	+0.2	−0.6	−0.6
				3.5 (8^{2b})	3.6 (8^{2b})		+0.1	+0.1	0.0
B–C	10	7.9	6.4	7.6	7.2	−1.5	−0.4	−0.3	+0.8
ring C	12^1	1.6	2.0 (12^{1a})	2.2 (12^{1a})	2.1 (12^{1a})	+0.4	−0.1	+0.6	+0.1
			2.4 (12^{1b})	3.5 (12^{1b})	3.6 (12^{1b})	+0.8	+0.1	+1.9	+1.2
	12^2	3.4	3.4	3.5	3.3	0.0	−0.2	+0.1	−0.1
	13^1	2.0	2.0	2.1	2.1	0.0	0.0	+0.1	+0.1
C–D	15	6.2 (15^a)	5.1	5.7	5.3	−1.1	−0.4	−0.5	+0.2
		6.9 (15^b)				−1.8		−1.2	
ring D	17^1	2.1	2.4	2.3	2.5	+0.3	+0.2	+0.2	+0.1
	18^1	1.8 (18^{1a})	2.0	1.4 (18^{1a})	2.1	+0.2	+0.7	−0.4	+0.1
		2.3 (18^{1b})		2.1 (18^{1b})		−0.3	0.0	−0.2	
	18^2	1.4	1.9	1.9	1.9	+0.5	0.0	+0.5	0.0

Table S3. ^{1}H chemical shifts of the protons bound to tetrapyrrole nitrogens in PCB chromophore as incorporated in All2699GAF1 and All2699(GAF1–PHY) in their Pr dark states as lyophilized powder. Published ^{1}H data of frozen All2699GAF1 and All2699(GAF1–PHY) solutions are listed for reference. The ^{1}H chemical shift values obtained from All2699GAF1 and All2699(GAF1–PHY) as lyophilized powder are compared with those from the frozen solution samples. The ^{1}H chemical shift differences of protons bound to tetrapyrrole nitrogens in the two photoreceptors between the lyophilized and frozen solution states are listed and illustrated in Figure 4e.

NH21–N24H		^{1}H chemical shift (ppm)				^{1}H chemical shift difference (ppm)			
		All2699GAF1		All2699(GAF1–PHY)		$\Delta_{\text{Lyopholized state – Frozen solution}}$		$\Delta_{\text{All2699(GAF1–PHY) – All2699GAF1}}$	
		Frozen solution	Lyophilized state	Frozen solution	Lyophilized state	GAF1	GAF1–PHY	Frozen solution	Lyophilized state
ring A	**N21H**	12.3 (N21H[a]) 12.8 (N21H[b])	12.2 (N21H[a]) 12.5 (N21H[b])	12.0	11.8	**−0.1** **−0.3**	**−0.1**	**−0.3** **−0.8**	**−0.2**
ring B	**N22H**	9.9	9.1 (N22H[a]) 9.9 (N22H[b])	10.2	10.3	**−0.8** **0.0**	**+0.1**	**+0.3**	**+1.2** **+0.4**
ring C	**N23H**	10.7	10.6	11.6	11.0	**−0.1**	**−0.6**	**+0.9**	**−0.6**
ring D	**N24H**	11.6	11.6	9.6	9.4	**0.0**	**−0.2**	**−2.0**	**−0.2**

Table S4. ^{15}N chemical shifts of the PCB chromophore incorporated in All2699GAF1 and All2699(GAF1–PHY) in their Pr dark states as lyophilized powder. Published ^{15}N data of frozen All2699GAF1 and All2699(GAF1–PHY) solutions are listed for reference. The ^{15}N chemical shift values obtained from All2699GAF1 and All2699(GAF1–PHY) as lyophilized powder are compared with those from the frozen solution samples. The ^{15}N chemical shift differences of PCB tetrapyrrole nitrogens in the two photoreceptors between the lyophilized and frozen solution states are listed and illustrated in Figure 4f.

Pyrrole nitrogens		^{15}N chemical shift (ppm)				^{15}N chemical shift difference (ppm)			
		All2699GAF1		All2699(GAF1–PHY)		$\Delta_{\text{Lyopholized state − Frozen solution}}$		$\Delta_{\text{All2699(GAF1–PHY) − All2699GAF1}}$	
		Frozen solution	Lyophilized state	Frozen solution	Lyophilized state	GAF1	GAF1–PHY	Frozen solution	Lyophilized state
ring A	**N21**	161.2	160.0 (N21[a])	160.2	158.8 (N21[a])	**−1.2**	**−1.4**	**−1.0**	**−1.2**
			163.2 (N21[b])		159.9 (N21[b])	**+2.0**	**−0.3**		**−3.3**
ring B	**N22**	144.7	145.2 (N22[a])	144.9	144.7	**+0.5**	**−0.2**	**+0.2**	**−0.5**
			146.4 (N22[b])			**+1.7**			**−1.7**
ring C	**N23**	155.8	154.6	156.7	156.9	**−1.2**	**+0.2**	**+0.9**	**+2.3**
ring D	**N24**	130.9	130.4	131.9	131.5	**−0.5**	**−0.4**	**+1.0**	**+1.1**