Supporting Information

Transient de-protonation of the chromophore affects protein dynamics proximal and distal to the linear tetrapyrrole chromophore in phytochrome Cph1

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Content

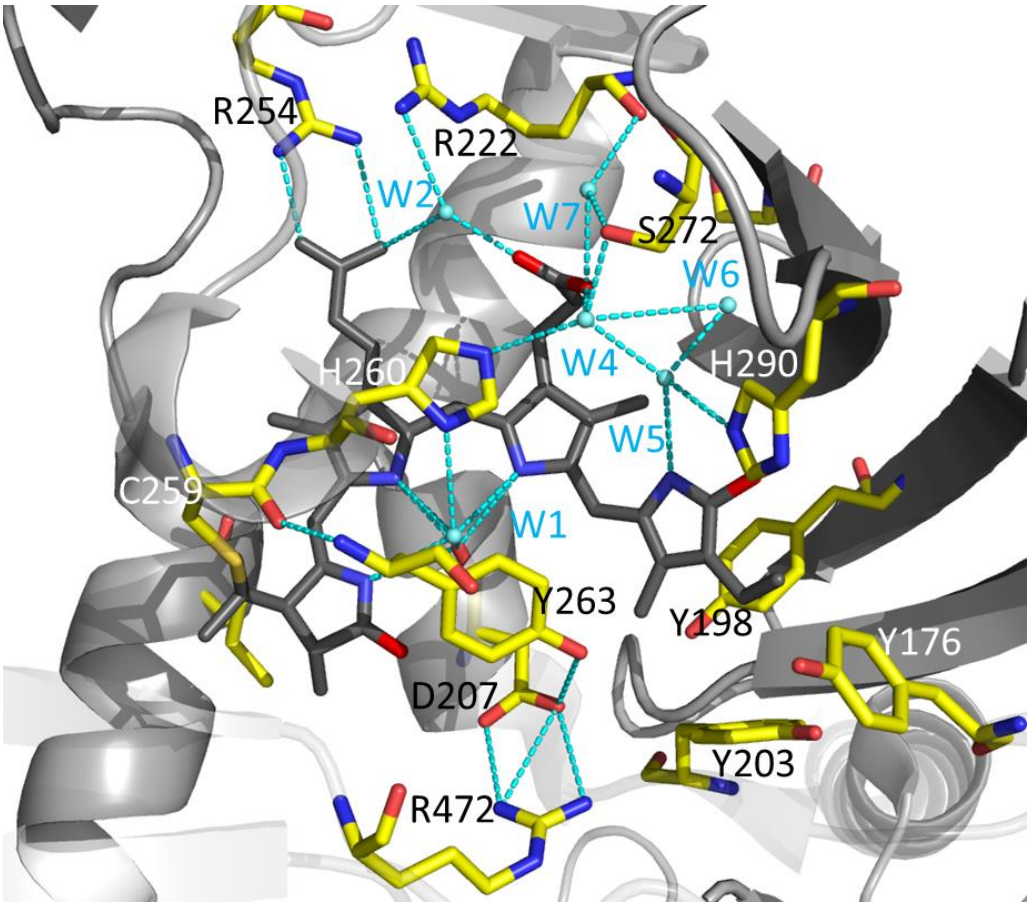

Figure S1: Structure of the chromophore binding pocket. Chromophore binding pocket of Cph1-PGP structure (pdb: 2VEA) with residues. Chromophore is shown in dark gray and for the formation of the binding pocket important residues are shown in yellow. Crystal waters (W1−W7) are plotted as spheres in cyan. Possible water bridges are shown as cyan dashed lines. Figure was created with PyMol.

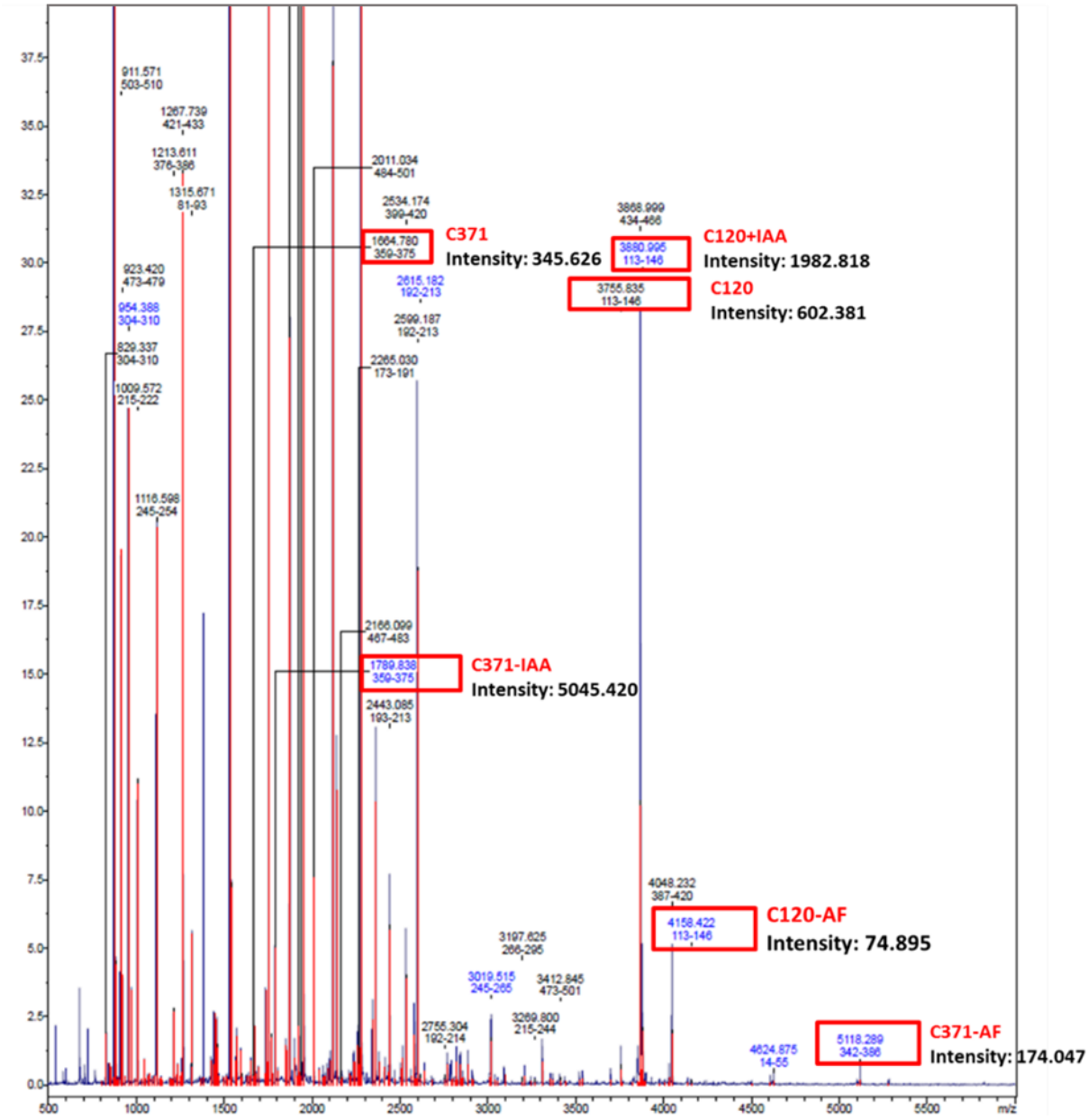

Figure S2: MALDI-MS analysis of Cph1-PGP-AF. The intensity and the mass of the peptides, which include C371-IAA, C371-AF, and C120-AF are marked with red. Based on the MS analysis the Cph1-PGP-AF construct is labeled with IAF at position 371 (the most exposed cystein according to the structure) and at position 120 (rather buried). Table S1 summarizes the respective tryptic peptides.

Table S1: Cph1 tryptic peptides detected by MALDI-MS sorted by their position in the protein sequence. *Peak #* indicates the peak number in the spectrum shown in Fig. S1; *M+H (expt)* is the mass determined experimentally; *M+H (calc)* is the peptide mass calculated from the Cph1 protein sequence; *ppm* is the relative deviation of the experimental mass from the theoretical value given in parts per million; *intensity* gives the intensity value from the mass spectrum for the respective peak; *#MC* is the maximal number of allowed missed cleavages by trypsin (incomplete cleavage); *position* gives the start and the end of the peptide; *peptide sequence* shows the underlying Cph 1 peptide sequence, and *modifications* lists side chain modifications taken into account in the peptide assignment. Search parameters used: Peptide tolerance: 150 ppm, specificity: Trypsin/P, optional modifications: N-ethylmaleimide (NEM,) 5-iodoacetamidofluorescein (5-IAF). IAF modifications are marked in bold.

Peak #	M+H (expt)	M+H (calc)	ppm	Intensity	#MC	Position	Peptide sequence	Cysteine Modifications
194	4.624.875	4.624.492	83	137	0	014-055	QLETLAIHTAHLIQPHGLVVVLQEPDLTISQISAN**C**TGILGR	C49 NEM
9	886.435	886.463	-32	4420	0	056-063	SPEDLLGR	
99	1.951.928	1.951.976	-25	47666	0	064-080	TLGEVFDSFQIDPIQSR	
46	1.315.671	1.315.722	-38.76	663.491	0	81 - 93	LTAGQISSLNPSK	
79	1.753.782	1.753.837	-31.43	42.174.456	0	98 - 112	VMGDDFVIFDGVFHR	
80	1.769.789	1.769.832	-24.19	1.755.126	0	98 - 112	VMGDDFVIFDGVFHR	
182	3.880.995	3.880.831	42.244	1.982.818	0	113 - 146	NSDGLLV**C**ELEPAYTSDNLPFLGFYHMANAALNR	C120 NEM
191	4.158.422	4.158.852	-103.5	74.895	0	113 - 146	NSDGLLV**C**ELEPAYTSDNLPFLGFYHMANAALNR	**C120-IAF**
175	3.755.835	3.755.783	13.813	602.381	0	113 - 146	NSDGLLVCELEPAYTSDNLPFLGFYHMANAALNR	
62	1.539.741	1539.78	-25.16	7.247.262	0	155 - 166	DFYDVIVEEVRR	
121	2265.03	2265.06	-13.48	783.111	1	173 - 191	VMLYRFDENNHGDVIAEDK	
68	1.602.641	1.602.703	-39	298.068	1	178 - 191	FDENNHGDVIAEDK	
141	2.615.182	2.615.219	-14.38	246.053	0	192 - 213	RDDMEPYLGLHYPESDIPQPAR	
144	2.755.304	2.755.326	-7.93	225.742	1	192 - 214	RDDMEPYLGLHYPESDIPQPARR	
140	2.599.187	2.599.225	-14.35	18.767.377	2	192 - 213	RDDMEPYLGLHYPESDIPQPAR	
130	2.443.085	2.443.123	-15.91	5.699.839	1	193 - 213	DDMEPYLGLHYPESDIPQPAR	
140	2.599.187	2.599.225	-14.35	18.767.377	0	193 - 214	DDMEPYLGLHYPESDIPQPARR	
141	2.615.182	2.615.219	-14.38	246.053	1	193 - 214	DDMEPYLGLHYPESDIPQPARR	
166	3269.8	3.269.816	-4.892	110.718	1	215 - 244	LFIHNPIRVIPDVYGVAVPLTPAVNPSTNR	
20	1.009.572	1.009.594	-21.96	11.033.526	1	215 - 222	LFIHNPIR	
123	2.279.189	2.279.239	-22.22	43.297.877	0	223 - 244	VIPDVYGVAVPLTPAVNPSTNR	
159	3.019.515	3.019.539	-7.793	1.606.118	0	245 - 265	AVDLTESILRSAYHCHLTYLK	
32	1.116.598	1.116.626	-25.29	20.363.386	1	245 - 254	AVDLTESILR	
93	1.921.826	1.921.931	-54.38	2.176.281	0	255 - 265	SAYHCHLTYLK	
163	3.197.625	3.197.671	-14.47	140.218	0	266 - 295	NMGVGASLTISLIKDGHLWGLIACHHQTPK	
96	1.937.672	1.937.944	-140.4	384.921	1	280 - 295	DGHLWGLIA**C**HHQTPK	C289 NEM
7	873.481	873.519	-44.12	48.800.021	0	296 - 302	VIPFELR	
16	954.388	954.414	-26.76	25.226.176	0	304 - 310	A**C**EFFGR	C305 NEM
3	829.337	829.366	-35.01	1930.18	0	304 - 310	ACEFFGR	
110	2.120.919	2.120.977	-27.3	37.227.899	0	311 - 328	VVFSNISAQEDTETFDYR	
56	1.464.743	1.464.806	-43.08	1.388.157	0	329 - 341	VQLAEHEAVLLDK	
196	5.118.289	5.118.439	-29.27	174.047	0	342 - 386	MTTAADFVEGLTNHPDRLLGLTGSQGAAI**C**FGEKLILVGETPDEK	**C371-IAF**
89	1.874.805	1874.87	-34.54	27.302.294	2	342 - 358	MTTAADFVEGLTNHPDR	
82	1.789.838	1.789.915	-43.02	5045.42	0	359 - 375	LLGLTGSQGAAI**C**FGEK	C371 NEM
72	1664.78	1.664.868	-52.73	345.626	0	359 - 375	LLGLTGSQGAAICFGEK	
39	1.213.611	1.213.667	-46.26	2.678.918	0	376 - 386	LILVGETPDEK	
61	1.532.781	1.532.822	-26.96	43.839.352	0	387 - 398	AVQYLLQWLENR	
189	4.048.232	4.048.049	45.087	1.881.463	0	387 - 420	AVQYLLQWLENREVQDVFFTSSLSQIYPDAVNFK	
134	2.534.174	2.534.245	-28	3901.73	1	387 - 420	EVQDVFFTSSLSQIYPDAVNFK	
196	5.118.289	5.117.602	134.25	174.047	0	421 - 466	SVASGLLAIPIARHNFLLWFRPEVLQTVNWGGDPNHAYEATQED GK	
45	1.267.739	1.267.773	-26.79	33.265.674	2	421 - 433	SVASGLLAIPIAR	
180	3.868.999	3.868.847	39.284	10.246.094	0	434 - 466	HNFLLWFRPEVLQTVNWGGDPNHAYEATQEDGK	
144	2.755.304	2.755.259	16.122	225.742	1	442 - 466	PEVLQTVNWGGDPNHAYEATQEDGK	
112	2.166.099	2.166.182	-38.19	250.195	0	467 - 483	IELHPRQSFDLWKEIVR	
170	3.412.845	3.412.863	-5.211	176.442	2	473 - 501	QSFDLWKEIVRLQSLPWQSVEIQSALALK	
13	923.42	923.462	-46	3997.27	2	473 - 479	QSFDLWK	
101	2.011.034	2.011.122	-43.7	7.627.675	0	484 - 501	LQSLPWQSVEIQSALALK	
12	911.571	911.604	-36.2	19.536.075	0	503 - 510	AIVNLILR	

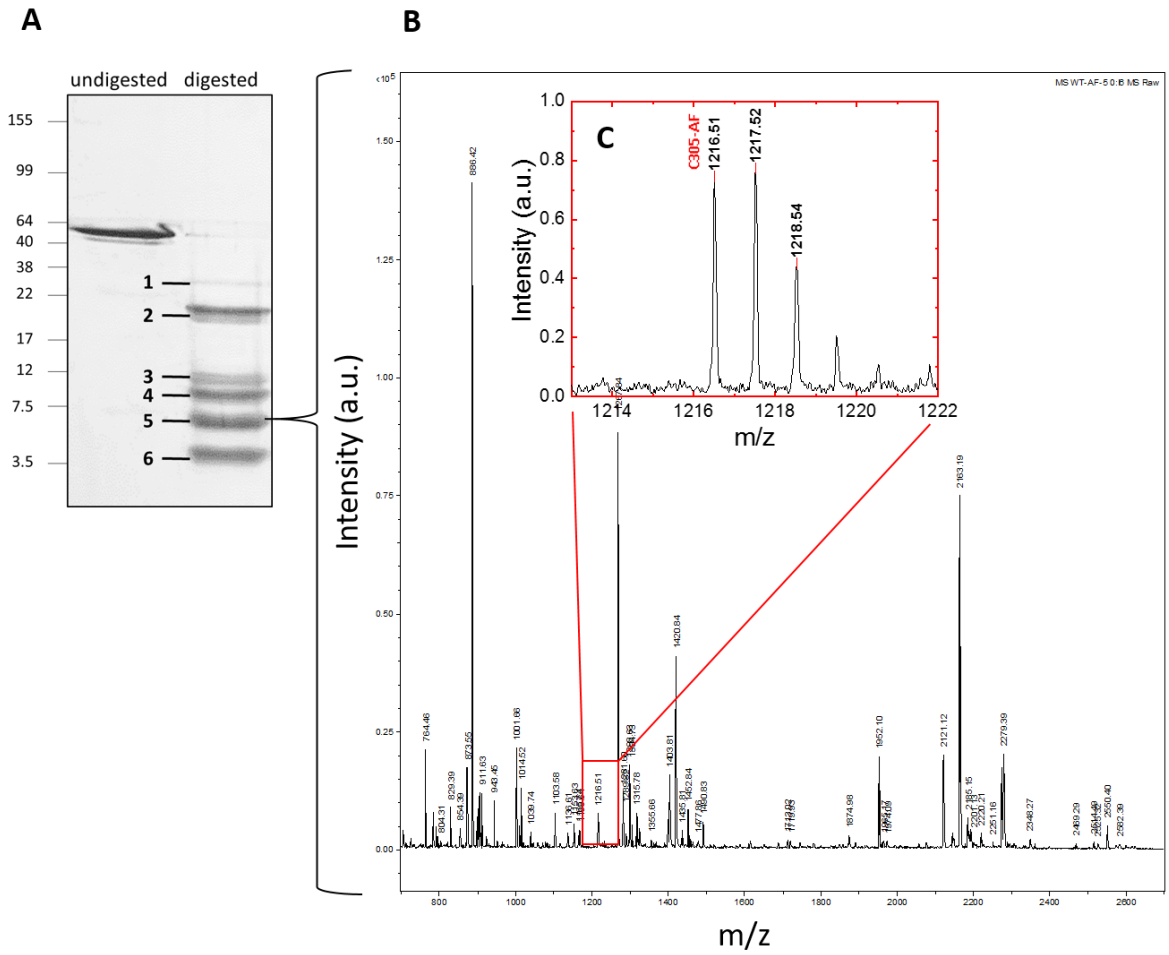

Figure S3: MS analysis of Cph1-PGP-C371S-AF. A) 10-20% acrylamide gradient SDS-PAGE of Cph1-PGP-C371S-AF undigested and in-solution digested with Lys-C. B) Mass spectrum of in-gel digest of band (fraction) 5 with Trypsin in the m/z range of 700-2700. C) Mass spectrum zoom into the m/z region from 1213 to 1222. The mass 1216.51 can be identified with the peptide pos. 304-310 (sequence ACEFFGR theoretical mass 829.37 Da) with the cysteine residue 'C305' modified with IAF (+387. 07 Da). Based on the MS analysis of the LysC/tryptic digest, the corresponding mass spectrum reveals IAF labeling at position 305. The other digest fractions do not contain IAF-labeled peptides.

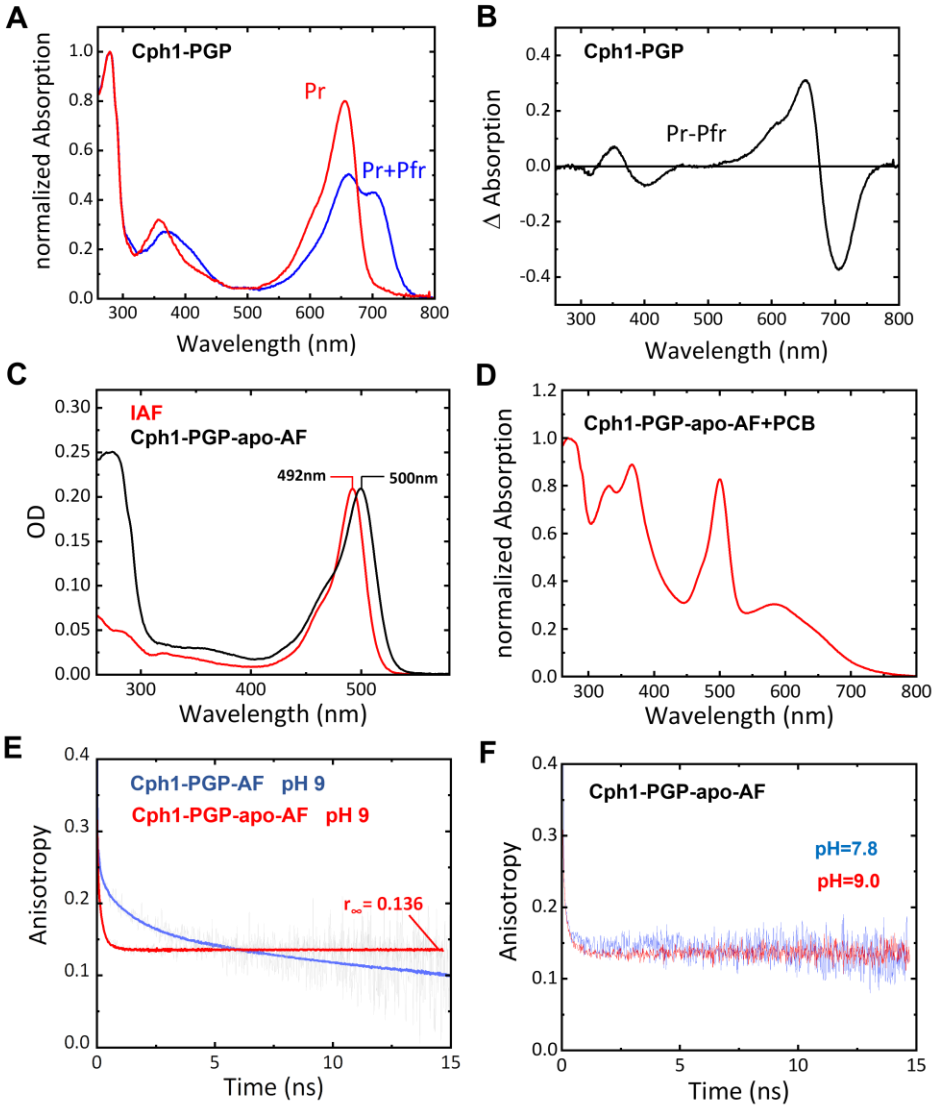

Figure S4: Cph1-PGP-apo-protein as control. A) UV-vis absorption spectra of purified Cph1-PGP after assembling PCB with the apo-protein overnight on ice, shown in the Pr-state (red curve) and the Pr+Pfr photoequilibrium mixture (blue curve) in 50 mM Tris-HCl, 150 mM NaCl pH 7.8. B) Absorption difference between Pr and Pr+Pfr state as shown in A). C) UV-vis absorption spectra of IAF-labeled Cph1-PGP apoprotein (black curve) and IAF (red curve) in 50 mM Tris-HCl, 150 mM NaCl pH 7.8. D) UV-Vis absorption spectrum of IAF-labeled Cph1-PGP-apoprotein together with PCB. No chromophore assembly occurs with the IAF-labeled apoprotein. E) Anisotropy decay of IAF-labeled Cph1-PGP-apo- and holoprotein at pH 9 (red and blue curves, respectively). F) Anisotropy decays of IAF-labeled Cph1-PGP-apoprotein at pH 7.8 and 9.0 (blue and red curves, respectively).

Table S2: Time-resolved anisotropy fit results of Cph1-PGP-AF. r_0 is initial anisotropy and the amplitudes β_1 and β_2 indicate the degree of depolarization of the anisotropy decay components with the correlation times ϕ_1 and ϕ_2. The reduced χ_2 (χ_{red}^2) is given as a measure of the goodness of the fit. The corresponding fluorescence lifetimes of bound IAF were 0.2 ns, 1.3 ns, and 3.9 ns (pH 8). The fit error is 10%.

pH	r_0	ϕ_1(ns)	ϕ_2 (ns)	ϕ_3 (ns)[a]	β_1	β_2	β_3	χ_{red}^2
6.6	0.30	0.11	2.8	30	0.063	0.051	0.183	0.98
7.0	0.37	0.09	1.4	30	0.104	0.056	0.211	1.07
7.5	0.35	0.08	1.3	30	0.091	0.060	0.199	1.26
8.0	0.34	0.07	1.2	30	0.090	0.059	0.191	1.12
8.5	0.33	0.10	1.3	30	0.088	0.063	0.179	1.02
9.4	0.31	0.12	1.5	30	0.078	0.068	0.164	1.00

[a]The rotational correlation time of the whole protein was fixed to 30 ns for better comparison. (The mean ϕ_3-value from the individual fits is 34±4 ns, the SD is given, n=6.)

Table S3: Time-resolved anisotropy fit results of Cph1-PGP-C371S-AF. r_0 is initial anisotropy and the amplitudes β_1 and β_2 indicate the degree of depolarization of the anisotropy decay components with the correlation times ϕ_1 and ϕ_2. The reduced χ^2 (χ_{red}^2) is given as a measure of the goodness of the fit. The corresponding fluorescence lifetimes of bound IAF were 0.2 ns, 1.0 ns, and 3.9 ns (pH 8). The fit error is 10%.

pH	r_0	ϕ_1(ns)	ϕ_2 (ns)	ϕ_3 (ns) [a]	β_1	β_2	β_3	χ_{red}^2
7.0	0.38	0.11	0.9	30	0.176	0.058	0.150	0.99
7.5	0.39	0.10	0.8	30	0.176	0.067	0.149	0.92
8.0	0.39	0.10	0.8	30	0.181	0.067	0.140	0.93
8.5	0.37	0.08	0.7	30	0.178	0.074	0.120	0.95
9.0	0.37	0.11	1.0	30	0.201	0.067	0.110	0.97
9.5	0.28	0.07	1.0	30	0.098	0.070	0.112	1.10

[a]The rotational correlation time of the whole protein was fixed to 30 ns for better comparison. (The mean ϕ_3-value from the individual fits is 28±3 ns, the SD is given, n=6.)

Table S4: Time-resolved anisotropy fit results of Cph1-PG-AF. r_0 is initial anisotropy and the amplitudes β_1 and β_2 indicate the degree of depolarization of the anisotropy decay components with the correlation times ϕ_1 and ϕ_2. The reduced χ^2 (χ_{red}^2) is given as a measure of the goodness of the fit. The corresponding fluorescence lifetimes of bound IAF were 0.3 ns, 1.5 ns, and 4.1 ns (pH 7.5). The fit error is 10%.

pH	r_0	ϕ_1(ns)	ϕ_2 (ns)	ϕ_3 (ns)[a]	β_1	β_2	β_3	χ_{red}^2
7.0	0.38	0.03	0.37	15	0.103	0.057	0.220	0.93
7.5	0.34	0.05	0.58	15	0.085	0.054	0.200	1.00
8.2	0.30	0.09	0.60	15	0.075	0.049	0.176	1.01
10	0.30	0.07	0.55	15	0.073	0.051	0.175	1.04

[a]The rotational correlation time of the whole protein was fixed to 15 ns for better comparison. (The mean ϕ_3-value from individual fits is 15±4 ns, the SD is given, n=4.)