Near infrared fluorescent biliproteins generated from bacteriophytochrome AphB of *Nostoc* sp. PCC 7120

Supplement

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Supplementary material

(1 figures, 4 tables)

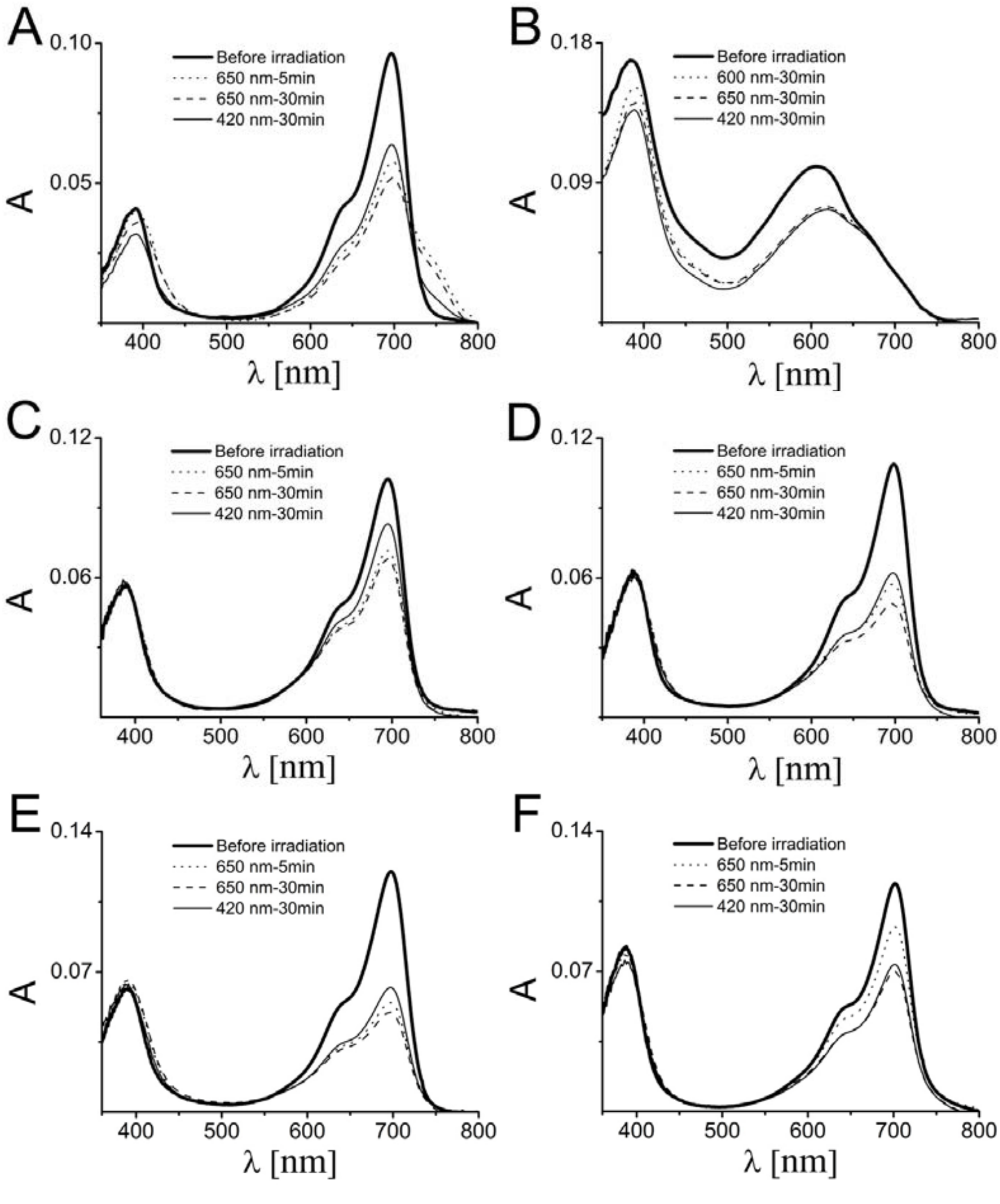

Fig. S1: **Photochemistry of PAS-GAF variants**. (A): BV-PAS-GAF-1, (B): BV-PAS-GAF-64, (C): BV-PAS-GAF-36, (D): BV-PAS-GAF-52, (E): BV-PAS-GAF-53, and (F): BV-PAS-GAF-60 (see Table S1). After recording the spectrum of each sample *as isolated*; its photochemistry was generally realized by an irradiation cycle of 650-420 nm, with the irradiation times specified in the inset. The 2^{nd} irradiation at 650 nm for 30 min has been carried out to ensure saturating conditions (see materials and methods). In the case of PAS-GAF-64, an additional irradiation has been carried out at 600 nm in order to check the photoactivity of the component with an absorption band in the 600 nm range.

Table S1: Primers for site-directed variants.

Primer	Sequence	DNA
P1	5'- CTAGATCTGGTGAACATCAACGATATCACTA -3'	*pas-gaf-1*
P2	5'- ACTAAGCTTAATCTTCTTTGGCACTCATTT -3'	
P3	5'- ACCGTTCCACAACAAGCTAGAAAACTC -3'	*pas-gaf-29*
P4	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGA -3'	
P5	5'- ACTATTCCACAACAAGCTAGAAAACTCTATAG -3'	*pas-gaf-36*
P6	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGA -3'	
P7	5'- GCTATTCCACAACAAGCTAGAAAACTCTATAGCC -3'	*pas-gaf-52*
P8	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGATA -3'	
P9	5'- GCTATTCCACAACAAGCTAGAAAACTCTATAGCC -3'	*pas-gaf-53*
P10	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATG -3'	
P11	5'- TTTATTCCACAACAAGCTAGAAAACTCTATAG -3'	*pas-gaf-55*
P12	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGA -3'	
P13	5'- ACACCAAAATATATACCCTATGAAATAC -3'	*pas-gaf-56*
P14	5'- TTGATGATGGCACAATGAGTCC -3'	
P15	5'- GGTATTCCACAACAAGCTAGAAAACTCTATAG -3'	*pas-gaf-60*
P16	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGA -3'	
P17	5'- TTCTCCCCATTACACATTGAATATATGCAGAA-3	*pas-gaf-41*
P18	5'- GCTGCGAAGAACTGAGCGACTTAAATC -3	
P19	5'- TATTATATACCCTATGAAATACGGAGCGCCTG -3'	*pas-gaf-57*
P20	5'- TGGTGATTGATGATGGCAGGCAATG -3'	
P21	5'- CATATTCCACAACAAGCTAGAAAACTCTATAGCC -3	*pas-gaf-58*
P22	5'- GCTAGCTGGATAATTTAACCCTAAATAAGATGTAAGATA -3	
P23	5'- TGTTCACCAAAATATATACCCTATGAAATAC -3'	*pas-gaf-61*
P24	5'- ATGATGGCAGGCAATGAGTCCCATAA -3'	
P25	5'- CCTGCCATCATCACAAATGACTTCTGTAGAAATG -3	*pas-gaf-62*

P26	5'- CACAGGCGCTCCGTATTTCATAGG -3	
P27	5'- ACGCGGATCCACTATTCCCTTTCAGGTAGACC -3'	For random mutations
P28	5'- ACCGCTCGAGTTAATCTTCTTTGGCACTCATT -3'	

Table S2: AphB(1-321) (i.e. PAS-GAF of AphB) variants. (a) site-directed mutations. (b) the unique H260Y variant and its ancestor. Following the parental protein, PAS-GAF-1 (= WT AphB(1-321), the various multiple mutants are numbered and listed in order of decaying fluorescence quantum yield (see Table S3).

PAS-GAF Variant no.	Sequence variations
1	AphB(1-321)
2	AphB(7-321/E74V/Q89R/N92D/L139R/D207T/I208V/Y263F/T270A/A288V/I301T)
3	AphB(7-321/L37F/S100T/L139H/D207T/I208V/Y263F/N266S/I287V/A288V)
4	AphB(7-321/E74V/I90S/N116D/L139H/D207T/I208V/Y263F/M278V)
5	AphB(7-321/T51P/E74V/L139H/D207T/I208V/I234V/Y263F/A288V)
6	AphB(7-321/E74V/L139H/Q181L/D207T/I208V/Y263F/A288V)
7	AphB(7-321/S132P/K166R/D207T/I208V/Y263F/A288V)
8	AphB(7-321/V36A/E74A/L126S/K129E/V140A/V190I/K191R/D207T/I208V/Y263F /A288V/T312A)
9	AphB(7-321/E74V/K80E/L139H/M144V/L145P/D207T/I208V/Y263F/A288V)
10	AphB(7-321/E74V/L139H/K141R/I159V/S206N/D207T/I208V/Y263F/A288V)
11	AphB(7-321/L37F/V47G/E74V/L142P/K146R/V173A/D207T/I208V/Q211R/L215P /Y263H/A288V)
12	AphB(7-321/E74V/K80E/F133S/L139H/K141R/D207T/I208V/Y263F/A288V)
13	AphB(7-321/I28V/E74V/L139H/L142P/D207T/I208V/Y263F/A288V)
14	AphB(7-321/E74V/D207T/I208V/Y263F/A288V)
15	AphB(7-321/E74V/L139H/S157N/D207T/I208V/Q210R/Y263F/A288V/P294S /Q310R/T312A)
16	AphB(7-321/E74V/H112Q/L139H/D207T/I208V/Y263F/A288V/I297M)
17	AphB(7-321/E74V/L139H/D207T/I208V/S249G/Y263F/A288V)
18	AphB(7-321/E74V/D108E/L139H/D207T/I208V/Y263F/A288V)
19	AphB(7-321/E74V/E103G/L139H/D207T/I208V/I224T/Y263F/A288V)
20	AphB(7-321/F52L/F72G/I91T/E95A/N102S/K129T/F134L/I156S/M174A/D207T /I208V/Y263F/A288V/F307L)

21	AphB(7-321/E74V/S100P/H131Q/L139H/D207T/I208V/Q210R/Y263F/A288V)
22	AphB(7-321/E74V/L139H/K146R/I159M/D207T/I208V/Q244R/Y263F/A288V)
23	AphB(7-321/S106G/L139H/S206G/D207T/I208V/Y263F/K282E/A288V/T312A)
24	AphB(7-321/E74V/L139H/D207T/I208V/Y263F/A288V)
25	AphB(7-321/E74V/L139H/D207T/I208V/M264L/A288V)
26	AphB(7-321/F134L/D207T/I208V/A288V)
27	AphB(7-321/Y137F/A151V/D207T/I208V/V256F/I261L/A288V/S293T)
28	AphB(7-321/S114P/D207T/I208V/V256F/A288V/S293T)
29 [a]	AphB(7-321/D207T/I208V/A288V)
30	AphB(7-321/L14V/V36A/E74V/F136S/D207T/I208V/A288V)
31	AphB(7-321/E74V/F96L/F136I/D207T/I208V/A288V)
32	AphB(7-321/L142P/E193G/D207T/I208V/V256F/A288V/S293T/I297M/T312A)
33	AphB(7-321/D207T/I208V/V256F/A288V/S293T)
34	AphB(7-321/E74V/D207T/I208V/Y263H/A288V)
35	AphB(7-321/L126S/Q162R/D207T/I208V/S276P/A288V)
36 [a]	AphB(7-321/D207T/A288V)
37 [b]	AphB(7-321/E74V/D207H/I208T/Y263F/A288V)
38	AphB(7-321/E74V/L139H/D207T/I208V/M264L/A288V)
39	AphB(7-321/E74V/S127L/D207T/I208V/A288V)
40	AphB(7-321/Q61R/L126S/S132L/D207T/I208V/A288V)
41 [a]	AphB(7-321/D207A/V256F/A288V)
42	AphB(7-321/E74V/Y137F/A151V/D207T/I208V/V256F/I261L/K281R/A288V/S293T)
43	AphB(7-321/E74V/C82W/I105T/K129R/L139R/D207T/I208V/L259S/Y263F/A288V)
44	AphB(7-321/D207A/V256F/A288V/E306A)
45	AphB(7-321/E74V/L195M/T196Q/D207H/I208T/Y216H/Y263F/A288V)
46	AphB(7-321/S15C/E74V/L139H/D207T/I208V/Y263F/A288V)
47	AphB(7-321/E74V/D207T/I208V/A288V)
48	AphB(7-321/P59S/H101Y/A111V/D128G/D207A/A288V/K295R)
49	AphB(7-321/N115D/K129E/F136V/H138L/L139R/I187M/D207A/A288V)

50	AphB(7-321H138L/L139R/D207A/A288V)
51	AphB(7-321/I90F/T130A/H138L/L139R/N183I/D207A/A288V)
52 [a]	AphB(7-321/D207A/A288V)
53 [a]	AphB(7-321/D207H/A288V)
54	AphB(7-321/F136L/D207A/L221Q/A288V/K295T)
55 [a]	AphB(7-321/D207F/A288V)
56 [a]	AphB(7-321/D207A/S293T)
57 [a]	AphB(7-321/D207H/K295Y)
58 [a]	AphB(7-321/D207H)
59	AphB(7-321/E86G/L126S/F136L/D207A/L221Q/K228N/N280D/A288V/K295T)
60 [a]	AphB(7-321/D207G/A288V)
61 [a]	AphB(7-321/D207H/Q292C)
62 [a]	AphB(7-321/D207A/A288V/E306A)
63	AphB(7-321/E74V/D207W/I208T/Y263F/A288V)
64 [b]	AphB(7-321/E74V/D207H/I208T/H260Y/Y263F/A288V)
65	AphB(7-321/E74V/D207T/I208V/Y263W/A288V)

Table S3: Quantitative absorption and fluorescence data of near infrared fluorescent biliproteins derived from PAS-GAF of AphB. Spectra in KPB (20 mM, pH 7.0) containing 0.5 M NaCl were obtained after purification by Ni^{2+} affinity chromatography. Unless otherwise stated, the fluorescence spectra were obtained under excitation at 600 nm. Transients were fitted with a bi-exponential sequential model. (a) site-directed mutations. (b) the unique H260Y variant and its ancestor. (c) fluorescence emission spectrum obtained under excitation at 640 nm. (d) brightness of the BV-variants relative to that of wt BV-PAS-GAF-1 ($\varepsilon \bullet \Phi_{fl}$ = 1.5 $mM^{-1}cm^{-1}$).

BV-PAS-GAF Variant no.	Absorption		Fluorescence				
	λ_{max} [nm]	$\varepsilon \bullet 10^{-4}$ [$M^{-1}cm^{-1}$]	λ_{max} [nm]	Φ_{fl}	Stokes shift [nm]	Bright-ness [d] [%]	Life time [ns] τ_1 (%), τ_2 (%); χ^2
1	697	6.4	721	0.023	24	100	0.48(97.7), 3.0(2.3); 1.86
2	693	8.1	717	0.060	24	330	0.62(98.1), 4.0(1.9); 1.94
3	691	7.7	717	0.060	26	314	0.61(97.6), 3.4(2.4); 1.81
4	693	8.1	717	0.059	24	325	0.62(98.2), 3.9(1.8); 2.11
5	693	7.9	717	0.057	24	306	0.61(98.0), 3.7(2.0); 1.84
6	693	8.0	718	0.057	25	310	0.61(97.6), 3.3(2.4); 2.15
7	693	8.0	717	0.057	24	310	0.61(98.1), 4.1(1.9); 1.82
8	693	7.9	718	0.057	25	306	0.60(98.1), 4.1(1.9); 1.86
9	693	8.4	717	0.057	24	326	0.61(97.8), 3.4(2.2); 2.00
10	694	8.1	718	0.057	24	314	0.60(97.8), 3.6(2.2); 1.84
11	693	7.9	717	0.056	24	301	0.59(97.9), 3.6(2.1); 1.85
12	693	7.9	717	0.056	24	301	0.62(98.1), 3.8(1.9); 2.00
13	693	8.1	716	0.056	23	308	0.61(97.8), 3.5(2.2); 2.16
14	693	8.2	718	0.056	25	312	0.60(98.6), 5.4(1.4); 1.83
15	693	8.6	718	0.055	25	321	0.60(97.8), 3.4(2.2); 2.05
16	693	9.2	718	0.055	25	344	0.60(97.4), 3.2(2.6); 1.61

17	693	7.7	718	0.055	25	288	0.60(98.1), 3.9(1.9); 2.02
18	693	8.4	717	0.054	24	308	0.61(97.7), 3.2(2.3); 1.67
19	692	7.7	718	0.054	26	283	0.59(97.3), 3.0(2.7); 1.56
20	695	9.6	718	0.053	23	346	0.61(98.0), 3.6(2.0); 2.02
21	693	8.7	717	0.053	24	314	0.61(98.3), 4.2(1.7); 1.63
22	693	8.4	717	0.052	24	297	0.61(98.1), 4.1(1.9); 1.92
23	695	7.4	719	0.052	24	262	0.58(98.0), 4.0(2.0); 1.90
24	693	8.9	718	0.051	25	308	0.61(97.0), 3.0(3.0); 1.58
25	694	7.4	719	0.051	25	257	0.58(98.1), 3.7(1.9); 2.17
26	694	7.4	718	0.051	24	257	0.56(98.2), 4.4(1.8); 1.94
27	694	7.3	719	0.050	25	248	0.55(98.4), 5.6(1.6); 1.83
28	692	7.2	718	0.050	26	245	0.55(98.7), 5.9(1.3); 1.76
29[a]	694	7.0	718	0.050	24	238	0.57(98.0), 4.1(2.0); 1.65
30	694	7.3	718	0.050	24	248	0.56(98.7), 6.0(1.3); 1.75
31	694	7.6	719	0.050	25	258	0.56(98.7), 6.3(1.3); 1.80
32	693	7.5	717	0.049	24	250	0.54(98.7), 5.8(1.3); 1.81
33	693	7.3	719	0.049	26	243	0.54(98.7), 5.4(1.3); 1.75
34	693	6.9	720	0.049	27	230	0.59(95.8), 2.4(4.2); 1.71
35	694	7.2	719	0.049	25	240	0.55(98.6), 5.4(1.4); 1.80
36[a]	695	7.0	719	0.049	24	233	0.59(98.0), 4.1(2.0); 1.51
37[b]	693	7.0	719	0.048	26	228	0.59(97.6), 3.6(2.4); 1.70
38	694	6.7	719	0.048	25	219	0.57(98.6), 5.5(1.4); 1.73
39	694	7.3	719	0.048	25	238	0.56(98.6), 5.5(1.4); 1.78
40	694	7.6	718	0.048	24	248	0.58(98.5), 5.0(1.5); 1.48
41[a]	697	7.0	721	0.048	24	228	0.53(98.2), 4.5(1.8); 1.70
42	694	7.4	719	0.048	25	242	0.57(98.1), 3.9(1.9); 1.60
43	692	8.7	717	0.047	25	278	0.57(97.2), 3.4(2.8); 2.06
44	696	7.0	720	0.047	24	224	0.53(98.4), 4.7(1.6); 1.81
45	692	7.2	719	0.047	27	230	0.60(97.8), 3.9(2.2); 1.67

46	693	9.2	717	0.047	24	294	0.57(96.0), 2.5(4.0); 2.34
47	694	7.3	720	0.047	26	233	0.55(98.7), 6.3(1.3); 1.72
48	698	8.3	721	0.046	23	260	0.55(98.7), 5.9(1.3); 1.77
49	699	8.3	721	0.046	22	260	0.54(98.6), 5.8(1.4); 1.70
50	698	6.8	722	0.045	24	208	0.54(97.7), 5.1(2.3); 1.86
51	700	7.9	722	0.045	22	242	0.52(98.2), 4.4(1.8); 1.83
52[a]	698	7.6	722	0.045	24	232	0.55(98.0), 4.0(2.0); 1.56
53[a]	698	7.2	722	0.044	24	215	0.56(98.0), 5.8(2.0); 1.62
54	698	7.9	720	0.044	22	236	0.52(98.4), 1.1(1.6); 1.81
55[a]	699	6.2	723	0.043	24	181	0.58(97.5), 6.8(2.5); 1.69
56[a]	700	7.2	723	0.042	23	206	0.56(98.4), 4.4(1.6); 1.48
57[a]	699	7.3	722	0.042	23	208	0.54(98.5), 5.4(1.5); 1.75
58[a]	699	6.4	722	0.041	23	178	0.54(98.1), 5.2(1.9); 1.89
59	697	7.0	720	0.041	23	195	0.57(97.8), 3.6(2.2); 2.15
60[a]	701	6.4	723	0.040	22	174	0.54(98.2), 4.8(1.8); 1.69
61[a]	699	6.5	722	0.039	23	172	0.54(98.4), 5.9(1.6); 1.73
62[a]	697	6.7	722	0.039	25	178	0.53(98.0), 3.4(2.0); 1.81
63	689	6.2	717	0.036	28	152	0.46(94.6), 1.8(5.4); 1.97
64[b]	606/692	2.2	648/712	0.031	20	46	0.27(96.7), 8.4(3.3); 2.28
65	703	7.1	724	0.025	21	121	0.36(94.2), 1.3(5.8); 1.94
64[b,c]	606/692	2.2	718	0.017	26	25	0.27(96.8), 9.1(3.2); 2.39

Table S4: Plasmids used. The pET30 and pACYCDuet, from Novagen, are T7 promoter expression vectors. pACYCDuet is designed to co-express two target proteins in *E. coli*. Using the two vector-derivatives together with compatible replicons and antibiotic resistance, 3 proteins could be co-expressed in the same cell, thereby generating the respective BV-variants in *E. coli*.

Antibiotic resistance	Plasmids with P15A replicon	Plasmids with ColE1 replicon
	pACYCDuet derivatives	pET30 derivatives
Kanamycin		pET-*pas-gaf-1* pET-*pas-gaf-2* pET-*pas-gaf-3* pET-*pas-gaf-4* pET-*pas-gaf-5* pET-*pas-gaf-6* pET-*pas-gaf-7* pET-*pas-gaf-8* pET-*pas-gaf-9* pET-*pas-gaf-10* pET-*pas-gaf-11* pET-*pas-gaf-12* pET-*pas-gaf-13* pET-*pas-gaf-14* pET-*pas-gaf-15* pET-*pas-gaf-16* pET-*pas-gaf-17* pET-*pas-gaf-18* pET-*pas-gaf-19* pET-*pas-gaf-20* pET-*pas-gaf-21* pET-*pas-gaf-22*

		pET-*pas-gaf-23* pET-*pas-gaf-24* pET-*pas-gaf-25* pET-*pas-gaf-26* pET-*pas-gaf-27* pET-*pas-gaf-28* pET-*pas-gaf-29* pET-*pas-gaf-30* pET-*pas-gaf-31* pET-*pas-gaf-32* pET-*pas-gaf-33* pET-*pas-gaf-34* pET-*pas-gaf-35* pET-*pas-gaf-36* pET-*pas-gaf-37* pET-*pas-gaf-38* pET-*pas-gaf-39* pET-*pas-gaf-40* pET-*pas-gaf-41* pET-*pas-gaf-42* pET-*pas-gaf-43* pET-*pas-gaf-44* pET-*pas-gaf-45* pET-*pas-gaf-46* pET-*pas-gaf-47* pET-*pas-gaf-48* pET-*pas-gaf-49* pET-*pas-gaf-50* pET-*pas-gaf-51*

		pET-*pas-gaf-52* pET-*pas-gaf-53* pET-*pas-gaf-54* pET-*pas-gaf-55* pET-*pas-gaf-56* pET-*pas-gaf-57* pET-*pas-gaf-58* pET-*pas-gaf-59* pET-*pas-gaf-60* pET-*pas-gaf-61* pET-*pas-gaf-62* pET-*pas-gaf-63* pET-*pas-gaf-64* pET-*pas-gaf-65*
Chloramphenicol	pACYC-*ho1*	