

**Orange fluorescent proteins shift constructed from cyanobacteriochromes  
chromophorylated with phycoerythrobilin**

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**Supplementary Material**

(2 tables, 7 figures)

**Table S1: Primers for GAFs and its fusion with affinity tags or *ho1* and *pebS*.**

Primer	Sequence	DNA
P1	5'-CCCGGATCCCAATCGCTGAATCTACAAAAT-3'	<i>all1280 gaf2</i>
P2	5'-GGCGTCGACTTACAATTCATTCTGGGCTTG-3'	
P3	5'-CGCGATATCATCTTGTTAATGTTGTT-3'	<i>all3691 gaf2</i>
P4	5'-ATTAAGCTTTTATTTTTGCGTTTGGAT-3'	
P5	5'-GCCGGATCCCAGCAATATCAACACACTTTAC-3'	<i>alr2279</i>
P6	5'-TTAGTCGACGGCTTGCCTAGTTTCCTGAGC-3'	
P7	5'-ATAGGATCCATGCAAATTCATTCTCACGCAG-3'	<i>alr3356</i>
P8	5'-GCAAGCTTCTCAACTTTCCAAAATATAAGGCGA-3'	
P9	5'-TCTGGATCCACTCAAGCTGAACTC-3'	<i>tlr0911</i>
P10	5'-ATAGTCGACTTATTCCTGATGTAGCGC-3'	
P11	5'-GCGGAATTCGAGTCTTTGGATTTAGAAAC-3'	<i>alr5272 gaf1</i>
P12	5'-CTGGTCGACTTTCGTATTTTGAATGGCTA-3'	
P13	5'-CTAGGATCCTCAGACAAATTTCAAGCA-3'	<i>alr5272 gaf2</i>
P14	5'-GCCAAGCTTTAATTTTTTCAGTCGTG-3'	
P15	5'-CCAGGATCCGATACACCACCAGAAGAAGA-3'	<i>all1280 gaf1</i>
P16	5'-TATGTCGACTCTGATGACTTGACGCGCTAA-3'	
P17	5'-CCGGAATTCTTTGCCTCTATCTT-3'	<i>all3691 gaf1</i>
P18	5'-CTGCTCGAGTTATGTTTGAGATTCTT-3'	
P19	5'-CCCGGATCCTCTCTAAGTTTACAAACCATT-3'	<i>alr3120 gaf1</i>
P20	5'-AATGTCGACTAACTGATCCTGCAATTTTTGG-3'	
P21	5'-CGTGAATTCCAACAGGTAGAAAGGGAGA-3'	<i>alr3120 gaf2</i>
P22	5'-TTTGTGCGACTCTTTCGGCAAGTTCTTG-3'	
P23	5'-TCACAAGCTTATCAAGGTAATTTAAAAACAG-3'	<i>all0729 gaf1</i>
P24	5'-TAGCTCGAGTTATTCTAAGGCTAAGGACAC-3'	
P25	5'-TAAGATATCAAGCAAGCCGAAGCGGAAAGC-3'	<i>all0729 gaf2</i>
P26	5'-GGGGTCGACTCATTGTAAACGTTCTTCGCT-3'	

P27	5'-CACGGATCCACCCTAGATTTAGATACC-3'	
P28	5'-CCCGTCGACCTAAACTTCTAACTGATT-3'	<i>all0729 gaf3</i>
P29	5'-AAAGAATTCAAAAGTCGGCAACAGCAACA-3'	
P30	5'-CGCCTCGAGCTATTGTAGTTCCGTCTGTAT-3'	<i>all2239</i>
P31	5'-ATAGGATCCCGGATTCGTCAATCCTTGGAG-3'	
P32	5'-CGCCTCGAGTTAGATCGCTACTGCAAGTTG-3'	<i>GCN4-tag::all2699 gaf1</i>
P33	5'-GCCGGTACCCAATCGCTGAATCTACAAAATATTCTCAATGCC-3'	
P34	5'-CTAGAATTCTCCAGAACCACCAGATCCCGCTAAGGCACTTCT-3'	<i>all1280 gaf2::ho1::pebS</i>
P35	5'-GCGGGTACCTTGGAGTTGGAGGATATTATCACAGCGACAA-3'	
P36	5'-CAAGAATTCACCAGATCCACCTGATCCAATGATGGCTTCTTT-3'	<i>all2699 gaf1::ho1::pebS</i>

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**Table S2: Plasmids used.** The pACYCDuet and pET30 (or pET28), from Novagen, are T7 promoter expression vectors. pACYCDuet are 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 designed biliproteins in *E. coli*. Subscripts indicate the strain of the parent organisms. a) *pebS* was synthesized according to the related gene sequence of *Prochlorococcus* phage P-SSM2 <sup>1</sup>. The “-” in “ho1<sub>PCC7120</sub>-pebS” denotes that the two genes (*ho1* and *pebS*) were inserted respectively at the two cloning sites of pACYCDuet. The “::” in “ho1<sub>PCC7120</sub>::pebS” denotes that the two genes were fused into one DNA segment.

Antibiotic resistance	Plasmids with P15A replicon	Plasmids with ColE1 replicon
	pACYCDuet derivatives	pET30 derivatives
Kanamycin		pET-all1280 <i>gaf2</i> <sub>PCC7120</sub>
		pET-all2699 <i>gaf1</i> <sub>PCC7120</sub>
		pET-all2699 <i>gaf3</i> <sub>PCC7120</sub>
		pET-all3691 <i>gaf2</i> <sub>PCC7120</sub>
		pET-alr2279 <sub>PCC7120</sub>
		pET-alr3356 <sub>PCC7120</sub>
		pET-slr1393 <i>gaf3</i> <sub>PCC6803</sub>
		pET-tlr0911 <sub><i>Thermosynechococcus elongatus</i> BP-1</sub>
		pET-all1280 <i>gaf1</i> <sub>PCC7120</sub>
		pET-all3691 <i>gaf1</i> <sub>PCC7120</sub>
		pET-all0729 <i>gaf1</i> <sub>PCC7120</sub>
		pET-all0729 <i>gaf2</i> <sub>PCC7120</sub>
		pET-all0729 <i>gaf3</i> <sub>PCC7120</sub>
		pET-all2239 <sub>PCC7120</sub>
		pET-alr5272 <i>gaf1</i> <sub>PCC7120</sub>
		pET-alr5272 <i>gaf2</i> <sub>PCC7120</sub>

		<p>pET-<i>alr3120</i> <i>gaf1</i><sub>PCC7120</sub></p> <p>pET-<i>alr3120</i> <i>gaf2</i><sub>PCC7120</sub></p> <p>pET28-GCN4-tag::<i>all2699</i> <i>gaf1</i><sub>PCC7120</sub></p> <p>pET-<i>all1280</i> <i>gaf2</i><sub>PCC7120</sub>::<i>ho1</i>::<i>pebS</i></p> <p>pET-<i>all2699</i> <i>gaf1</i><sub>PCC7120</sub>::<i>ho1</i>::<i>pebS</i></p> <p>pET-<i>slr1393</i> <i>gaf3</i><sub>PCC6803</sub>::<i>ho1</i>::<i>pebS</i></p>
Chloramphenicol	pACYC- <i>ho1</i> <sub>PCC7120</sub> - <i>pcyA</i> <sub>PCC7120</sub>	
Chloramphenicol	pACYC- <i>ho1</i> <sub>PCC7120</sub> - <i>pebS</i> <sup>a</sup>	
Streptomycin	pCDF- <i>ho1</i> <sub>PCC7120</sub> :: <i>pebS</i> <sup>a</sup>	

## A

### All10729(1645aa), two-component hybrid sensor and regulator

QSLNSQSLVSSNNKITRSKNNTQEQYQINAVKLYKHNLVLTCLAKNQVLYQGNLKTALAEI→240  
TNVAAHNLGIERASIWLYDETLTKIQCADLFEYSRNQHSAGLSLSAKEYPTYFQALHQDE→300  
AIAVDDAYTDPRTKEFAQSYLTPLNITAMLDTPIRLAGKTVGVLCLEAVKLPHHWTPEDQ→360  
NFARSLTNLVSLALEARERQRAEAAHRISEQKLASAFRASPDPIALCTYPETRYIEVND→420  
FCRLFGYSRSQVIGNTDKELNIWVNLEECHFLSQILEKAKAIRNHEVDFRTSNGEIKTTL→480  
FSAEMIEIDGQKYILGTAKDITERKQAEAESRLLLTTQAIARAVDVKSALTTLVRVICQ→540  
TIGWDFGEAWTPNRESHVLEHSLVCYCEEASLEEFCHQSQNLRIAPGEGLPGRVWQTKQS→600  
EWIEDVSLVKQGQFLRSPQAQKVGKAGFGIPIIAGKEVLAVLIFFKRSSIPVDKRLML→660  
LGAVATQLGSLIERKLIESAHRNSEERLQLALEASDLGLWDWNITTKGIYRDWQWQKMLG→720  
YTQEDIEDDERVIEQLLHPEDLATVKSAITAHLQGVTPVYEMEFRMCAAGGWKVVQSRG→780  
QIVERNEQGQPLRMTGTTKDITERKILEKELELREARLNAFFSGAPVGMSSILDNQLRFVQ→840  
INELIAHIHGKPAQEHIGKTLLEIAPRIAPLVTPCQKVLLTGQPIILNVELSLPAVNQAD→900  
SLRHFLVSYFPIPGEGNQLSGVGKIVVEISDVYDELRLRKRAEEALKESIERERAIQVI→960  
QRMRQTLDDLDTIFAATTEELRQVLNCDRAVVYRFDSQSGGEFVAESWGKGWISLIEKQKN→1020  
VPHLTENTAKDQSCAKLLEYTPQATPKNFCLVADIYQAGFGSCYIEFLERLQAKAYII→1080  
VPILSGDQVWGLLATYQNSGPRQWKTGEINIVIQIGNQLEVALQQAQLLTQTQRQSQALE→1140

### All1280(941 aa), annotated as two-component sensor histidine kinase

MQHIINFSSNKAIMLFNDESSRLETLYQYRIIDTPPEEEFDDLVLNLAADSCNTPIALIS→60  
ILDAQREWFKSKVGI TELEIPRNICGIHTIGQNDILIPDTPWQDERFVENPLVRQKPTA→120  
FRFYAGVPLINSEGFALGCLAVIDSTPRNLSLKEQRILKRLARQVIRQLELHRKQISHES→180  
SLYVHLLFTNNPRPIWICERRTLQILDVNQAAITQYGYRAEFLEMQLAQVVFPEFISDL→240  
IRDIEQENFQFPFLMECQHRLSSGQVIDVELAINYIEHSGYKACLVDAINITEHIQIERN→300  
LQKSETRVRTILEAIPVPLVISRVDDGLILYTNSEFLQTFQLSGNDLINHYAADLYENPE→360  
DRQQILEALSQHGSLQNYDIQFKKSDGTSFWAIASIQYLNFNNEYAILTVLYDITERKNI→420  
EAKLQEKNALQSIFTGIPLMIALISPEGQIQWVNQELDRLLGWSLRDYQTLDFIAELYP→480  
QPEYRQLVINFIQSGECIWCDFRTQTRYQVLDTSWINIKLADGRIIGIGQEITKRKQTE→540  
RALKGQVEREQLMRAVAQRIRQSLNLQNILNATVKEIKDLLDVRVVVYQFAPDMSGKIV→600  
AESVKPGWKIALGADIQDNCFOSGAGADYROGHKRAIANIYTAELTDCHLRLLEQFOVKA→660  
NLVVPILLEVSEGNTVPQLWGLLIAHOCSTPRDWEAHELDLLDQLSVPIAIAIQSSILQ→720  
QAQNELAERQKVEVRLRSALAEKEVLLKEVHHRVKNNLQIVSGLLLLHSQTLKDPPELIRT→780

### All12239(1707 aa), annotated as two-component hybrid sensor and regulator

QSTIHYAVERMLLTRQLEKSRQQQQLMVAIALIIRQSLKLEDILSVTVAEVRQFLOADR→180  
LTIYQFQPDMSGIVVAESILPGWIATKGAQIEDKCFQDSAGHNHQLKKRAINDIYQAGLTN→240  
CYLELLEQFOVKATLIVPIMVTNKSGLLIAHOCAPHHWQSFELDLLEQLAVQIAIAIQ→300  
QASAYEQIQTELQERKRVEATLRESEQRFRQMADTAPVLIWMSGLDKLCYYFNKTWLDFT→360

### All12699(920aa), two-component sensor histidine kinase

MSPTAKPNSQVSLNQESVLRRTARIRQSLELEDIITATTAEVRALLGTDRVMIYKFHPD→60  
 GSGQVIAESIYENRPLSLGLNFPADDIPPOARELLVKSIVDVATGMIGOSPVHDL→120  
 ETGELISEDICYRPVDSCHVEYLTAMGVKSSVVAPIFCODELWGLLVSHHSENRTVSEDE→180  
 LEAMQMIVDQLAVAIAQSHLLTQARKKAQKEAIINRIITLLHSLPTIVLKPAAEAVGAF→240  
 AGVGGRLCLRNQAVESQHVLRSLAECLIPGNSCVQLYTCGQQPITPEQTIYPLIEQYRV→300  
 WQEHYTSHHDIWAIADIYQDSTLRSLQAVFQPTKIRGILIIIPLEYRQQLLGYLSIFRNEI→360  
 DTETLWAGRIDQDQRQMFPRVSNLWRDAKKSQAQEWTSSEEIELAKEIGQHFASAIQQYE→420  
 LYQQVQAFNENLEKQVQKRTLELRHTSEQQQAVFGVISKIR~~ESLDTNTIFQITTK~~EACOL→480  
 IKADRVSVYRFDNEWGGEFVGDFEATSPHWSNESKISINTVWNDTYLONTQGGRYRYNET→540  
 FAVDDIYKVGFTQCHVENLEQFOIYAFVLAPIFVGOKLWGLLATYOHSGPROWKPSEVNF→600  
 LTQIAAQGLIALQQAELLNQTQQQAQKLTQALHHLQQTQTQLIQTEKMSSLGQLVAGVAH→660

**All3691(2021 aa), annotated as serine/threonine kinase with two-component sensor domain**

YPQLLQPILQERQFKFNQETI~~AVPGTSSSTLSSTL~~GSTSI~~SDTLD~~FASILKAAQVISTS→1380  
 LELDELITNLTEIILENSGAKKSALLLPQEDIWQIKAMTLSNFQPNSSSESTQTILESQPL→1440  
 ETCEDIPKNI~~IYYVKN~~TQQT~~VVIDNL~~QTDI~~PGLIGEYMLQHQP~~QSVFCTPMMNQGLVGI→1500  
 LYLENRLTRGVFTSDRLEVIRLLSAQAAVSLEKARLYQESQTKAQQLKQLS~~KQQK~~ILFNV→1560  
 VNQMRQSLDLNAIFCVVTONIRRI~~LDVDRVGIYQFHLDVNYEYGEFVAEDVSPAFPSALA~~→1620  
 VKVQ~~DHCF~~GENYANLYKQGRICAITDVOSSEILD~~CH~~ROILAOFHVRASLVVPIMQEEELW→1680  
 GLLCIHQCDRPRQWEPELMOFAQOVGAQMGI~~ALKOTD~~LLIQTQKQATQLEHTLQHLQQTQ→1740

**Alr2279(1299 aa), annotated as two-component hybrid sensor and regulator**

QAEQDLQ~~QQYQHTLLLKQITEEIRQSLQWEKILKTTVTEVORILQVDRALIFQINSDGSG~~→480  
 KVVQEA~~VMPGWSVTLDQDIY~~~~DPCT~~KDGYLNMYRDGRITAIADVYQGGPK~~CY~~VEFLOQFO→540  
 VKANLVVPIRVRNLWGLLIVHOC~~DRPROWTELELDLLKHLADOMGIALTOSOLLAQETR~~→600  
 QAQLLALQNEELNVAKQVAEKANMAKSNFLATMSHEIRTPMNAIIGMSGLLLD~~TTLKPEQ~~→660

**Alr3120(1286 aa), annotated as two-component hybrid sensor and regulator**

RS~~SLSLQ~~TILD~~TVEQVRQLGCDRVNIWYFETEWESIVMAESTTSSTSL~~LGERIE~~ELCF~~→420  
 QQHSAEIYRQGRIRVVPDIYTIEMTD~~CH~~REFLIRLQTRAKILVPLLCGDELWGLLN~~VSES~~→480  
 QHPREWQTEEVELLQGLSVHLAIAIHQATTHQKLQEQ~~LIALQQRSDQLRGSEQRYITLSQ~~→540  
 NLEAKIEERTAE~~LQAREAQLKQTS~~DRLSISLKS~~GAI~~GCWDWDIVENNI~~FWDERMYELYGV~~→600  
 TKTSDSRLVYDTWANRLHPDDRANIEVLVQQAILGQAEYDTEFRVIHPDGSIHFIKAYGV→660  
 VVRDAEGNPQKMIGVHFDISDRKKSEEIIR~~QOVEREKLLREITQRI~~RQSLDLQ~~TIFDIAC~~→720  
 QEICQVIQADRVGIFK~~FYPESNFNDGEFVAESV~~VKEFPSVIAIRVH~~DHCF~~GENYSSLYAL→780  
 GRSYVDDIYHSDMTT~~CH~~TDILAQFHVRANVIMPLLCGAELWGLLCIHQCATTRHWQ~~QSE~~→840  
 IDFTQQLATQLAIAIQQASLVEQLQ~~QELAER~~HQAE~~LKLTQSNQQLAISNQELARATRLK~~D→900

**Alr3356(179 aa), annotated as similar to phytochrome**

MQIHSHAEFD~~PSSNKPTEQGLQKVLQRLVQTMQRDALVRQTTNQLRESLQVDRVVLYFY~~→60

WQWHGQVTFEALSSEEFSSILGSTGAD~~ECT~~NDEYAALYLAGRTKAIADIESEPITTC~~HRDF~~→120  
LRTLQVRANLVVPLVLPKGLWGLLVAHHCQTHDWKESDIELMQAGAKTLATSPYILE~~S~~

**Alr5272(751 aa), annotated as two-component sensor histidine kinase**

STWWS~~T~~TLPLRDTNSKIYRLIGTSSNITAVKQTAQAREIQAEQAQILGAIQRIQESL~~D~~→180  
LETILYQTAKDLRQCLKCDRLIYQIKSDNNGAIVAESTILPNVSLGKHFR~~DPCFTGKY~~→240  
KERQGRCCLEI~~I~~EDIYAAGVKP~~C~~ORDFLASM~~O~~VRANIVVPIALKSDLWGLLIAQYCDEPH→300  
QWQOIEIDLKQLATQLGIAIQNTK~~L~~QQQLKQLQTKLATHKHEQQAQVKQSDKFQALVLD→360  
ITEKTRDYS~~D~~ETQVLATVTQELANLFQLESCYIELYNTDCTAVSVVCEYAAANQPTYQGLT→420  
RQIADFSEIYQPLLQKQHFQ~~T~~IEIVPGWHPQLLVITQLACPIFDAQGMGLGNLWLRPIQQ→480  
MLSQSEICLVQTLANLCAIAIRQAKLDATN~~Q~~ARLKK~~L~~EQRESLTNKFLRKL~~S~~QELRTPIT→540

**Slr1393(975aa), phytochrome-like protein, two-component sensor histidine kinase**

IAEERALTRVIEGIRQTL~~E~~LONIFRATSDEVRHLLSCDRVLVYRFNPDSGFEFIHESVA→480  
QMWEPKDLQNNFPLWQD~~T~~YLOENEGGRYRNHESLAVGDVETAGFTD~~CHLDNLR~~RFEIRA→540  
FLTVPV~~F~~VGEQLWGLL~~G~~AYONGAPRHWOAREIHLHQIANQLGVAVYQAOLLARFQ~~E~~QSK→600

**Tlr0911(1240 aa), annotated as two-component hybrid sensor and regulator**

NQWFAVRVFP~~T~~SRGLAIFCQ~~N~~ITLQVDSSTSLQRRQ~~T~~QAE~~L~~LHRLTIKIR~~R~~SLDLE~~T~~VLK→540  
TAL~~E~~EIRQLLNVDRTLIFQ~~F~~CADGTGEVVAESVAAPPFSLMHR~~T~~FH~~DPCFH~~HRESAEAYVQ→600  
GRVLAIA~~D~~INTATL~~A~~Q~~C~~HRDFLSQLOVRALLAVPIIQEERLWGLFLCHHCSSARPWANDE→660  
VELI~~R~~OLGEOLSFGI~~H~~RAELV~~S~~ALHQ~~E~~KERYRRVLEAQTELLYR~~C~~TPEGHLTFGNPAFFR→720

**B**

**His<sub>6</sub>-tag::All12699 GAF1::HO1::PebS**

MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTD~~DDDDKAMADIGT~~LELEDIIITATTA~~E~~VRALLGTDR  
VMIYKFHPDGSGQVIAESIHENRLP~~S~~LLGLNFPADDIPPQARELLVKS~~K~~VRSIVDVATGMIGQSPVHDLETG  
ELISEDICYRPVDSCHVEY~~L~~TAMGVKSSV~~V~~APIFCQDELWGLLVSHHSENRTVSEDELEAMQMI~~V~~DQLAVAI  
AQSHLLTQARKKAQKEAIGSGSGEFMSSNLANKLRVGT~~K~~KAHTMAENVGFVKCFLKGVAEKSSYRKL~~V~~AN  
FYYVYSAMEEEMEKHSQHP~~I~~VSKINFSQLNRKQ~~T~~LEQDLSYYYGANWREQIQLSPAGEAYVQRI~~R~~EISATEP  
ELLIAHSYTRYLGDLSGGQILKNIAVTAMN~~L~~NDGQGTAFYEFADISDEKAFKAKYRPTLDELAIDEATGDRI  
VDEANA~~A~~AFGMN~~M~~KMFQ~~E~~LEGNLIKAIGMMLFN~~T~~LTRKRTRGATELATAEYPMADIAMTKNPRNNKPKKILDS  
SYKSKTIWQNYIDALFETFPQLEISEVWAKWDGGNVTKDGGDAKLTANIR~~T~~GEHFLKAREAHIVDPNSDIYN  
TILYPKTGADLPCFGMDLMKFS~~D~~KKVIVFDFQHPREKYLFSVDGLPEDDGKYRFFEMGNHFSKNI~~F~~VRYCK  
PDEV~~D~~QYLDTFKLYLTKYKEMIDNNKPVGEDTTVYSDFDTY~~M~~TELDPV~~R~~GYMKNKFGEGRSEAFVND~~F~~LSY  
K

**His<sub>6</sub>-tag::All1280 GAF2::HO1::PebS**

MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTD~~DDDDKAMADIGT~~QSLNLQNILNATVKEIKDLLDV  
DRVVVYQFAPDMSGKIVAESVKPGWKIALGADIQDNC~~F~~QSGAGADYRQGHKRAIANIYTAELTDCHLRLLEQ

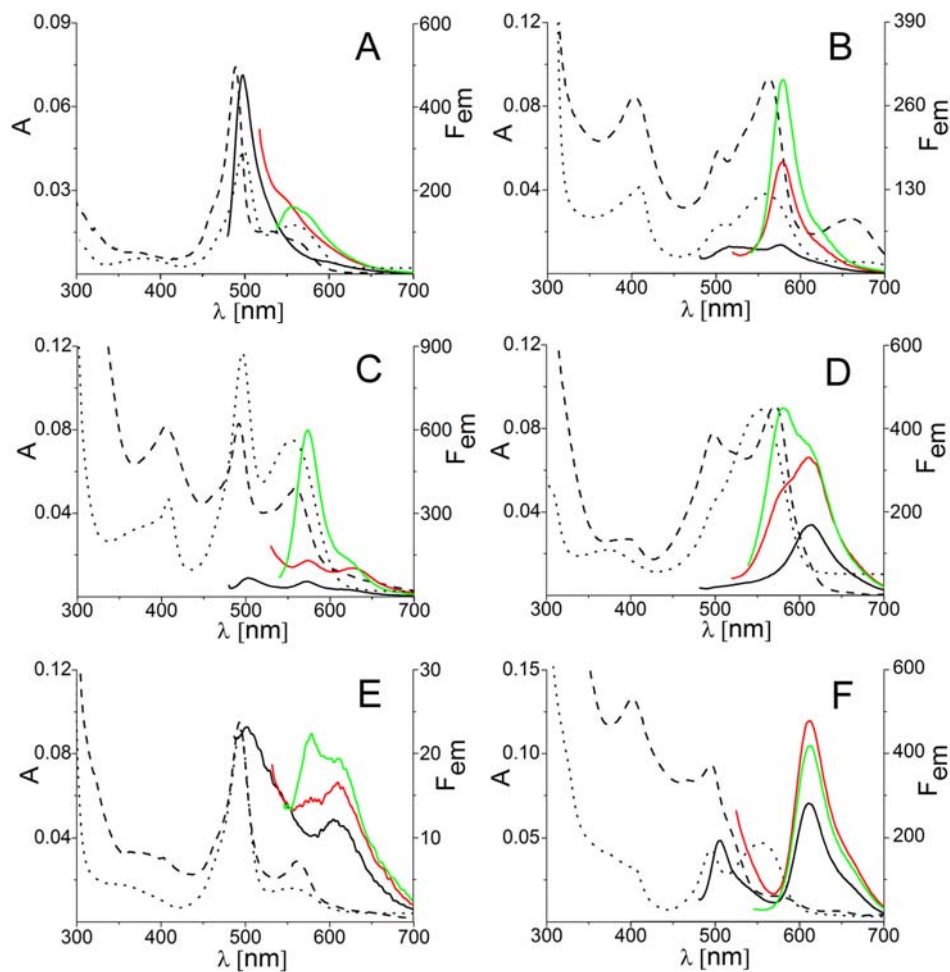


FQVKANLVVPIILLEVSEGNTPVQLWGLLIAHQ CSTPRDWEAHELDLLDQLSVPIAIAIQSSILQQAQNELA  
ERQKVEVRLRSALAGSGGSGEFMSSNLANKLRVGTCKKAHTMAENVGFVKCFLKGVAEKSSYRKLVANFYVYVY  
 SAMEEEMEKHSQHPIVSKINFSQLNRKQTLEQDLSYYYGANWREQIQLSPAGEAYVQRIREISATEPELLIA  
 HSYTRYLGDLSGGQILKNIAVTAMNLDGQGTAFYEFADISDEKAFKAKYRPTLDELAIDEATGDRIVDEAN  
 AAFGMNMKMFQELEGNLIKAI GMMLFNTLTRKRTRGATELATAE YPMADIAMTKNPRNNKPKKILDSSYKSK  
 TIWQNYIDALFETFPQLEISEVWAKWDGGNVTKDGGDAKLTANIRTGEHFLKAREAHIVDPNSDIYNTILYP  
 KTGADLPCFGMDLMKFSKDKKVIIVFDFQHPREKYLFSVDGLPEDDGKYRFFEMGNHFSKNIFVRYCKPDEVD  
 QYLDTFKLYLT KYKEMIDNNKPVGEDTTVYSDFD TYMTELPVRYGMKNKFGGRSEAFVNDFLFSYK

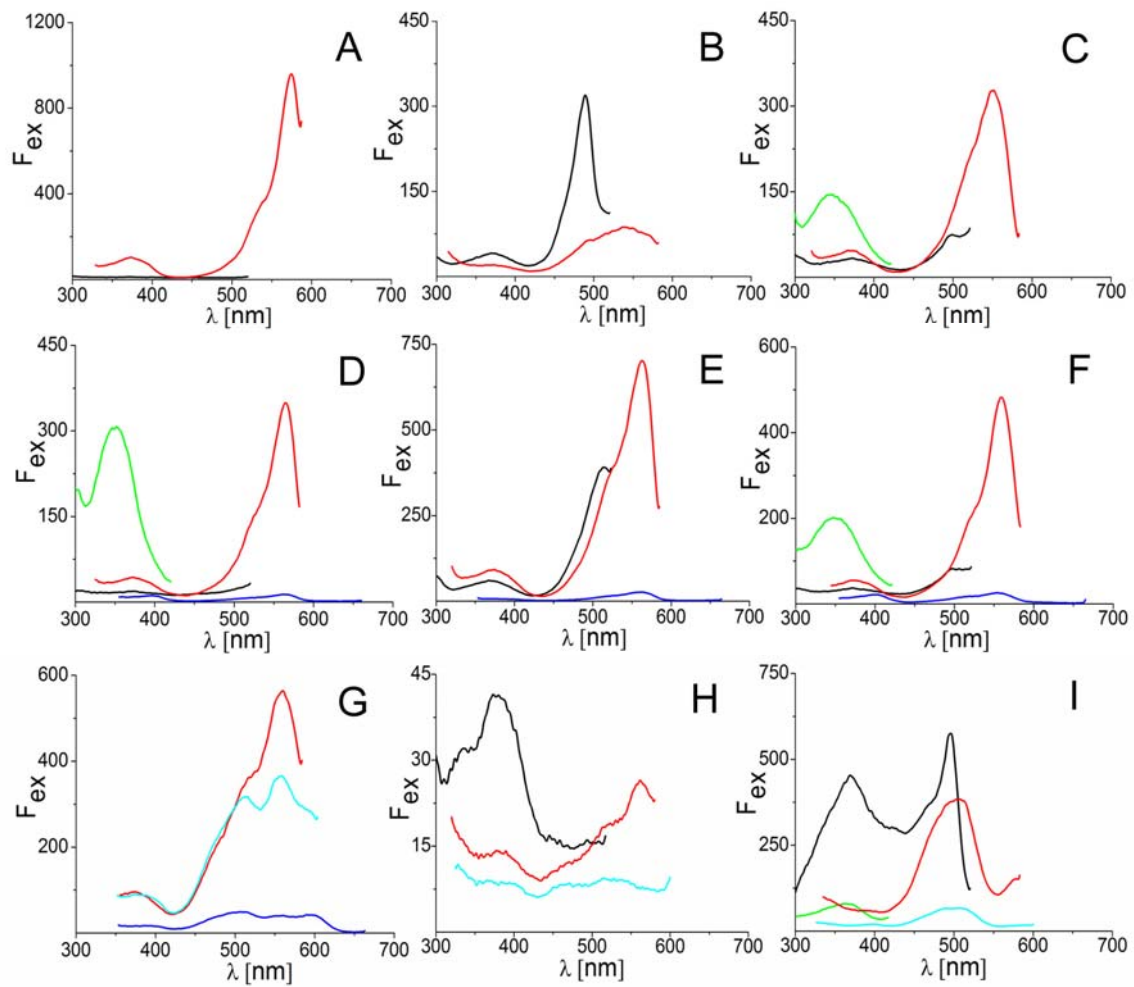
**His<sub>6</sub>-tag::Slr1393 GAF3::HO1::PebS**

MGSSHHHHHSSGLVPRGSHMLQNI FRATSDEVRLHLLSCDRVLVYRFNPDWSGEFIHESVAQMWEPLKDLQN  
 NFPLWQD TYLQENEGGRYRNHESLAVGDVETAGFTDCHLDNLRRFEIRAFLTVPVFVGEQLWGLLGAYQNGA  
 PRHWQAREIHLLHQIANQLGVAVYQAQLLARF EQSKTIDLGGGRSRTSGSPGVMSSNLANKLRVGTCKKAHT  
 MAENVGFVKCFLKGVAEKSSYRKLVANFYVYSAMEEEMEKHSQHPIVSKINFSQLNRKQTLEQDLSYYYGA  
 NWREQIQLSPAGEAYVQRIREISATEPELLIAHSYTRYLGDLSGGQILKNIAVTAMNLDGQGTAFYEFADI  
 SDEKAFKAKYRPTLDELAIDEATGDRIVDEANAAFGMNMKMFQELEGNLIKAI GMMLFNTLTRKRTRGATEL  
 ATA E YPMADIAMTKNPRNNKPKKILDSSYKSKTIWQNYIDALFETFPQLEISEVWAKWDGGNVTKDGGDAKL  
 TANIRTGEHFLKAREAHIVDPNSDIYNTILYPKTGADLPCFGMDLMKFSKDKKVIIVFDFQHPREKYLFSVDG  
 LPEDDGKYRFFEMGNHFSKNIFVRYCKPDEVDQYLDTFKLYLT KYKEMIDNNKPVGEDTTVYSDFD TYMTELPVRYGMKNKFGGRSEAFVNDFLFSYK

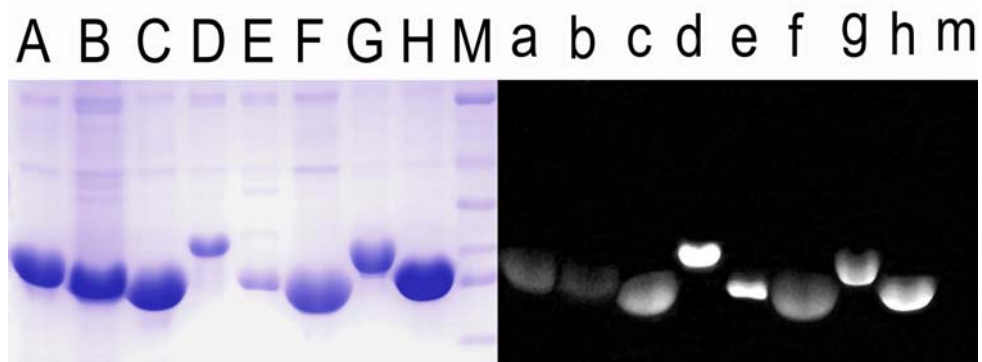
**Fig. S1: A: Amino acid sequences containing GAF-domains.** The cloned and expressed segments are boxed. The heavily underlined segments could be PEB-chromophorylated, the lightly underlined ones could not. DXCF and like motifs were marked in red, and CH (chromophore binding position) and like motifs in magenta. **B: Amino acid sequences of the fusion proteins.** Black, blue and green characters denote the segments of the original genes, designed linkage and His<sub>6</sub>-tags, respectively.



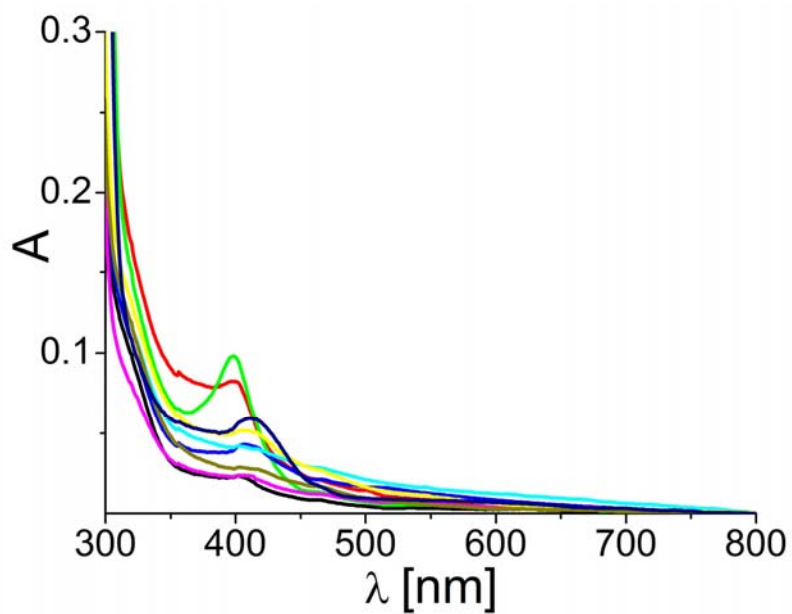
**Fig. S2: Absorption (- - -) and fluorescence emission-spectra (—) of chromophorylated AII3691 GAF2 (A), AII2699 GAF3 (B), Tlr0911 (C), AII3356 (D), AII2279 (E) and AII5272 GAF1 (F).** Samples were reconstituted in *E. coli* (Table S2), purified with Ni<sup>2+</sup> affinity chromatography and then kept in KPB (20 mM, 0.5 M NaCl, pH 7.0). Emission spectra were obtained by excitation at 460 nm (black), 500 nm (red) and 520 nm (green). Denatured absorption spectra (. . .) were done with 8 M urea (pH 2.0) to show the prosthetic chromophore. Spectra of PEB-AII2699 GAF1, PEB-AII1280 GAF2 and PEB-Slr1393 GAF3 are shown in Fig. 1



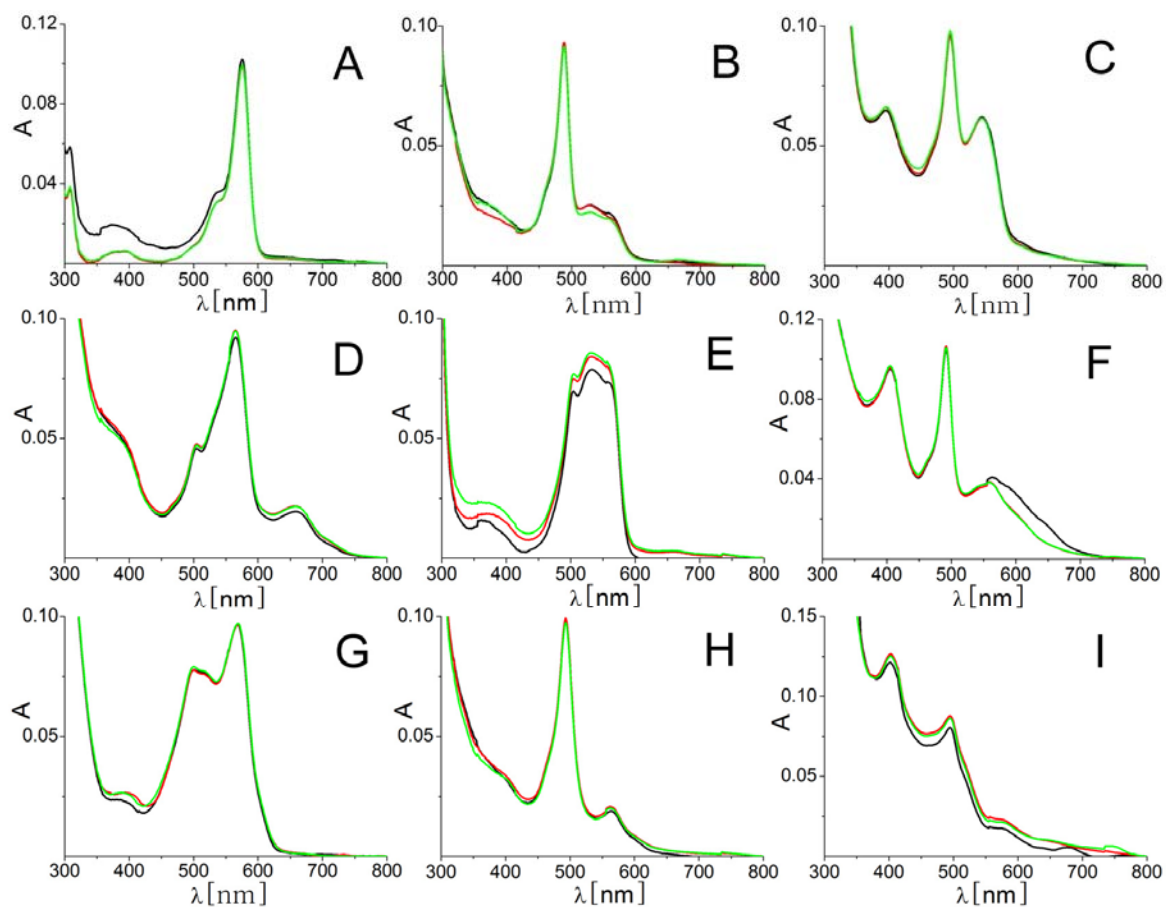
**Fig. S3: Fluorescence excitation-spectra of chromophorylated AII2699 GAF1 (A), AII3691 GAF2 (B), AII1280 GAF2 (C), AII2699 GAF3 (D), Slr1393 GAF3 (E), Tlr0911 (F), Alr3356 (G), Alr2279 (H), and Alr5272 GAF1 (I).** Samples were reconstituted in *E. coli* (Table S2), purified with Ni<sup>2+</sup> affinity chromatography and then kept in KPB (20 mM, 0.5 M NaCl, pH 7.0). Excitation spectra were obtained by monitoring fluorescence at 540 nm (black), 600 nm (red), 440 nm (green), 620 nm (cyan) and 680 nm (blue).



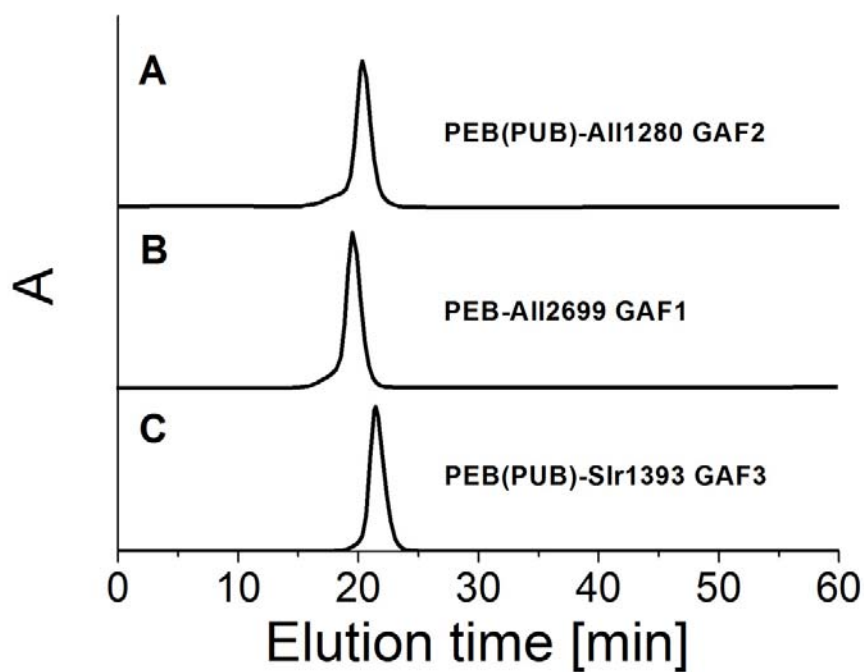
**Fig. S4: SDS-PAGE of PEB-chromophorylated GAFs stained by Coomassie Blue (left panel) and  $Zn^{2+}$  induced fluorescence (right panel). A,a: Alr2279 ; B,b: All2699 GAF3; C,c: Tir0911; D,d: Alr3356; E,e: All3691 GAF2; F,f: All1280 GAF2; G,g: All2699 GAF1; H,h: Slr1393 GAF3; M,m: molecular marker (66, 45, 36, 29, 24, 20 kDa from top to bottom).**



**Fig. S5: Absorptions of nonchromophorylated GAFs.** All3691 GAF1 (black), All0729 GAF1 (red), All0729 GAF2 (green), All0729 GAF3 (blue), Alr3120 GAF1 (cyan), Alr3120 GAF2 (magenta), All1280 GAF1 (yellow), All2239 (dark yellow) and Alr5272 GAF2 (navy); obviously they could not be PEB(PUB)-chromophorylated.



**Fig. S6: Absorption of chromophorylated AII2699 GAF1 (A), AII3691 GAF2 (B), AII1280 GAF2 (C), AII2699 GAF3 (D), Sir1393 GAF3 (E), Tir0911 (F), Alr3356 (G), Alr2279 (H), and Alr5272 GAF1 (I).** The biliproteins showed the same absorption spectra after purification (black) and subsequent irradiation with 500 (red) and 570 (green) nm light; thus they are not photochromic



**Fig. S7: Oligomerization of PEB chromophorylated GAFs.** A: PEB(PUB)-AII1280; B: PEB-AII2699; and C: PEB(PUB)-Sir1393 GAF3. The samples were analyzed with gel filtration on a Superdex 75 gel filtration column, developed with KPB buffer. Their peaks eluting at 20.3, 19.7 and 21.4 min correspond to mw of 44.2, 49.2 and 36.8 kDa, corresponding to a dimer (calculated 47.4, 53.1 and 49.6 kDa), respectively.

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