Minimal domain of bacterial phytochrome required for chromophore binding and fluorescence

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Supplementary Materials

Supplementary Figures

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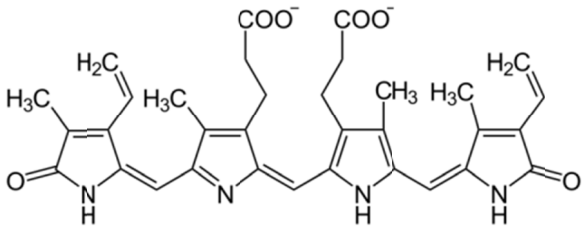
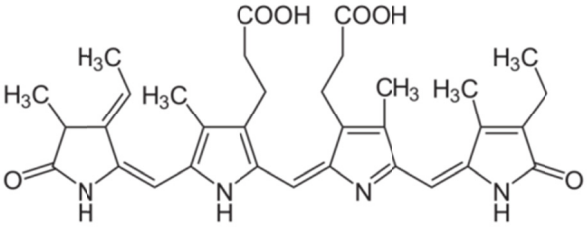
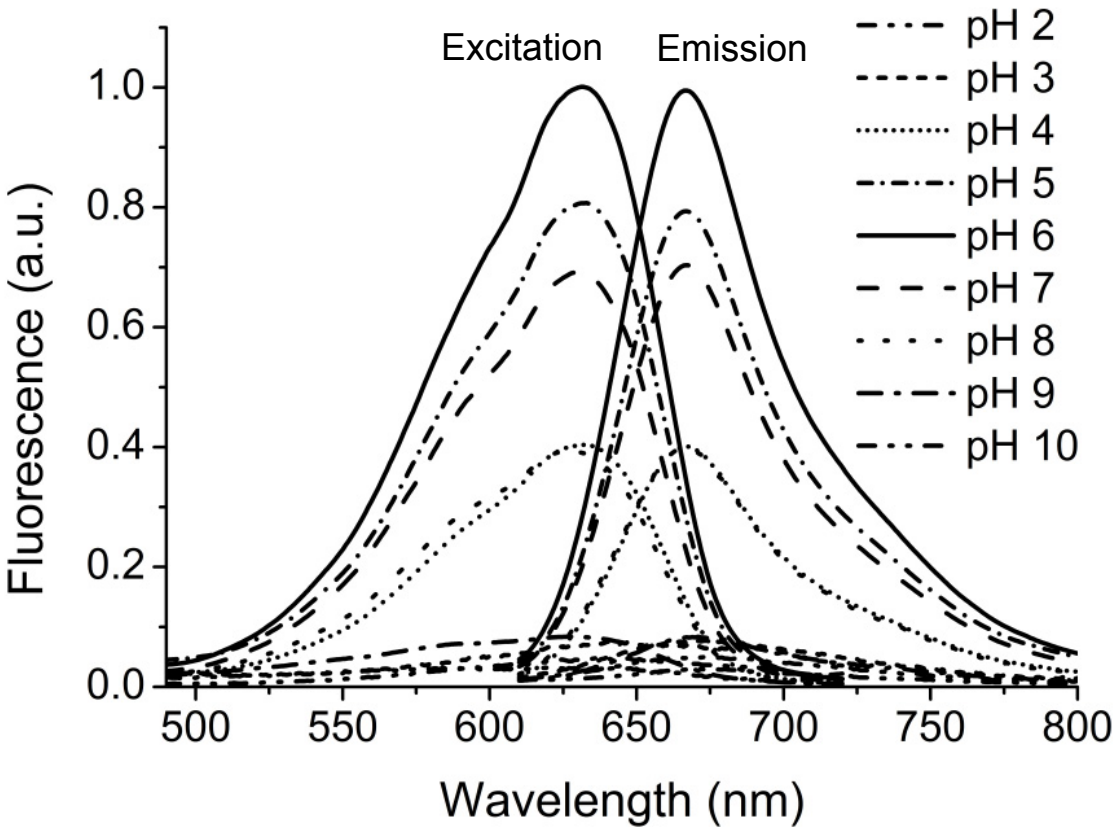
Biliverdin IXα



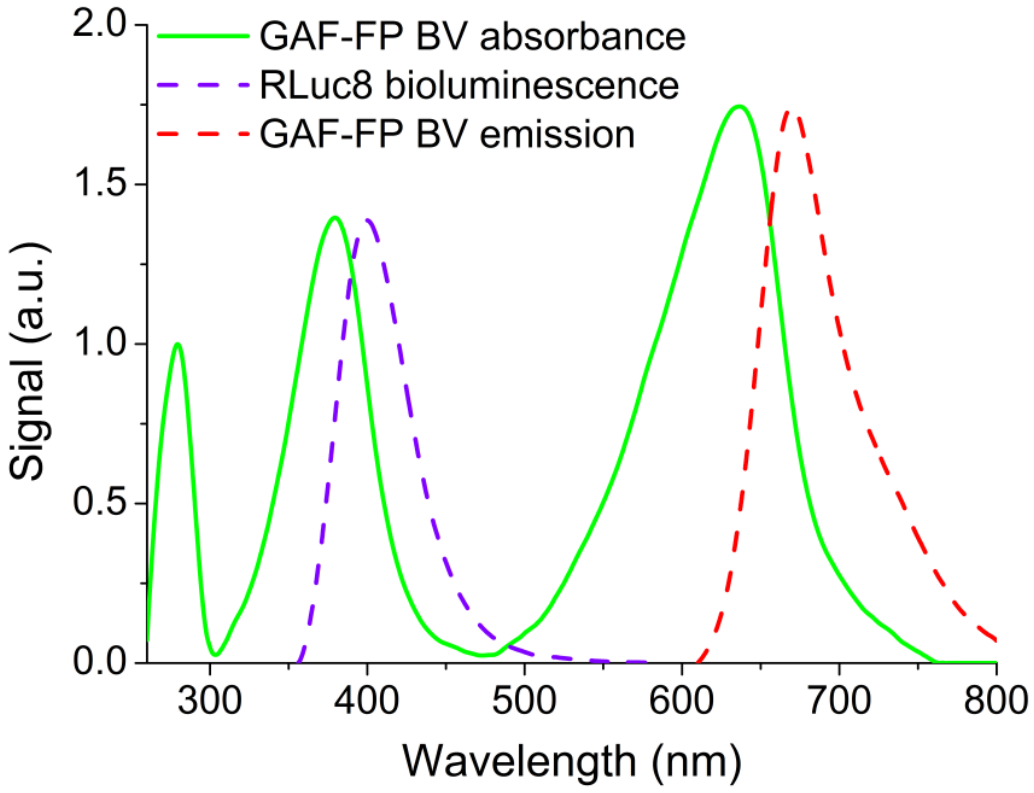
Phycocyanobilin



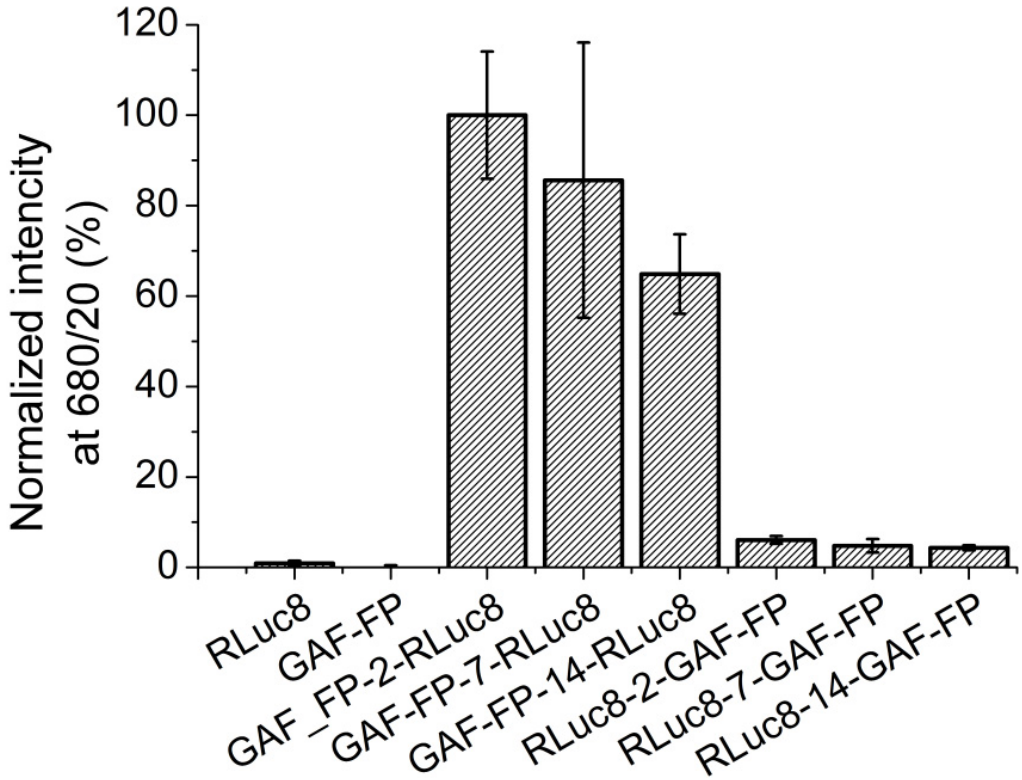
Supplementary Figure 1. The structural formula of GAF-FP chromophores. Biliverdin IXα (BV) and phycocyanobilin (PCB) are linear tetrapyrroles enzymatically derived from a heme.



Supplementary Figure 2. The pH dependence of the normalized fluorescence excitation and emission spectra of GAF-FP bound to BV.



Supplementary Figure 3. Bioluminescence resonance energy transfer between *R*Luc8 and GAF-FP. Energy of the substrate oxidized by *R*Luc8 migrates via BRET to Soret band of GAF-FP. As a result, GAF-FP emits photons in its normal emission spectrum, leading to the appearance of NIR bioluminescence.



Supplementary Figure 4. Screening for optimal chimeric fusion construct between *R*Luc8 and GAF-FP. All the proteins were compared for near-infrared signal intensity when expressed in bacteria. The sample with the highest signal was taken as 100%, while the lowest as 0%. Error bars, s.d. (n = 3).

Supplementary Table 1. Spectral and biochemical properties of the GAF-FP insertion variants.

Insertion variant	Excitation maximum (nm)	Emission maximum (nm)	Extinction coefficient (M^{-1} cm^{-1})	Quantum yield (%)	Molecular brightness relative to GAF-FP BV (%)	Maturation $t_{50\%}$ at 37°C (h)	p*K*a$_1$	p*K*a$_2$	Brightness in *E. coli* at 37°C relative to GAF-FP BV (%)
GAF-FP i1 BV	635	670	45,650	5.7	72	4.1	4.8	8.1	58
GAF-FP i2 BV	635	670	52,300	6.5	94	3.7	4.7	8.1	111
GAF-FP i3 BV	635	670	49.600	6.2	85	5.4	4.7	8.0	56
GAF-FP i4 BV	635	670	47.400	5.5	72	5.1	4.6	7.8	60
GAF-FP i1 PCB	625	657	66,500	12.4	227	3.0	4.4	9.1	111
GAF-FP i2 PCB	625	657	83,000	12.7	290	3.3	4.6	8.6	182
GAF-FP i3 PCB	625	657	81,500	12.8	287	4.4	4.7	8.2	55
GAF-FP i4 PCB	625	657	72,500	11.4	227	3.7	4.8	8.4	158