

Supplementary information.

A Novel Near-Infrared Fluorescent Protein for Biliverdin Biosensing with a Hydrogel Scaffold

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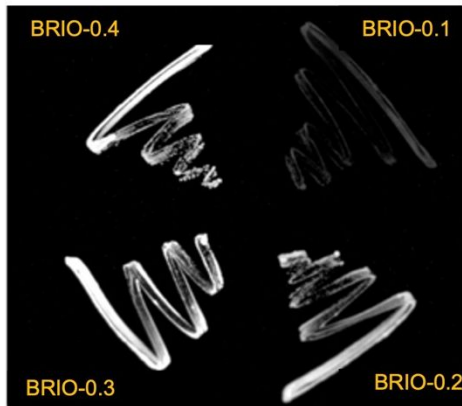
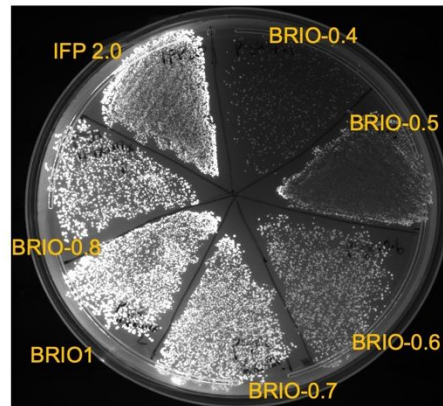
a**b**

Fig. S1. Fluorescence images of *E. coli* on a 100 mm LB agar plate expressing BRIO-0.1-0.4 (a) and BRIO0.4-1 with IFP2.0 (b).

PcyA_WT	MAVTDLSLTNSSLMPTLNPMIQQLALAI AASWQSLPLKPYQLPEDLG YVEGRLEGEK	57
BRIO-0.4	MAVTDLSLTNSSLMPTLNPMIQQLALAI AASWQSLPLKPYQLPEDLG YVEGRLEGEK	57
BRIO1	MAVTDLSLTNSSLMPTLNPMIQQLALAI AASWQSLPLKPYQLPEDLG YVEGRLEGEK	57
PcyA_WT	LV IENRCYQTPQFRKMHELEAKVGKGLD I LHCVMFPEPELYGLPLFGCD I VAGPGGVS	114
BRIO-0.4	LV IENRCYQTPQFRKMHELEAKVGKGLD I LHCVMFPEPELYGLPLFGCG I VAGPGGVS	114
BRIO1	LV IENRCYQTPQFRKMHELEAKVGKGLD V LHCVMFPEPELYGLPLFGCG I VAGPGGVS	114
PcyA_WT	AA IADLSPTQSDRQLPAAYQKSLAELGQ PEFEQQRELPPWGE I FSEYCLFIRPSNVT	171
BRIO-0.4	AA IADLSPTQSDRQLPAAYQKSLAELGR PEFEQQRELPPWGE I FSEYCLFIRPSNVT	171
BRIO1	AA IADLSPTQSDRQLPAAYQKSLAELGR PEFEQQKELPPWGE I FSEYCLFIRPSNVT	171
PcyA_WT	EEERFVQRVVD F LQIHCHQS IVAEPLSEAQTLEH RQGQ IHYCQQQQKND KTRRVLEK	228
BRIO-0.4	EEERFVQRVVD LLQIHCHQS IVAEPLSEAQTLEH HQGQ IHYCQQQQKND SPRRVLEK	228
BRIO1	EEERFVQRVVD LLQIHCH LS IVAEPLSEAQTLEH HQGQ IHYCQQQQKND ACRRVLEK	228
PcyA_WT	AFGEAWAERYMSQVLF DVIQ	248
BRIO-0.4	AFGEAWAERYMSQVLY DVIQ	248
BRIO1	AFGEAWAERYMSQVLY DVIQ	248

Fig. S2. Sequence alignment of wild type *PcyA*, *BRIO-0.4* and *BRIO1*.

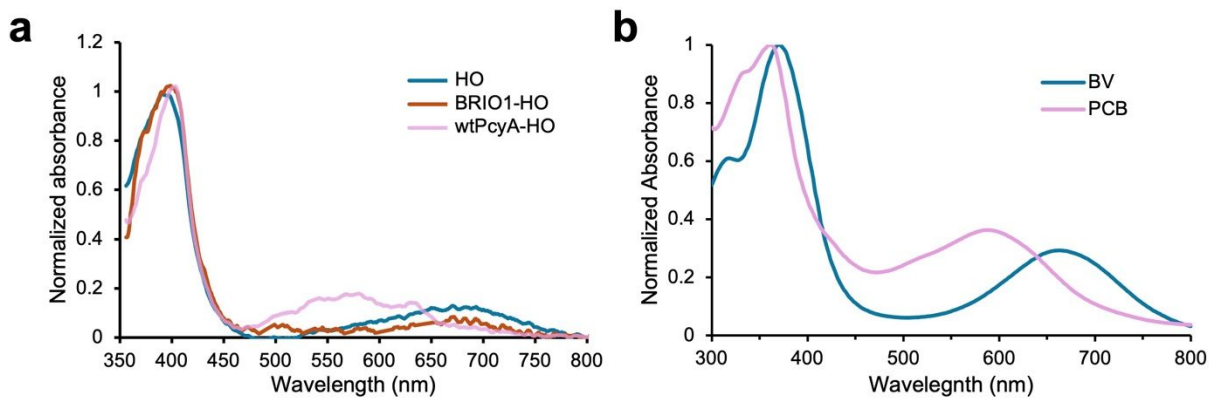


Fig. S3. (a) Absorption spectra of the LB medium after overnight culture of *E. coli* expressing wtPcyA-HO, BRIO1-HO, or HO. (b) Absorption spectra of 100 μ M pure BV or PCB in PBS. Each spectrum was normalized to its own maximum.

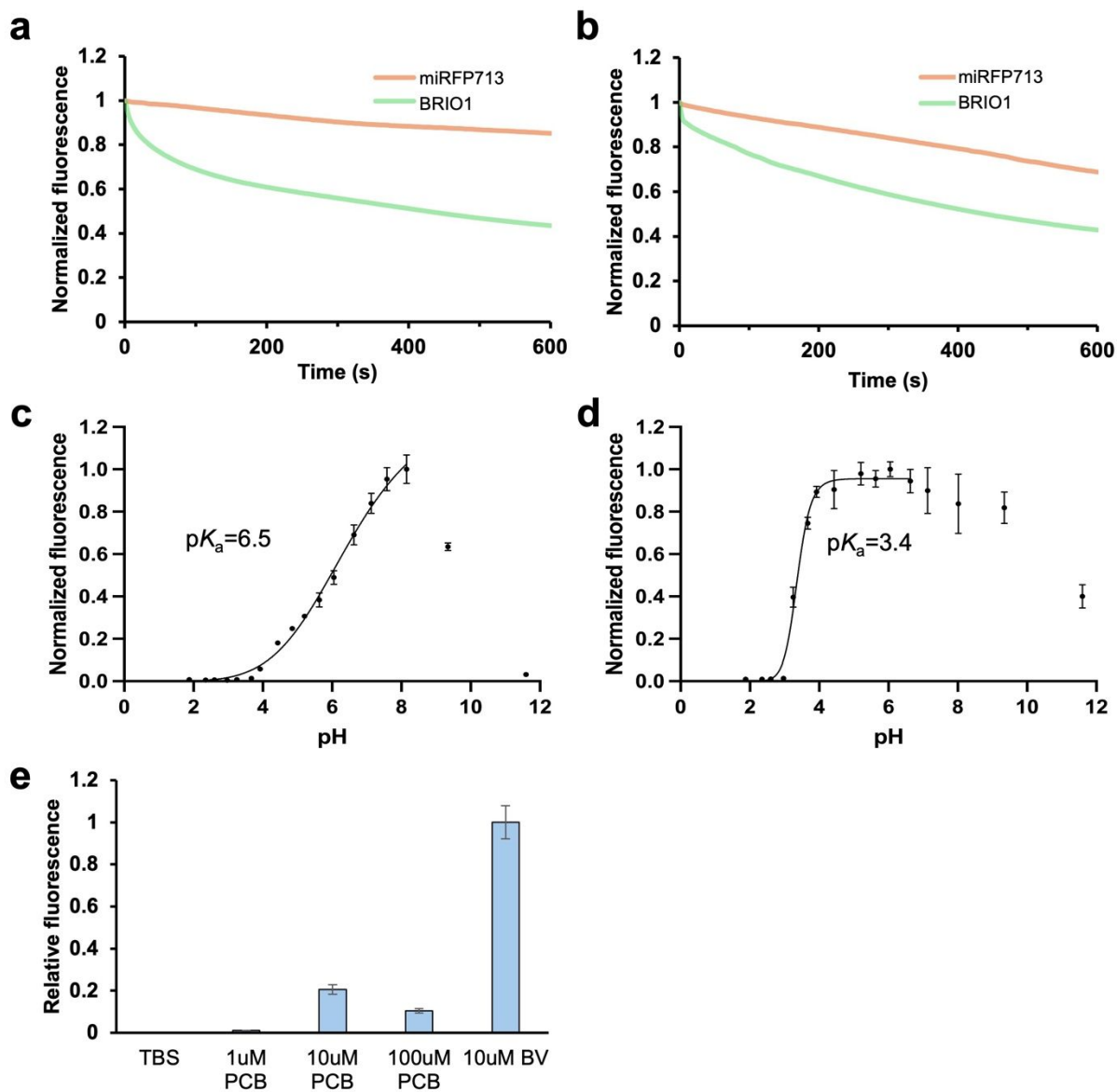


Fig. S4. Additional characterization of BRIO1 and miRFP713. (a-b). Photobleaching curves of BRIO1 and miRFP713 in *E. coli* (a) and as purified proteins in PBS (b). (c-d) pH titration of purified BRIO1 (c) and miRFP713 (d). $N=4$. (e) Specificity of purified BRIO1 for PCB. $N=4$. All error bars represent standard deviation.

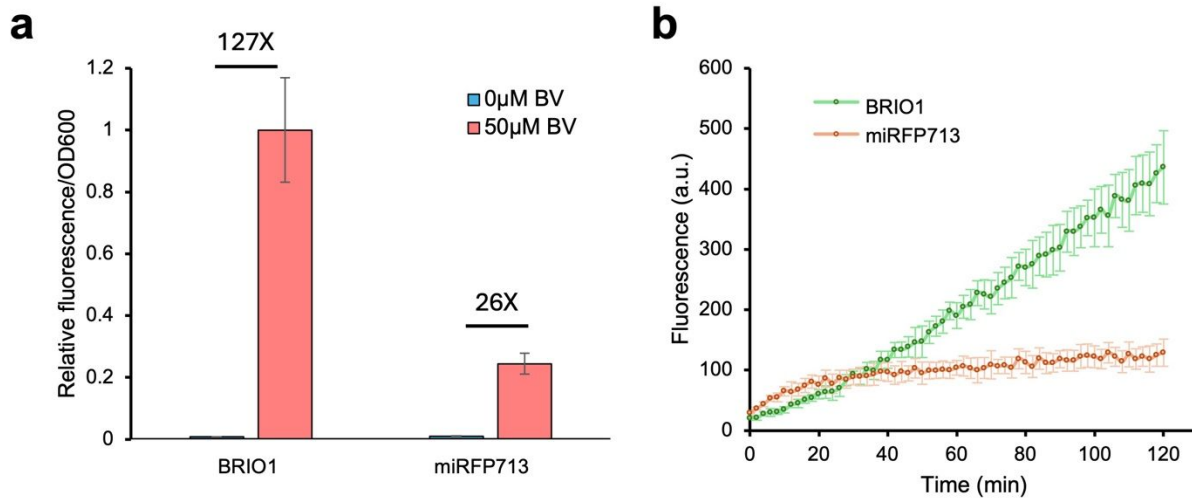


Fig. S5. Comparing BRIO1 and apo-miRFP713 as whole cell sensors for BV. (a) End-point fluorescence response of whole-cell sensors to 50 μM BV, normalized to the same OD600. Three culture tubes were used for each sensor for comparison. End-point signals at 2 h after mixing cells with buffer were used for calculation. (b) Kinetics of signals from whole-cell sensors after mixing with 50 μM BV buffer. All error bars represent standard deviation ($N = 3$).

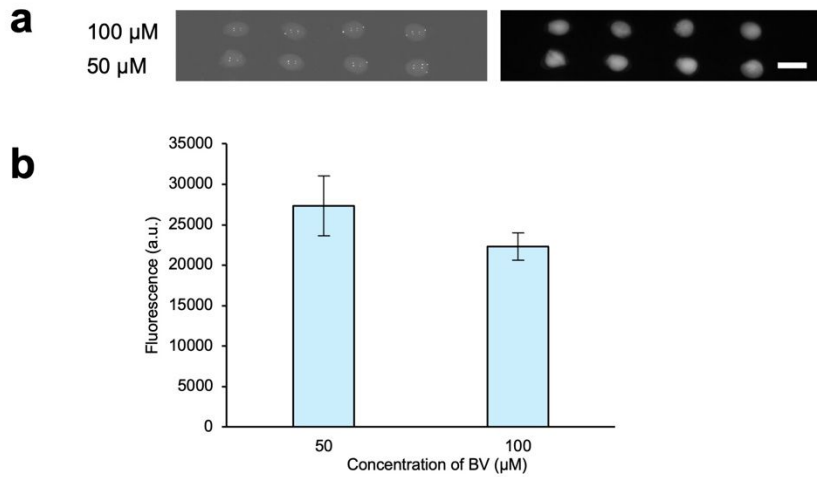


Fig. S6. Assessing the upper limit of alginate encapsulated BRIO1 sensor in standard PBS solutions. (a) Bright field (left) and fluorescence (right) image of sensors incubated with 50 (lower row) or 100 μM (upper row) BV. Scale bar: 5 mm. (b) Average fluorescence of the sensors in (a). Error bars represent standard deviation ($N = 4$).

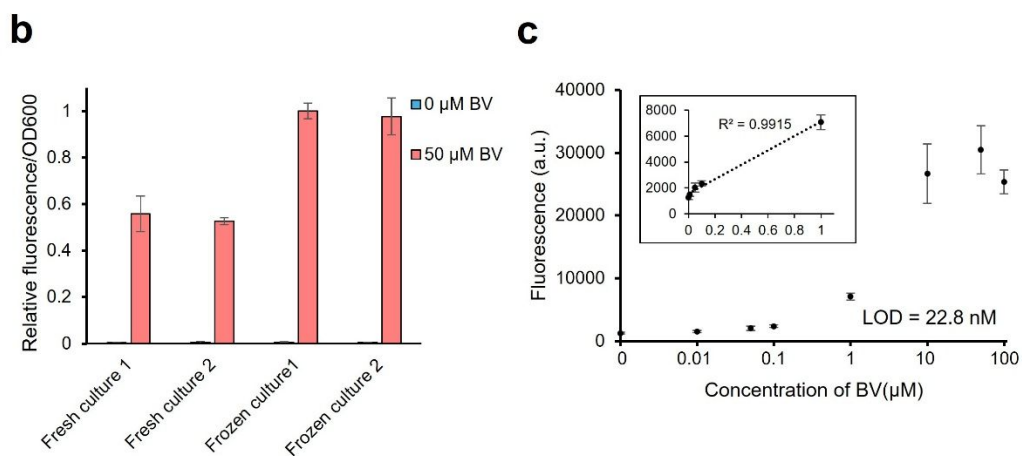
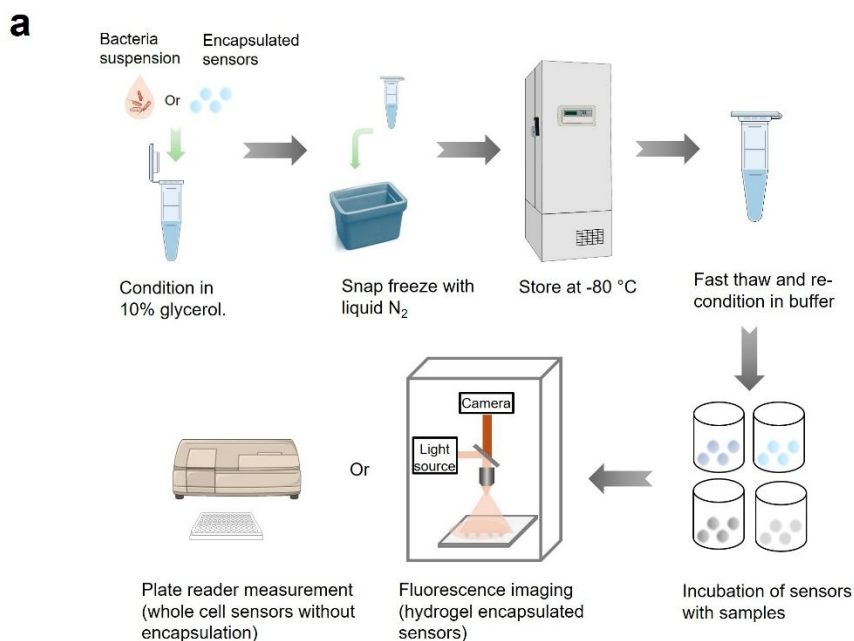


Fig. S7. Freezing whole-cell or hydrogel-encapsulated sensors for storage and use. (a) Workflow of freezing sensors and subsequent use for detection after thawing. (b) Comparison of frozen whole-cell sensors with fresh cultures in standard BV solutions ($N = 3$). (c) Use of frozen alginate-encapsulated sensors for detecting BV in FBS samples ($N = 4$ or 5). Inset: linear range up to $1 \mu\text{M}$ with fitted curve. Error bars represent standard deviation.

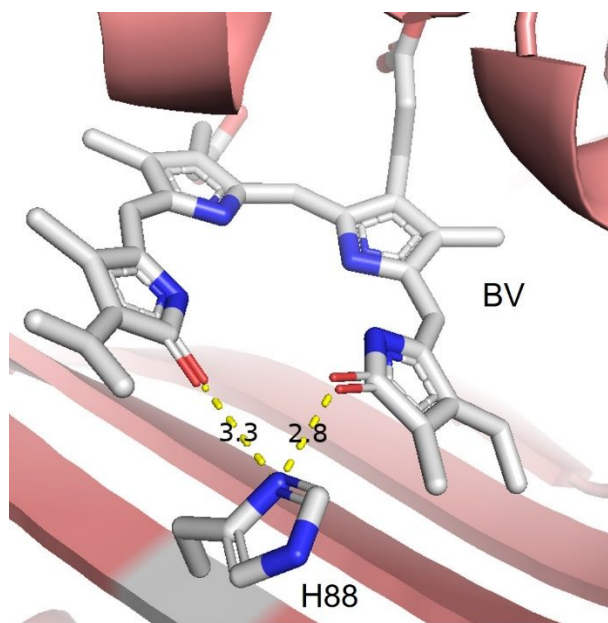


Fig. S8. Zoomed-in image of the PcyA-BV complex structure illustrating the interaction between H88 and BV. Yellow dashed lines represent hydrogen bonds, with distances indicated in Å.