Supplementary Information

Programming Microbe-Computer Interaction for Customized Life-Nonlife and Cross-Life Communication

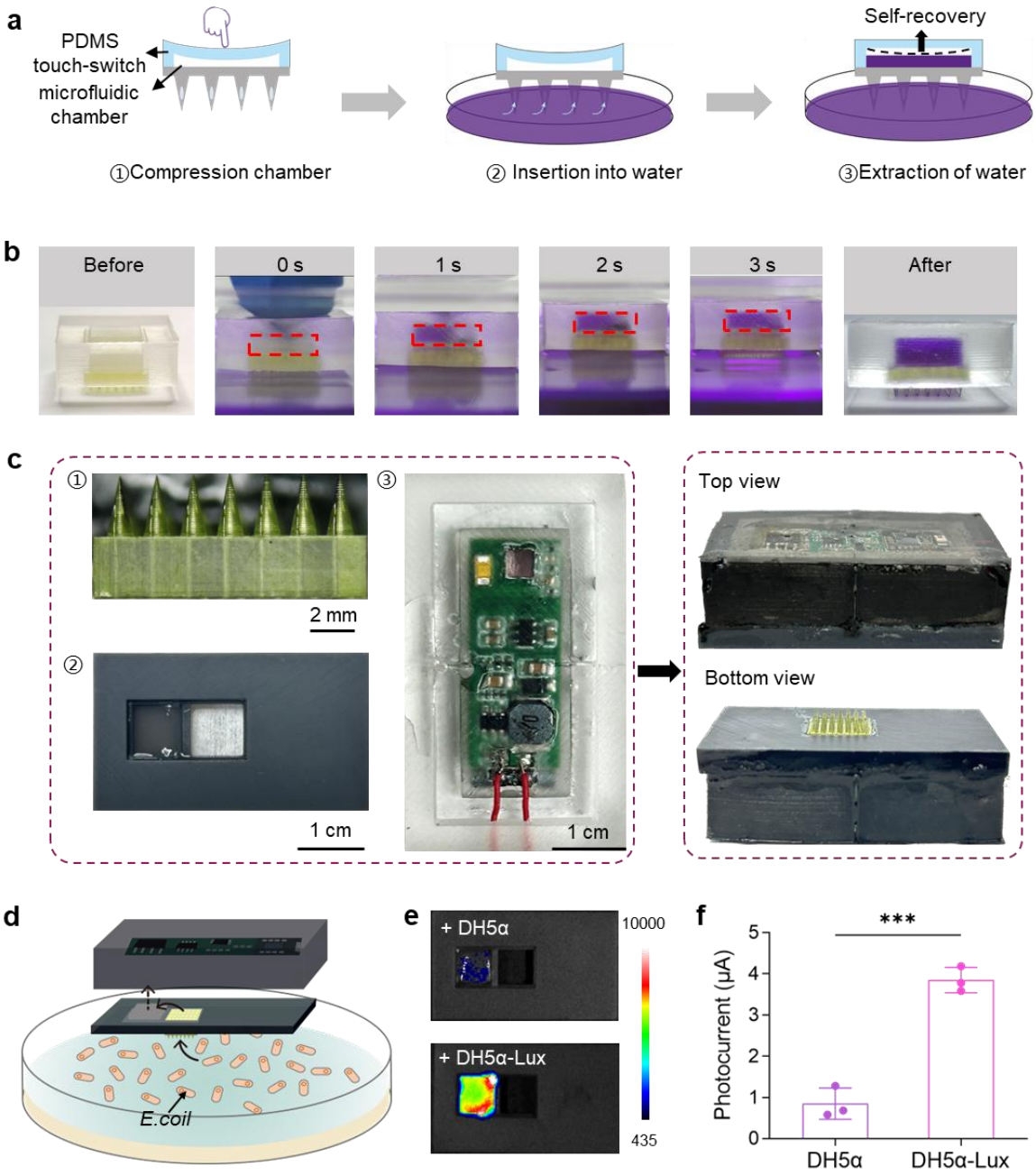

Figure S1. Expansion of MCU functions for detecting the sampled bioluminescent bacteria. **a**, Diagram of the sample uptake process using a touch-switch microfluidic chamber. **b**, Photographs of the chamber in **a** and the sample uptake process. **c**, Illustration of assembling MCU unit consisting of the microneedle, light-proof enclosure, and integrated electronic circuit board. **d**, Illustration of sampling from the liquid containing *E. coli* strains through the microneedle interface of the MCU unit. **e**, Imaging of bioluminescence after respective uptake of different strains using the setup in **d**. **f**, Photocurrent detection results of the experiment in **d**. All the

measurements were taken from three distinct biological replicates. Significance was determined by unpaired two-tailed Student's t-test for **f**. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.

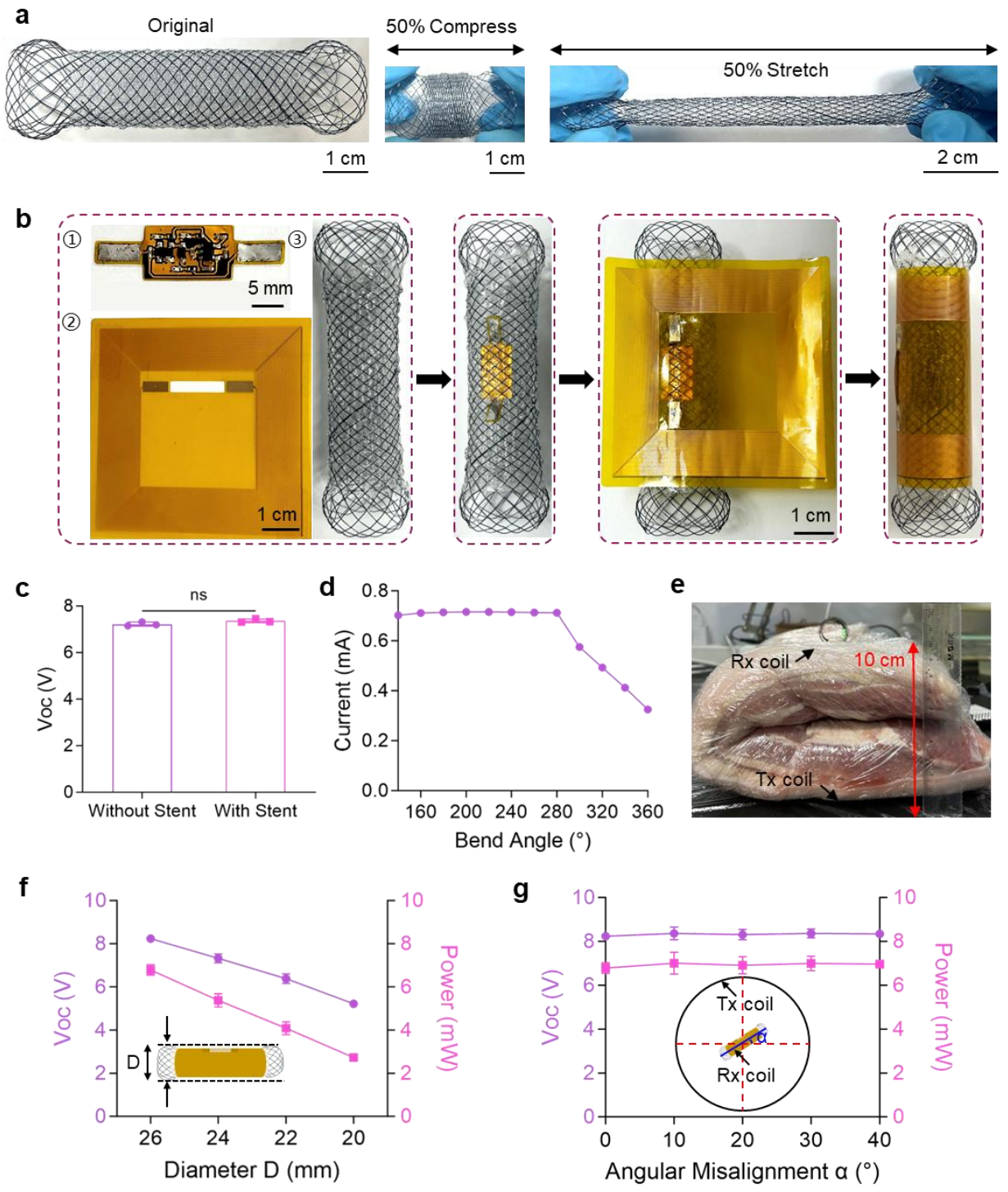

Figure S2. Assembly of the stent-form CMU actuator and electrical test. a, Feasibility test of 50% compression or 50% stretching of a stent encapsulated with biocompatible PVA material. **b**, Photograph of the fully assembled stent-configured MCU, consisting of the PVA layer (pre-loaded with optogenetic engineered bacteria as needed), stent structure, and electronic modules. **c**,

Measurement of the open-circuit voltage of the receiving coil under different stent configurations. **d**, Effect of bending angle of the receiving coil on the operating current of the electronic circuit. **e**, Diagram of the experimental setup for wirelessly charging the MCU stent through a 10 cm-thick porcine tissue section using an external transmitter (Tx) coil and evaluating circuit performance. **f**, Test of the variations in open-circuit voltage and output power of the electronic circuit under stent compression. **g**, Test of the variations in open-circuit voltage and output power of the electronic circuit as the stent is rotated at different angles relative to the transmitter coil. Significance was determined by unpaired two-tailed Student's t-test for **c**.

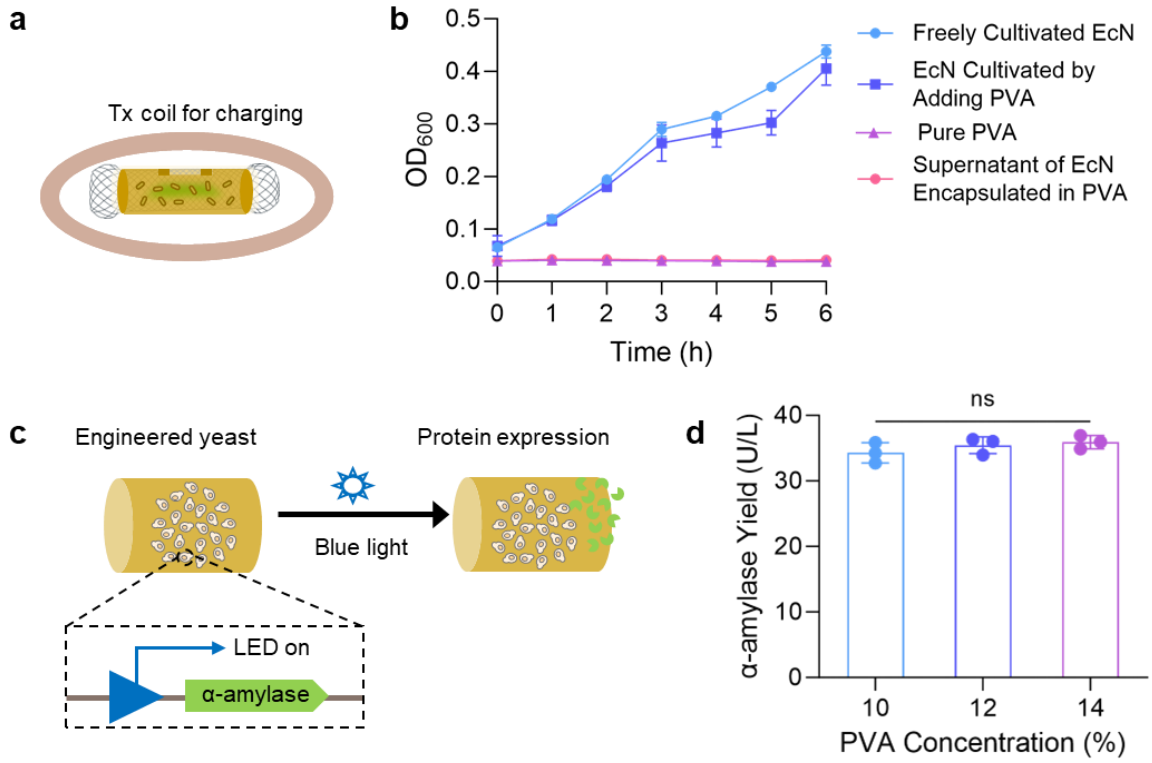

Figure S3. Test of opto-activation of microbial protein expression using stent-form CMU actuator. a, Diagram of wireless charging and activation of the stent-formed MCU actuator via a Tx coil. **b**, Test of the effect of PVA material on the growth performance of engineered EcN bacteria in culture medium, along with the assessment of potential bacterial leakage from PVA-encapsulated EcN. **c**, Diagram of the activation of α-amylase expression in PVA-encapsulated engineered yeast upon LED switching. **d**, Effect of varying PVA concentrations on the expression and secretion of α-amylase in encapsulated yeast. All the measurements were taken from three distinct biological replicates. Significance was determined by one-way ANOVA with Dunnett's post-test for **d**.

Table S1. The engineered microbial strains in this study.

Name	Key characteristics
EcN	*E. coli* Nissle 1917 ΔpMUT1 ΔpMUT2 (cryptic plasmids)
B. subtilis 168	ATCC 23857, Ind^- Tyr^+
S. cerevisiae BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
EcN-CSG-Lux	EcN Δ*csgA*, Δ*csgD*::pJ23101-*csgEFG*, pJ23100-*csgBC*, Δ*lacZ*::pJ23100-*LuxBCDE*
DH5α-Lux	DH5α harboring pCons-LuxABCDE
B. subtilis-Lux	*B. subtilis* 168 integrated with Ppen-*luxABCDE* cistron at *wprA* site
S. cerevisiae BY4741-Lux	*S. cerevisiae* BY4741 transformed with pRS425 plasmid inserted by P_{TEF1}-nanoluc-T_{GPM1}
DH5α-Ara	DH5α transformed with pAra-LuxABCDE
EcN-Nitrate	EcN-CSG-Lux transformed with pNitrate-LuxA
EcN-Tet	EcN-CSG-Lux transformed with pTet-LuxA
EcN-Heme	EcN-CSG-Lux transformed with pHeme-LuxA
DH5α-Hypoxia	DH5α transformed with pHypoxia-LuxCDABE
EcN-Pb	EcN-CSG-Lux transformed with pPb-LuxA
EcN-As	EcN-CSG-Lux transformed with pAs-LuxA
EcN-Opto-GFP	EcN harboring p15A-ccaS-PCB and pGreen-sfGFP
EcN-Opto-Lux	EcN-CSG-Lux harboring p15A-ccaS-PCB and pGreen-LuxA
EcN-Opto-secGFP	EcN harboring pHlyBD, p15A-ccaS-PCB and pGreen-sfGFP-H60
EcN-Opto-secCherry	EcN harboring pHlyBD, p15A-ccaS-PCB and pGreen-mCherry-H60
EcN-Opto-secPD-L1nb	EcN harboring pHlyBD, p15A-ccaS-PCB and pGreen-Nb-PD-L1nb-H60
EcN-Opto-secMtnB	EcN harboring pHlyBD, p15A-ccaS-PCB and pGreen-Nb-MtnB-H60
EcN-Opto-secIL-10	EcN harboring pHlyBD, p15A-ccaS-PCB and pGreen-Nb-IL-10-H60

BY4741-Opto-secAmylase	*S. cerevisiae* BY4741 harboring pBlue-Amylase

Table S2. The constructed plasmids in this study.

Name	Key characteristics
pCons-LuxABCDE	p15A ori, Kan^R, T5-*luxABCDE*
pAra-LuxABCDE	p15A ori, Chl^R, P_{BAD}-*luxABCDE*, P_C-*AraC*
pNitrate-LuxA	pRSF ori, Amp^R, P_{YeaR}-Toehold switch-*luxA*, P_{YeaR}-Trigger
pTet-LuxA	p15A ori, Kan^R, P_{JKR}-tetR-P_{LtetO}-*luxA*
pHeme-LuxA	Plasmid 1: P_{j23104}-*chuA*, P_{taco2}-*luxC-lacI*, P_{j23100}-hrtR; Plasmid 2: P_{tac}-*luxA-luxB*, P_{tac}-*luxD-luxE*
pHypoxia-LuxCDABE	pYfiD-*luxCDABE*
pPb-LuxA	pRSF ori, Amp^R, P
pAs-LuxA	p15A ori, Kan^R, P_{probe}-NT, P_{lacV}-*arsR*, P_{arsOC2}-*luxA*
p15A-ccaS-PCB	p15A ori, Chl^R, P_{J23106}-*mini ccaS*, P_{J23108}-*HO1-pcyA*-L3S1P22
pGreen-GFP	pUC ori, Kan^R, P_{J23100}-*ccaR*-T_{B0015}, P_{cpcG2}-*sfGFP*-T_{rrnB}
pGreen-luxA	pUC ori, Kan^R, P_{J23100}-*ccaR*-T_{B0015}, P_{cpcG2}-*luxA*-T_{rrnB}
pHlyBD	pSC101 ori, Amp^R, P_{J23100}-*HlyBD*-T_{B0015}
pGreen-GFP-H60	pUC ori, Kan^R, P_{J23100}-*ccaR*-B0015, PcpcG2-*sfGFP-H60*-rrnB T1
pGreen-mCherry-H60	pUC ori, Kan^R, P_{J23100}-*ccaR*-B0015, P_{cpcG2}-*sfGFP-H60*-rrnB T1
pGreen-PD-L1nb-H60	pUC ori, Kan^R, P_{J23100}-*ccaR*-B0015, P_{cpcG2}-*PD-L1nb-H60*-rrnB T1
pGreen-MtnB-H60	pUC ori, Kan^R, P_{J23100}-*ccaR*-B0015, P_{cpcG2}-*MtnB-H60*-rrnB T1
pGreen-IL-10-H60	pUC ori, Kan^R, P_{J23100}-*ccaR*-B0015, P_{cpcG2}-*IL-10-H60*-rrnB T1
pBlue-Amylase	Plasmid 1: pRS425-P_{TEF1}-CIBN-T_{ADH1}-P_{GAP}-CRY2-T_{GPM1}; Plasmid 2: pRS413-EL222-nanoluc

Movie S1. Implantation of CMU stent in live pig rectum.

Movie S2. Endoscopic visualization of CMU stent implantation in live pig rectum.

Movie S3. Endoscopic visualization of the externally-controlled LED switching.

Movie S4. Simulation of the USV sampling, sensing and activating the alert LED.