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SUPPORTING INFORMATION

Fluorescence-based microbial sensor for the selective detection of gold

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Materials and Methods

Bacterial strains, growth conditions, and cloning

R. eutropha and *C. metallidurans* cells were cultured at 30℃ in lysogeny broth (LB) medium overnight in a shaker (200 rpm). *E. coli* cells were propagated at 37℃ with 200 rpm agitation. All liquid cultures were inoculated from single colonies and freshly diluted (1:1000) in LB media supplemented with any appropriate antibiotics for 6 h for analysis. Strains and plasmids used are listed in Table S1, S2 and S3. Standard protocols for conjugation with *E. coli* strain S17-1 were used to generate *R. eutropha* H16[1] and *C. metallidurans* CH34 strains. Cloning was performed in *E. coli* using standard procedures.

Response of gold biosensor to exogenous metal ions

The modified *cupR* regulon with RFP was expressed in *R. eutropha* and *C. metallidurans.* Metal induction analysis was performed at exponential phase (OD_{600}=0.6). Cells were then induced with 50 or 100 μM of Au^{3+} ($HAuCl_4 \cdot 3H_2O$, Acros Organics), Cu^{2+} ($CuCl_2$, Acros Organics), Zn^{2+} ($ZnCl_2$, Acros Organics), Ni^{2+} ($NiSO_4 \cdot 6H_2O$, Acros Organics), and Fe^{3+} ($FeCl_3$, Acros Organics) for 24 h. The cells were harvested and re-suspended in PBS buffer (pH 7.4). All buffers are prepared using metal-free reagents and water that has been purified by a MilliQ purification system (Millipore).

Response of gold biosensor to gradient concentration of gold ions

For gold ion dose-response measurements, the strains bearing *cupR* regulon were induced by gradient concentrations (0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 10, 25, 50, 60, 75, 90, and 100 μM) of Au^{3+} for 24 h, and the cells were harvested and fluorescence intensity / OD_{600} ratio were normalized for each sample. For fluorescence determinations, 300 μL aliquots of three biological replicates were measured by Multi-Mode Microplate Reader (BioTek) and 530 nm and 590 nm filters for excitation

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and emission wavelengths. The lower limit of detection was obtained from the formula: LOD = 3(SD/S) (SD: standard deviation of the response, S: the slope of the calibration curve).

Interference effect of gold biosensor to metal ions

To examine the effects of interference to metal ions on the *cupR* regulon, we mixed solutions of Au^{3+} (with final concentrations: 0, 0.1, 0.5, and 25μM) with each of the other metal ions (with final concentration of 50μM of Zn^{2+}, Ni^{2+}, Fe^{3+}, or Cu^{2+}) as well as with all three/four of the metals for 24 h. After incubation, cells were harvested by centrifugation, washed and recorded as described before.

Fluorescence microscopy

Cells grown in the presence and absence of Au^{3+} were immobilized on a thin layer of 1% agarose in PBS medium. The differential interference contrast and fluorescence microscopy images were obtained using a confocal spectral microscope (Leica TCS-SP2) connected to digital camera and computer.

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Table S1: Strains used in this study

Strain	Relevant genotype	Construction and source
C. metallidurans CH34	Wild type	Purchased from BCRC (bioresource collection and research center, Taiwan)
YCY_074	*C. metallidurans cupR(rev)-PcupA/R(rev)-PcupC-rfp*	pYCY_064 conjugated into *C. metallidurans* CH34
YCY_155	*C. metallidurans cupR(rev)-PcupA/R(rev)-PcupC-rfp-cupC*	pYCY_148 conjugated into *C. metallidurans* CH34
YCY_191	*C. metallidurans cupR(rev)-PcupA/R(rev)-*P_{Rmet_6382}*-rfp-bbr*	pYCY_143 conjugated into *C. metallidurans* CH34
R. eutropha H16	Wild type	DSM 428 Purchased from BCRC (bioresource collection and research center, Taiwan)
YCY_102	*R. eutropha cupR(rev)-PcupA/R(rev)-PcupC-rfp*	pYCY_064 conjugated into *R. eutropha* H16
YCY_156	*R. eutropha cupR(rev) -PcupA/R(rev)-PcupC-rfp-cupC*	pYCY_148 conjugated into *R. eutropha* H16
YCY_162	*R. eutropha PcupC-rfp*	pYCY_142 conjugated into *R. eutropha* H16
YCY_188	*R. eutropha cupR(rev)-PcupA/R(rev)-*P_{Rmet_6382}*-rfp-bbr*	pYCY_143 conjugated into *R. eutropha* H16

Table S2. Plasmids used in this study

Plasmids	Relevant genotype	Source
pYC_006	pBBRMCS plasmid with *pBAD-rfp*	[2]
pYCY_041	pBBRMCS plasmid with *pBAD-PcupA/R(rev)-PcupC-rfp*	PCR fragments were amplified with primers 15 and 20. The PCR products were treated with EcoRI and BglII, and cloned into plasmid pYC_006.
pYCY_064	pBBRMCS plasmid with *cupR(rev)-PcupA/R(rev)-PcupC-rfp*	PCR fragments were amplified with primers 62 and 34. The PCR products were treated with EcoRV and EcoRI, and cloned into plasmid pYCY_041.
pYCY_142	pBBRMCS plasmid with *PcupC-rfp*	PCR fragments were amplified with primers 59 and 20. The PCR products were treated with EcoRI and BglII, and cloned into plasmid pYC_006.
pYCY_143	pBBRMCS plasmid with *CupR(rev)-PcupA/R(rev)-*P_{Rmet_6382} *-rfp-bbr*	PCR fragments were amplified with primer 15 and 53. The PCR products were treated with EcoRI and BglII, and cloned into plasmid pYCY_64, then get the plasmid cupR(rev)-PcupR(rev)-rfp. PCR fragment were amplified with primer 54 and 55. The PCR products were treated with BglII and NdeI, and cloned into plasmid cupR(rev)-PcupR(rev)-rfp.
pYCY_148	pBBRMCS plasmid with *cupR(rev)-PcupA/R(rev)-PcupC -rfp-cupC*	PCR fragments were amplified with primers 60 and 61. The PCR products were treated with BamHI and XhoI, and cloned into plasmid pYC_064.

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Table S3. Primers used in this study

Primers	Sequence
011_rfp_NdeI fw	TTTTcatatggcgagtagcgaagacgttatcaaaga
012_rfp_BamHI rv	TTTTggatccttaagcaccggtggagtg
062_cupR(rev)_EcoRV fw	TTTGATATCttagtgatggcaggccggg
034_cupR(rev)_EcoRI rv	TTTgaattcatgaacatcggagaagccgcgg
015_PcupR(rev)_EcoRI fw	TTTgaattcaaatccttgaccttccaatggtggtaaggttcacagtgcagttgtcgcgctccg tcgctcagattccacgactccgatgaacatcggagaagccgcg
053_PcupR(rev)_BglII rv	TTTTAGATCTtgtcgcgtgcgactcaacaaat
059_PcupC_EcoRI fw	TTTgaattccttgcgcttcccatggtgg
020_PcupC_BglII rv	TTTTAGATCTggtgatgactcctgttgggttggatcg
054_P_{Rmet_6382}_BglII fw	TTTTAGATCTcttgaccttcccatggcggg
055_P_{Rmet_6382}_RBS _ NdeI rv	TTTTCATATGtatatctccttcttaaagacgtcacctttcagacaattagc
060_cupC_BamHI fw	TTTTGGATCCtttaagaaggagatatacatatgatccagttccaagtcgaagg
061_cupC_XhoI rv	TTTTCTCGAGtcagcttgccgacttgaccg

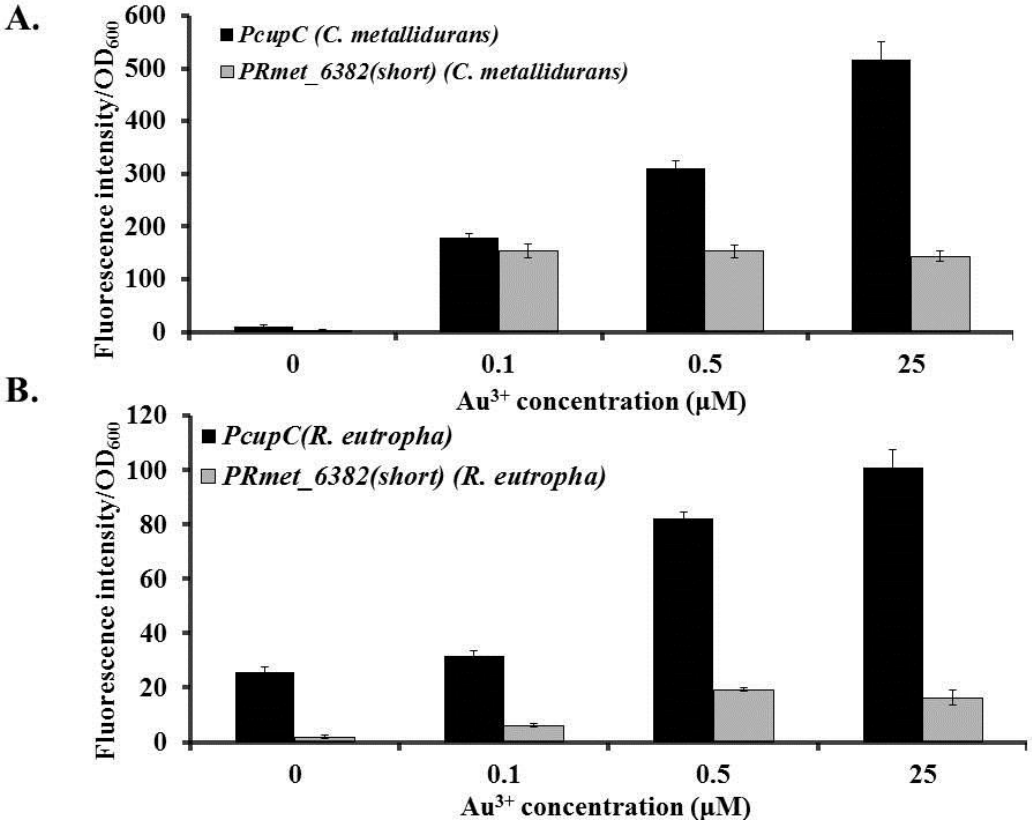

Figure S1. Quantitative analysis of the fluorescence response of cells carrying PcupC and $P_{Rmet\ 6382}$ promoters. We tested a plasmid carrying the *rfp* gene under the control of P*cupC* or P*Rmet_6382* of (A) *C. metallidurans* and (B) *R. eutropha* cells with Au^{3+} ions at 0, 0.1, 0.5 or 25 µM. Bar heights represent the average fluorescence intensity/OD_{600} of at least three independent biological replicates and error bars represent one standard deviation.

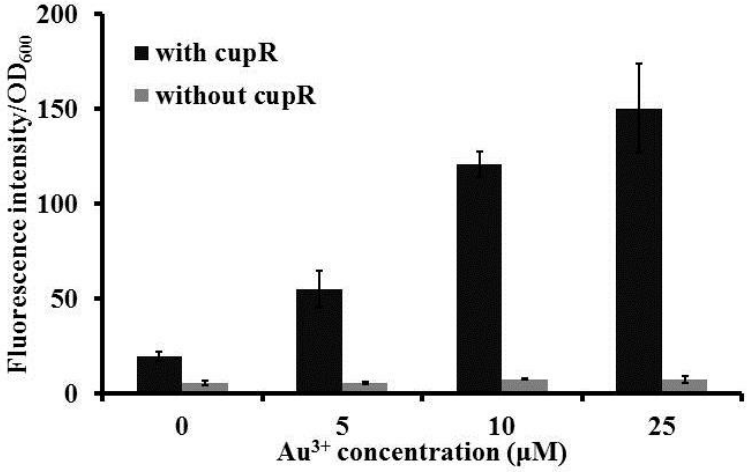

Figure S2. Quantitative analysis of the fluorescence response of *R. eutropha* cells in the presence or absence of CupR regulation.

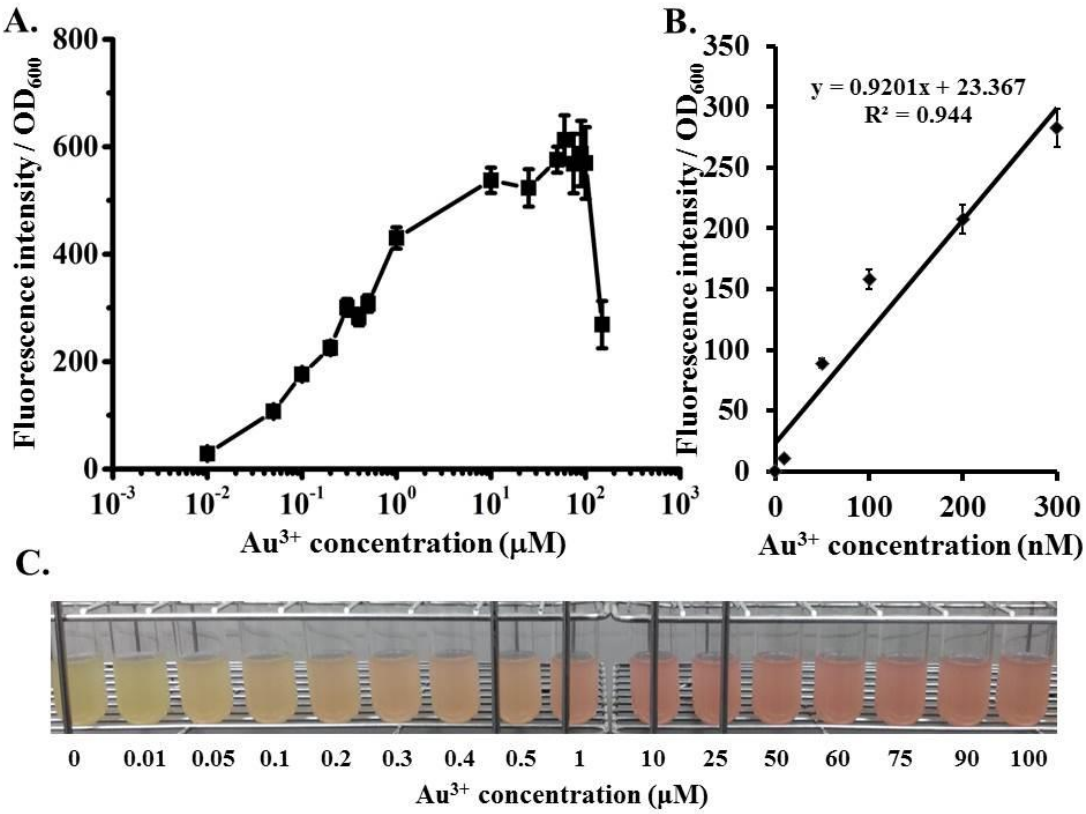

Figure S3. Quantitative analysis of the dose-response curve of *C. metallidurans* cells to Au^{3+}. (A) Following incubation with Au^{3+} ranging from 0 to 100μM, the cells were washed and analyzed. Error bars represent one standard deviation from three replicate experiments. (B) The dose-response curves showed a linear relationship in the concentration range of 0-300 nM. Linear regression provided a correlation coefficient (R^2) of 0.944. (C) Photograph of *C. metallidurans* cells of (A).

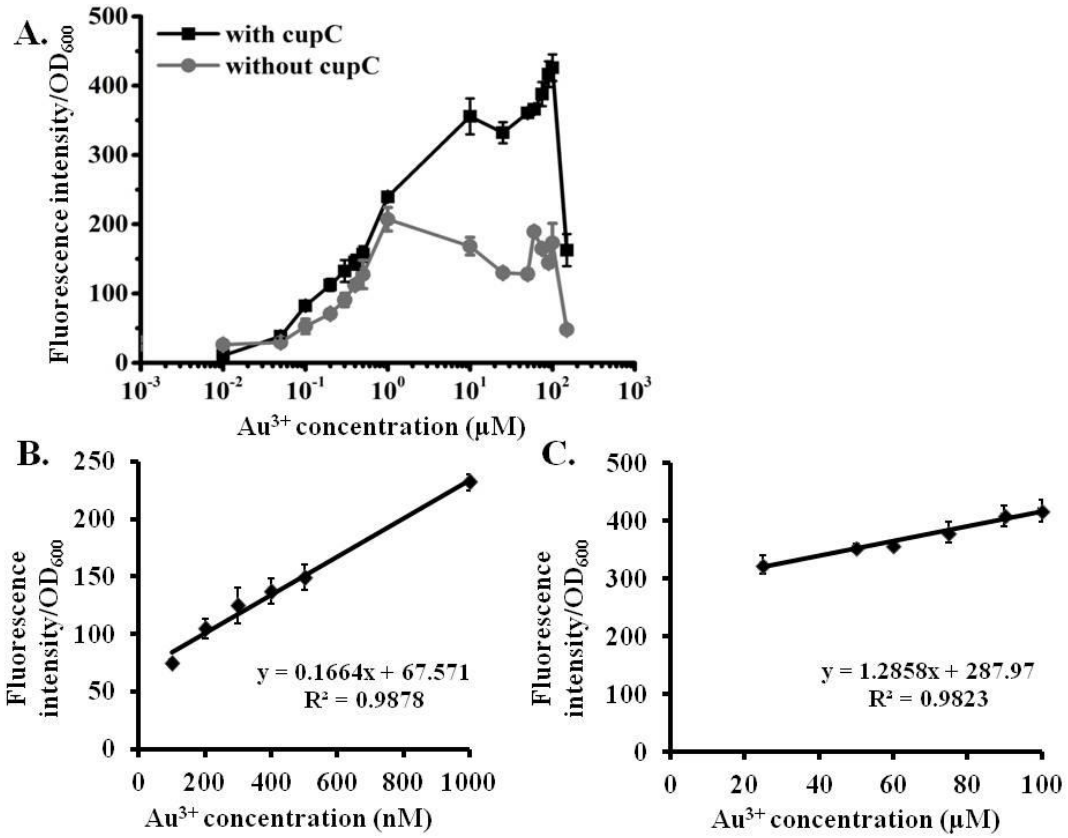

Figure S4. (A) Comparison of the dose-response curve of *R. eutropha* cells in the presence or absence of CupC protein. The dose-response curves of cells co-expressing *cupC* gene showed a linear relationship when the concentrations of Au^{3+} are range from (B) 100 to 1000 nM. Linear regression provided a correlation coefficient (R^2) of 0.987. (C) 25 to 100 μM, R^2 of 0.982.

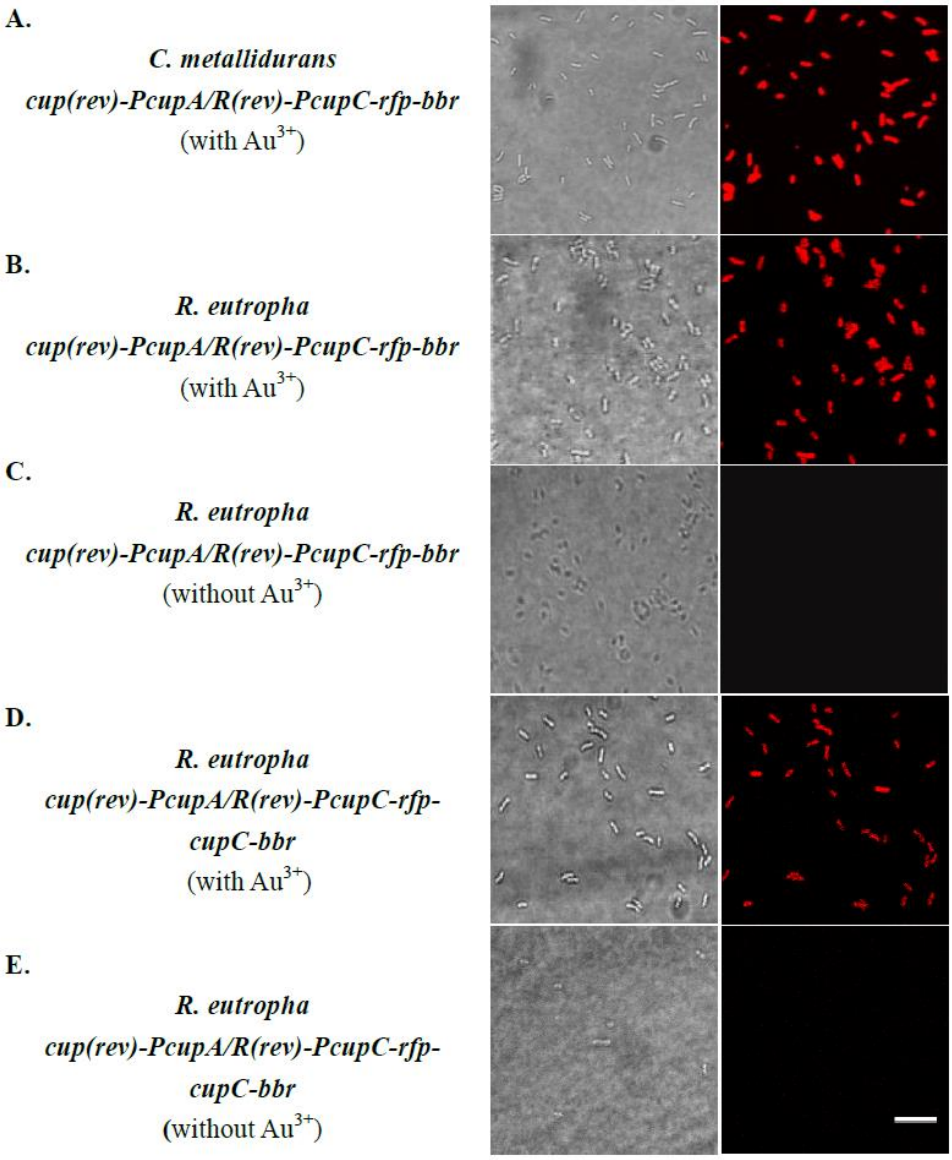

Figure S5. Differential interference contrast and fluorescence microscopy images of (A) *C. metallidurans* cells carrying *cupR(rev)-PcupA/R(rev)-PcupC-rfp* plasmid in the presence of 1μM of Au^{3+}. *R. eutropha* cells with the same plasmid (B) in the presence and (C) in the absence of 1μM of Au^{3+}. *R. eutropha* cells co-expressed *cupC* in conjunction with *rfp* (D) in the presence and (E) in the absence of 1μM of Au^{3+}. Scale bar: 100 μm.

Reference:

1. C. J. Brigham, C. F. Budde, J. W. Holder, Q. Zeng, A. E. Mahan, C. Rha and A. J. Sinskey, *J Bacteriol.*, 2010, **192**, 5454-5464.

2. Y. C. Yeh, J. Muller, C. Bi, N. J. Hillson, H. R. Beller, S. R. Chhabra and S. W. Singer, *Chemical communications*, 2013, **49**, 910-912.