Electronic Supplementary Material (ESI) for Chemical Science
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Supporting Information

Engineering a Gold-specific Regulon for Cell-based Visual Detection and Recovery of Gold

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Experimental Section

S1 Gold selective sensing by engineered *E.coli* cell

The BioBrick Vector pSB1A2 and the gene encoding RFP were graciously provided by 2010 The International Genetically Engineered Machine competition (iGEM) organization. The gold selective sensing plasmid was constructed by two steps and all DNA fragment were amplified from *Salmonella typhimurium* genomic DNA. First a 200bp DNA fragment including the gene encoding *PgolTS* was amplified by the primers PgolS1 and PgolS2. The gene encoding *golS* and *PgolB* was amplified by the primers GolS1 and PgolB2. Then these two fragments were connected by overlap PCR using PgolS1 and PgolB2. After confirmed by sequencing, the PCR product was digested by *Eco*RI and *Xba*I, and then inserted into pSB1A2 vector. The gene encoding RFP was amplified by primers RFP1 and RFP2, and then digested by *Xba*I and *Spe*I and insert the plasmid including the modified *gol* regulon.

The modified *gol* regulon with RFP was expressed in *E. coli* strain DH5α with concentration of 20 μM $HAuCl_4$ (Au^{3+}) or 20 μM $CuSO_4$ (Cu^{2+}), or 20 μM $CdCl_2$ (Cd^{2+}), 20 μM $ZnCl_2$ (Zn^{2+}), or 20 μM $NiCl_2$ (Ni^{2+}), or without metal ions induction overnight, and then the cells were harvested and re-suspended in PBS buffer (pH 7.4), respectively. For gold concentration sensitivity measurement, the *E. coli* strain containing the plasmid was also induced by gradient concentrations (0 μM, 0.25 μM, 1 μM, 5 μM, 10 μM and 20 μM) of $HAuCl_4$ (Au^{3+}) induction overnight, and then the cells were harvested and normalized to an OD_{600} =1.0 with PBS buffer (pH 7.4) respectively. For fluorescence determinations, 300 μL aliquots of each sample were applied in triplicate into a 96-well flat bottom black plates (Corning). Fluorescence was recorded using a Multi-Mode Microplate Reader (BioTek) and 558 nm and 583 nm filters for excitation and emission wavelengths, respectively.

S2 Expression and purification of recombinant GolB protein from *E. coli*

The expression plasmid of *Salmonella typhimurium golb* gene is a generous gift from Prof. Chuan He at The University of Chicago. The gene was sub-cloned into pGEX-4T-1 Vector between *BamH1* and *Sal1* restriction site with an N-terminal GST tag. Then the plasmid was transformed into *E. coli* BL21 (DE3) cells. A single colony of transformed *E. coli* cell was cultured in 10 mL LB media at 37 °C over

night with 50 µg mL^{-1} ampiciline. Overnight culture was diluted 100X in 1000 mL LB containing 50 µg mL^{-1} ampiciline and grown at 37 °C. Protein expression was induced with 0.5 mM IPTG (isopropyl-beta-D-1-thioglactopyranoside) at optical density of OD_{600}=0.6 and continued expression for additional 4 h at 30 °C. Cells were harvested by centrifugation at 4 °C, stored at -80 °C until further use. Cell pellet was thawed on ice and re-suspended in 1X PBS buffer with 1 mM final concentration of PMSF. After sonication, the lysate was centrifuged at 14, 000 rpm for 20 min and the supernatant was filtered through sterile 0.22 mm filter. The filtrate was loaded onto 5 mL GSTrap column packed with glutathione sepharose 4B resin. Column was washed with 80 mL of 1X PBS buffer and GST-GolB was eluted with linear gradient of freshly prepared 50 mM Tris-HCl, pH 8.0 containing 10 mM glutathione reduced. The fractions containing GST-GolB were pulled out to exchange buffer to 1X PBS and concentrated by 10K Amicon Ultra-15 centrifugal filters to 2 mL. Then the GST-GolB protein was digested by thrombin (1U thrombin/0.2 mg target protein) for 24 h at room temperature. The digested protein was concentrated to less than 2 mL and loaded onto Superdex-200 column (Hiload 16/60 superdex-200, GE healthcare) pre-equilibrated with degassed buffer (100 mM NaCl, 100mM Tris-HCl, pH 7.5). After this purification step the protein was showed single band (Figure S1) and the protein concentration was measured by UV-Vis Spectrophotometer. The protein can be stored with 30% glycerol at -80 °C for up to one month.

S3 UV-visible measurement of metal binding experiment

For gold (I) binding assay, protein was reduced with 5 mM DTT overnight at 4 °C and passed through 10 DG desalting column using 100 mM NaCl, 100 mM Tris-HCl, pH 7.5. About 50 µM of GolB protein was used immediately for gold (I) binding experiment in the same buffer. For all measurements, baseline was recorded with protein itself and UV spectra differences were recorded for each measurement. Gold(I) binding was monitored as an increase in absorption band at 250-255 nm and cyanide mediated gold depletion was monitored as decrease in absorption in this region. Concentration and absorbance were corrected for final volume. The binding ratio between GolB and Au(I) was obtained by the Job's plot method (Figure S2).[1] Equilibrium concentration of ligand, and metal-protein complex was determined

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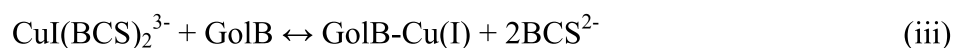
using non-linear curve fitting software. The dissociation constants was calculated based on the published standard values of protonation constant of HCN (logK = 9.04 at 0.1 M ionic strength and 25 °C),[2] and the stability constant of $[Au(CN)_2]$, $\log\beta^2 = 36.5$.[3] Apparent association constant for $Au(CN)_2$ at pH 7.5 was calculated using equation (i).[4] This value along with the equilibrium concentration of [GolB], [GolB-Au(I)], and $[CN^-]$ at half equivalence point were used to calculate dissociation constant of GolB for gold using equation (ii).

$$\beta'_2 = \beta_2 \, (\alpha_{H\text{-}CN})^2 \qquad \text{(i)}$$

$$GolB\text{-}Au^I + 2CN^- \leftrightarrow Au(CN)_2 + GolB \qquad \text{(ii)}$$

Cu (I) binding experiment was carried out using BCS (bathocuproine disulfonate sodium salt). Stock solution of tetrakis (acetonitrile) copper (I) hexafluorophosphate was prepared anaerobically in nitrogen purged acetonitrile. A 20 µM of $[Cu(I)(bcs)_2]^{3-}$ complex was prepared by adding concentrated BCS solution directly into the concentrated solution of tetrakis (acetonitrile) copper (I) hexafluorophosphate in deoxygenated acetonitrile and diluting with tris buffer to make required final ration of 1 : 10. Exact concentration of $[Cu(I)(bcs)_2]^{3-}$, GolB-Cu(I), free Cu(I), and GolB-Cu(I) were determined based on the absorption maximum at 483 nm ($\varepsilon = 13300\ M^{-1}\ cm^{-1}$) and association constant $\beta^{2'} = 10^{19.8}$.[5] Individual samples were prepared using increasing concentration of GolB (freshly reduced and purified) to a fixed concentration of $[Cu(I)(BCS)_2]^{3-}$ or in a reverse way where Cu(I) was titrated to a solution of protein-BCS complex (Figure S3). A change in absorption at 483 nm was recorded to determine the dissociation constant of Cu(I) -GolB considering the formation of 1 : 1 complex with protein.



$$CuI(BCS)_2^{3-} + GolB \leftrightarrow GolB\text{-}Cu(I) + 2BCS^{2-} \qquad \text{(iii)}$$

S4 Mass Spectrometry

Protein total mass was determined on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer with electrospray ionization (ESI, Micromass). GolB-Au(I) adduct was desalted with ddH_2O and acquired on an automated ESI/MS system (CapLC-ESI-Q-TOF)

S5 Construction of plasmids for surface display on *E.coli*

The pBAD-OmpA plasmid with a HA-tag following the OmpA signal peptide was graciously provided by Dr. Xi Ge at Prof. Zengyi Chang's lab at Peking University. The expression gene of OmpA-GolB fusion protein was constructed by two steps PCR. The gene encoding OmpA 1-159 amino acids was amplified by the primers Ompa1 and Ompa2, and the gene encoding the full-length GolB with a FLAG-tag at the C-terminus was amplified by the primers Golba1 and Golba2. Then these two fragments were connected by overlap PCR using Ompa1 and Golba2. After confirmed by sequencing, the PCR product was digested by *Nco*I and *Hind*III, and then inserted into pBAD vector for displaying GolB protein on *E. coli* cell surface. As an undisplayed control, the pBAD-OmpA plasmid without displayed items was used.

S6 Mutagenesis

As a negative control, the possible metal binding sites (Cys10 and Cys13) were mutated to serine by site-directed mutagenesis with primers Golbm1 and Golbm2. (Table S1).

Table S1. Primers for Construction of plasmids for surface display on *E. coli* and mutagenesis

Primers	Sequences
Ompa1 (forward)	5'- CATGCCATGGGCATGAAAAAGACAGCT -3'
Ompa2 (reverse)	5'- CTGCATGTCGACTCCGTTGTCCGGACGAGT -3'
Golba1 (forward)	5'- GACAACGGAGTCGACATGCAGTTCCATATT -3'
Golba2 (reverse)	5'-CCCAAGCTTTCAGATCTTATCGTCGTCATCCTTGTAATCCTCGAGCCTCTCGCG CGGC -3'
Golbm1 (forward)	5'-ATATTGATGACATGACCTCCGGCGGCTC-3'
Golbm2 (reverse)	5'-TACTGGCGGAGCCGCCGGAGGTCATGT-3'

S7 Bioadsorption and recovery of gold by GolB displayed cells

The OmpA-GolB or mutant OmpA-GolB fusion proteins were expressed in *E. coli* strain DH10B. Cells were grown in LB medium containing ampicilline (50 μg mL^{-1}) with shaking overnight at 37 °C. After 1 : 100 dilution in LB medium containing ampicilline (50 μg mL^{-1}), the culture was grown at 37 °C to an OD_{600}=0.6~0.8. Protein expression was induced by the addition of arabinose to the final concentration of

0.002% and incubated at 37 °C overnight. For metal ions adsorption, appropriate concentration of different metal ions was added in LB medium or LB agar plate respectively during the induction. To measure the metal ions adsorption ability of GolB displayed *E. coli*, cells were harvested from LB medium by centrifugation (4500 rpm, 10 min) or collected from LB Agar plates, and then washed by ddH_2O at least three times. The cells were lyophilized for measuring dry weight and analyzed by wet-ashing followed by inductively coupled plasma-atomic emission spectrometer (ICP-AES; J-A1100, Jarrell-Ash, USA). To recover gold ions, gold adsorbed cells were incubated in PBS (pH 7.4) containing 10 mg mL^{-1} papain at 30 °C for 12 h with gentle shaking. [6] After centrifugation, the supernatant from each sample was analyzed by ICP-AES, the treated cells were harvested and then re-cultured for repeating adsorption and recovery up to three cycles.

S8 Analysis of SDS-PAGE and Western blotting

GolB displayed *E. coli* cells were harvested by centrifugation (4500 rpm, 5 min) and re-suspended in lysis buffer (PBS, pH 7.4). After sonication, the two fractions supernatant and cell membrane were separated by centrifugation (14000 rpm, 30 min, 4 °C). The cell membrane fraction was re-suspended in 100 μL PBS and 100 μL supernatant fraction were both mixed with 10 μL 10X loading buffer and heating at 95 °C for 10 min. After centrifugation (14000 rpm, 10 min, 4 °C), the samples were loaded onto 15% SDS-PAGE gels and electrophoresed for 30 min at 80 V and 50 min at 150 V. For Western blotting analysis, the separated proteins were transferred onto polyvinylidene difluoride membranes (Bio-Rad) at 250 mA, 4 °C 2 h. After blocking at room temperature for 1 h in Blotto (5% nonfat dry milk in 1×TBST), the membranes were performed using 1 : 1000 dilutions of monoclonal anti HA-tag (Santa Cruz) or monoclonal anti FLAG-tag (Santa Cruz) as primary antibody overnight at 4 °C followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Santa Cruz) at room temperature for 2 h. Antibodies were detected with ECL reagents (Pierce).

S9 Immunofluorescence microscopy and transmission electron microscopy (TEM)

To confirm the display of GolB on the *E. coli* cell surface, immunofluorescence labeling of cells was performed using the FLAG-tag as described below. GolB displayed cells were fixed with 4% (v/v) formaldehyde/PBS (pH7.4) for 1.5 h and incubated in PBS (pH 7.4) containing 1% (w/v) horse blood serum for 30 min prior to immunostaining. Mouse monoclonal anti FLAG-tag antibody (Santa Cruz) was used as the primary antibody at a dilution rate of 1 : 200. A mixture of cells and the antibody was incubated using a rotator for 4 h at room temperature or overnight at 4 °C. The cells were then washed with PBS (pH 7.4) three times. FITC-conjugated goat anti-mouse IgG antibody (Santa Cruz) diluted at 1 : 300 was then reacted with the cells using a rotator for 1.5 h at room temperature.[6] After washing with PBS (pH 7.4), the cells were suspended in appropriate volume of PBS (pH 7.4) and observed by microscopy. Fluorescence was detected using an inverted confocal microscope A1 (Nikon, Tokyo, Japan).

TEM of unstained gold adsorbed cells attached to the carbon-coated grids was conducted using a Hitachi TEM system, at magnifications ranging from X 10,000 to X 100,000 at 80 kV.

S10 Plate sensitivity assays of GolB displayed *E.coli*

The *E. coli* (DH10B) strains containing OmpA-GolB plasmid and the negative control were grown in LB medium containing ampicilline (50 μg mL^{-1}) with shaking overnight at 37 °C. After 1 : 100 dilution in LB medium containing ampicilline (50 μg mL^{-1}), the cultures were grown at 37 °C to an OD_{600}=0.6~0.8. The numbers of bacterial cells from each strain of bacterial samples were normalized to approximately 5×10^{8} CFU ml^{-1} with fresh LB, followed by six 10-fold serial dilutions. Then 5 μl of each strain of bacterial samples (from 10^{-3} to 10^{-6} dilution) were spotted onto the LB agar plates containing ampicilline (50 mg mL^{-1}), arabinose (0.002%) and gold ions (0~30 μM).[7] All plates were incubated at 37 °C for 18 h before being read.

S11 Figures in Supporting Information

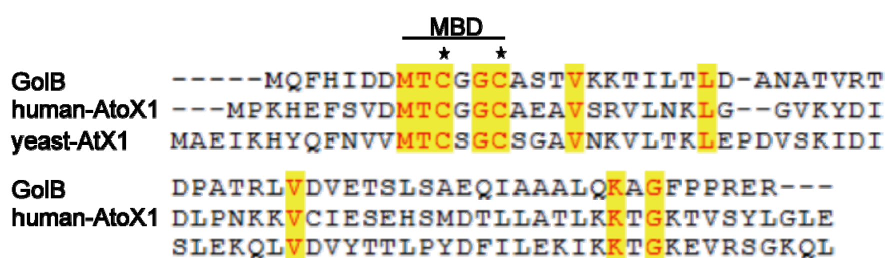


Fig. S1 Sequence alignment of GolB (*S. typhimurium*), AtoX1 (*Human*) and AtX1 (*Yeast*) with the conserved amino acids colored in yellow and the metal binding domain (MBD) labeled. ★indicates the two conserved cystine residues among these three proteins.

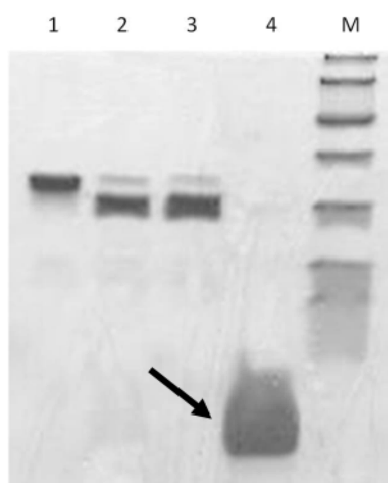


Fig. S2 SDS-PAGE analysis of GolB expression. Lane1 GST-GolB after purification; lane2 and lane3 before and after purification of thrombin digested GST-GolB; lane4 GolB after final purification and concentration (The black arrow indicated). M: Protein marker.

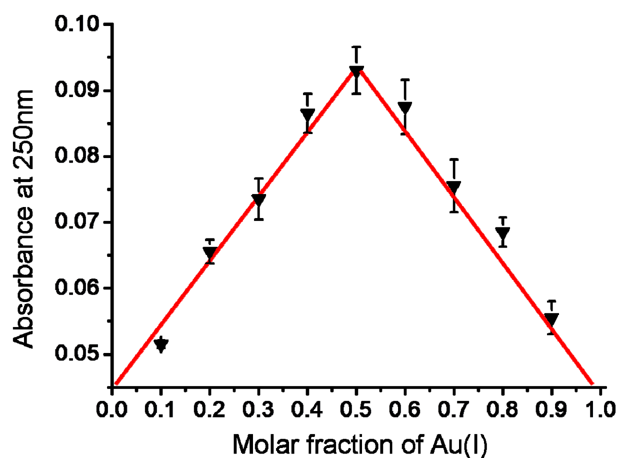


Fig. S3 Job's plot of Au (I) binding to GolB.

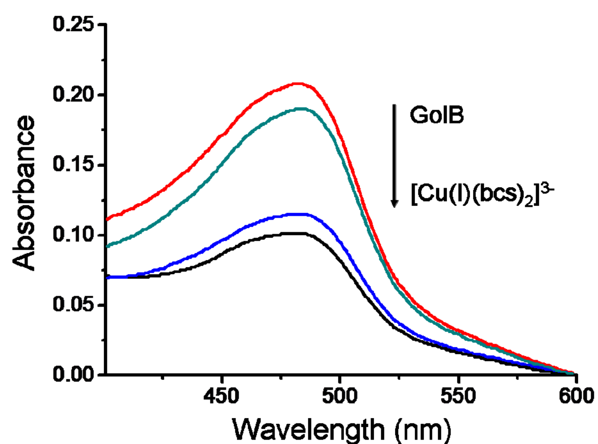


Fig. S4 Absorption spectra of Cu(I) (20 μM) and BCS (200 μM) in in 0.1M Tris-HCl, 0.1M NaCl pH 7.5 (top red) and after addition of 10, 20, and 40 μM apo-GolB. All samples were prepared separately and incubated for 5 minutes before recording absorbance spectra.

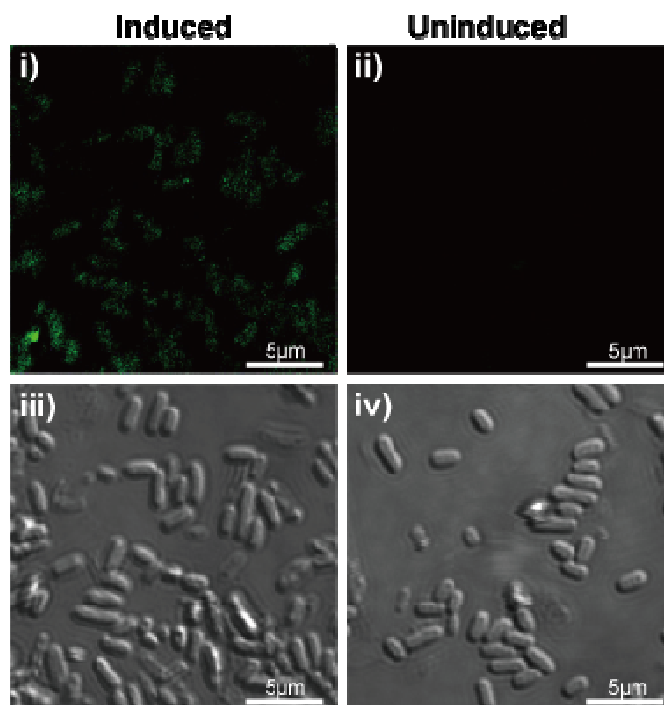


Fig. S5 Immunofluorescence labeling of *E. coli* cells using anti-FLAG antibody and FITC conjugated anti-mouse IgG antibody, immunofluorescence images (i, ii) and brightfield images (iii, iv) of arabinose induced GolB displayed on *E. coli* cell surface. The uninduced *E. coli* bacteria were used as negative control.

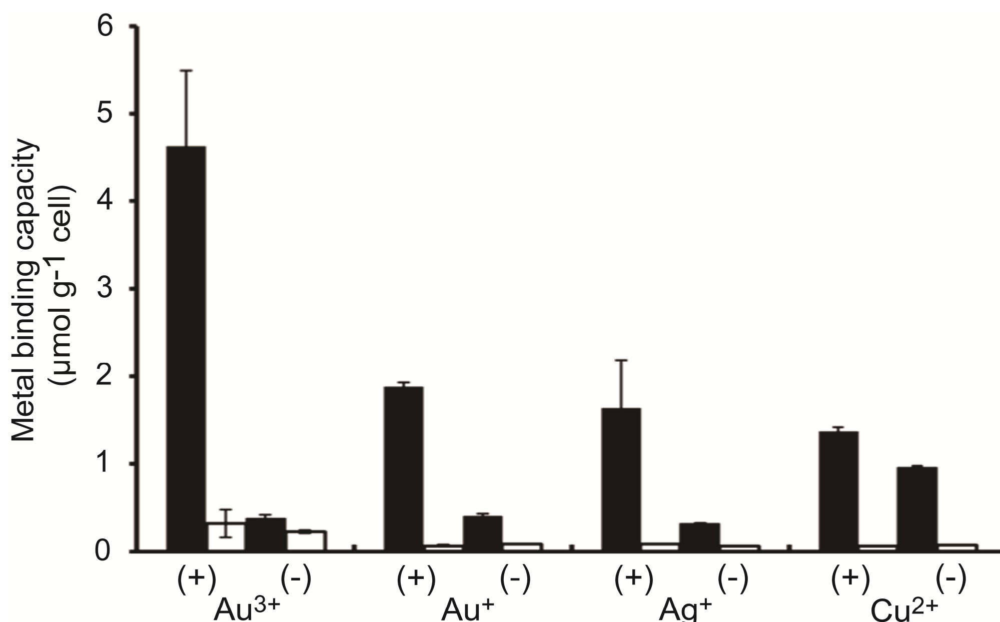


Fig. S6 Metal selective adsorption of GolB displayed *E. coli*. *E. coli* (DH10B) strains containing OmpA-GolB plasmid (+) or not (-) were induced by 0.002% arabinose (black columns) or not (white columns), then grown overnight in LB with an addition of 50 µM $HAuCl_4$ (Au^{3+}) or 50µM $KAu(CN)_2$ (Au^{+}) or 50µM $AgNO_3$ (Ag^{+}) or 50µM $CuSO_4$ (Cu^{2+}).

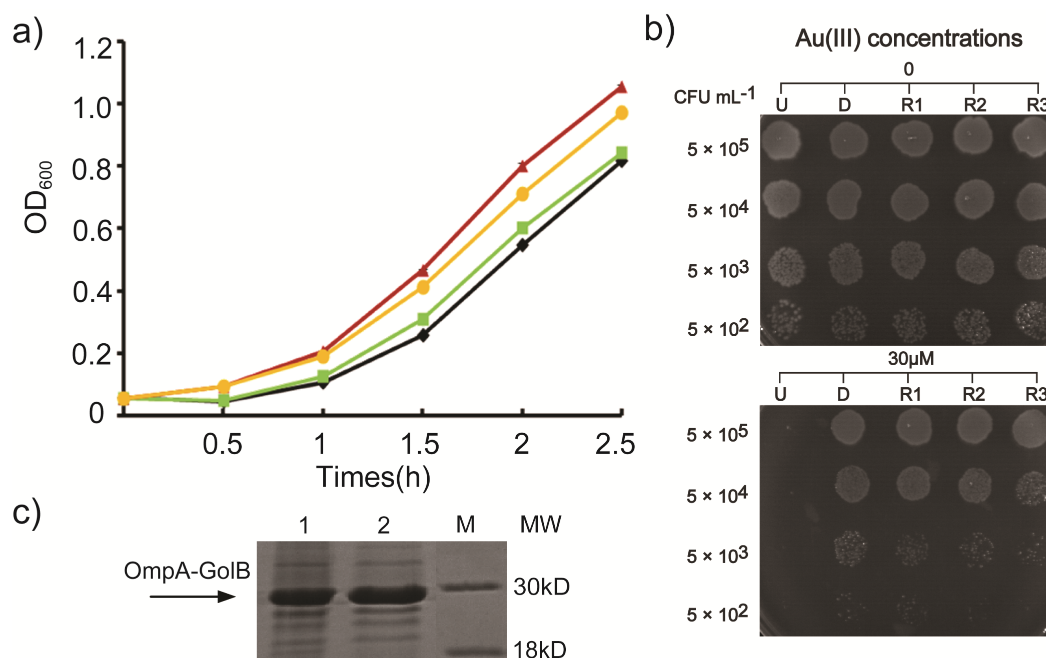


Fig. S7 a) Growth curves of *E. coli* cells bearing the surface displayed GolB re-cultured in LB medium before (black, ◆) and after the repeated adsorption and recovery process including papain treatment once(green, ■), twice(red, ▲) and third time(yellow, ●). b) Plate sensitive assay measuring the gold tolerance of *E. coli* cells with GolB undisplayed (U), displayed (D), and GolB displayed *E. coli* after a repeated adsorption and recovery process including papain treatment: 1st cycle(R1), 2nd cycle(R2) and 3rd cycle(R3). All plates were incubated at 37 °C for 18 h before being read. c) Reuse of GolB displayed *E. coli.* SDS-PAGE analysis of surface-displayed GolB expression before and after recovery. GolB displayed *E. coli* before (lane1) or after papain treatment (lane2) were re-cultured in LB medium and induced by 0.002% arabinose. The black arrow indicates OmpA-GolB expressed.

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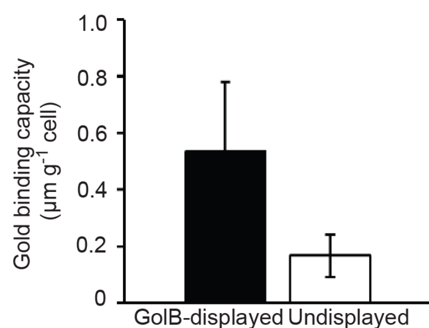

Fig. S8 Gold adsorption by GolB displayed *E. coli* from LB Agar medium. *E. coli* (DH10B) strains containing OmpA-GolB plasmid or not were grown until OD_{600}=0.6, After diluted the cells to $5X10^5$ CFU mL^{-1} , pipetted 1mL and evenly coated on LB agar medium with the addition of 50μM $HAuCl_4$ (Au^{3+}) and 0.002% arabinose, then grown 18h at 37 °C.

References

1. C. J. Fahrni and T. V. O'Halloran, *J. Am. Chem. Soc.*, 1999, **121**, 11448-11458.
2. A. Changela, K. Chen, Y. Xue, J. Holschen, C. E. Outten, T. V. O'Halloran and A. Mondragon, *Science*, 2003, **301**, 1383-1387.
3. R. D. Hancock, N. P. Finkelstein and A. Evers, *Journal of Inorganic and Nuclear Chemistry*, 1972, **34**, 3747-3751.
4. Z. Xiao and A. G. Wedd, *Nat Prod Rep*, 2010, **27**, 768-789.
5. Z. Xiao, F. Loughlin, G. N. George, G. J. Howlett and A. G. Wedd, *J. Am. Chem. Soc.*, 2004, **126**, 3081-3090.
6. T. Nishitani, M. Shimada, K. Kuroda and M. Ueda, *Appl Microbiol Biotechnol*, 2010, **86**, 641-648.
7. P. R. Chen, T. Bae, W. A. Williams, E. M. Duguid, P. A. Rice, O. Schneewind and C. He, *Nat. Chem. Biol.*, 2006, **2**, 591-595.