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SUPPORTING INFORMATION

Bacterial surface display of metal binding peptides as whole-cell biocatalysts for 4-nitroaniline reduction

Dong-Yu Tsai, Yi-Jung Tsai, Chia-Ho Yen, Chun-Yu Ouyang, and Yi-Chun Yeh*
Department of Chemistry, National Taiwan Normal University

Materials and methods

Bacterial strains, growth conditions, and cloning

R. eutropha cells were cultured overnight at 30°C in lysogeny broth (LB) or H3 medium with shaking at 200 rpm. *E. coli* cells were incubated in lysogeny broth or M9 medium at 37°C (250 rpm). Strains, plasmids, primers, and construct details are listed in Table S1, S2 and S3.

Analysis of RFP localization by fluorescence microscopy

For localization studies, the overnight *E. coli* culture was diluted in LB media for 3 h until the OD_{600} reached 0.6. Then, 0.2% arabinose was used to induce expression of fluorescent protein fusions for 5 h. Similarly, *R. eutropha* strains were induced for 15 h with 0.2% arabinose. Cells were immobilized using a thin layer of 1% agarose in PBS medium. Phase contrast and fluorescence microscopy images were obtained using a Zeiss Axio A1 Microscope with an EC Plan-Neofluar 100x/1.30, and oil PH3 objective.

Synthesis of nanoparticles

The gold nanoparticles were synthesized by citrate reduction of $HAuCl_4$.[1] The $HAuCl_4$ solution (1.0 mM, 250 mL) was boiled, and added sodium citrate tribasic hydrate (38.8 mM, 25 mL) rapidly for 15 minutes until color change to deep red. The size of Au nanoparticles was determined by TEM analysis (JEOL JEM-1200 EXII). The absorption of Au nanoparticles solution was recorded by Multi-Mode Microplate Reader (BioTeK). The concentration of the Au nanoparticles was determined using an extinction coefficient of ca. 2.0×10^8 $M^{-1}cm^{-1}$ at 520 nm from Beer's law.[2]

Analysis of binding affinity of various surface display systems by gold binding peptide

R. eutropha strains were induced to express gold binding peptides. The induced cells were incubated with freshly prepared Au nanoparticles solution for 2 h at 28°C. The

cell pellets were collected by centrifugation (12,225 g, 1 min) and washed three times with PBS buffer. The cells were dropped on the copper grid and stained with 2% phosphotungstic acid for 10 seconds for TEM analysis.

Biosynthesis of Au and Pt nanoparticles

The strains were diluted and induced to express the metal binding peptides. The $HAuCl_4 \cdot 3H_2O$ (Acros organics) or $H_2PtCl_6 \cdot H_2O$ (Acros organics) solution was then added into the culture (final concentration: 1 mM), and incubated for 1 h. The cell pellets were collected by centrifugation (12,225 g, 1 min) and resuspended in PBS buffer. The sample was dropped on the copper grid for TEM analysis. *E. coli* and *R. eutropha* cells were incubated for 18 h and 26 h in M9 and H3 media for UV-vis spectrum analyses. Energy dispersive spectroscopy (EDS) was used to identify the elemental composition of samples. To get the sample for SEM/EDS analysis, the aforementioned cells with nanoparticles were lyophilized by VirTis Freeze Dryer 5SL. EDS was analyzed by using JEOL JSM-7600F Field Emission Scanning Electron Microscope with INCA X-Max EDS.

Reduction of 4-nitroaniline to 4-aminoaniline

The cell pellets with metal NPs were collected by centrifugation (4,293 g, 1 min), and washed three times with distilled deionized water (DDW). The cells binding Au or Pt nanoparticles on the surface were used as catalysts for the reduction of 4-nitroaniline by $NaBH_4$. 100 μL mixtures of methanol and DDW (1:1) containing 0.012 mg of 4-nitroaniline (final concentration: 0.9 mM), 0.78 mg cell pellets (wet weight), and 0.34 mg $NaBH_4$ (final concentration: 90 mM) were analyzed. Absorption spectra were measured by UV-vis spectrophotometer (Hewlett-Packard, 8453) and Multi-Mode Microplate Reader. Kinetic analyses were generated by measuring the decrease of absorbance at 385 nm.

Table S1 Bacterial strains used in this study

Strains	Relevant genotype	Construction and source
***Ralstonia eutropha* H16**	Wild type	DSM 428
pYCY_281	*R. eutropha pBAD-rfp bbr*	pYCY_037 conjugated into *R. eutropha* H16
pYCY_052	*R. eutropha pBAD-rfp-gbp bbr*	pYCY_038 conjugated into *R. eutropha* H16
pYCY_067	*R. eutropha pBAD-fhuA-gbp bbr*	pYCY_063 conjugated into *R. eutropha* H16
pYCY_107	*R. eutropha pBAD-fhuA-rfp bbr*	pYCY_027 conjugated into *R. eutropha* H16
pYCY_051	*R. eutropha pBAD-fhuA-rfp-gbp bbr*	pYCY_039 conjugated into *R. eutropha* H16
pYCY_060	*R. eutropha pBAD-ompA-gbp bbr*	pYCY_049 conjugated into *R. eutropha* H16
pYCY_080	*R. eutropha pBAD-ompA-rfp bbr*	pYCY_082 conjugated into *R. eutropha* H16
pYCY_079	*R. eutropha pBAD-ompA-rfp-gbp bbr*	pYCY_081 conjugated into *R. eutropha* H16
pYCY_068	*R. eutropha pBAD-ss'-rfp-igA bbr*	pYCY_007 conjugated into *R. eutropha* H16
pYCY_073	*R. eutropha pBAD-ss'-gbp-igA bbr*	pYCY_071 conjugated into *R. eutropha* H16
pYCY_303	*R.eutropha pBAD-ss-lpp-ompA$_{46\sim159}$-rfp bbr*	pYCY_294 conjugated into *R. eutropha* H16
pYCY_315	*R.eutropha pBAD-ss'-gbp-rfp-igA bbr*	pYCY_297 conjugated into *R. eutropha* H16
pYCY_323	*R.eutropha pBAD-ss-lpp-ompA$_{46\sim159}$-rfp-gbp bbr*	pYCY_319 conjugated into *R. eutropha* H16
pYCY_324	*R.eutropha pBAD-ss-lpp-ompA$_{46\sim159}$ bbr*	pYCY_316 conjugated into *R. eutropha* H16
pYCY_360	*R. eutropha pBAD-ss-lpp-ompA$_{46\sim159}$-gbp bbr*	pYCY_320 conjugated into *R. eutropha* H16
pYCY_399	*R. eutropha pBAD-ss'-s7-igA bbr*	pYCY_393 conjugated into *R. eutropha* H16

Table S1 (continued)

Strains	Relevant genotype	Construction and source
***E. coli* DH5α**		
pYCY_038	*E. coli pBAD-rfp-gbp bbr*	pYCY_038 transformed into *E. coli* DH5α.
pYCY_039	*E. coli pBAD-fhuA-rfp-gbp bbr*	pYCY_039 transformed into *E. coli* DH5α.
pYCY_082	*E. coli pBAD-ompA-rfp bbr*	pYCY_082 transformed into *E. coli* DH5α.
pYCY_081	*E. coli pBAD-ompA-rfp-gbp bbr*	pYCY_081 transformed into *E. coli* DH5α.
pYCY_294	*E. coli pBAD-ss-lpp-ompA*$_{46\sim159}$*-rfp bbr*	pYCY_294 transformed into *E. coli* DH5α.
pYCY_297	*E. coli pBAD-ss'-gbp-rfp-igA bbr*	pYCY_297 transformed into *E. coli* DH5α
pYCY_393	*E. coli pBAD-ss'-s7-igA bbr*	pYCY_393 transformed into *E. coli* DH5α.

Table S2 Plasmids used in this study

Plasmids	Relevant genotype	Construction and source
pYCY_037	pBBRMCS plasmid with *pBAD-rfp, Kan*^{R}	
pBbE5k-rfp		Lee TS et al., 2011[3]
pYCY_004	pBBRMCS plasmid with *pTrc-ss'-gbp-igA, Kan*^{R}	
pYCY_033	pBbE5K plasmid with *rfp-gbp, Kan*^{R}	PCR fragments were amplified with primers 11 and 12, using pYCY_037 as a template. The PCR products were treated with NdeI and BamHI, and cloned into plasmid pYCY_020.
pYCY_038	pBBRMCS plasmid with *pBAD-rfp-gbp, Kan*^{R}	*rfp-gbp* fragments were treated with EcoRI and BamHI from pYCY_033, and cloned into plasmid

		pYCY_037.
Plasmids	Relevant genotype	Construction and source
pYCY_013	pBBRMCS plasmid with *pBAD-fhuA, Kan^R*	PCR fragments were amplified with primers 09 and 10, using pYCY_027 as a template. The PCR products were treated with EcoRI and XhoI, and cloned into plasmid pYCY_037.
pYCY_063	pBBRMCS plasmid with *pBAD-fhuA-gbp, Kan^R*	*gbp* fragments were amplified with primers 35 and 36 by annealing. The *gbp* fragments were treated with NdeI and BamHI, and cloned into plasmid pYCY_027.
pYCY_027	pBBRMCS plasmid with *pBAD-fhuA-rfp, Kan^R*	Yeh YC et al 2013[4]
pYCY_039	pBBRMCS plasmid with *pBAD-fhuA-rfp-gbp, Kan^R*	PCR fragments were amplified with primers 11 and 12, using pYCY_037 as a template. The PCR products were treated with NdeI and BamHI, and cloned into plasmid pYCY_027.
pYCY_049	pBBRMCS plasmid with *pBAD-ompA-gbp, Kan^R*	*ompA* fragments were amplified with primers 23 and 24, using *E. coli* colony as a template. The PCR products were treated with NdeI and BamHI, and cloned into plasmid pYCY_037.

Plasmids	Relevant genotype	Construction and source
pYCY_082	pBBRMCS plasmid with *pBAD-ompA-rfp, Kan^R*	PCR fragments were amplified with primers 41 and 42, using pYCY_049 as a template. The PCR products were treated with NdeI and BglII, and cloned into plasmid pYCY_037.
pYCY_081	pBBRMCS plasmid with *pBAD-ompA-gbp-rfp, Kan^R*	PCR fragments were amplified with primers 40 and 42, using pYCY_049 as a template. The PCR products were treated with NdeI and BglII, and cloned into plasmid pYCY_037.
pYCY_007	pBBRMCS plasmid with *pBAD-ss'- rfp-igA , Kan^R*	
pYCY_071	pBBRMCS plasmid with *pBAD-ss'-gbp-igA ,Kan^R*	*pBAD* fragments were treated with PstI and EcoRI from pYCY_037, and cloned into plasmid pYCY_004.
pYCY_292	pBBRMCS plasmid with *pBAD-ompA$_{46\sim159}$-rfp, Kan^R*	PCR fragments were amplified with primers 165and 166, using pYCY_049 as a template. The PCR products were treated with BglII and XhoI, and cloned into plasmid pYCY_037.
pYCY_294	pBBRMCS plasmid with *pBAD-ss-lpp-ompA$_{46\sim159}$-rfp, Kan^R*	*ss-lpp* fragments were amplified with primers 167, 168, 169 and 170 by annealing. The *ss-lpp* fragments were treated

		with NdeI and BamHI, and cloned into plasmid pYCY_292.
Plasmids	Relevant genotype	Construction and source
pYCY_297	pBBRMCS plasmid with *pBAD-ss'-gbp-rfp-igA, Kan^R*	*ss'-gbp* fragments were amplified with primers 171, 172, 173 and 174 by annealing. The *ss'-gbp* fragments were treated with NdeI and SmaI, and cloned into plasmid pYCY_007.
pYCY_316	pBBRMCS plasmid with *pBAD-ss-lpp-ompA$_{46\sim159}$, Kan^R*	PCR fragments were amplified with primers 180 and 166, using pYCY_294 as a template. The PCR products were treated with BglII and XhoI, and cloned into plasmid pYCY_037.
pYCY_319	pBBRMCS plasmid with *pBAD-ss-lpp-ompA$_{46\sim159}$-rfp-gbp, Kan^R*	PCR fragments were amplified with primers 222 and 223, using pYCY_294 as a template. The PCR products were treated with EcoRI and BamHI, and cloned into plasmid pYCY_037.
pYCY_320	pBBRMCS plasmid with *pBAD-ss-lpp-ompA$_{46\sim159}$-gbp, Kan^R*	PCR fragments were amplified with primers 222 and 224, using pYCY_294 as a template. The PCR products were treated with EcoRI and BamHI, and cloned into plasmid pYCY_037.

Plasmids	Relevant genotype	Construction and source
pYCY_393	pBBRMCS plasmid with *pBAD-ss'-s7-iga , Kan^R*	PCR fragments were amplified with primers 4 and 273, using pYCY_071 as a template. The PCR products were treated with SmaI and BamHI, and cloned into plasmid pYCY_071.

Table S3 Primers used in this study

Primers	Sequence
004_biobrick rv	cgttttatttgatgcctggagatcc
009_fhuA EcoRI fw	ttttgaattcaaaagatctagatcttttaagaaggagatataatggc
010_fhuA XhoI rv	ttttctcgagtcaagagtccggagaagagccagaaacgaagcggaa
011_rfp NdeI fw	ttttcatatggcgagtagcgaagacgttatcaaaga
012_rfp-gbp BamHI rv	ttttggatccttaagagtccggagaagagccagaaacagcaccggtgga
023_ ompA NdeI fw	ttttcatatgaaaaagacagctatcgcgattgcagt
024_ompA159 gbp SmaI BamHI rv	ggatccttaagagtccggagaagagccagaaaccccggggttgtccggacg
035_gbp oligo NdeI/BamHI	tatggtttctggctcttctccggactcttaag
036_gbp oligo com NdeI/BamHI	gatccttaagagtccggagaagagccagaaacca
040_ompA-gbp rfp NdeI rv	ttttcatatgagagtccggagaagagccagaa
041_ompA-rfp NdeI rv	ttttcatatggttgtccggacgagtgccgatg
042_ompA-gbp-rfp BglII fw	ttttagatcttttaagaaggagatataatgaaaaagacagctatcgcgattg
111_s7 oligo NdeI/XhoI	tatgtcgtcctttccgcagccgaactaac
112_s7 oligo NdeI/XhoI	tcgagttagttcggctgcggaaaggacgaca
165_ompA(46-159)BglII fw	ttttagatcttttaagaaggagatatagctagctctagaaacccgtatgttggcttgaaatg
166_ompA(46-159)XhoI rv	ttttctcgaggttgtccggacgagtgccg
167_ss-lpp oligo	ctagcatgaaagctactaaactggtactgggcgcggtaatcctgggttctact

NheI/BamHI 5’-1	
168_ss-lpp oligo NheI/BamHI 5’-2	ctgctggcaggttgctccagcaacgctaaaatcgatcagt

Primers	Sequence
169_ss-lpp oligo NheI/BamHI 3’-1	ctagactgatcgattttagcgttgctggagcaacctgccagcagagtagaacc
170_ss-lpp oligo NdeI/BamHI 3’-2	caggattaccgcgcccagtaccagtttagtagctttcatg
171_ss'-gbp oligo NdeI/SmaI 5’-1	tatgaaatacctgctgccgaccgctgctgctggtctgctgctcctcgctgccca
172_ss'-gbp oligo NdeI/SmaI 5’-2	gccggcgatggccgtttctggctcttctccggactctccc
173_ss'-gbp oligo NdeI/SmaI 3’-1	gggagagtccggagaagagccagaaacggccatcgccggctgggcagcgagg
174_ss'-gbp oligo NdeI/SmaI 3’-2	agcagcagaccagcagcagcggtcggcagcaggtatttca
180_ss-lpp BglII fw	ttttagatcttttaagaaggagatatacatatgaaagctactaaactggtactggc
222_ss-lpp EcoRI fw	ttttgaattctttaagaaggagatatacatatgaaagctactaaactggtactggc
223_rfp-gbp BamHI rv	ttttggatccttaagagtccggagaagagccagaaacagcaccggtggagtgacgac
224_ompA-gbp BamHI rv	ttttggatccttaagagtccggagaagagccagaaacgttgtccggacgagtgccgat
237_ompA BamHI rv	ttttggatccttacccggggttgtccggac
273_s7 SmaI fw	ttttcccgggtcgtcctttccgcagccgaacggttctggttctaccatggggag

Table S4 The number of gold-nanoparticles labeling observed on cell surface

	FhuA-GBP	OmpA-GBP	ss-Lpp-$OmpA_{46\sim159}$-GBP	ss'-GBP-IgA
Number of particle per cell	**35±9**	**35±20**	**27±6**	**57±16**

n=20

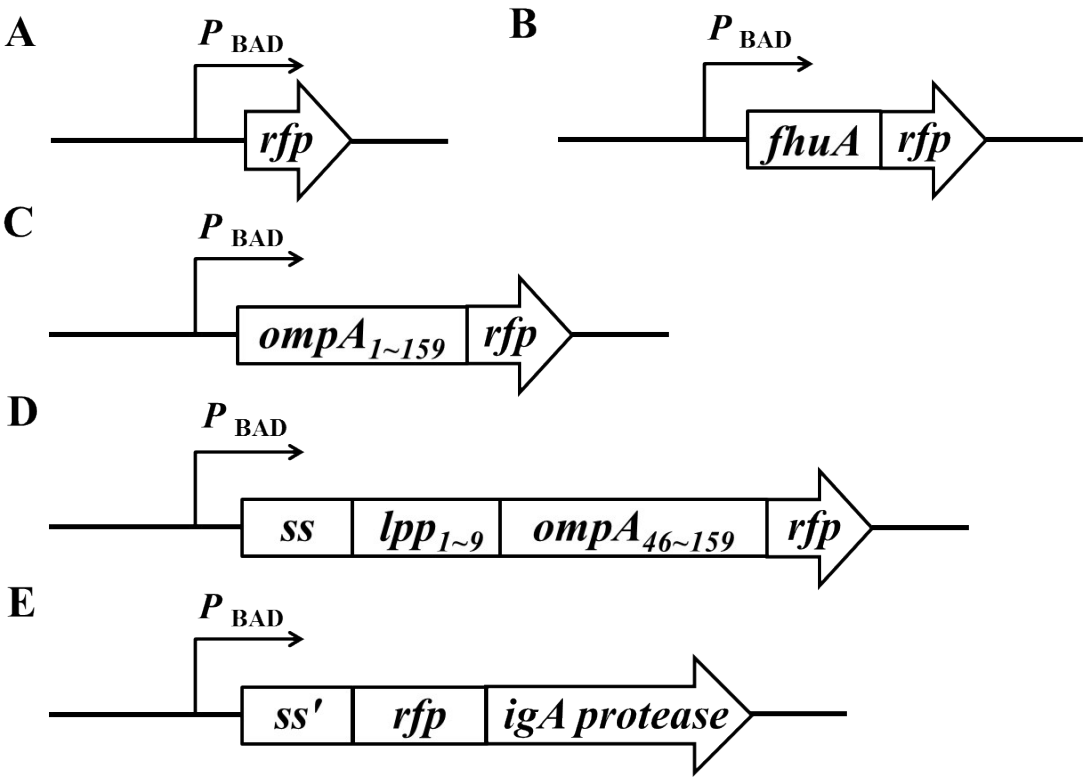

Figure S1 Schematic representation of the constructs used in this work. The arabinose-inducible (A) RFP only, (B) FhuA-RFP, (C) $OmpA_{1\sim159}$-RFP, (D) ss (signal-sequence of Lpp)-Lpp-$OmpA_{46\sim159}$-RFP, and (E) ss' (signal-sequence of pelB)-RFP-IgA expression.

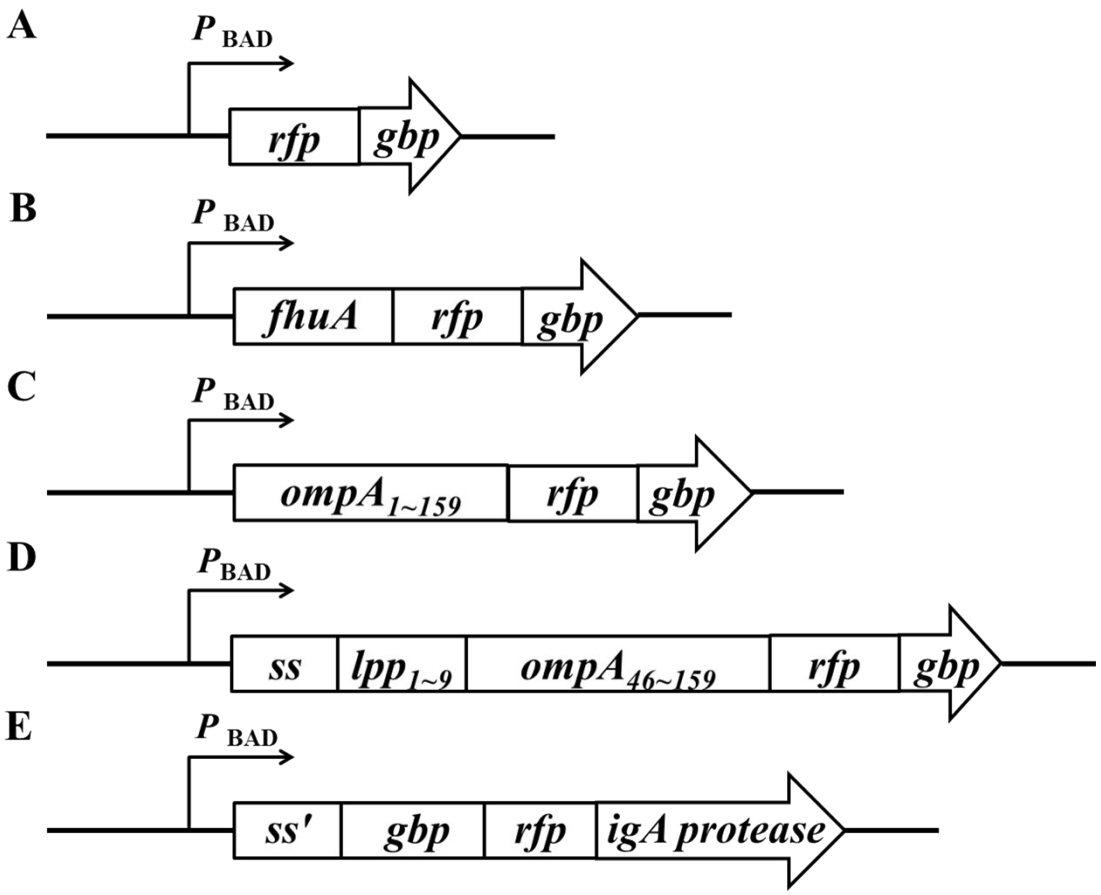

Figure S2 Schematic representation of the constructs used in this study. The gold-binding-peptide (GBP) is fused with surface-display-carrier-RFP.

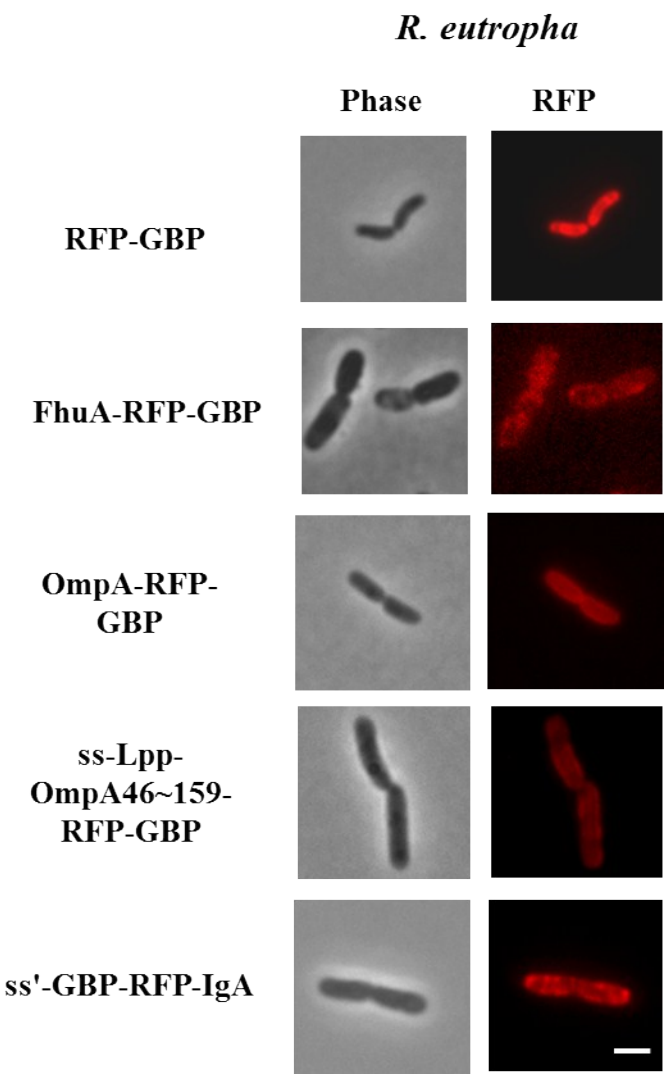

Figure S3 Fluorescence micrograph of *R. eutropha* expressing RFP-GBP and surface display carrier-RFP-GBP (Scale bar: 2 μm).

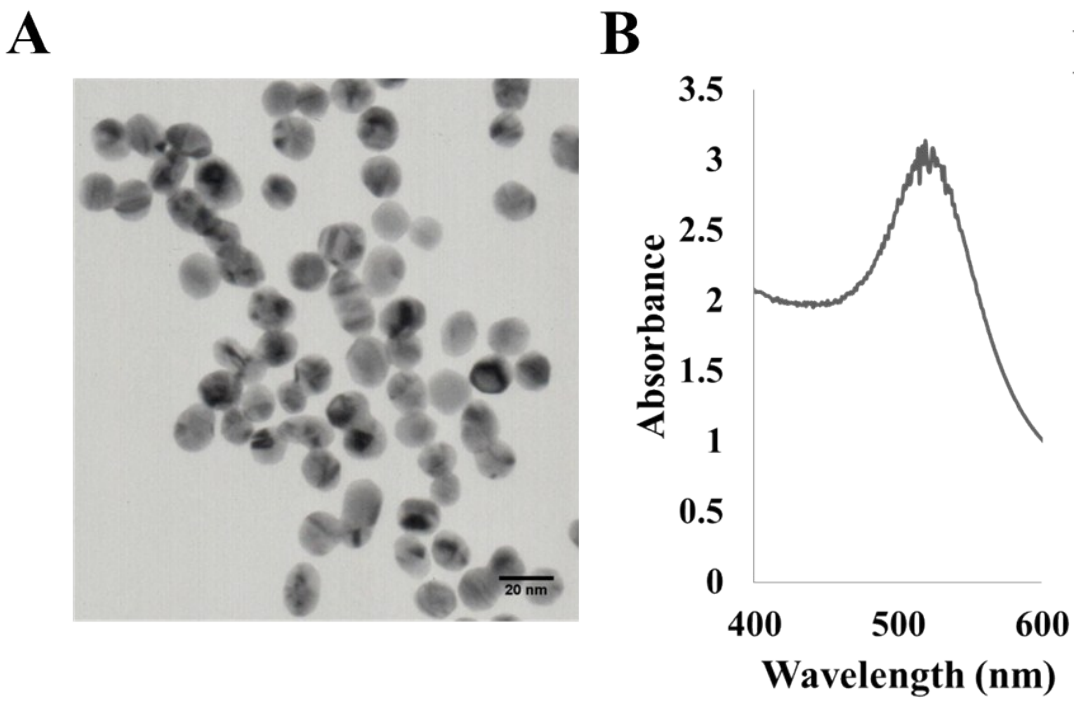

Figure S4 Characterization of Au nanoparticles synthesized for GBP labeling.
(A) TEM image of Au nanoparticles, and (B) UV/Vis spectrum.

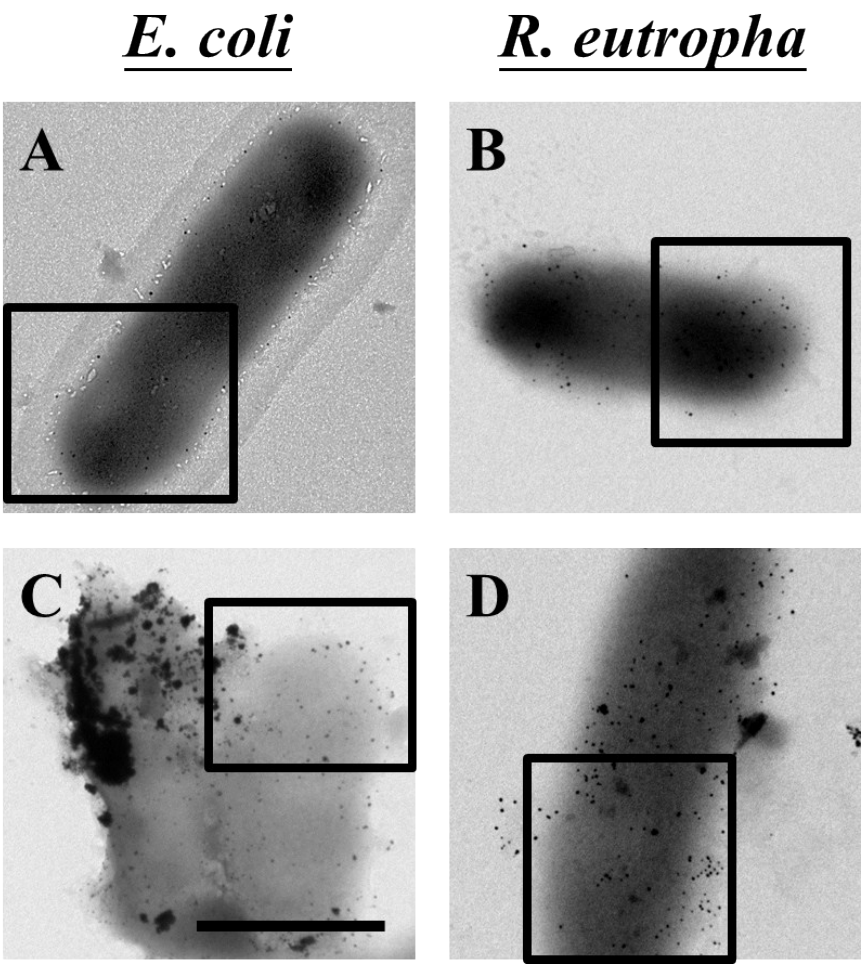

Figure S5 The representative TEM images at a lower magnification of the *E. coli* and *R. eutropha* cells displaying (A)(B) Au and (C)(D) Pt NPs (Scale bar: 1 μm).

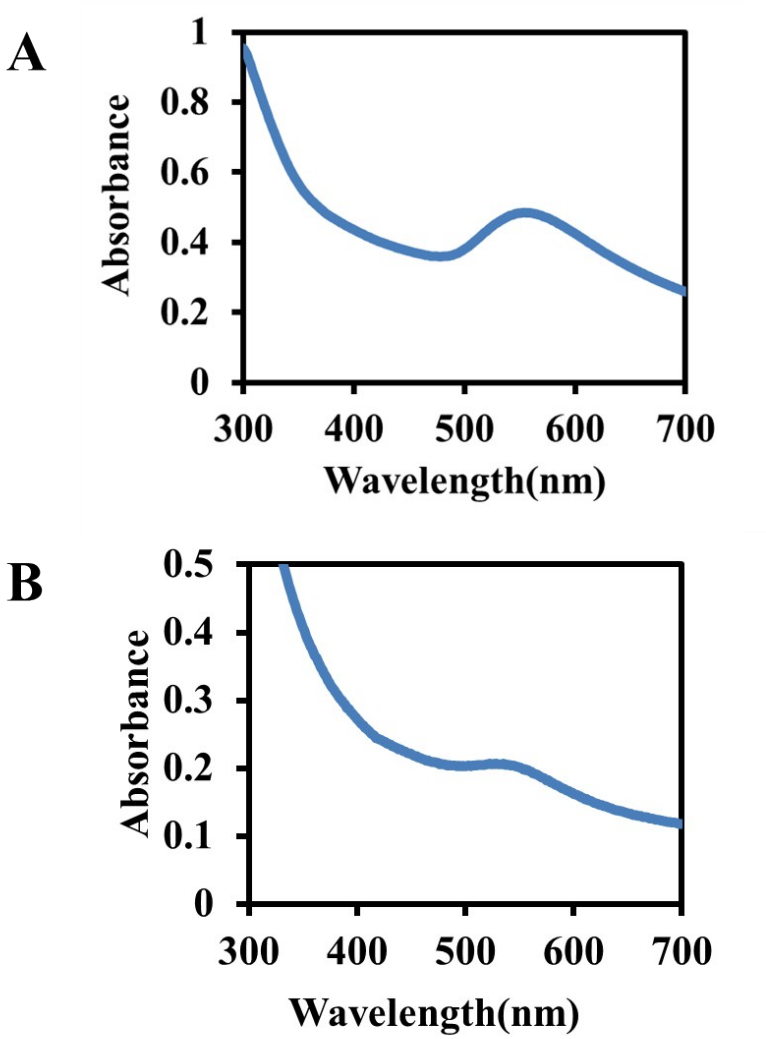

Figure S6 UV-vis spectra of the gold NPs synthesized by (A) *E. coli* (B) *R. eutropha* expressing GBP-IgA.

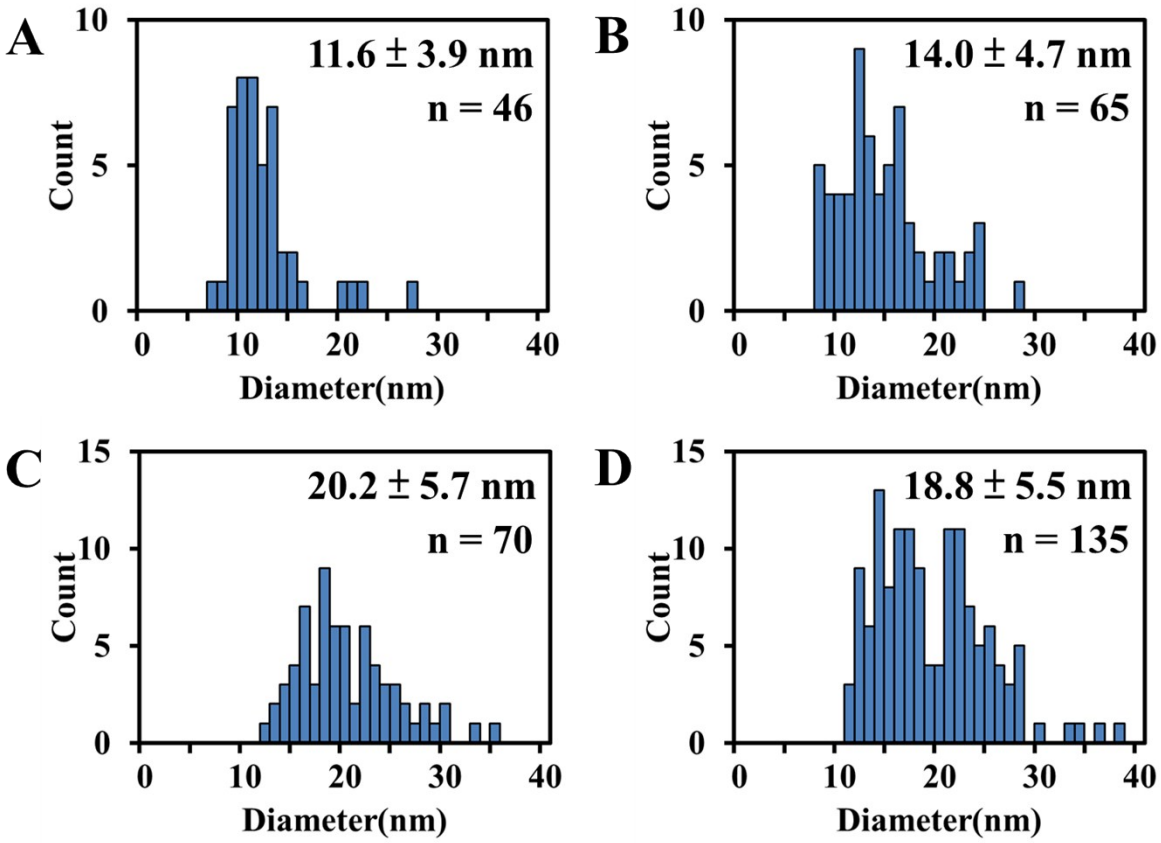

Figure S7 Histograms of size distribution analysis of Au and Pt NPs from (A) Figure 3A, (B) Figure 3B, (C) Figure 3C, and (D) Figure 3D.

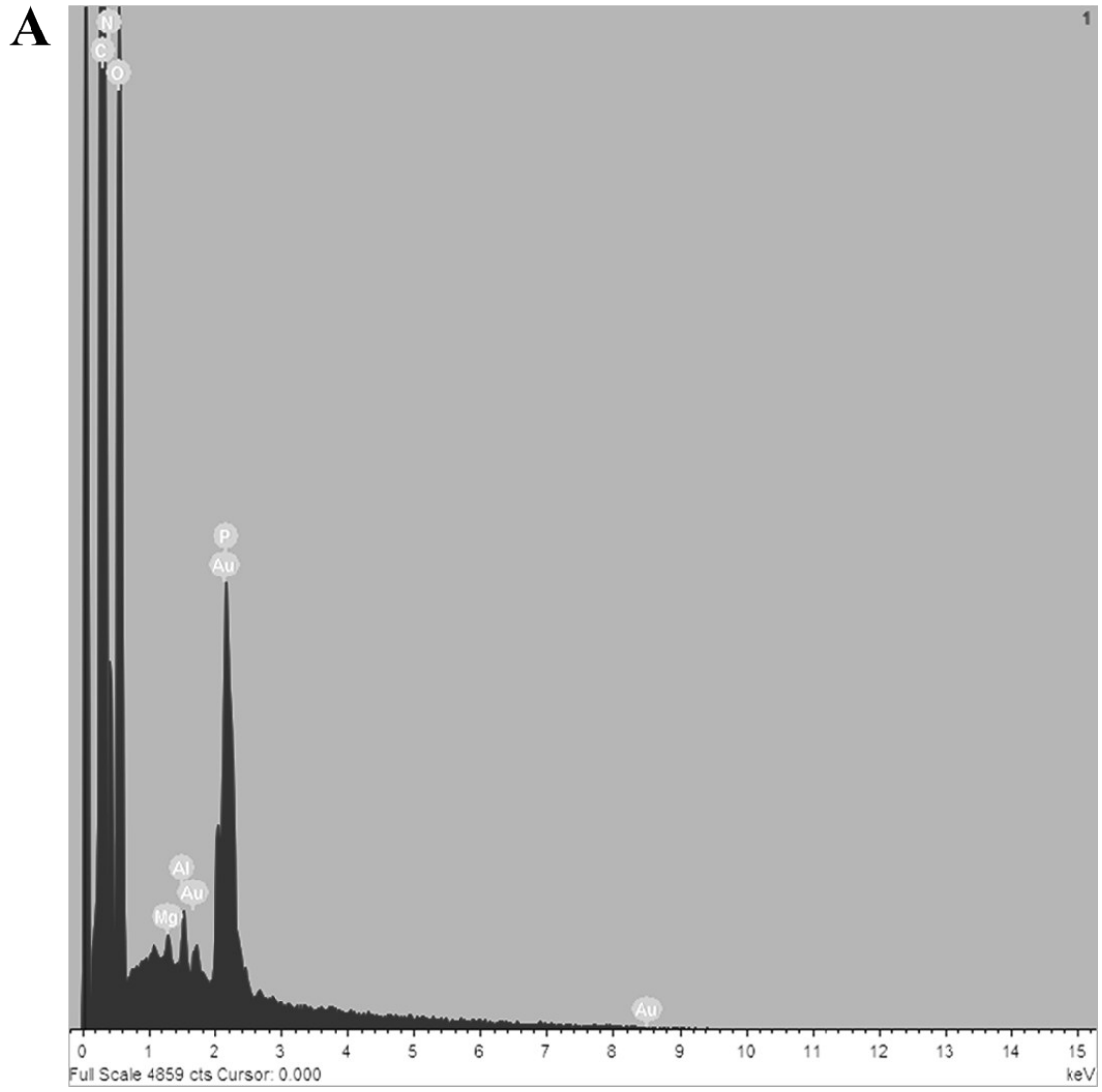

Figure S8 EDS analysis of Au (A and B) and Pt (C and D) nanoparticles synthesized by a recombinant strain of (A, C) *E. coli* and (B, D) *R. eutropha*.

B

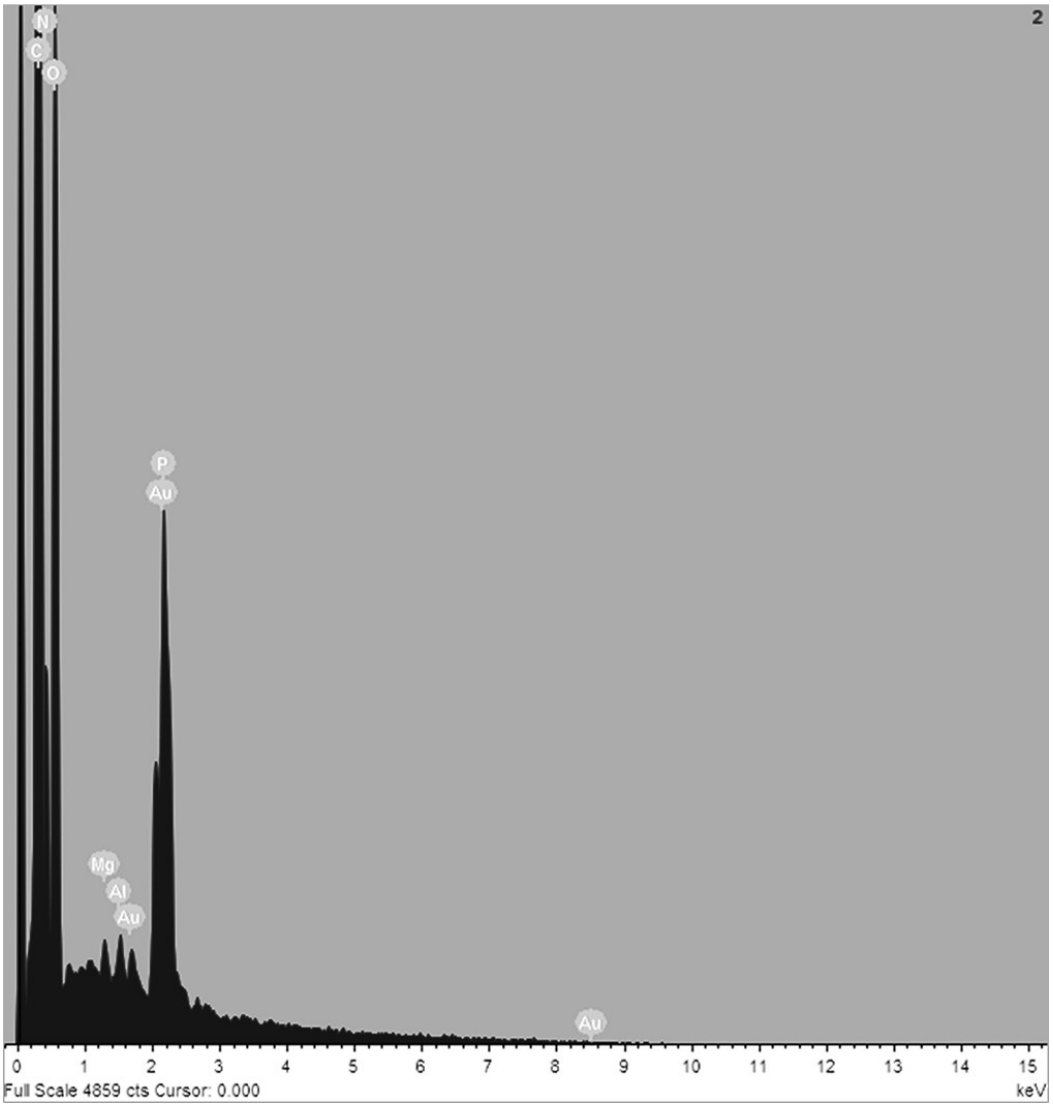

Figure S8 (continued)

C

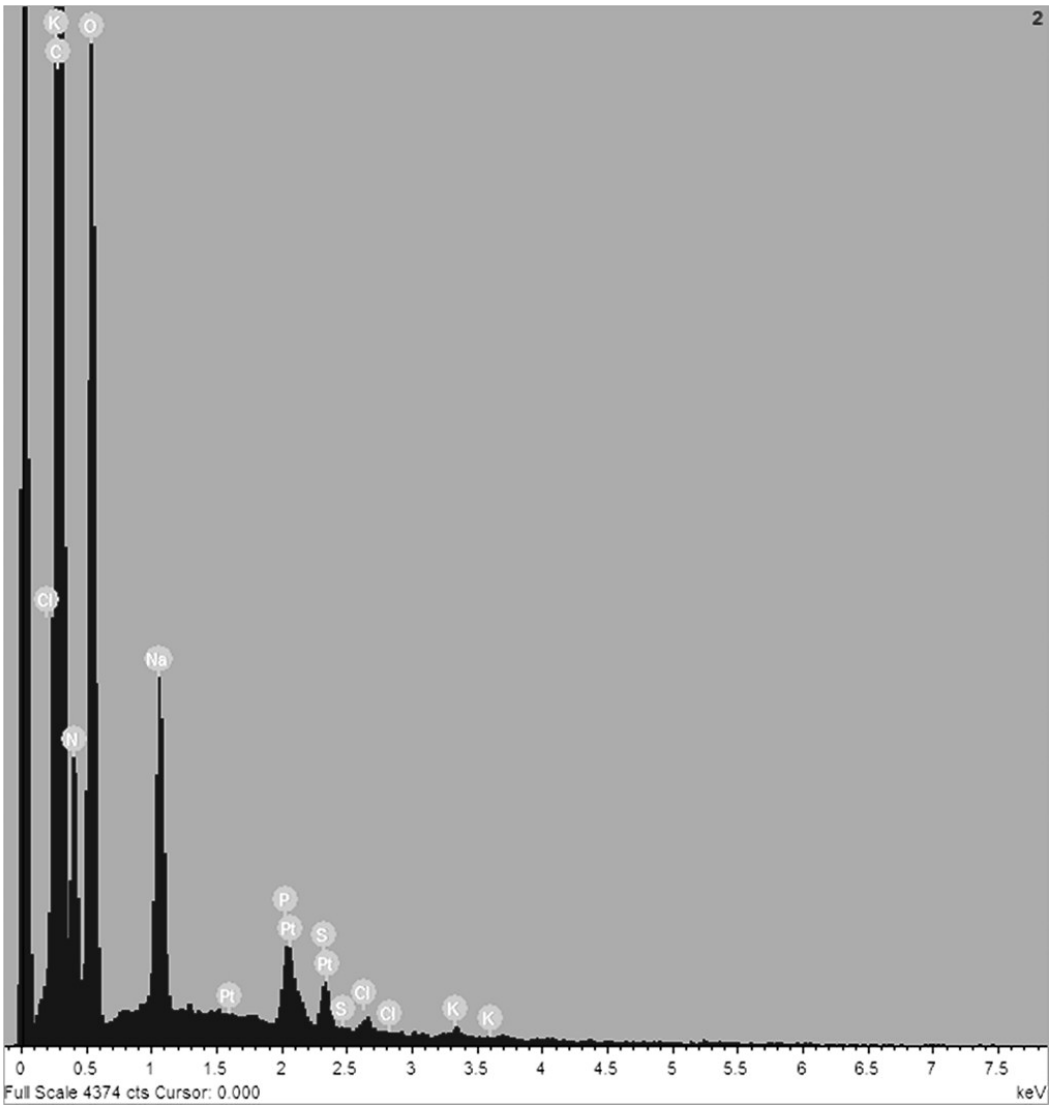

Figure S8 (continued)

D

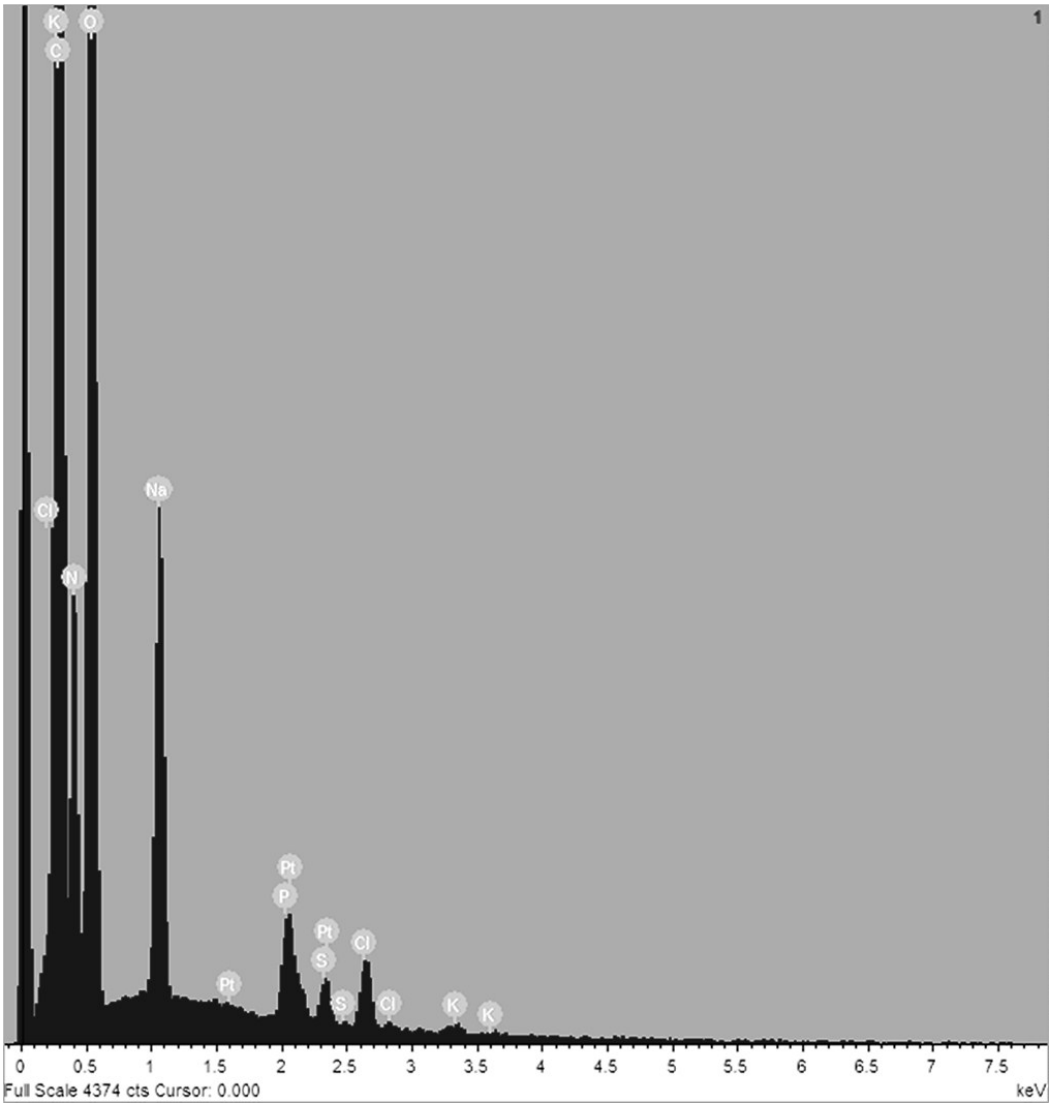

Figure S8 (continued)

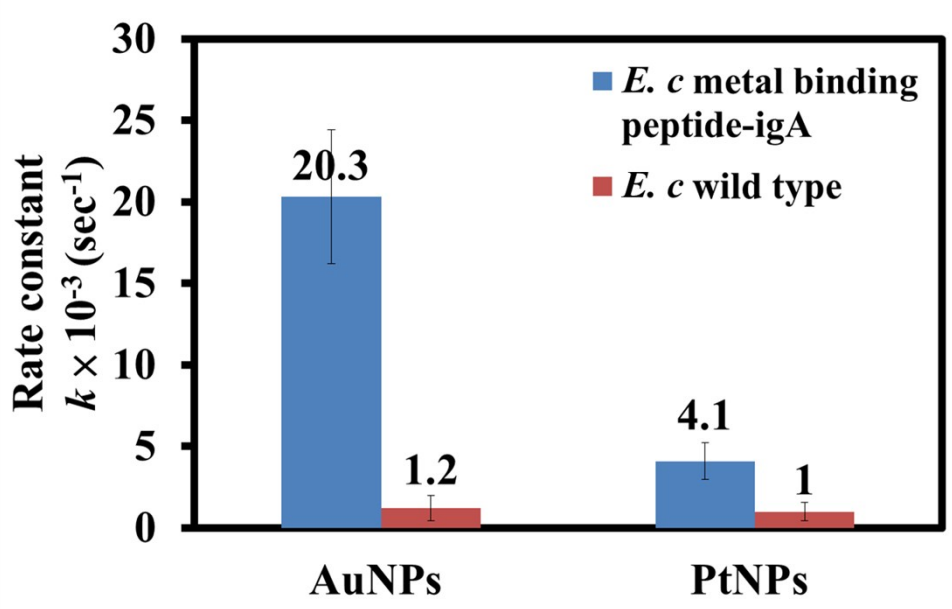

Figure S9 The comparison of rate constants for the reduction of 4-nitroaniline by wild type *E. coli* and metal binding peptide-IgA cells anaerobically treated with 1mM of Au^{3+}/Pt^{4+}.

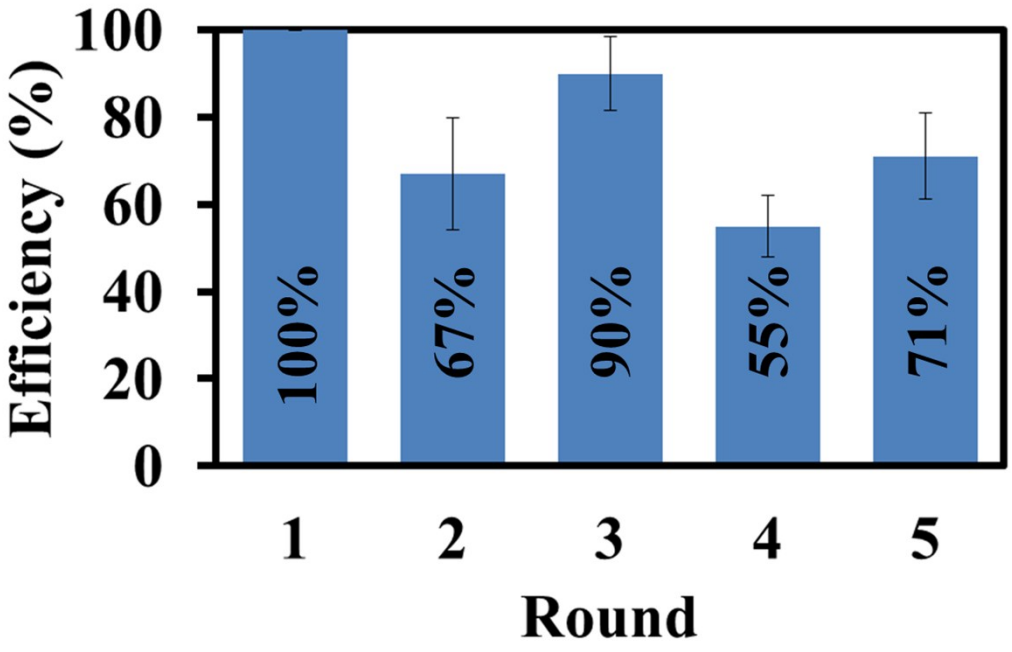

Figure S10 The rate constants of the *E. coli* AuNP whole-cell biocatalyst for the reduction of 4-nitroaniline for five rounds of reuse (efficiency is relative to the rate constant of the first round).

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2. **J.-W. Jian and C.-C. Huang, *Chemistry – A European Journal*, 2011, 17, 2374-2380.**
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