Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Biosynthesis and display of diverse metal nanoparticles by recombinant *Escherichia coli*

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Materials and Methods

Bacterial strains, growth conditions, and cloning

Rhizobium etli cells were grown at 30°C in TY medium overnight with 200 rpm agitation. *E. coli* cells were cultured in lysogeny broth medium at 37°C (250 rpm). Strains, plasmids, and primers used are listed in Table S1, S2 and S3. Cloning was performed in *E. coli* in a standard technique.

Melanin-mediated gold nanoparticles synthesis in *Rhizobium etli*

Rhizobium etli were inoculated from single colonies and freshly diluted (2%) in TY medium supplemented with 1 mM 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA, Acros Organics) for overnight. Cells were then centrifuged at 12,225 g for 15 min and the supernatant was retained, followed by adding 2mM Au^{3+} ($HAuCl_4 \cdot 3H_2O$, Acros Organics).

Expression of *melA* in *E. coli* for various nanoparticles synthesis

Recombinant *E. coli* DH5α strain, YCY_203, was inoculated from single colonies and freshly diluted (2%) in M9 medium supplemented with 0.2% glucose and kanamycin (50 μg/mL) and induced with 100 nM of IPTG and 1 mM L-DOPA for 12 hours. Then, cells were centrifuged at 12,225 g for 15 min and the supernatant was retained for reactions. We then incubated the supernatant with 2.0 mM of Au^{3+} ($HAuCl_4 \cdot 3H_2O$, Acros Organics), Ag^{+} ($AgNO_3$, Acros Organics), Cu^{2+} ($CuCl_2$, Acros Organics), Mn^{2+} ($MnCl_2 \cdot 4H_2O$, Acros Organics), Pt^{4+} ($H_2PtCl_6 \cdot 6H_2O$, Acros Organics) or Ni^{2+} ($NiSO_4 \cdot 6H_2O$, Acros Organics).

Quantification of eumelanin

Purification of melanin produced in liquid cultures was performed following the procedure as described previously.[1-3] Briefly, the supernatant was collected (centrifuged cells at 12,225 g, 15 min). Before measuring optical density at 400 nm, the solutions were adjusted to pH=7. The concentration of melanin was calculated from standard curves of chemically produced melanin (Sigma) as references (Fig. S2).

Size control with different concentrations of metal salt or melanin

The supernatants containing eumelanin were prepared as described above.[1] Then, the eumelanin solution was diluted with deionized water to 0.1X or 0.5X and incubated with 2mM Au^{3+} for reaction. In addition, 0.5, 1.0 and 2.0 mM Au^{3+} ($HAuCl_4 \cdot 3H_2O$) were tested with the overnight eumelanin solution, respectively.

Characterization of nanoparticles

For TEM analysis (JEOL JEM-1200EX II), all samples were placed on carbon-coated copper grids. Elemental analyses were performed on samples that had been previously air-dried on silicon wafers. Field emission scanning electron microscope with INCA X-Max EDS (JEOL JSM-7600F) was used. For EDS acquisition, single specific point on the electron image is selected.

Selected sample image file was imported to Image J for size distribution analysis. We first cropped the part of desired area and adjusted the image brightness. We used the "Threshold" function to remove background information, and left behind the particles. "Analyze particles" function was used to obtain the area of particles. The following formula was used to obtain the diameter of particles. (d, diameter and A, area)

$d = 2\sqrt{A/\pi}$

Antibacterial investigation of EuMel-mediated metal nanoparticles

The supernatants containing eumelanin were prepared as described above. We then incubated the supernatant with 1 mM Au^{3+} ($HAuCl_4 \cdot 3H_2O$) , Ag^{+} ($AgNO_3$) and Pt^{4+} ($H_2PtCl_6 \cdot 6H_2O$). These Au, Ag and Pt nanoparticles were obtained by centrifuged with 12,225 g for 30 min and washed three times with deionized water and dried. Chemical synthesis of silver nanoparticles was prepared as describe previously. Briefly, 1mM of Ag^{+} ($AgNO_3$) was mixed with 2mM of ice-chilled $NaBH_4$ under vigorous stirring. *E. coli* DH10B strain was inoculated from single colonies and freshly diluted (2%) in LB medium supplemented with 150 μg/mL of Au, Ag and Pt nanoparticles for 8 hours, respectively. All samples were sequentially diluted, and 8μL were taken on for colony forming unit test.

Display metal nanoparticles *via* FhuA-GBP of bacteria

Recombinant *E. coli* DH5α strains, YCY_201, were inoculated from single colonies and freshly diluted (2%) in M9 medium supplemented with 0.2% glucose and kanamycin (50 μg/mL) and induced with 100 nM of IPTG, 1 mM L-DOPA and 0.2% arabinose, followed by adding 2mM Au^{3+} ($HAuCl_4 \cdot 3H_2O$) and further cultivation for 2 - 3 h. Cells were harvested by centrifugation at 12,225 g for 1 min and washed three times with deionized water. For TEM analysis, all samples were placed on carbon-coated copper grids and stained with 2% phosphotungstic acid for 10 seconds.

Table S1 Strains used in this study

Strain	Relevant genotype	Construction and source
***E. coli* DH5α**	F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169 hsdR17(rK- mK+) λ⁻	Purchased from Protech Technology
***E. coli* DH10B**	F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG λ⁻	Purchased from Invitrogen
Rhizobium etli	Wild type	Purchased from BCRC (Bioresource Collection and Research Center, Taiwan)
YCY_203	*E. coli* DH5α *pBbE1k-melA*	pYCY_203 transformed into *E. coli* DH5α
YCY_201	*E. coli* DH5α *pBbE1k-melA* and *pKT230-fhuA-gbp*	pYCY_195 and pYCY_203 transformed into *E. coli* DH5α

Table S2 Plasmids used in this study

Plasmids	Relevant genotype	Construction and source
pYCY_002	pBBRMCS plasmid *pTrc-rfp*, *Kan^R*	Yeh *et al*, 2013[4]
pYCY_077	Broad host-range plasmid pKT230, IncQ group, *Cm^R*	Bi *et al*, 2013[5]
pYCY_203	pYCY_002 derivative, *pBbE1k-melA, Kan^R*	PCR fragments were amplified with primers 77-1 and 78-1 from pTrc-melA template[3]. The PCR products were treated with NdeI and XhoI, and cloned into plasmid pYCY_002.
pYCY_063	pBBRMCS plasmid, *pBAD-fhuA-gbp*, *Kan^R*	*gbp* fragments were inserted with oligos 35 and 36 by annealing and cloned into plasmid pYCY_027.
pYCY_195	pYCY_077 derivative, *pKT230-fhuA-gbp, Cm^R*	*fhuA-gbp* fragments were treated with EcoRI and BamHI from pYCY_063, and cloned into plasmid pYCY_077.

Table S3 Primers used in this study

Primers	Sequence
035_GBP oligo NdeI/BamHI	tatggtttctggctcttctccggactcttaag
036_GBP oligo com NdeI/BamHI	gatccttaagagtccggagaagagccagaaacca
077-1_melARe NdeI fw	ttttcatatgccgtggctggtcggcaagc
078-1_melARe_XhoI rv	ttttctcgagttaggcggacactatggctatttctagctttgc

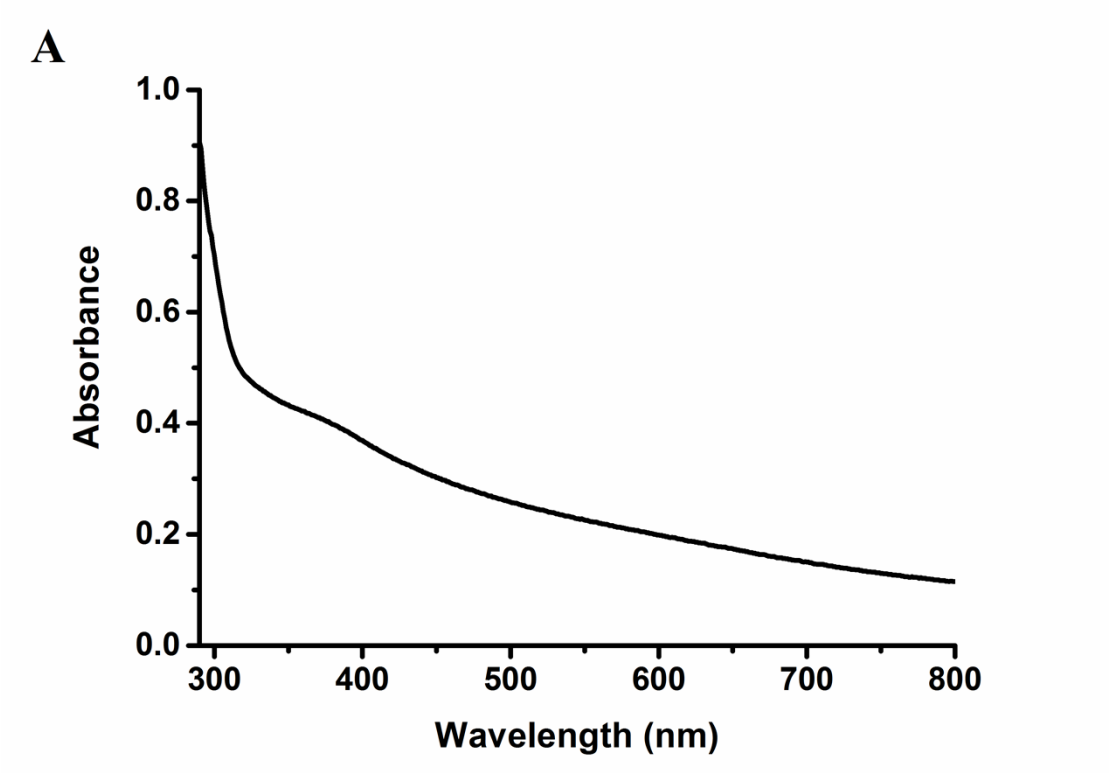
Table S4 List of metal particles synthesized and its average diameters

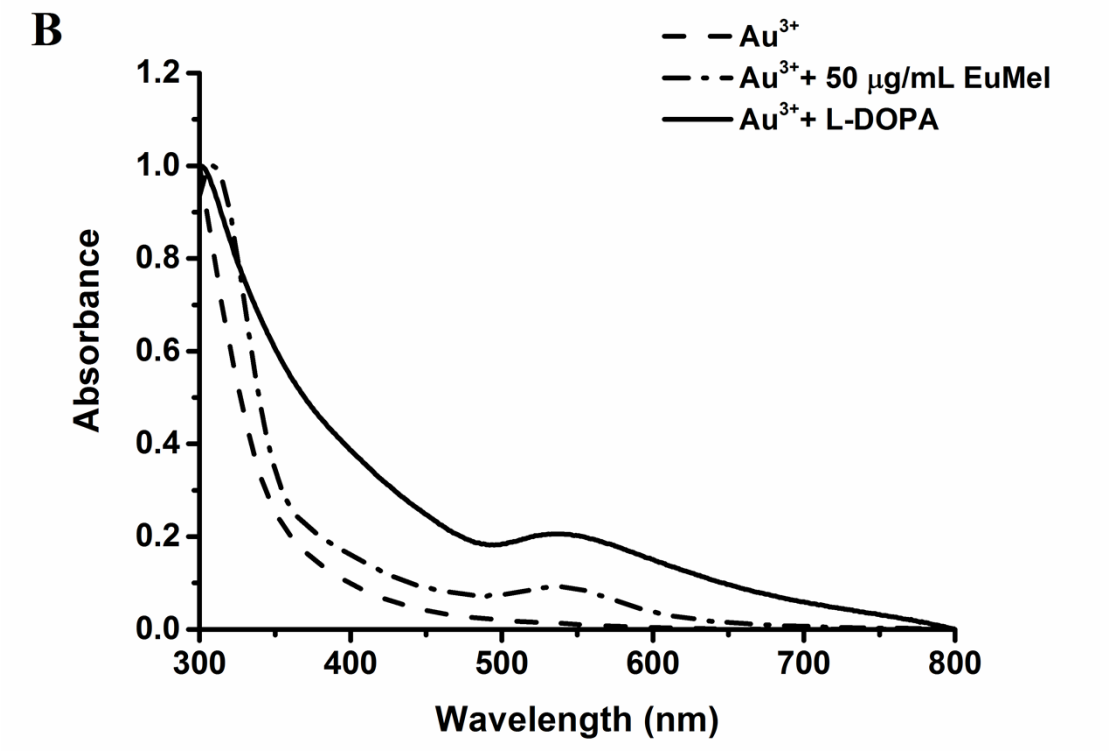
Types of nanoparticles	Au	Ag	Cu
Average diameters (nm)	13.37 ± 4.10	19.89 ± 6.53	11.78 ± 6.60
Types of nanoparticles	Mn	Pt	Ni
Average diameters (nm)	13.65 ± 8.60	14.82 ± 4.74	12.50 ± 6.69

n = 50

Table S5 The average diameters of gold nanoparticles synthesized from different (A) Au^{3+} and (B) eumelanin concentrations.

A	**Eumelanin concentration (μg/mL)**	**69.78 ± 2.02**	**69.78 ± 2.02**	**69.78 ± 2.02**
	Au^{3+} concentration (mM)	**0.5**	**1**	**2**
	Average diameters (nm)	**7.29 ± 2.41**	**9.83 ± 4.32**	**13.37 ± 4.10**
B	**Eumelanin concentration (μg/mL)**	**6.98 ± 0.20**	**34.89 ± 1.01**	**69.78 ± 2.02**
	Au^{3+} concentration (mM)	**2**	**2**	**2**
	Average diameters (nm)	**7.54 ± 3.11**	**10.78 ± 3.39**	**13.37 ± 4.10**


A
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800


B
Au3+
Au3+ + 50 μg/mL EuMel
Au3+ + L-DOPA
Absorbance
Wavelength (nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800

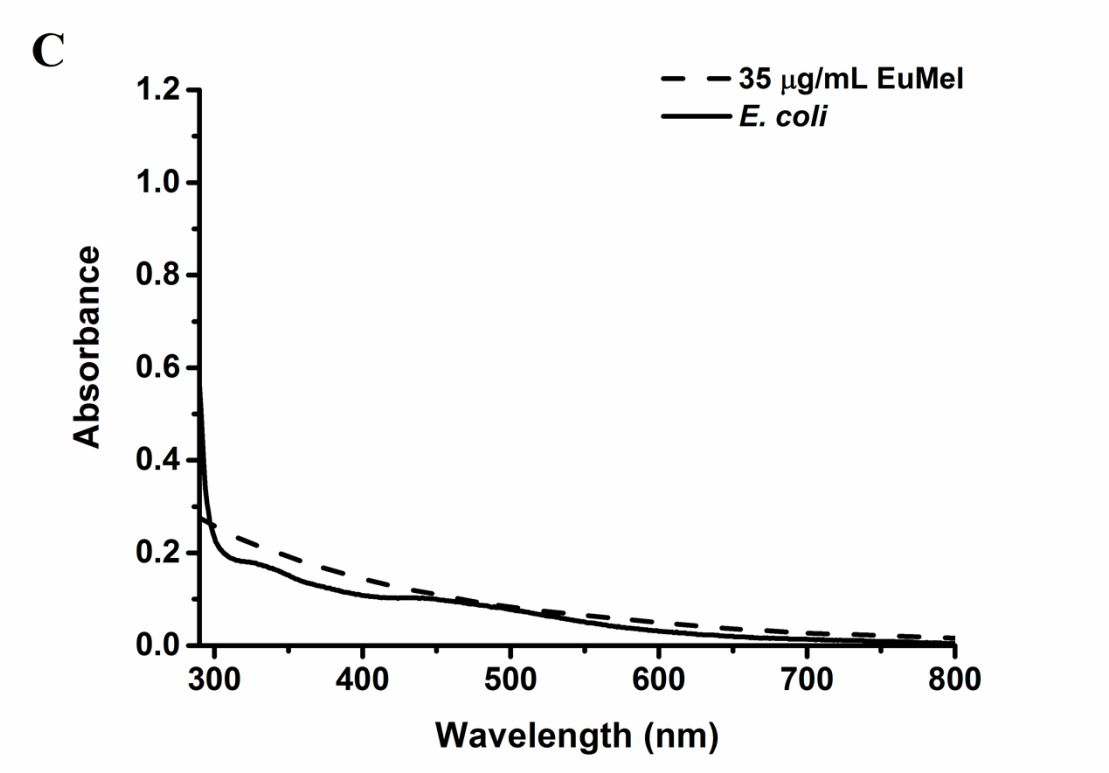


Figure S1 (A) UV/Vis spectrum of eumelanin formed in *R. etli* with 1mM of L-DOPA. (B) The absorbance spectra of solutions containing Au^{3+}, Au^{3+}+ L-DOPA, or Au^{3+}+ 50 μg/mL commercial eumelanin. The data is normalized to [0,1]. (C) The absorbance spectra of solutions containing eumelanin formed in *E. coli* and 35 μg/mL commercial eumelanin.

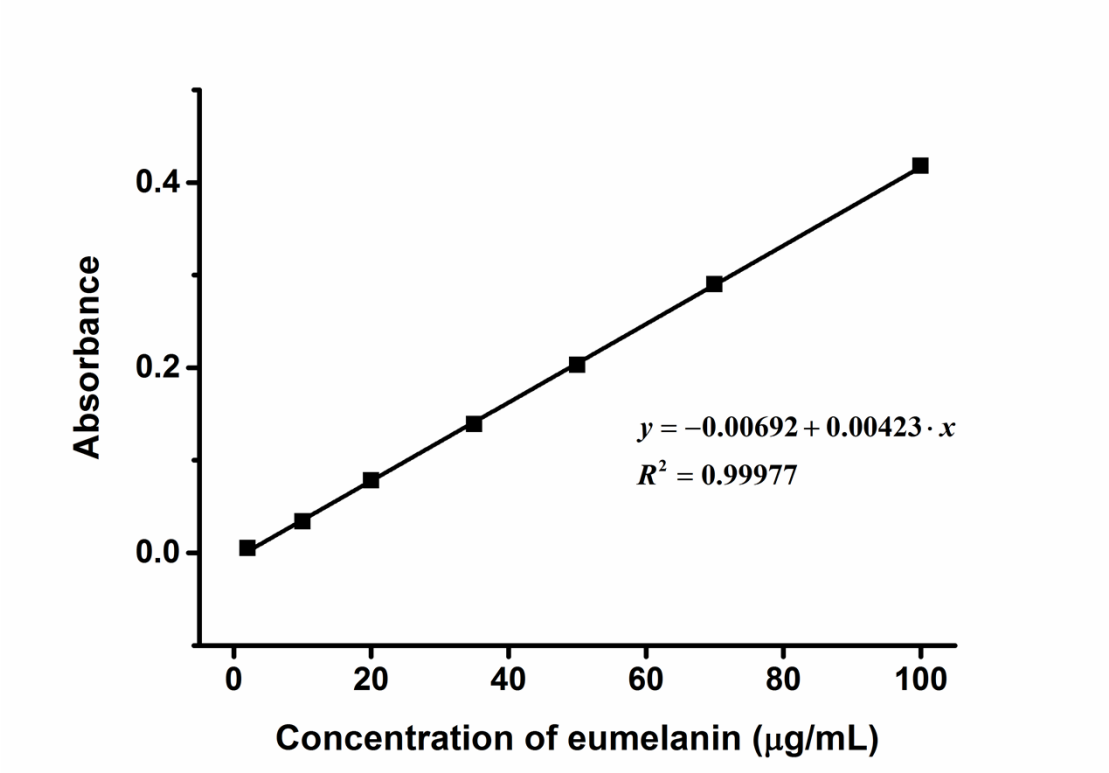


Figure S2 Eumelanin concentration was determined using optical density at 400 nm. The calibration curve was prepared by measuring absorbance of commercial eumelanin in M9 at different concentrations: 2, 10, 20, 35, 50, 70, 100 μg/mL.

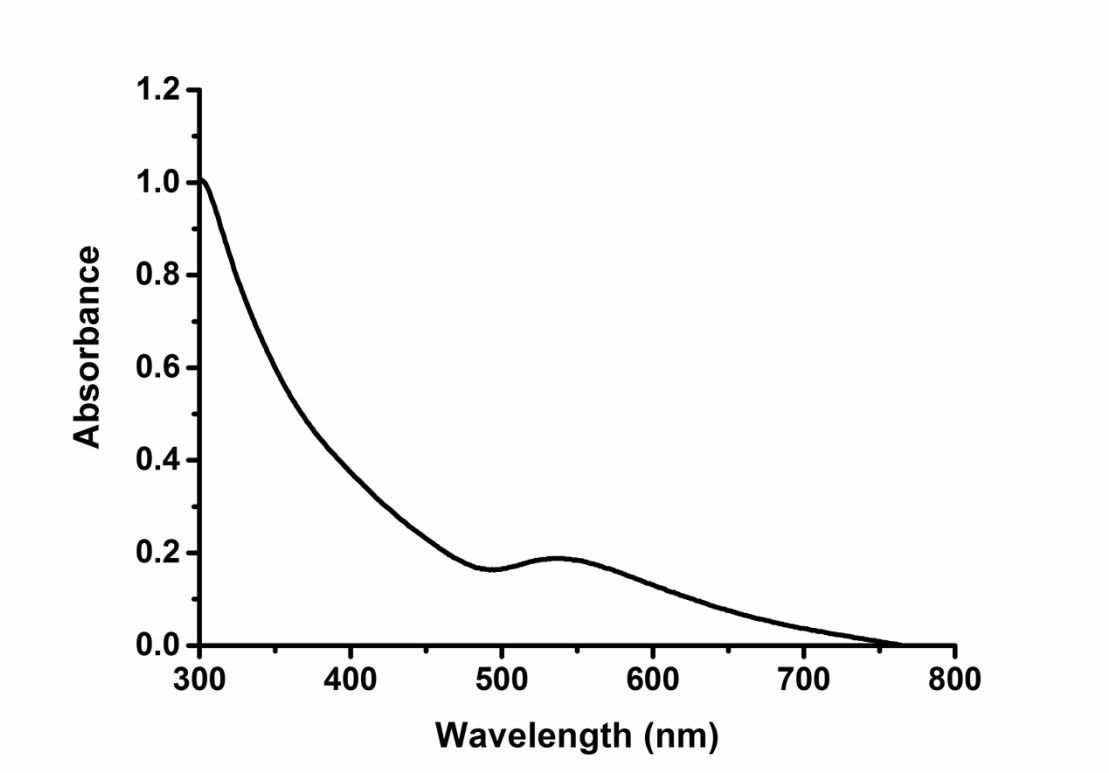
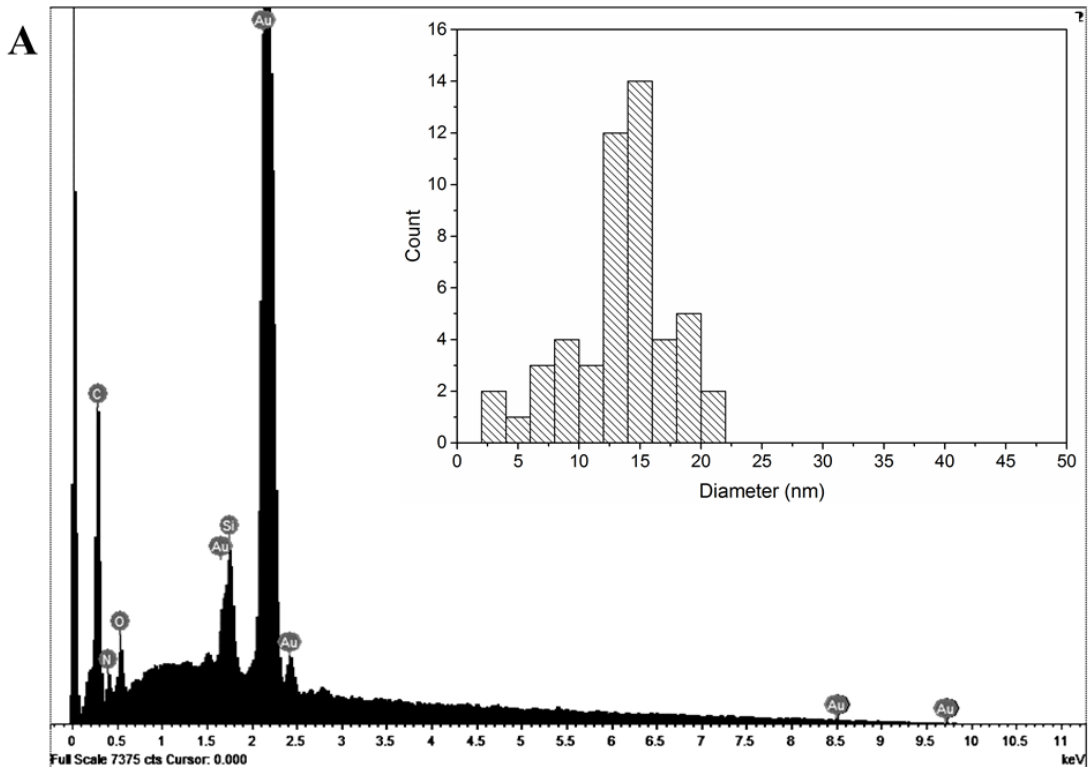
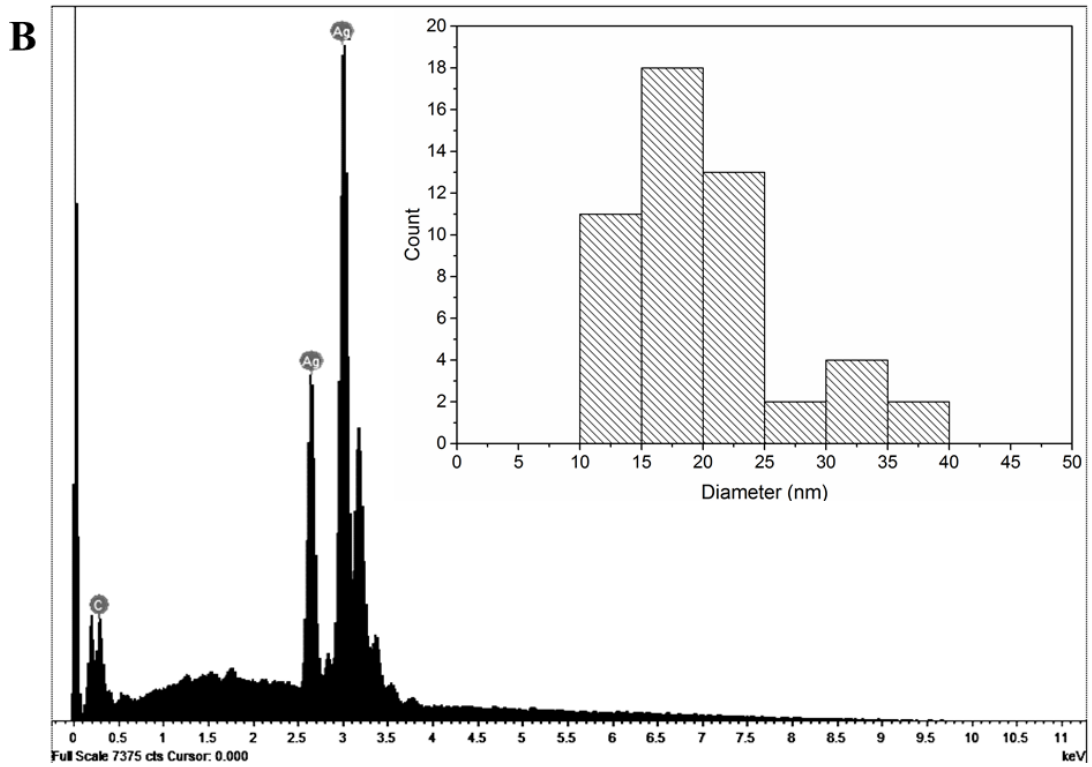
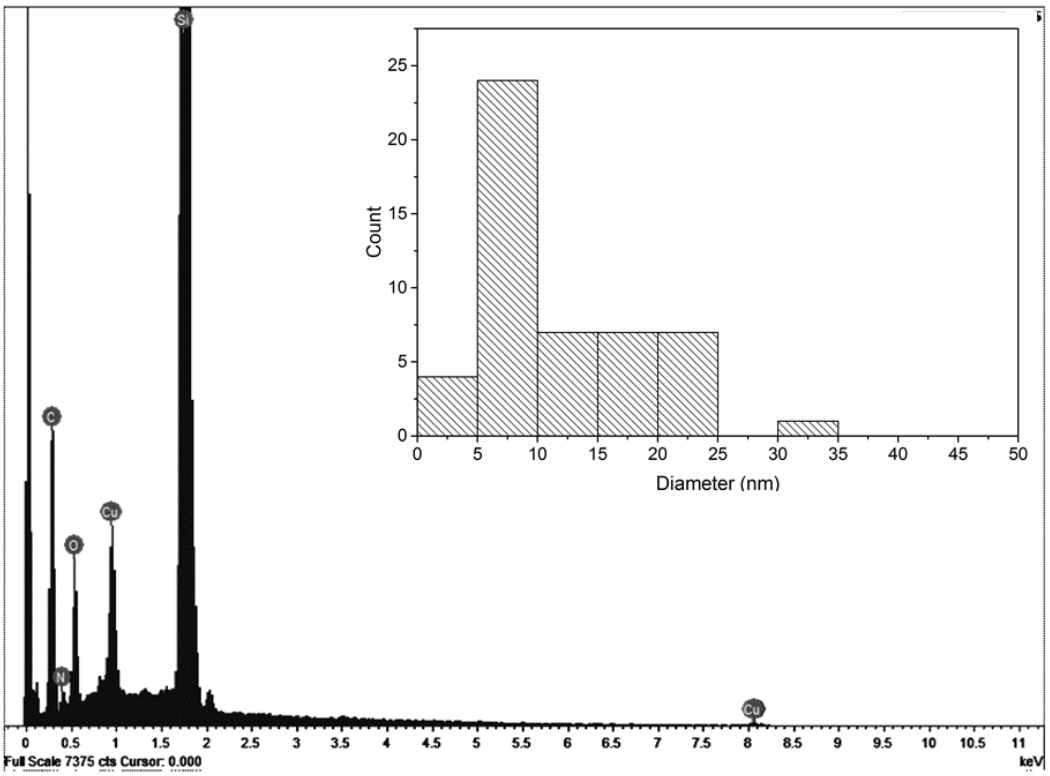


Figure S3 UV/Vis spectrum of Au nanoparticles synthesized by EuMel with 2mM of Au^{3+}.

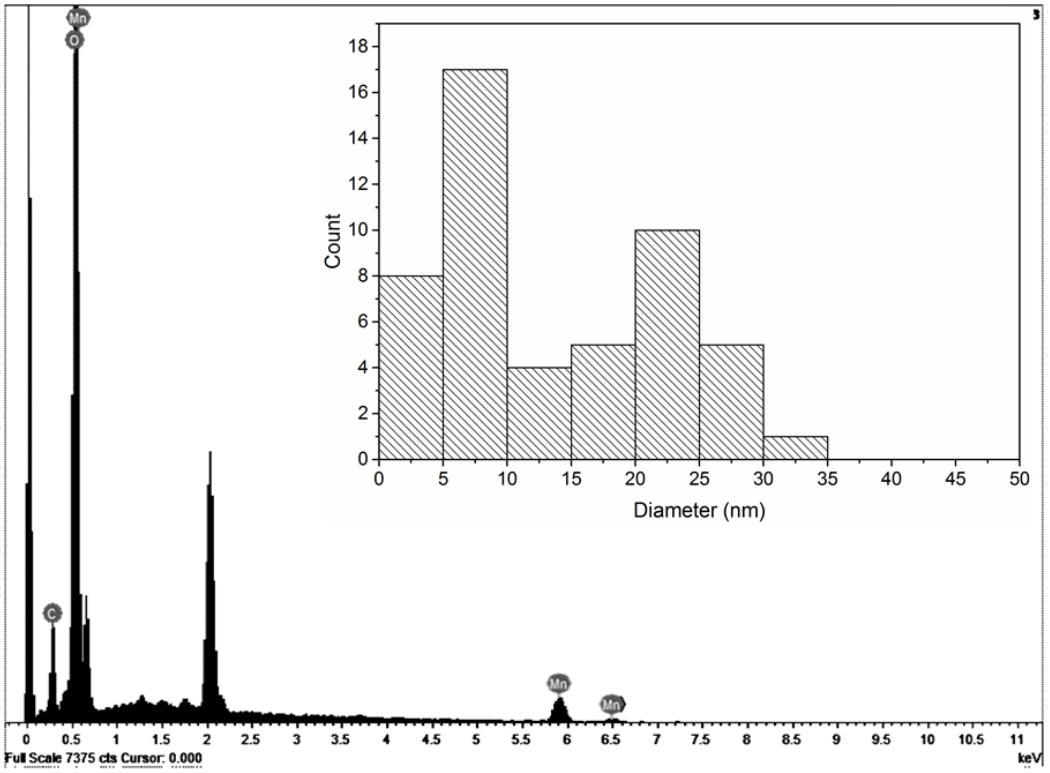

A
Au
C
Si
Au
O
N
Au
Au
Au
Count
Diameter (nm)
Full Scale 7375 cts Cursor: 0.000
keV

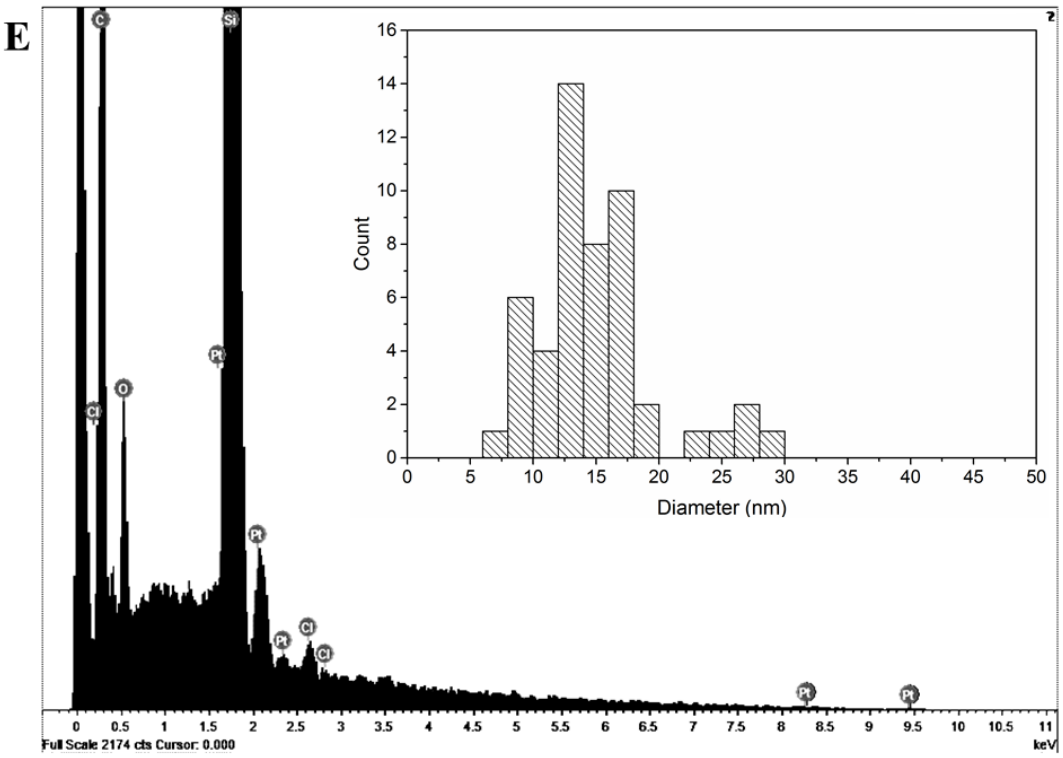

B
Ag
Ag
C
Count
Diameter (nm)
Full Scale 7375 cts Cursor: 0.000
keV

C


Si
C
O
Cu
N
Cu
Count
Diameter (nm)
Full Scale 7375 cts Cursor: 0.000
keV

D


Mn
O
C
Mn
Mn
Count
Diameter (nm)
Full Scale 7375 cts Cursor: 0.000
keV



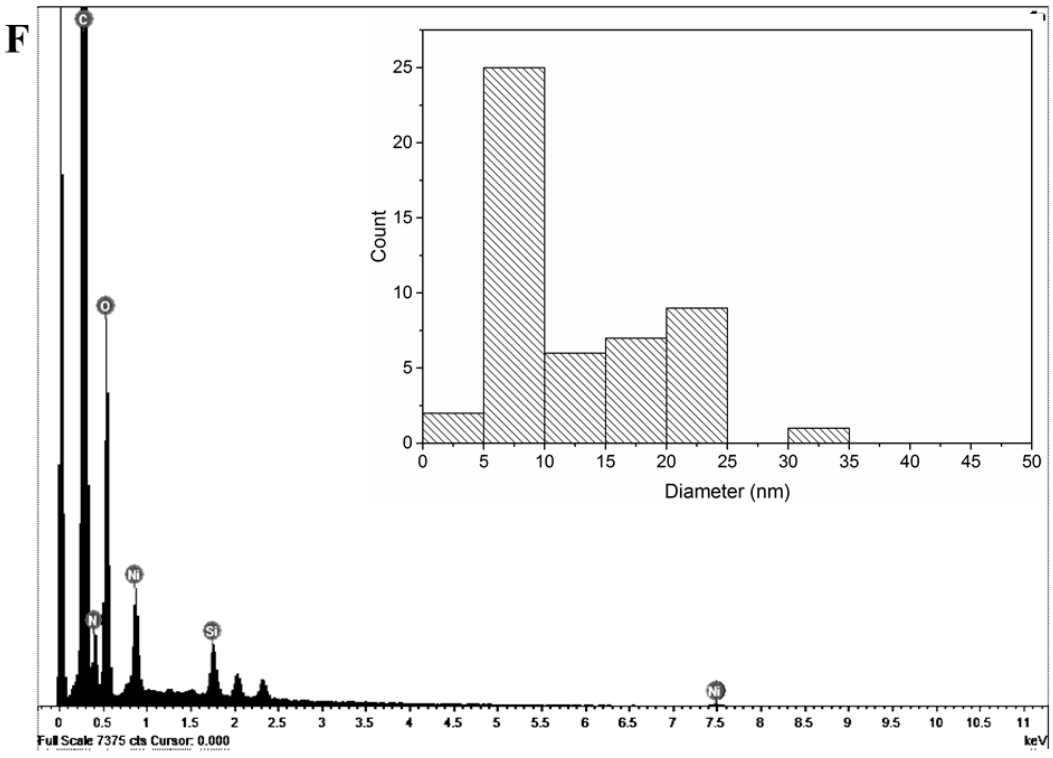
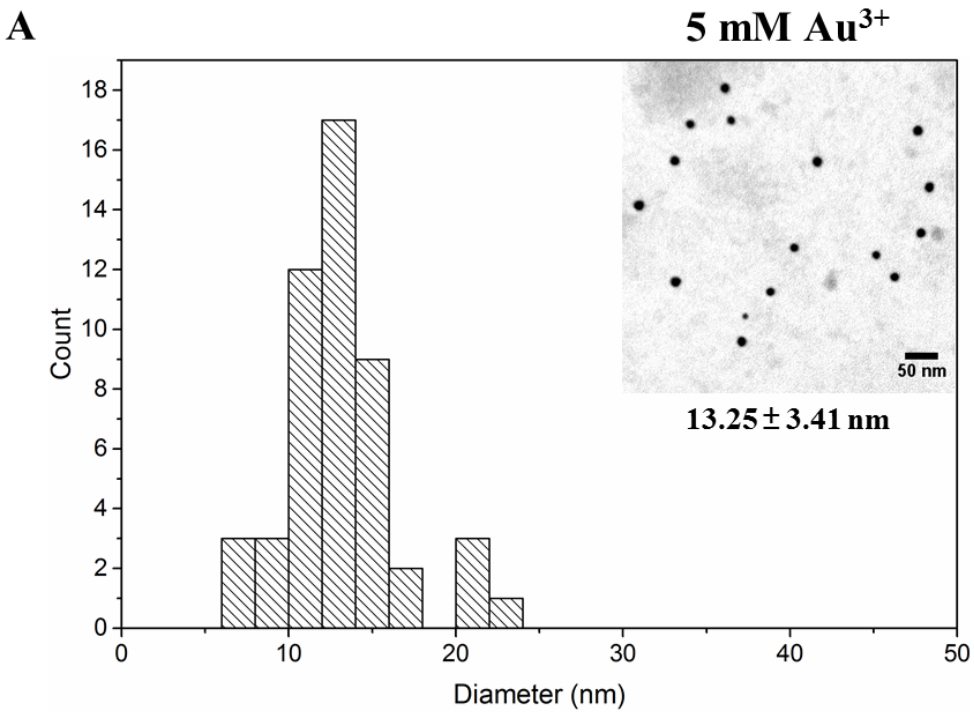
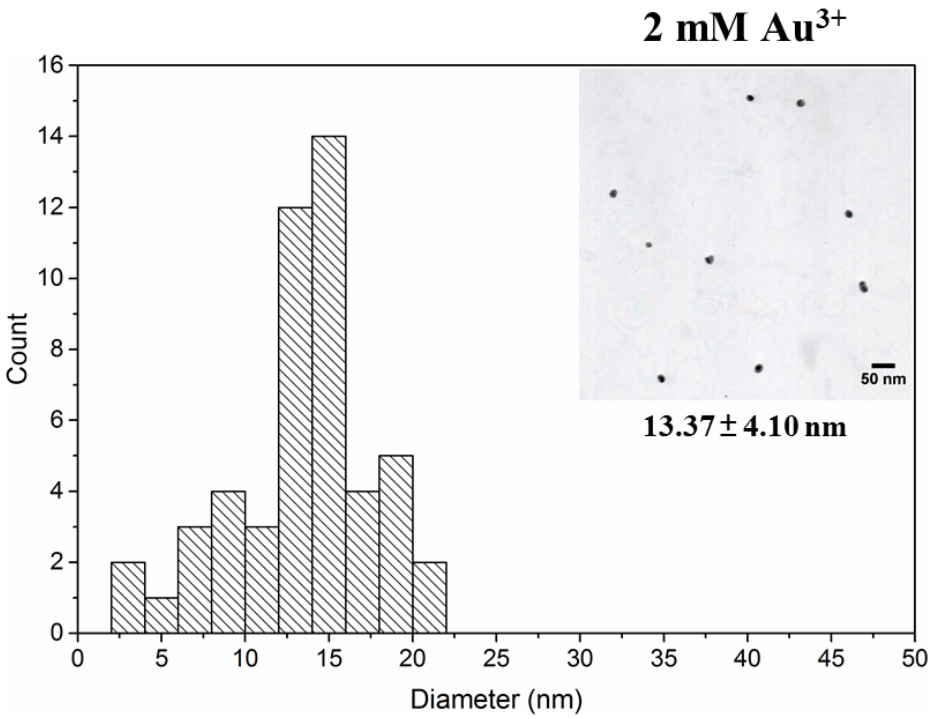
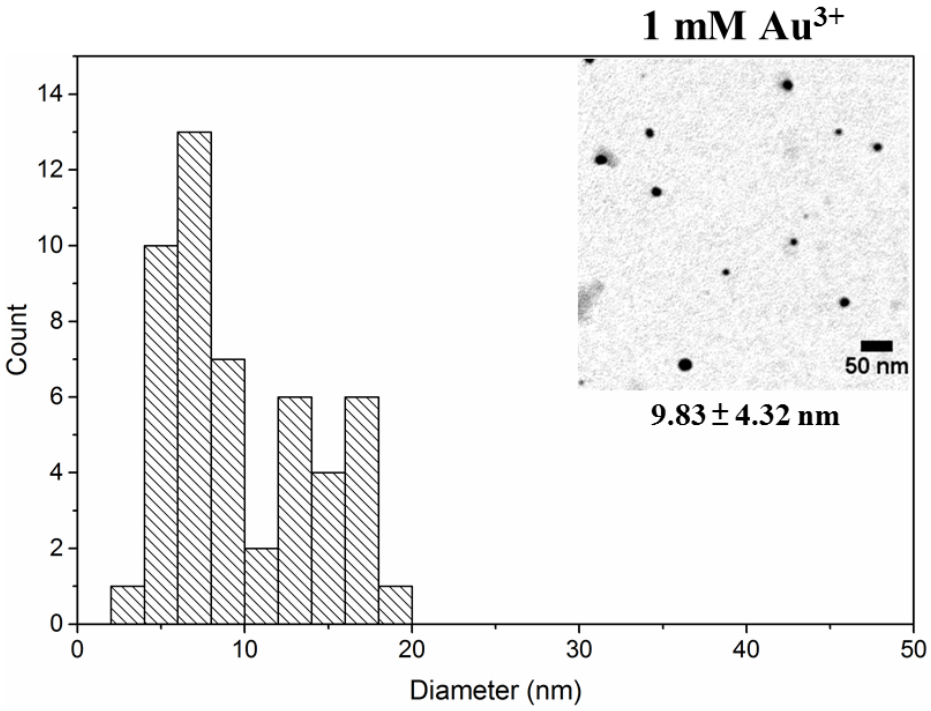
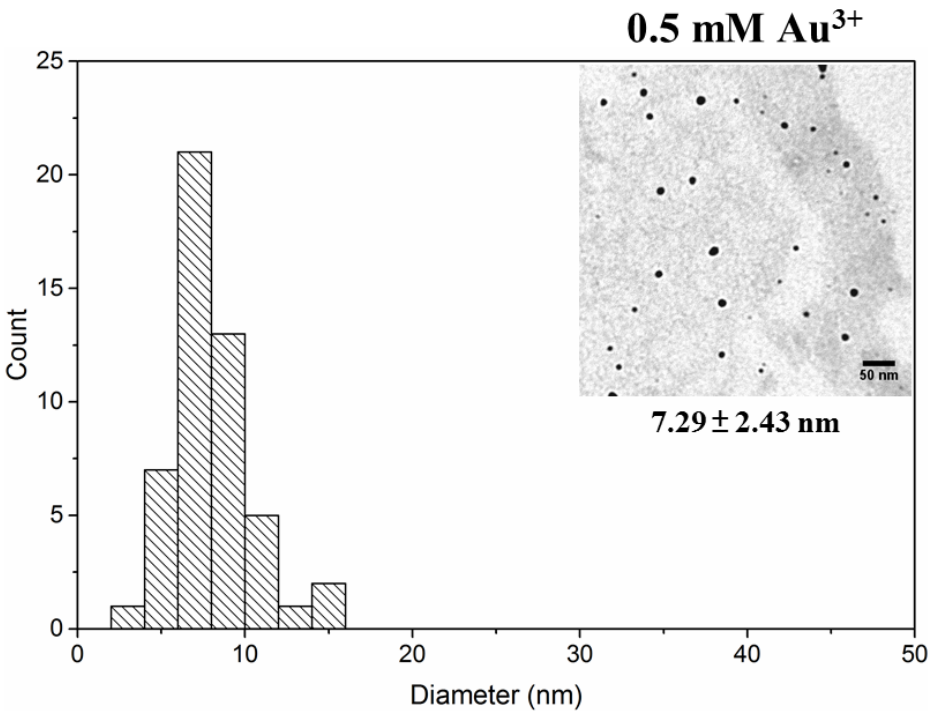


Figure S4 EDS spectra show the presence of (A) Au in Figure 2A, (B) Ag in Figure 2B, (C) Cu in Figure 2C, (D) Mn in Figure 2D, (E) Pt in Figure 2E, (F) Ni in Figure 2F. Inset: Histograms of size distribution of metal nanoparticles from corresponding TEM images.


A
5 mM Au3+
Count
Diameter (nm)
50 nm
13.25 ± 3.41 nm


2 mM Au3+
Count
Diameter (nm)
50 nm
13.37 ± 4.10 nm


1 mM Au3+
Count
Diameter (nm)
50 nm
9.83 ± 4.32 nm


0.5 mM Au3+
Count
Diameter (nm)
50 nm
7.29 ± 2.43 nm

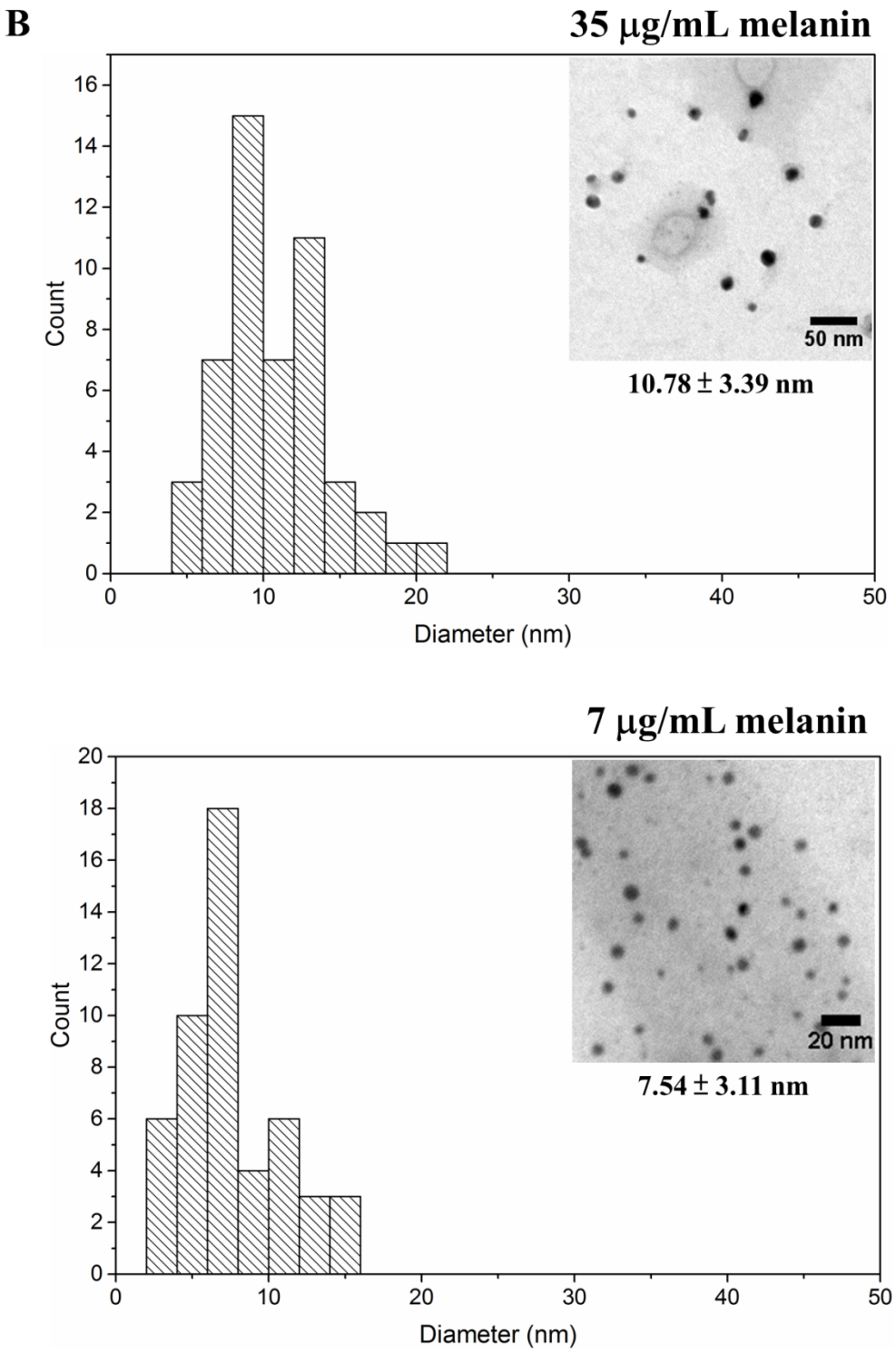


Figure S5 Histograms of size distribution analysis of Au nanoparticles from corresponding TEM images (A) at the different concentrations of Au^{3+} (B) at the different concentrations of eumelanin.

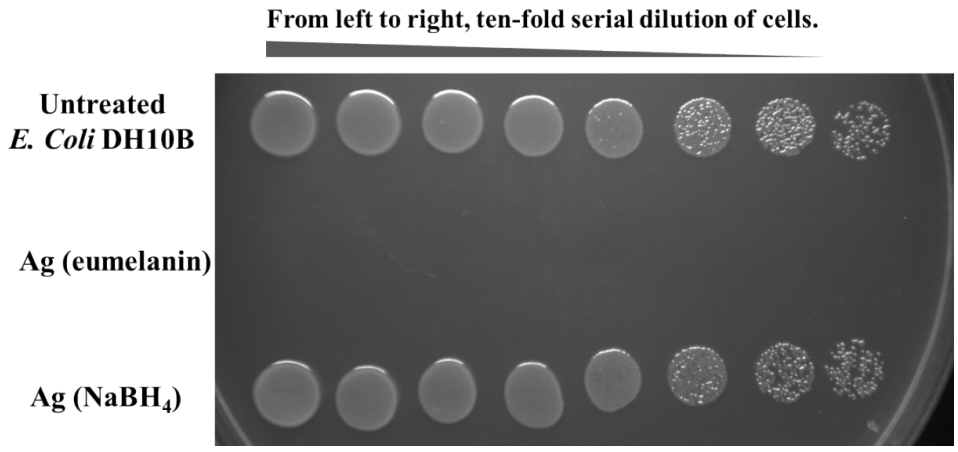


Figure S6 Growth and comparison of various metal nanoparticles treated cells. Quantitative survival measurements of *E. coli* DH10B in the presence or absence of 150μg/mL of Ag nanoparticles prepared by EuMel or $NaBH_4$.

References

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3. N. Cabrera-Valladares, A. Martínez, S. Piñero, V. H. Lagunas-Muñoz, R. Tinoco, R. de Anda, R. Vázquez-Duhalt, F. Bolívar and G. Gosset, *Enzyme and Microbial Technology*, 2006, **38**, 772-779.
4. 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.
5. C. Bi, P. Su, J. Muller, Y.-C. Yeh, S. Chhabra, H. Beller, S. Singer and N. Hillson, *Microbial cell factories*, 2013, **12**, 107.