SUPPORTING INFORMATION

for

AES and ToF-SIMS combination for single cell chemical imaging of gold nanoparticle-labeled *Escherichia coli.*

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1. Gold nanoparticles synthesis.

A gelatin solution was prepared by dissolving 1 mg of gelatin A from porcine skin (300 bloom, Sigma) in 15 ml of milli Q water at 50 °C. 0.01g of ascorbic acid dissolved in 1 ml of milli Q water was then added to the gelatin solution and mixed for 30 seconds.

An addition of 2 x 1mL (one every 2 s) of an aqueous solution (0.25 g/100 ml) of $HAuCl_4$ (Sigma) heated separately at 50°C was done in the previous solution. The color of the solution turned rapidly purple to red indicating the formation of gold nanoparticles. The suspension was stirred for 5 mins at 50 °C before cooling at room temperature under stirring. Finally, the particles were centrifuged and washed twice before characterization. The concentration of gold metal within the suspension was estimated to 0.7 mM.

2. Bacterial strain, plasmids and growth conditions

E. coli strain used in this study is a DH5alpha carrying the pFLO1 plasmid. pFLO1 plasmid was constructed using classical digestion and ligation procedures (Table S1). Plasmids used for the construct are listed in Table S1. Cells were grown under shaking at 250 rpm at 37°C in LB medium supplemented with 7 µg/ml chloramphenicol.

Table S1. Plasmids used in this study

Name	Construct and Usage[a]	Source or reference
pSEVA237R_Pem 7	Carries *mCherry* gene under Pem7 promoter, *kan* gene and pBBR1 replication origin	Gift from de Lorenzo's lab
pSEVA637	Carries *gfp* gene, *gen* gene and pBBR1 replication origin	Gift from de Lorenzo's lab
pSEVA338	Carries XylS/Pm regulator/promoter system, *cat* gene and pBBR1 replication origin	Gift from de Lorenzo's lab
pSEVA337R Pem7	Replacement of *kan* gene from pSEVA237R_Pem7 by *cat* gene from pSEVA338 using the double digestion SwaI - PshaI	This work
pFLO1	Replacement of *mCherry* gene from pSEVA337R_Pem7 by *gfp* gene from pSEVA637 using the double digestion HindIII - SpeI	This work

[a] The abbreviations *kan*, *cat*, and *gen* refer to insertions conferring resistance to kanamycin, chloramphenicol, and gentamycin, respectively.

3. Incubation of gold nanoparticles with bacteria and substrate adhesion conditions

Cells from a 10 ml culture at an OD_{600} of 0.5 were harvested by centrifugation 10 min at 28366g, washed with milli Q water and incubated 15 min at room temperature with 1.5 ml of gold nanoparticules solution (see above). The bacteria were centrifuged at 6037g for 1 min 30 s and washed with 1ml of milli Q water . The solution was then incubated with silicon wafers (5 mm x 5 mm) as substrates for 90 mins at room temperature. The substrates were finally washed with

milli Q water and bacteria adhesion was confirmed by fluorescent microscopy imaging using a Zeiss AxioObserver Z1 microscope with a Plan-Apochromat 63x/1,4 Oil DIC M27 objective.

4. Sample preparation for Auger and ToF-SIMS analyses.

The samples were prepared followed the protocol of Hua *et al*.[1] The silicon substrates with adhering bacteria and gold nanoparticles were first rinsed 30 seconds in a solution of milli Q water. The excess of water was then removed and absorbed using Kimtech wipes. To enhance the ionization yield for ToF-SMS analysis, a drop (10 µL) of 2,5-dihydroxybenzoic acid (DHB, 0.1 mg mL^{-1}, from Sigma) solution was deposited on top of the substrates. These one were then rapidly transferred into liquid nitrogen for flash freezing at −196 °C to maintain the cell shape. The samples were finally lyophilized for 12 h before ToF-SIMS and Auger analysis.

5. Characterization of gold nanoparticles

Transmission electron microscopy (TEM) images of gold nanoparticles were taken with a Philips CM 200 (200 kV) instrument equipped with a LaB6 source. The particles dispersed in water were dropped onto a carbon copper grid and dried at room temperature before analysis.

X-ray photoelectron spectroscopy analyses (XPS) were performed with the Thermo K-alpha spectrometer with a hemispherical analyzer and a microfocused monochromatized radiation Al Kα line (1486.6 eV) operating at 75 W under a residual pressure of 1 x 10^{-7} mBar with 400 µm spot size. A pass energy of 20 eV was used for core peak records. The neutralizer gun, which sprays low energy electrons and Ar^+ ions over the sample surface to minimize the surface charging, was used. All binding energies were referenced to the C 1s peak at 285.0 eV originating from the adventitious carbon (always detected at the surface of materials). Core peaks were analysed using a Shirley background and peak positions and areas were obtained

by a weighted least-squares fitting of model curves (70% Gaussian, 30% Lorentzian) to the experimental data. Quantification of surface composition was based on Scofield's relative sensitivity factors.[2]

6. Characterization of cells and gold nanoparticles/cells samples.

Auger Electron Spectroscopy (AES). Auger analyses and Scanning Electron Microscopy (SEM) images were carried out with a JEOL JAMP 9500F Auger Microprobe spectrometer (JEOL Ltd, Tokyo, Japan) working under UHV conditions (pressure < 2 $.10^{-7}$ Pa). The UHV equipment was a Schottky field emission Auger electron spectrometer (FE-AES) dedicated to very high spatial resolution analysis and high brightness. The hemi-spherical electron analyzer coupled with a multichannel detector (7 channeltrons) offered ideal settings for energy resolved Auger analysis.

High resolution SEM images were obtained using an acceleration voltage of 30 keV, a probe current of 2 nA and a working distance at 23 mm (sample tilt angle = 30°).

Auger acquisitions were recorded using an acceleration voltage of 20 keV and a probe current of 5 nA (working distance = 23 mm and sample tilt angle = 30°).

Spectra (kinetic energy from 0 to 1000 eV for control cells and 0 to 2200 eV for gold-labelled cells) were carried out at a constant retarding ratio (CRR) mode with dE/E = 0.5% (high sensibility).

The line scan and chemical Scanning Auger Mapping (SAM) analyses were performed in constant retarding ratio (CAE) operating conditions to enable defining the useful energy width needed to obtain a significant peak background-intensity difference with respect to the Auger transition and the background shape. An "auto probe tracking" correction was also used to

control and compensate for the drift due to the balance of sample surface charge effects and instrument-dependent fluctuations.

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). ToF-SIMS analysis were performed using a TRIFT V nanoToF II Tandem MS (Physical Electronics, Chanhassen (MN), US) instrument equipped with a 30 kV Bi_n^{q+} LMIG primary ion gun. All mass spectra were acquired at room temperature in the negative polarity with the same experimental conditions, so that to perform semi-quantitative analysis (comparing the ratio of peaks intensities). For surface analysis, the LMIG gun was tuned to deliver Bi_3^{++} bismuth clusters primary ions with a dc current of 12 nA over a 50 µm raster size, the mass range was fixed between 0 and 2000 uma and the number of frames was set to 50 (dose: 5.4 $x10^{12}$ ions/cm^2). For 2D imaging, a resolution of 256x256 image pixels, with a time per channel of 128, over 30x30 $µm^2$ field-of-view was used and the PIDD (primary ion dose density) never exceeded the static limit of 2.10^{13} ion/cm^2. For Tandem MS analyses, the selected precursor ion was sent into the CID activation cell and analyzed in MS^2 with a duty cycle of 100%. Note that dual charge (15eV e^-/10eV Ar^+) compensation was used for all analyses. Data processing was performed using ToF-DR software provided by Physical Electronics. All negative polarity mass spectra were calibrated using CH^-(m/z 13), NH^- (m/z 14) and OH^- (m/z 17) peaks.

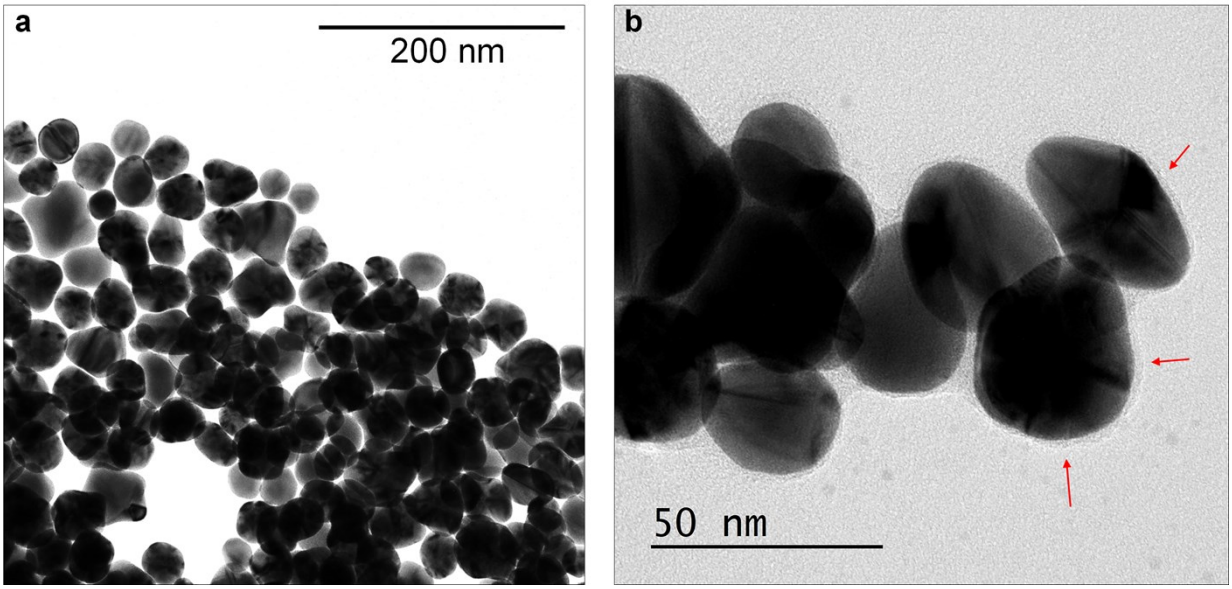

Figure S1. TEM images of the gelatin coated gold nanoparticles at (a) low magnification and (b) high magnification (red arrows show gelatin coating on particles).

7. Results of XPS analysis of gold nanoparticles

Assignment of the chemical environments of gelatin has been made in agreement with our previous work.[3]

On figure S2, the Au 4f core peak exhibits two main components due the spin-orbit coupling located at 84.0 eV and 87.7 eV attributed to Au $4f_{7/2}$ and Au $4f_{5/2}$ respectively. The energy position and the FWHM (~ 0.7 eV) indicate that the gold is present only under the metallic state in gold nanoparticles. Concerning O 1s, N 1s and C 1s core peaks, characteristic chemical environments of the gelatin molecule can be identified, which proves the gelatin coating on the surface of gold nanoparticles. Indeed, three components can be detected on the O 1s core peak: the main one at 532.0 eV is assigned to an oxygen in **O**=C-O and **O**=C-N environments (Table S2, 9.8 at.%), the one at 533.5 eV is attributed to **O**-C=O environments (Table S2, 3.7 at.%) and the one at 530.0 eV is related to oxide environment from sample holder. This O=C-N environment can also be identified in the N 1s core peak at a binding energy of 400.2 eV (Table S2, 4.6 at.%); the minor component located at 398.7 eV is attributed to N=C-NH and N-C environments. Concerning C 1s core peak, four components can be observed: the one at 285 eV is related to the aliphatic carbon of the gelatin and the adventitious carbon, the one at 286.6 eV refers to C-O/C-N environments from the gelatin molecule, the one at 288.2 eV to the O=C-N environment and the one at 289.0 eV is attributed to O=C-O groups. The quantification issued from these XPS analysis is consistent with the assignment. Indeed, the proportion of O=C-N groups (Table S2, 5.0 %) calculated from C1s core peak is close to the one originated from N 1s core peak (Table S2, 4.6 %).

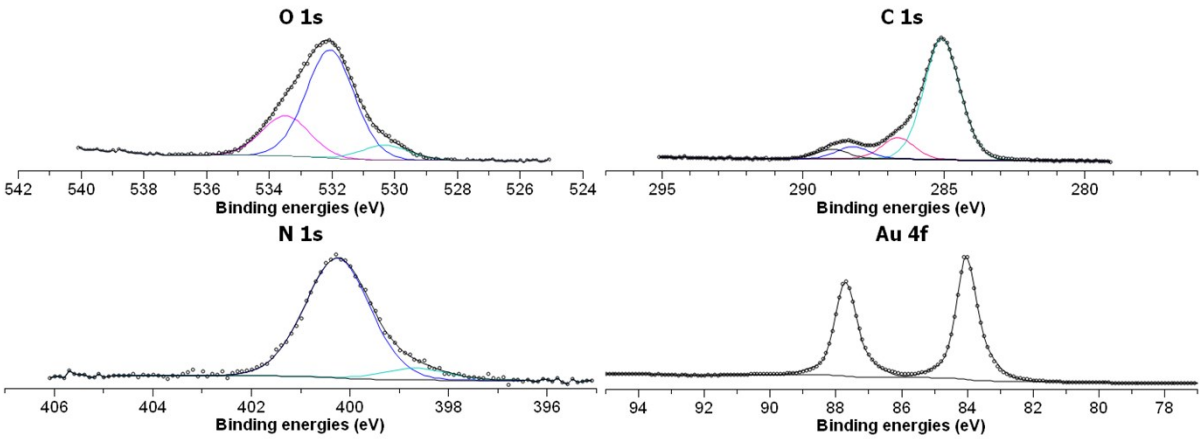

Figure S2: XPS O 1s, C 1s, N 1s and Au 4f core peaks of gelatin coated gold nanoparticles.

Table S2: XPS binding Energies (eV) and atomic percentages (at. %) of gelatin coated gold nanoparticles.

Orbitals	Position	%At. Conc.	assignments
Au 4f	84.0	9.1	
C 1s	285.0	50.0	**C**-C
	286.6	8.3	**C**-O, **C**-N
	288.2	4.3	O=**C**-N
	288.9	3.8	**C**O2
		66.4	
N 1s	400.2	4.6	O=C-**N**
	398.7	0.5	**N**=C-NH, N-C
		5.1	
O 1s	530.3	0.9	Oxide from sample holder
	532.1	9.8	**O**=C-O/**O**=C-N
	533.5	3.7	O=C-**O**
		14.4	

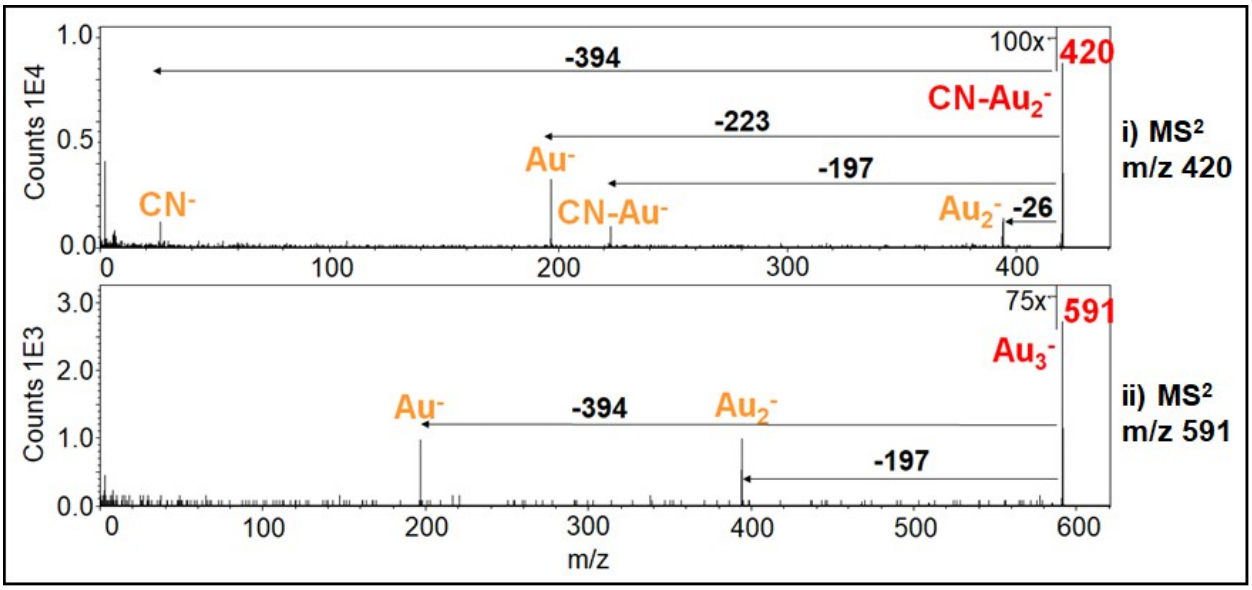

Figure S3. ToF-SIMS MS^2 mass spectra of m/z 420 and m/z 591 precursor ions (in red) of *E. coli* cells interacting with gelatin-coated gold nanoparticles; the detected peaks following the fragmentation are indicated in orange on the mass spectra (arrows with black negative values on top indicate the different mass losses from the precursor ion). Intensity scale expender is indicated for each precursor ion peak.

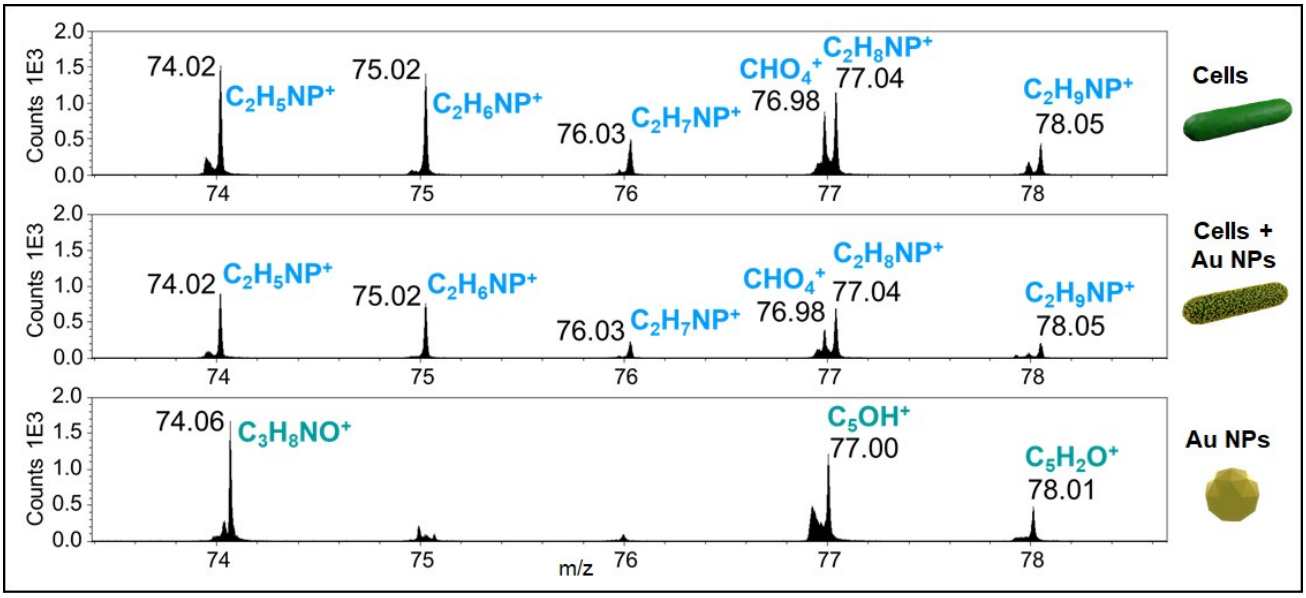

Figure S4. Positive ToF-SIMS mass spectra from m/z 70 to m/z 84 range of *E. coli* cells (top line), *E. coli* cells incubated with gelatin-coated gold nanoparticles (middle line) and gelatin-coated gold nanoparticles (bottom line).

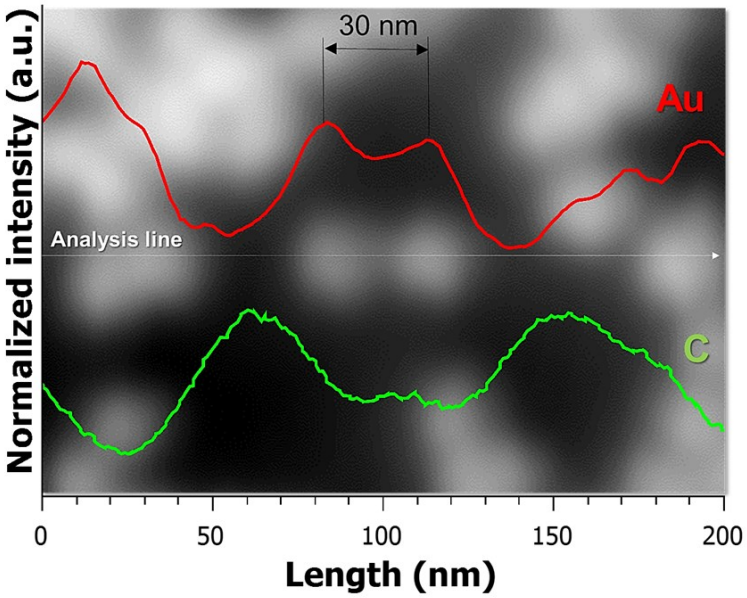

Figure S5. AES line profile analysis of Au and C on *E. coli* surface.

The carbon signal in green exhibits maxima outside gold nanoparticles on the cell outer membrane. The shape of the Au curve (in red) displays two peaks corresponding to the chemical signature of two gold nanoparticles separated from near 30 nm which corresponds the chemical lateral resolution ie the minimal distance that allows to distinguish chemically two points.

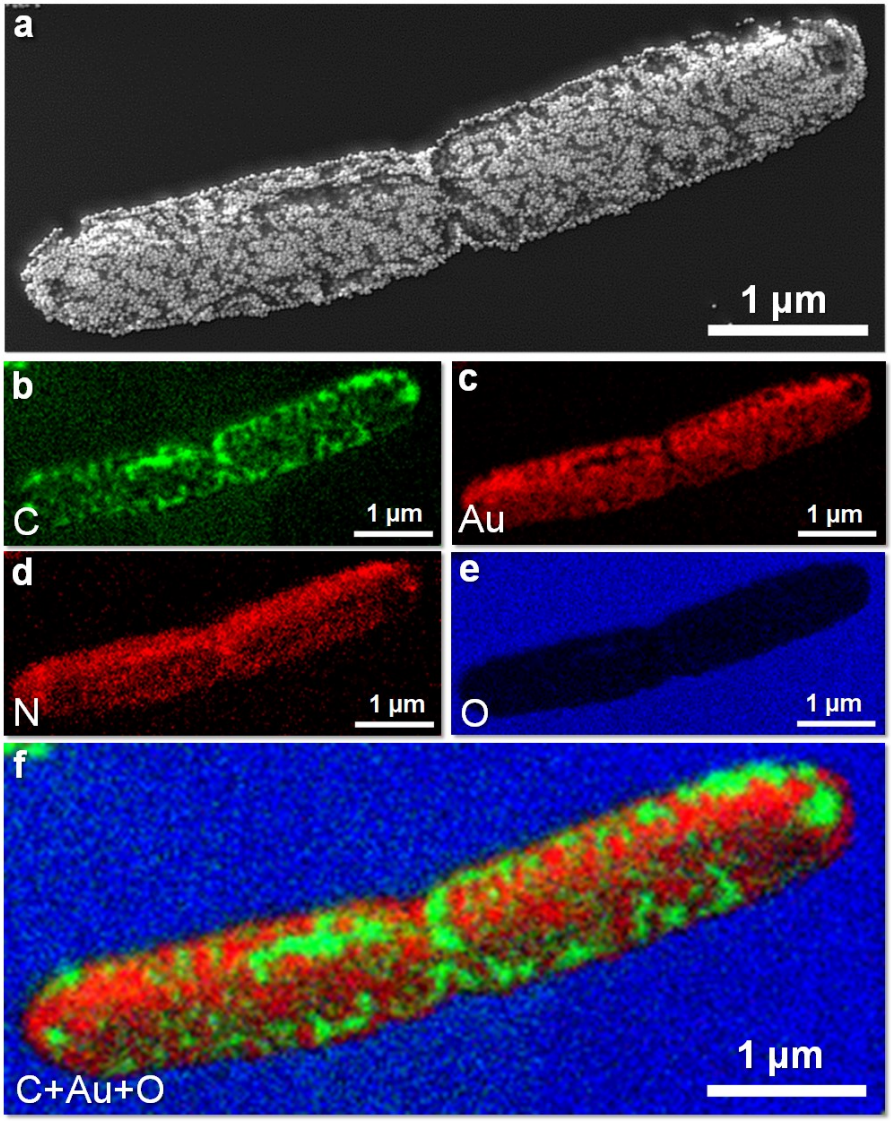

Figure S6. Low magnification SAM elemental mappings of *E. coli* cells interacting with gelatin coated gold nanoparticles. (a) SEM images of cells and related elemental mappings of (b) C, (c) Au, (d) N, (e) O and (f) C+Au+O overlay.

References

1 X. Hua, H.-W. Li and Y.-T. Long, *Anal. Chem.*, 2018, **90**, 1072–1076.
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3 S. Soulé, A.-L. Bulteau, S. Faucher, B. Haye, C. Aimé, J. Allouche, J.-C. Dupin, G. Lespes, T. Coradin and H. Martinez, *Langmuir*, 2016, **32**, 10073–10082.