

Supporting Information

Experimental section

Chemicals. Sodium borohydride (NaBH₄) and all of the metal salts used in this study were purchased from Aldrich (Milwaukee, WI, USA). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). Sodium tetraborate and hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄·3H₂O) were obtained from Acros (Geel, Belgium). The bacterial strains *E. coli* DH 5 α , *E. coli* K12 (BCRC 12438), *S. aureus* (BCRC 10780), and *S. enterica* (BCRC 10744) were obtained from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Mouse monoclonal antibodies against *E. coli*, *E. coli* K99, *S. aureus*, and *Salmonella* flagella were supplied by Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Amersham Hybond-C Extra NCM (pore size: 0.45 μ m) was purchased from GE Healthcare Bioscience (Buckinghamshire, UK). Milli-Q ultrapure water was used in each experiment.

Synthesis of Au NPs and Ag NPs. The Au NPs were synthesized via reduction of HAuCl₄ with NaBH₄, according to a slight modification in a reported procedure.¹ An aqueous solution (50 mL) was prepared containing 0.25 mM HAuCl₄ and 0.25 mM trisodium citrate. Next, 0.01 M NaBH₄ solution (1.5 mL) was added in one portion to the HAuCl₄ solution under constant stirring. After stirring for 3 min, the color changed from yellow to brown, thereby indicating the formation of the Au NPs. The solution was incubated at 4 °C for 24 h prior to further use. The average size of the as-prepared Au NPs was determined by transmission electron microscopy (Tecnai 20 G2 S-Twin TEM, Philips/FEI, Hillsboro, Oregon) to be 5.5 (\pm 0.5) nm from 100 counts. The particle concentration of the as-prepared Au NP solution was determined to be 36 nM using the equation $n = 3 m/4\pi r^3 s$, assuming the presence of ideal spherical particles. In the equation, n denotes the amount of Au NPs per mL, m is the molar mass of gold in substance [g/mol], r is the particle radius [cm], and s is the specific gravity of colloidal gold [19.3 g/cm³]. The m and r values were determined via inductively coupled plasma mass spectroscopy (ICP-MS) with an Elan 6000 instrument (Perkin-Elmer) and TEM measurements, respectively. This formula gives the number of Au NPs per mL. This concentration was then converted into number of Au NPs per L and divided by Avogadro's number (6.023×10^{23}) to determine the final molar concentration of Au NPs.

The Ag NPs were synthesized via reduction of AgNO₃ with NaBH₄ according to a slight modification of a reported procedure.² An aqueous solution (50 mL) was prepared containing 0.25 mM AgNO₃ and 0.25 mM trisodium citrate. Next, 0.005 M NaBH₄ solution (3 mL) was added in one portion into the AgNO₃ solution under constant stirring. After stirring, the color changed to yellow, indicating the formation of Ag NPs. The solution was incubated at 4 °C for 24 h prior to further use. The average size of the as-prepared Ag NPs was determined via TEM to be 12.2 (± 0.7) nm from 200 counts. The particle concentration of the as-prepared Ag NPs solution was determined to be 3 nM.

Preparation and Characterization of Antibody-Modified Au NPs and BSA-Capped Ag NPs.

To prepare an antibody-conjugated Au NPs (Ab–Au NPs) stock solution, a mixture of the as-prepared Au NPs (1.0 nM) and an antibody (5.0 nM) in 5.0 mM sodium phosphate (pH 9.0) was maintained at room temperature for 1 h. The antibody molecules readily conjugated to the Au NPs through electrostatic and hydrophobic interactions.³ By conducting the Coomassie blue assay,⁴ we estimated that ~95% of the antibody molecules have been conjugated on Au NPs. Subsequently, the Ab–Au NPs (0.8 nM) were passivated with 100 µM BSA for 1 h. The BSA-passivated Ab–Au NPs sample was stable for at least two weeks when stored at 4 °C in the dark. The dynamic light scattering (DLS) and Zeta potentials of the Ab–Au NPs were measured using a Zetasizer 3000HS analyzer (Malvern Instruments, Malvern, UK). A Synergy 4 Multi-Mode Microplate Reader (Biotek Instruments, Winooski, Vermont, USA) was used to measure the absorbance of the Ab–Au NPs solution. The BSA-conjugated Ag NPs (Ab–Ag NPs) stock solution was readily prepared by mixing the as-prepared Ag NPs (250 pM) and BSA (100 µM) in 5.0 mM sodium phosphate (pH 7.0) and then incubating the mixture at room temperature for 1 h. BSA molecules readily conjugated to Au NPs through electrostatic and hydrophobic interactions.

Bacterial Growth. *E. coli* DH 5α and K12, *S. aureus*, and *S. enterica* were separately grown in sterile Luria-Bertani (LB) media [containing bacto-tryptone (2.5 g), bacto-yeast extract (1.25 g), and NaCl (1.25 g) in 250 mL of deionized water]. A single colony of each strain was lifted from LB agar plates and inoculated in LB media (10 mL), and the culture was grown at 37 °C with shaking (200 rpm) until the value of the absorption at 600 nm (OD₆₀₀) reached 1.0. A portion of each of the cell mixtures (1 mL) was centrifuged (12,000 g, 5 min, 4 °C) and washed twice with 5.0 mM phosphate buffer solution (adjusted to pH 7.0) for further use.

Bacterial Assay. The cells, diluted to 0–1.0 × 10⁷ colony-forming units (CFU)/mL, were incubated with Ab–Au NPs (100 pM) for 1 h in a sodium phosphate buffer (5.0 mM, pH 7.0) in the presence

of BSA (100 μM) while shaking mildly. After centrifugation (1000 g , 10 min, 4 $^{\circ}\text{C}$) and washing twice with sodium phosphate buffer (5 mM, 500 μL , pH 7.0), a portion of each re-suspended solution (20 μL) was deposited on the NCM (0.3 cm \times 0.3 cm) and then dried on a hot plate (60 $^{\circ}\text{C}$) for 1 min prior to LDI-MS analysis. The membrane substrates were then attached to a matrix-assisted LDI (MALDI) plate using an adhesive polyimide film tape.

MS experiments were performed in the reflectron positive-ion mode using an Autoflex III MALDI time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany). The samples were irradiated with an Nd:YAG (355 nm) laser at 100 Hz. The ions produced by laser desorption were stabilized energetically during a delayed extraction period of 30 ns, and then accelerated through the TOF chamber in the reflection mode before entering the mass analyzer. The available accelerating voltages ranged from +20 to -20 kV. The instruments were calibrated with Au clusters using their theoretical mass values ($[\text{Au}_x]^+$; $x = 1, 2$). A total of 2000 pulsed laser shots were applied to accumulate signals from 10 MALDI target positions under a laser fluence of 50 μJ .

Analysis of Real Samples. Apple juice and milk samples were purchased from a traditional supermarket (Taipei, Taiwan). The urine sample was collected from a healthy male adult (25 years old). To prepare the real samples, 1 mL aliquots of 25% (wt/wt) tap water, 25% (wt/wt) apple juice, 25% cow milk (wt/wt), or 25% urine (wt/wt) were incubated with the desired concentrations of *E. coli* (from 0 to 1×10^7 CFU/mL). The *E. coli* spiked samples were analyzed by the plate-count method and by our Ab-Au NPs-based assay, as described above for bacteria detection.

References:

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- 2 S. D. Solomon, M. Bahadory, A. V. Jeyarajasingam, S. A. Rutkowsky and C. Boritz, *J. Chem. Educ.*, 2007, **84**, 322–325.
- 3 S. Fazekas De St. Groth, R.G. Webster and A. Datyner, *Biochim. Biophys. Acta*, 1963, **71**, 377–391.
- 4 (a) P. M. Tessier, J. Jinkoji, Y.-C. Cheng, J. L. Prentice and A. M. Lenhoff, *J. Am. Chem. Soc.*, 2008, **130**, 3106–3112; (b) F. Cañaveras, R. Madueño, J. M. Sevilla, M. Blázquez and T. Pineda, *J. Phys. Chem. C*, 2012, **116**, 10430–10437.

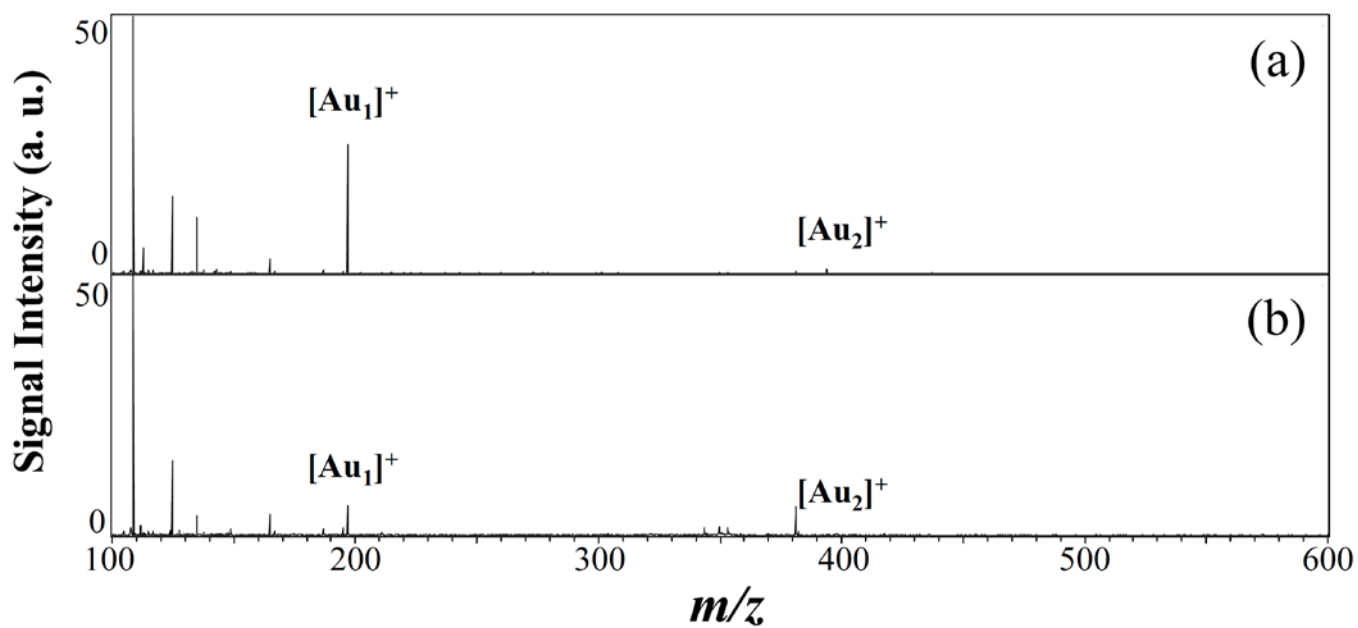


Fig. S1 LDI-MS of (a) 5.5 nm-Au NPs (100 pM) and (b) Ab-Au NPs (100 pM). The peaks at $m/z = 196.967$ and 393.933 are assigned to $[\text{Au}_1]^+$ and $[\text{Au}_2]^+$, respectively. A total of 2000 pulsed laser shots were applied under a laser fluence set at $50 \mu\text{J}$. The peak intensities are plotted in arbitrary units (a. u.).

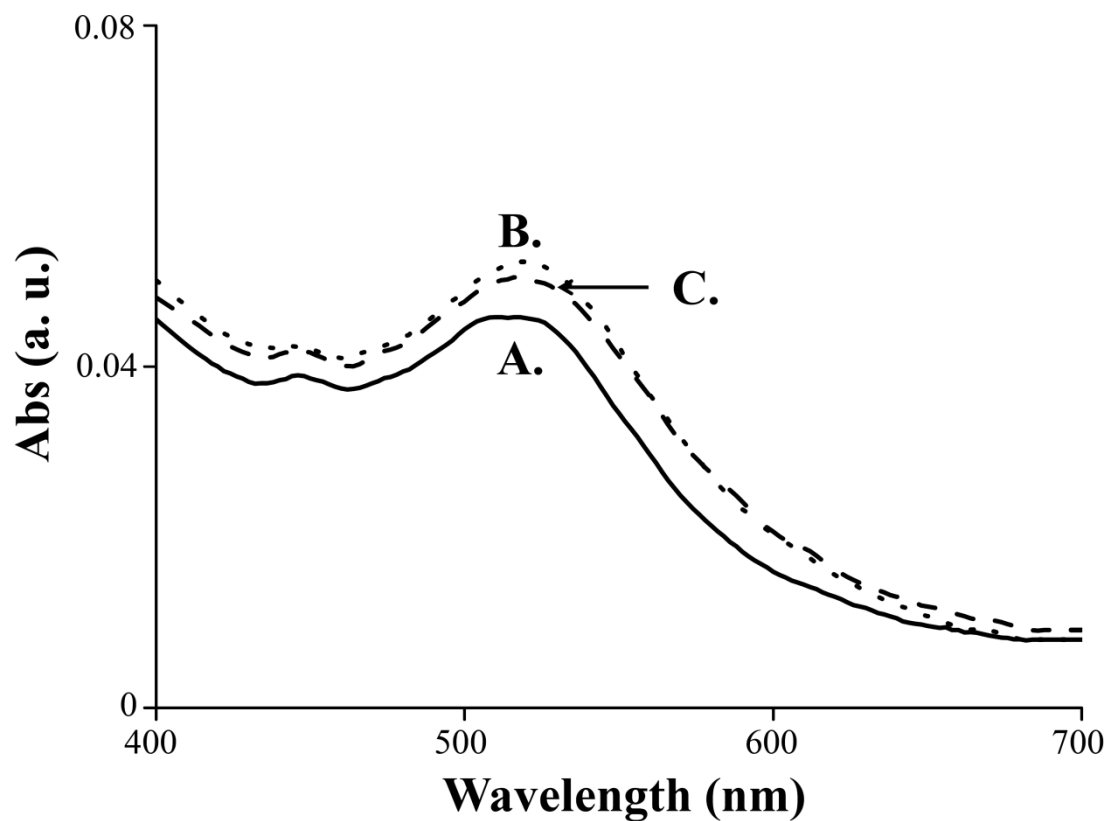


Fig. S2 UV-vis absorption spectra of (A) 5.5 nm-Au NPs (1.0 nM), (B, C) Ab-Au NPs (1.0 nM) in 5.0 mM sodium phosphate buffer (pH 7.0) in the (B) absence and (C) presence of 200 mM NaCl. The absorbance (Abs) was plotted in arbitrary units (a. u.).

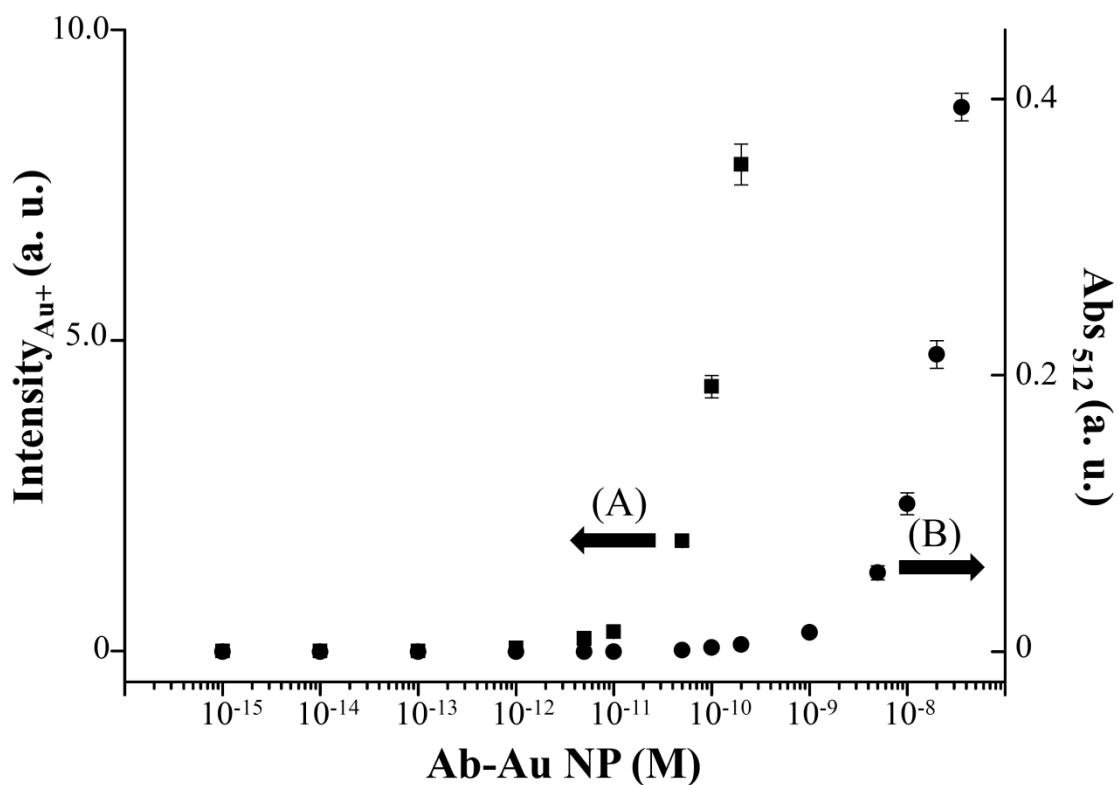


Fig. S3 (A) Signal intensities of the $[\text{Au}_1]^+$ ions recorded on the Ab–Au NPs/NCM substrate by LDI-MS for different Ab–Au NPs (0–100 pM, 20 μL) solutions deposited on the NCM. (B) UV-vis absorption recorded in the surface plasmon resonance absorption bands at 512 nm (Abs_{512}) for Ab–Au NPs (200 μL) at concentrations from 0 pM to 36 nM. Error bars represent standard deviations from four repeated experiments. Other conditions were the same as those described in Figures S1 and S2.

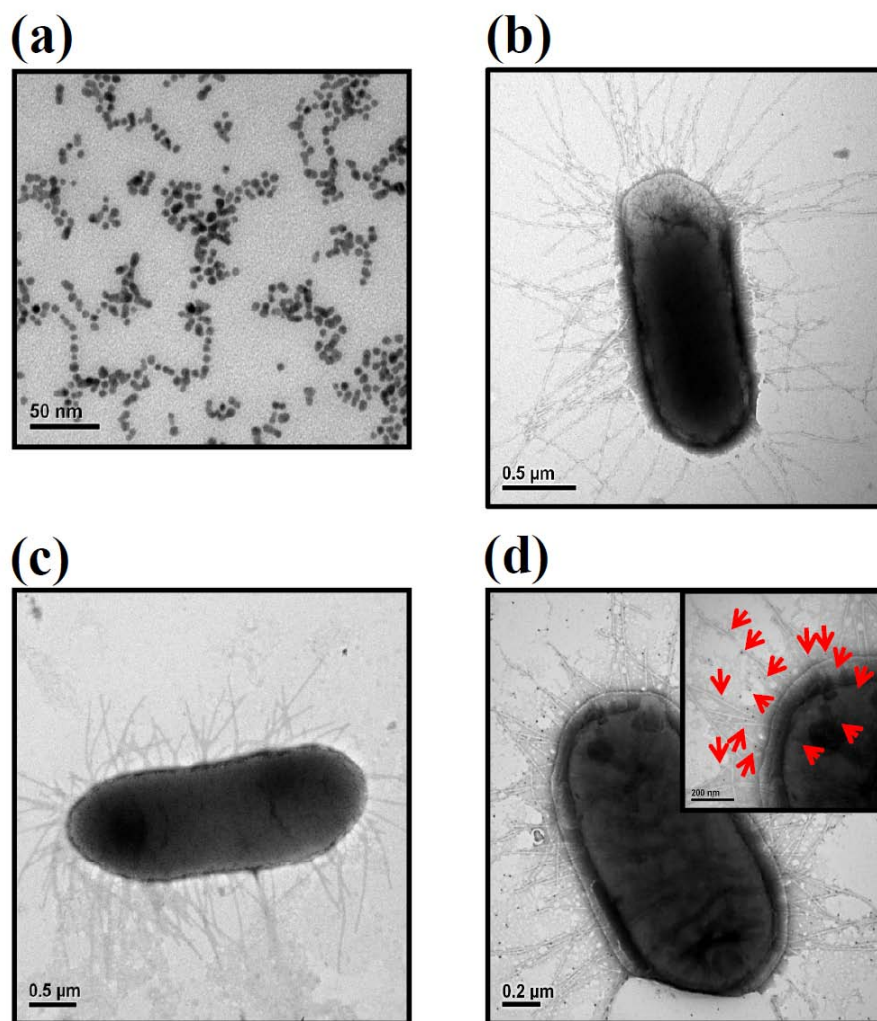


Fig. S4 TEM images of (a) the Au NPs, (b) *E. coli*, (c) mixture of BSA–Au NPs and *E. coli*, and (d) Ab–Au NPs/*E. coli* conjugates. The arrows indicate the Ab–Au NPs in (d). Other conditions were the same as those described in Figure 1d.

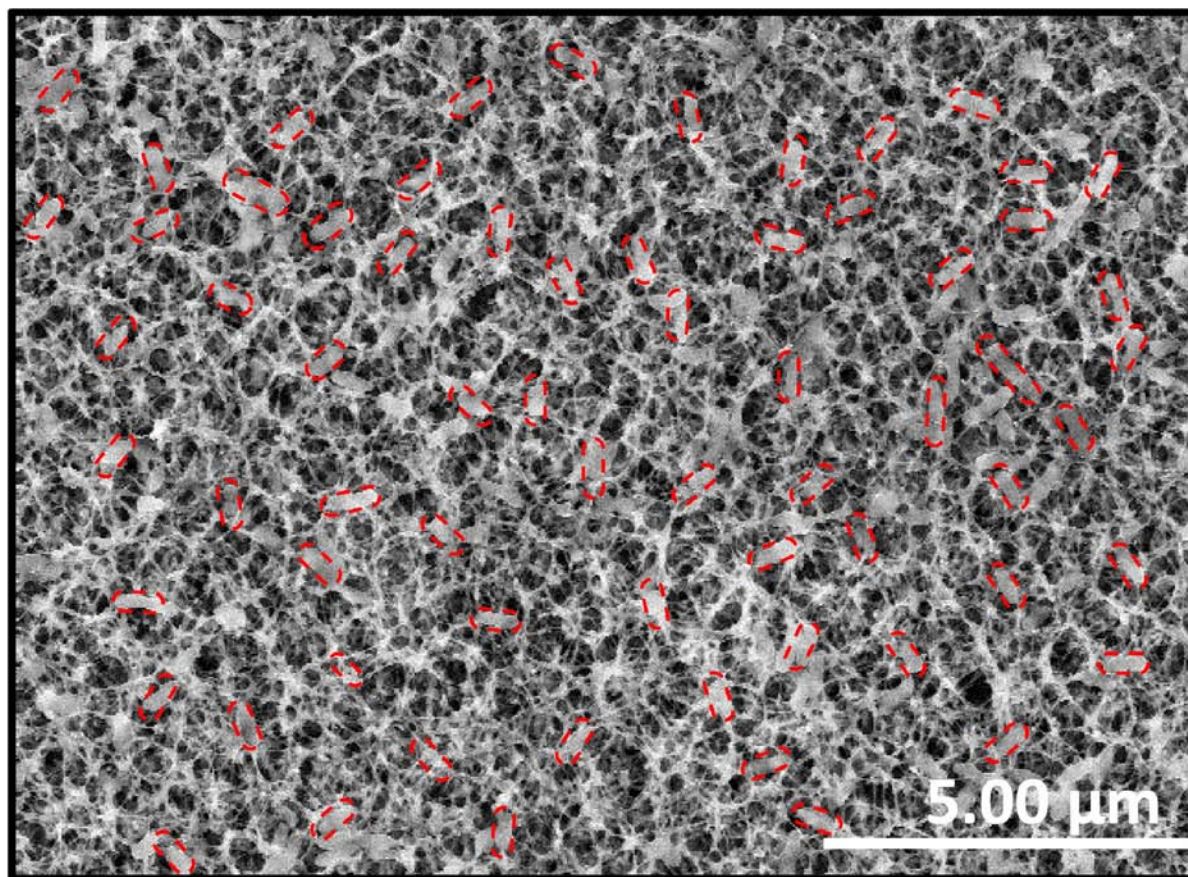


Fig. S5 Scanning electron microscope (SEM) image of Ab–Au NPs/*E. coli* conjugates on the NCM. The *E. coli* cells are marked with dashed circles. Other conditions were the same as those described in Figure 1d.

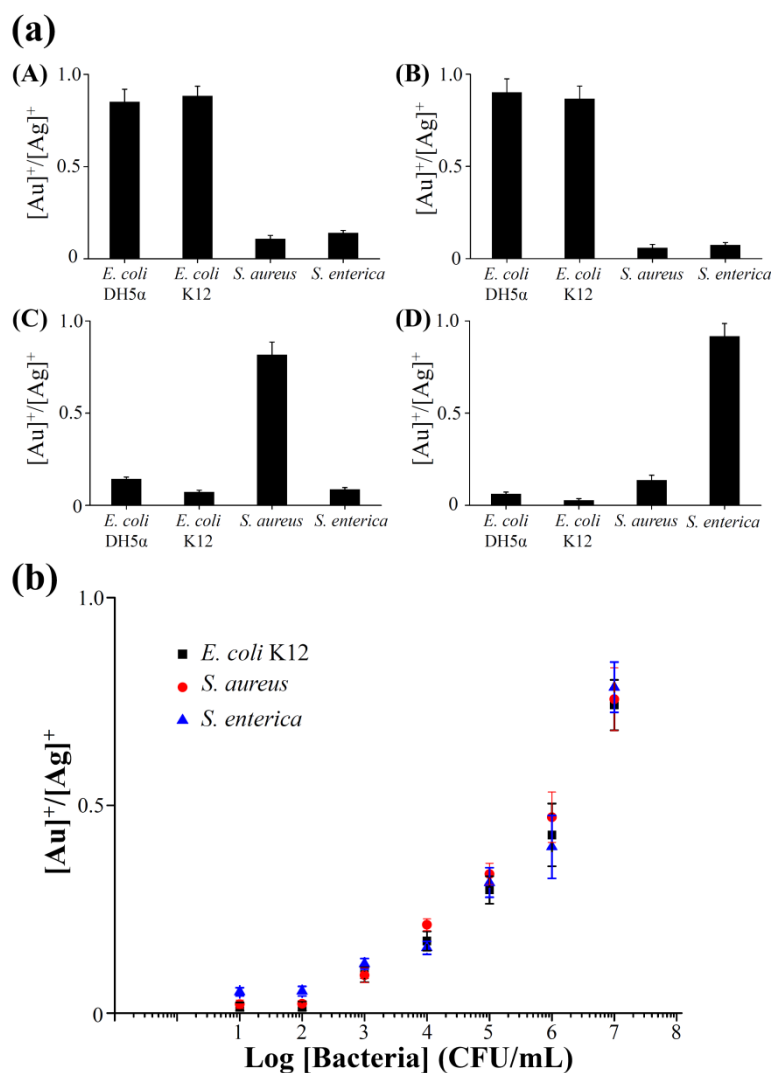


Fig. S6 (a) Selectivities of the (A) *E. coli*-against antibody (for full *E. coli*), (B) *E. coli* K99-against antibody (for *E. coli* pili), (C) *S. aureus*-against antibody (for *S. aureus*), and (D) *Salmonella* flagella-against antibody (for *S. enterica* flagella) conjugated Au NPs for the detection of *E. coli*, *S. aureus*, and *S. enterica* through Ab–Au NPs/NCM-based LDI-MS measurements. (b) Detection of bacteria with concentrations from 0 to 1.0×10^7 CFU/mL in a physiological buffer containing BSA (100 μM) by specific Ab–Au NPs/NCM coupled with LDI-MS measurements. The concentration of bacteria in (a) is 1.0×10^7 CFU/mL. Error bars represent standard deviations from four repeated experiments. Other conditions were the same as those described in Figure 2

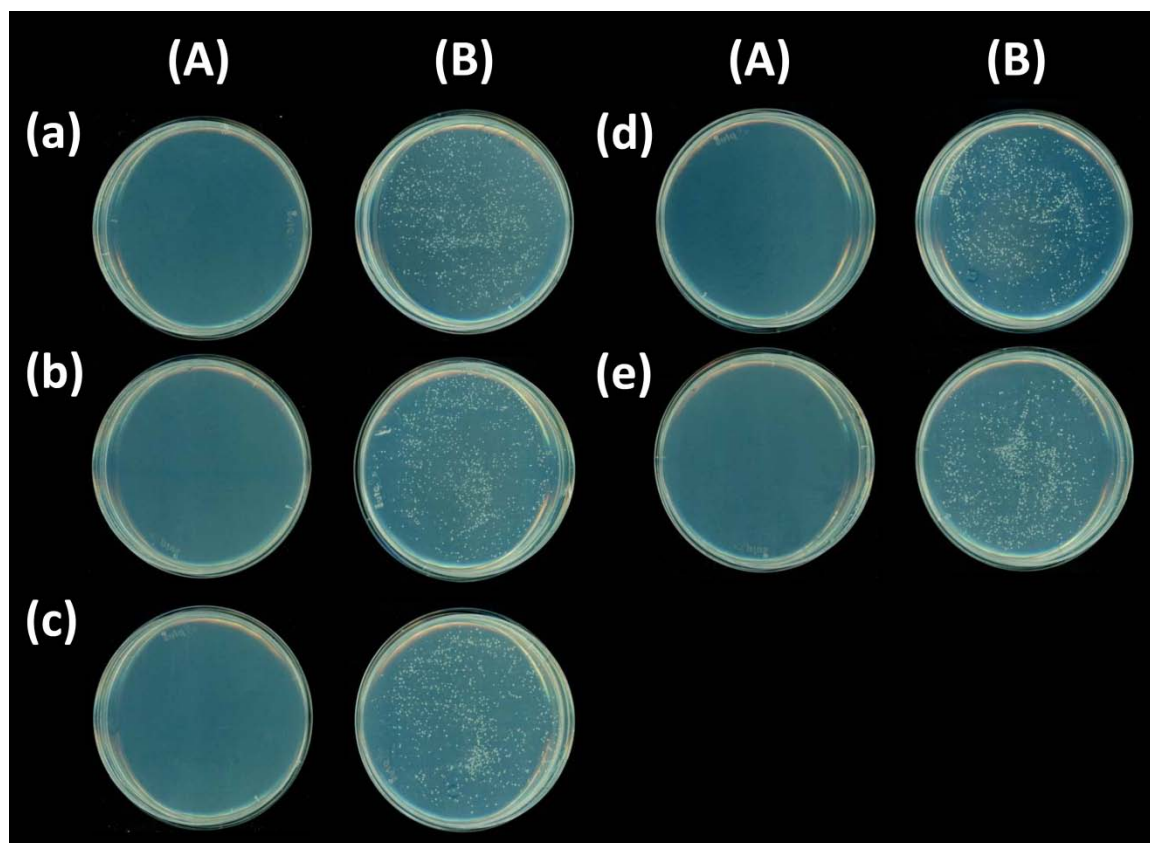


Fig. S7 Colony formation of *E. coli* on LB agar plates of (a) DI H₂O, (b) tap water (25% w/w), (c) apple juice (25% w/w), (d) cow's milk (25% W/W), and (e) human urine (25% W/W) samples (1 mL) with a spike of (A) 0 and (B) 300 *E. coli* cells. The number of colonies on the *E. coli* (300 cells) spiked plates a, b, c, d, and e was 285, 311, 378, 282, and 321, respectively.

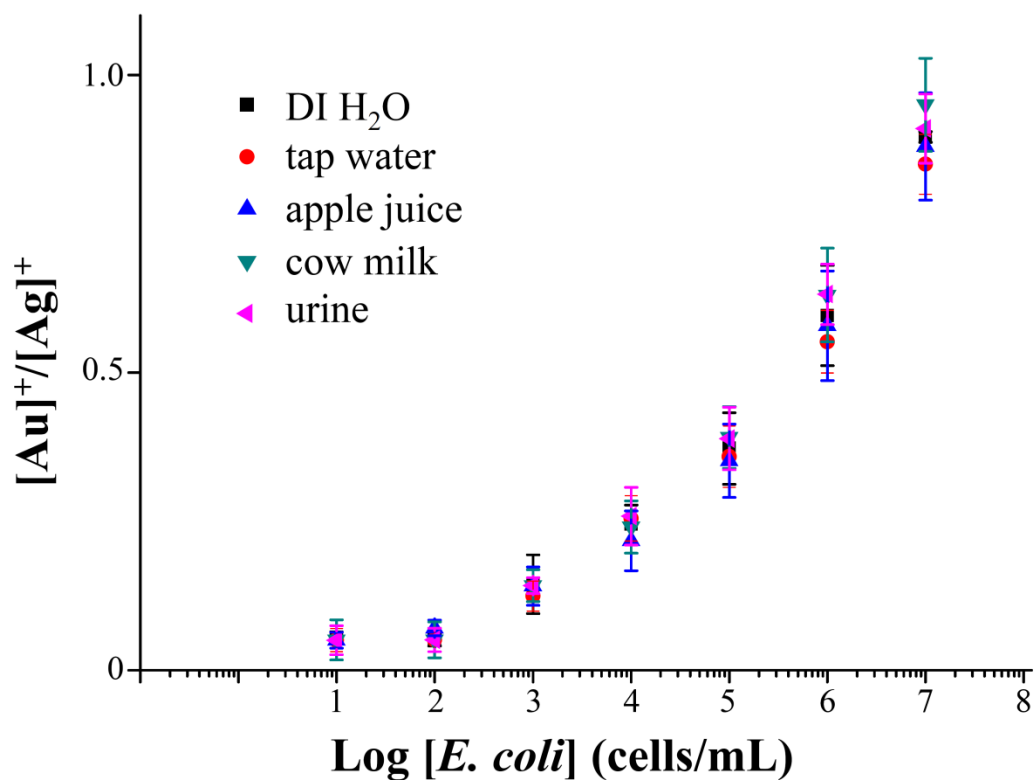


Fig. S8 Analysis of representative DI H₂O (black), tap water (25% w/w), apple juice (25% w/w), cow's milk (25% W/W), and human urine (25% W/W) samples using the Ab–Au NPs/NCM probes coupled with LDI-MS for *E. coli*. The samples (25% W/W) were spiked with *E. coli* (from 0 to 1.0×10^7 CFU/mL). Other conditions were the same as those described in Figure 1d.