Supporting Information for

Bacteria-templated fabrication of a charge heterogeneous polymeric interface for highly specific bacterial recognition

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1 Materials: 3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate (DMAPS, 97%), and methacrylatoethyl trimethyl ammonium chloride (DMC, 80 wt % in H_2O) were purchased from Sigma Aldrich. To remove the stabilizer (MEHQ), DMC was passed through an aluminum oxide column before use. 2-bromoisobutyryl bromide (>98%), $_L$-ascorbic acid (>99%) and tris(2-pyridylmethyl)amine (TPMA, >98%) were purchased from TCI Reagent Co. Ltd . $CuBr_2$ (99%), N,N′-methylenebis(acrylamide) (99%), 11-mercapto-1-undecanol (97%), other solvents and inorganic salts were purchased from Aladdin Chemistry Co. Ltd.

2 Bacterial cultivation: Four strains of bacteria, *Escherichia coli* expressing a green fluorescent protein (GFP-*E. coli*), *Shewanella oneidensis* MR-1 (*S. oneidensis* MR-1), *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecium* (*E. faecium*) were used as templates in the BIP fabrication. GFP-*E. coli* cells (using a pET28a vector) were inoculated in 25 mL lysogeny broth (LB) medium with 100 μg mL^{-1} kanamycin and 100 μM IPTG (isopropyl–β-D-thiogalactopyranoside) at 37°C with shaking (200 rpm). The expression of GFP was examined under a fluorescence microscope. *S. oneidensis* MR-1 was from our laboratory stock at -80°C. *Enterococcus faecium* (*E. faecium*) and *Staphylococcus aureus* (*S. aureus*) was purchased from China Center of Industrial Culture Collection (CICC). Cells were cultivated in LB medium at 37°C with shaking (200 rpm). All cells were harvested at late exponential growth phase by centrifugation at 5000 G for 5 min. The cells were sequentially resuspended and washed with 0.1 M phosphate buffer solution (PBS, pH=7.2) for 3 times. Finally, the harvested cells were

prepared as bacterial suspension at approximately 5×10^8 cfu mL^{-1} for further dilution. The surface charge of cells was determined using a Zetasizer (Malvern, Nano ZS90) using a bacterial suspension of 10^7 cfu mL^{-1} in 0.1 M PBS. Each scan was conducted in triplicates.

3 BIP fabrication: A glass slide with a deposited gold pattern (200 nm thick, gold area=1.6 mm^2) was cleaned by sonication in acetone for 15 min and dried under nitrogen stream. The slide was sequentially immersed in 20 mL fresh-prepared piranha solution (7:3, 98% H_2SO_4: 30% H_2O_2, v:v) for 15 min to remove organic residues (*caution: piranha solution reacts violently with organic matter and should be carefully handled*). The slide was washed with deionized water and dried under nitrogen stream. The self-assembled monolayer of ATRP initiator was formed by immersing the cleaned slide in 1 mM ethanol solution of ω-mercaptoundecyl bromoisobutyrate overnight at room temperature. The ω-mercaptoundecyl bromoisobutyrate was synthesized following a literature method.[30] The initiator-coated gold substrate (effective area 1.6 mm^2) was rinsed with several aliquots of tetrahydrofuran and ethanol sequentially to remove unbound initiator and dried under nitrogen stream. To immobilize the template bacteria, cells at late exponential growth phase were harvested and washed by centrifugation in PBS for 3 times. The bacteria concentration was adjusted to approximately 5×10^8 cfu mL^{-1}. 100 μL bacterial suspension was dripped and spread on the initiator-coated gold substrate. The slide was left for bacteria attachment at 4 ^oC for 1h. Four strains of bacteria, GFP-*E. coli*, *S. oneidensis* MR-1, *S. aureus* and *E. faecium*

were used as templates in the BIP fabrication. Their corresponding BIPs were termed *Es*-BIP, *Sh*-BIP, *St*-BIP and *En*-BIP, respectively. As a control, a non-imprinted polymer, which was prepared without template bacteria, was also fabricated and abbreviated as NIP. For fabricating NIP, the template bacteria immobilization step was skipped. 65 mg DMC, 1400 mg DMAPS and 7 mg BIS were dissolved in 5 mL DMSO/H_2O mixture (1:9, v:v). Then, 11.2 mg $CuBr_2$ and 14.5 mg TMPA was sequentially added into the mixture. The mixture was degassed by three consecutive freeze-pump-thaw cycles. The slide with immobilized template bacteria was placed into a three-necked flask and the reactor was bubbled with nitrogen continuously. The degassed mixture was then transferred into the flask to immerse the slide. 0.3 mL $_L$-Ascorbic Acid solution (5 mg in 1 mL degassed DI water) was injected into the reactor. The reactants were left to polymerization at 30 ^oC. After 12h, the reaction was terminated by exposing to the air. The polymer grafted gold substrate was washed with DI water to remove the unreacted monomer. The template bacteria were removed by rinsing with 0.5% sodium dodecyl sulfate and DI water for several times. The brief fabrication procedure was presented in Figure 1.

4 BIPs characterization: The film thickness of fabricated BIPs on gold substrates in air was measured by an ellipsometer (Gaeriner Scienfific Corporation, 03-AK) with a laser (λ=632.8 nm) at a fixed incidence angle of 70^o. The fabricated BIPs were characterized by attenuated total reflection (ATR) infrared spectra using a FT-IR spectrometer (Thermo Scientific, Nicolet iS50). Wavenumbers were given in cm^{-1}

ranging from 500-4000 with 128 scans. To investigate the surface hydrophobicity, contact angle goniometry was performed under ambient conditions. The contact angle with ultrapure water was measured with sessile drop method and analyzed using video-based contact angle measuring device (Dataphysics, OCA 20). The surface morphology of BIPs was observed using AFM (Veeco MultiMode) in tapping mode.

5 The Microfluidic chip for Bacterial recognition: The microfluidic chip for bacterial recognition was assembled with a glass slide and a commercial available polydimethylsiloxane (PDMS) channel (Wenhao Chip Tech., China). The PDMS channel was 50 μm in height, 200 μm in width and 2 cm in length. The volume of the channel was 0.2 μL. On the glass slides, two gold electrodes were deposited and they are next to each other along the direction of flow (each effective area of 1.6 mm^2). One gold electrode is grafted with fabricated BIPs, and the other is a bare gold electrode. The two gold electrodes are connected to the electrochemical workstation in the electrochemical impedance spectroscopy (EIS) experiment via deposited gold lines on the slides. In bacterial recognition experiments, a blunt needle with a Luer connector was inserted into the microfluidic channel inlet as the bacterial suspension reservoir. The microfluidic channel outlet was connected to a syringe and the fluid was withdrew by a syringe pump.

6 Bacterial recognition performance evaluations: 100 μL bacterial suspension (approximately 5×10^7 cfu mL^{-1}) was dripped into the reservoir. In each test, the bacterial

suspension was infused into the microfluidic channel via the syringe pump for 30 min at the speed of 1 mm min^{-1} (*i.e.*, hydraulic retention time=20 min). PBS was sequentially infused into the microfluidic channel at the same speed to rinse free cells. GFP-*E. coli* could emit green fluorescence on cell wall; while for other bacteria, Rhodamine 123 dye was used as the fluorescence dye to label cells before microscope inspection. The excitation peak wavelengths of GFP were 395 nm and 475 nm, the emission peak wavelength was 509 nm. The excitation peak wavelength of Rhodamine 123 dye was 511 nm, the emission peak wavelength was 534 nm. The competitive binding experiments were conducted using GFP-*E. coli* and *S. aureus* mixed suspension with the same density (approximately 2.5×10^7 cfu mL^{-1}) on the *Es*-BIP and *St*-BIP assembled chips. Before mixing the two bacteria, *S. aureus* was stained with CellTrack Red dye, a fluorescent dye that only stained the cell's interior without affecting the bacterial outer surface. The excitation peak wavelength of CellTrack Red dye is 577 nm, the emission peak wavelength is 602 nm. The mixed bacterial suspension was dripped into the reservoir and the rest procedure remained the same. For bacteria counting, a biological microscope (Nikon, eclipse E200) with a florescence module (Mshot, MF-BG-LED) was used. The excitation wavelength of the florescence module for observing GFP and Rhodamine 123 was 460-490 nm, the wavelength of the filter was 510 nm. The excitation wavelength of the florescence module for observing CellTrack Red stained *S. aureus* was 510-550 nm, the wavelength of the filter was 590 nm. Fluorescence images were captured through a CCD camera (Mshot, MS31). In each test, cells in five random microscopic fields with an area of 80×80 μm were

counted. The five microscopic fields were captured by successively moving the objective table with a certain distance along the channel direction once the first field was randomly selected. The quantity of bacteria on the images was manually counted according to the area of colored pixel assemblies.

7 Cell adhesion force probing: To quantify the cell adhesion forces at the BIP surfaces, single-cell force spectroscopy (SCFS) was employed using a single living cell immobilized AFM probe. The force-distance curves were performed and recorded by an AFM (Asylum Research, MFP-3D-BIO). The procedure is generally following a literature method. Briefly, a silica microsphere of 20 μm in diameter was glued on the cantilever of an AFM probe (Bruker, RTESP) via UV-curable glue. Polydopamine was then modified onto the microsphere for immobilizing a single GFP-*E. coli* cell. By employing this single-cell probe, the force-distance curves were performed on gold substrates grafted with different BIPs. For each test, 10 random locations were investigated and each location was repeatedly recorded for 5 times. The AFM software (Asylum Research, MFP-3D) was used to calculate the maximum adhesion force for each curve and generated the adhesion-force histograms on different substrates.

8 Electrochemical impedance spectroscopy test: EIS tests were performed in two-electrode model with an electrochemical work station (Biologic, VSP-300). The *Es*-BIP grafted gold electrode used as the working electrode and the bare gold electrode in the channel used as the counter electrode. After GFP-*E. coli* suspension flowed over

the BIP in the chip, 5 mM solution of redox couple $[Fe(CN)_6]^{4-/3-}$ prepared in 100 mM PBS was infused in the channel to rinse free cells and provide redox probe. EIS measurements were performed in the frequency range of 50 mHz to 50 kHz at the open circuit potential and the amplitude of applied sinusoidal wave potential was 20 mV. All experiments were conducted in triplicate. The EIS measurement with mixing bacterial suspension was conducted using GFP-*E. coli* and CellTrack Red stained *S. aureus* mixed suspension with the same density (approximately 2.5×10^7 cfu mL^{-1}) on the *Es*-BIP assembled chip. The other operation conditions remained the same. After EIS measurement, the chip was examined under the fluorescence microscopy for selectivity.

Table S1. Bacteria strains used in this study

	GFP-*E. coli*	*S. oneidensis* MR-1	*S. aureus*	*E. faecalis*
Zeta potential[a] (mv)	-48.6±3.3	-7.40±0.35	-25.9±1.0	-33.8±5.8
Gram-class	negative	negative	positive	positive
Shape	rod	rod	sphere	sphere
Size (μm)[b]	Φ(0.4–0.8) ×2.0	Φ(0.4–0.7) ×(2.0-3.0)	Ca. Φ1.0	Ca. Φ0.8

[a] Zeta potential was determined by using bacterial suspension at 10^7 cfu mL^{-1} in 0.1M PBS at pH=7.2. [b] Bacteria size was according to the literature data.

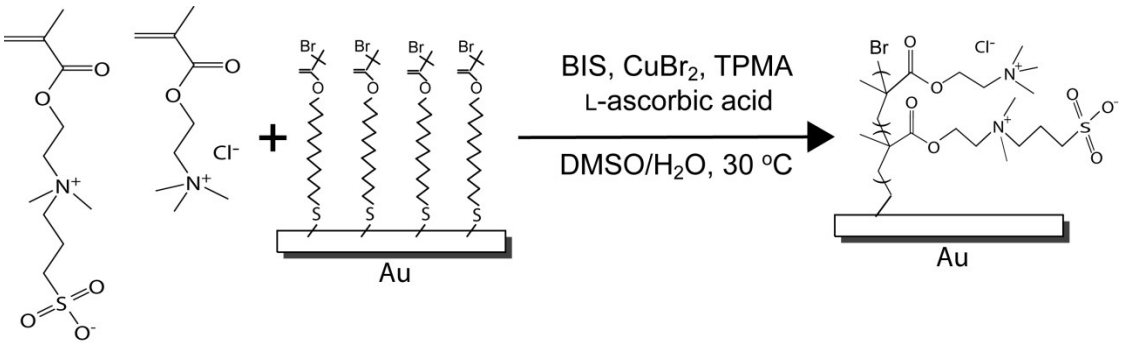

Fig. S1 Schematic diagram for SI-ATRP process. 3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate and methacrylatoethyl trimethyl ammonium chloride were used as monomers to fabricate the polymeric matrix.

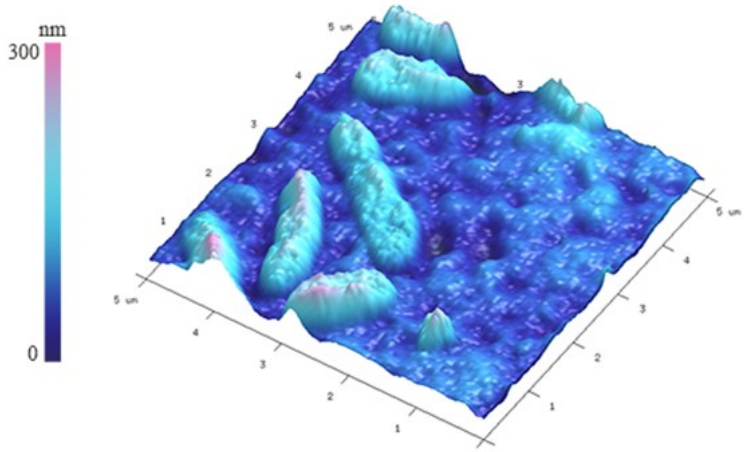

Fig. S2 Surface morphology of *Es*-BIP fabricated with a DMC/DMAPS feed ratio of 1:5. After template bacteria removal operation, most GFP-*E. coli* cells could not be removed from the polymeric matrix due to the strong interaction between the BIP and *Es*-BIP cell. This indicated the DMC amount was the key factor that influenced the BIP-bacteria binding force. The DMC/DMAPS feed ratio of 1:5 was too high for the BIP fabrication.

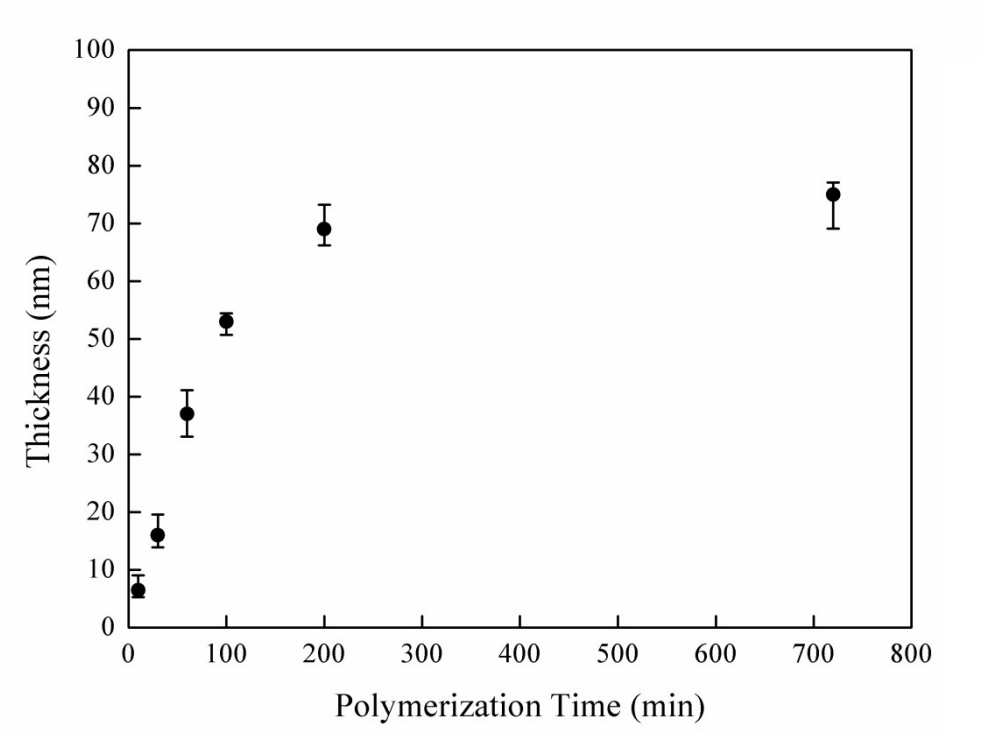

Fig. S3 *Es*-BIP layer thickness measurement with polymerization time.

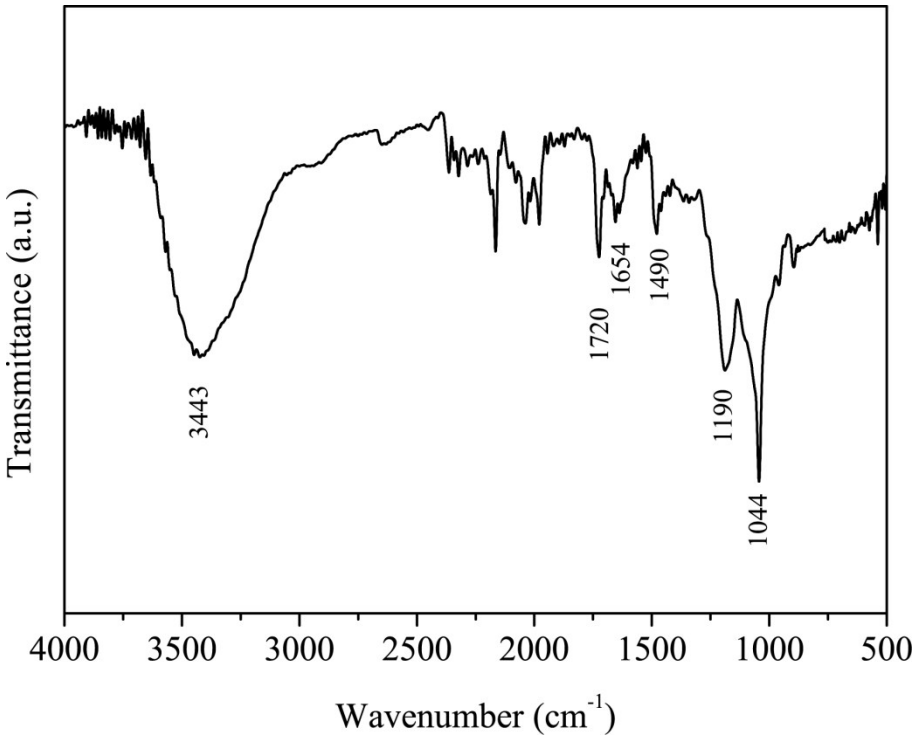

Fig. S4 ATR/FT-IR spectra of *Es*-BIP. The fabricated BIPs were characterized by attenuated total reflection infrared spectra using a FT-IR spectrometer. Wavenumbers were given in cm^{-1} ranging from 500-4000 with 128 scans. The spectrum showed the absorption peaks at around 1720 cm^{-1} (O-C=O stretching), 1654 cm^{-1} (C=O stretching) and 1490 cm^{-1} (quaternary ammonium) could be from DMC. The absorption peaks at around 1044 and 1190 cm^{-1} (sulfonate stretching vibration) could be from DMAPS.

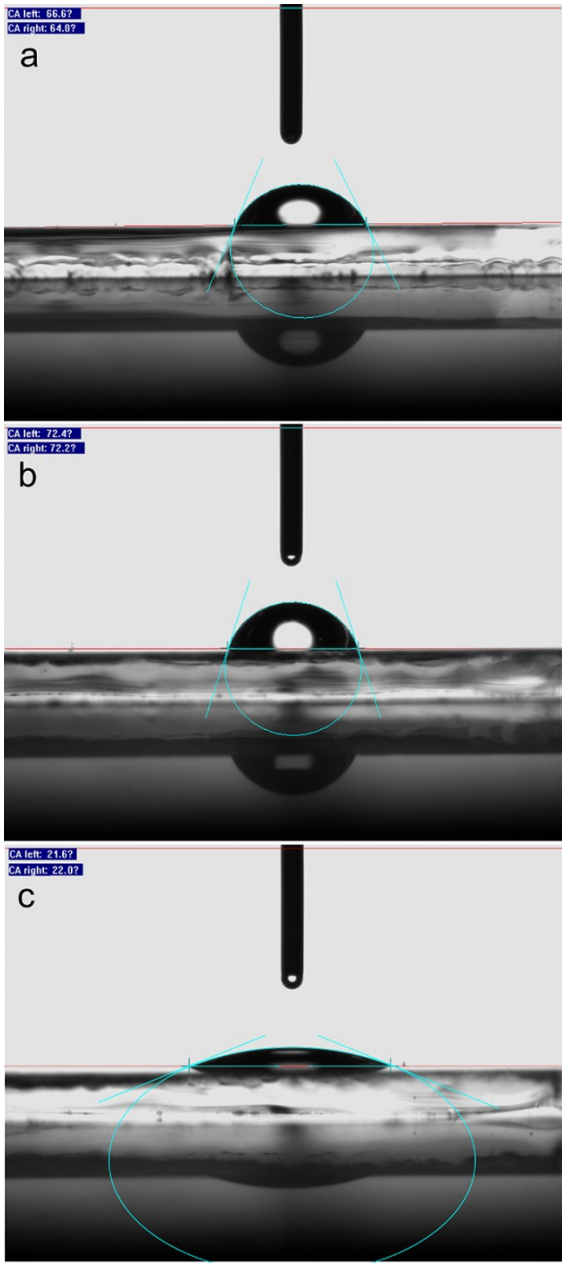

Fig. S5 Contact angle and static images of a water drop on bare gold surface (a), initiator-coated gold surface (b) and BIP grafted gold surface (c). The contact angle with ultrapure water was measured with sessile drop method and analyzed using video-based contact angle measuring device. The results indicated the BIP grafted surface was much more hydrophilic than the bare gold surface and the initiator-coated surface.

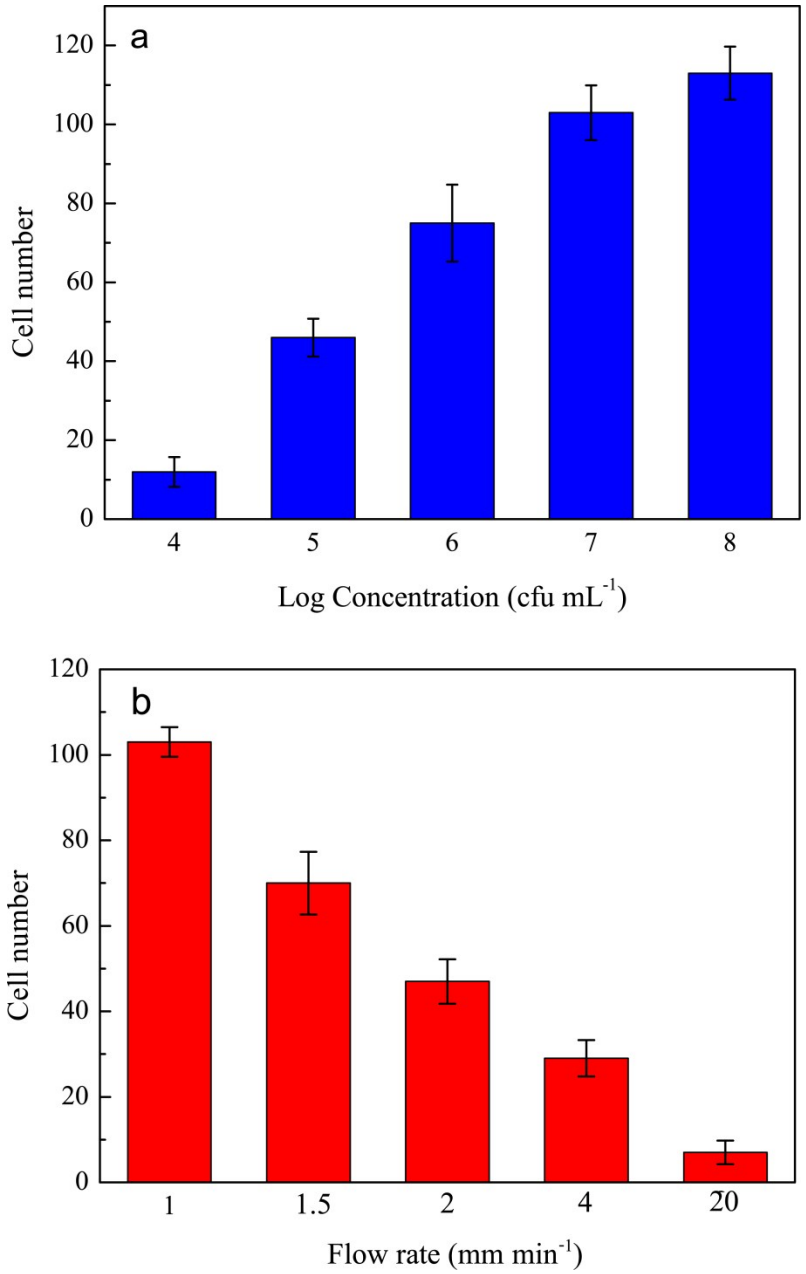

Fig. S6 (a) Numbers of GFP-*E. coli* cells adhered on *Es*-BIP with different cell densities. 100 μL bacterial suspension was infused into the microfluidic chip channel at the flow rate of 1 mm min^{-1} for 30 min followed by PBS rinse. (b) Numbers of GFP-*E. coli* cells adhered on *Es*-BIP at different flow rates. 100 μL bacterial suspension with the density of 5×10^7 cfu mL^{-1} was infused into the microfluidic chip channel for 30 min followed by PBS rinse.

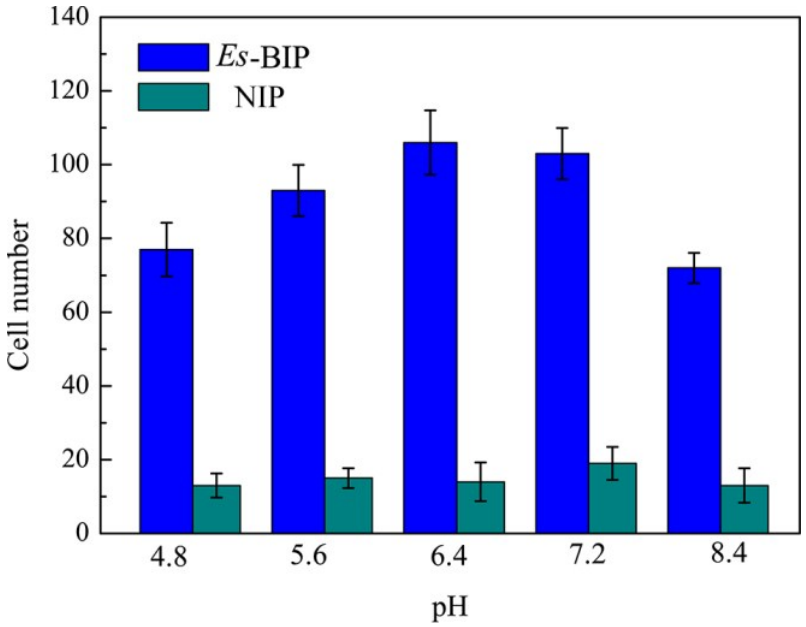

Fig. S7 Numbers of GFP-*E. coli* cells adhered on *Es*-BIP and NIP at different pH. 100 μL bacterial suspension (approximately cell density of 5×10^7 cfu mL^{-1}) was infused into the microfluidic channel at the flow rate of 1 mm min^{-1} for 30 min followed by PBS rinse. The pH of the bacterial suspensions was adjusted by hydrochloric acid solution or sodium hydroxide solution. The results indicated that a pH neutral environmental was optimal for bacterial recognition.

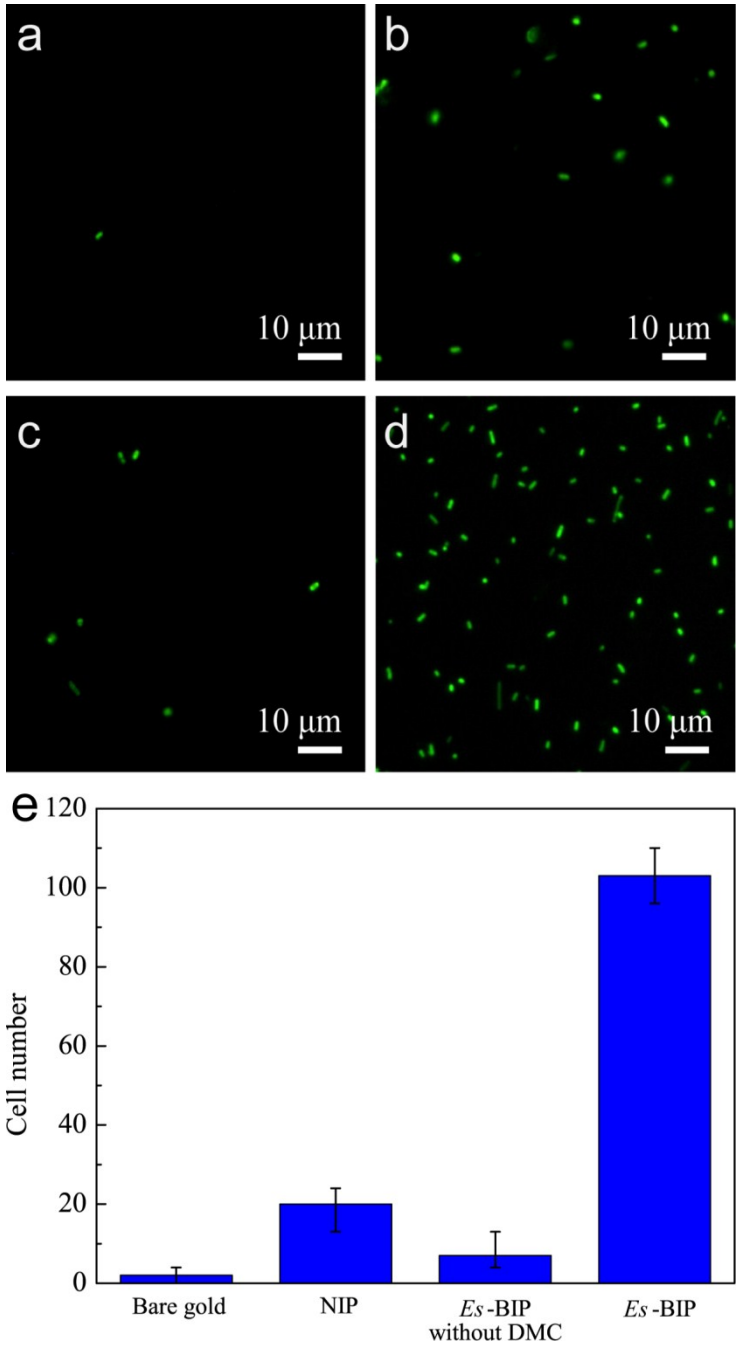

Fig. S8 Representative fluorescence images of GFP-*E. coli* cells adhered on bare gold substrate (a), NIP (b), *Es*-BIP without DMC (c) and *Es*-BIP (d). (e) Histogram of cell numbers of GFP-*E. coli* captured on the four substrates. 100 μL bacterial suspension (approximately cell density of 5×10^7 cfu mL^{-1}) was infused into the microfluidic chip channel at the flow rate of 1 mm min^{-1} for 30 min followed by PBS rinse. Statistical data was based on five random microscopic fields with an area of 80×80 μm for each substrate.

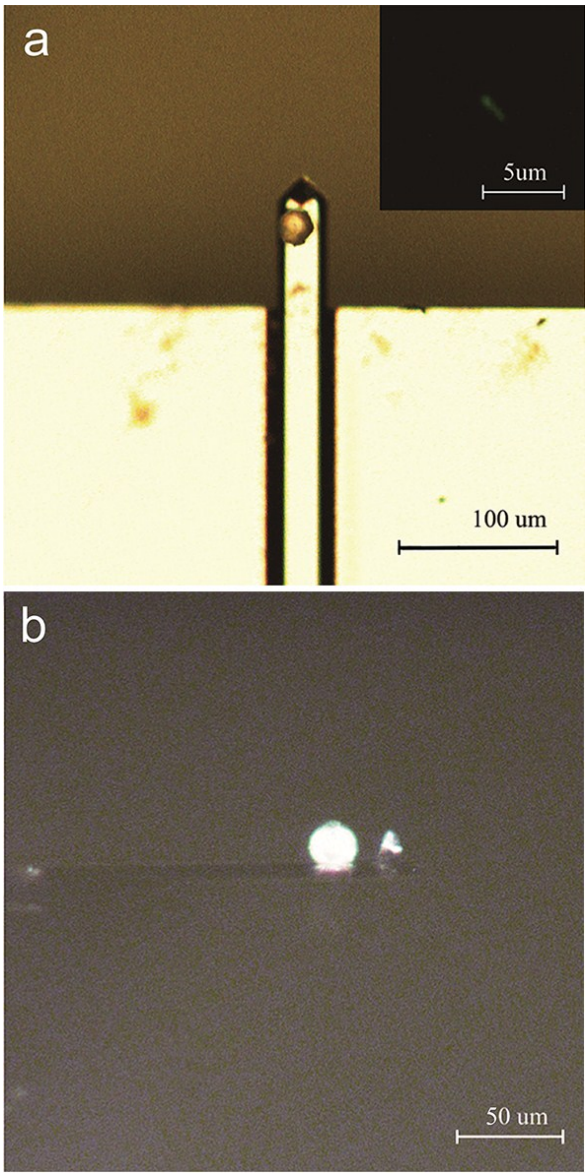

Fig. S9 Microscopic photographs of SCFS probe in front view (a) and side view (b). Insert shows fluorescent image of single GFP-*E. coli* cell on silica microsphere.

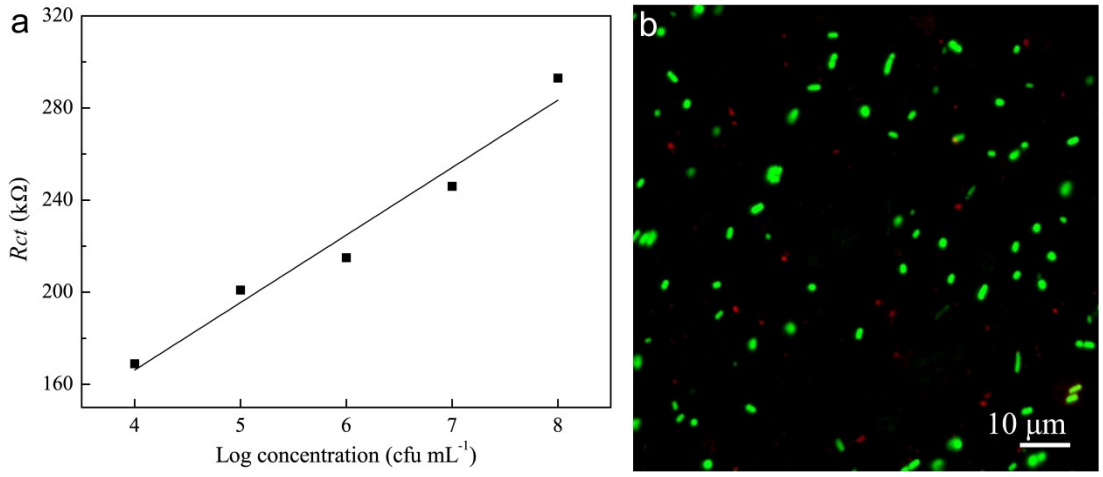

Fig. S10 (a) The linear relationship between R_{ct} and the logarithm of bacterial concentration. R_{ct} was measured at different bacterial concentrations in EIS measurements after selectively binding cells from the GFP-*E. coli* and *S. aureus* mixing suspension on the *Es*-BIP grafted gold electrode in the microfluidic chip; (b) Representative fluorescent image of selectively binding *S. aureus* and GFP-*E. coli* mixing suspension on *Es*-BIP after EIS measurements. Averaged by 5 random fields, the number ratio of GFP-*E. coli* / *S. aureus* is about 3.6.

Table S2 Examples of impedimetric electrochemical biosensors for bacterial detection

Bacteria	Transducer	Receptor	Medium	Detection Limit	Ref.
E. coli K-12	Interdigitated gold microelectrode	Bacteriophage	PBS	$10^{4\text{-}7}$ CFU/ml	1
E. coli	Interdigitated micro-hole array electrode	None	DI	10-1000 cells/ml	2
E. coli O157:H7	Gold nanoparticles modified graphene paper	Antibody	PBS	1.5×10^{2} CFU/ml	3
E. coli O157:H7	Interdigitated gold microelectrode	Antibody	PBS	$2.5\times10^{4\text{-}7}$ CFU/ml	4
E. coli O157:H7	Indium tin oxide electrode	Antibody	PBS	1 CFU/ml	5
Salmonella	Graphene oxide/gold nanoparticles modified glassy carbon	Aptamer	PBS	3 cells/ml	6
Salmonella typhimurium	Gold on printed circuit board	Monoclonal antibody	PBS	10 CFU in 100 µl	7
S. aureus	Nanoporous alumina membrane	Antibody	PBS	$10^{2\text{-}5}$ CFU/ml	8
Campylobacter jejuni	Modified Fe_3O_4 nanoparticles on glassy carbon	Monoclonal antibody	PBS	$10^{3\text{-}7}$ CFU/mL	9
GFP-*E. coli*	Gold electrode	*Es*-BIP	PBS	$10^{4\text{-}8}$ CFU/mL	This work

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