Electronic Supplementary Information

Construction of nanoantennas on the outer bacterial membrane

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Table of Contents

Experimental

Chemicals. All chemicals were reagent grade. Ultrapure water (>18 MΩ cm) sterilized using UV light was used for all experiments. Chloroauric acid ($HAuCl_4$), sodium citrate, 2-aminoethanethiol, sodium borohydride ($NaBH_4$) and aniline used to prepare gold nanoparticles (AuNPs) were purchased from Wako Pure Chemical Industries, Ltd., Japan.

Microscopic observations. SEM observations were performed using a field emission-scanning electron microscope (S-4700, Hitachi) at an applied voltage of 10 kV. SEM images of the labeled bacteria were obtained after adding 2 μL of a suspension on a conducting Si (111) wafer and drying in atmospheric air. The surface potentials of bacteria on a Si wafer were measured using a scanning-probe microscope in KFM mode (Nanocute SS, Seiko Instruments Inc., Japan).

Dark-field observations. Dark-field microscopy detects only the light scattered by a structure, whereas directly transmitted light is blocked using a dark-field condenser. Dark-field light-scattering images were acquired using an optical microscope (Eclipse 80i, Nikon, Japan) with a dark field condenser, a 100 W halogen lamp, and a CCD camera. Scattering spectra were obtained using a miniature grating spectrometer (USB4000, Ocean Optics), which was connected to the microscope using an optical fiber (core diameter 400 μm). Typical acquisition times were less than 1 s. Light-scattering spectra were corrected for spectral variations in the system response and the white-light intensity distribution through division by bright-field spectra recorded through the sample. The collection volume was nearly diffraction-limited (cross-sectional area, *ca*. 1 mm^2) for the 100× objective/fiber combination used here. Drops of nanoparticle dispersion, bacterial suspensions, and mixtures thereof were each pipetted onto a glass slide and dried in air.

Bacterial culture and sample preparation. A strain of *P. aeruginosa* was cultured in agar growth medium (E-MC35, Eiken Chemical Co., Japan) at 30 °C for 48 h. Colonies were suspended in liquid growth medium (30 mL) and cultured at 30 °C for 24 h. After centrifugation at 6,500 rpm for 15 min, the supernatant was removed. The precipitate was suspended in fresh ultrapure water by shaking for 1 min, respectively. The suspension was centrifuged using the same conditions described above. After these procedures were repeated three times, the final precipitate was suspended in aqueous 5 mM phosphate buffer systems (pH 2.8-9.3). The resulting suspension (9.2×10^9 cells mL^{-1}) was diluted by ultrapure water and/or the AuNP dispersions for the following experiments.

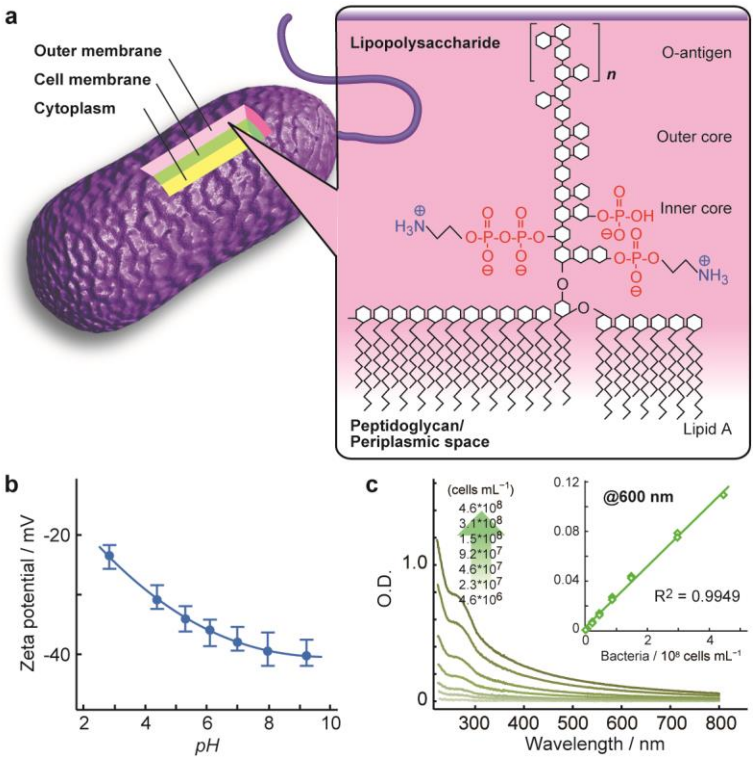

Fig. S1 (a) Schematic of the chemical structure of the outer membrane of a Gram-negative bacterium. (b) Dependence of zeta potential of *P. aeruginosa* on the pH in an aqueous buffer system (ionic strength, 2.6 mM). (c) Photometric spectra of *P. aeruginosa* by different concentrations of bacteria (4.6×10^6 to 4.6×10^8 cells mL^{-1}) each suspended in l mL of ultrapure water. The inset shows a calibration of the absorbance at 600 nm.

Preparation and characterization of AuNPs. Metal NPs used here were prepared using conventional chemical reduction in aqueous media. AuNPs were characterized using a zeta−potential and particle-size analyzer (ELSZ-2Plus, Otsuka Electronics Co., Ltd., Japan) and a transmission electron microscope (TEM, JEM-2000FX, JEOL, Japan).

Negatively charged AuNPs. Negatively-charged AuNPs were prepared by reduction of aqueous $HAuCl_4$ with sodium citrate as follows[1]: An aliquot (1 mL) of 1 wt% $HAuCl_4$ was added to 176 mL of 0.23 wt% sodium citrate and stirred at 80 °C for 20 min. The resulting solution was centrifuged at 8,500 rpm at 278 K. The precipitate was redispersed in 30 mL of ultrapure water, and the suspension was centrifuged using the same conditions described above. These procedures were repeated three times. The resulting precipitate that did not include unreacted species was redispersed in ultrapure water (0.028 wt%). The mean diameter of the AuNPs was 28.2 ± 4.2 nm and their zeta potential was –24.2 mV.

Positively charged AuNPs. Positively-charged AuNPs were prepared by reducing an aqueous solution of $HAuCl_4$ with $NaBH_4$, as follows[2]: $HAuCl_4$ (1 wt%, 2.34 mL) and 450 mM 2-aminoethanethiol (189 μL) were added to 40 mL of ultrapure water and stirred for 20 min at room temperature. $NaBH_4$ was added (10 μL, 10 mM), and the mixture was stirred vigorously for 10 min at room temperature in the dark. The resulting solution was centrifuged at 8,500 rpm at 278 K. The precipitate was redispersed in 30 mL of ultrapure water, and then the suspension was centrifuged using the same conditions described above. These procedures were repeated three times. The precipitate that had no unreacted species was redispersed in ultrapure water (0.028 wt%). The mean diameter of the AuNPs was 24.9 ± 5.7 nm with a zeta potential of +38.8 mV.

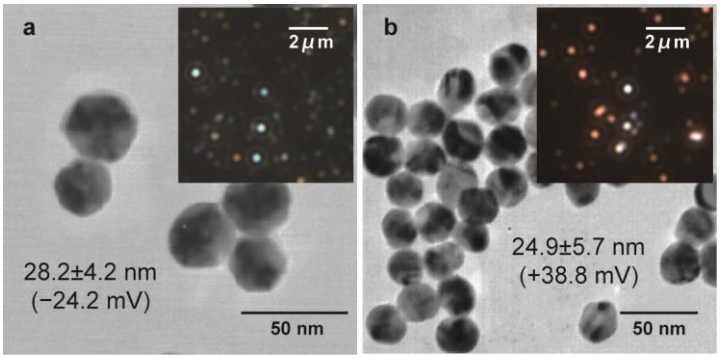

Fig. S2 TEM and dark-field (inset) images of AuNPs. (a) Negatively and (b) positively charged AuNPs. Respective mean diameter (nm) and zeta potential (mV) are indicated.

Preparation and characterization of nanoraspberries. An aqueous aniline solution (0.1 M) was added to an aqueous solution of chloroauric acid (0.01%), stirred at 353 K for 20 min centrifuged at 8,500 rpm (278 K). The resulting precipitate that did not include unreacted species was dispersed into 30 mL of ultrapure water stored in a glass bottle. The resulting nanoraspberries were characterized SEM, TEM and a zeta-potential & particle size analyzer. The mean diameter of the nanoraspberries (0.038 wt%) was approximately 100 nm with a zeta potential of +16.7 mV. SEM and TEM images show the formation of a raspberry-like hybrid with uniform shape and size (Fig. S3a).[3,4] A homogeneous nanoraspberry with a mean diameter of approximately 100 nm comprises numerous AuNPs with a mean diameter of approximately 5.0 nm. The AuNPs did not contact each other (refer to Fig. S3b). The aniline oligomers form molecular bridges not only between adjacent AuNPs within the raspberry, but also with those surrounding the raspberry.

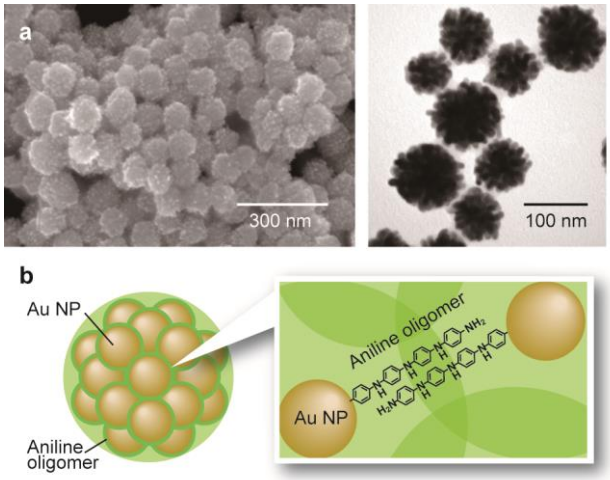

Fig. S3 (a) SEM and TEM images, (b) model illustration of nanoraspberry.

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