Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Aggregation-Based Detection of *M. smegmatis* using D-Arabinose-functionalized Silica Nanoparticles

Kalana W. Jayawardana, Samurdhi A. Wijesundera and Mingdi Yan*

Department of Chemistry, University of Massachusetts, Lowell, Lowell, MA 01854

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Materials and Methods

Tetraethyl orthosilicate (TEOS), ethanol (200-proof), ammonium hydroxide (25%), D-arabinose (99%), β-cyclodextrin (≥ 97%), fluorescein isothiocyanate (>90%), (3-aminopropyl)trimethoxysilane were purchased from Sigma-Aldrich. Water used was from a Milli-Q water ultrapure water purification system. 4-Azido-2,3,5,6-tetrafluoro-*N*-(3-(trimethoxysilyl)propyl)benzamide (PFPA-silane) were synthesized following previously developed procedures.[1-3]

Middlebrook 7H9 broth with OADC enrichment was prepared by mixing Middlebrook 7H9 (4.7 g, BD bioscience), glycerol (2.0 mL, Acros Organics), Tween® 80 (2.5 mL, 20%) and Milli-Q water (900 mL) together and sterilized by autoclaving (Tuttnauer EZ 10, Hauppauge, NY) before use. OADC enrichment (100 mL, BD Bioscience) was added to sterilized Middlebrook 7H9 broth at room temperature. The phosphate buffered saline (PBS) (pH 7.4) was prepared by dissolving a PBS tablet (Sigma-Aldrich) in Milli-Q water (200 mL). 200-Mesh Cu grids were purchased from Electron Microscopy Sciences (Hatfield, PA). *M. smegmatis* strain mc^2 155, DPBS 1X buffer were purchased from ATCC (Manassas, VA). SYTO® 61 red fluorescent nucleic acid stain was purchased from Life Technologies (Carlsbad, CA).

Dynamic light scattering (DLS) measurements were taken using Horiba SZ-100 particle size analyzer (Horiba Scientific Ltd., Kyoto, Japan). Transmission electron microscopy (TEM) images were obtained on a Phillips EM-400 TEM microscope operating at an accelerating voltage of 100 kV. Laser scanning confocal microscopy (LSCM) images were obtained on an Olympus LSCM FV300 microscope.

Synthesis of PFPA-functionalized dye-doped silica nanoparticles (FSNPs). FSNPs were synthesized following a previously developed procedure (Scheme S1).[4-6] Fluorescein isothiocynate (FITC) (15 mL, 12 mM in ethanol) was stirred overnight with (3-aminopropyl)trimethoxysilane (APTMS) (20 □L) in anhydrous ethanol (15 mL) at 42 ^{o}C to obtain the FITC-APTMS precursor. This precursor solution (5.0 mL) was then added to anhydrous ethanol (34 mL) containing ammonium hydroxide (2.0 mL, 6.25%) and TEOS (2.0 mL). The mixture was stirred for 2 days to obtain FSNPs. To synthesize PFPA-functionalized FSNP, a solution of PFPA-silane in toluene (8 mL, 12.6 mM) was added to FSNPs solution and the mixture was stirred overnight followed by refluxing at 78 ^{o}C for 1 h. The mixture was centrifuged at 12,000 rpm for 20 min, and the precipitate was re-dispersed and washed in ethanol for 3 times followed by in acetone for 3 times. Finally, the purified PFPA-FSNPs were dispersed in acetone.

Conjugation of carbohydrates on FSNPs. To a glass bottle containing an acetone solution of PFPA-FSNPs (1 mL, 2 mg/mL), an aqueous solution of D-arabinose (Ara) or β-cyclodextrin (CD) (200 μL, 10.0 mg/mL) was added. A 280 nm long-pass optical filter (Schott Glass Technologies Inc., Duryea, PA) was placed on top of the glass bottle, and the mixture was irradiated with a 450 W medium-pressure mercury vapor lamp (Ace Glass Inc., Vineland, NJ) for 30 min while stirring (3.2 mW/cm^2 measured by a 254 nm sensor). The resulting carbohydrate-conjugated nanoparticles were purified by centrifugation and re-dispersion in autoclaved Milli-Q water for 6 times followed by dialysis overnight.

Determination of carbohydrate density via thermal gravimetric analysis (TGA). All carbohydrate conjugated-nanoparticles and PFPA-functionalized nanoparticles were first

heated to 100 °C and held it for 10 min to remove volatiles, and then cooled to 30 °C using ultra-high purity argon gas (99.99 %). Then samples were heated at a rate of 10 °C /min to 1000 °C.

Colony counting assay to determine the number of bacteria. Bacteria were grown in OADC-enriched Middlebrook 7H9 broth at 37 °C until an OD_{650} of 0.3 was attained. Then a 10 μL aliquot of this bacterial suspension was taken and serially diluted 10^5 folds in OADC-enriched Middlebrook 7H9 broth. From the diluted solution, 20 μL was spread on OADC-enriched Middlebrook 7H10 agar plates. Colonies were counted after 36 h of incubation at 37 ^oC, and the colony counts are reported as log CFU/mL.

SYTO® 61 staining of *M. smegmatis.* *M. smegmatis* **(**mc^2 155) was grown until OD_{650} of 0.3 (1 x 10^8 bacteria cells/mL) was reached. The bacteria cells (10 mL, ~ 1 x 10^9) were then harvested, centrifuged at 5,000 rpm, and re-dispersed in pH 7.4 PBS buffer (1.0 mL). Then SYTO® 61 (5 μL) was added and incubated at 37 °C for 1 h. To remove the excess dye, SYTO61® stained bacteria was centrifuged at 5000 rpm for 10 min and the supernatant was discarded. The pellet was redispersed in fresh PBS buffer (10 mL).

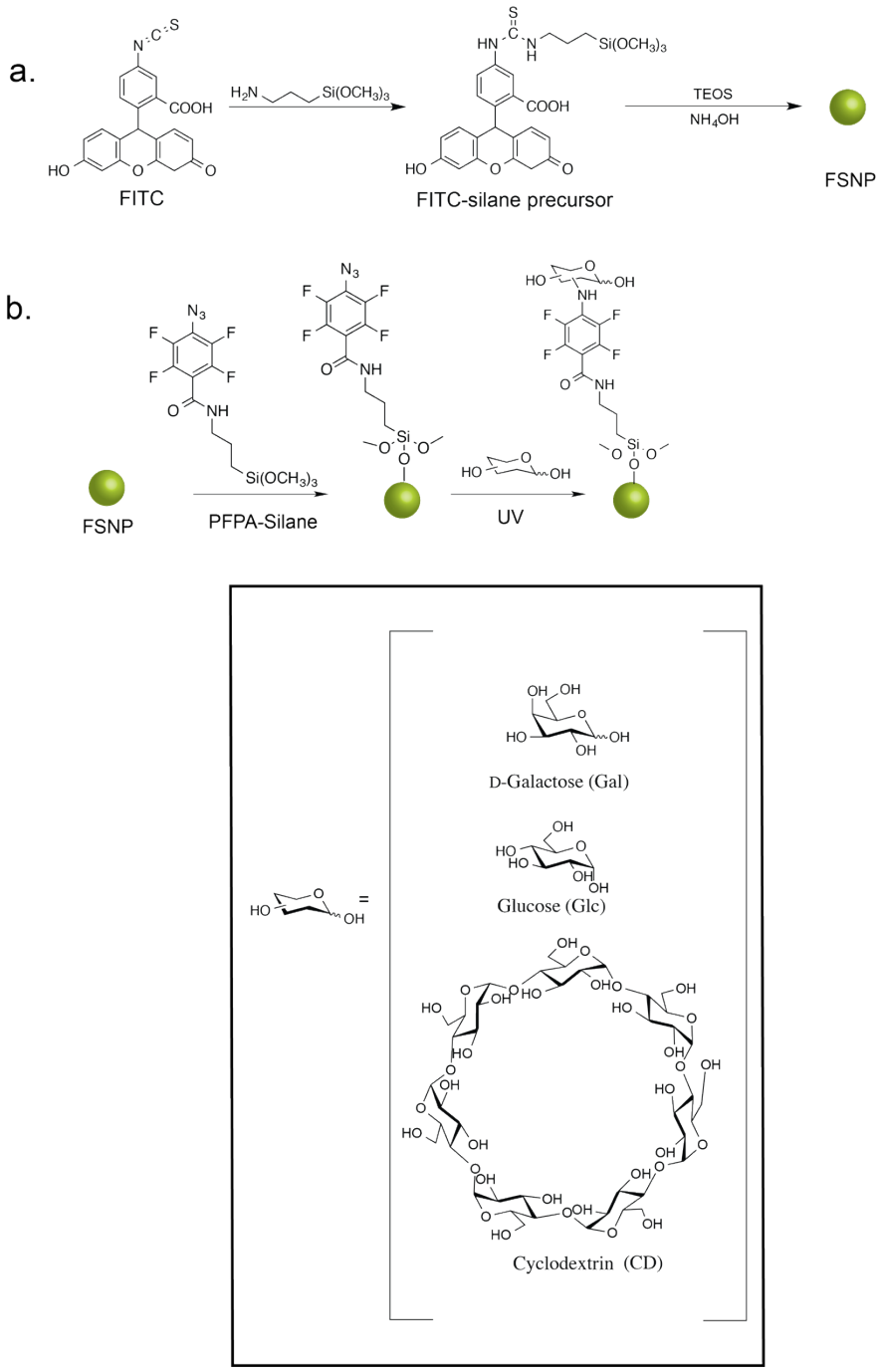
Treatment of carbohydrate-conjugated nanoparticles with *M. smegmatis*. SYTO® 61- stained *M. smegmatis* bacteria suspension was serially diluted until final bacteria cell count of 10^8-10^3 CFU/mL. To each sample, carbohydrate-conjugated nanoparticles (200 μL, ~1 mg/mL) were added to an aliquot of *M. smegmatis* (500 μL) from each dilution, and the mixture was incubated at 37 °C for 6 h while shaking at 180 rpm. The mixture was then centrifuged at 1,500 rpm for 10 minutes, and the supernatant containing nanoparticles was discarded. The pellet was then re-dispersed in autoclaved PBS. This centrifugation/ re-dispersion step was repeated for 3 times.

Treatment of carbohydrate-conjugated nanoparticles with *E. coli.* *E. coli* (ORN 178) was grown until OD_{600} of 0.3 (1 x 10^8 bacteria cells/mL) was reached. The bacteria cells (10 mL, ~ 1 x 10^9 bacteria cells) were then harvested, centrifuged at 5,000 rpm, and re-dispersed in pH 7.4 PBS buffer (1.0 mL). The carbohydrate-conjugated nanoparticles (200 μL, ~1 mg/mL) were added to an aliquot of *E. coli* (500 μL, ~ 1 x 10^8 CFU/mL), and the mixture was incubated at 37 °C for 6 h while shaking at 180 rpm. The mixture was then centrifuged at 1,500 rpm for 10 minutes, and the supernatant containing nanoparticles was discarded. The pellet was then re-dispersed in autoclaved PBS. This centrifugation/ re-dispersion step was repeated for 3 times.

Treatment of carbohydrate-conjugated nanoparticles with *S. epidermidis*.

S. epidermidis was grown until OD_{600} of 0.3 (1 x 10^8 bacteria cells/mL) was reached. The bacteria cells (10 mL, ~ 1 x 10^9 bacteria cells) were then harvested, centrifuged at 5,000 rpm, and re-dispersed in pH 7.4 PBS buffer (1.0 mL). The carbohydrate-conjugated nanoparticles (200 μL, ~1 mg/mL) were added to an aliquot of *S. epidermidis* (500 μL, ~ 1 x 10^8 CFU/mL), and the mixture was incubated at 37 °C for 6 h while shaking at 180 rpm. The mixture was then centrifuged at 1,500 rpm for 10 minutes, and the supernatant containing nanoparticles was discarded. The pellet was then re-dispersed in autoclaved PBS. This centrifugation/ re-dispersion step was repeated for 3 times.

TEM measurements. Samples for TEM were prepared by dropping the suspension of nanoparticles (few drops) or nanoparticle-treated bacteria cells (few drops in PBS buffer) onto a Cu grid (200- mesh), and was vacuum dried for few hours. The particle size was estimated by averaging the diameters of over 100 nanoparticles.



Scheme S1. (a) Synthesis of FSNP, (b) Synthesis of PFPA-functionalized FSNP and Conjugation of carbohydrates to FSNP.

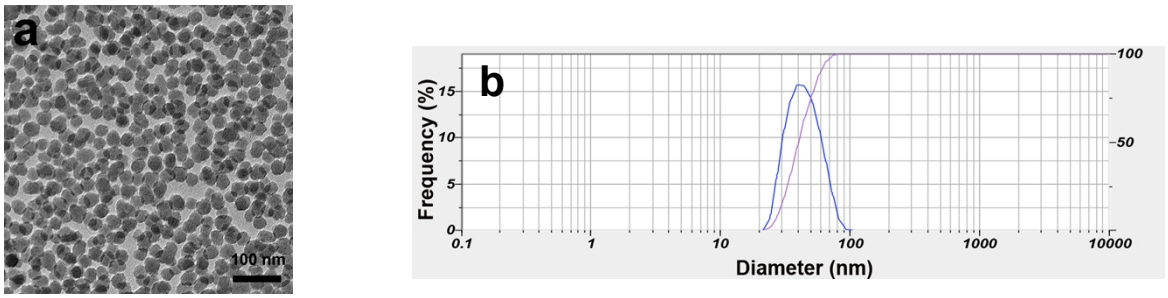


Figure S1. (a) TEM image and (b) DLS graph of FSNP.

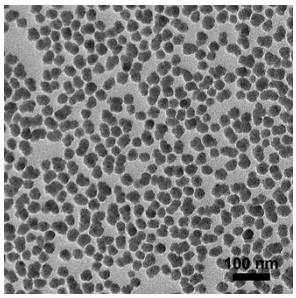


Figure S2. TEM image of PFPA-FSNP.

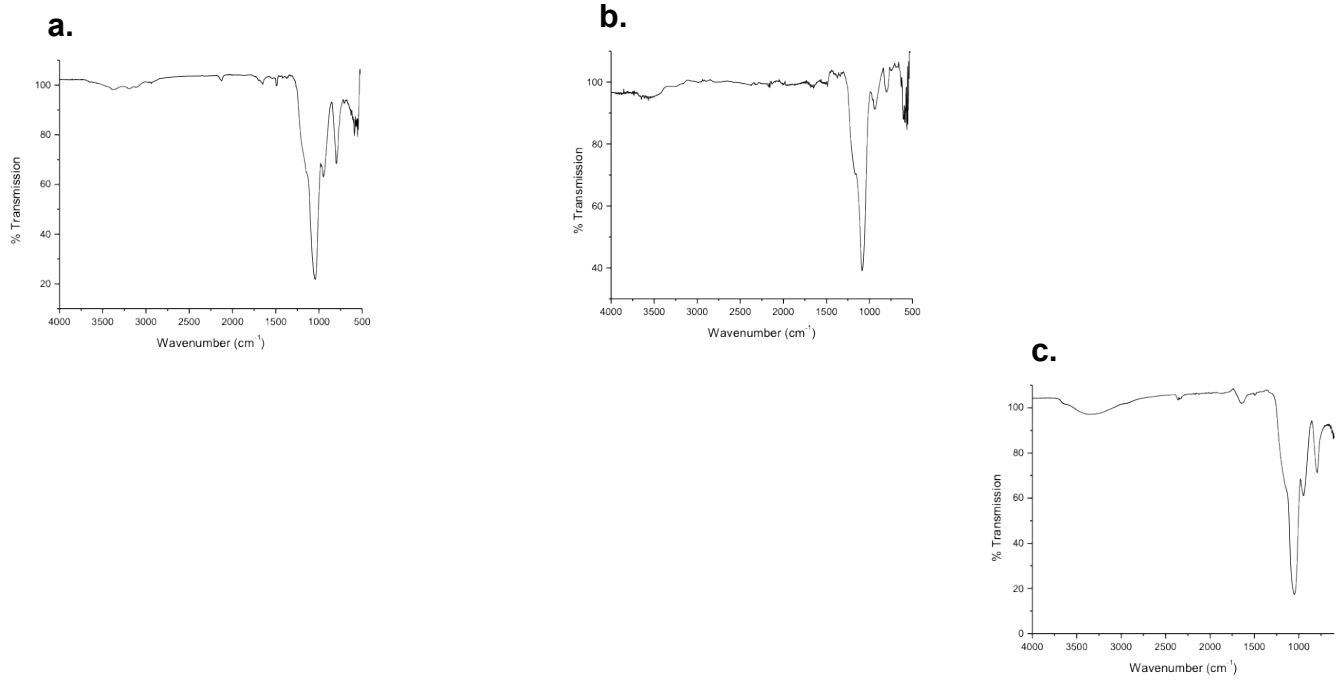


Figure S3. FTIR spectra of (a) PFPA-FSNP, (b) Ara-FSNP, (c) CD-FSNP.

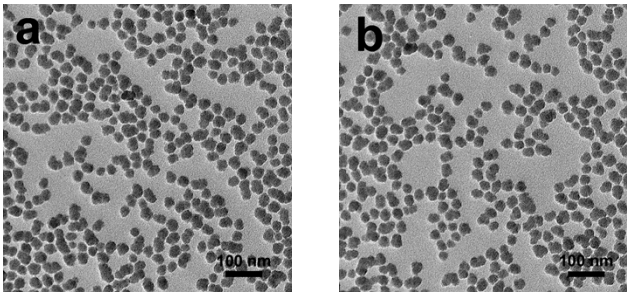


Figure S4. TEM images of (a) Ara-FSNP, (b) CD-FSNP.

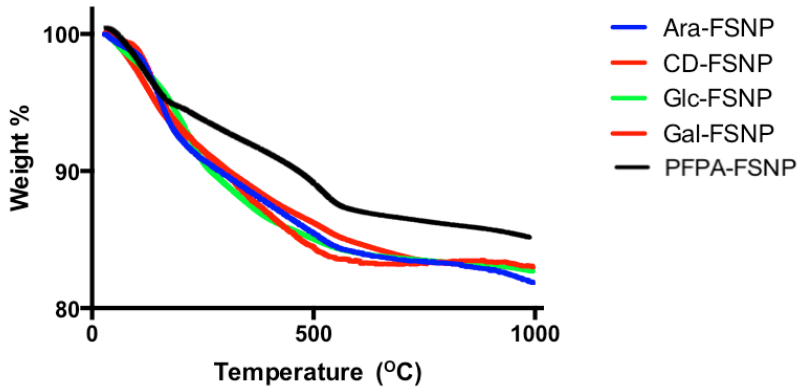


Figure S5. A typical TGA curve of functionalized FSNPs.

Table S1. Surface coverage of carbohydrates on nanoparticles. Each data is the average of 3 repeats.

carbohydrate	surface coverage density (μg/nm^2)	% (Experimental/Theoretical)
Ara	$3.8\times10^{-16} \pm 0.2\times10^{-16}$	28% ±1.5%
CD	$2.0\times10^{-16} \pm 0.1\times10^{-16}$	12% ±0.6%
Gal	$2.1\times10^{-16} \pm 0.1\times10^{-16}$	18% ±0.9%
Glc	$2.6\times10^{-16} \pm 0.1\times10^{-16}$	22% ±0.8%

Carbohydrate density was calculated according to the following equation: $\frac{W_x \frac{4}{3}\pi r^3 \rho}{W_y 4\pi r^2} = \frac{W_x}{3W_y}\rho r$, where w_x is the percent (%) weight difference between carbohydrate-FSNP and PFPA-FSNP at the cut-off temperature, w_y is the percent (%) weight of carbohydrate-FSNP at the cut-off temperature, ρ is the density of the nanoparticles and r is the DLS radius of the nanoparticles. Experimental (molecules/nm^2) is calculated by $\frac{(Carbonhydrate\ density \times 10^6) \times L}{M_W}$ where M_W is the molecular weight of the carbohydrate, L is the Avogadro number. Theoretical (molecules/nm^2) is calculated by $\frac{1}{S_A}$ where S_A is the average surface area of the carbohydrate by projecting a molecule of Ara, CD, Gal and Glc on a surface using Chem3D. They were 0.2 nm^2, 1.12 nm^2, 0.26 nm^2 and 0.25 nm^2, respectively. The cut-off temperature is chosen at 1000 °C.

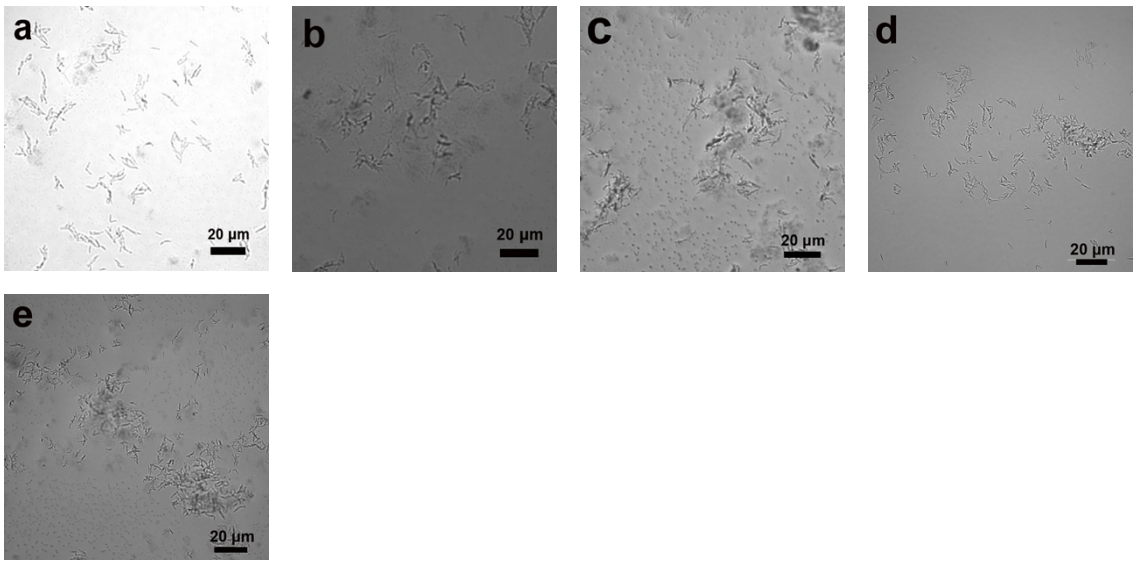


Figure S6. Optical images of *M. smegmatis* after incubating with Ara-FSNP for (a) 2 h, (b) 3 h, (c) 4 h, (d) 5 h, (e) 6 h. Bacteria concentrations were 10^8 CFU/mL.

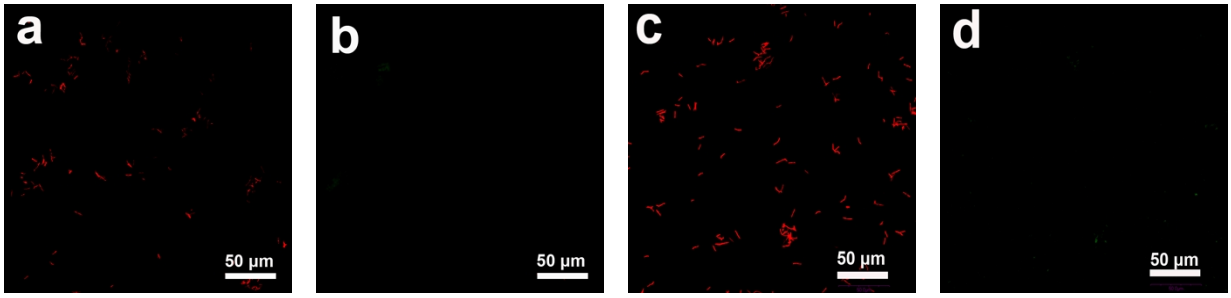


Figure S7. LSCM images of SYTO® 61 dye stained *M. smegmatis* after incubating with Gal-FSNP (a, b) and CD-FSNP (c, d). Bacteria concentration of the samples was 10^8 CFU/mL. (a, c) At 633 nm excitation showing SYTO® 61-stained *M. smegmatis*. (b, d) At 488 nm excitation showing FSNPs.

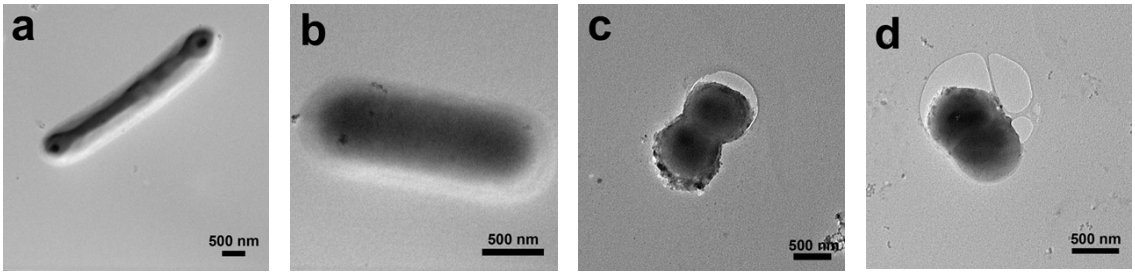


Figure S8. TEM images of *E. coli* strain ORN 178 after incubating 6 h with (a) Ara-FSNP, (b) CD-FSNP and TEM images of *S. epidermidis* 35984 after incubating 6 h with (c) Ara-FSNP, (d) CD-FSNP.

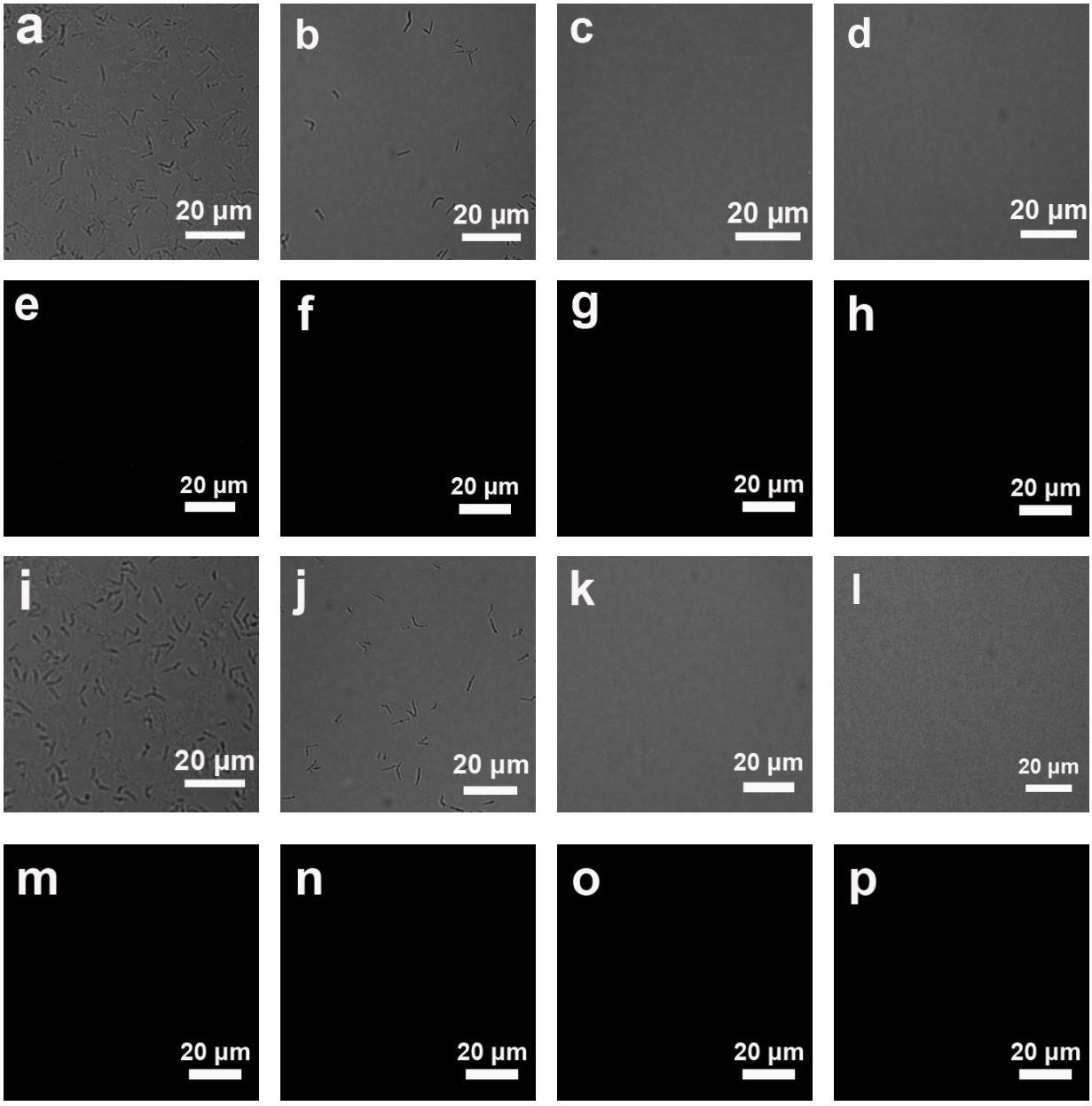


Figure S9. Optical images of *M. smegmatis* after incubating with CD-FSNP (a-d), Gal-FSNP (i-l). LSCM images after incubating with CD-FSNP (e-h), Gal-FSNP (m-p).

Bacteria concentrations of the samples were (a, e, i, m) 10^7 CFU/mL, (b, f, j, n) 10^6 CFU/mL, (c, g, k, o) 10^5 CFU/mL, (d, h, l, p) 10^4 CFU/mL.

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