Electronic Supplementary Information

Maltoheptaose Promotes Nanoparticle Internalization by *Escherichia coli*

*H. Surangi N. Jayawardena, Kalana W. Jayawardana, Xuan Chen, Mingdi Yan**

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

Table of Contents

Experimental Section

Materials. 1,2-Hexadecanediol (85%), tetraethyl orthosilicate (TEOS), IGEPAL® CO-520, oleylamine (70%), dibenzyl ether, octadecylamine (90%), sulfur (99.5%), selenium (99.5%), tri-*n*-butylphosphine (99%), zinc oxide (99%), stearic acid (95%), cadmium oxide (99.5%), trioctylphosphine oxide (90%), 1-octadecene (90%), anthrone (97%), ethanol (200-proof), ammonium hydroxide (25%), D-(+)-maltoheptaose, D-(+)-mannose, β-cyclodextrin (≥97%), resazurin sodium salt and sulfuric acid (98%) were purchased from Sigma-Aldrich. Water used was from a Milli-Q water ultrapure water purification system. (3-Aminopropyl)trimethoxysilane (APS) was acquired from TCI America (Portland, OR). Iron (III) acetylacetonate was purchased from Acros Organics (Fair Lawn, NJ). Luria-Bertani (LB) medium was prepared from LB (2 g, Sigma-Aldrich) in Milli-Q water (100 mL), and was sterilized before use. The phosphate buffer saline (PBS) (pH 7.4) was prepared by dissolving a PBS tablet (Sigma-Aldrich) in Milli-Q water (200 mL). Glutaraldehyde (8%), OsO_4 (4%), Embed 812, Araldite 502, DDSA, DMP-30, 200-mesh Cu grids were purchased from Electron Microscopy Sciences (Hatfield, PA). *E. coli* strains ORN 178 and ORN 208 were kindly provided by Professor Paul E. Orndorff of North Carolina State University. *E. coli* strain ATCC 33456 was purchased from ATCC™ (Manassas, VA), and the strain JW3992-1 was acquired from the Yale University Coli Genetic Stock Center (New Haven, CT). The UV source used was a 450 W medium-pressure quartz mercury vapor lamp (Ace Glass Inc., Vineland, NJ). 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. Fluorescence spectra were obtained on a Cary Eclipse fluorimeter (Agilent Technologies, Walnut Creek, CA).

Synthesis and functionalization of SMNPs with PFPA. The synthesis of MNPs followed a previously reported procedure of Sun *et al*.[1] Iron (III) acetylacetonate (0.706 g, 2.0 mmol), 1,2-hexadecanediol (2.584 g, 10.0 mmol), oleic acid (2.239 mL, 6.0 mmol) and oleylamine (2.820 mL, 6 mmol) in dibenzyl ether (20 mL) were stirred under a blanket of nitrogen. The mixture was then heated to 200 °C for 2 h, followed by heating at 280 °C for 1 h. After cooling down to room temperature, ethanol (200-proof, 40 mL) was added and the mixture was centrifuged at 7000 rpm for 10 min. The black precipitate was re-dispersed in hexane (30 mL) containing oleic acid (0.05 mL) and oleylamine (0.05 mL), and the mixture was centrifuged at 6000 rpm for 10 min. The precipitate was discarded, and the supernatant was collected and ethanol was added. After centrifugation, the precipitate was re-dispersed in hexane.

The reverse micelle method was employed to introduce silica coating on MNPs.[2] MNPs (0.5 mg) were dispersed in cyclohexane (1 mL), and a solution of APS in cyclohexane (60 μL, 0.1 mM) was added and the mixture was stirred at room temperature for 30 min. The following reagents, IGEPAL (0.225 g) in cyclohexane (5 mL), NH_4OH (25%, 100 μL) and TEOS (100 μL), were added sequentially and the mixture was stirred for 30 min, 1 h and 8-10 h, respectively after the addition of each reagent. The product was purified by centrifugation and re-dispersion in ethanol for 3 times to give SMNPs.

To a dispersion of SMNPs in ethanol (5 mL, 1 mg/mL) was added PFPA-silane in toluene (2 mL, 12.5 mM), and the mixture was stirred overnight at room temperature followed by refluxing at 78 °C for 1 h. The resulting PFPA-functionalized SMNPs were purified by repeated centrifugation (13,300 rpm, 30 min) and re-dispersion by sonication in ethanol (3 times) followed by acetone (3 times).

Synthesis of PFPA-functionalized MNPs. 2-(2′-(2″-(4-Azido-2,3,5,6-tetrafluorobenzamido)ethoxy)-ethoxy)ethyl dihydrogen phosphate (PFPA-phosphate, Scheme 1) was synthesized following a previously reported procedure.[3] A solution of MNPs in $CHCl_3$ (2 mL, 10 mg/mL) was added to a solution of PFPA-phosphate in $CHCl_3$ (2 mL, 6 mg/mL), and the mixture was stirred at room temperature overnight. Hexane was added until the solution became turbid, and the mixture was centrifuged at 7000 rpm for 15 min. The supernatant was discarded and the pellet was re-dispersed in acetone. This washing/centrifugation cycle was repeated for 5 times.

Synthesis of PFPA-functionalized SQDs. QDs were synthesized following the procedure of Peng *et al*.[4] Cadmium oxide (256.4 mg, 2.0 mmol), stearic acid (2.2754 g, 8.0 mmol) and 1-octadecene (6.0 g) were mixed and heated to 270 °C under argon until all cadmium oxide was dissolved, and the solution was cooled to room temperature. Octadecylamine (4.5 g) and tributylphosphine oxide (1.50 g) were added and the mixture heated up to 300 °C until it became a transparent solution. After the temperature was reduced to 250 °C, the Se precursor prepared by dissolving selenium (157.9 mg, 2.0 mmol) in tributylphosphine (0.50 g) was injected. The mixture was kept at 250 °C for 5 min before cooling down slowly to ~100 °C, and toluene (30 mL) was injected. After cooling to room temperature, methanol (40 mL) was added, and the resulting CdSe nanoparticles were purified by centrifugation and decantation with toluene and methanol for 3 times each, and were finally re-dispersed in toluene.

CdS and ZnS coatings were introduced using the successive ionic layer adsorption and reaction method.[5] The cadmium precursor solution (0.1 M) was prepared by dissolving cadmium oxide (320.8 mg, 2.5 mmol) in oleic acid (6.180 g, 6.9 mL) and 1-octadecene (18.0 mL) at 250 °C. The sulfur precursor solution (0.1 M) was prepared by dissolving sulfur (0.1128 g, 3.6 mmol) in 1-

octadecene (36.0 mL) at 180 °C. The zinc precursor solution (0.1 M) was prepared by dissolving zinc oxide (203.4 mg, 2.5 mmol) in oleic acid (6.180 g, 6.9 mL) and 1-octadecene (18.0 mL) at 300 °C. The Cd and Zn precursor solutions were used at about 90 °C and the sulfur solution was used at room temperature with argon protection.

A solution of 1-octadecene (3.00 mL) and octadecylamine (1.023 g) was vacuumed for 1 h at 100 °C. The solution of CdSe nanoparticles in toluene (4 mL, 2 mg/mL) was added after the mixture was cooled to room temperature. Toluene was then removed by heating the mixture to 100 °C for 1 h under vacuum, after which, the temperature was raised to 235 °C. Cd, Se, Zn and S precursors were injected sequentially. For a better coating quality, the quantum dots were annealed at 260 °C for 30 min. The reaction was then cooled to room temperature, and toluene (30 mL) and methanol (40 mL) were added. After centrifugation, CdSe/CdS/ZnS core/shell QDs were obtained, and the particles were re-dispersed in toluene for subsequent use.

Silica coating was introduced on QDs following the same reverse micelle method described above.[2] Briefly, CdSe/ZnS/ZnS core/shell QDs in toluene were dried under vacuum, and a solution of APS (50.0 μL, 0.1 M) in cyclohexane (1.00 mL) was added. The solution was then introduced to a solution of IGEPAL (0.225 g) in cyclohexane (4.38 mL), and the mixture was stirred for 30 min. NH_4OH (25%, 50 μL) followed by TEOS (30 μL) was added and the mixture was stirred overnight. Acetone was added to break the micelles and the solution was centrifuged at 12000 rpm for 30 min. SQDs were obtained after re-dispersing the precipitate in ethanol followed by repeated centrifugation and re-dispersion in ethanol.

To the SQDs re-dispersed in ethanol (5 mL, 1 mg/mL), a solution of *N*-(3-trimethoxysilylpropyl)-4-azido-2,3,5,6-tetrafluorobenzamide (PFPA-silane, Scheme 1, synthesized following a previous reported procedure[6]) in toluene (2 mL, 12.5 mM) was added.

The mixture was stirred overnight at room temperature followed by refluxing at 78 °C for 1 h. The resulting particles were purified by centrifugation (13000 rpm, 30 min) and re-dispersion in ethanol and acetone for 3 times, respectively.

Synthesis of PFPA-functionalized SNPs. Silica nanoparticles were synthesized following a slightly modified Stöber protocol.[7] A solution of 200-proof ethanol (34 mL), TEOS (2.80 mL) and NH_4OH (6.25%, 2.80 mL) was vigorously stirred at room temperature for at least 8 h. A solution of PFPA-silane in toluene (17 mL, 12.5 mM) was added directly to the Stöber solution, and the solution was stirred overnight at room temperature and then refluxed at 78 °C for 1 h. The product was purified by centrifugation and re-dispersion by sonication for 3 times in 200-proof ethanol and 3 times in acetone to afford PFPA-functionalized SNPs. Particle size was determined using TEM and DLS (Figs. S1g and S2).

Conjugation of carbohydrates to PFPA-functionalized SNPs, SMNPs, SQDs and MNPs. To a glass bottle containing an acetone solution of SNPs (1 mL, 4 mg/mL), SMNPs (1 mL, 1 mg/mL), SQDs (1 mL, 1 mg/mL) or MNPs (1 mL, 1 mg/mL) was added an aqueous solution of carbohydrate Man or G7 or CD (200 μL, 10.0 mg/mL). A 280 nm long-pass optical filter was placed on top of the glass bottle, and the mixture was irradiated with the 450 W medium-pressure Hg lamp (3.2 mW/cm^2 measured by a 254 nm sensor) for 7 min while stirring. The resulting carbohydrate-conjugated nanoparticles were purified by centrifugation and re-dispersion in Milli-Q water followed by dialysis overnight.

Determination of carbohydrate density on SNPs. A colorimetry assay based on anthrone/sulfuric acid was used to determine the sugar density on SNPs.[8] A solution of anthrone in concentrated sulfuric acid (1.50 mL, 0.048 M) was added slowly to an aqueous solution of carbohydrate-modified SNPs (0.50 mL, 1.0 mg/mL) under vigorously stirring in an ice bath. The

mixture was then boiled for 10 min. After cooling to room temperature, the solution was subjected to UV-vis spectroscopy measurement, and the absorbance at 620 nm was recorded. The measured absorbance was compared to the calibration curve obtained by performing the same assay on the free carbohydrate. The density, reported as the number of carbohydrate molecules on a single SNP, was then calculated (Table S1). The surface coverage, defined as the percentage of carbohydrate on SNP vs. the theoretical value computed assuming a densely packed carbohydrate monolayer on SNP, was calculated following a previously developed method (Table S1).[8b]

Incubation of carbohydrate-conjugated NPs with *E. coli*. *E. coli* strains ATCC 33456, JW 3992-1, ORN 178 and ORN 208 were each inoculated overnight in the LB medium at 37 °C and 150 rpm. They were re-inoculated in the fresh LB medium and grown until an OD_{600} of 0.5 was attained (OD of 0.1 corresponds to ~ 10^8 bacteria cells). The bacteria cells (30 mL) were then harvested, centrifuged at 5000 rpm, and re-dispersed in pH 7.4 PBS buffer (5.0 mL). The carbohydrate-conjugated NPs (200 μL, ~1 mg/mL) were added to an aliquot of *E. coli* (500 μL) and the mixture was incubated at 37 °C for 2 h while shaking at 150 rpm. The mixture was then centrifuged at 2000 rpm for 10 minutes, and the supernatant containing NPs was discarded. The pellet was then re-dispersed in PBS for subsequent studies.

TEM measurements. TEM samples were prepared by dropping the suspension of NPs or NP-treated bacteria cells (20.0 μL) onto a 200-mesh Cu grid followed by vacuum drying overnight. Particle size was measured by averaging the diameters of over 25 nanoparticles. The thin section samples were prepared as follows. A suspension of MNP-treated bacterial cells or untreated bacterial cells (1 mL) was centrifuged at 2000 rpm for 5 min and the supernatant removed. The pellet was then immersed in a solution of glutaraldehyde in PBS (1.00 mL, 2.5%) in an

Eppendorf tube at 4 °C overnight. The glutaraldehyde-fixed pellet was washed 3 times in PBS by incubating the pellet in PBS (1 mL) at 4 °C for 10 min. The pellet was then incubated in a solution of OsO_4 in PBS (2%) at 4 °C for 30 min. The excess OsO_4 was washed with PBS for 3 times by incubating the pellet in PBS (1 mL) at 4 °C for 10 min and removing the supernatant. The pellet was then dehydrated by incubating the pellet in 1 mL of 25%, 30%, 50% and 75% ethanol at 4 °C for 10 min each, followed by 95% ethanol (twice) and 100% ethanol (3 times) at room temperature for 10 min each. Finally, the pellet was incubated in 1 mL of propylene oxide (twice) at room temperature for 10 min each. The embedding resin medium was prepared by mixing Embed-812 (5.0 mL), Araldite 502 (heated to 60 °C, 3.0 mL), DDSA (heated to 60 °C, 11.0 mL) and DMP-30 (0.50 mL), and cooling to room temperature. The pellet was then infiltrated sequentially in propylene oxide/resin (3:1, 2:1, 1:1, 1:2 vol/vol) at room temperature for 15 min each, and finally in the embedding resin. A portion of the pellet with embedding resin was transferred to a BEEM capsule and was allowed to cure overnight at 60 °C in a vacuum oven. The BEEM capsule was then removed and the resin-embedded pellet was cut with an ultramicrotome (Leica EM UC6, Buffalo Grove, IL) into ~100 nm thick slices which were then placed on 200-mesh Cu grids for TEM imaging.

Resazurin cell viability assay. *E. coli* cells (ATCC 33456) in pH 7.4 PBS buffer (500 μL) was incubated with nanoparticle suspension (200 µL) for 2 h. An aqueous solution of resazurin (20.0 µL, 4 mM) was added and the mixture was incubated at room temperature for 3 h. The fluorescence intensity was measured at 589 nm (excitation 570 nm).

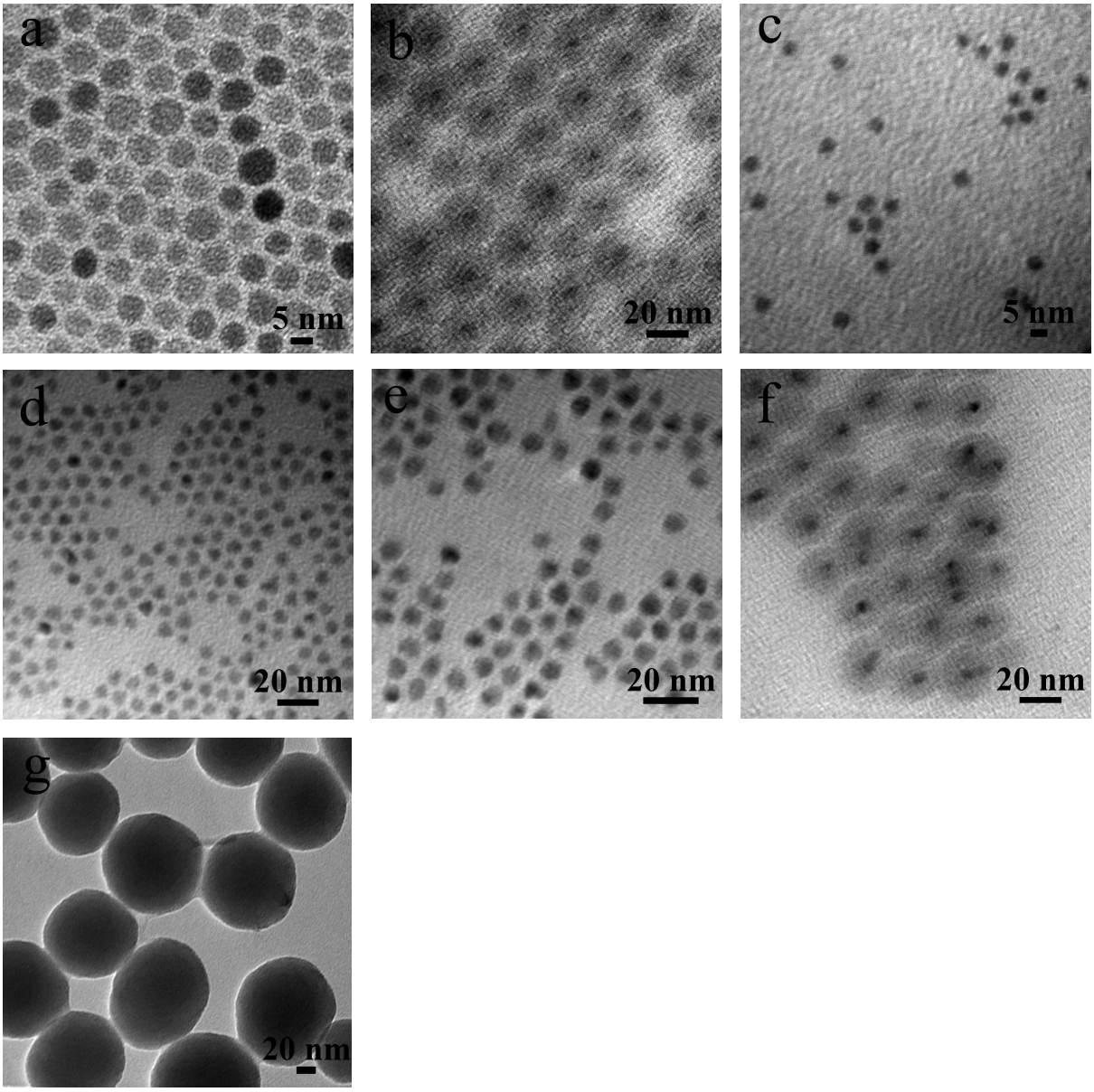

Figure S1. TEM images of (a) MNPs, (b) SMNPs, (c) CdSe NPs, (d) CdSe/CdS QDs, (e) CdSe/CdS/ZnS QDs, (f) SQDs, (g) SNPs.

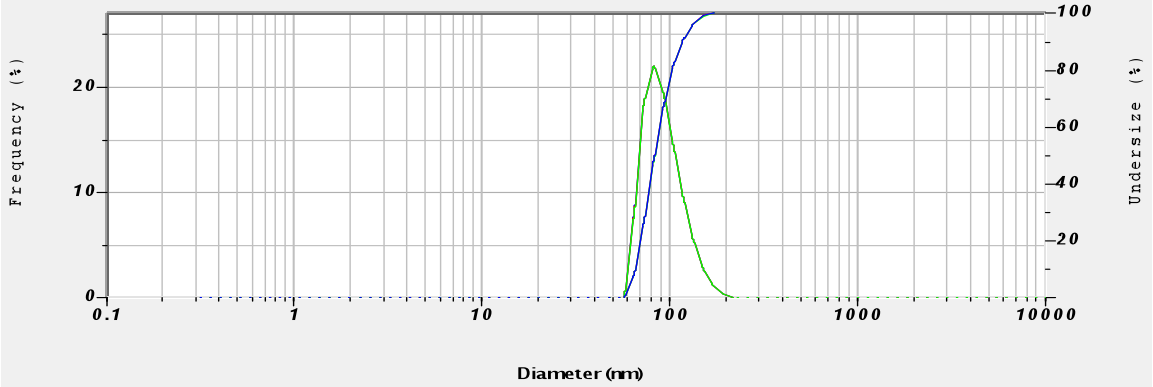

Figure S2. DLS characterization of SNPs.

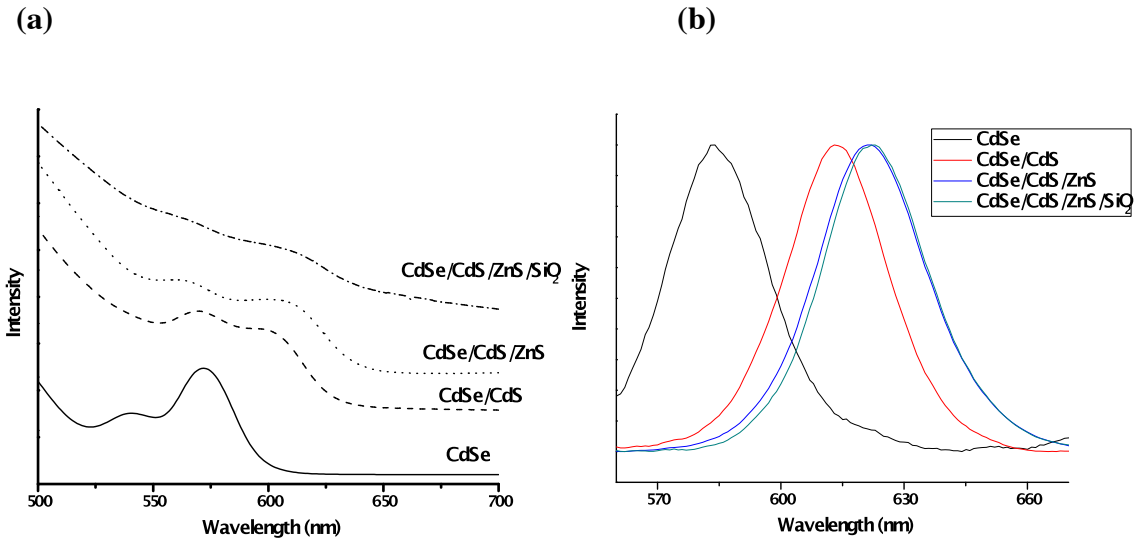

Figure S3. (a) Absorption and (b) emission spectra (excitation: 450 nm) of various QDs.

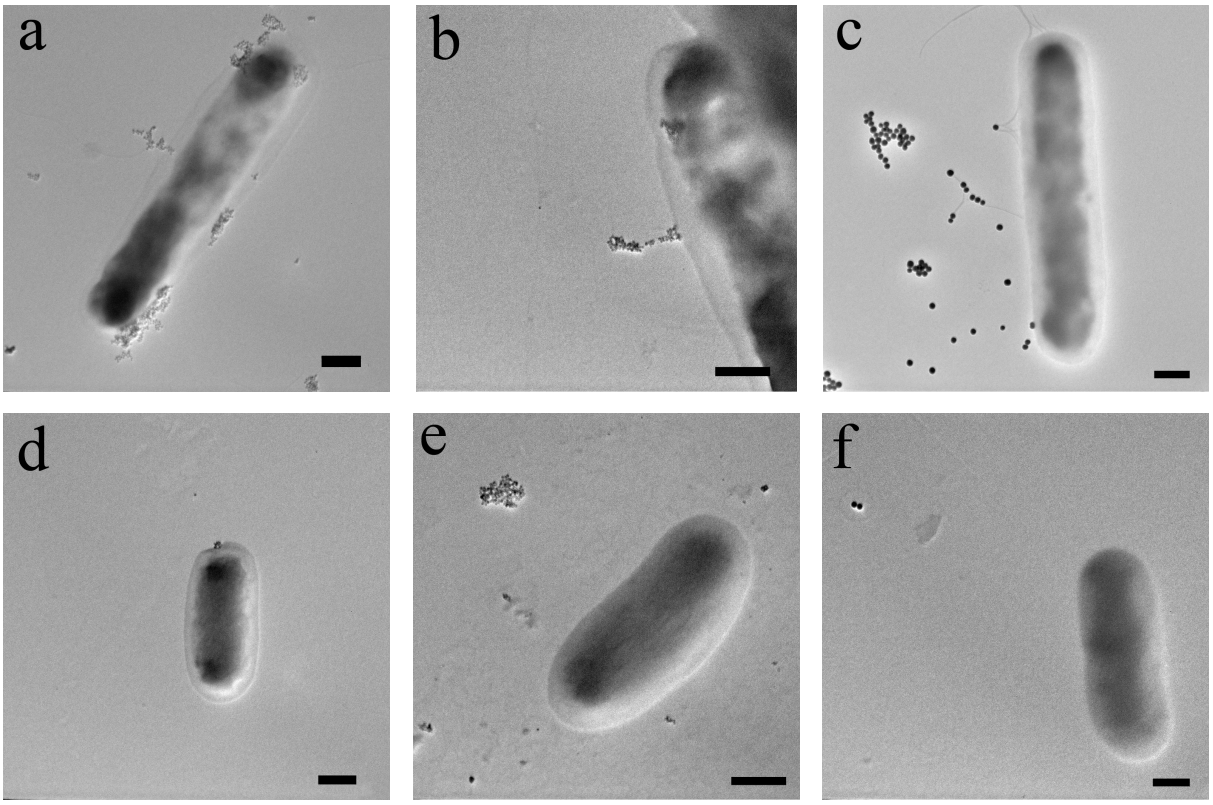

Figure S4. TEM images of ORN 178 incubated with (a) Man-SMNP, (b) Man-SQD, (c) Man-SNPs, and ORN 208 incubated with (d) Man-SMNP, (e) Man-SQD, (f) Man-SNPs. Scale bars: 500 nm.

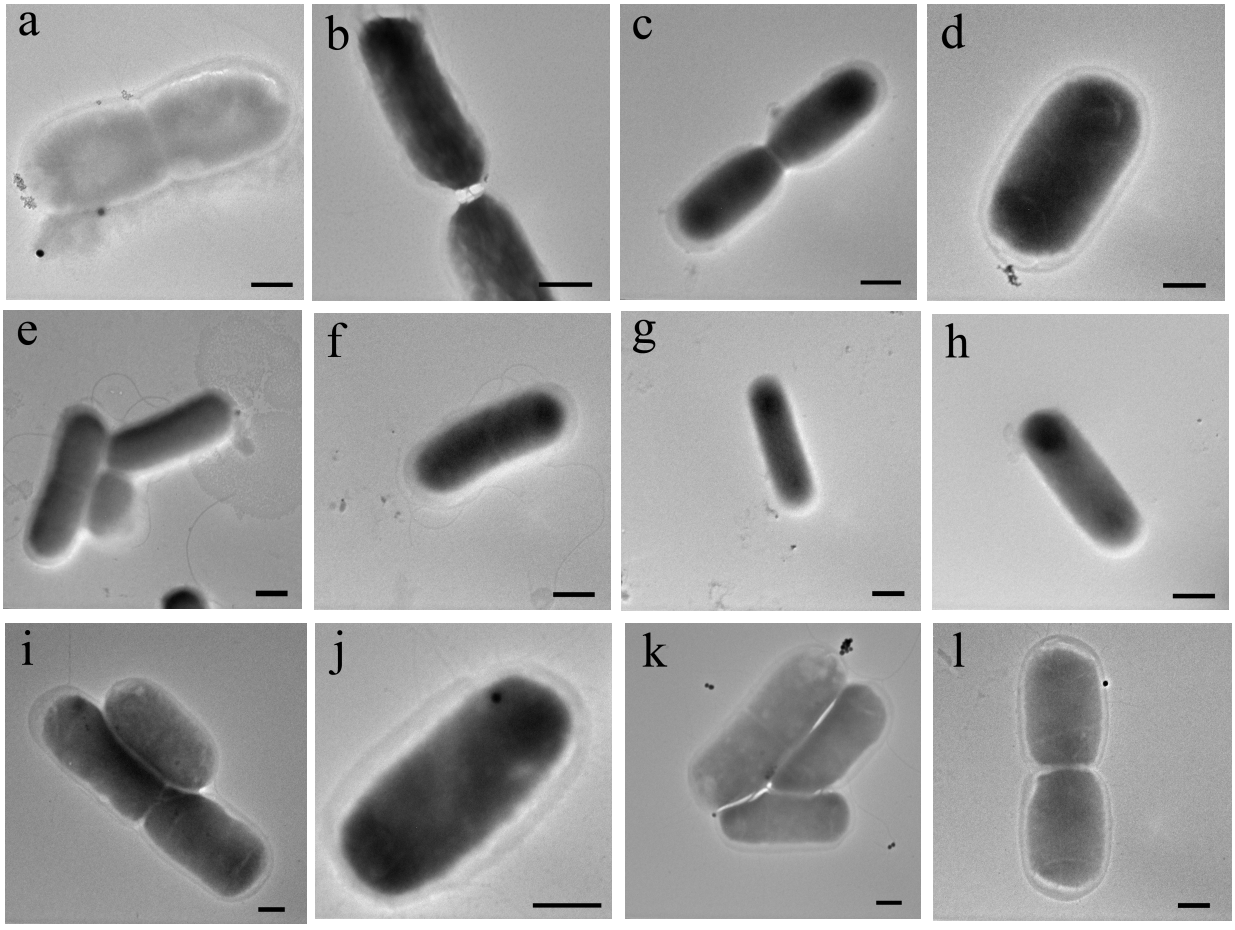

Figure S5. TEM images of CD-SMNPs incubated with *E. coli* (a) ORN 178, (b) ORN 208, (c) ATCC 33456, (d) JW3392-1; CD-SQDs incubated with *E. coli* (e) ORN 178, (f) ORN 208, (g) ATCC 33456, (h) JW3392-1; CD-SNPs incubated with *E. coli* (i) ORN 178, (j) ORN 208, (k) ATCC 33456, (l) JW3392-1. Scale bars: 500 nm.

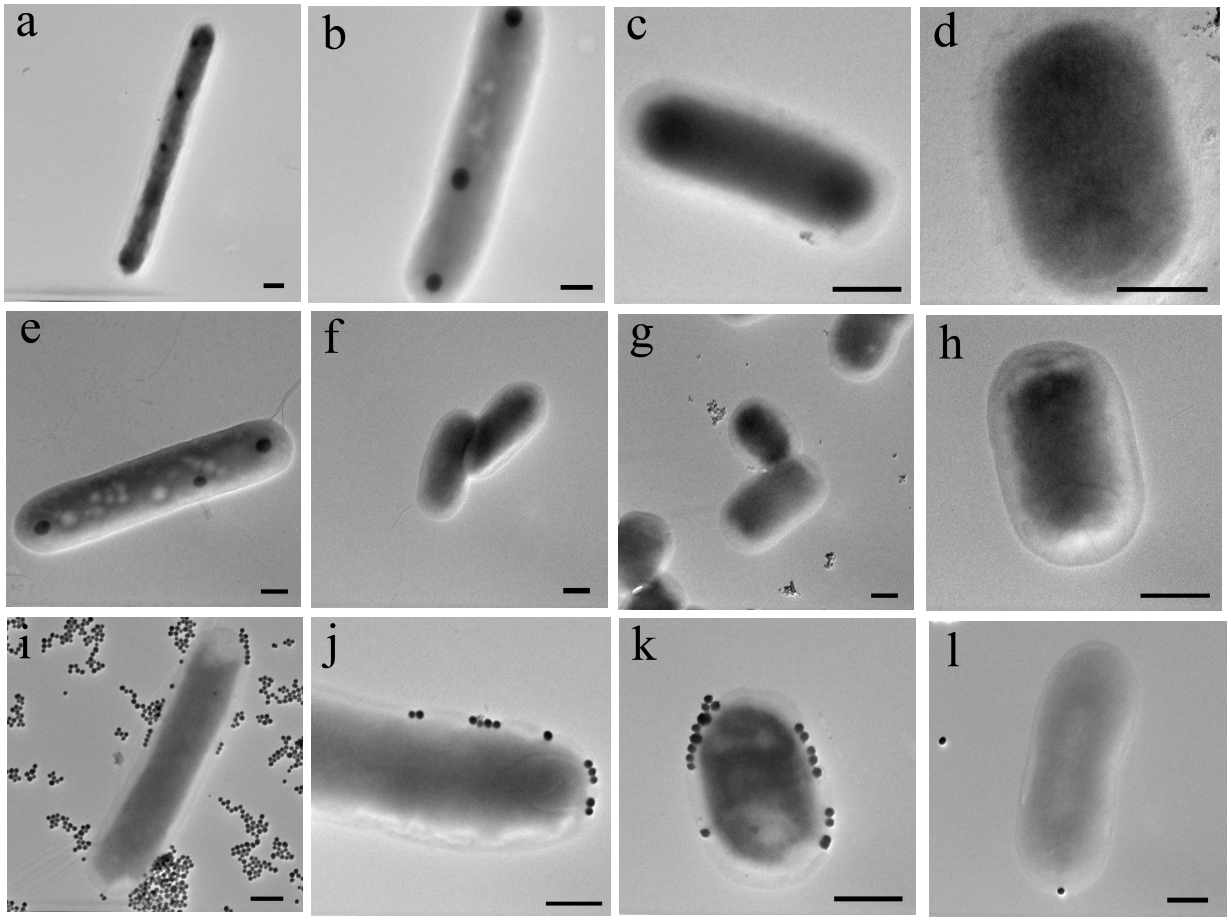

Figure S6. TEM images of SMNPs incubated with *E. coli* (a) ORN 178, (b) ORN 208, (c) ATCC 33456, (d) JW3392-1; SQDs incubated with *E. coli* (e) ORN 178, (f) ORN 208, (g) ATCC 33456, (h) JW3392-1; SNPs incubated with *E. coli* (i) ORN 178, (j) ORN 208, (k) ATCC 33456, (l) JW3392-1. Scale bars: 500 nm.

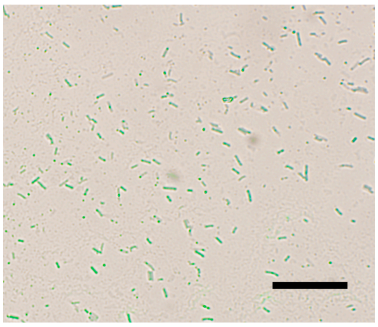

Figure S7. Overlay of confocal laser scanning microscopy image and bright field image of *E. coli* ATCC 33456 treated with G7-SNP.[8b] SNPs were doped with fluorescein. Scale bar: 10 μm.

Table S1. Ligand density and surface coverage of carbohydrate conjugated on SNPs

Carbohydrate	No. of carbohydrate /SNP (Experimental)*	No. of carbohydrate /SNP (Theoretical)	Surface coverage
Man	68,623 ± 6176	134,556	51 ± 9%
G7	11,517 ± 1497	23,993	48 ± 13%
CD	14,657 ± 879	28,187	52 ± 6%

* Each data was an average of 5 assays.

Table S2. Viability of *E. coli* ATCC 33456 after incubating with nanoparticles

Nanoparticles	*Fluorescence intensity at 589 nm*[*]	*Percent viability*[**]
G7-SMNPs	58.33805 ± 0.37093	94.3 %
G7-SQDs	61.19452 ± 0.88657	98.9 %
G7-SNPs	54.41646 ± 1.04457	87.9 %
SMNP	59.93642 ± 0.69806	96.9 %
SQD	60.82896 ± 1.82130	98.4 %
SNP	60.49563 ± 2.20434	97.8 %

*All data are an average of 3 repetitions.

**Percent viability was computed by taking the ratio the fluorescence intensity of the sample against the control which was the bacterial cells incubated for 2 h in the absence of the nanoparticles.

References

1. S. Sun, H. Zeng, D. B. Robinson, S. Raoux, P. M. Rice, S. X. Wang and G. Li, *J. Am. Chem. Soc*., 2003, **126**, 273-279.
2. S. T. Selvan, T. T. Tan and J. Y. Ying, *Adv. Mater*., 2005, **17**, 1620-1625.
3. L.-H. Liu, H. Dietsch, P. Schurtenberger and M. Yan, *Bioconjugate Chem*., 2009, **20**, 1349-1355.
4. J. J. Li, Y. A. Wang, W. Guo, J. C. Keay, T. D. Mishima, M. B. Johnson and X. Peng, *J. Am. Chem. Soc*., 2003, **125**, 12567-12575.
5. (a) M. Ristov, G. Sinadinovski, I. Grozdanov and M. Mitreski, *Thin Solid Films*, 1989, **173**, 53-58; (b) S. Park, B. L. Clark, D. A. Keszler, J. P. Bender, J. F. Wager, T. A. Reynolds and G. S. Herman, *Science*, 2002, **297**, 65.
6. M. Yan and J. Ren, *Chem. Mater*., 2004, **16**, 1627-1632.
7. J. P. Gann and M. Yan, *Langmuir*, 2008, **24**, 5319-5323.
8. (a) X. Wang, O. Ramstrom and M. Yan, *Chem. Commun*., 2011, **47**, 4261-4263; (b) Q. Tong, X. Wang, H. Wang, T. Kubo and M. Yan, *Anal. Chem*., 2012, **84**, 3049-3052; (c) X. Wang, O. Ramstro¨m and M. Yan, *Anal. Chem*., 2010, **82**, 9082-9089.