Supporting Information

Synthetic polymer nanoparticles conjugated with $FimH_A$ from *E. coli* pili to emulate the bacterial mode of epithelial internalization

Lily Yun Lin,[†] Kristin M. Tiemann,[‡] Yali Li,[§] Jerome S. Pinkner,[#] Jennifer N. Walker,[‡]

Scott J. Hultgren,[#] David A. Hunstad[‡#]* and Karen L. Wooley[†]*

[†]*Departments of Chemistry and Chemical Engineering, Texas A&M University, P.O. Box 30012, College Station, TX 77840, United States*

Departments of [‡]Pediatrics and [#]Molecular Microbiology and Center for Women's Infectious Disease Research, Washington University School of Medicine, St. Louis, MO 63110, United States

[§]*Center for Molecular Imaging Research, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02129, United States*

Materials and instruments:

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received unless otherwise indicated. PAA_{120}-*b*-PS_{100} (Mn^{NMR} = 19,500 Da, PDI = 1.1, from $PtBA_{120}$-*b*-PS_{100} precursor) polymer precursor was synthesized as previously reported.[1] 5-((5-aminopentyl)thioureidyl)fluorescein, dihydrobromide salt (5-FITC cadaverine) (Invitrogen, Carlsbad, CA) and sulfonated *N*-hydroxysuccinimide (Sulfo-NHS) (Thermo Scientific, Waltham, MA) were used as received. Spectra/Por membrane tubes purchased from Spectrum Laboratory Inc. (Rancho Dominguez, CA) were used for dialysis. Amicon® ultra centrifugal filter devices (100 kDa MWCO) were purchased from Millipore (Bedford, MA). Nanopure water (18 MΩ•cm) was acquired by means of a Milli-Q water filtration system (Millipore, Billerica, MA).

UV-Vis spectra were collected at 37 °C in the region of 200 - 800 nm, using a Varian Cary 100 Bio UV-visible spectrophotometer. Dynamic light scattering measurements were conducted with a Brookhaven Instruments (Holtsville, NY) DLS system equipped with a model BI-200SM goniometer, BI-9000AT digital correlator, a model EMI-9865 photomultiplier, and an Innova 300 Argon laser operated at 514.5 nm (Coherent Inc., Santa Clara, CA). Measurements were made at 25 ± 1 °C. Prior to analysis, solutions were filtered through a 0.45 μm Millex®-GV PVDF membrane filter (Millipore, Medford, MA) to remove dust particles. Scattered light was collected at a fixed angle of 90°. The digital correlator was operated with 522 ratio spaced channels, and initial delay of 5 μs, a final delay of 50 ms, and a duration of 8 min. A photomultiplier aperture of 400 μm was used, and incident laser intensity was adjusted to obtain photon counting between 200 and 300 kcps. Calculations of particle size distributions and distribution averages were performed with the ISDA software package (Brookhaven Instruments), which employed single-exponential fitting, Cumulants analysis, and CONTIN particle size distribution analysis routines. All determinations were average values from ten measurements, with the standard deviations being calculated as the breadth of the distributions. Transmission electron microscopy (TEM) bright-field imaging was conducted on a Hitachi H-7500 microscope, operating at 80 kV. Immunogold EM samples

were viewed on a JEOL 1200EX II microscope (JEOL USA, Peabody, MA). Fluorescent and confocal images were collected with an LSM510 fluorescent confocal microscope (Carl Zeiss Inc., Thornwood, NY).

Experimental procedures:

General procedure for the preparation of the micelles and SCKs: PAA_{120}-*b*-PS_{100} block copolymer precursor (*ca*. 50 mg) was dissolved in THF (50 mL) in a 250-mL round bottom flask and allowed to stir for 30 min at room temperature. To this solution, an equal volume of nanopure water was added dropwise *via* a syringe pump over a period of 3 h. The reaction mixture was allowed to stir for an additional 24 h at room temperature and dialyzed against nanopure water for 4 d in presoaked dialysis tubing (MWCO *ca*. 6 - 8 kDa) to afford a micelle solution with a final polymer concentration of *ca*. 0.25 mg/mL. To the micelle solution of PAA_{120}-*b*-PS_{100} was added a solution of 2,2′-(ethylenedioxy)bis(ethylamine) (EDDA) in nanopure water (*ca*. 15 mg/mL, 1.1 eq, nominal 20% crosslinking) dropwise. To this solution, 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDCI) in nanopure water (*ca*. 14 mg/mL, 1.4 eq) was added dropwise *via* a syringe pump over 20 min, and the resulting mixture was allowed to stir overnight before dialysis against nanopure water for 4 d in presoaked dialysis tubing (MWCO.*ca*. 6 - 8 kDa) to afford SCK solutions with a final polymer concentration of *ca*. 0.25 mg/mL. Samples for TEM were prepared as follows: 4 μL of the SCK solution (with a polymer concentration of *ca.* 0.25 mg/mL) was deposited onto a carbon-coated copper grid, which was pre-treated with absolute ethanol to increase the surface hydrophilicity. After 5 min, the excess of the solution was quickly wicked away by a piece of filter paper. The samples were then negatively stained with 4 μL of 1 wt% phosphotungstic acid (PTA) aqueous solution. After 1 min, the excess PTA solution was quickly wicked away by a piece of filter paper and the samples were left to dry under ambient conditions overnight. Diameter (TEM) = 18 ± 2 nm, $D_{h(intensity)}$ = 100 ± 30 nm, $D_{h(volume)}$ = 70 ± 20 nm, $D_{h(number)}$ = 40 ± 10 nm.

Preparation of 5-FITC cadaverine-labeled SCKs: A solution of EDCI in nanopure water (1.8 mg, 18 mg/mL, 30 eq., 6 μmol) was added dropwise over five minutes to a solution of SCK in nanopure water

(15 mL, 0.25 mg/mL polymer concentration). The reaction mixture was stirred for 30 min at room temperature. To this solution, a solution of 5-FITC cadaverine in nanopure water (0.6 mg, 1.8 mg/mL, 1.1 μmol) was added dropwise over 2 min. The reaction was allowed to proceed for 24 h at room temperature before being transferred to presoaked dialysis tubing (MWCO *ca.* 6 - 8 kDa), then dialyzed against nanopure water for 4 d to remove unconjugated chromophores and other impurities, yielding a 5-FITC cadaverine-labeled SCK solution with a final concentration of *ca.* 0.21 mg/mL.

Conjugation of $FimH_A$ to 5-FITC cadaverine-labeled SCKs: A solution of 5-FITC cadaverine-labeled SCKs (3 mL, 0.21 mg/mL polymer concentration) was cooled to 4 °C while the pH of the solution was adjusted to *ca.* 5 - 6 with the dropwise addition of 0.1 N acetic acid. Sulfo-NHS (*ca.* 1 mg, 12 mg/mL, 4.4 μmol, 1.2 eq.) and EDCI (*ca.* 1.3 mg, 13 mg/mL, 4.4 μmol, 1.2 eq.) were added to the SCK solution and the reaction mixture was allowed to stir at 4 °C for another 30 minutes. The solution pH was then adjusted to 7 - 8 with dropwise addition of saturated sodium phosphate followed by addition of $FimH_A$ in phosphate buffer saline (PBS) (optimal results obtained with 2.0 mL, 0.65 mg/mL, 0.080 μmol). The reaction was allowed to proceed at 4 °C and slowly warmed to room temperature over 24 h. Unconjugated $FimH_A$ and other impurities were removed by extensive washing using a centrifugal filtration device (MWCO 100 kDa) with PBS (pH 7.4, 5 mM), and the final volume was reconstituted to 5 mL with PBS to give a $FimH_A$-conjugated, 5-FITC cadaverine-labeled SCK solution (final polymer concentration *ca.* 0.13 mg/mL, 5-FITC cadaverine concentration 0.033 mg/mL, *ca.* 5 dyes per particle; calculated by UV-vis spectroscopy using the extinction coefficient of 5-FITC cadaverine at pH 7, ε = 82000 ± 6000 L/mol·cm). Diameter (TEM) = 20 ± 3 nm, $D_{h(intensity)}$ = 80 ± 30 nm, $D_{h(volume)}$ = 50 ± 20 nm, $D_{h(number)}$ = 40 ± 10 nm.

Immunogold electron microscopy: $FimH_A$-conjugated and naked SCKs were allowed to adsorb onto glow discharged, formvar/carbon-coated copper grids for 10 min. The grids were washed twice with PBS, blocked with 1% fetal bovine serum (FBS) for 5 min, and subsequently incubated with rabbit anti-FimH antibody for 20 min at room temperature. A set of comparator SCK-$FimH_A$ grids received no primary antibody. Grids were again washed twice with PBS, blocked with 1% FBS for 5 min, and

incubated with 12-nm colloidal gold-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 20 min at room temperature. Following three washes with distilled H_2O, the grids were stained with 1% aqueous uranyl acetate (Ted Pella Inc., Redding, CA) for 1 min. Excess liquid was gently wicked off and the grids were allowed to air dry. Ten random frames were captured for each experimental condition, and *ca*. 500 SCKs per condition were assessed for immunogold labeling.

Fluorescence and confocal microscopy: For fluorescence microscopy, 5637 bladder epithelial cells (American Type Culture Collection HTB-9, Manassas, VA) were grown to sub-confluence on sterile glass coverslips in RPMI 1640 medium with 10% FBS. On the day of experiment, cells were washed with PBS, inoculated with SCK solution as described above, and incubated for 1 h at 37 °C. After additional washing, cells were fixed with 3% paraformaldehyde (PFA) (Electron Microscopy Sciences, Hatfield, PA) for 10 min, then stained with 1:1000 AlexaFluor 594-conjugated wheat germ agglutinin (WGA) (Molecular Probes). For confocal microscopy, after SCK inoculation as above, cells were liberated by application of 0.05% trypsin-0.02% EDTA for 15 min, collected by gentle centrifugation, and fixed with 3% PFA for 10 min. Subsequently, cell suspensions were washed 3 times with PBS, then stained by resuspending in PBS with 1:1000 AlexaFluor 594-conjugated WGA. After a final PBS wash, cells were applied to poly-L-lysine coated slides by cytocentrifugation before microscopy.

References:

(1) Lin, L. Y.; Lee, N. S.; Zhu, J.; Nyström, A. M.; Pochan, D. J.; Dorshow, R. B.; Wooley, K. L. *J. Controlled Release* **2011**, *152*, 37-48.

(2) Hung, C. S.; Dodson, K. W.; Hultgren, S. J. *Nat. Protoc.* **2009**, *4*, 1230-43.