Supporting Information

Fabrication of core-shell nanoparticles via controlled aggregation of semi-flexible conjugated polymer and hyaluronic acid

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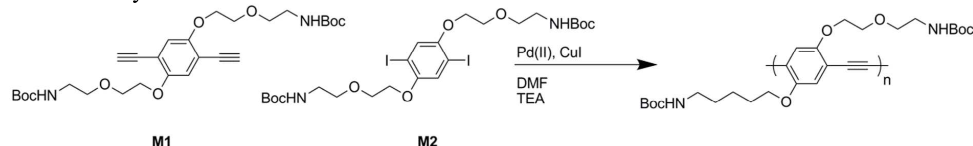
General: Chemicals and solvents were purchased from Fisher Scientific and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). Molecular weight was determined using gel permeation chromatography (GPC) against

polystyrene standards on a Shimadzu HPLC system equipped with PLgel 5 µm MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector. The purification of the CP was conducted using an Ultrafiltration Stirred Cell (Millipore) with a 30 kDa molecular weight cut-off (MWCO) membrane (Ultracel ultrafiltration disc). UV-Vis spectrum was recorded using Varian Cary 50 Bio spectrophotometer. Fluorescence spectrum was obtained using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). NMR spectrum was recorded on a 600 MHz Avance Bruker NMR spectrometer using a 5 mm BBI probe at 298 K. Fourier transform infrared (FT-IR) spectrum was obtained on a PerkinElmer Spectrum 100 FT-IR Spectrometer, using sample mounted on an attenuated total reflection cell.

Synthesis control PPE without flexible linker. A Schlenk flask was charged with M1 (41 mg, 0.056, 1.0 equiv) and M2 (30 mg, 0.056 mmol, 1.0 eqiv) and, along with Pd[$(PPh_3)_2Cl_2$] (4 mg, 0.0056 mmol ,0.1 equiv) and CuI (0.3 mg, 0.0028 mmol , 0.05 equiv), as shown in **Scheme S1**.

Scheme S1. Synthesis of control PPE without flexible linker



The Schlenk flask was evacuated and filled with N_2 three times. A solution of anhydrous dimethylformamide (DMF) (4 mL) and freshly distilled triethylamine (1 mL) was degassed, and 1 mL of the mixed solution was transferred to the Schlenk flask using a cannular needle. The reaction was heated at 70 °C for 14 h. The solution was then cooled to room temperature and transferred dropwise to cold ether, resulting in precipitation. After centrifugation (5 min, 4000 rpm), the supernatant was decanted, the precipitate was collected and dried to yield product.

Yield: 44mg, 73%. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.16 (s, 1H, Ar-H), 6.72 (s, 0.2H, NH-Boc), 4.21 (br, 1.84H, Ar-OCH_2), 3.80 (br, 2.16H, CH_2O), 3.66 (br, 1.34H, OCH_2), 3.52 (br, 2.22H, CH_2NH), 1.37 (s, 7.33H, $C(CH_3)_3$); FT-IR (Neat): ν = 3366 (br), 2933 (w), 2865 (s), 1704 (vs; C=O), 1507 (vs), 1457 (w), 1423 (w), 1365 (s), 1277 (w), 1247 (w), 1219 (s), 1172 (w), 1108 (s), 1057 (s), 1026 (w), 945 (s), 860 cm^{-1} (m); UV-vis (DMF): λ_{max} 432 nm; fluo λ_{max}(400nm ex) =475 nm; QY = 37% ; GPC Mn: 11.6 kDa, Mw: 16.4 kDa , PDI: 1.41.

Deprotection of Boc groups was carried out by mixing the polymer solution with acetic acid (2 mL) and trifluoroacetic acid (1 mL) and allowed to stir at room temperature for 14 days. The mixture was then added to acetic acid (20 mL), allowed to stir overnight, and centrifuged, and supernatant was added drop wise (2 drops/s) to 500 mL water (18 Ω) while stirring. Using a solvent-resistant stir cell fitted with a 30 kDa-MWCO membrane, the solution was concentrated to approximately 10 mL, and dialyzed against 1 L of water. The resulting solution was further dialyzed in a 10 KDa membrane for 3 days. ^{1}H NMR (600 MHz, D_2O, δ): 7.03 (br, 1H, Ar-H), 4.24-2.76 (br, 10.74H, $CH_2CH_2OCH_2CH_2$), 1.36 (s, 0.46H); FT-IR (Neat): ν = 3417 (br), 2925 (w), 2865 (s), 1647 (br), 1495 (w), 1462 (w), 1418 (w), 1357 (w), 1283 (w), 1205 (s), 1094 (s), 1040 (w), 936 (w), 841 (w) cm^{-1}; UV-vis (H_2O): λ_{max} 427 nm; fluo λ_{max}(400nm ex) =492 nm; QY = 2%.

CP/HA complex formation. Sodium hyaluronate (MW 100 K) was purchased from Lifecore and used as received. A stock solution was prepared by dissolving 2 mg of HA in 1 mL of deionized water. The CP/HA complex was formed by mixing CP (10 μM) with various molar equivalents of HA (i.e., 1:1, 1:3, and 1:9) for 1 h.

Dynamic Light Scattering: Dynamic light scattering and zeta potential experiments were performed by Zetasizer nano–ZS (Zen 3600, Malvern Instruments Ltd.) using a microcuvette and a folded capillary cell (Catalog # DTS1060), respectively, at room temperature. For hydrodynamic measurements, samples were analyzed three times. For zeta potential measurements, samples were analyzed six times.

Atomic Force Microscopy: (3-aminopropyl)triethoxysilane (APTES) was vapor deposited to a freshly cleaved mica surface for 80 min in a desiccator filled with dry Argon. 30 µL of CP/HA complex in deionized (DI) water was placed on the APTES coated mica and incubated for 30-45 min in a laminar flow hood. The droplet was then rinsed away with ~1 mL DI water (18 M ohm) and dried gently with dry argon. Images were acquired with a MultiMode5 AFM microscope (Bruker, Santa Barbara, CA) operated in tapping mode in air using a 1.58-1.62 V oscillation amplitude with uncoated silicon AFM tips (T190, vistaprobes, k ~ 40N/m) at a resonance frequency of 190 kHz. Typically, areas of 1 × 1 μm^2 were scanned at a rate of 0.5-1 Hz and a resolution of 512 × 512 pixels. All the experiments were performed at room temperature. The images were further processed by Image Analysis Software Gwyddion.

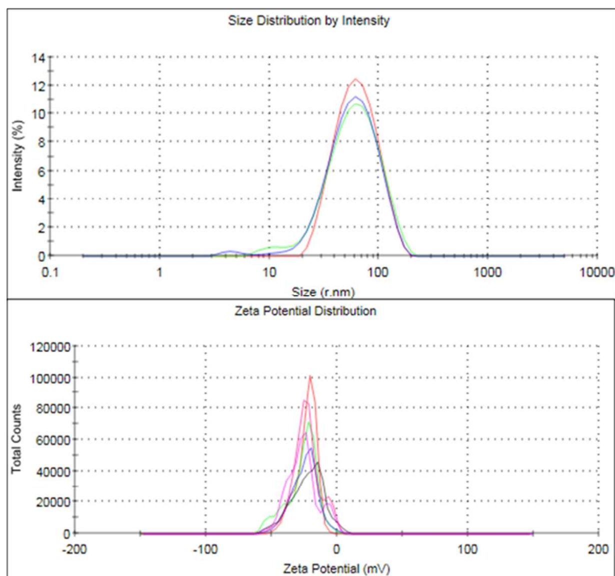


Figure S1. DLS (top) and zeta potential (bottom) of the semi-flexible PPB/HA complex. Hydrodynamic radius was measured with three replicates and zeta potential was measured with six replicates.

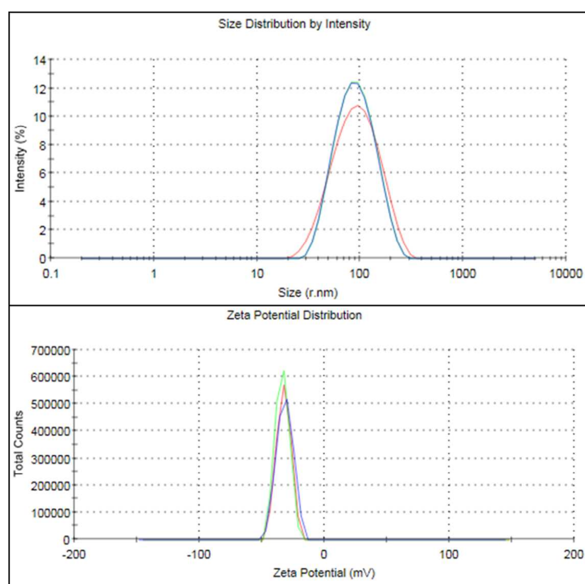
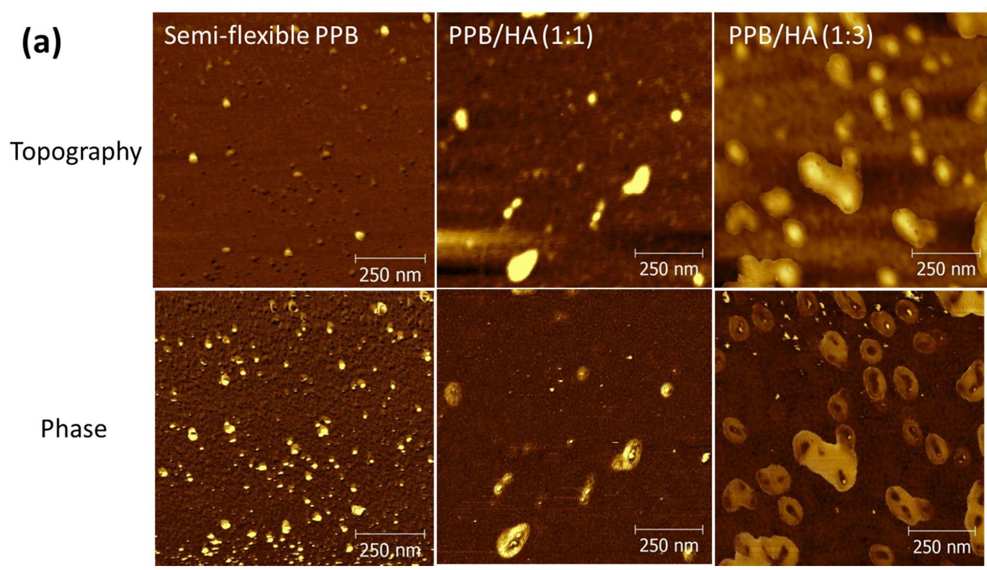


Figure S2. DLS (top) and zeta potential (bottom) of the control PPE/HA complex. Hydrodynamic radius was measured with three replicates and zeta potential was measured with six replicates.



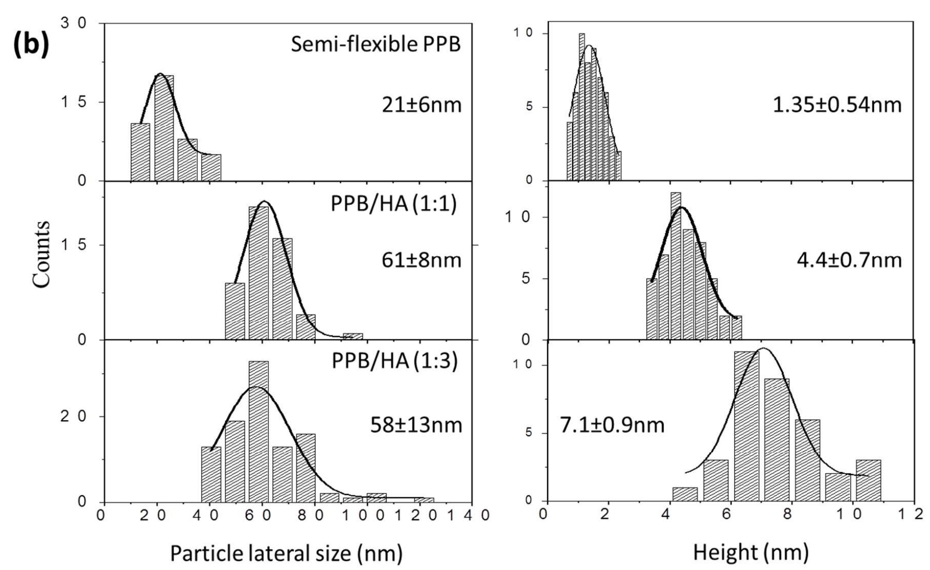


Figure S3. Topography and phase images (a) and size analysis histograms (b) of semi-flexible PPB, semi-flexible PPB/HA (1:1), and semi-flexible PPB/HA (1:3), respectively.

Comment [MT1]: Changed CPN-f annotation

Comment [MT2]: Change CPN-f annotation

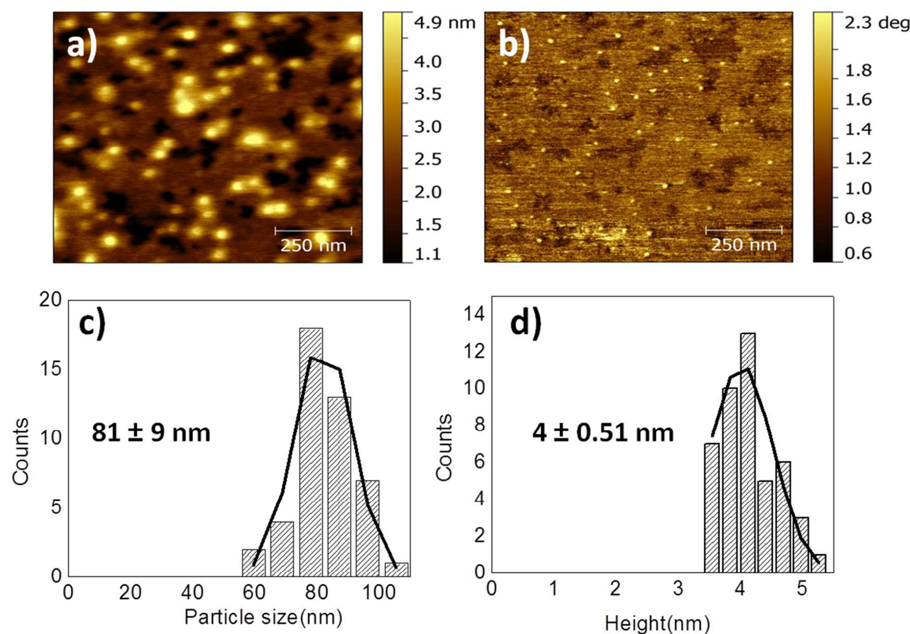


Figure S4. Topography (a) and (b) phase images, size distribution (c), and height (d) histograms for control PPE/HA.

Cell Culture: Human Embryonic Kidney (HEK) and human pancreatic cancer (Panc-1) cells were maintained in DMEM (Hyclone, Logan, UT). Human cervical cancer (HeLa) and human breast cancer (MCF7) cells were maintained in MEM/EBSS (Hyclone, Logan, UT). Both media contain 10% FBS (Hyclone, Logan, UT) and 100 U/ml penicillin. All cells were incubated in a 5% CO_2 incubator at 37°C. Cells were seeded in a 12 well-plate and allowed to attach overnight (1×10^5) before incubation with core-shell nanoparticles for the time course experiments.

Cytotoxicity: CellTiter-Glo® Luminescent Cell Viability assay (Promega, Madison, WI) was used to determine cytotoxicity of the core-shell nanoparticles. HeLa cells (~5×10^3 cells/well) were seeded into a 96 well-plate for 1 day. The cells were then incubated with various concentrations of core-shell nanoparticle for 24 h. After core-shell nanoparticle incubation and

following the manufacturer's protocol, 100 µL of CellTiter-Glo reagent was added to each well and the plate was incubated for 2 minutes. Cell viability was monitored by measuring luminescence values of each well using a microplate well reader with a 528/20 emission filter (Synergy 2, BioTek, USA). Relative cell viability as a function of core-shell nanoparticle concentration was obtained by dividing the luminescence value of each sample by the control value. All measurements were triplicated and standard deviation was included in the error bar.

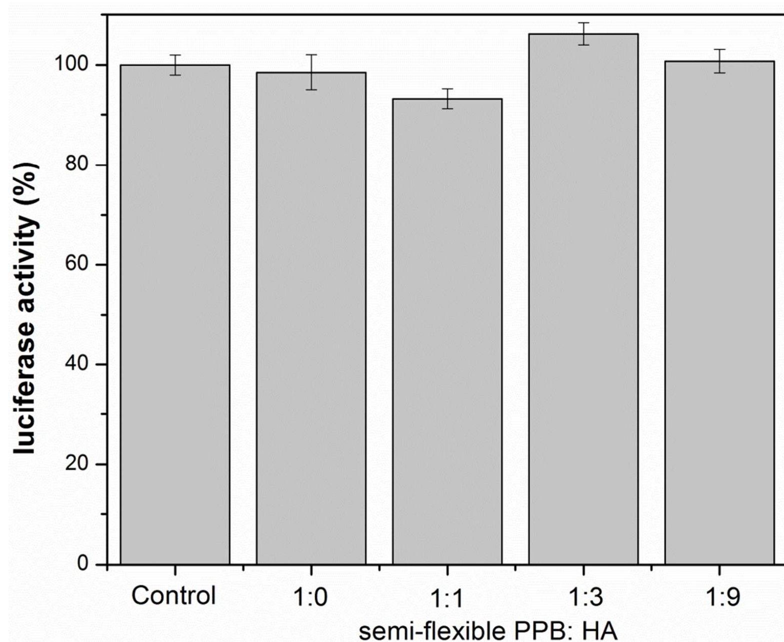


Figure S5. Cytotoxicity of the core-shell nanoparticles measured by CellTiter-Glo® Luminescent Cell Viability assay. No toxic effects of were observed under the experimental conditions.

Flow Cytometry: Accuri™ C6 Flow Cytometer (BD Biosciences, San Jose, CA) equipped with a fluorescence detector was used to analyze the fluorescence intensity of cells incubated with core-shell nanoparticles. After incubation, cells were rinsed with PBS two times followed by centrifugation (Eppendorf Centrifuge 5430 R) at 1,000 relative centrifugal force (rcf) for 7 min

and resuspension in 400 µL FACS buffer. The mean fluorescent intensity of cells treated with the core-shell nanoparticles was normalized to that of untreated control cells. All measurements were triplicated and standard deviation was included in the error bar. For each measurement, 10,000 events were recorded and analyzed.

Evaluation of CD44 expression levels: Cells were seeded in an appropriate medium at a density of 1 x 10^5 cells/ml in a 12-well plate and cultured for overnight. Each cell line was incubated with FITC-labeled mouse anti-human CD44 (BD Pharmingen, San Diego, CA) for 30 min at 4°C, according to the protocol provided by the manufacturer. The cells were washed three times with cold PBS and resuspended in 400 µL FACS buffer for flow cytometry.

Microscopic imaging of mixed HeLa and HEK cells: To distinguish HeLa cells co-cultured with HEK cells, HeLa cells were pre-labeled with the CellTracker™ Red CMPTX (Invitrogen, Grand Island, NY) before co-culturing with HEK cells. 1.75 µM of CellTracker Red was incubated with HeLa cells in a 12-well plate (1 x 10^5 cells/ml) for 30 min at 37 °C. After replacing the labeling solution with a fresh medium (~1 ml), the cells were further incubated for 30 min at 37°C. The HeLa cells labeled with CellTracker were then trypsinized, washed, and re-suspended in 500 µL of culture medium. Equal amounts (1 x 10^5 cells/ml) of both HeLa and HEK cells were mixed in DMEM medium (400 µL) containing 10% fetal bovine serum (FBS) and 100 U/mL penicillin, and seeded in a glass-bottomed eight-well chamber slide (Lab-Tek, Thermo Scientific). After overnight co-culture, cells were incubated with core-shell nanoparticles (final 10 µM) for 1 h. The cells were rinsed with PBS and were incubated with a Hoechst dye (5 µg/µL) (Invitrogen, Grand Island, NY) for 10 min. Finally, the cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. Fluorescence images of the cells were obtained using a DeltaVison microscope equipped with 40x lens. Band-pass filters

were used for imaging nucleus (410-460 nm), core-shell nanoparticles (500-550 nm), and CellTracker Red (575-620 nm).