SUPPORTING INFORMATION

Convective Polymer Assembly for the Deposition of Nanostructures and Polymer Thin Films on Immobilized Particles

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MATERIALS AND METHODS

Materials. Poly(styrene sulfonate) (PSS, Mw ~15 kDa) was purchased from Fluka. Poly(allylamine hydrochloride) (PAH, Mw ~15 kDa), PSS (Mw ~70 kDa), fluorescein isothyocyanate (FITC), sodium citrate, tannic acid, sodium chloride (NaCl), sodium acetate, naphthofluorescein, gold (III) chloride trihydrate, calcium chloride ($CaCl_2$), sodium carbonate (Na_2CO_3), ultra-low gelling agarose, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and Triton X-100 were purchased from Sigma-Aldrich. High purity de-ionized water with a resistivity greater than 18 MΩ·cm was obtained from an inline Millipore RiOs/Origin water purification system. Colloidal silica particles (1.11 and 4.99 μm diameter) were purchased from Microparticles GmbH. 3DKUBEs were purchased from Kiyatec. The syringe pump (PHD2000) was purchased from Harvard Apparatus and used with a 50 mL glass syringe. The 6-port valve was purchased from IDEX Health and Science. A diameter polyether ether ketone tube with a length of 115 cm and an inner diameter of 1.02 mm (0.04") (~1 mL volume) was used for loading the polymer solutions. 1,2-dimyristoyl-*sn*-*glycero*-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphocholine (DPPC) and the fluorescent lipid 1-myristoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-*glycero*-3-phosphocholine (NBD-PC) were obtained from Avanti Polar Lipids.

Synthesis of calcined calcium carbonate ($CaCO_3$) particles. $CaCO_3$ particles were obtained by vigorously stirring 24 mM $CaCl_2$ and 12 mM Na_2CO_3 in the presence of 1 mg mL^{-1} PSS (Mw ~70 kDa) for 30 s. The solution was left to incubate overnight and the resultant particles were then calcined at 550 °C for 6 h to remove the stabilizing PSS, thereby yielding porous $CaCO_3$ particles capable of being dissolved in 40 mM sodium acetate, pH 4.

Synthesis of citric acid/tannic acid-stabilized gold nanoparticles (Au NPs). Gold (III) chloride trihydrate was dissolved in ultrapure water (1/80 (w/v), 80 mL) and heated to 60 °C. Sodium citrate solution (1% (w/v), 4 mL), tannic acid solution (1% (w/v), 57 μL) and ultrapure water (16 mL) were mixed and heated to 60 °C. The citric acid/tannic acid solution was rapidly added to the gold solution and stirred at 60 °C until the color of the solution turned red. The solution was refluxed for 10 min for Ostwald ripening. After cooling to room temperature, the gold nanoparticle solution was obtained and stored in the dark at 4 °C.

Liposome preparation. Fluorescently labeled liposomes were prepared by dissolving lipid solution in chloroform (2 mg of DMPC, 0.5 mg of DPPC, and 0.04 mg of NBD-PC). A thin lipid layer was formed inside a round-bottomed flask and the chloroform was then evaporated under nitrogen for 45 min. The liposomes were hydrated with 1 mL of HEPES buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) and then extruded through 100 nm filters (31 times).

Analysis of flow-through. Calibration curves were prepared through serial dilutions for both PAH and PSS. Fractions of the flow-through were collected in 96-well plates every minute for the first 153 min and every 5 min afterward. The polymers were injected every 75 min. The flow-through was then analyzed using an Infinite 200 PRO NanoQuant (TECAN) and its inbuilt settings.

Atomic force microscopy (AFM). 2 μL of the capsule solution was dropped onto a clean glass slide and allowed to dry in air. This was imaged with a NanoWizard II BioAFM (JPK Instruments) in intermittent contact mode with MikroMasch silicon cantilevers (NSC/CSC).

Transmission electron microscopy (TEM). 2 μL of the capsule solution was dropped onto a carbon-coated Formvar film mounted on 300-mesh UV-treated copper (ProSciTech, Australia) and allowed to air dry. This was imaged and analyzed using a Tecnai F20 instrument (FEI

Company). High angle annular dark-field (HAADF) imaging in combination with energy dispersive X-ray spectroscopy (EDX) was used to characterize the composition of the capsules and detect the loaded Au NPs.

Differential interference contrast (DIC) and fluorescence microscopy. Using a 60× lens, images were obtained with white light or a green excitation filter with an inverted Olympus IX71 microscope. Images were captured with a mounted CCD camera.

Flow cytometry. Experiments were performed using a Partec CyFlow Space instrument, and FlowJo 8.7 software was used to analyze the data. Thresholds for forward and side scattering were adjusted to maximize the signal-to-noise ratio.

SUPPORTING FIGURES

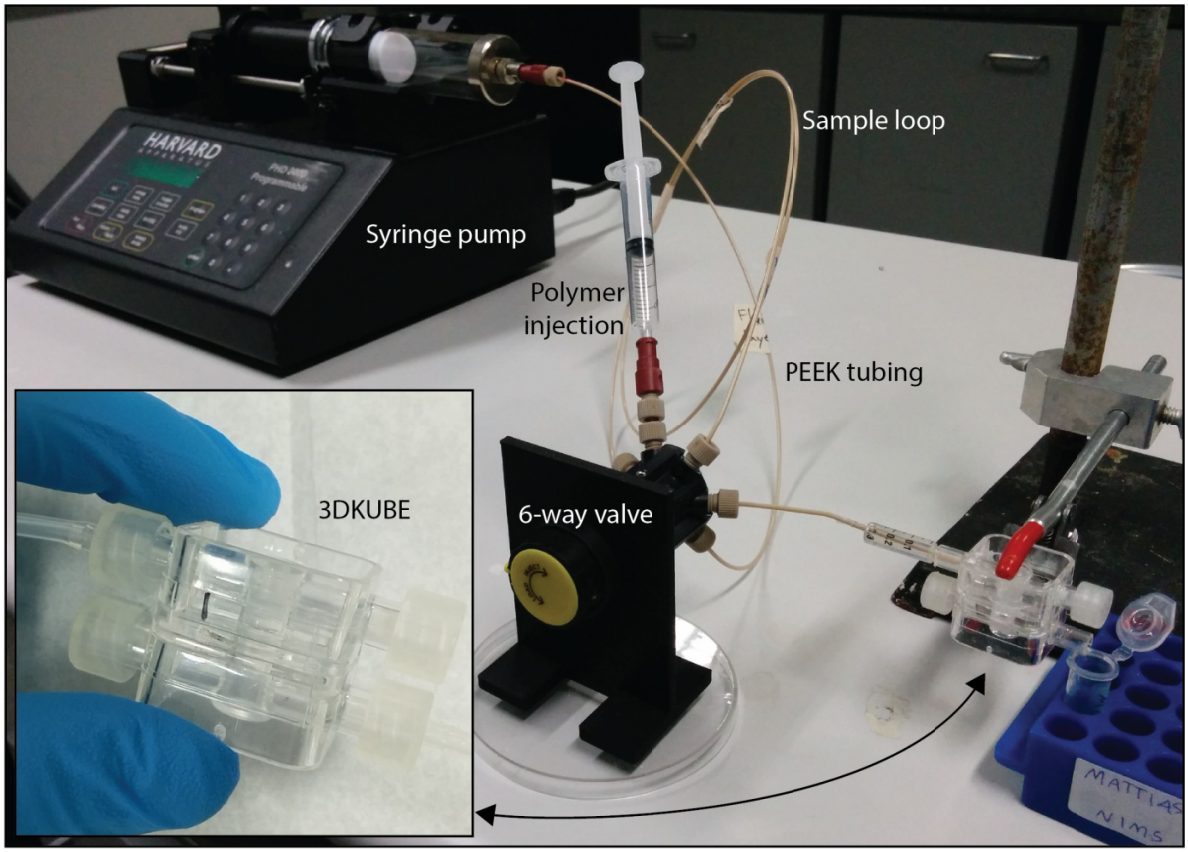

Figure S1. A 3DKUBE containing agarose with the HPLC-like set-up used for CPA.

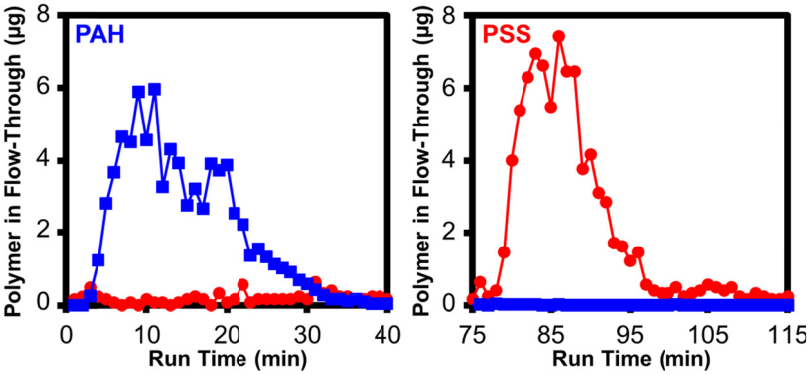

Figure S2. Polymer in the flow-through solution for the first bilayer of CPA, as measured by UV-Vis spectrophotometry. This is the magnified data of Figure 1.

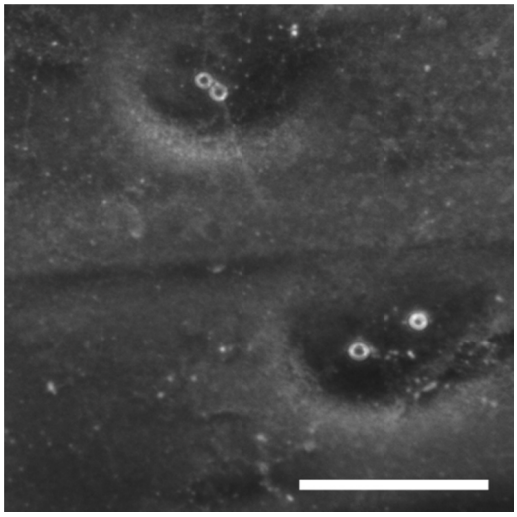

Figure S3. 1.11 µm silica template particles immobilized in agarose, imaged after one layer of PAH prior to the washing step. Note the decrease in the fluorescence intensity, corresponding to the decreased polymer concentration, around the template particles. The scale bar is 10 µm.

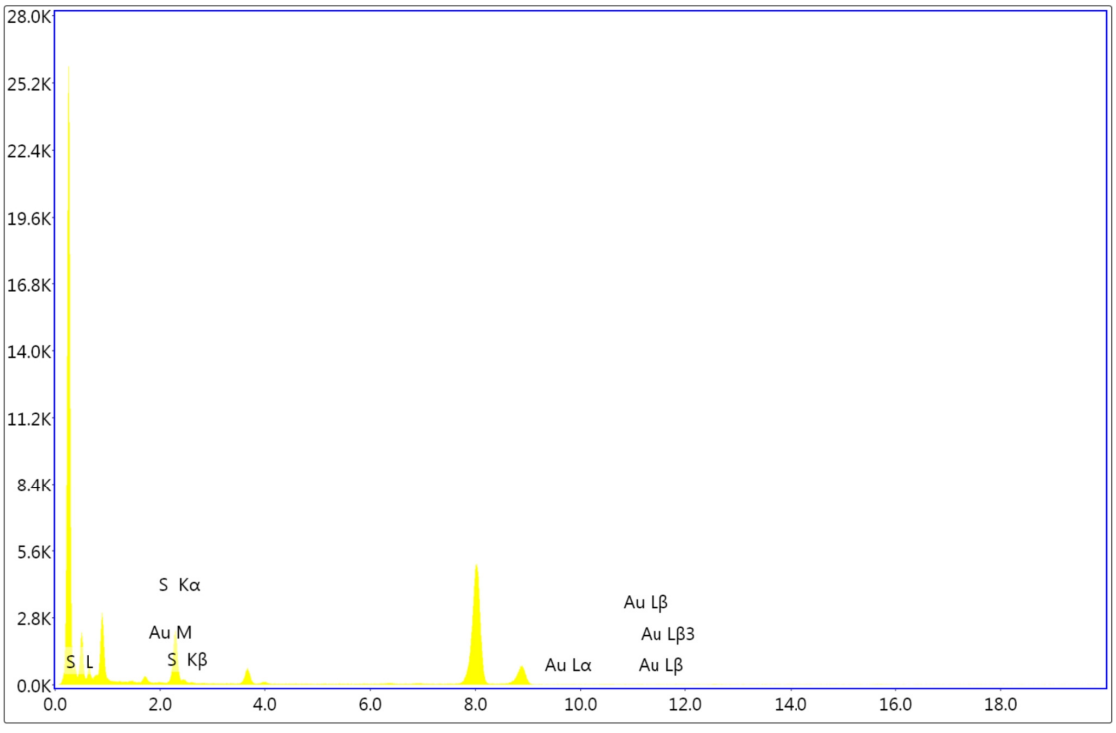

Figure S4. EDX detection of gold in the Au NP-loaded PAH/PSS capsules prepared via CPA.

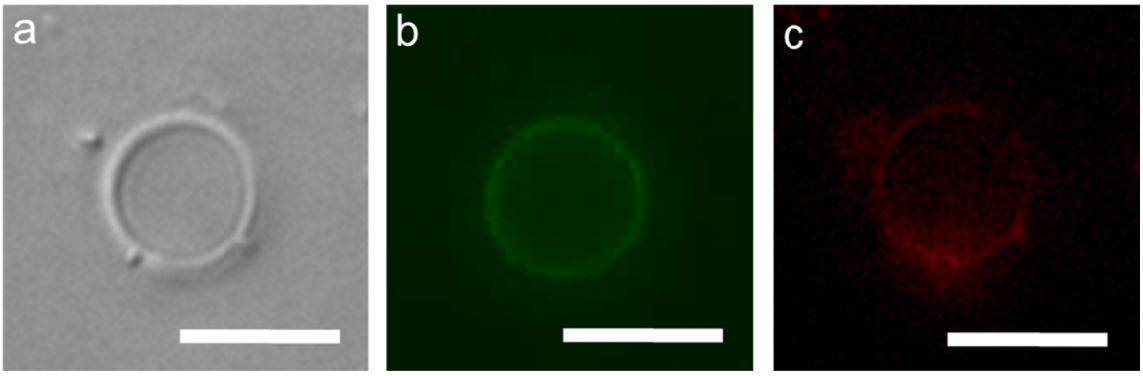

Figure S5. A PAH/PSS-liposome capsule loaded with naphthofluorescein. (a) DIC image, (b) fluorescence microscopy image of the labeled liposomes (green), and (c) fluorescence microscopy image of the naphthofluorescein (red). The scale bars are 5 μm.

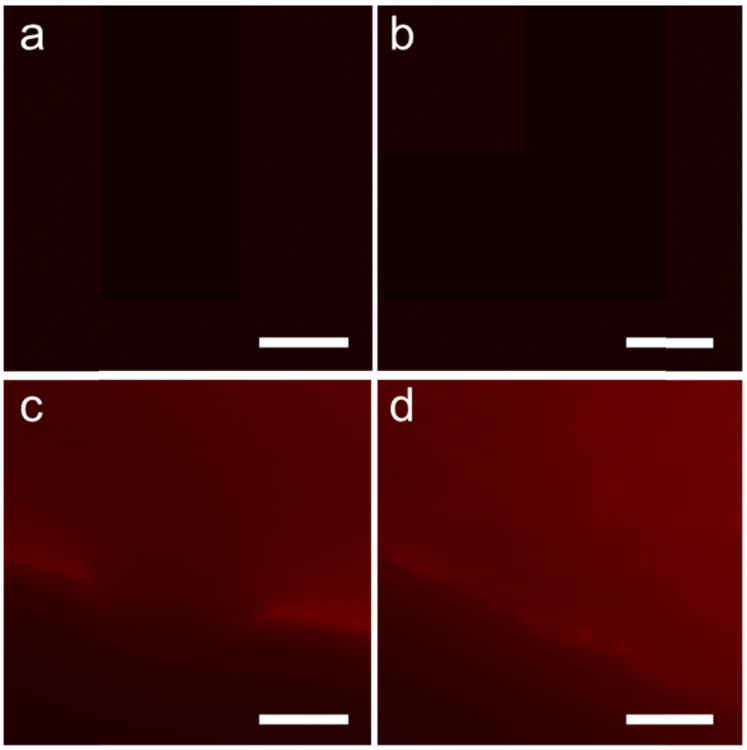

Figure S6. Loading of liposome-containing capsules with a hydrophobic dye, naphthofluorescein. Fluorescence microscopy images of the edge of the solution containing PAH/PSS-liposome capsules (a) before and (b) after the addition of naphthofluorescein and (c) after the subsequent addition of Triton X-100. (d) Fluorescence microscopy image of liposome-free PAH/PSS capsules after the addition of naphthofluorescein (control experiment). The scale bars are 20 μm.

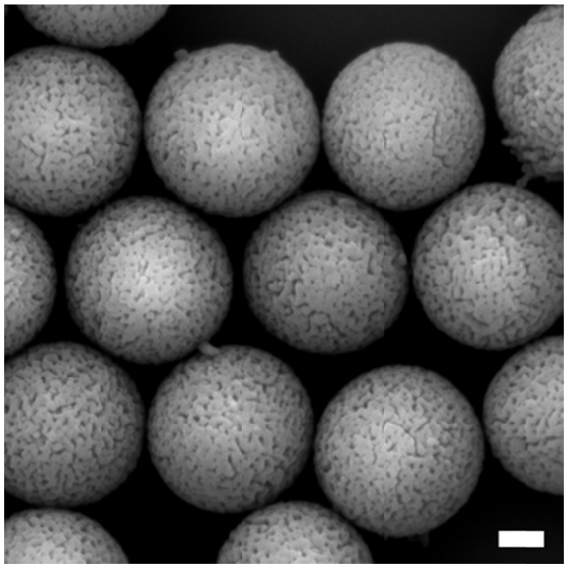

Figure S7. SEM of calcined $CaCO_3$ template particles. The scale bar is 1 μm.