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Supporting Information to Continuous fabrication of polymeric vesicles and nanotubes with fluidic channels

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EXPERIMENTAL SECTION

Materials: Styrene, N, N, N', N", N"-pentamethyl diethylene triamine (PMDETA), methoxy poly(ethylene glycol) (MW 2000 Da), poly(vinyl pyrrolidone) (MW 10000 Da), potassium tetrachloro platinate (II), L (+) ascorbic acid were purchased from Sigma-Aldrich. Nile red was obtained from Chem-Impex. Doxorubicin hydrochloride was purchased from LC Laboratories. Styrene was purified by distillation under reduced vacuum prior to use. Copper bromide was obtained from Fluka. Anisole and tetrahydrofuran (THF) were purchased from Acros. Dioxane was from Biosolve. All chemicals are used as received unless otherwise indicated. Aqueous solutions were prepared with MilliQ water, which was double deionized with a MilliQ QPOD purification system (18.2 MΩ).

Instruments: ^{1}H nuclear magnetic resonance spectra (^{1}H NMR) were measured on a Varian Inova 400 MHz spectrometer, with $CDCl_3$ as solvent. Gel permeation chromatography (GPC) was performed on a Shodex GPC column equipped with UV SPD 20A detectors (254 nm, 215 nm). THF was used as eluent and the flow rate was kept constant at 1 mL/min. Polystyrene standards were used for calibration. The hydrodynamic diameter of polymeric vesicles was determined with a Malvern Zetasizer Nano S using the following conditions: temperature 25°C, He-Ne laser wavelength 633 nm and detector angle 173°. Transmission electron microscopy was performed on a JEOL 1010 Transmission Electron Microscope with MegaView Soft Imaging camera at an acceleration voltage of 60 kV. Scanning electron microscopy images were obtained with JEOL 6330 Cryo Field Emission Scanning Electron Microscope (FESEM). For nanoparticle tracking measurements, a NanoSight NS500 (Malvern Instruments) was used.

Synthesis of poly(ethylene glycol)$_{44}$-b-polystyrene$_{190}$ (PEG$_{44}$-b-PS$_{190}$)

Poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ was synthesized by atom transfer radical polymerization, via a protocol reported in literature.[1] To monitor the polymerization process, ^{1}H NMR were used for determining the molecular weight of the block copolymer. The reaction was terminated once the desired MW 21000 Da was reached. The crude product was purified by precipitation three times from dichloromethane in methanol. The ^{1}H NMR spectra of the PEG initiator and purified copolymer were shown in Figure S1. Gel permeation chromatography traces

(UV detector) of poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ and PEG initiator were shown in Figure S2.

Fabrication of platinum nanoparticles/PtNPs with PVP coating

Platinum nanoparticles capped with poly(vinyl pyrrolidone) (PVP) were synthesized according to a previously reported method.[2] Briefly, PVP (MW = 10000 Da, 20 mg) was mixed with an aqueous solution of K_2PtCl_4 (20 mM, 2 mL). After magnetic stirring for 48 hours at room temperature, 1 mL of reducing agent ascorbic acid (0.2 mM) was rapidly injected to the above solution. After stirring for 1 min, the resulting solution was incubated in a sonication bath (VWR Ultrasonic Cleaner Model 75D) at room temperature for 40 min. The appearance of a black color indicated the completion of reduction. The obtained platinum nanoparticles were centrifuged (12000 rpm, 10 min) and washed 3 times with MilliQ water to remove remaining salt. Before each use, the above obtained platinum solution was sonicated for 20 seconds and diluted with MilliQ water. The concentration and size of platinum nanoparticles were determined by the Nanosight.

Preparation of polymer vesicles with co-solvent method

10 mg poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ was dissolved in 1 mL THF/dioxane (4:1, v:v) and stirred for 30 min. Then MilliQ water (3 mL) was slowly added into the polymer organic solvent through a syringe pump (Fusion 100, KR analytical) with a rate of 1 mL/h. After addition of water, the solution was immediately transferred to a dialysis bag (MW cutoff 12000-14000 Da) and dialyzed against MilliQ water (800 mL) for at least 48 hours to remove remaining organic solvent. For monitoring the kinetic process of vesicle formation, MilliQ water was delivered to the polymer organic solution in flow cuvette. The plot of scattered light intensity along time was recorded under the flow mode of dynamic light scattering (DLS).

Preparation of platinum nanoparticles/PtNPs loaded stomatocytes with co-solvent method

Poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ (10 mg) was allowed to be fully dissolved in 1 mL THF/dioxane (4:1) solvent. Then MilliQ water (0.35 mL), followed by a platinum nanoparticle solution (0.65 mL) was slowly delivered into the polymer organic solvent through a syringe pump (Fusion 100, KR analytical) (1 mL/h) with vigorous stirring. After 1 h, the solution was transferred

to a dialysis bag (MW cutoff 12000-14000 Da) and dialyzed against MilliQ water (800 mL) for at least 48 hours with frequent change of water.

Preparation of polymer vesicles with fluidic channels

The fluidic channel (glass/plastic) consisted of three inlet channels (0.4 mm deep, 1 mm wide, 12 mm long) and a main channel (0.4 mm deep, 3 mm wide, 24 mm long). Poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ was dissolved in THF/dioxane (4:1, v:v) to obtain the desired polymer concentration, and injected into the main channel via the middle inlet channel, while water was injected into the two side channels using syringe pumps (Fusion 100, KR analytical) at a set flow rate. For Nile red loading, 20 µL of Nile red in DMF (6 mg/mL) was added to the polymer solution. In case doxorubicin hydrochloride was loaded, a 0.5 mg/mL aqueous solution instead of water was pumped through the two side channels. For the loading of platinum nanoparticles an aqueous PtNPs solution (PtNPs concentration 5.06 ± 0.1 × 10^{11} particles/mL) instead of water was injected into the side channels. For polymeric nanotubes collection with shearing force, outflow was collected while magnetically stirred at 0, 500, 1000 rpm (IKA® Big Squid magnetic stirrers).

Transmission electron microscopy (TEM)

Sample specimens were prepared by casting 6 µL of diluted fresh sample aliquots onto 300 mesh copper grids coated with carbon films. The grid was allowed to dry for at least 8 hours at room temperature. For the sample preparation in Fig S7, 6 µL of channel outflow was quickly casted onto the carbon film coated grid and transferred to liquid nitrogen (approximately 3 s) for structure “freezing”. Then the grid was left to dry under vacuum for an overnight. Image acquisition was operated with iTEM software (Olympus). For diameter and membrane thickness measurement, ImageJ software plot profile plugin was used. 80 vesicles or nanotubes were measured to determine an average diameter/membrane thickness.

Scanning electron microscopy (SEM)

6 µL of aqueous sample was applied on a copper grid and allowed to dry overnight. The grid was placed on a SEM mount with a double sided tape, followed by gold (60%)/palladium (40%) sputtering for 30 seconds in a Cressington 208 HR sputter coater. Then the grid was transferred to the microscope chamber for imaging.

Dynamic light scattering (DLS)

Dynamic light scattering analysis of samples was performed on a Malvern Zetasizer Nano S instrument. Samples were typically loaded in Malvern microcuvettes with a minimum volume of 60 µL. The data acquired was processed with Malvern Instruments Dispersion Technology software.

Nanoparticle tracking analysis technique (NTA)

The sample solution was diluted 2000 times and pumped into the NanoSight NS500 sample chamber. Five videos (each video duration time 60 seconds, rate 30 frames/sec) were recorded and the particle movement was analyzed with nanoparticle tracking technique software. Particle concentration was based on the average particle number in a frame. After averaging the values obtained from 5 videos, the concentration of the platinum nanoparticles solution before dilution was determined to be $5.06 \pm 0.1 \times 10^{11}$ particles/mL while the mean size of the particles was obtained as 48.9 ± 3.3 nm.

Confocal laser scanning microscope (CLSM)

Fluorescent Nile red/doxorubicin hydrochloride loaded polymer structures were visualized with a confocal fluorescence laser scanning microscope (Leica TCS SP2 AOBS). To observe Nile red, an argon laser line (514 nm) with a detection window of 590 – 650 nm was used. Doxorubicin hydrochloride was excited with an argon laser line (488 nm) and the resulted emission was collected between 550-650 nm. The laser beam was focused on the sample through a 63× oil immersion lens. A pinhole of 400 µm was selected. An average of two scans was used.

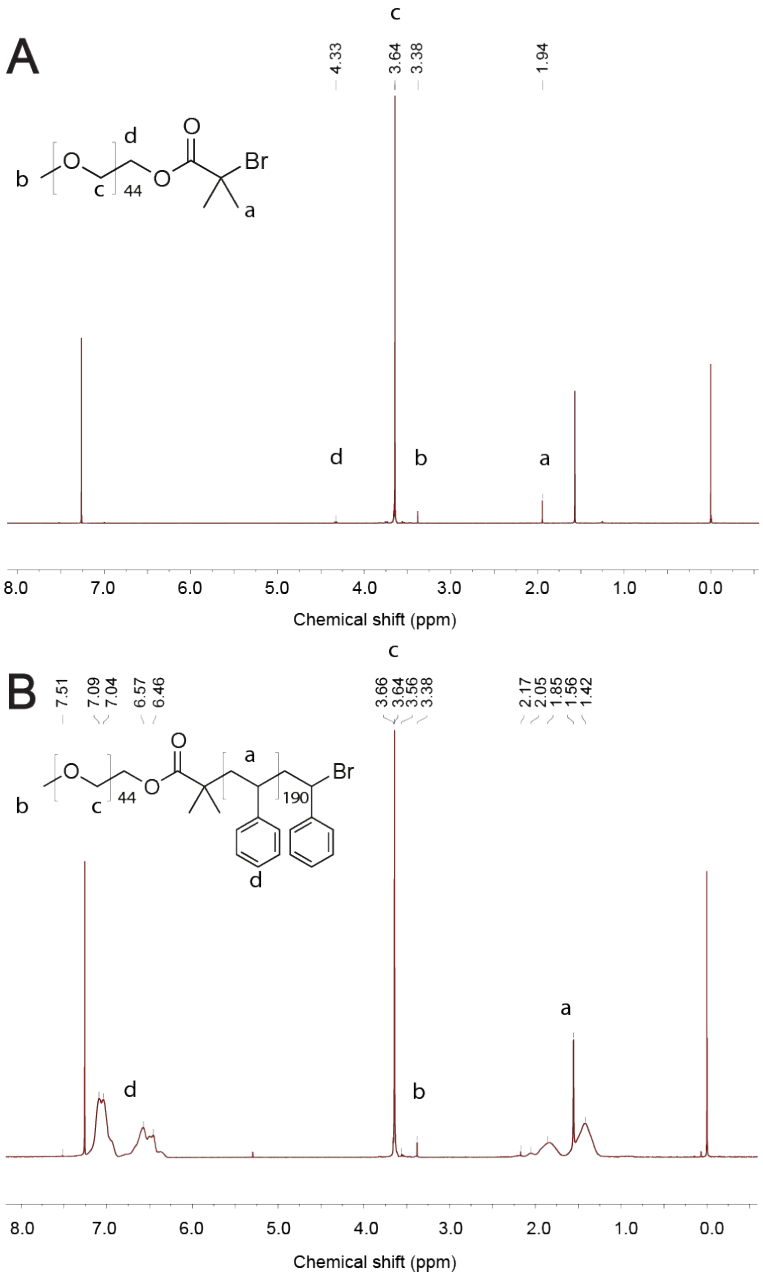

Figure S1. ^{1}H NMR in $CDCl_3$ at room temperature of (A) PEG initiator δ: 1.94 ppm (s, 6H, $(CH_3)_2CBr$), 3.38 ppm (s, 3H, CH_3OCH_2), 3.64 ppm (br. s, PEG backbone), 4.33 ppm (t, 2H, $CH_2CH_2OC(O)C(CH_3)_2Br$); (B) poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ 1.42-2.17 ppm (br. m, PS backbone), 3.38 ppm (s, 3H), 3.56 – 3.66 ppm (br. s, PEG backbone), 6.46 – 7.51 ppm (br. m, aromatic ring of PS).

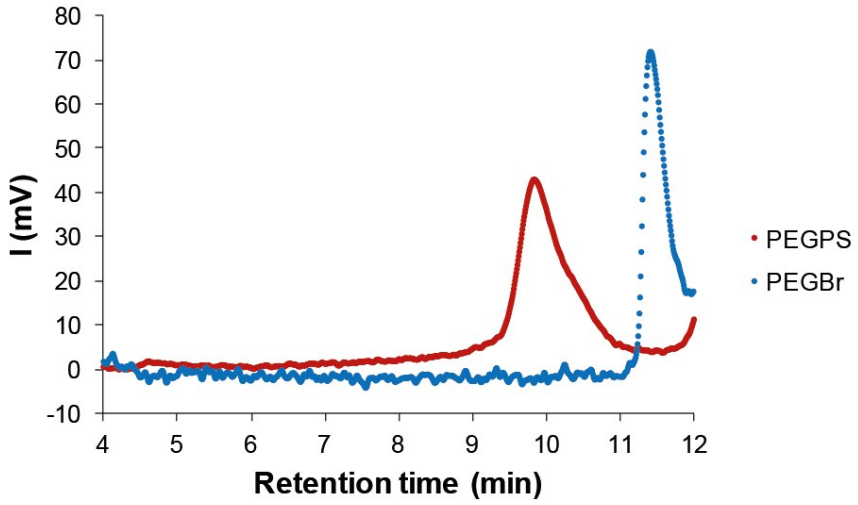

Figure S2. Gel permeation chromatography traces (UV detector 254 nm) of PEG initiator (PEGBr) and poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ in THF. Polydispersity index (PDI) of the PEG initiator and purified poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ was determined to be 1.02 and 1.06 respectively with gel permeation chromatography. The rentention time of the PEG initiator and purified poly(ethylene glycol)$_{44}$-*b*-polystyrene$_{190}$ was 11.4 min and 9.8 min, respectively.

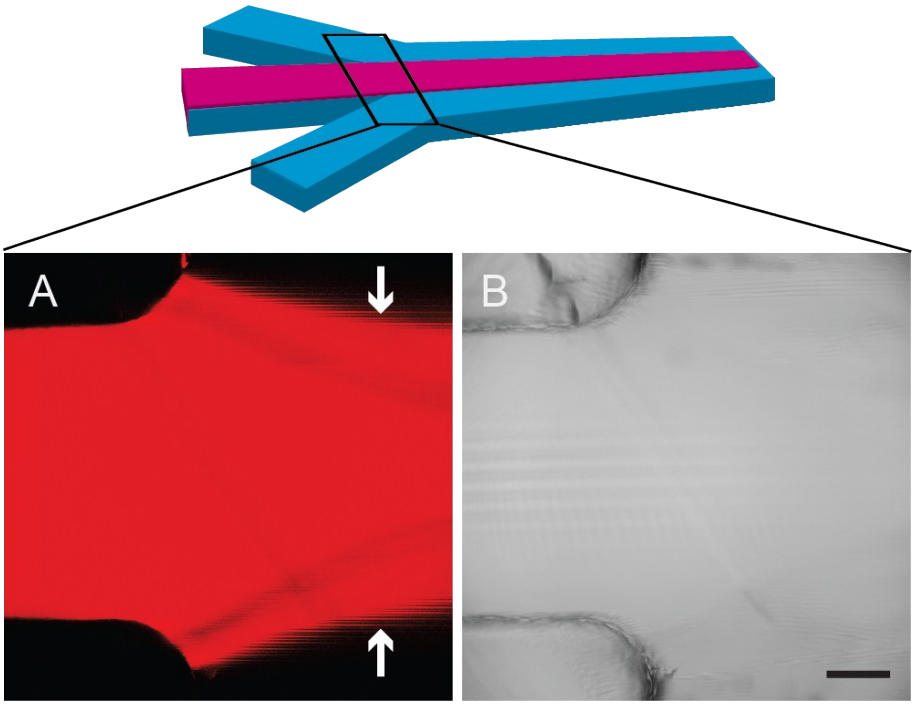

Figure S3. (A) confocal microscopy image; (B) corresponding bright field image of the section where flow from 3 inlets meets, the white arrows point to the phase boundary. The scale bar represents 200 μm. Polymer/Nile red organic solution was injected through the middle inlet while aqueous solution was pumped in through 2 side inlets. Polymeric structures were formed at the periphery of the two phases.

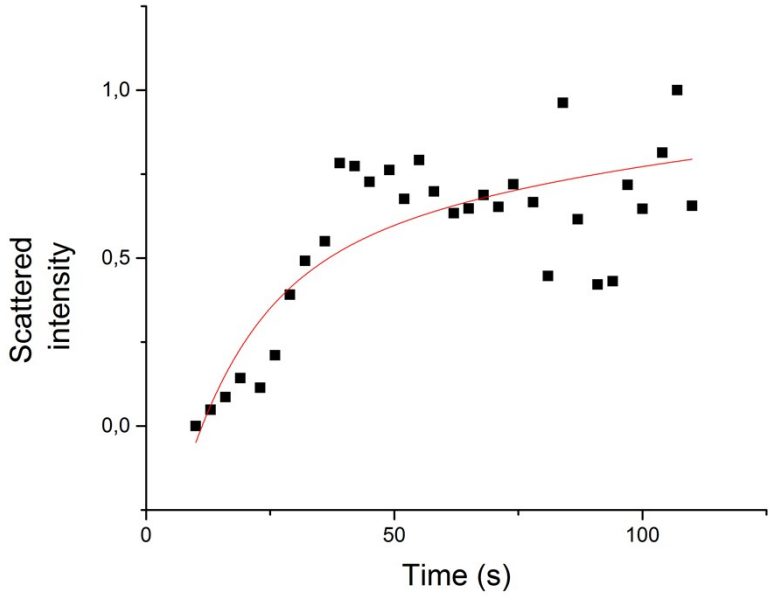

Figure S4. Scattered light intensity as a function of time (black squares); the plot was fitted with a double exponential function (red curve) I=1-(C1*exp(-t/ τ1)+C2*exp(-t/τ2)). Relaxation approach is very useful for kinetics determination in liquid phase. In this experiment, the curve can be fitted with a double exponential function indicating a two step process involved in the initial stages of vesicle formation before it experience a longer process towards final equilibrium. The τ1 was derived as 33.7 s while τ2 was obtained as 152.1 s. The time was higher than the mixing time (in the range of 500 ms – 3 s) in fluidic channel.

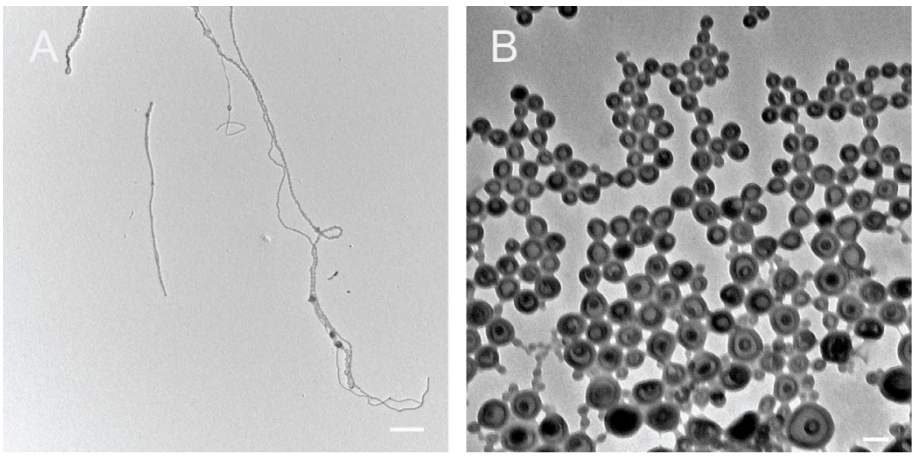

Figure S5. Transmission electron microscopy images of (A) necklace like nanotubes, scale bar represents 2.5 μm; (B) stomatocytes and vesicles, scale bar represents 100 nm.

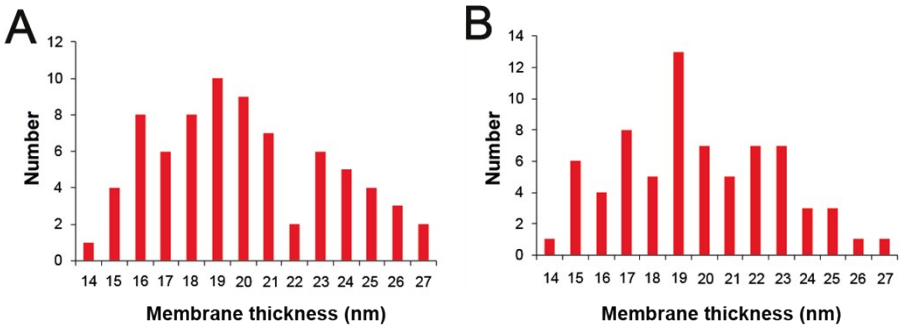

Figure S6. TEM analysis of (A) polymeric nanotubes membrane thickness; (B) vesicles membrane thickness. For each analysis 80 assemblies were counted.

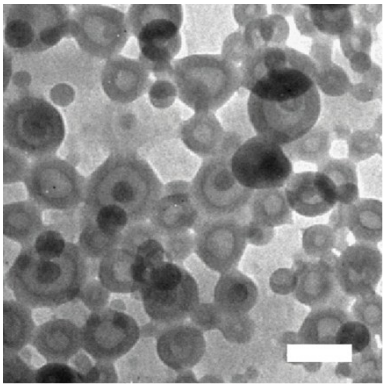

Figure S7. Transmission electron microscopy image of the outflow from the fluidic channel after immediate freeze-dry step， scale bar = 150 nm.

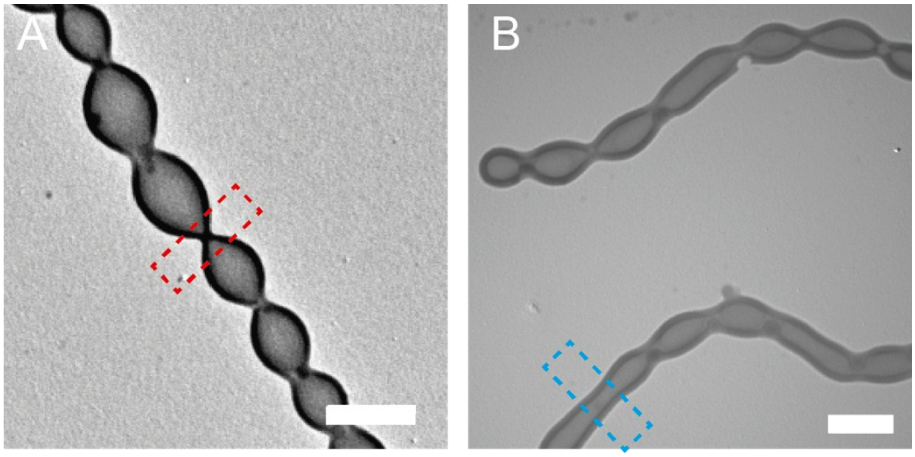

Figure S8. Transmission electron microscopy image of polymer nanotubes. (A) Shown in red rectangle is the connecting section of two neighbouring vesicle in hemifusion state; (B) within the blue rectangle is the area where two neighbouring vesicles are more connected. The scale bar represents 300 nm.

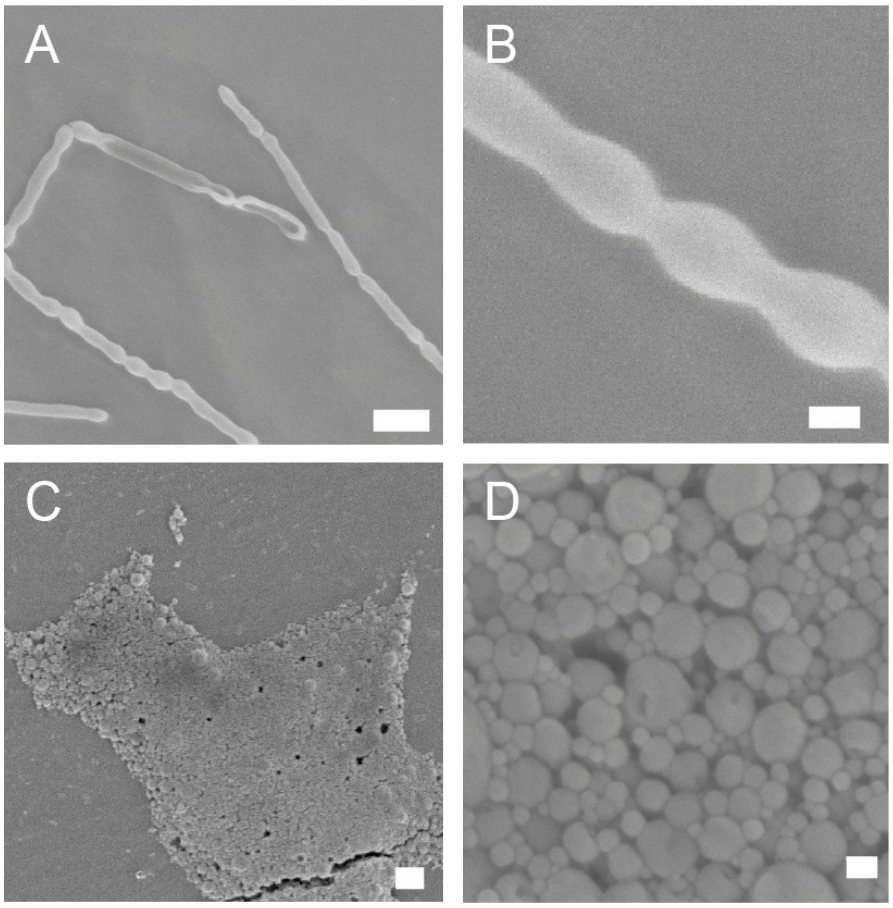

Figure S9. Scanning electron microscopy images of (A) necklace like nanotubes (scale bar represents 500 nm) (B) necklace like nanotubes (scale bar represents 100 nm) obtained using dripping method with initial polymer concentration of 20 mg/mL; (C) stomatocytes and vesicles (scale bar represents 1 μm) (D) stomatocytes and vesicles (scale bar represents 100 nm)

obtained using dripping method with initial polymer concentration of 1 mg/mL.

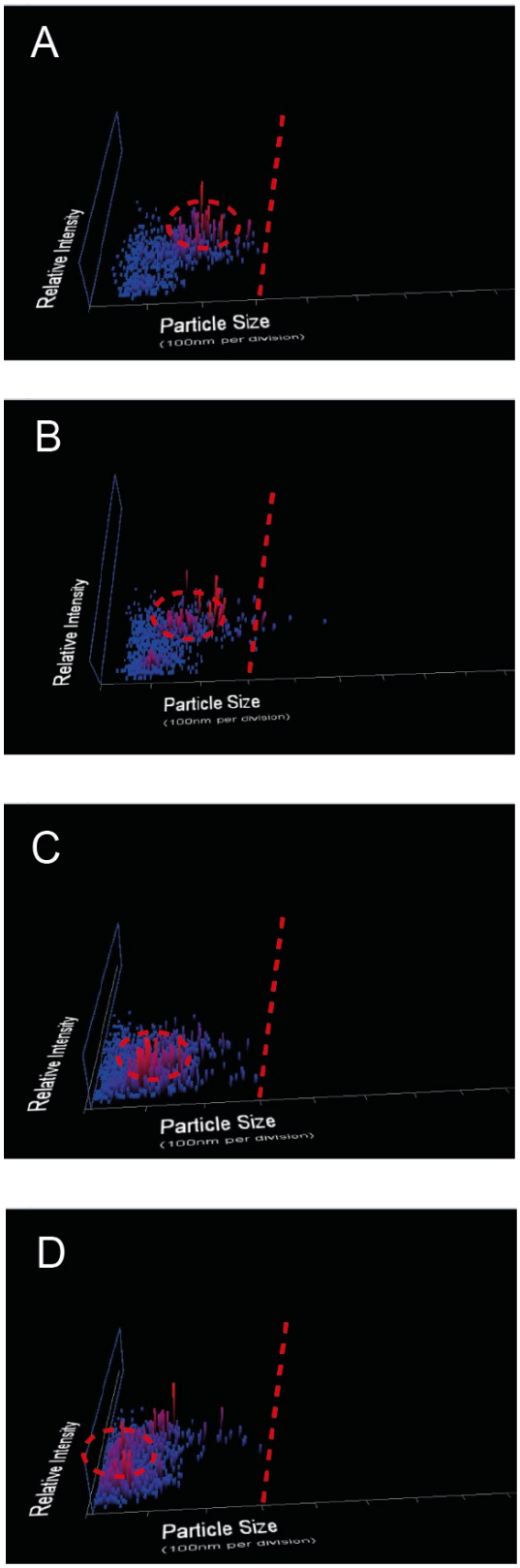

Figure S10. Vesicle size experienced a decrease by increasing flow rate (A) 5 mL/h; (B) 10 mL/h; (C) 20 mL/h; (D) 30 mL/h (nanoparticle tracking analysis/NTA data). Shown above are 3D plots of particle size (x axis) vs. intensity (y axis, 100 nm per division) vs. particle concentration (z axis).The red bars (within the red dashed circle) represent the main particle population. The NTA technique is based on particle-by-particle tracking and can translate the obtained diffusion data to size using Stokes- Einstein equation. The NTA approach is not biased towards

subgroups in a particle population.

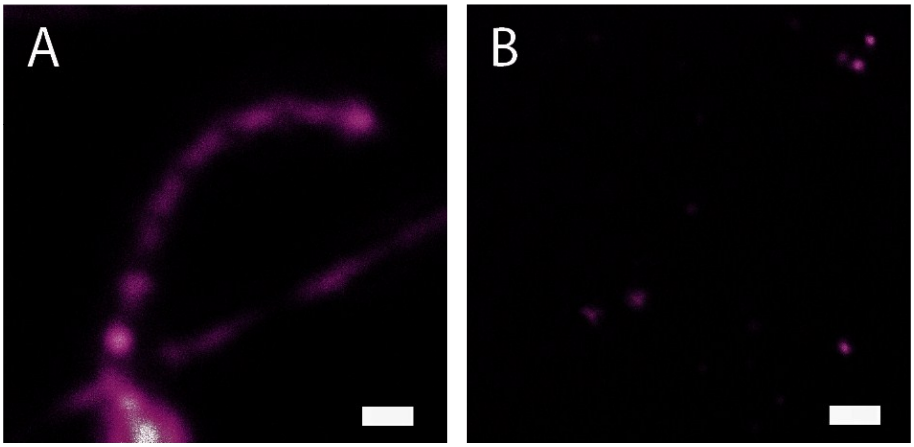

Figure S11. Confocal microscopy fluorescent images of hydrophilic doxorubicin hydrochloride loaded (A) nanotubes, scale bar = 500 nm; (B) vesicles, scale bar = 2 μm.

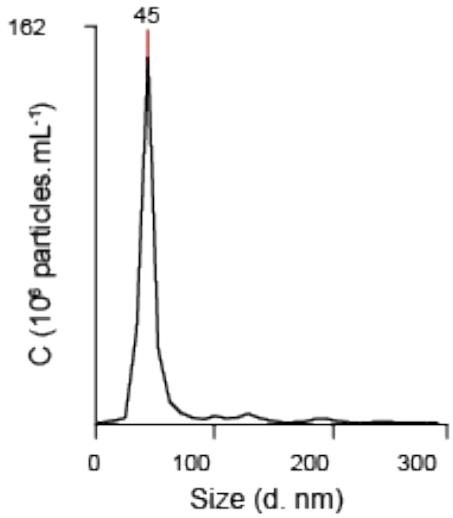

Figure S12. Averaged size/diameter distributions of platinum nanoparticles solution determined by nanoparticle tracking analysis technique.

References

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2 D. A. Wilson, R. J. M. Nolte, and J. C. M. van Hest, J. Am. Chem. Soc. 2012, 134, 9894–9897