Supporting Information for

Nanopod Formation Through Gold Nanoparticle Templated and Catalyzed Crosslinking of Polymers Bearing Pendant Propargyl Ethers

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Experimental details

All materials were purchased from Sigma-Aldrich Co., MO, USA, and used without further purification unless otherwise indicated. TEM characterization was conducted on a Hitachi H8100 electron microscope (Hitachi High-Tech Co., Japan). NMR experiments were performed using a Bruker Avance III 500 MHz coupled with a DCH CryoProbe (Bruker BioSpin Co., MA, USA). DLS data were acquired from a MALVERN Zetasizer, Nano-ZS (Malvern Instruments, UK). IR results were obtained from a Bruker TENSOR 37, and analyzed using the OPUS software (Bruker Optics Inc., MA, USA). MALDI-ToF measurements were carried out on a Bruker Autoflex III SmartBeam mass spectrometer (Burker Daltonics Inc., MA, USA). Tetrahydrofuran-based gel permeation chromatography (THF GPC) was conducted on a Waters Chromatography, Inc. (MA, USA) model 1515 GPC, equipped with a Waters model 5414 differential refractometer, a Precision Detectors, Inc. (MA, USA) model PD-2026 dual-angle (15˚ and 90˚) light scattering detector, and Polymer Laboratories, Inc. (MA, USA) gel mixed-bed styrene-divinylbenzene columns (PLgel 5µm Mixed C, 500 Å, and 104 Å, 300 x 7.5 mm columns). Fluorescence measurements were carried out with a Fluorolog-3 system (HORIBA Jobin Yvon Inc., NJ, USA). UV-Vis data were obtained on a Cary 5000 UV-Vis spectrophotometer (Varian Inc., CA, USA). XPS spectra were taken using an Omicron ESCA Probe X-ray photoelectron spectrometer (Omicron NanoTechnology GmbH, Germany).

1. Synthesis of poly(*N*-(2-(3-(prop-2-ynyloxy)propanamido)ethyl)acrylamide) **1**:

Polyacrylamidoethylamine$_{120}$ (PAEA$_{120}$) was prepared following literature reported methods with minor modifications.[1,2] Briefly, poly(acrylic acid)$_{120}$ (synthesized *via* atom transfer radical polymerization of *t*butyl acrylate followed by deprotection with trimethyl iodide) is activated by O-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate /hydroxybenzotriazole in DMF, followed by amidation using *N*-Boc ethylene diamine to afford PAEA-Boc$_{120}$. THF GPC of PAEA-Boc$_{120}$ indicates a narrow molecular weight distribution (PDI = 1.18). Excess aqueous HCl (2.0 M) was used to remove the Boc protecting group (4 h in RT), giving PAEA$_{120}$. PAEA$_{120}$ (67.5 mg, 4.9 µmol) was dissolved in anhydrous DMSO (2 mL), and stirred for 3 h, before 1 mL of a DMSO solution containing propargyl-dPEG$_1$-NHS ester (150 mg, 660 µmol, Quanta Biodesign Ltd., OH, USA) and diisopropylethylamine (DIPEA, 204 µL 1.17 mmol) was added. The reaction mixture was allowed to stir overnight, diluted by the addition of DMSO (10 mL), transferred to pre-soaked dialysis tubing (MWCO = 3.5 kDa), and dialyzed against Nanopure™ water (>18.0 MΩ•cm) for 3 days. The solution was then lyophilized and re-suspended in water (15 mL). A small amount of cloudiness was observed, which was removed by filtering through a 0.2 µm syringe filter. No residual amine groups were detected by a ninhydrin test. The polymer exhibits a lowest critical solution temperature (LCST) of 28.8 ˚C as determined by light scattering while monitoring by UV-Vis spectroscopy at 600 nm. The solubility of the polymer increased with decreasing temperature (room temp- 0 ˚C). Calculated molecular weight: 27.0 kDa. ^{1}H-^{13}C HSQC NMR and IR spectra of **1** are shown in Fig. S1-A and S1-D, respectively.

2. Synthesis of methyl-terminated poly(ethylene glycol)-propargyl ether conjugate **5**:

Monodisperse $mPEG_{24}$-amine (39.0 mg, 34.6 μmol, Quanta Biodesign) was dissolved in 1.0 mL pH = 8.0 phosphate buffer, to which propargyl-$dPEG_1$-NHS ester (12.1 mg, 53.7 μmol) was added. The mixture was allowed to be shaken for 12 hours at 4 °C. The desired conjugate was isolated from the reaction mixture by reverse phase HPLC (water/acetonitrile, Varian DYNAMAX C18 column (250 × 10.0 mm)). MALDI-ToF: 1220.553 $[M+Na]^+$. ^1H-^{13}C HSQC: Fig. S2.

3. Synthesis of 13 nm AuNPs.

An aqueous solution of $HAuCl_4$ (1 mM, 500 mL) was brought to reflux while stirring, and then trisodium citrate solution (50 mL, 77.6 mM) was added quickly to the boiling mixture. The solution was refluxed for an additional 15 min, and allowed to cool to room temperature. The average diameter of the gold nanoparticles was determined by TEM (12.8 ± 1.2 nm). AuNPs of other sizes used in this study were purchased from Ted Pella Inc., CA, USA.

4. General method for the preparation of nanopods.

To 10 mL AuNP solution (10 nM), 10 μL of 10% sodium dodecyl sulfate solution was added. Then, an aqueous solution containing **1** was added to give a final concentration of 20 nM. The solution was stirred for 2 days, before being subjected to centrifugation using an Eppendorf 5424 centrifuge at 15,000 rpm for 30 min. Supernatant was removed by careful pipetting, and the AuNP was resuspended in Nanopure™water. The process was repeated 3 times to ensure complete removal of excess polymers. After the final centrifugation, the polymer-coated AuNP were concentrated to 1 mL, and 50 μL of 1.0 M KCN aqueous solution was added to remove the gold core. The resulting solution was then dialyzed against Nanopure™water (>18.0 MΩ•cm) using pre-soaked dialysis tubing (MWCO = 6-8 kDa) for 3 days. The final nanopod solution appeared clear and slightly yellow. A large volume of gold nanoparticle templates (>500 mL) was required to prepare sufficient quantity of nanopods for NMR and IR analyses. The concentration of citrate capped gold nanoparticles prior to the addition of polymer **1** must not exceed 0.5 μM (optimal 10-100 nM) in order for the resulting nanopods to be aggregate-free.

5. Functionalization of polymer **1** with Alexa Fluor 488.

To 3.0 mL of a 3.54 mg/mL aqueous polymer **1**, 0.50 mg Alexa Fluor 488-azide (Invitrogen, OR, USA) was added as a 1.5 mL aqueous solution. Sodium ascorbate (4.5 mg) was dissolved in this mixture to give a concentration of 5.0 mM. Tris-(hydroxypropyltriazolylmethyl)amine (synthesized following literature reported method)[3] and $CuSO_4$ were mixed in 5:1 molar ratio in water, and added to the polymer mixture to give a final copper ion concentration of 0.1 mM. The solution was stirred at room temperature (21 ˚C) overnight. After the reaction, the mixture was dialyzed against Nanopure™ water (>18.0 MΩ•cm) using pre-soaked dialysis tubing (MWCO = 3.5 kDa) for 3 days to remove copper ions and unreacted Alexa Fluor 488-azide. The purified polymer was lyophilized and resuspended in water to give a final concentration of 2.37 mg/mL.

6. General procedure for the quantification of AuNP surface-bound polymers.

AuNPs (10.0 nM) were separated into 16×1.0 mL aliquots, which were each concentrated by centrifugation to a final volume of 100 μL (100 nM). To each of the concentrated solutions, 0.2 – 20.0 μL of the AF488-labeled polymer **1** solution (87.4 μM) were added. The mixtures were allowed to react overnight. After the reaction, the solutions were diluted to 2.0 mL, and their fluorescence was measured (excitation: 488 nm; emission: 515 nm). Fluorescence intensities were plotted against polymer concentration. Fluorescence increased with regard to polymer concentration at a lower rate before the AuNP surface was consumed, and at a higher rate thereafter. Each stage appeared to have a linear relationship. The inflection point, that is,

when the surface of the AuNP becomes completely covered by polymer chains, can be identified from the plot as the onset of the second stage. The number of polymers per AuNP can be calculated by:

Number of polymers per AuNP = $[\text{polymer}]_{\text{inflection point}}/[\text{AuNP}]$

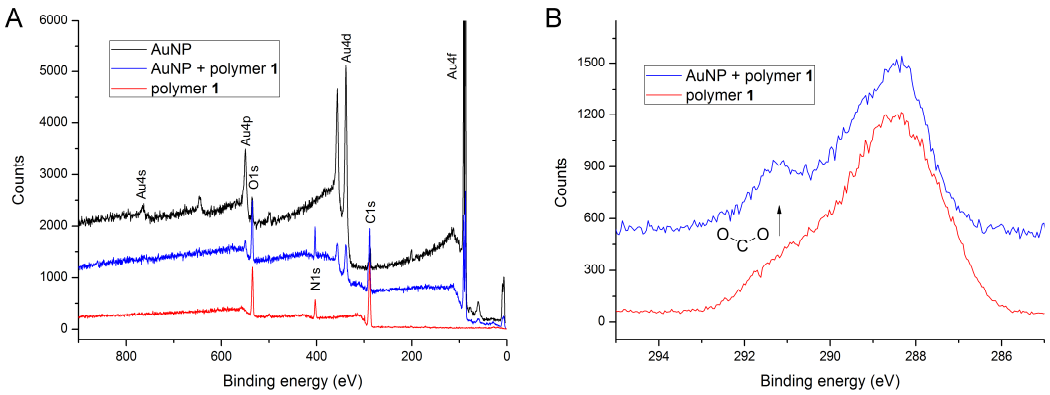

Figure S1. **A**. XPS survey spectra of AuNPs, AuNP-polymer complexes and polymer **1** on silicon wafer. B. Detailed scan of the C1s region.

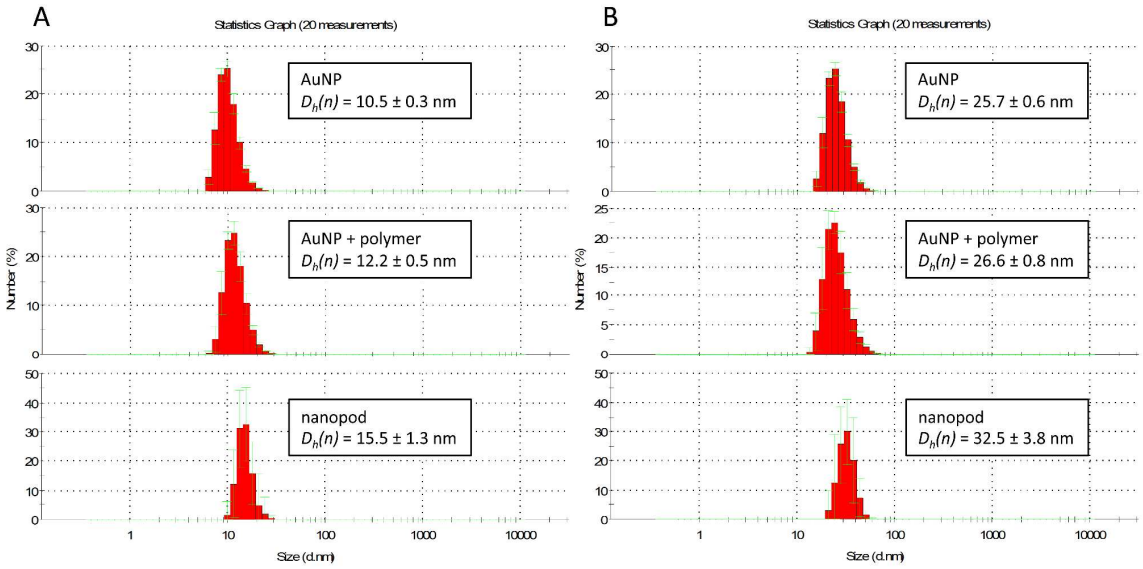

Figure S2. DLS number-average hydrodynamic diameter distribution graphs for AuNPs, AuNP-polymer complexes, and polymer nanopods based on **A**) 13 nm and **B**) 30 nm AuNP cores. Standard deviation is calculated from 20 independent measurements. The unimodal distributions indicate no aggregation.

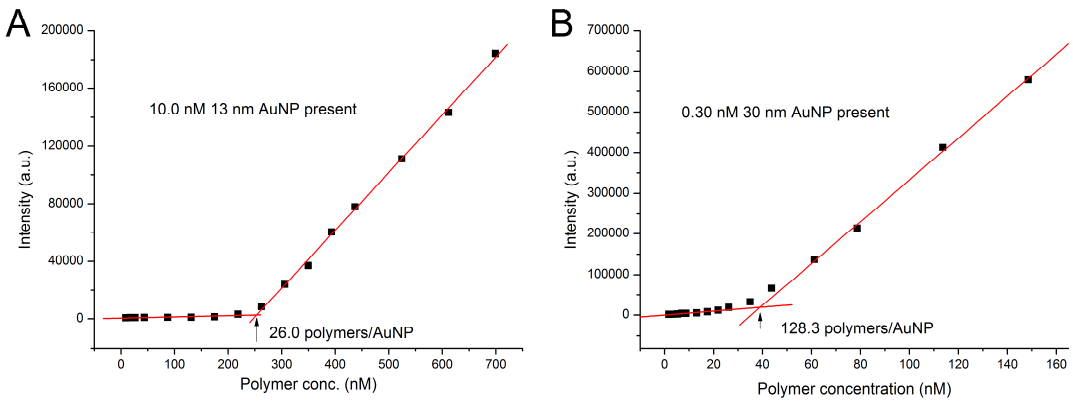

Figure S3. Titration of AuNP surface (**A**. 13 nm **B**. 30 nm) with AF488-labeled polymer **1**. Fluorescence intensities increase with regard to polymer concentration at a lower rate before the AuNP surface is saturated with polymer chains, and at a higher rate thereafter (two stages). The onset of the second stage indicates complete coverage of the AuNP's surface. For 13 nm AuNP, it is determined that an average of 26.0 polymers are required to completely cover the surface of each particle, giving a surface density of 2.27 mg/m^2 (TEM number-averaged diameter of 12.8 nm used in calculation). For 13 nm AuNP, 128.3 polymers are required to completely cover the surface of each particle. Polymer surface density is 2.32 mg/m^2 (TEM number-averaged diameter of 28.1 nm used in calculation).

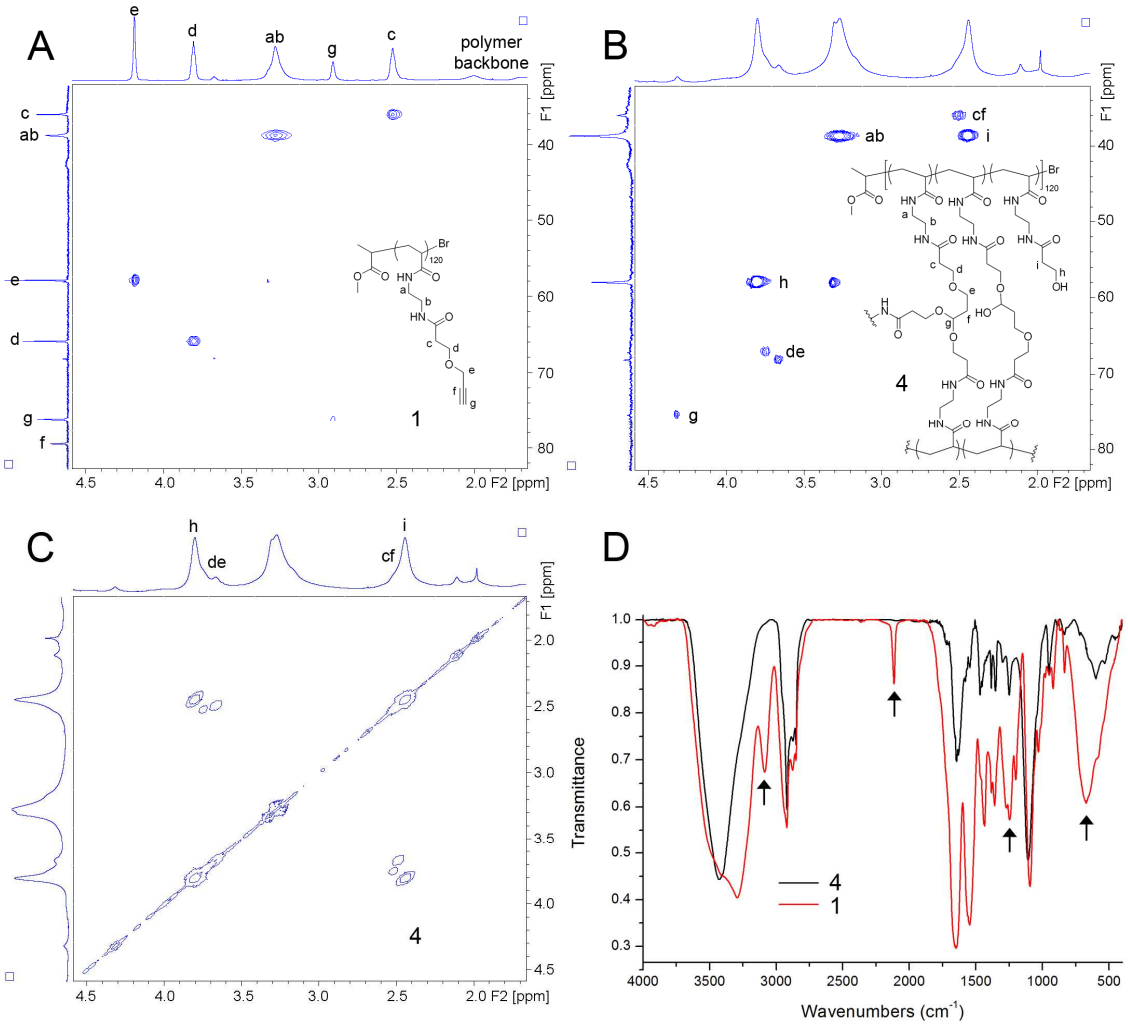

Figure S4. **A**. ^{1}H-^{13}C HSQC of **1** in D_2O. **B**. ^{1}H-^{13}C HSQC of **4** in D_2O. **C**. ^{1}H-^{1}H COSY of **4** in D_2O, showing h-i, c-d and e-f correlations. **D**. IR spectra of **1** (red) and **4** (black), showing the loss of alkyne-related vibrations, most notably the C ≡ C stretching (2114 cm^{-1}).

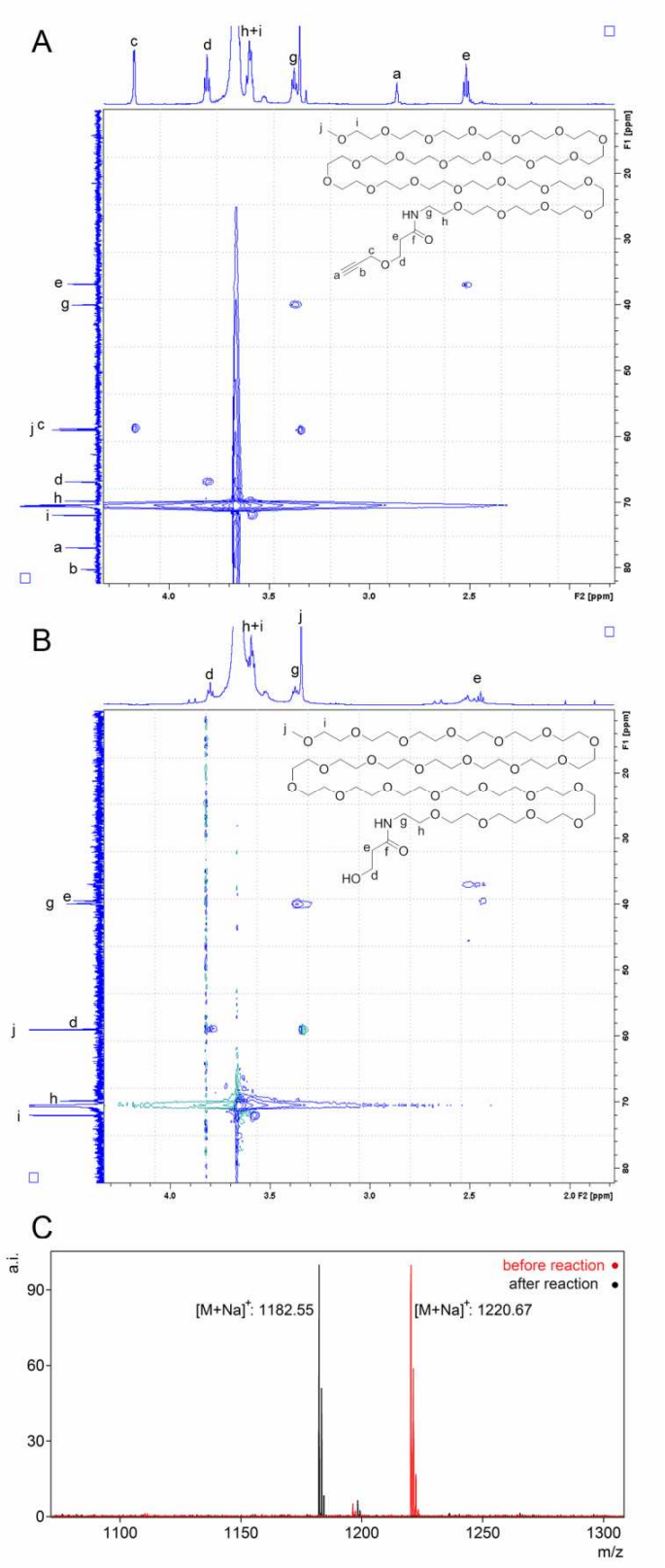

Figure S5. **A**. ^{1}H-^{13}C HSQC for **5** in D_2O. **B**. ^{1}H-^{13}C HSQC for **5** in D_2O after reaction. **C**. MALDI-ToF MS for **5** before and after reaction.

(1) Zhang, K.; Fang, H. F.; Wang, Z. H.; Li, Z.; Taylor, J. S. A.; Wooley, K. L. *Biomaterials* **2010**, *31*, 1805.
(2) Zhang, K.; Fang, H. F.; Wang, Z. H.; Taylor, J. S. A.; Wooley, K. L. *Biomaterials* **2009**, *30*, 968.
(3) Hong, V.; Presolski, S.; Ma, C.; Finn, M. *Angew. Chem. Int. Ed.* **2009**, *48*, 9879.