Electronic Supplementary Material (ESI) for Polymer Chemistry

Supplemental Information

Single-chain Polymer Nanoparticles via Reversible Disulfide Bridges

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Experimental Section

General Methods. Reagents were purchased from Aldrich and used as received except for *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride, which although commercially available was synthesized from the *endo* isomer (purchased from Acros) according to the literature.[1] Size exclusion chromatography (SEC) was performed on a Tosoh EcoSEC dual detection (RI and UV) GPC system coupled to an external Wyatt Technologies miniDAWN Treos multi angle light scattering (MALS) detector and a Wyatt Technologies ViscoStarII differential viscometer. Samples were run in THF at 40 ºC at a flow rate of 0.35 mL/min. The column set was two Tosoh TSKgel SuperMultipore HZ-M columns (4.6x150 mm), one Tosoh TSKgel SuperH3000 column (6x150mm) and one Tosoh TSKgel SuperH4000 column (6x150mm). (**Note:** SEC experiments on the poly(norbornene anhydride) homopolymer **P1** and nanoparticles **N1** were run using only the SuperMultipore columns without the external detectors). Increment refractive index values (dn/dc) were calculated online assuming 100% mass recovery (RI as the concentration detector) using the Astra 6 software package (Wyatt Technologies) by selecting the entire trace from analyte peak onset to the onset of the solvent peak or flow marker. Absolute molecular weights and molecular weight distributions were calculated using the Astra 6 software package. Relative molecular weights were obtained vs. polystyrene standards (PStQuick MP-M, Tosoh) and calculated using the EcoSEC software package (Tosoh). Intrinsic viscosity ($[\eta]$) and viscometric hydrodynamic radii (Rh) were calculated from the differential viscometer detector trace and processed using the Astra 6 software. Transmission Electron Microscopy (TEM) images were recorded using a Zeiss LEO 922Ω operating at 120kV with a Gatan Multi-scan bottom mount digital camera. Samples were prepared by drop casting 1.5 μL of a nanoparticle solution (1.8 x10-4 mg/mL) on to Formvar carbon film coated 300 square mesh copper grids. ^{1}H NMR (400 MHz) spectra were recorded on a Varian Associates Mercury 400 spectrometer. Solvents ($CDCl_3$ or *d8*-THF) contained 0.03% v/v TMS as an internal reference, chemical shifts (δ) are reported in ppm relative to TMS. Peak abbreviations are used as follows: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad. Infrared spectra were recorded on a Thermo Scientific Nicolet iS10 FT-IR spectrometer with Smart iTR ATR accessory.

Polymer Synthesis and Nanoparticle Fabrication

Poly(norbornene-*exo*-anhydride) (P1):

To a 100 mL three neck RBF was added Grubbs' third generation catalyst (0.009 g, 0.01 mmol) and 4 mL of dry THF. The solution was degassed via nitrogen purge (30 minutes). Norbornene-*exo*-anhydride monomer (0.492 g, 0.003 mmol) was dissolved in 6 mL of dry THF and added to an addition funnel and degassed via argon purge for ten minutes. The monomer solution was then added dropwise over 15 minutes into the flask with medium stirring at 25 °C. The reaction was run for one hour then quenched with excess ethyl vinyl ether. The polymer was then purified via precipitation into cold, dry hexanes and isolated as slightly grey powder ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.15-8.80 (br s), 8.05 (m), 5.75-5.63 (m), 5.54-5.52 (m), 5.39-5.25 (br m), 5.13 (s), 4.38-4.25 (br m), 4.21-3.83 (br m), 3.75-3.63 (br m), 2.5 (m), 2.25 (m), 2.21-1.62 (br m). IR ν (cm^{-1}) 2933 (br CH), 1858 & 1770 (anhydride C=O), 908 (=C-H bending) SEC (THF vs. polystyrene) M_W = 31.8 kDa, PDI = 1.39.

Poly(cyclooctadiene-*co*-norbornene-*exo*-anhydride) (P2):

Cyclooctadiene (1.436 g, 13.3 mmol) was dissolved in 86 mL of dry THF and added to a dry 250 mL three neck flask sealed with rubber septa. The solution was degassed via nitrogen purge for 30 minutes. Norbornene-*exo*-anhydride (0.935 g, 5.7 mmol) was dissolved in 5 mL of dry THF solution, which was degassed for 5 minutes via argon purge. Grubbs' first generation catalyst (0.034 g, 0.041 mmol) was dissolved in 0.5 mL of dry THF and was added to the stirring COD solution via gas tight syringe. Once the catalyst was added the norbornene-*exo*-anhydride monomer was added dropwise to the reaction over the course of 5 minutes. This helps circumvent the tendency for alternation previously reported for these two monomers.[2] The reaction was run for two hours then quenched with excess ethyl vinyl ether. The polymer solution was purified via precipitation into cold, dry hexanes yielding a gray adhesive gum. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 6.29 (s), 3.75 (t), 3.45 (s), 2.95 (s), 1.85-1.79 (br m), 1.65 (d) 1.45 (d), 1.25 (t). IR ν (cm^{-1}) 2933 (br CH), 1858 & 1770 (anhydride C=O), 908 (=C-H bending) SEC: M_W = 50.1kDa (MALS, dn/dc = 0.1667); PDI = 1.22 (MALS)

General Crosslinking Procedure (N1 and N2):

Polymer was dissolved in dry THF under dilute conditions (1 mg/mL). The solution was then added to a dry three neck flask capped with rubber septa. The solution was degassed via nitrogen purge for 30 minutes. Depending on the polymer being cross-linked, varying molar percentages of 4-aminophenyl disulfide was added to the stirring polymer solution via a gas tight syringe (for **N1** cross-linking percentages reported were calculated based on the percentage of backbone anhydride units consumed. For **N2** 0.5 equivalents of 4-aminophenyl disulfide per anhydride unit was used). The solutions were stirred at 25 °C for 12 hours, aliquots taken for direct SEC analysis, then purified by dialysis against THF (MWCO = 3500) for 18 hours and isolated via solvent evaporation. Nanoparticles remain stable in solution for weeks based on repeated SEC measurements. **N1**: IR ν (cm^{-1}): 3356 (carboxylic –OH), 2917 (br –CH), 1683 (amide C=O), 820 (ArH bending). **N2:** ^{1}H NMR (400 MHz, d-THF): δ ppm 7.30-7.00 (br m, ArH), 6.65-6.37 (br m, ArH), 5.47 (s,), 5.29-5.13 (br d,), 4.13 (t,), 3.70-3.63 (m,), 2.25 (t,), 2.12-1.71 (br m,), 1.17 (s,). IR ν (cm^{-1}): 3356 (carboxylic –OH), 2917 (br –CH), 1683 (amide C=O), 820 (ArH bending). SEC: M_W = 50.1kDa (MALS dn/dc = 0.1526); PDI = 1.22 (MALS).

General Unfolding Procedure:

The SCNPs (**N1** or **N2**) were dissolved in dry THF under dilute conditions (1 mg/mL) and added to a dry three neck flask capped with rubber septa. The solution was degassed via nitrogen purge for 30 minutes. A large excess (3 mmol) of dithiothreitol (DTT) in THF was added to the SCNP solution and stirred at 25 °C for 1 hour. Aliquots were taken for direct SEC analysis, followed by dialysis against THF for 18 hours (MWCO = 3500) and isolation via solvent evaporation. **N1**: IR ν (cm^{-1}): 3353 (-OH), 3300 (-OH), 2230 (-SH), 2090 (-SH), 1644 (amide C=O), 819 (ArH bending).**uN2** ^{1}H NMR (400 MHz, d-THF): δ ppm 10.75 (s,), 7.50-6.95 (br m,), 6.59-6.37 (br m), 5.25 (s,), 4.13 (t,), 3.74-3.65 (br m,), 2.45-2.31 (br s, -SH), 2.25 (t,), 2.00-1.69 (br m,), 1.18 (s). IR ν (cm^{-1}): 3353 (-OH), 3300 (-OH), 2230 (-SH), 2090 (-SH), 1644 (amide C=O), 819 (ArH bending).

General Disulfide Oxidation (refolding) Procedure:

The unfolded SCNPs (**uN1**) were dissolved in dry THF under dilute conditions (1 mg/mL) and added to a three neck flask capped with rubber septa. A catalytic amount of $FeCl_3$ in dry THF and was added via a gas tight syringe. The solution was stirred at 60 °C for 12 hours. Aliquots were then taken from this solution for direct SEC analysis.

Supplemental Figures:

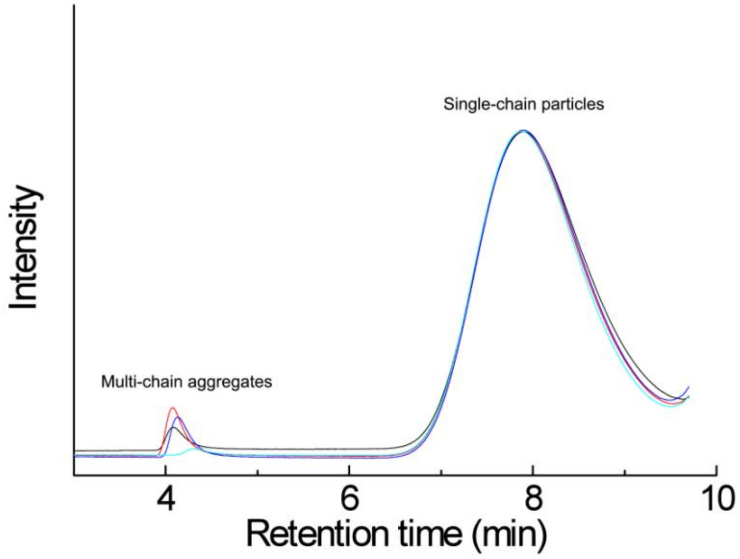

Figure S1: Full SEC trace for unfolded chains **uN1,** showing a small amount of intermolecular particle-particle coupling (see main text for details).

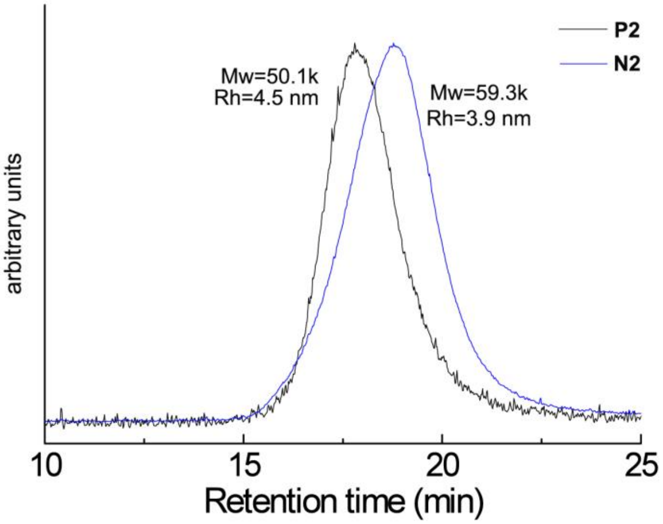

Figure S2: SEC overlay of MALS detector trace for **N2** and **P2** for which data in Table 1 (see main text) was derived.

Example Spectra.

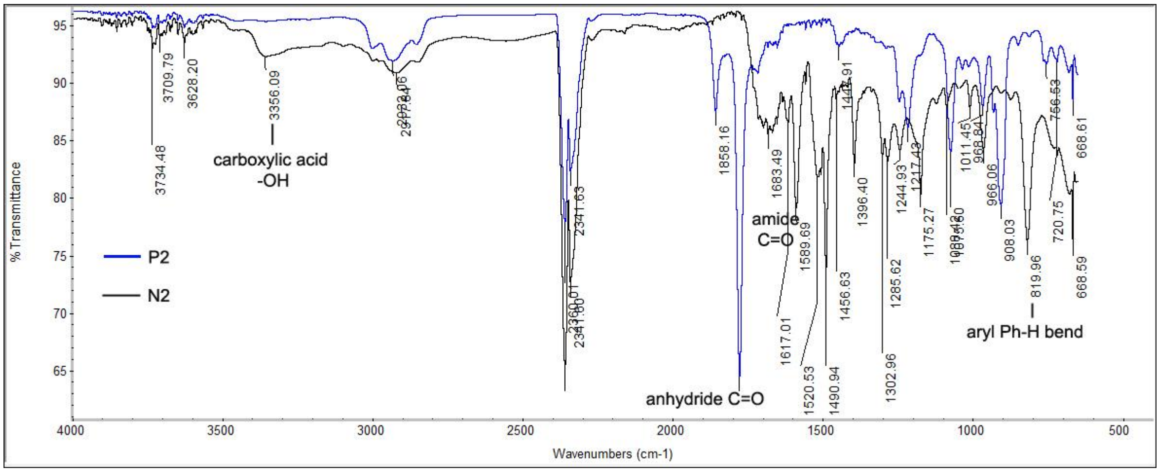

Figure S3: IR overlay for **P2** and **N2**. Addition of cross-linker is confirmed by the shift in the anydride C=O stretch to lower wavenumber, as well as the appearance of the Ph-H bend and the carboxylic acid –OH.

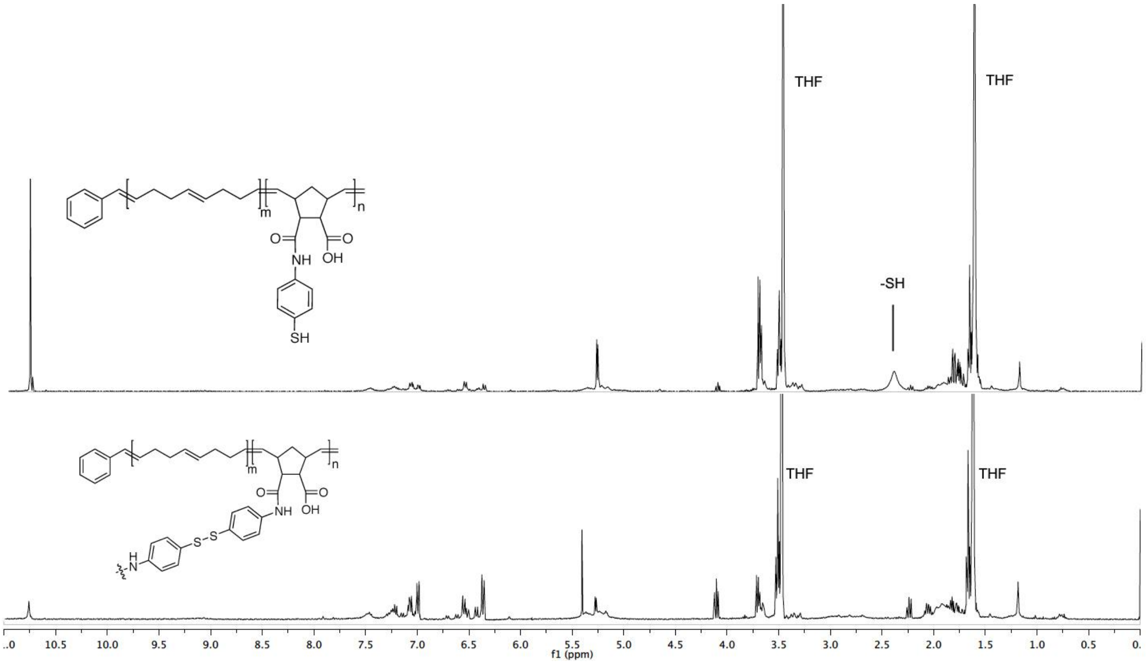

Figure S4: NMR overlay of **N2** before and after treatment with DTT showing the appearance of the –SH peak confirming disulfide reduction.

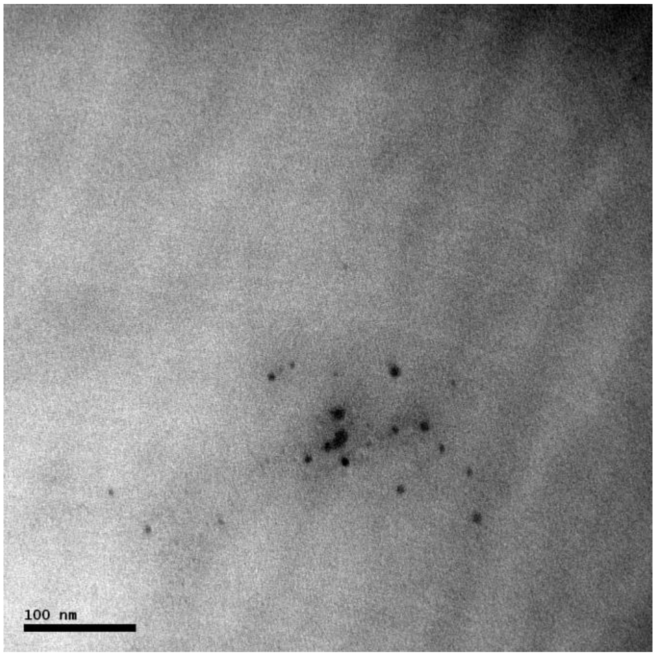

Figure S5: Full TEM image of SCNPs **N2** from which Figure 4 was taken.

References

1. J. B. Matson and R. H. Grubbs, *Journal of the American Chemical Society*, 2008, **130**, 6731-6733.
2. M. F. Ilker and E. B. Coughlin, *Macromolecules*, 2001, **35**, 54-58.