Supporting Information

Monovalent Organic Nanoparticles from Sequential Ring-Opening Metathesis Polymerization and Intramolecular Ring Closing Metathesis

Yugang Bai,[†] Hang Xing,[†,‡] Gretchen A. Vincil,[†] Jennifer Lee,[†] Essence J. Henderson,[†] Yi Lu,[†,‡] N. Gabriel Lemcoff,[§] and Steven C. Zimmerman*[,†]

[†]Department of Chemistry and [‡]Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

[§] Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Table of Contents

General Methods

Materials. All reagents were purchased from Acros Organics, Fisher Scientific, AK Scientific, TCI America, or Sigma-Aldrich, and used without further purification unless otherwise noted. Dichloromethane (DCM), pyridine, THF, toluene, DMSO and DMF were stored over activated 4 Å molecular sieves. Pyridine-modified Grubbs 2nd Generation catalyst for ROMP polymerization,[1] *N*-glycine *cis*-5-norbornene-*endo*-2,3-dicarboximide,[2] *N*-benzyl *cis*-5-norbornene-*endo*-2,3-dicarboximide (**M2**),[3] and *N*-methyl *cis*-5-norbornene-*endo*-2,3-dicarboximide (**M3**)[4] were each prepared according to literature procedures. The preparation of *N*-(6-amino)hexyl *cis*-5-norbornene-*endo*-2,3-dicarboximide was similar to that reported by Hanson and coworkers[5], but replacing the diamine (ethylenediamine) with hexamethylenediamine and further purifying the crude product with flash chromatography (DCM-MeOH, 10:1 to 5:1). *N,N'*-DiBoc *cis*-1,4-di(4-aminoethylphenoxyl)-2-butene (**CTA2**)[6] and tris(allyloxymethyl)aminomethane (triallyl-Tris)[7] were synthesized according to procedures in literature. Thiolated mPEG-10,000 was purchased from Laysan Bio Inc. and used as received.

Instrumentation. NMR spectra were recorded using a Varian U400, UI400, U500 or VXR500 spectrometer in the NMR Laboratory, School of Chemical Science, University of Illinois. The data was processed in MestReC v4.8.1.1. Some spectra were treated with the smoothing function of the software to obtain better baseline. NMR images were screenshots taken from MestReC and aligned and annotated in Adobe Photoshop CS4 (Figure 2a) or Microsoft Paint of Windows 7 (Figures in ESI). Mass spectral analyses were provided by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois, using ESI on a Waters Micromass Q-Tof spectrometer, FD on a Waters 70-VSE spectrometer. Analytical gel permeation chromatography (GPC) experiments were performed on a Waters system equipped with an Waters 1515 isocratic pump, a Waters 2414 refractive index detector, and a Waters 2998 photodiode array detector. Separations were performed at 50 °C using DMF containing 0.1 M LiBr as the mobile phase. Relative molecular weights and PDIs were calculated by Breeze 2 software from Waters Corporation, based on conventional calibration method. Absolute molecular weights were collected on a GPC system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS 18-angle laser light scattering detector, a multi-angle laser light scattering (MALLS) detector, (Wyatt Technology) and an Optilab rEX refractive index detector (Wyatt Technology). The detection wavelength of HELEOS was set at 658 nm. Separations were performed at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene and used for the determination of the absolute

molecular weights. The molecular weights of all polymers were determined based on the *d*n/*d*c value of each sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology). GPC data points were exported as ASCII files, re-imported into OriginPro 8, plotted, saved as vector image files (*.ai) and colored and annotated in Adobe Illustrator CS6. TEM experiments were conducted with a JEOL 2100 Cryo transmission electron microscope. Negative staining of polymer nanoparticles with ammonium molybdate was applied when preparing TEM samples in order to achieve better contrast. Samples were prepared by drop casting polymer nanoparticle and ammonium molybdate mixture solutions onto a carbon-coated copper TEM grid or a silicon wafer (Ted Pella), followed by removing excess solution with filter paper after 10 min. TEM grids were then left dried for another 15 min before imaging. DLS analysis was performed on a Brookhaven ZetaPALS instrument.

Synthetic Procedures

***N*-Glycine *cis*-5-norbornene-*endo*-2,3-dicarboximide NHS ester (M1).** *N*-Glycine *cis*-5-norbornene-*endo*-2,3-dicarboximide (2.21 g, 10 mmol) was dissolved in anhydrous dichloromethane (40 mL) in an ice bath. Oxalyl chloride (0.93 mL, 11 mmol, 1.1 eq) and one drop of DMF as catalyst was added to the solution. The reaction was stirred for 3 h with the temperature gradually increased to 25 ^{o}C. Volatiles were removed under reduced pressure to yield a white powder. The solid was dissolved in 40 mL of dry dichloromethane, and *N*-hydroxysuccinimide (1.27 g, 11 mmol, 1.1 eq) and K_2CO_3 (2.07 g, 15 mmol, 1.5 eq) were added. The suspension was stirred overnight. To the suspension was added 100 mL of dichloromethane and the organic solution washed with water twice and dried over Na_2SO_4. The solvent was removed under reduced pressure and a white solid was obtained. The crude product was dissolved in anhydrous acetone and crystallized by adding a layer of hexane which afforded **M1** as a fluffy white solid. Yield: 2.4 g. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.11 (s, 2H), 4.40 (s, 2H), 3.41 (m, 2H), 3.35 (m, 2H), 2.81 (s, 4H), 1.73 (d, J = 8.8 Hz, 1H), 1.53 (d, J = 8.8 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 176.2, 168.4, 162.8, 134.8, 52.4, 46.4, 45.3, 37.0, 25.7. ESI-MS: calculated 318.1, found 319.1 [M+H^{+}].

***N*-(2,2-Dimethoxy)ethyl *cis*-5-norbornene-*endo*-2,3-dicarboximide (M4).** To 2-amino-acetaldehyde dimethyl acetal (3.15 g, 30 mmol) was slowly added a solution of carbic anhydride (4.92 g, 30 mmol) in 40 mL of warm toluene. The suspension was heated to reflux on a heating mantle overnight. Solvent was removed under reduced pressure. The crude product was purified

by column chromatography (silica, EtOAc-hexanes, 1:1 v/v) followed by recrystallization in EtOAc-hexanes to yield a colorless crystal. Yield: 4.2 g (55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 2H), 4.54 (t, *J* = 5.8 Hz, 1H), 3.47 (d, *J* = 5.8 Hz, 2H), 3.39 (m, 2H), 3.30 (s, 6H), 3.26 (m, 2H), 1.73 (d, *J* = 8.7 Hz, 1H), 1.53 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 177.7, 134.6, 99.5, 53.1, 52.4, 46.0, 54.2, 39.2. ESI-MS: calculated 251.1, found 274.1 [M+Na^+].

***N*-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl *cis*-5-norbornene-*endo*-2,3-dicarboximide (M5).** 3-Amino-1,2-propanediol (1.92 g, 21 mmol, 1.05 eq) was dissolved in warm toluene-dioxane mixture (50 mL, 10:1 v/v) and *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride (3.28 g, 20 mmol, 1 eq) was added. The mixture was heated at reflux overnight. The solution was decanted while hot to remove insoluble residue (if present) and cooled to 50 ^{o}C. To the solution was added *p*-toluenesulfonic acid monohydrate (0.38 g, 2 mmol, 0.1 eq) and 2,2-dimethoxypropane (10 mL, excess). The reaction was monitored by TLC, and the volatiles were removed under reduced pressure once the protection was complete. The residue was dissolved in 40 mL of EtOAc, and washed by saturated aqueous $NaHCO_3$, water, and brine. The organic phase was collected, dried over Na_2SO_4, and the solvent removed under reduced pressure to give the crude product, which could be further purified by flash column chromatography (silica, EtOAc-hexanes, 1:3 v/v) to give a white solid. Yield: 5.3 g (96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.08 (s, 2H), 4.16 (m, 1H), 3.93 (dd, *J* = 8.5, 6.1 Hz, 1H), 3.57-3.67 (m, 2H), 3.33-3.40 (m, 3H), 3.27 (m, 2H), 1.72 (d, *J* = 8.8 Hz, 1H), 1.53 (d, *J* = 8.8 Hz, 1H), 1.40 (s, 3H), 1.28 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 177.7, 134.7, 109.8, 72.8, 67.8, 52.4, 46.0, 45.2, 41.3, 27.0, 25.7. ESI-MS: calculated 277.1, found 300.1 [M+Na^+].

5(6)-Carboxyfluorescein monomer (M6). Commercially available 5(6)-carboxyfluorescein (0.92 g, 2.4 mmol, 1.0 eq) was dissolved in DMF (10 mL). EDC hydrochloride (0.50 g, 2.6 mmol, 1.1 eq) and *N*-hydroxysuccinimide (0.30 g, 2.6 mmol, 1.1 eq) were added and the mixture was stirred for 10 min and *N*-(6-amino)hexyl *cis*-5-norbornene-*endo*-2,3-dicarboximide (0.68 g, 2.6 mmol, 1.1 eq) was added. The solution was stirred at room temperature overnight. The resulting solution was diluted with 50 mL of EtOAc and washed three time with water and once each with aqueous HCl (0.1 M) and brine. The organic solution was dried over Na_2SO_4 and evaporated to a residue, which was purified by column chromatography (silica, DCM-MeOH, 10:1) to give approximately 1.0 g of product as a yellow solid. Note: due to the lactone-acid equilibrium of fluorescein moiety, it is difficult to obtain this intermediate in very high purity, but the subsequent acetyl protection locks the fluorescein in its lactone form and greatly reduces the polarity of the compound, facilitating its purification. The solid was suspended in 5 mL of acetic

anhydride and heated at 50 ^{o}C overnight to give a homogeneous, dark red solution. Acetic anhydride was removed under reduced pressure. The residue was purified by column chromatography using silica that was dried in a vacuum oven at 150 ^{o}C for 48 h and pre-treated with EtOAc-hexanes (3:2 v/v) to give a white, non-fluorescent solid, which was approximately a 4:3 mixture of 5-isomer and 6-isomers. Yield: 0.7 g (50%). ^{1}H NMR (400 MHz, $CDCl_3$): 5-isomer: δ 8.42 (s, 1H), 8.21 (dd, *J* = 8.1 Hz, *J* = 1.5 Hz, 1H), 7.27 (d, *J* = 8.1 Hz), 7.09 (m, 2H), 6.81 (m, 4H), 6.61 (t, *J* = 5.4 Hz), 6.10 (s, 2H), 3.16-3.51 (m, 8H), 2.30 (s, 6H), 1.16-1.74 (m, 10H). 6-isomer: δ 8.05-8.12 (m, 2H), 7.52 (s, 1H), 7.08 (m, 2H), 6.80 (m, 4H), 6.42 (t, *J* = 5.5 Hz, 1H), 6.04 (s, 2H), 3.16-3.51 (m, 8H), 2.30 (s, 6H), 1.16-1.74 (m, 10H). ^{13}C NMR was not attempted as the product was a mixture. High resolution ESI-MS: calculated for [M+H^{+}]: 705.2673; found: 705.2672. Elemental analysis: calculated for $C_{40}H_{36}N_2O_{10}$: 68.17% C, 5.15% H, 3.98% N; found: 68.03% C, 5.07% H, 3.96% N.

***Cis*-2-Butene-1,4-diol bis(5-azidovaleric acid) ester (CTA1).** A solution of 5-bromovaleric acid (3.7 g, 20 mmol) and oxalyl chloride (1.8 mL, 21 mmol, 1.05 eq) in 30 mL of dichloromethane was cooled in an ice bath and one drop of DMF added. The ice bath was removed and the solution stirred for 3 h. Solvent was removed under reduced pressure, and the resulting residue was dissolved in 5 mL of dry dichloromethane and added to a mixture of *cis*-1,4-dihydroxy-2-butene (0.80 g, 9.1 mmol, 0.91 eq) and K_2CO_3 (4.0 g, 29 mmol, 1.45 eq) in THF (25 mL). The mixture was stirred at room temperature overnight. Volatiles were removed under reduced pressure and the residue was partitioned between EtOAc and water. The organic layer was washed with water and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product purified by column (silica, EtOAc-hexanes, 1:5 v/v) to give a olorless oil. The oil was dissolved in 20 mL of DMF, added NaN_3 (1.3 g, 20 mmol), and stirred at 50 ^{o}C for 24 h. The mixture was partitioned between EtOAc and water. Organic phase was collected and washed further by water and brine. Crude product was obtained after evaporation, and was further purified by column (silica, EtOAc-hexanes, v/v 1:5) to give a colorless oil. Overall yield: 1.6 g (52%, relative to the diol). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75 (t, *J* = 4.3 Hz, 2H), 4.70 (d, *J* = 4.3 Hz, 4H), 3.30 (t, *J* = 6.6 Hz, 4H), 2.37 (t, *J* = 7.2 Hz, 4H), 1.73 (m, 4H), 1.64 (m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 173.0, 128.3, 60.2, 51.3, 33.8, 28.5, 22.3. ESI-MS: calculated 338.2, found 339.2 [M+H^{+}].

***Cis*-1,4-Bis(4-(2-azidoethyl)phenoxy)-2-butene (CTA3).** To a suspension formed from 2-(4-hydroxyphenyl)ethanol (2.8 g, 20 mmol), K_2CO_3 (4.0 g, 29 mmol) and *cis*-1,4-dichloro-2-butene (1.0 mL, 9.5 mmol) in anhydrous DMF (10 mL), was added tetrabutylammonium iodide (10 mg,

catalytic) and the mixture stirred at 40 ^{o}C for 24 h. The resulting mixture was partitioned between diethyl ether and water, separated, and the organic phase was washed with water and brine. The solution was dried over Na_2SO_4 and filtered, then cooled in a -20 ^{o}C freezer. The crystalline white solid was collected by filtration and dried under vacuum to get the pure intermediate compound *cis*-1,4-bis(4-(2-hydroxyethyl)phenoxy)-2-butene.

The intermediate *cis*-1,4-bis(4-(2-hydroxyethyl)phenoxy)-2-butene (1.7 g, 5.2 mmol) was dissolved in 40 mL of DMF-CCl_4 (4:1 v/v). To the solution was added NaN_3 (0.9 g, 14 mmol) and PPh_3 (2.7g, 10.3 mmol) and the mixture was heated to 90 ^{o}C for 5 h and cooled to room temperature. The mixture was poured into 350 mL of water and extracted by diethyl ether. The organic layer was collected and washed with water and brine, dried over Na_2SO_4, and the solvent removed under reduced pressure to give a crude oil. The crude product was purified by column chromatography (EtOAc-hexanes, 1:4 v/v) to give the pure product as a colorless or light-yellow oil (0.7 g, 19% from *cis*-1,4-dichloro-2-butene). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, J = 8.5 Hz, 4H), 6.84 (d, J = 8.5 Hz, 4H), 5.91 (t, J = 3.6 Hz, 2H), 4.64 (d, J = 3.6 Hz, 4H), 3.44 (dd, J = 7.2 Hz, 4H), 2.81 (t, J = 7.2 Hz, 4H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 157.5, 130.7, 130.1, 128.8, 115.1, 64.5, 52.9, 34.7. ESI-MS: calculated 378.2, found 401.2 [M+Na^+].

***N,N'*-Diallyl-1,6,7,12-tetrachloroperylenediimide (PDI-allyl).** To a suspension of 1,6,7,12-tetrachloroperylene tetracarboxylic acid dianhydride (0.3 g, 0.56 mmol) in 8 mL of pyridine was added allylamine (0.2 mL, 2.7 mmol, 4.8 eq), and the suspension heated to reflux under nitrogen for 16 h. Solvent was removed under reduced pressure and the residue was purified by column (silica, DCM-hexanes, 4:1 to 5:1 v/v) to yield a dark orange-red powder. Yield: 0.11 g (31%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (s, 4H), 6.00 (ddt, J = 10.2 Hz, J = 17.1 Hz, J = 5.9 Hz, 2H), 5.41 (dd, J = 1.3 Hz, J = 17.1 Hz, 2H), 5.38 (dd, J = 1.3 Hz, J = 10.2 Hz, 2H), 4.85 (d, J = 5.9 Hz, 4H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 162.2, 135.7, 133.3, 133.3, 131.7, 128.9, 123.6, 123.4, 118.8, 43.0. MALDI-TOF: calculated 610.0, found 610.1 [M^+].

Typical procedure of polymerization without a CTA. M1 (95 mg, 0.3 mmol, 50 eq) and M4 (151 mg, 0.6 mmol, 100 eq) wre dissolved in anhydrous DCM (10 mL). The solution was degassed with three freeze-pump-thaw cycles to remove oxygen. Under nitrogen protection, pyridine-modified Grubbs 2nd Generation catalyst (0.04 M in DCM, 0.15mL, 0.006 mmol, 1 eq) was added. The solution was stirred at room temperature for 4 h before butyl vinyl ether (1 mL) was added to quench the catalyst. Solvent was removed under reduced pressure. The solid residue

was sonicated in ether and centrifuged for 3 times before it was dried under reduced pressure to give an off-white solid. Yield: 213 mg (86%).

Typical procedure of polymerization with a CTA. M1 (95 mg, 0.3 mmol, 50 eq) and M3 (106 mg, 0.6 mmol, 100 eq) were dissolved in anhydrous dichloromethane (10 mL). The solution was degassed with three freeze-pump-thaw cycles to remove oxygen. Under a nitrogen atmosphere, pyridine-modified Grubbs 2nd Generation catalyst (0.04 M in DCM, 0.15 mL, 0.006 mmol, 1 eq) was added. The solution was stirred at room temperature for 2.5 h and CTA2 (0.2 M in DCM, 0.15 mL, 5 eq) was added. The solution was stirred for 3 h and butyl vinyl ether (1 mL) was added to quench the catalyst. Solvent was removed under reduced pressure. The solid residue was sonicated in ether, centrifuged, and the supernatant discarded 3 times before the solid was dried under reduced pressure to give an off-white solid. Yield: 187 mg (93%).

Typical procedure for functionalization with triallyl-TRIS. The poly(activated ester) (180 mg) was dissolved in a mixture of DCM (8 mL) and nitrobenzene (1 mL) in a 20-mL vial. Triallyl-TRIS (0.2 mL) was added. The vial was capped and sealed by parafilm, and the solution was stirred in a 40 ^{o}C oil bath overnight. Most of the solvent was removed under reduced pressure, and the viscous residue was precipitated in 15 mL of a 2:1 (v/v) mixture of ether-hexanes and centrifuged. The precipitate was sonicated two times in ether (12 mL) and two times in methanol (10 mL) each time centrifuging and discarding the supernatant to remove triallyl-TRIS, NHS and nitrobenzene. The remaining solid was dried under reduced pressure to give the product as an off-white powder. Yield: 160 mg.

Typical procedure for intramolecular crosslinking with or without PDI-allyl. In a 1 L round-bottom flask, 50 mg of polymer P5-b and 3.3 mg of PDI-allyl (if needed, 5 eq relative to the amount of polymer chains) was dissolved in 600 mL of anhydrous DCM under a nitrogen atmosphere, and the solution was stirred at room temperature for 15 min. To the solution was added 12 mg of 1st Generation Grubbs catalyst in 1 mL of DCM and the lower 1/3 of the flask immersed in a 35 ^{o}C oil bath. The solution was stirred for 6 h and 6 mg catalyst added and stirred 18 h and 6 mg catalyst added. The mixture was stirred for a total of 48 h after the first catalyst addition and 3 mL of butyl vinyl ether was added to quench the catalyst. The solution was stirred 30 min and evaporated under reduced pressure. The solid residue was dissolved in DCM and purified by passing through silica gel eluted by DCM. Solvent was evaporated and the residue was sonicated and triturated in diethyl ether. Decantation gave a grey (or reddish if PDI was added) powder after vacuum drying.

Typical procedure for dihydroxylation of the alkene ONPs. In a 20 mL glass vial, 40 mg of polymer P3-c was suspended in a mixture of water (3 mL) and acetone (7 mL). NMO (50% wt in H_2O, 0.4 mL) and K_2OsO_4 (catalytic amount) was added to the mixture. The vial was loosely capped and heated in a 40 ^oC oil bath for several hours, before the cap was removed and the vial was covered by a piece of chemical paper wipe. Acetone was allowed to evaporate over time and 4 mL of water was added when the mixture became almost homogeneous. Heating and stirring was continued for 5-6 h and the solution decanted to remove any insoluble residue. The supernatant was stirred with Smopex-105 metal scavenger at 50 ^oC. The solution could be further purified by dialysis against water using a 1 kDa cutoff membrane and the solution lyophilized to yield a grayish-white solid. Yield: 32 mg.

Typical procedure for dihydroxylation and hydrolysis of acetal groups in one pot. The crosslinked polymer (40 mg, from the linear polymer in which M1:M5:M6 = 25:50:4) was suspended in a mixture of acetone and water (10 mL, 7:3 v/v) in a 20-mL vial. NMO (0.4 mL, 50% w/w in water) was added, followed by K_2OsO_4 (catalytic amount). The vial was loosely capped and heated in a 40 ^oC oil bath for several hours, before the cap was removed and the vial was covered by a piece of chemical paper wipe. Acetone was allowed to evaporate over time and 4 mL of water was added when the mixture became almost homogeneous. Heating and stirring was continued for 5-6 h and the solution was cooled to room temperature and quenched by adding $NaHSO_3$ (200 mg) and stirring for 30 min. Concentrated HCl was added dropwise to the fast-stirring solution until the pH of the solution dropped below 1 as measured by pH test paper. Precipitates formed during this process because the polymer before hydrolysis was not soluble in aqueous solution with high ionic strength. The reaction was stirred at 35 ^oC for several hours until the solution became almost clear, then filtered or centrifuged to remove any insoluble residue and purified by dialysis using dilute aqueous $NaHCO_3$ once and water once. The purified solution was lyophilized to give a yellow solid (29 mg).

Example of Large Scale ONP Preparation.

(1) ROMP polymerization. M1 (1.7 g, 6 mmol, 100 eq), **M5** (958 mg, 3.01 mmol, 50 eq.) and **M6** (165 mg, 0.244 mmol, 4 eq.) were dissolved in 100 mL of chloroform in a 500 mL round bottom flask. The solution and flask were purged with argon for 5 min. A solution of pyridine-modified Grubbs 2nd Generation catalyst (43.6 mg, 59.9 μmol, 1 eq. in 4 mL deoxygenated DCM) was added and the mixture stirred at room temperature for 20 min. CTA2 (1.097 g, 2.08 mmol, 35 eq.) was added and the solution stirred 4 h. Butyl vinyl ether (15 mL, 116 mmol) was added

and the mixture stirred for 45 min. The solvent was removed under reduced pressure and the residue dissolved in 30 mL of DCM. The solution was transferred to two 50 mL tubes and polymer precipitated with 30 mL diethyl ether and centrifuged (10 min x 4000 rpm). The brown supernatant was removed and the polymer dissolved and combined (total vol. 28 mL) and precipitated with 22 mL of diethyl ether. The polymer was pulsed for 30 s in the centrifuge and the supernatant poured off. The polymer was dried under high vacuum to yield an off-white solid. Yield: 2.48 g, 94 %.

(2) Attachment of TriallylTris. The ROMP polymer (2.48 g, 50.5 μmol, 2.54 mmol activated ester) was dissolved in 200 mL chloroform:nitrobenzene (19:1 v/v) in a 500 mL round bottom flask. Triallyltris (2.9 g, 12 mmol, 4.7 eq.) was added and the flask sealed with a septum. The reaction was heated to 55 °C for 12 h. The solvent was removed under reduced pressure and the residue transferred with DCM to a 50 mL tube (total volume 22 mL). The polymer was precipitated with 25 mL diethylether:hexane (2:1 v/v) and centrifuged (10 min x 4000 rpm). The supernatant was removed and the precipitate dissolved with 10 mL DCM and precipitated with 40 mL diethyl ether. The polymer was pulse centrifuged and the supernatant removed. The polymer was dissolved in 20 mL DCM precipitated with 30 mL MeOH, sonicated for 5 min then centrifuged and the supernatant decanted. The polymer was then dried on high vacuum to yield an off-white solid. Yield: 1.76 g, 62 %.

(3) **RCM.** The above polymer (1.76 g, 35 μmol) was dissolved in 5 L of DCM in a 3-neck RBF. A solution of Grubbs Generation 1 catalyst (445 mg, 0.54 mmol) in 5 mL DCM was added. The reaction was performed under a nitrogen atmosphere and the internal temperature monitored to maintain 30 °C via heating mantle. Additional catalyst was added (223 mg, 0.27 mmol in 4 mL DCM) at 20 h and the mixture heated for a total time of 68 h. The reaction was quenched with 25 mL butyl vinyl ether (193 mmol) and stirred for 45 min. The solvent was removed under reduced pressure and the crude polymer purified by silica gel plug eluting with DCM to remove the bulk of the catalyst. The solvent was removed and the dark brown polymer carried forward

(4) **Dihydroxylation and Hydrolysis.** To the RCM polymer product in a 300 mL round bottom flask was added 200 mL acetone:water (7:3 v/v), K_2OsO_4 dihydrate (26 mg, 70.6 μmol) and NMO (50% w/w in water, 16 mL, 77.2 mmol). The flask opening was covered by a chemical paper wipe and allowed to stir with the bottom half of the flask heated in an oil bath at 50 °C. After 12 h most of the polymer dissolved off the sides of the flask. The temperature was increased to 65 °C and the mixture stirred for a total of 40 h. Sodium bisulfite (sold as mixture with sodium

metabisulfite, 8.0 g) was added to the greenish mixture and stirred for 1 h. Several large black clumps were present. The reaction was then carefully acidified to pH 1 by drop-wise addition of 5 mL of concentrated HCl. The solution was stirred for 3 h, basified to pH 12 with 4 M NaOH momentarily, and neutralized with 2 M HCl. The black clumps dissolved with the addition of ~150 mL DI water. The reaction was vacuum filtered and most of the solvent removed under reduced pressure. When ~50 mL water remains solid starts to form on the sides of the evaporation flask and the contents were transferred to dialysis tubing. The polymer was dialyzed using a Spectra/Por dialysis membrane with a molecular weight cut-off of 1000 kDa against 4 L water for 14 h, and three times for 2 h. The volume of solvent was decreased via rotary evaporation then dried via lyophilization. Yield: 1.51 g (88% for three steps).

Typical procedure for mPEG conjugation. To demonstrate the function of amine group on ONP surface, a 10 kDa thiolated polyethylene glycol (mPEG-SH) was conjugated with non-water-soluble amine-ONP. Amine-ONPs (5 mg) were dispersed with 0.1 M sodium phosphate buffer (with 0.1 M NaCl, pH 7.3) and DMF to form a 0.2 mM suspension. Sulfo-SMCC (1 mg) was added into 500 μL of the amine-ONP suspension, and then placed on a shaker for 2 h at room temperature. The excess sulfo-SMCC was removed and the sulfo-SMCC-activated amine-ONPs were purified by Amicon-30K passing 0.1 M sodium phosphate buffer (with 0.1 M NaCl, pH 7.3) through 8 times. Thiolated mPEG-10,000 (2 mg) was activated by using tris(2-carboxyethyl) phosphine (TCEP) in sodium acetate buffer (pH 5.2). The purified sulfo-SMCC-activated ONPs solution and the activated thiolated mPEG-10,000 solution were mixed together and vortexed for 1 min. The mixture was then incubated at room temperature under shaking for 48 h. After incubation, non-reacted ONPs were not water-soluble and can be centrifuged down as precipitates and removed by filtration. The reacted PNPs formed water-soluble PNP-mPEG conjugates and stayed in supernatant. The conjugate was purified by 8 passes through an Amicon-30K centrifugal filter using 0.1 M sodium phosphate buffer (with 0.1 M NaCl, pH 7.3) to remove any un-reacted thiolated mPEG-10,000.

Fluorescent ONP Photobleaching experiment. Free fluorescein and fluorescent nanoparticle(s) were dissolved in 0.1 M phosphate buffer (pH = 7.4) to give 1 μM solutions in 7 mL vials. The vials were placed in a black box equipped with a power-adjustable 470 nm LED. The distance between the vials and LED was set at 20 cm. After the LED was turned on (t = 0), the fluorescence intensity of the solutions were examined every 2 h by a fluorospectrometer until enough data points were obtained.

Cell culture experiment. HeLa cells used for ONP uptake experiments were cultured in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, on 25 cm^2 culture flasks in a humidified 5% CO_2 incubator at 37°C. Hela cells were plated onto 35 mm glass-bottom dishes (MatTek) and grown to 70-90% confluence before confocal microscopy imaging.

ONP uptake, imaging and flow cytometry study. HeLa cells were incubated with 2 mL of Opti-MEM containing 5 μM ONPs in glass-bottom dish. After 6 hours, cells were washed with PBS 3 times and fresh DMEM was added. ONPs treated cells were then stained with Hoechst 33258 (Invitrogen) of 2.5 ng/mL for 20 minutes. After Hoechst staining, Lysotracker Red (Invitrogen) of 50 nM was added to cells and incubated for 30 minutes.

Confocal images were obtained using a Zeiss LSM 710 NLO confocal microscope at 63x magnification equipped with a Mai-Tai Ti-Sapphire laser. Fluorescence emission was obtained over 450-520 nm (DAPI), 510-570 nm (ONP), and 575-620 nm (LysoTracker Red) ranges, with excitation at 401 nm, 488 nm, and 561 nm, respectively. The pinhole and gain settings were kept constant throughout the whole imaging process.

For flow cytometry study, after 6-hour incubation with ONPs, Hela cells were washed with PBS and detached by 0.05% trypsin. The suspended cell solution was collected by centrifugation at 2,000 g for 5 min and further washed with PBS three times. Flow cytometry was performed using a BD FACSCanto system under 488 nm excitation. Control cells without any treatment were used to set the gating. Each measurement set was performed using 10,000 cells.

^{1}H NMR spectra of selected polymers

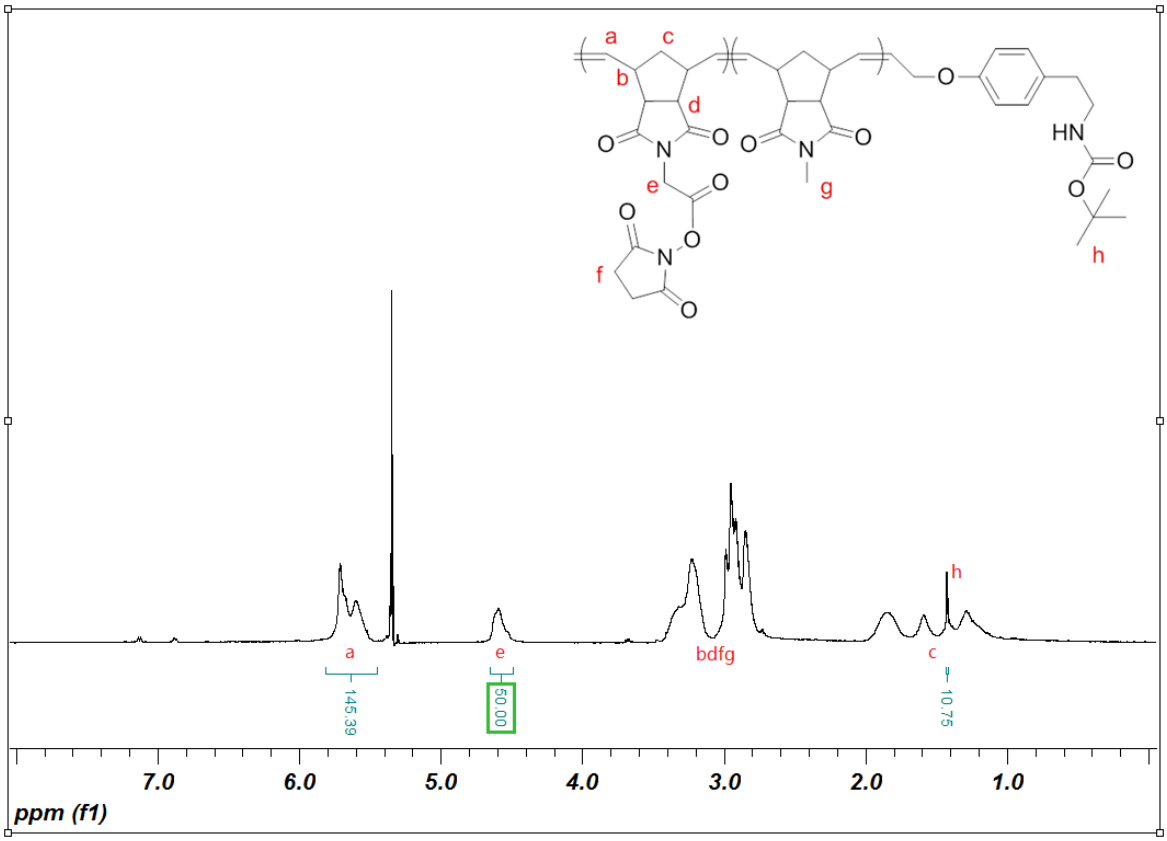


Figure S1. ^{1}H NMR spectrum of poly(activated ester) (Entry 4, Polymer **3** of Table 1 in main text) in CD_2Cl_2.

Calculated integration ratio: a:e:h = 150 **:** 50 **:** 9

Obtained integration ratio: a:e:h = 145.4 **:** 50.0 **:** 10.7

Due to the overlapping of h and c, the integration value of h is larger than expected.

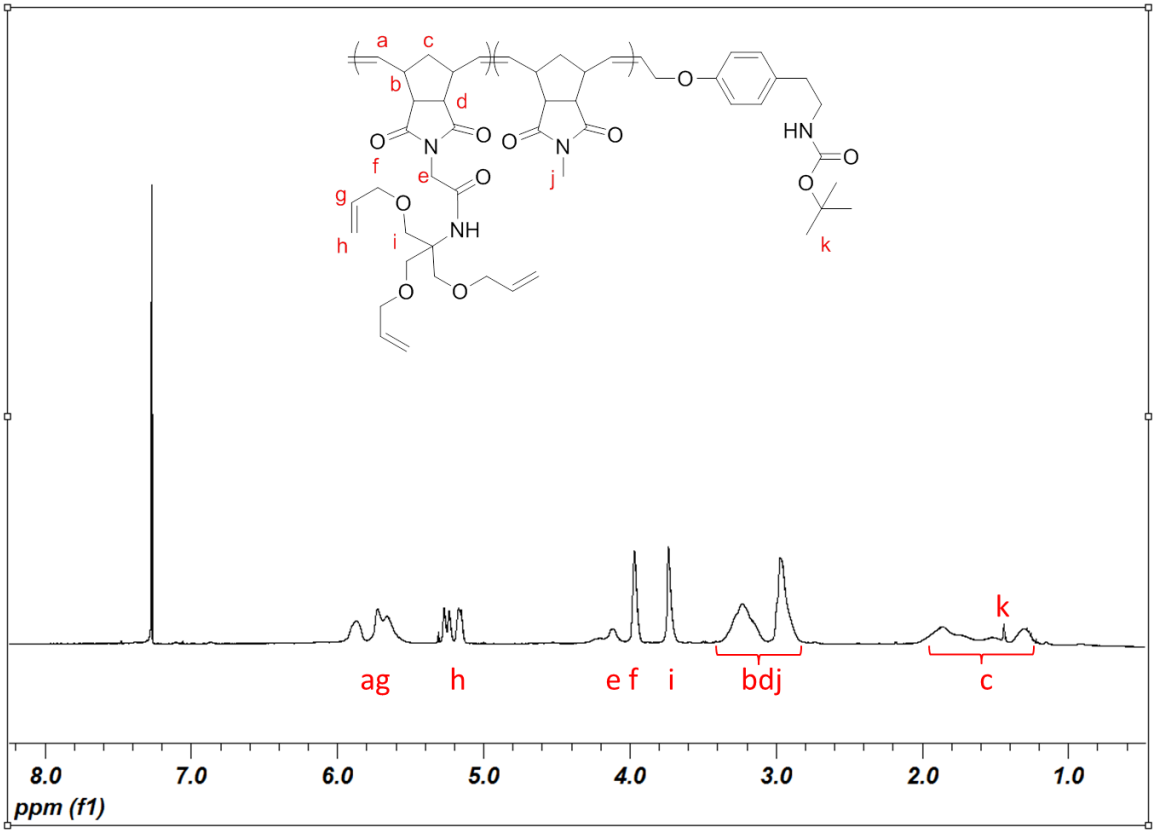


Figure S2. ^{1}H NMR spectrum of allylated polymer (Entry 4, Polymer **5** of Table 1 in main text) in $CDCl_3$.

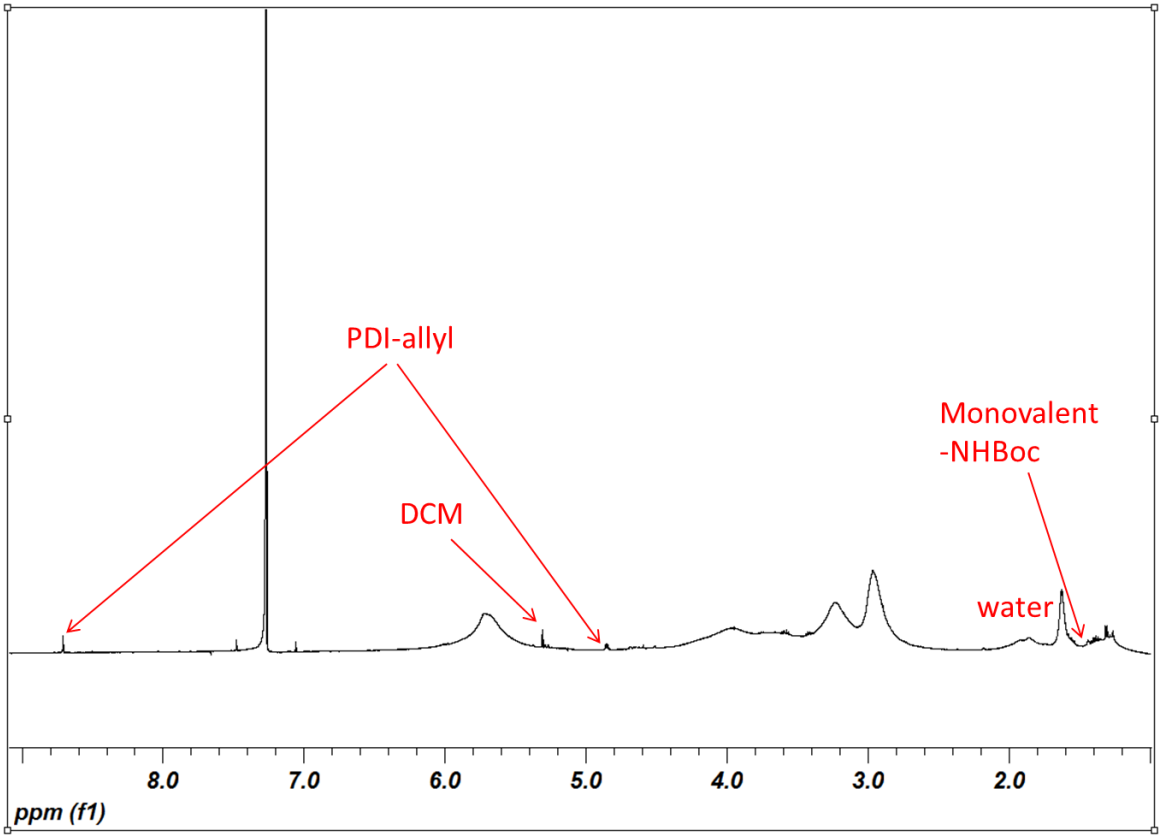


Figure S3. ^{1}H NMR spectrum of intramolecularly crosslinked polymer (Entry 5, ONP **7** of Table 1 in main text) in $CDCl_3$.

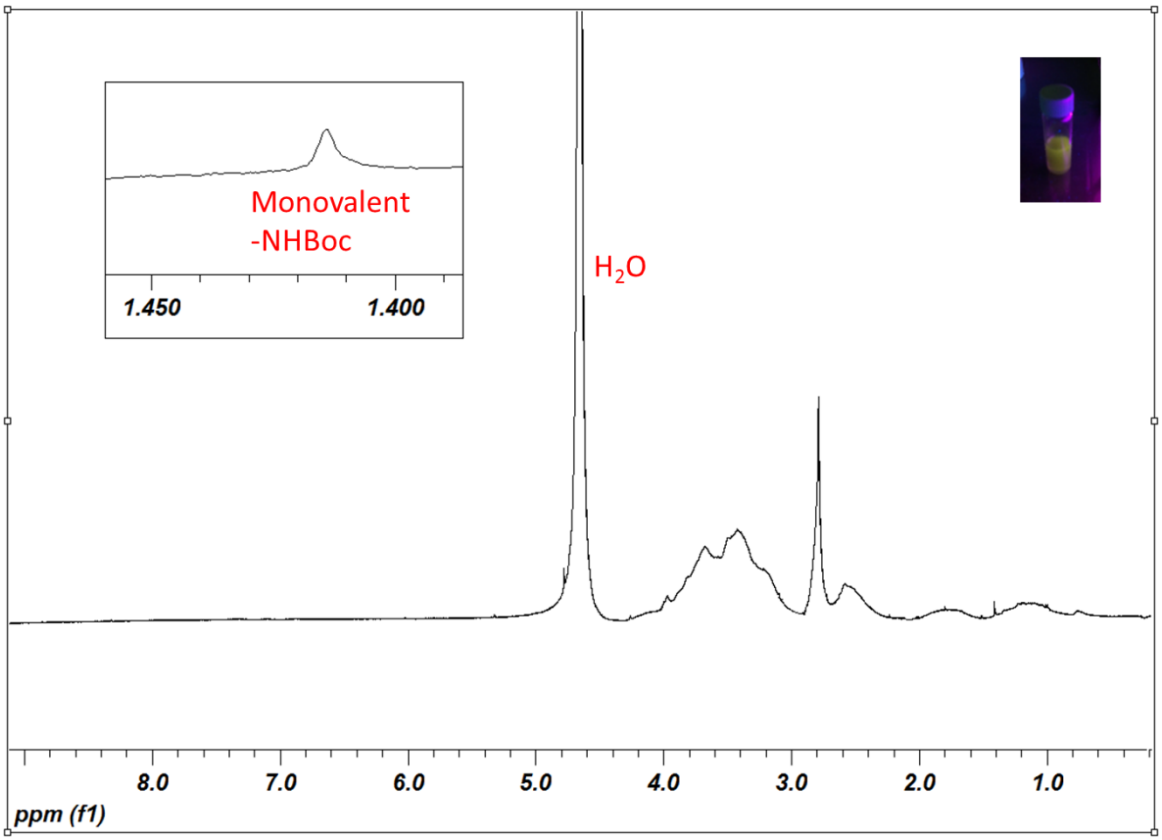


Figure S4. ^{1}H NMR spectrum of water-soluble ONP (dihydroxylated Entry 5, ONP **7** of Table 1 in main text) in D_2O. PDI was not seen in the spectrum presumably because its peaks were too small and broad. The presence of the PDI moieties in the polymer was observed by fluorescence of the aqueous solution under UV irradiation (upper right) which did not diminish during dialysis. Further, the dihydroxylation of PDI-allyl under the same conditions did not give a fluorescent aqueous solution because the product was insoluble in water.

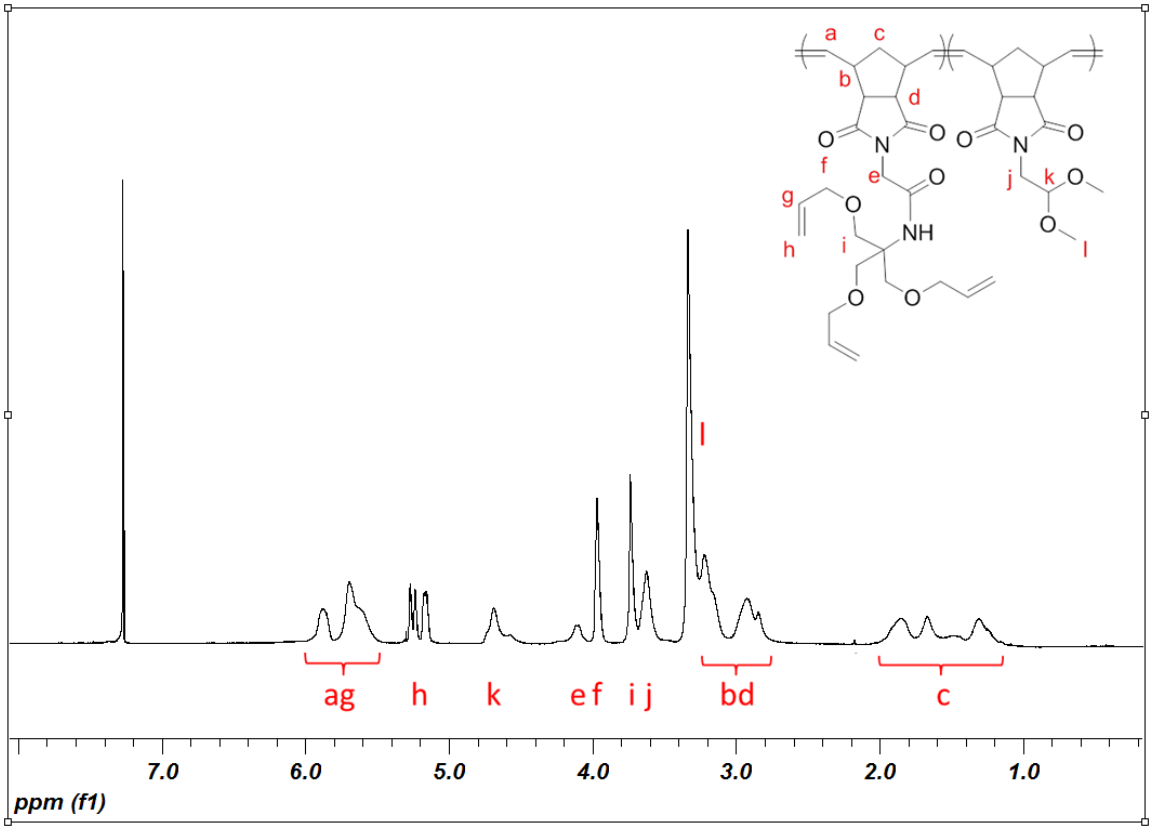


Figure S5. ^{1}H NMR spectrum of aldehyde-functionalized allyl polymer (Entry 7, Polymer **5** of Table 1 in main text) in $CDCl_3$.

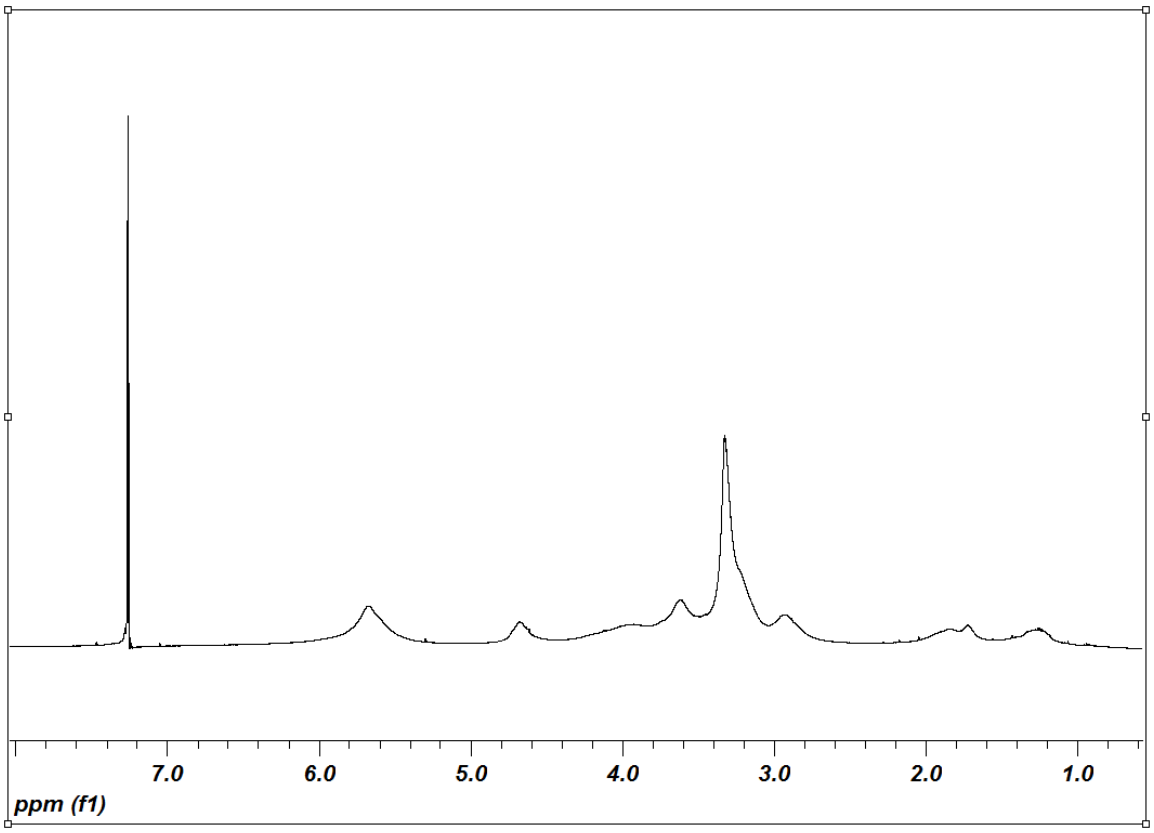


Figure S6. ^{1}H NMR spectrum of aldehyde-functionalized ONP (Entry 7, ONP **7** of Table 1 in main text) in $CDCl_3$.

TEM images of organic nanoparticles

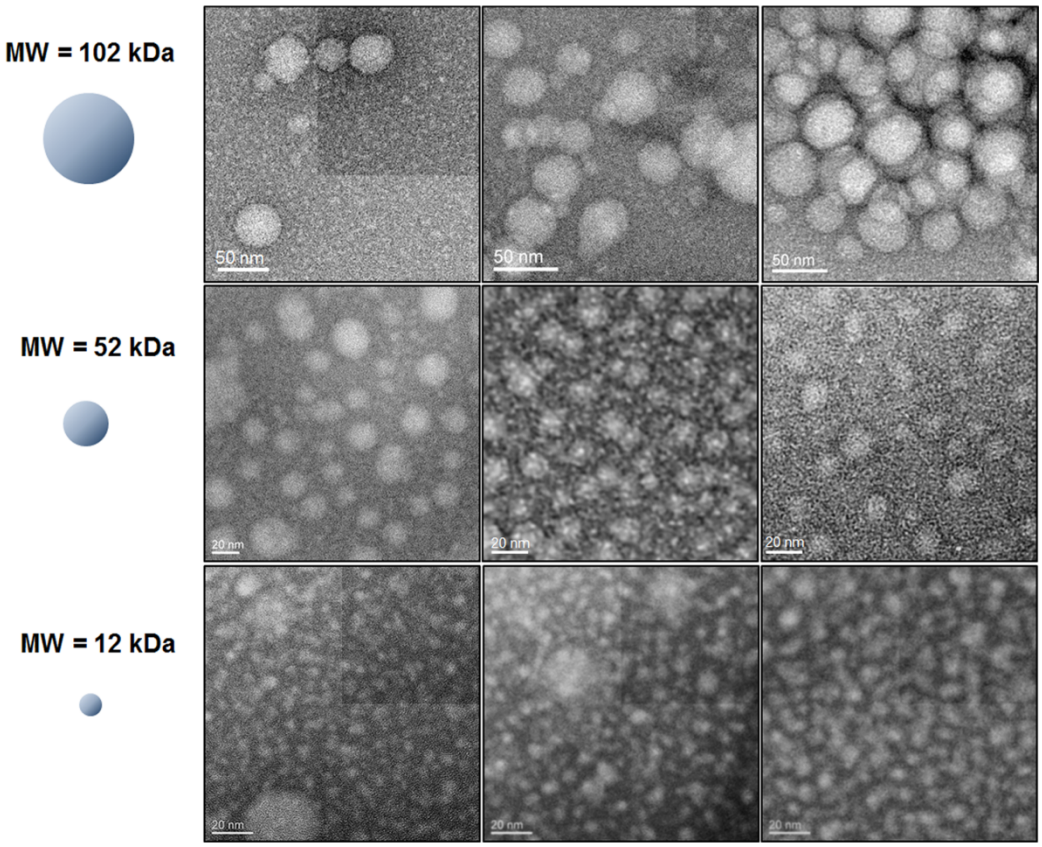
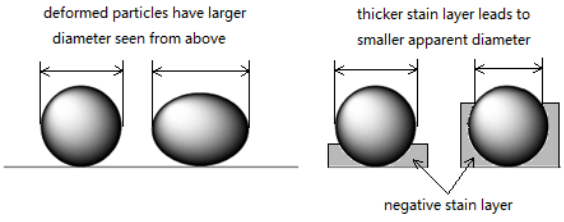


Figure S7. TEM images of organic nanoparticles of different molecular weights.

The organic nanoparticles tend to aggregate when completely dry, especially when they are dried from a more concentrated solution. Aggregation can be clearly seen on the TEM images.



In addition, large particles show broader size distribution on TEM, presumably because: (1) these particles are soft in nature and can easily be deformed. Solution DLS analysis shows less size discrepancy (next page); (2) we had to use negative stain to visualise these small particles with very low density, and the thickness of the stain can affect the size on the images. These two combined could result much broader apparent size distribution on TEM.

Dynamic light scattering (DLS) analysis

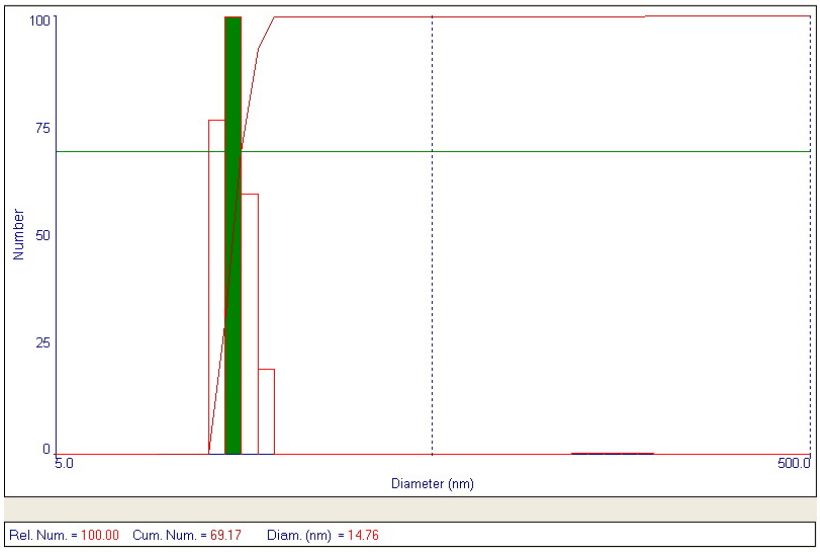


Figure S8. DLS analysis of 52 kDa dihydroxylated organic nanoparticles.

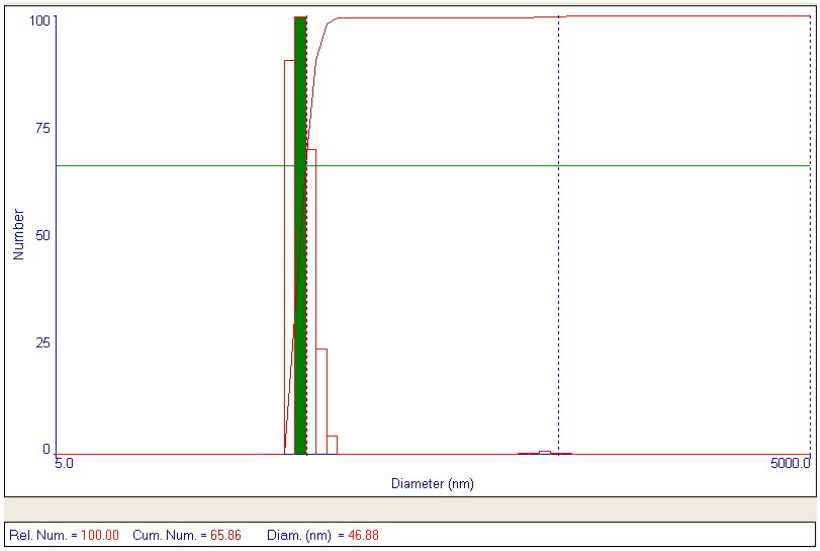


Figure S9. DLS analysis of 102 kDa dihydroxylated organic nanoparticles.

DLS analysis also showed broader size distribution compared to GPC analysis (but better than TEM), mainly because the light scattering signals was quite weak for these small organic particles with very low density, leading to lower accuracy. In fact, we had to do these analyses with quite concentrated samples repetitively to obtain meaningful results.

Additional GPC characterization

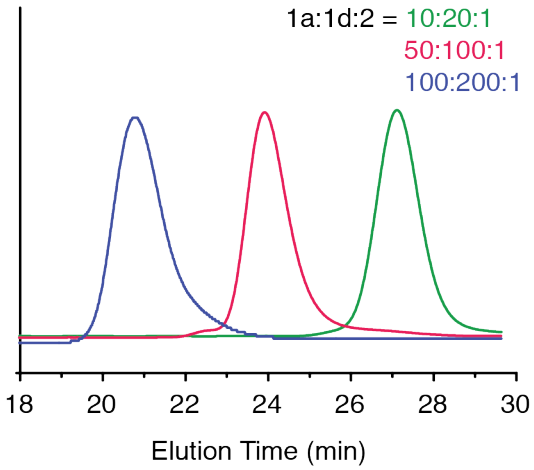


Figure S10. GPC curve overlay of polymers prepared with different M/I ratio.

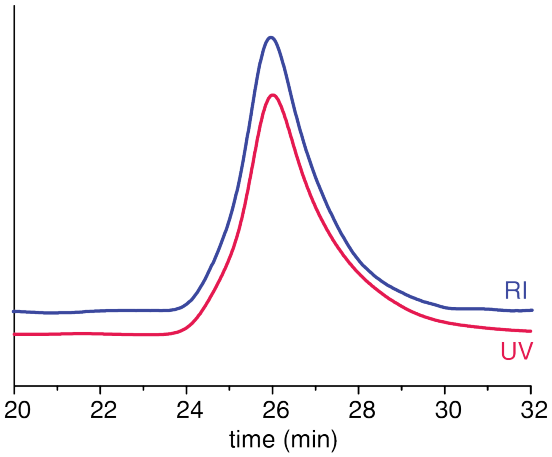


Figure S11. GPC characterization of perylenediimide-functionalized ONP. The RI and UV curve overlay confirmed the loading of the dye in the ONP.

MTT cytotoxicity study

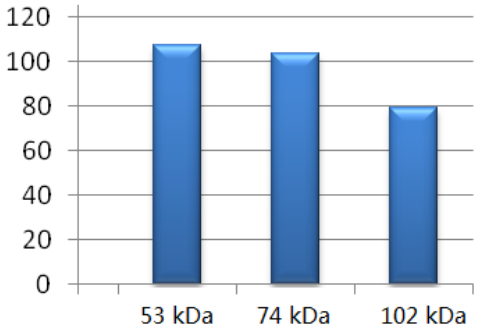


Figure S12. Preliminary results of MTT toxicity study of the organic nanoparticles of different molecular weights. The Y-axis shows % cell viability (see procedure below).

MTT Cell Viability Assay: Cellular toxicity of the organic nanoparticles (ONPs) was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. HeLa cells were seeded into 96-well plates at a density of 2,000 cells/well in DMEM media with 10 % fetal bovine serum (200 µL) and grown in a humidified 5 % CO_2 atmosphere at 37 ^{o}C for 24 h. The media was removed, cells were rinsed with 200 µL of PBS, and incubated for 24 hrs in the presence of ONP (60 µL/well). Next, 25 mg of MTT in 5 mL of PBS was added to each well (20 µL/well) and incubated for an additional 1.5 h protected from light. The solution was removed and the reduced formazan was solubilize with the addition of DMSO (200 µL/well) producing a purple color and mixed for 15 min. The absorbance of each well was read with a fluorescence plate reader at 590 nm. For each experiment, a control of cells that were not incubated with ONPs was also analyzed. All samples were run in quadruplet. The average absorbance for each sample was calculated and percent viability was determined using the following equation: % cell viability = A_{590} treated cells/A_{590} untreated cells × 100.

Reference

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