Electronic Supplementary Information for:

Light-Triggered Unfolding of Single Linear Molecular Bottlebrushes from Compact Globular to Wormlike Nano-Objects in Water

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Experimental Section

Materials. The synthesis of azide-bearing backbone polymer PTEGn3MA (Scheme 1) with a degree of polymerization (DP) of 800 and a degree of azide functionalization of 90% was described in a previous report.1 The $M_n,SEC$ and polydispersity index (PDI) were 313.4 kDa and 1.09, respectively, relative to polystyrene standards. Propargyl 2-bromoisobutyrate (PBiB) was synthesized by the reaction between propargyl alcohol and 2-bromo-2-methylpropionyl bromide in dry methylene chloride in the presence of triethylamine. The product was purified by column chromatography, and the molecular structure was confirmed by $^1$H and $^{13}$C NMR spectroscopy. Alkyne end-functionalized PEO with a molecular weight of 2000 Da (DP = 45) was synthesized from poly(ethylene oxide) monomethyl ether with a molecular weight of 2000 g/mol from Aldrich and 4-pentyloic acid as described previously;1 $^1$H NMR spectroscopy analysis showed that the end group functionalization was complete. Methoxydi(ethylene glycol) acrylate (DEGMA) and o-
nitrobenzyl acrylate (NBA) were synthesized by the reaction of acryloyl chloride (97%, Aldrich) with 2-(2-methoxyethoxy)ethanol (99%, Acros Organics) and o-nitrobenzyl alcohol (97%, Acros), respectively, in the presence of triethylamine (99%, Acros). The products were purified by column chromatography, and their molecular structures were verified by $^1$H and $^{13}$C NMR spectroscopy analysis. $N,N,N',N'',N'''$-Pentamethyldiethylenetriamine (PMDETA, 99%, Acros) was dried with CaH$_2$ and distilled under vacuum. Copper(I) chloride (≥ 99.99%, Aldrich) was used as received. All other chemicals were purchased from either Aldrich or Fisher and used as received.

**General Characterization.** Size exclusion chromatography (SEC) of PTEGN$_3$MA backbone polymer and all molecular brushes was performed at 25 ℃ on PL-GPC 50 Plus (Polymer Laboratories, Inc.), consisting of a differential refractive index detector, one PLgel 10 µm guard column (50 × 7.5 mm, Agilent), and three PLgel 10 µm Mixed-B columns (each 300 × 7.5 mm, molecular weight range from 500 to 10,000,000 Da, Agilent). $N,N$-Dimethylformamide (DMF) containing 50 mM LiBr was used as solvent at a flow rate of 1.0 mL/min. The data were processed with Cirrus™ GPC/SEC software (Polymer Laboratories). SEC of alkyne end-functionalized PEO, P(DEGMA-co-NBA), and PNBA side chain polymers was carried out using a PL-GPC 20 integrated GPC/SEC system from Polymer Laboratories equipped with a refractive index detector, one PLgel 5 µm guard column (50 × 7.5 mm, Agilent Technologies), and two PLgel 5 µm Mixed-C columns (each 300 × 7.5 mm, linear range of molecular weight from 200 to 2,000,000 Da, according to Agilent). THF was the carrier solvent and the flow rate was set at 1.0 mL/min for the molecular weight and PDI analysis. Both SEC systems were calibrated with near-monodisperse linear polystyrene standards (Polymer Laboratories, Inc.). $^1$H and $^{13}$C NMR spectroscopy analysis was performed using either a Varian Mercury 300 or a Varian VNMRS 500 NMR spectrometer. FT-IR spectra were recorded on a Varian 4100 FT-IR Excalibur Series spectrometer.
Synthesis of Dual Thermo- and Light-Responsive Side Chain Polymer P(DEGMA-co-NBA) by ATRP. Propargyl 2-bromoisobutyrate (59.0 mg, 0.288 mmol), DEGMA (5.015 g, 28.8 mmol), NBA (0.681 g, 3.29 mmol), CuCl (28.6 mg, 0.289 mmol), anisole (5.693 g), and PMDETA (0.121 g, 0.698 mmol) were added into a 25 mL two-neck round bottom flask equipped with a magnetic stir bar. The mixture was degassed by three freeze-pump-thaw cycles and placed in an 80 °C oil bath. After 21 h and 30 min, the reaction mixture was taken from the oil bath, opened to air, and passed through a neutral alumina/silica gel column to remove the copper catalyst. The polymer was purified by precipitation in hexanes three times and dried under high vacuum to obtain a light yellow, viscous polymer (1.569 g). The numbers of DEGMA and NBA monomer units in the copolymer were 42 and 5, respectively, giving a total DP of 47, determined by end group analysis from the ¹H NMR spectrum, using the integrals of the peaks at 4.62 ppm (HC≡CCH₂OOC- of the alkyne end group), 4.02-4.26 ppm (-COOCH₂- of DEGMA monomer units), and 5.33-5.56 ppm (-COOCH₂- of NBA monomer units). SEC results (THF as solvent): $M_n,_{SEC} = 8,800$ Da; PDI = 1.17.

Synthesis of Light-Responsive PNBA Side Chain Polymer. Propargyl 2-bromoisobutyrate (49.9 mg, 0.243 mmol), NBA (5.846 g, 28.2 mmol), CuCl (25.1 mg, 0.254 mmol), anisole (5.927 g), and PMDETA (71.1 mg, 0.410 mmol) were weighed into a 25 mL two-necked round bottom flask. The mixture was degassed by three freeze-pump-thaw cycles and placed in an 80 °C oil bath. The polymerization was monitored by ¹H NMR spectroscopy and SEC. After 9 h, the flask was removed from the oil bath and opened to air. The polymerization mixture was passed through an alumina/silica gel column to remove the catalyst. The polymer was purified by precipitation in diethyl ether four times and dried under high vacuum, yielding a light yellow, powdery polymer (1.217 g). The DP was calculated to be 36 by end group analysis from the ¹H NMR spectrum,
using the integrals of the peaks at 4.54 ppm (HC≡CCH2OOC- of the alkyne end group) and 5.09-
5.45 ppm (-COOCH2- of NBA monomer units). The results of SEC analysis using PL GPC-20
system with THF as carrier solvent: $M_{n,\text{SEC}} = 4,800$ Da; PDI = 1.29.

**Synthesis of Thermo- and Light-Responsive Homografted P(DEGMA-co-NBA) Molecular Brushes.** PTEGN3MA (5.28 mg, 0.0217 mmol monomer units, from a stock solution in THF) was added into a 3.7 mL vial containing a stir bar. THF was evaporated off with a stream of nitrogen, and DMF (0.5 mL) was added. P(DEGMA-co-NBA) (350.3 mg, 0.0412 mmol alkyne end groups) was weighed into a vial, dissolved in DMF (2.0 mL), and transferred to the vial containing PTEGN3MA. CuCl (5.4 mg, 0.055 mmol) was added, and a rubber septum was used to seal the reaction vial. The mixture was flushed with nitrogen via needles for 10 min before PMDETA (5.0 µL, 0.024 mmol) was injected using a microsyringe. The reaction progress was monitored by SEC. After 40 h, propargyl alcohol (100 µL, 1.72 mmol) was injected via a microsyringe under nitrogen to attempt to cap unreacted backbone azide units. The mixture was stirred for additional 4 h before the reaction mixture was opened to air, diluted with methylene chloride, and passed through a short neutral alumina/silica gel column to remove the catalyst. The unreacted side chains were removed by fractionation in a mixture of THF (good solvent) and hexanes (poor solvent) five times to yield a light yellow, viscous polymer (0.082 g). The grafting density, defined as the percentage of backbone monomer units that are grafted with a side chain polymer, was determined to be 79.6 % by comparison of the brush and the unreacted side chain polymer peak areas from SEC chromatogram of the reaction mixture at the end of the reaction. The results of SEC analysis of the purified brushes using PL-GPC 50 Plus system with DMF containing 50 mM LiBr as carrier solvent: $M_{n,\text{SEC}} = 1,380,800$; PDI = 1.14.
Synthesis of Light-Responsive Heterografted PEO/PNBA Molecular Brushes.

PTEGN₃MA (6.27 mg, 0.0258 mmol monomer units, from a stock solution in THF) was added into a 3.7 mL vial equipped with a stir bar. THF was evaporated off with a stream of nitrogen, and DMF (0.5 mL) was added. PEO (57.4 mg, 0.0273 mmol alkyne end groups) and PNBA (138.4 mg, 0.0182 mmol alkyne end groups, from a stock solution in THF) were weighed into a separate vial. The THF was evaporated off with a stream of nitrogen, DMF (2.0 mL) was added, and the side chain polymers were transferred to the vial containing PTEGN₃MA. CuCl (3.9 mg, 0.039 mmol) was added, and a rubber septum was used to seal the reaction vial. The mixture was flushed with nitrogen via needles for 15 min before PMDETA (5.0 µL, 0.024 mmol) was injected. The reaction was followed by SEC. After 41 h 20 min, propargyl alcohol (50 µL, 0.86 mmol) was injected under N₂ to attempt to cap unreacted azide units. The mixture was stirred for another 3 h 10 min, and then the mixture was opened to air, diluted with CH₂Cl₂, and passed through a short neutral alumina/silica gel column to remove the catalyst. Unreacted side chain polymers were removed by fractionation twice using THF as a good solvent and hexanes as a poor solvent to yield a light yellow, powdery polymer (39.2 mg). The grafting density was determined to be 77.2 % by comparing the brush and the unreacted side chains peak areas from SEC chromatogram of the reaction mixture at the end of the reaction. The results of SEC analysis of the purified brushes using PL-GPC 50 Plus system with Agilent Mixed-B columns in DMF containing 50 mM LiBr as carrier solvent: $M_n,_{SEC} = 933,300$; PDI = 1.16. The molar ratio of PEO to PNBA side chains in the purified brush was 0.62 : 0.38, determined from the $^1$H NMR spectrum using the integrals of the peaks at 5.18-5.45 ppm (-COOC₂H₅- of NBA units) and 3.55-3.68 ppm (-OC₂H₅CH₂- of PEO units).

Preparation of Collapsed Globular Nano-Objects of Heterografted PEO/PNBA Molecular Brushes in Water. An aqueous solution of collapsed PEO/PNBA binary heterografted
molecular brushes with a concentration of 0.1 mg/g was prepared by dropwise addition of a 1.0 mg/mL solution of brushes in DMF (1.0 mL) into rapidly stirred Milli-Q water (10 mL). The brush solution was dialyzed against pure Milli-Q water using a 3500 Da MWCO dialysis membrane to remove DMF, and the brush concentration was adjusted to 0.1 mg/g by the addition of Milli-Q water. The mixture was passed through a 0.2 μm PTFE filter prior to analysis.

Dynamic Light Scattering (DLS) Study of Homografted P(DEGMA-co-NBA) and Binary Heterografted PEO/PNBA Molecular Brushes Under Various Conditions. DLS studies were carried out using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. The reported DLS sizes in this work were the intensity mean diameters. An aqueous solution of thermo- and light-responsive P(DEGMA-co-NBA) molecular brushes with a concentration of 0.2 mg/g for DLS measurements were prepared by dissolving the brush molecules in Milli-Q water in an ice/water bath followed by storage in a refrigerator (4 °C) for at least one night to ensure complete dissolution. The temperature was gradually increased, and at each selected temperature the sample was equilibrated for 3 min. The sample in a quartz cuvette was then irradiated with 365 nm UV light from a Spectroline ENF-240C hand-held UV lamp (equipped with a 4 watt long wavelength tube filtered at 365 nm) at 30 °C for 70 min in a thermostated water bath. The increase in size with irradiation time was monitored by DLS at 30 °C at 10 min intervals until no further change in size was observed. After equilibrating at 30 °C in dark overnight, the sample was cooled to 1 °C, and the temperature was then gradually increased; the size at each selected temperature was measured by DLS after equilibration for 3 min.

For heterografted PEO/PNBA molecular brushes, DLS was performed using a 0.2 mg/g brush solution in DMF and also 0.1 mg/g aqueous solutions in Milli-Q water obtained by the procedure
in the above section. A portion (2 mL) of the 0.1 mg/g aqueous solution in a 3.7 mL vial was then irradiated with 365 nm UV light from a Spectroline ENF-240C hand-help UV lamp (equipped with a 4 watt long wavelength tube filtered at 365 nm) at ambient temperature for 24 h. DLS measurements were performed at 25 °C.

**Atomic Force Microscopy (AFM) Study of Molecular Brushes.** AFM was performed using a Digital Instruments Multimode IIIa Scanning Probe Microscope operated in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a nominal resonant frequency of 300 kHz and force constant of 40 N/m were used. Aqueous solutions of P(DEGMA-co-NBA) brushes with a concentration of 0.02 or 0.05 mg/g in Milli-Q water for AFM imaging were drop cast onto polystyrene-coated mica inside a covered dish containing a drop of Milli-Q water (for slow evaporation) in either a refrigerator (4 °C) or an oven (30 °C), depending on the desired casting temperature. P(DEGMA-co-AA) brush samples (obtained from UV irradiation of P(DEGMA-co-NBA) brushes) for AFM were prepared by either drop casting (30 °C sample) or spin coating (65 °C sample, 3k rpm) from a 0.02 mg/g aqueous solution onto polystyrene-coated mica. Polystyrene-coated mica was prepared by spin coating a solution of 1.0 wt % polystyrene (30 kDa) in chloroform onto freshly cleaved mica at 10,000 rpm. For the PEO/PNBA brush samples, aqueous solutions before (0.1 mg/g in Milli-Q water) and after UV irradiation (0.05 mg/g in 2 mM KH2PO4 solution at pH = 7.06) were either drop cast or spin coated at 3,000 rpm onto freshly cleaved bare mica. A solution of PEO/PNBA brushes was also prepared in chloroform with a concentration of 0.01 mg/g and spin coated onto freshly cleaved mica for AFM imaging. The quantitative image analysis was done with ImageJ software. For the wormlike morphology, at least 100 brush molecules were measured. For the collapsed globular morphology, 26 to 52 nano-objects were measured.
Calculation of Grafting Density of Molecular Bottlebrushes. The following is the calculation of grafting density for P(DEGMA-co-NBA) HMB after 44 h reaction time using the molar ratio of backbone monomer units to the side chain polymer in the feed and the ratio of peak areas for the brushes and the unreacted side chains from SEC analysis. The feed contained 5.28 mg of PTEGN₃MA backbone polymer (monomer unit molar mass = 243.27 g/mol, assuming 100 % functionalization with azide) and 350.3 mg of P(DEGMA-co-NBA) side chain polymer (DP_{DEGMA} = 42; DP_{NBA} = 5) with an absolute molecular weight of 8,500 Da (found by 42 \times 174.20 + 5 \times 207.19 + 160.60). For 100 % grafting density, 5.28 mg PTEGN₃MA can react with 184.5 mg of P(DEGMA-co-NBA) to give 189.8 mg brushes. After 44 h reaction time, we found from SEC that the reaction mixture contained 42.8 % brushes by mass using peak areas. The reaction mixture therefore contained (0.428) \times (355.58 \text{ mg total polymer}) = 152.19 \text{ mg brushes}. This means that 146.9 mg of side chains have reacted (found by 152.19 mg brushes – 5.28 mg backbone). This gives a grafting density of (146.9 \text{ mg side chains reacted})/(184.5 \text{ mg theoretical}) = 79.6 %. Note that we have previously confirmed that the peak areas in SEC are proportional to the masses of polymers.¹ The grafting density of heterografted PEO/PNBA molecular brushes was calculated by the same method using the assumption that the molar ratio of PEO and PNBA side chains is the same in the brushes as in the feed.

**Figure S1.** (A) SEC trace of P(DEGMA-co-NBA) side chain polymer with a total DP of 47 and (B) $^1$H NMR spectrum of P(DEGMA-co-NBA) in CDCl$_3$. SEC analysis was carried out on PL GPC-20 system using THF as carrier solvent.

**Figure S2.** (A) SEC trace of side chain polymer PNBA with a DP of 36 and (B) $^1$H NMR spectrum of PNBA in CDCl$_3$. SEC analysis was carried out on PL GPC-20 system using THF as carrier solvent.
Figure S3. Size distributions by intensity from DLS measurements of solutions of P(DEGMA-co-NBA) molecular brushes HMB in Milli-Q water with different polymer concentrations at (A) 1 °C, (B) 30 °C, and (C) 45 °C.
Figure S4. Additional AFM height images of P(DEGMA-co-NBA) molecular brushes HMB drop cast onto polystyrene-coated, freshly cleaved mica from an aqueous solution at 30 °C with a polymer concentration of 0.02 mg/g.

Figure S5. Additional AFM height images of P(DEGMA-co-AA) molecular brushes (obtained by irradiation of P(DEGMA-co-NBA) molecular brushes with 365 nm UV light at 30 °C for 70 min) drop cast onto polystyrene coated, freshly cleaved mica from an aqueous solution with a polymer concentration of 0.02 mg/g in a 30 °C oven.
Figure S6. Additional AFM height images of P(DEGMA-co-AA) molecular brushes (obtained by irradiation of P(DEGMA-co-NBA) HMB with 365 nm UV light at 30 °C for 70 min) spin cast (3k rpm) onto polystyrene coated mica from an aqueous solution at 65 °C with a polymer concentration of 0.02 mg/g.

Figure S7. Additional AFM height images of PEO/PNBA BMB spin coated onto freshly cleaved bare mica from a 0.01 mg/g solution in chloroform.
Figure S8. Additional AFM height images of PEO/PNBA BMB drop cast onto mica from a 0.1 mg/g solution in water obtained by solvent switching from DMF followed by dialysis against pure water.

Figure S9. Additional AFM height images of PEO/PAA BMB (obtained by irradiation of PEO/PNBA BMB with 365 nm UV light for 22 h) spin coated onto mica from an aqueous 2 mM KH$_2$PO$_4$ solution at pH = 7.06 with a polymer concentration of 0.05 mg/g.
Figure S10. FT-IR spectra of (A) PEO/PNBA BMB before UV irradiation and (B) PEO/PAA BMB (obtained by irradiation of PEO/PNBA BMB) drop cast from THF solutions (~1 mg/mL) onto NaCl plates followed by evaporation of THF with a gentle stream of nitrogen.