Supporting Information

Functionalization-Induced Self-Assembly Under Ambient Conditions via Thiol-Epoxide "Click" Chemistry

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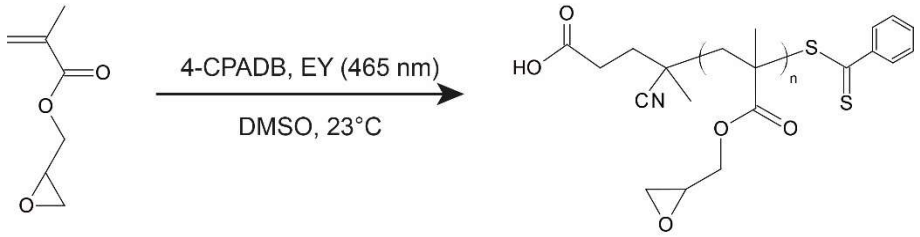
1. Experimental procedures, analytics, and methods

1.1 Reagents

All reagents were purchased from Sigma-Aldrich and used as received unless otherwise noted. Glycidyl methacrylate (GMA, 97 %, inhibited with 100 ppm 4-methoxyphenol (MEHQ)) and poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 300 g/mol, inhibited with 100 ppm MEHQ and 300 ppm butylated hydroxytoluene (BHT)) were filtered through activated basic alumina columns (60 mesh powder, Alfa Aesar) prior to use. Other compounds employed were dimethyl sulfoxide (DMSO, ≥ 99.9 % ACS reagent), tetrahydrofuran (THF, ≥ 99.9 % HPLC grade, Chromasolv), diisopropyl ether (DIE, ≥ 99 %, stabilized with BHT, Macron), chloroform-d ($CDCl_3$, 99.8 % (isotopic), containing 0.03 % v/v trimethylsilane Alfa Aesar), ethanol (reagent grade), 4-cyanopentanoic acid dithiobenzoate (4-CPADB, Aldrich), 2-naphthalenethiol (99 %, Alfa Aesar), thiophenol (97 %, VWR), 2,3,4,5,6-pentafluorothiophenol (97 %, Alfa Aesar), triphenylmethanethiol (97 %, TCI Chemicals), eosin Y (EY, 99 %, Aldrich), and potassium hydroxide (KOH, ACS grade pellets, Fisher Chemical).

1.2 PET-RAFT polymerization of GMA

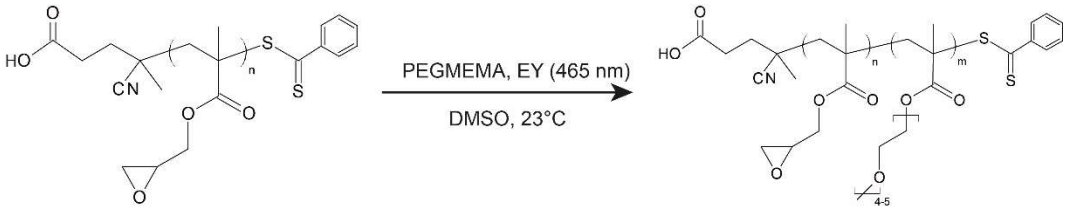


Formulation: A representative reaction formulation is as follows: Glycidyl methacrylate (GMA)|chain transfer agent (CTA)|eosin Y (EY) at a molar ratio of 40|1|0.004 with a monomer weight fraction of 40 %. For example, this resulted in 2.007 g GMA (14.11 mmol) together with 0.0982 g CPADB (0.351 mmol) and 0.00091 g EY (0.00140 mmol). The total DMSO used was 2.91 g to achieve the targeted weight fraction of monomer.

Preparation: The CTA and GMA were charged into a 20 mL scintillation vial under ambient conditions. EY was added from a stock solution made of 2 mg EY dissolved in approximately 5 g of DMSO. Additional DMSO was added to the reaction solution to obtain the targeted monomer weight fraction. The scintillation vial was covered with aluminum foil to protect against external irradiation and homogenized for 30 minutes using a vortex mixer (Vortex Genie 2, Scientific Industries). The reaction mixture was transferred into a 10 mL Schlenk flask with a stir-bar and then sealed with a rubber septum. The solution was purged under nitrogen for 30 minutes, during which the vessel was covered with aluminum foil. Blue LED stripes

(Mouser Electronics, SB-0465-CT Inspired LED, λ_{max} = 465 nm, 10.8 W) were attached on the outer wall of a 500 mL beaker inside of which was a 250 mL beaker to hold the Schlenk flask and water for cooling to prevent overheating by the LEDs. The setup was covered with a cardboard box for safety and to reduce irradiation exposure. The polymerization was allowed to stir for 24 hours. Kinetic samples were taken during the reaction with a nitrogen-purged syringe. The mixture was exposed to air after completion and purified by precipitation three times from THF into DIE, with product isolation aided by centrifugation using an Eppendorf 5702 Centrifuge at 4000 rpm for 5 minutes. Final samples were dried overnight in a vacuum oven. The poly(GMA) used for subsequent chain extensions and FISA experiments had a M_n = 5,645 g/mol and a $Đ$ = 1.16.

1.3 Chain extension of poly(GMA) to form poly(GMA-*b*-PEGMEMA)



Formulation: A representative chain extension reaction with PEGMEMA was formulated as follows: PEGMEMA|poly(GMA)|EY = 120|1|0.006 with a monomer weight fraction of 20 % from 2.184 g monomer (7.279 mmol), 0.301 g poly(GMA) (0.0570 mmol), 0.000249 g EY (0.000384 mmol), and 8.441 g DMSO.

Preparation: The preparation steps follow the procedure outlined previously (see section 1.2). The reaction solution was purged for 40 minutes under nitrogen. The chain extension was held for 6 hours. After purification, the produced batch was stored in the freezer prior to FISA. The poly(GMA-*b*-PEGMEMA) used for subsequent FISA experiments had a M_n = 27,175 g/mol and a $Đ$ = 1.20.

1.4 Thiol-epoxide FISA reactions of poly(GMA-*b*-PEGMEMA)

Formulation: All reactions were conducted in an ethanol solution under an epoxide:thiol ratio of 1:1.25 and a catalyst mole fraction of 10 % (moles KOH to moles of epoxide and thiol), with exception of real-time reactions, described in section 1.5. A representative thiol-epoxide reaction formulation (for Table 1, Entry 2) is described as follows: 0.0334 g of block copolymer (corresponding to 0.043 mmol of epoxide groups) and 0.0087 g 2-naphthalenethiol (0.054 mmol) were dissolved in approximately 0.50 g of ethanol. Next, a stock solution of ethanol containing 0.00055 g KOH (0.0097 mmol) and the remaining ethanol needed to reach a polymer concentration of 3 wt% (0.57 g EtOH) was added dropwise to commence FISA.

Preparation: A representative FISA process was conducted as follows: p(GMA-*b*-PEGMEMA) and the thiol were dissolved in ethanol using a stir-bar. Next, a stock solution of KOH in ethanol, was added dropwise. The reaction was then allowed to stir at room temperature for 20 hours prior to DLS and TEM analysis. Following this, the samples were purified via one precipitation from THF into DIE and dried overnight in a vacuum oven prior to NMR analysis.

1.5 "Real-time" FISA of poly(GMA-*b*-PEGMEMA) with 2-naphthalenethiol

Formulation: The experiment was carried out with a BCP weight fraction of 2.5 %, a 1.25 molar excess of thiol to epoxy, and a catalyst loading of 2 mol%. A representative real-time thiol-epoxide FISA formulation (Table 1, Entry 4) is described as follows: 0.0432 g of block copolymer (corresponding to 0.056 mmol of epoxide group) and 0.0113 g 2-naphthalenethiol (0.070 mmol) were dissolved in approximately 0.50 g of ethanol. Next, a stock solution of ethanol containing 0.00014 g KOH (0.0025 mmol) and the remaining ethanol needed to reach a polymer concentration of 2.5 wt% (1.22 g EtOH) were added dropwise to commence the reaction.

Preparation: The procedure follows the FISA reaction procedure described in section 1.4, except that immediately upon mixing the KOH stock with the solution of thiol and BCP, the mixture was transferred to a quartz cuvette and placed inside the DLS instrument. Additionally, kinetic samples were taken at time intervals typically of ~20 minutes and placed in separate vials containing ~3 drops of 3-bromo-1-propanol (~0.461 mmol) as a quenching agent. Upon collection of all kinetic samples, impurities were extracted in DIE and subsequent centrifugation allowed collection of the purified polymer from the supernatant. The kinetic samples were then dried in a vacuum oven prior to NMR analysis.

1.6 Size exclusion chromatography in THF (SEC-THF)

Preparation: Samples for size exclusion chromatography in tetrahydrofuran (SEC-THF) were prepared by dissolving 1 drop (ca. 15 - 20 mg) of crude reaction mixture in 1 mL of THF. The solution was homogenized via vortex mixing for ~3 minutes and filtered through a 0.20 µm filter (Millex-LG) into a 1.8 mL screw-thread vial.

Measurement: Information related to relative number-averaged molecular weight (M_n), weight-averaged molecular weight (M_w), and dispersity ($Đ$) were derived with a Shimadzu LC-20AD HPLC pump equipped with a Shimadzu RID-20A 120V refractive index detector using HPLC grade tetrahydrofuran as the mobile phase. Polymer analytes were separated at a flow rate of 1 mL/min at 35 °C using two PLgel mixed-B Agilent columns connected in series and calibrated against poly(methyl methacrylate) standards.

1.7 Nuclear magnetic resonance (NMR)

1.7.1 ^{1}H-NMR sample preparation and acquisition

Preparation: Proton nuclear magnetic resonance (^{1}H-NMR) samples of homopolymerizations and chain extensions were prepared by dissolving 2-3 drops (ca. 30-40 mg) of crude reaction mixture in 1 mL of $CDCl_3$ containing 0.03% v/v TMS (or, in the case of purified samples, dissolving approximately 10 mg of purified sample in 1 mL of the $CDCl_3$). All dissolved samples were homogenized via vortex mixer for 5-10 minutes and transferred into 5 mm precision NMR sample tubes.

Measurement: Conversions, as well as chemical structure analysis, were determined by using a Varian Unity Inova-500 MHz spectrometer (Varian, USA) at 25 °C. Typically, 128 scans were taken for each sample with a relaxation delay of 1 second. Chemical peak shifts were analyzed against a TMS standard peak at 0 ppm.

1.7.2 Monomer conversion calculations for poly(GMA)

Monomer conversion was calculated using equation 1 by comparing monomer and side-chain CH_2 resonances[1] as follows:

$$monomer\ conversion\ [\%] = \frac{\sum I_{c'}}{\sum I_{c'} + \sum I_c} * 100\% \qquad (1)$$

with $\Sigma I_{c'}$ being the integral sum of peaks at 3.8 and 4.3 ppm associated with the CH_2 proton signals of the p(GMA) side chain, and ΣI_c is the sum of integral values for peaks at 4.0 and 4.5 ppm from the monomer. The corresponding ^{1}H-NMR spectrum can be found below (Figure S1.).

1.7.3 Monomer conversion calculations for poly(GMA-*b*-PEGMEMA)

Monomer conversion was calculated using equation 2, employing the same concept used to calculate conversions for the homopolymer.

$$monomer\ conversion\ [\%] = \frac{I_{C'}}{I_{C'} + \sum I_D} * 100\% \quad (2)$$

$I_{C'}$ is the integral value of CH_2 (C′) protons (4.09 ppm) next to the oxygen of the ester group in the PEGMEMA side chain in the NMR spectrum of the final reaction solution. ΣI_D is the sum of the integrated peaks originating from the residual vinylic CH_2 (D) (5.58 and 6.13 ppm) protons of the monomer species in the NMR spectrum of the final reaction solution. Peak assignments are given in Figure S2.

1.7.4 Epoxide conversion calculations for post-polymerization modification of poly(GMA-*b*-PEGMEMA)

Epoxide conversion calculations were typically referenced to the PEGMEMA peak at 3.3-3.45 ppm for the purified poly(GMA-*b*-PEGMEMA) sample, labeled as "f" in Figure S3. Comparing the integrals of the three epoxide resonances from 2.5 ppm to 3.4 ppm (labeled as "a" and "b") to the reference PEGMEMA peak integral in the precursor establishes a point of comparison (e.g., when the PEGMEMA peak is set to 100, the typical epoxy peak integral value is equal to 10.38). This ratio allows quantification of the gradual disappearance of the identified epoxide samples in some instances as well as quantification of emergent peaks associated with protons of the functionalized product. For the majority of reactions, a CH_2 resonance at 3.0 ppm associated with the epoxide adduct can be easily normalized to the PEGMEMA peak at 3.3-3.45 ppm and quantification of this emergent peak provides a convenient handle for quantifying %*f*. The exception to this strategy was the triphenylmethanethiol adduct (depicted in Figure S9), for which the CH_2 peak was not visible. Instead, the aromatic region (~7–8 ppm) associated with the 15 new protons of the adduct was used to calculate %*f*, which required subtraction of the residual chloroform peak at 7.27 ppm from the remainder of the aromatic region peak integral.

1.7.5 Reaction quenching for kinetic ^{1}H-NMR sampling of real-time FISA reactions

Real-time FISA reactions, described in the main text, were kinetically sampled for ^{1}H-NMR analysis. Initially, it was found that the kinetically sampled aliquots for these real-time reactions continued to react after sampling so each time point converged to ~100 %*f* by the time ^{1}H-NMR analysis was carried out. However, by introducing an excess of 3-bromo-1-propanol as a strong electrophile for the preferential consumption of the thiolate nucleophile in the kinetic samples, it was possible to freeze these aliquots at approximately the %*f* at the time of their sampling. Quenching efficacy is demonstrated by the comparison of quenched and unquenched kinetic samples from equivalent reactions seen in Figure S7.

1.8 Dynamic light scattering (DLS)

1.8.1 DLS sample preparation

Samples for DLS were prepared either by transferring the raw reaction solution to a quartz cuvette, in the case of direct FISA observations, or by dissolving a purified polymer sample in a sufficient quantity of solvent required to meet a specified wt% and vortexing for 5-10 minutes.

1.8.2 DLS measurements

The hydrodynamic diameters (D_H) and polydispersity index (PDI) for FISA products and precursors were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments, USA). Measurements were carried out in a quartz cuvette with a 632 nm laser at a 173° backscattering configuration and a sample temperature of 25 °C. Each data point represents an average of 8-15 measurements, each measurement lasting for 8-10 seconds. The final D_H was calculated by taking the mean of three to five data points. For real-time FISA experiments, the D_H was continuously measured with delay times of 90 seconds in between each set of 8-15 measurements. The solvent settings for each measurement were selected from pre-programmed options containing solvent refractive indexes and viscosities at multiple temperatures. The refractive index for the polymer was set to 1.467, based upon a reported refractive index value for PEG-methacrylate, a structurally similar component to the major wt. fraction of the employed BCP.[2]

1.8.3 DLS for real-time FISA experiments

In order to execute this experiment, the reaction solution was prepared in a cuvette suited for DLS and initiated, like the other FISA reactions, by the combination of a catalyst-containing stock solution and the solution containing the polymer and thiol. The solution was sampled every ~20 minutes from the initial time point, measured from the time of catalyst addition, and stirred at these points to ensure homogeneity during DLS analysis. DLS measurements were taken continuously with approximately 90 seconds between measurements.

1.9 Transmission Electron Microscopy (TEM)

Preparation: TEM samples were prepared by adding one drop of crude reaction solution to a TEM grid (Ultrathin Carbon Film on Lacey Carbon Support Film, 400 mesh, Copper, Tedpella), waiting approximately 30 seconds, and dabbing the excess solution up with a Kim-wipe. The grids were then held under vacuum at room temperature for 5 minutes before being immediately analyzed via TEM.

Measurement: Conventional TEM imaging was performed on a JEOL 2100F microscope. The TEM was equipped with a spherical aberration coefficient, C_s, value of 1 mm and a Schottky electron emitter. The accelerating voltage was 200 kV. The images were acquired in bright-field mode with a defocus value of several microns.

2. Supporting figures and tables

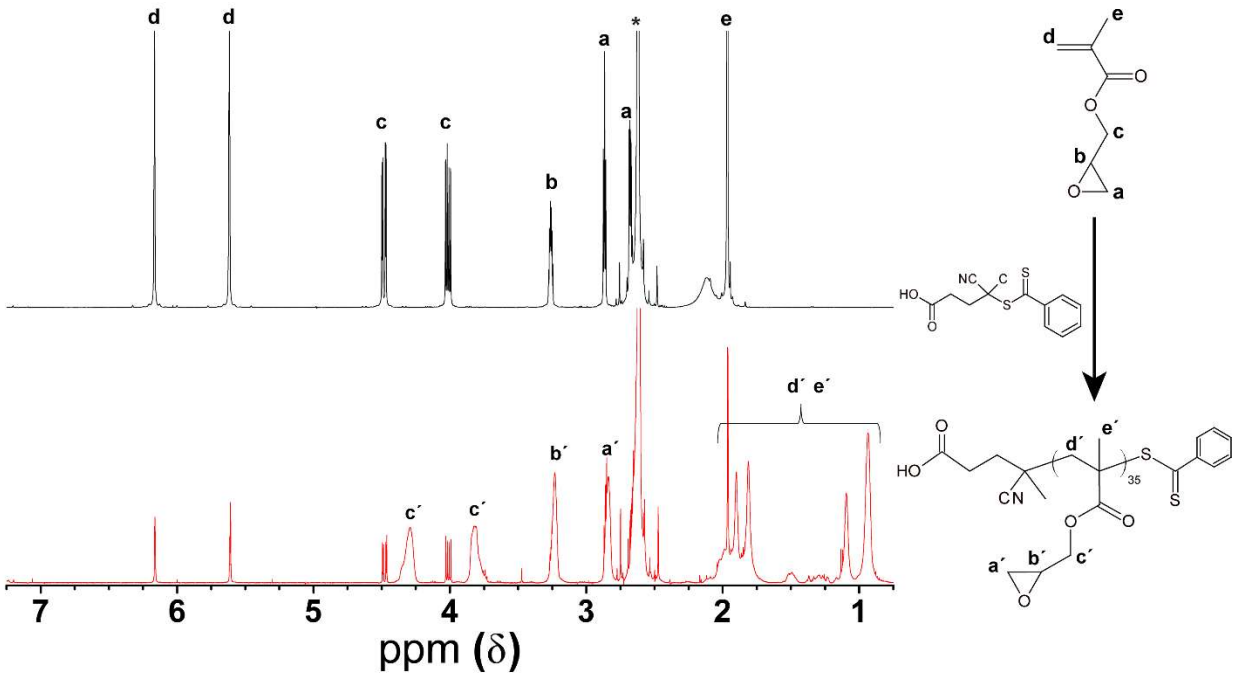


Figure S1. ^{1}H-NMR spectra in $CDCl_3$ with assignments for representative PET-RAFT polymerized GMA (top) to poly(GMA) (bottom) after 12 hours. Solvent resonances from DMSO are denoted with a "*".

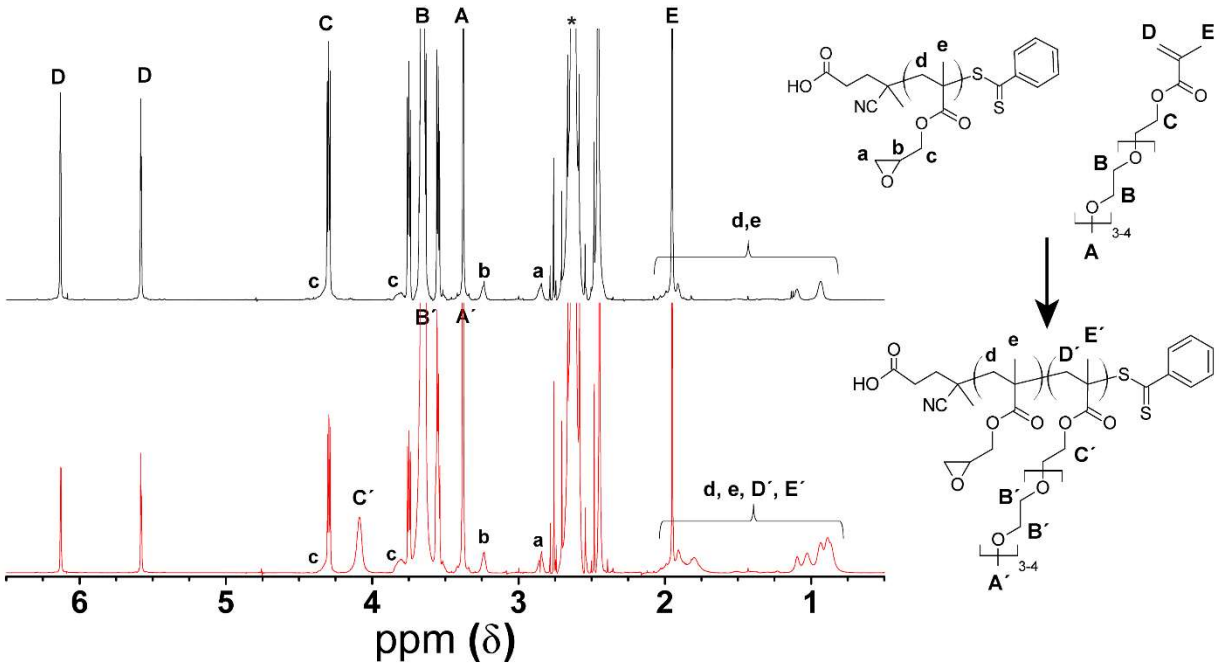


Figure S2. ^{1}H-NMR spectra in $CDCl_3$ with assignments for representative chain extension of p(GMA) (top) to poly(GMA-*b*-PEGMEMA) (bottom) after 9.5 hours. Solvent resonances from DMSO are denoted with a "*".

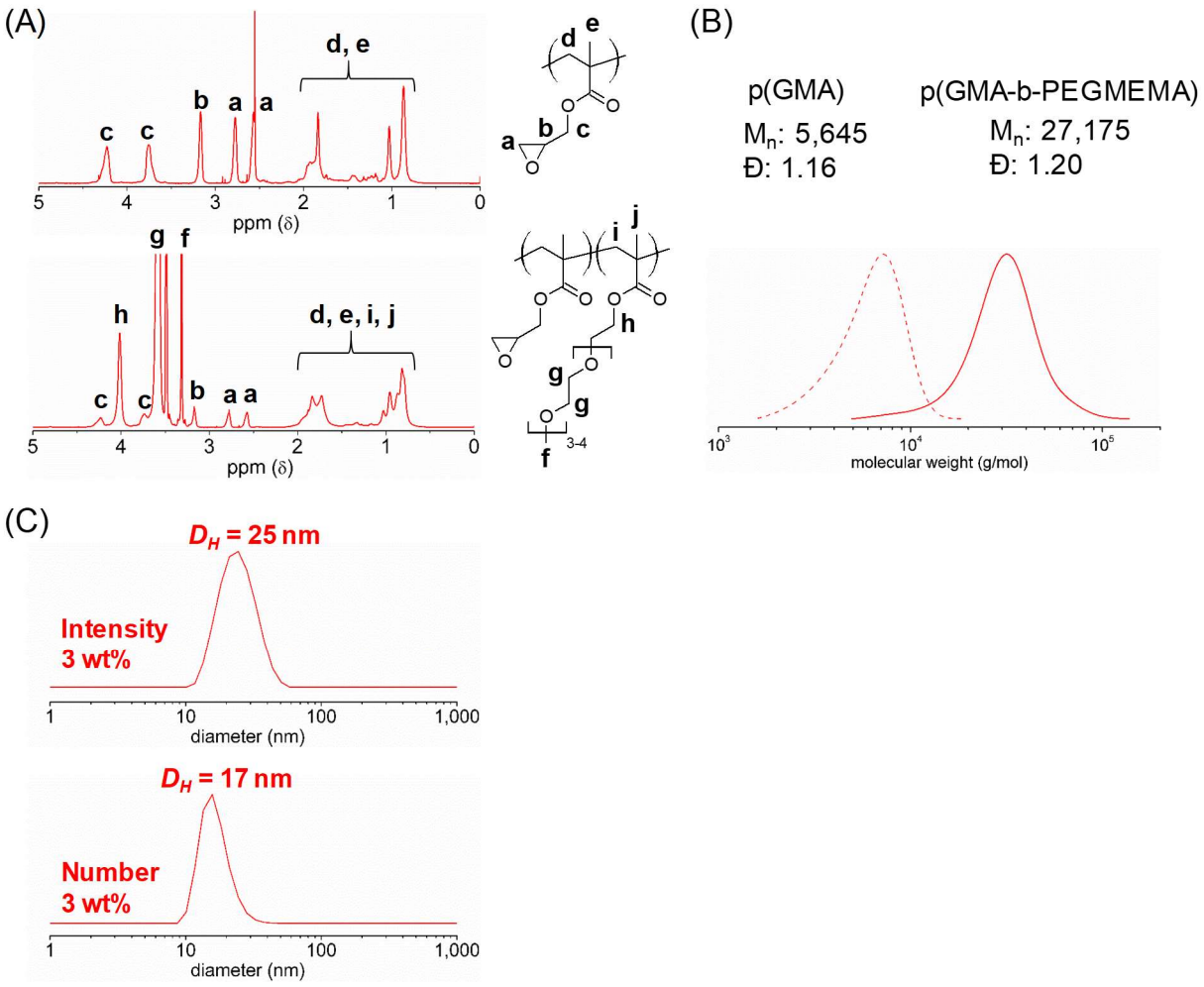


Figure S3. (A) ^{1}H-NMR of poly(GMA) macro-CTA (top) and of poly(GMA-*b*-PEGMEMA) (bottom). (B) THF-SEC traces for macro-CTA and chain-extended poly(GMA-*b*-PEGMEMA). (C) DLS intensity (top) and number (bottom) distributions for the BCP precursor in ethanol at 3 wt%. Details are reported in Table 1, Entry 1. This poly(GMA-*b*-PEGMEMA) was used as the precursor for all post-polymerization modification and FISA experiments.

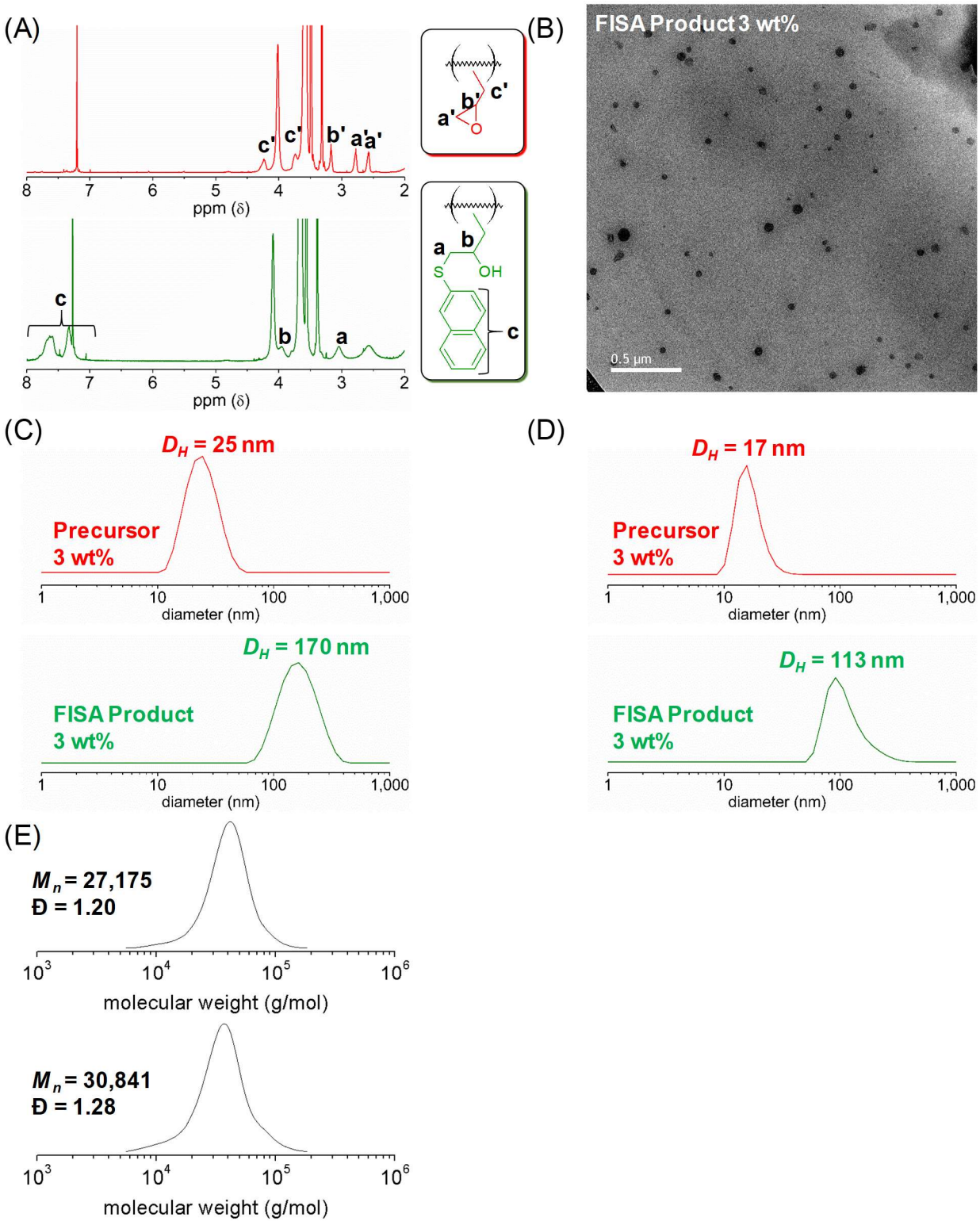


Figure S4. Data for Table 1, Entry 2: FISA with 2-naphthalenethiol in ethanol. (A) ^{1}H-NMR before (top) and after (bottom) functionalization, (B) TEM micrograph of final product, solvent cast from ethanol, (C) DLS intensity distribution of precursor (top) and final FISA product (bottom), (D) DLS number distribution of precursor (top) and final FISA product (bottom), and (E) SEC traces for precursor (top) and final FISA product (bottom).

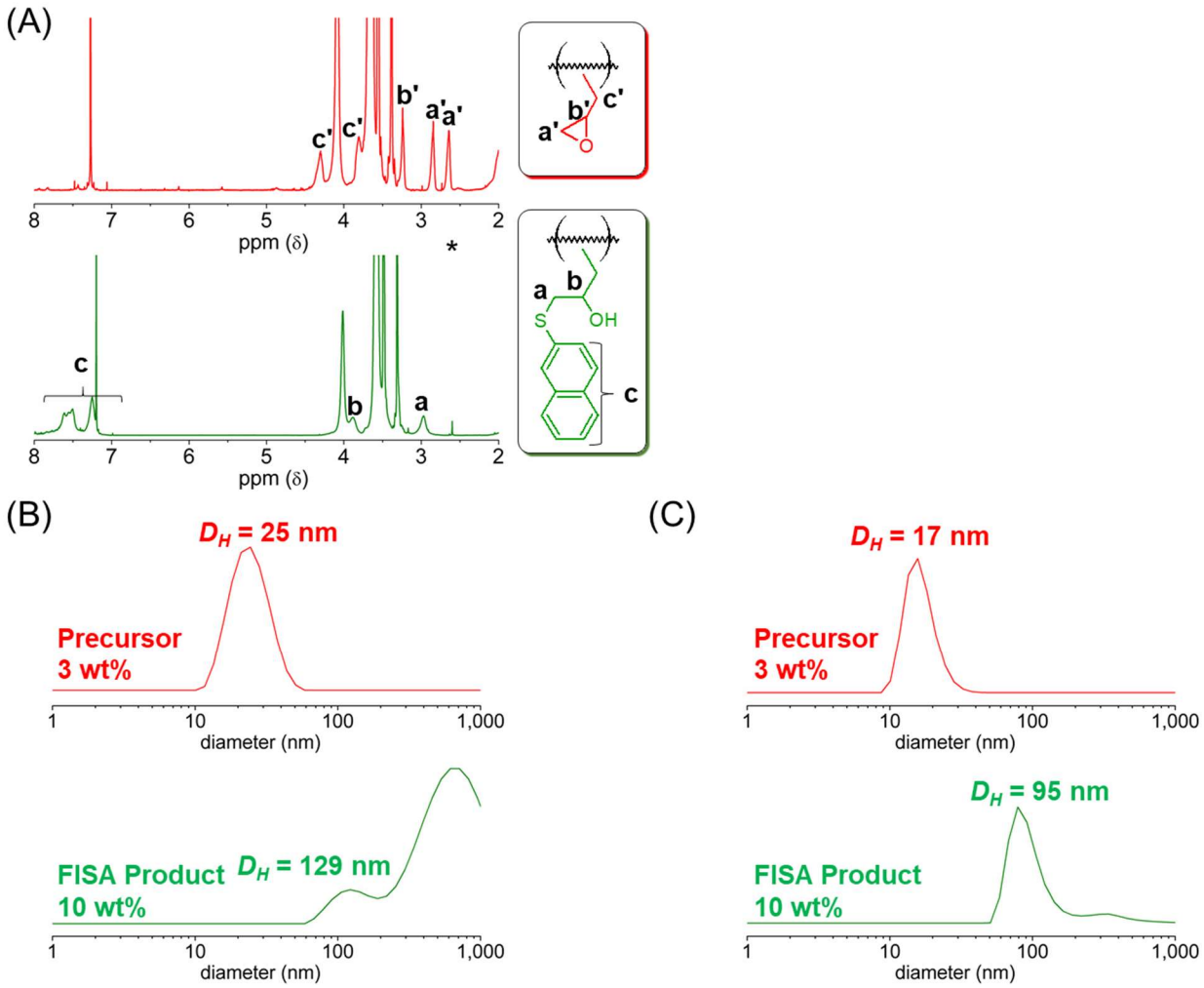


Figure S5. Data for Table 1, Entry 3: FISA with 2-naphthalenethiol at 10 wt% polymer. (A) ^{1}H-NMR before (top) and after (bottom) functionalization, (B) DLS intensity distribution of precursor (top) and final FISA product (bottom), and (C) DLS number distribution of precursor (top) and final FISA product (bottom).

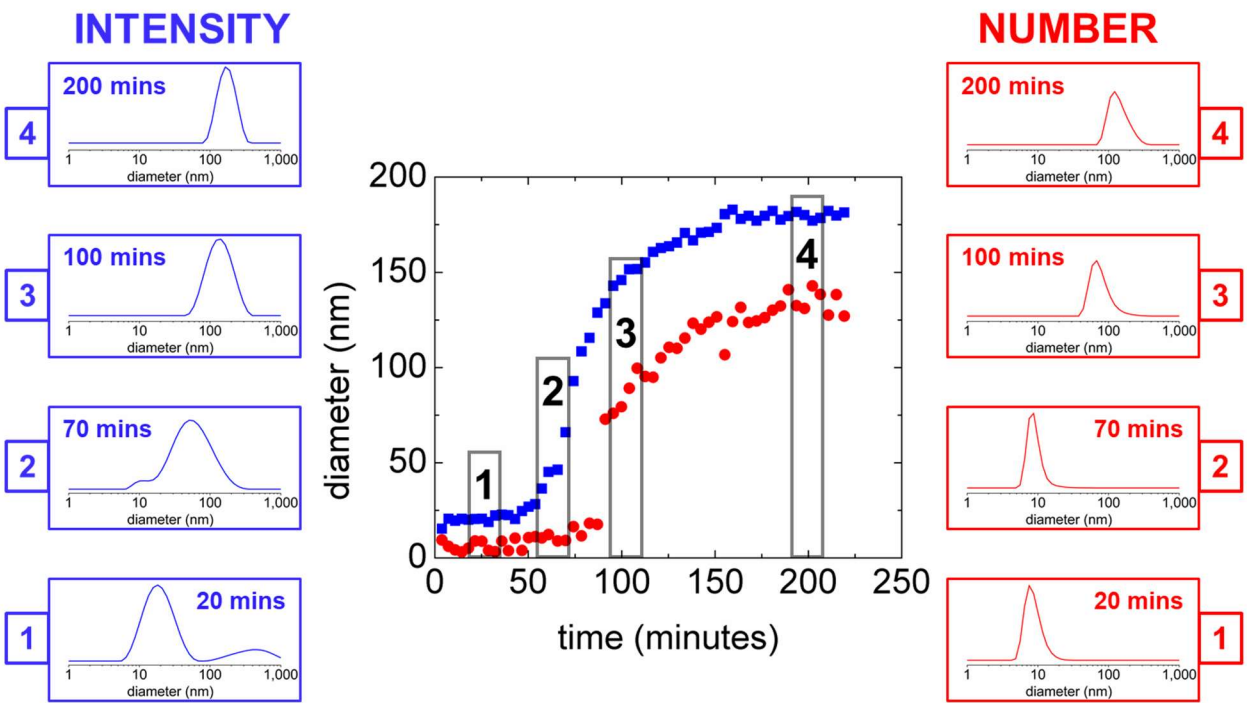


Figure S6. Table 1 Entry 4, DLS intensity (blue) and number (red) trends over the course of the reaction with selected example DLS profiles for individual time points.

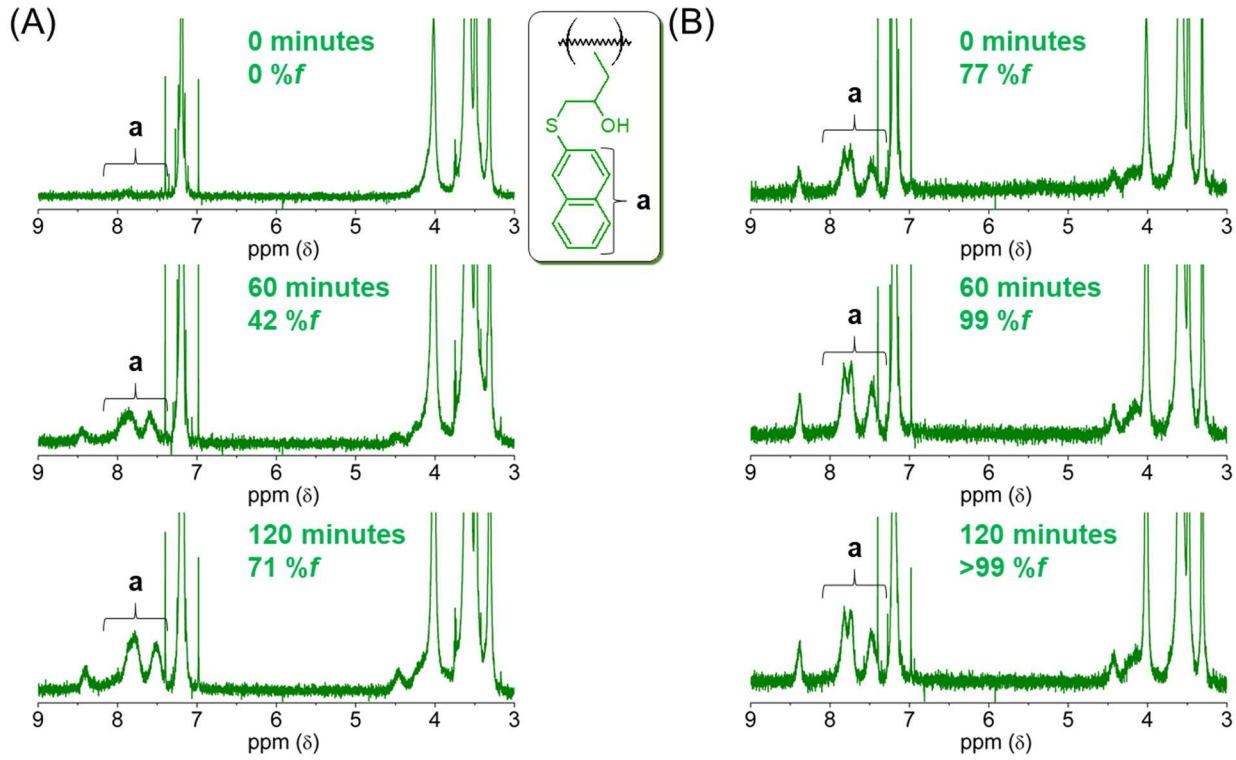


Figure S7. Table 1, Entry 4, NMR for selected kinetic samples of real-time 2-naphthalenethiol functionalization. (A) Quenched samples and (B) unquenched samples from an equivalent reaction.

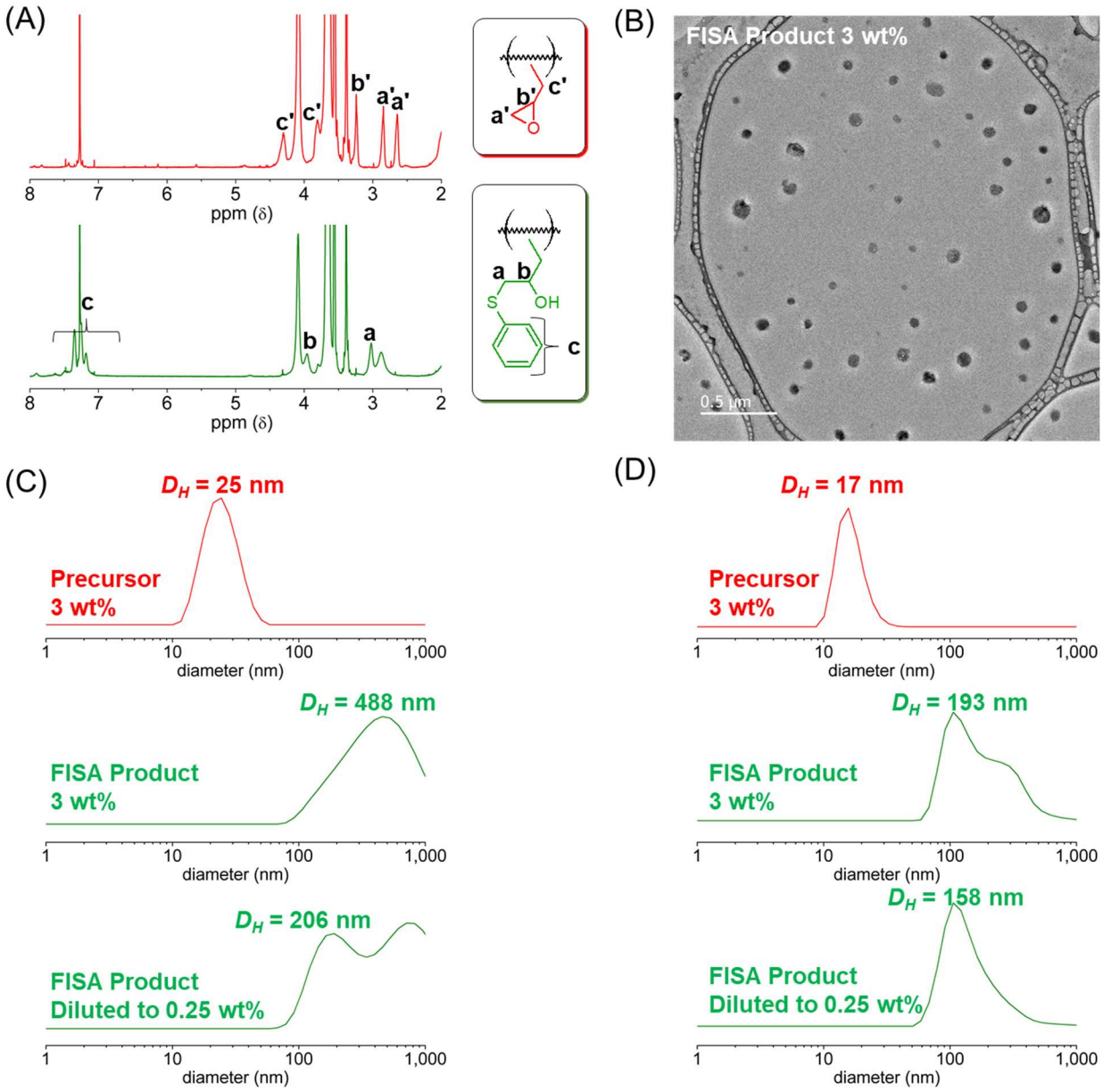


Figure S8. Table 1, Entry 5: FISA with thiophenol in ethanol. (A) NMR before (top) and after (bottom) functionalization, (B) TEM micrograph of final product, solvent cast from ethanol, (C) DLS intensity trace of precursor (top), final FISA product (middle), and final FISA product after a 12-fold dilution (bottom), and (D) DLS number trace of precursor (top), final FISA product (middle), and final FISA product after a 12-fold dilution (bottom).

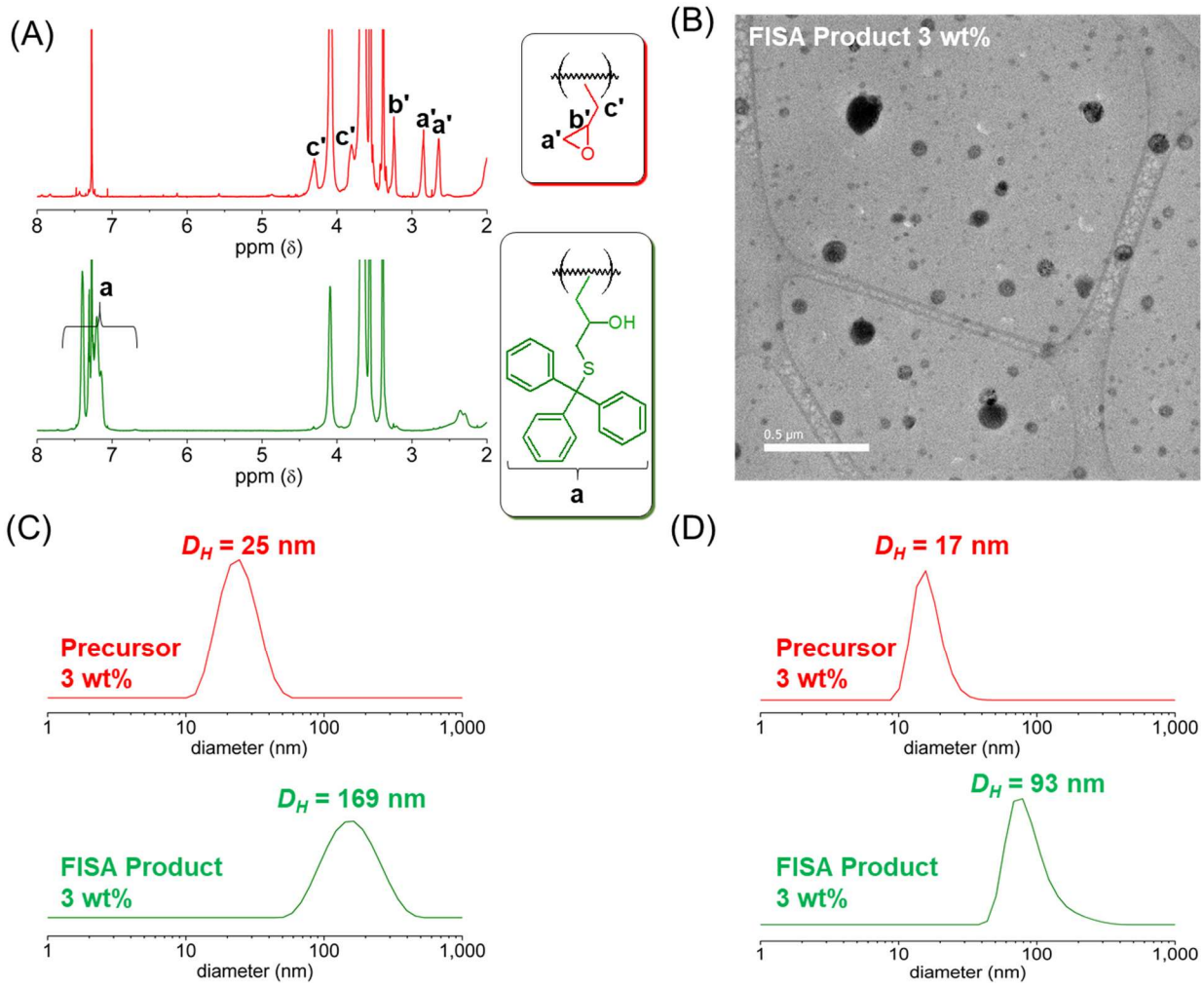


Figure S9. Table 1, Entry 6: FISA with triphenylmethanethiol in ethanol. (A) NMR before (top) and after (bottom) functionalization, (B) TEM micrograph of final product, solvent cast from ethanol (C) DLS intensity trace of precursor (top) and final FISA product (bottom), and (D) DLS number trace of precursor (top) and final FISA product (bottom).

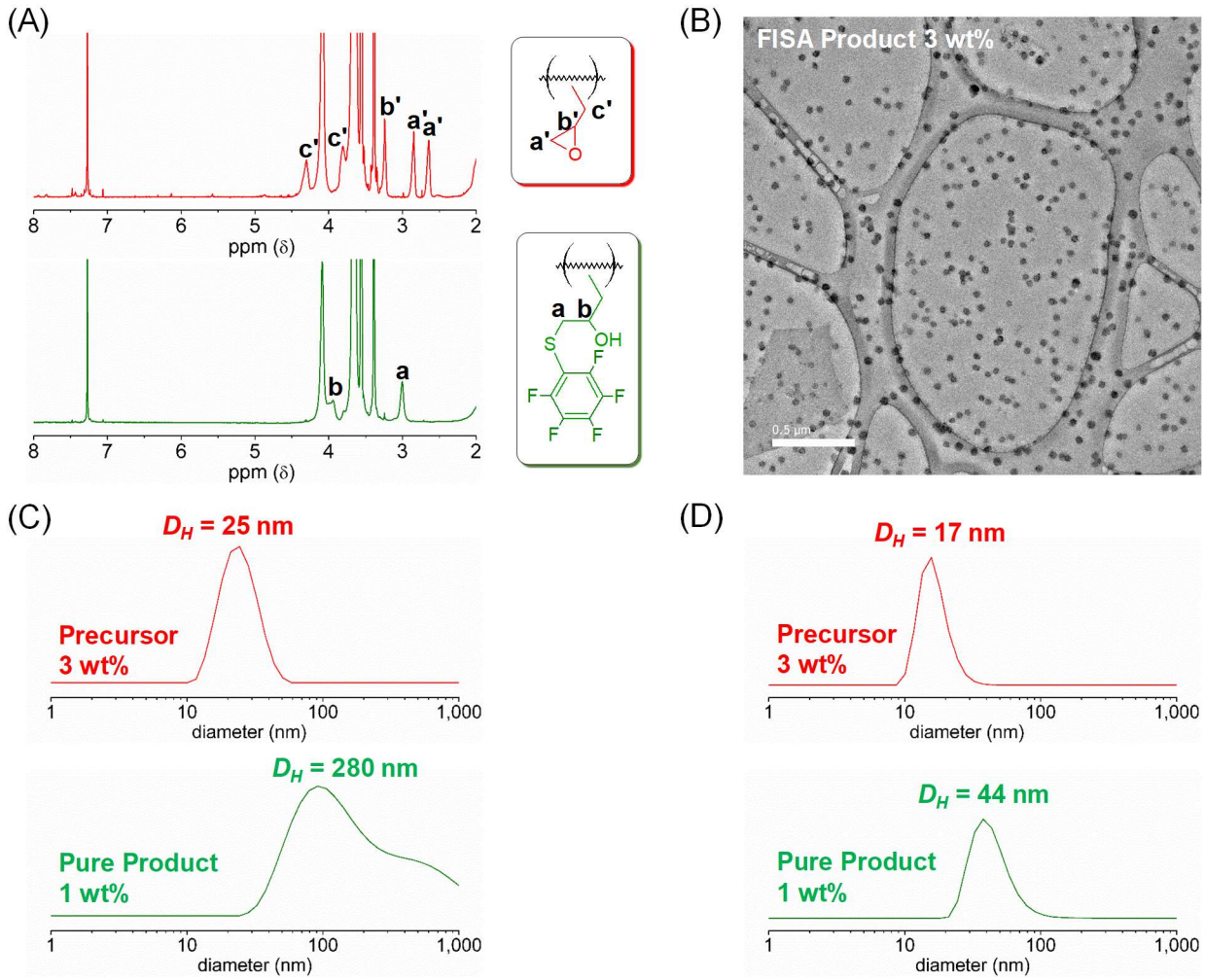


Figure S10. Table 1, Entry 7: FISA with pentafluorophenylthiol in ethanol. (A) NMR before (top) and after (bottom) functionalization, (B) TEM micrograph of final product, solvent cast from ethanol, (C) DLS intensity trace of precursor (top), final FISA product (middle), and purified FISA product (bottom), and (D) DLS number trace of trace of precursor (top), final FISA product (middle), and purified FISA product (bottom)

3. References

1 Gadwal, I., Stuparu, M. C. & Khan, A. Homopolymer bifunctionalization through sequential thiol--epoxy and esterification reactions: An optimization, quantification, and structural elucidation study. *Polym. Chem.* **6**, 1393-1404, doi:\url{10.1039/C4PY01453G} (2015).

2 Sigma, M. *Poly(ethylene glycol) methacrylate product description*, <https://www.sigmaaldrich.com/catalog/product/aldrich/409529?lang=en&region=US> (2019).