Electronic Supplementary Material (ESI) for Soft Matter.

Oxidative instability of boronic acid-installed polycarbonate nanoparticles

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Supporting Information

Materials

Unless stated otherwise, all chemicals and solvents were purchased from Sigma-Aldrich or Fisher Scientific and used as received. 4-(Hydroxymethyl)phenylboronic acid was purchased from Accela. High-purity water (Milli-Q or nanopure water) was obtained by purifying deionized water in a Barnsted Nanopure purification system to a final resistance of 18.2 mΩ. The synthesis of the pinacol-protected phenylboronic acid-installed cyclic carbonate monomer (PBC) and its polymerization from a monomethoxy poly(ethylene glycol) macroinitiator were carried out as described in a previously reported protocol.[1]

Characterization

To determine polymer polydispersity (PDI), gel permeation chromatography (GPC) was performed on a Waters 1515 Isocratic HPLC equipped with two Styragel® columns (HR4 and HR3, 300 mm x 7.8 mm) connected in series, equipped with a differential refractive index detector (Waters 2414) and a UV-visible detector (Waters 2489). HPLC grade tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 mL/min. Samples were filtered through 0.22 μm PVDF syringe filters (Millipore) before injection. All measurements were carried out at 30 °C. Molecular weights are reported referenced to polystyrene standards (Shodex SL-105). ^{1}H NMR spectra were recorded on a Bruker AV 400 MHz spectrometer in either CDCl3 or d_6-DMSO and referenced to CDCl3 (7.26 ppm) or d_6-DMSO (2.50 ppm). Dynamic light scattering (DLS) experiments were carried out on a Malvern Instruments Nano-ZS ZetaSizer equipped with a 4 mW He-Ne laser operating at 633 nm. Measurements were performed at 25 °C at a scattering angle of 173°. All measurements were carried out two times with a duration of 100 s each. Measurements of the ζ-potential were made inside folded capillary cells (Malvern, DTS 1060) and calculated using the Smoluchowski equation. Fluorescence spectroscopy was carried out on a Fluorolog-3 system (HORIBA Jobin Yvon Inc., NJ), with an excitation wavelength of λex= 538 nm. Excitation and emission bandwidths were set to 2 nm. Emission spectra were collected from 550 to 800 nm. UV/Vis measurements were obtained using Varian Cary 50 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA). Bright-field transmission electron microscopy (TEM) imaging was performed on a FEI Technai 12 Twin Transmission Electron Microscope operated at an acceleration voltage of 100 kV. All TEM images were recorded by a SIS Megaview III wide-angle CCD camera. TEM grids (carbon-coated copper, Electron Microscopy, Hatfield, PA) were ionized under plasma to render carbon films hydrophilic. Grids were placed on top of a droplet of nanoparticle suspension (50 μL) for 5 min, washed with 5 drops of doubly distilled water, and placed onto a drop (50 μL) of a 2 wt% aqueous uranyl acetate solution for 30 s. Excess solution was blotted off with filter paper and grids were allowed to dry at room temperature prior to imaging.

Methods

1. Monomer synthesis

PBC monomer precursors, 4-(hydroxymethyl)phenylboronic acid pinacol ester (HMPBAE)[1] and MTC-OH,[2] were synthesized according to previously reported protocols. HMPBAE (7.51 g, 0.0321 mol) was transferred to a schlenk flask and dissolved in a mixture of anhydrous tetrahydrofuran (THF) and dichloromethane (DCM) (75:25, v/v). MTC-OH (5.646 g, 0.0353 mol, 1.1 equiv.), 4-dimethylaminopyridine (DMAP, 0.784 g, 0.0064 mol, 0.2 equiv.), and 4-(N,N-dimethylamino)pyridinium-4-*p*-toluenesulfonate (DPTS, 2.384 g, 0.0080 mol, 0.25 equiv.) were added to the flask and the reagents were then dried at 40 °C under high vacuum (100 mTorr) to remove any traces of moisture. After 2 h of drying, the reaction mixture was cooled to 0 °C using an ice-salt bath. *N*,*N*′-dicyclohexylcarbodiimide (DCC, 7.149 g, 0.0346 mol, 1.08 equiv.) was separately dissolved in 20 mL of anhydrous DCM and then added dropwise to the reaction mixture over a period of 30 min. The reaction was then allowed to proceed for 24 h at room temperature. Although MTC-OH is slightly insoluble in the starting THF/DCM mixture, its solubility improves as DCC begins to activate the carboxylic acid. Upon reaction completion, insoluble DCU salts were filtered off and the solvent was removed under vacuum. To remove DMAP and DPTS, the crude product was passed through a short silica gel column with DCM as the eluent. It was further recrystallized from hexane/ethyl acetate (80:20, v/v), and purified again by column chromatography using silica gel and DCM/methanol from 100% DCM to a 97:3 mixture. The desired product was obtained in 60% yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.67 (d, 2H), 7.37 (d, 2H), 5.24 (s, 2H), 4.59 (d, 2H), 4.39 (d, 2H), 1.29 (s, 12H), 1.20 (s, 3H). Spectrum and peak assignments of the functional monomer are provided below (**S1**).

*2. Polymer synthesis (PEG_{45}-b-$PPBC_{26}$) (**P1**)*

Monomethoxy poly(ethylene glycol) (108 mg, 0.054 mmol) was added to a 10 mL schlenk flask equipped with a magnetic stir bar. Anhydrous toluene (5 mL) was added to the flask to perform azeotropic distillation. After complete removal of toluene, PEG polymer was further dried at 90 °C under high vacuum (100 mTorr) to remove any traces of moisture. After 2 h of drying, the flask was cooled down to room temperature and PBC monomer (692 mg, 1.84 mmol) was added to the flask under high argon flow. Both PEG macroinitiator and PBC monomer were immersed in an oil bath set at 50 °C under high vacuum and further dried for 2 h. After completion of the drying process, the flask was cooled down to room temperature under argon. Anhydrous dichloromethane (6.5 mL) was added *via* a syringe and the reagents were allowed to dissolve completely prior to the addition of DBU (1,8- diazabicycloundec-7-ene, 13.8 μL, 0.092 mmol). The reaction was allowed to proceed at room temperature under argon for 3.5 h and was subsequently quenched with an excess of benzoic acid. ^{1}H NMR of the raw product was used to calculate the percent of monomer conversion based on the ratio of the signal from polymer (d) and from unreacted monomer (d'). Conversion was estimated to be ~82% (Figure **S2** inset). Raw polymer was further purified by precipitation (3 times) in 2-propanol, and pure product was obtained in 79% yield based on monomer conversion. The degree of polymerization (DP) was calculated using the end-group analysis by comparing the signal from the methoxy peak of PEG (h) to the signal from polymer (d), and estimated to be 26 (**S2**). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.63 (d, 52H), 7.26 (d, 52H), 5.09 (s, 52H), 4.19 (s, 104H), 3.50 (s, 180H), 3.23 (s, 3H), 1.22 (s, 312H), 1.12 (s, 78H). PDI: 1.15. Gel permeation chromatography was also performed on this

polymer and, as shown in **S3**, a single peak was observed with no apparent trace of unreacted macroinitiator.

3. Monomer oxidation in deuterated phosphate buffer

Monomer (**1**), initially dissolved in d_6-DMSO (4.3 mg/mL), was diluted with deuterated phosphate buffer (0.5×) (3.8 mg/mL) and the mixture was incubated at 37 °C for ~5 min. Subsequently, hydrogen peroxide solutions raging in concentration from 50 to 1960 mM H_2O_2 (4.85 to 98 molar equivalents of H_2O_2 to boronic acid) were added to each individual monomer sample in d_6-DMSO/PBS, and ^{1}H NMR spectra were recorded at 37 °C *in situ* at different time intervals. Monomer stability in d_6-DMSO/0.5×PBS-D_2O in the absence of H_2O_2 was also tracked and used as reference. This sample was prepared as detailed above (excluding the oxidant) and incubated at 37 °C for 11 days.

4. Monomer oxidation in deuterated water

Monomer (**1**), initially dissolved in d_6-DMSO (4.3 mg/mL), was diluted with deuterium oxide (3.8 mg/mL) and the mixture was incubated at 37 °C for ~5 min. Subsequently, 50 mM hydrogen peroxide solution (4.85 molar equivalents of H_2O_2 to boronic acid) was added to the monomer sample and ^{1}H NMR spectra were recorded at 37 °C *in situ* at different time intervals. Monomer stability in d_6-DMSO/D_2O in the absence of H_2O_2 was also tracked and used as reference. This sample was prepared as detailed above (excluding the oxidant) and incubated at 37 °C for 10 days.

5. Self-assembly of PEG_{45}-b-$PPBC_{26}$ nanoparticles

Self-assembly of the amphiphilic copolymer PEG_{45}-*b*-$PPBC_{26}$ was triggered by a large and rapid change in solvent quality inside a multi-inlet vortex mixer (MIVM) in which micromixing occurs in the *ms* range. Nanopure water was charged into three 50 mL syringes (Hamilton, NJ) and the organic solution into a 10 mL syringe, which were mounted on two separate syringe drivers (PHD Ultra, Harvard Apparatus). Flow rates of water and organic streams were 108 mL/min (water) and 12 mL/min (THF) to achieve a 10% (v/v) THF concentration. The initial amphiphile concentration in the organic phase (THF) was kept at 5 mg/mL, which after the 10x dilution inside the mixer resulted in 0.5% w_p/w nanoparticle suspensions. To remove the remaining organic solvent, nanoparticles were dialyzed (6-8 kDa MWCO, Fisherbrand) against Nanopure water for 24 h at 20 °C. Water was replenished every 4 h throughout the dialysis process. After dialysis in water, micelles were transferred to 1× PBS and dialyzed for another 4 h.

6. Nile red-loaded nanoparticles

Nile red (NR) nanoparticles, stabilized by PEG_{45}-*b*-$PPBC_{26}$, were prepared by flash nanoprecipitation inside a multi-inlet vortex mixer using the same protocol described above. However, in this case both the polymer and the solute were dissolved together in tetrahydrofuran at 5 mg/mL and 5 μg/mL respectively, resulting in 0.5% w_p/w nanoparticle suspensions with nile red at 0.1% w/w_p.

7. Determination of Critical Micelle Concentration for PEG_{45}-b-PCC_{26}

Freshly prepared PEG_{45}-*b*-$PPBC_{26}$ nanoparticles (0.41% w_p/w) were transferred to 0.1× PBS and incubated at 37 °C for 18 h in the presence of 100 mM H_2O_2. Fully oxidized nanoparticles were then dialyzed for 24 h against Nanopure water to remove PBS salts, free pinacol, and 4-(hydroxymethyl) phenol (resulting from the oxidation). Sample was then frozen and lyophilized

to obtain a fully oxidized PEG_{45}-*b*-PCC_{26} polymer. Critical micelle concentration (C_{CMC}) of the oxidized copolymer was measured by fluorescence spectroscopy using pyrene as a probe. For each sample, 50 μL of pyrene solution (6.0×10^{-5} M in acetone) was added to a 7 mL scintillation vial and the solvent was allowed to evaporate completely. After 1 h of evaporation, 1 mL of polymer solution of known concentration (0.05 – 1000 μg/mL in H_2O) was added to each vial containing pyrene. The samples were vigorously stirred with a vortex mixer for 1 min and left uncovered overnight to evaporate any remaining traces of acetone. Pyrene concentration in each sample was kept constant at 3.0×10^{-6} M. Pyrene excitation was recorded from 300 to 360 nm with the bandwidth set at 2 nm. The intensity ratio from signals at 335 nm and 334 nm (I_{335}/I_{334}) were plotted against the logarithm of polymer concentration. C_{CMC} values were read from the intersection between curve tangents at low and high concentrations (S4).

8. Polymer Oxidation by H_2O_2

Filtered (0.45 μm PVDF syringe filters, Genesee Scientific) nanoparticle suspensions in water (0.42% w_p/w) were first incubated at 37 °C for 1 h, after which an aliquot was taken and used as reference. Hydrogen peroxide was added to achieve a final concentration of 500 μM (0.55 molar equivalents of H_2O_2 to boronic acid). The sample was incubated at 37 °C and aliquots were taken at 1, 2.5, 4.5, 7, 20 and 30 h time intervals. Each aliquot was immediately frozen, lyophilized and subsequently redissolved in d_6-DMSO for ^{1}H NMR analysis.

9. Oxidation of nile red nanoparticles at high concentration (above the C_{CMC} of the oxidized polymer)

Nile Red encapsulated PEG_{45}-*b*-$PPBC_{26}$ nanoparticles were incubated *in-situ* in 1X PBS at pH 7.4 and 37 °C. Nanoparticles were prepared according to the method described above (point 6), resulting in 0.41% w_p/w nanoparticle suspensions with Nile Red at 0.1% *w/wp*. Freshly prepared nanoparticles were first dialyzed against 1X PBS for 4 h, and then transferred to six clean scintillation vials and incubated in 1X PBS at 37 °C for 30 min (time 0). After equilibration, six different hydrogen peroxide concentrations raging from 0 to 1000 μM H_2O_2 (0 to 1.16 molar equivalents of H_2O_2 to boronic acid) were added to the samples and further incubated at 37 °C. Aliquots were taken at predetermined time points and analyzed by Dynamic Light Scattering, Fluorescence Microscopy and Transmission Electron Microscopy. Oxidation of nanoparticles in water was carried using an identical procedure, with the exception of dialysis in 1X PBS.

10. Oxidation of nile red nanoparticles at low concentration (below the C_{CMC} of the oxidized polymer)

Nile Red encapsulated PEG_{45}-*b*-$PPBC_{26}$ nanoparticles were incubated *in-situ* in H2O at 37 °C. Samples were prepared according to the method described above (point 6), resulting in 0.41% w_p/w nanoparticle suspensions with Nile Red at 0.1% *w/wp*. Freshly prepared nanoparticles were filtered (0.45 μm PVDF syringe filters, Genesee Scientific) and then diluted with nanopure water to three different concentrations: 0.00045, 0.00089 and 0.00223% w_p/w. Samples were equilibrated at 37 °C for 30 min prior to the addition of hydrogen peroxide at three different concentrations of 50, 100 and 250 μM H_2O_2, keeping the initial ratio of H_2O_2 to boronic acid at 5 molar equivalents. A sample at 0.00223% w_p/w was used as a control with 0 μM H_2O_2. All four nanoparticle suspensions were further incubated at 37 °C and aliquots were taken at predetermined time points (0 h refers to time immediately after H_2O_2 addition) to be analyzed by Dynamic Light Scattering, Fluorescence Microscopy and Transmission Electron Microscopy.

Oxidation in water at a 2-molar excess of H_2O_2 was carried using an identical procedure, with the exception that nanoparticles were prepared at 0.2% w_p/w and further diluted to three different concentrations: 0.0011, 0.0022 and 0.0056% w_p/w.

11. Effect of H_2O_2 on Nile Red Fluorescence

Nile Red (NR) was dissolved in THF at a concentration of 0.421 μg/mL. Subsequently, 50 μL ofH2O2 solution of varying concentration, ranging from 0 to 1000 μM H_2O_2, was added to 950 μL of the NR solution in THF, keeping the total volume constant at 1 mL. NR fluorescence was recorded from 550 to 750 nm with the slit width set to 2 nm and an excitation wavelength λ_{ex} = 538 nm.

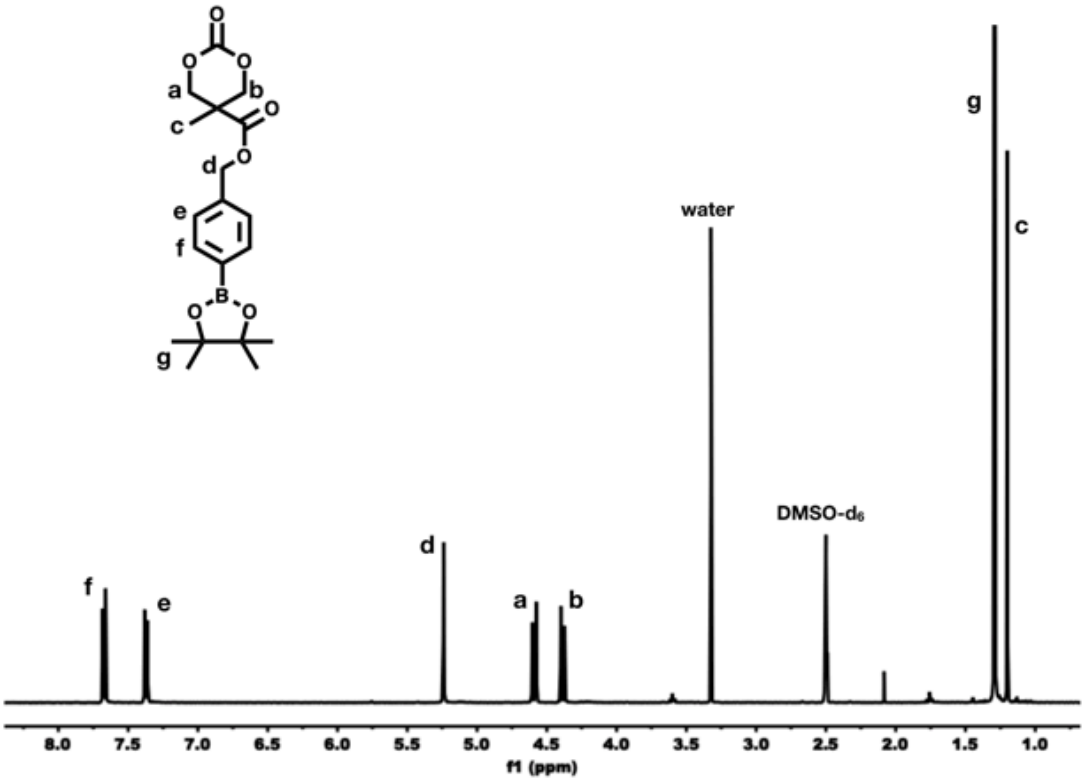

Figure S1. ^{1}H NMR spectrum and peak assignment of monomer **1**.

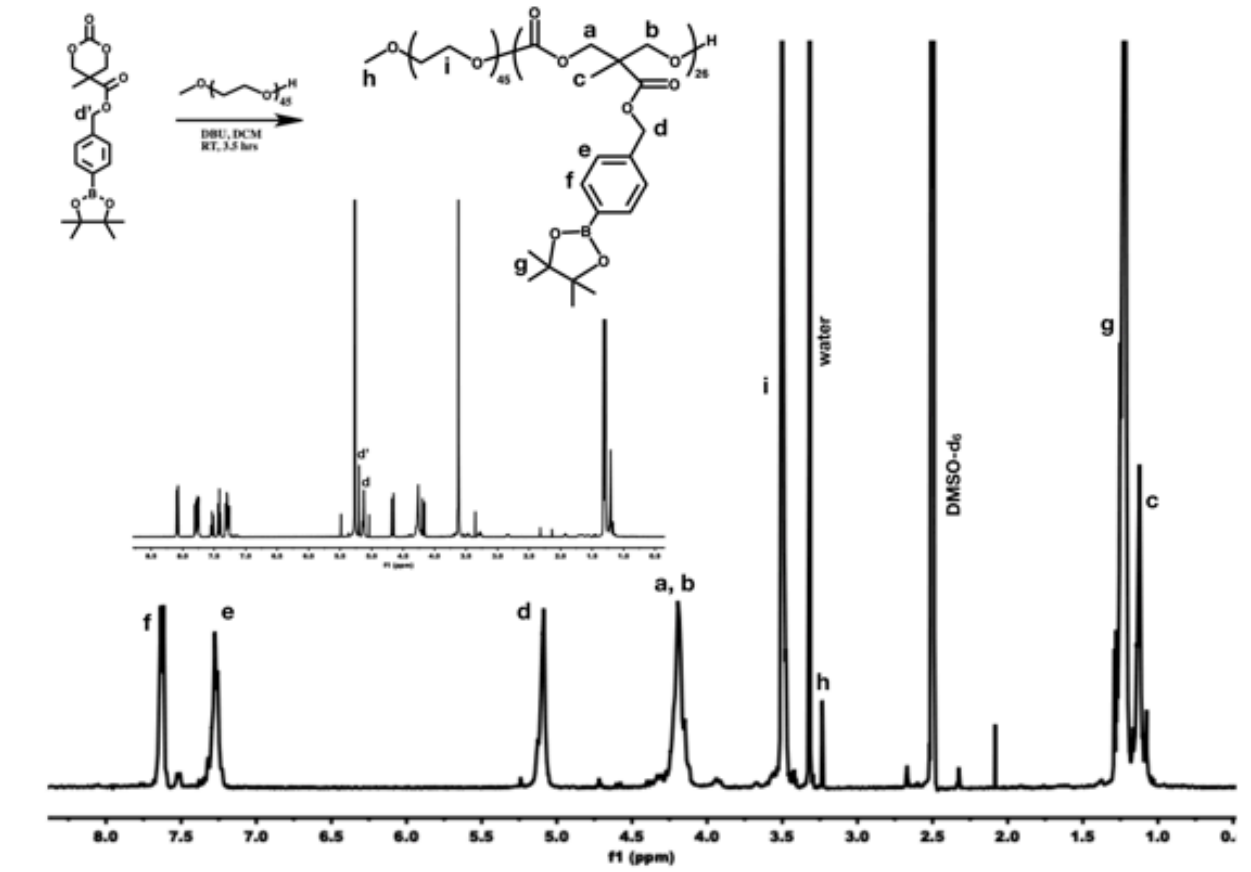

Figure S2. ^{1}H NMR spectra and peak assignments of purified PEG_{45}-*b*-$PPBC_{26}$ (**P1**) in d_6-DMSO. The insert corresponds to the raw material containing unreacted monomer (in $CDCl_3$).

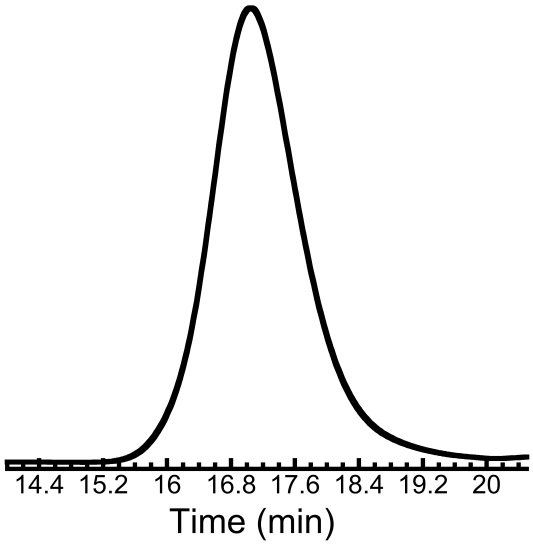

Figure S3. Chromatograph of purified PEG_{45}-*b*-$PPBC_{26}$ in tetrahydrofuran at 35 °C.

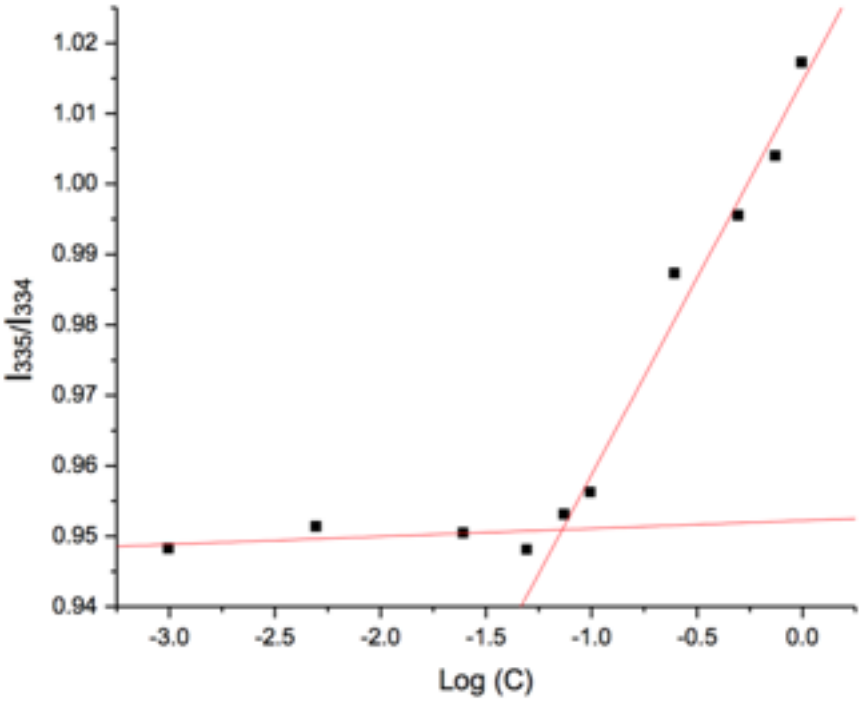

Figure S4. Critical micelle concentration for the fully oxidized copolymer (**P4**).

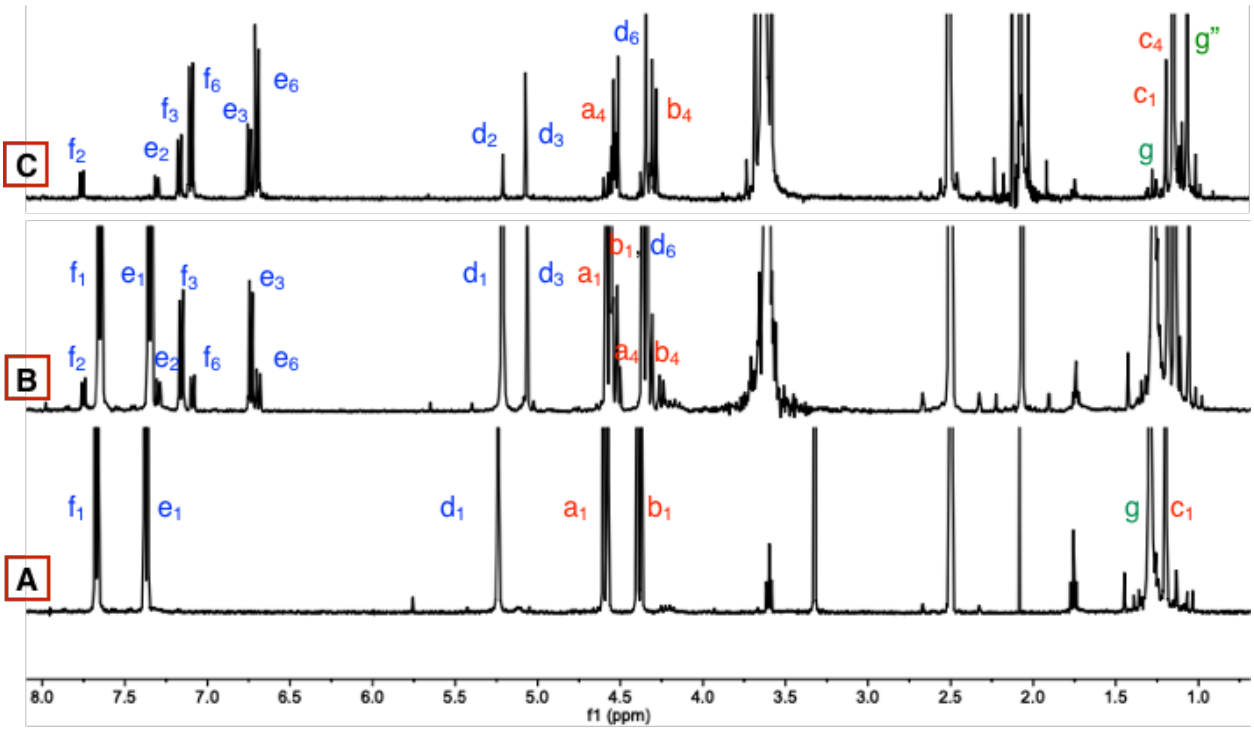

Figure S5. Oxidation of monomer **1** at 50 mM H_2O_2 in *d*$_6$-DMSO/D_2O tracked by ^{1}H NMR. Spectrum A corresponds to monomer **1**; spectra B and C correspond to oxidation periods of 5 h and 6 days, respectively. Peak assignments are provided in **Figure 1**.

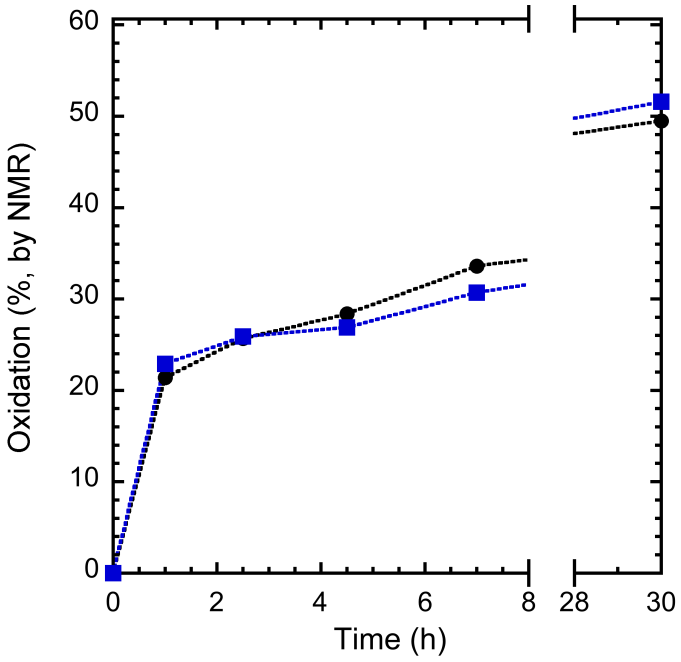

Figure S6. Oxidation of **P1** nanoparticles in water (circles) and PBS (squares) tracked by ^{1}H NMR.

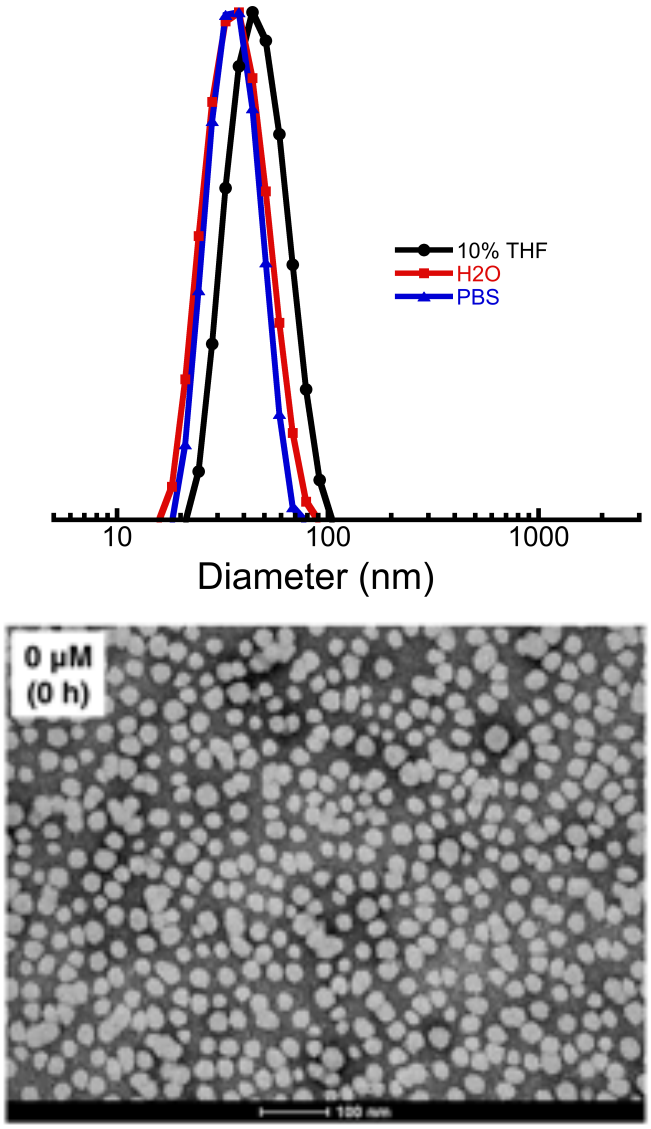

Figure S7. Size distributions and transmission electron microscopy of **P1** nanoparticles loaded with nile red.

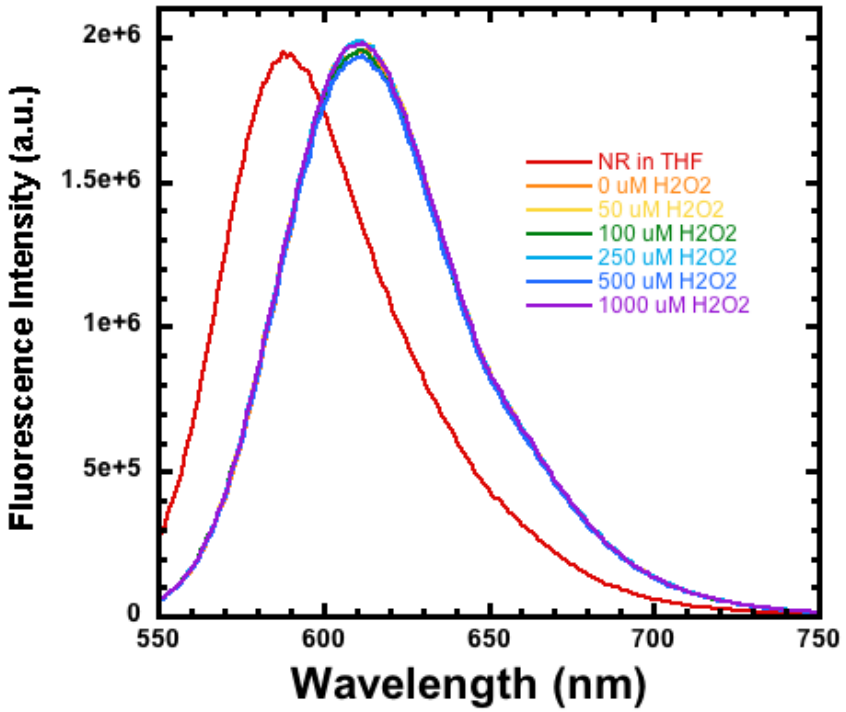

Figure S8. Effect of hydrogen peroxide concentration on the fluorescence of nile red.

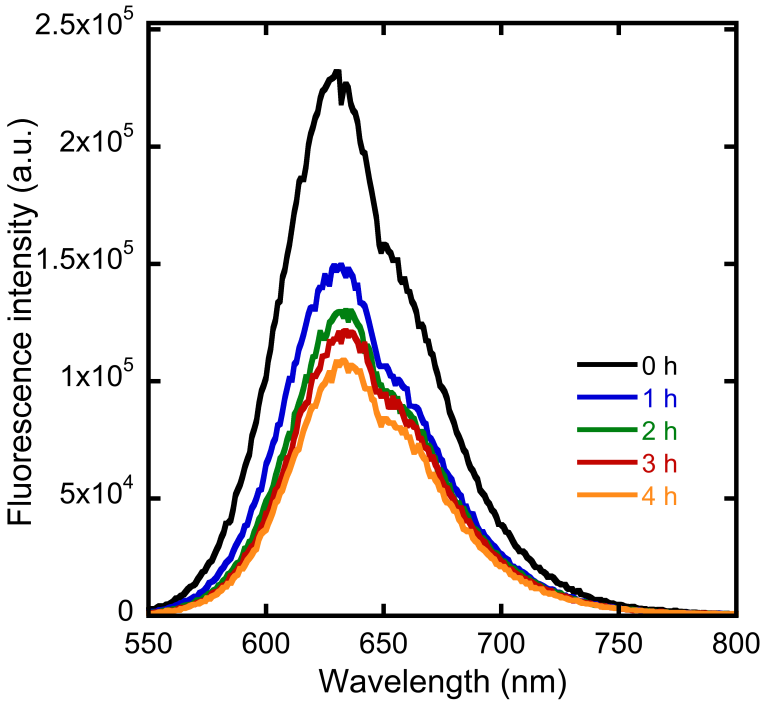

Figure S9. Time dependent change in fluorescence intensity of NR encapsulated PEG_{45}-*b*-$PPBC_{26}$ nanoparticles as a result of in-situ oxidation in 1X PBS at 250 μM H_2O_2.

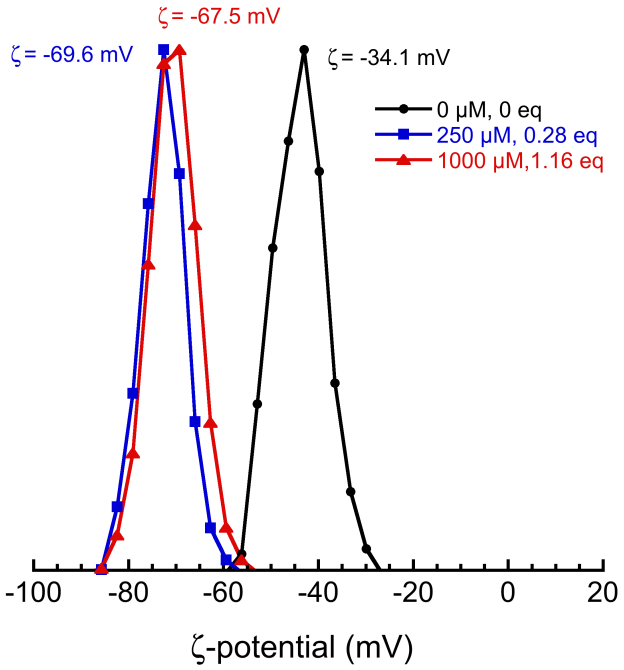

Figure S10. ζ-potential of nile red encapsulated PEG_{45}-*b*-$PPBC_{26}$ nanoparticles during in-situ oxidation in 0.1X PBS in the H_2O_2-limited regime at concentrations above polymer C_{CMC}.

References

1. Aguirre-Chagala, Y. E.; Santos, J. L.; Aguilar-Castillo, B. A.; Herrera-Alonso, M., Synthesis of Copolymers from Phenylboronic Acid-Installed Cyclic Carbonates. *Acs Macro Letters* **2014,** *3* (4), 353-358.
2. Pratt, R. C.; Nederberg, F.; Waymouth, R. M.; Hedrick, J. L., Tagging alcohols with cyclic carbonate: a versatile equivalent of (meth)acrylate for ring-opening polymerization. *Chemical Communications* **2008,** (1), 114-116.