

Supporting information

Highly Sensitive Visual Detection of Mutant DNA Based on
Polymeric Nanoparticles-Participating Amplification

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Materials.

4-Cyanopentanoic acid dithiobenzoate (CPADTBA) as chain transfer reagent in RAFT and monoamino polyethylene glycol (PEGNH₂) were prepared according to the reported method.¹⁻³ Biotinyl-*N*-hydroxyl succinimide ester (Biotin-NHS) was synthesized as the previously reported method.⁴ Monomer benzyl-protecting ascorbyl acrylate (BnAA) was fabricated according to our described method.^{5,6} 2,2'-Azobis(isobutyronitrile) (AIBN, purity >98%) purchased from Sinopharm Chemical (China) was recrystallized from methanol. Triethylamine (TEA) was dehydrated with KOH overnight and distilled. Toluene and tetrahydrofuran (THF) were dried using sodium with benzophenone as color indicator. Palladium on carbon (10 wt%, from Aldrich) was used as received.

Characterization.

Proton nuclear magnetic resonance (¹H NMR) spectra were carried out on a 400 MHz spectrometer (Bruker, Germany) at room temperature using CDCl₃ or deuterated DMSO as solvent. The chemical shifts were measured against the solvent signals of CDCl₃ or deuterated DMSO as internal standard. The molecular weights and polydispersity index of the polymers were determined with Waters gel permeation chromatograph (GPC) instrument equipped with Styragel HR4E-HR5E chromatographic column following a guard column and a differential refractive-index detector.

Synthesis of block polymer PAA-*b*-PS. Four steps were included as followed. *PBnAA preparation.* In a typical RAFT polymerization procedure, a 25 mL Schlenk flask charged with monomer BnAA (1.24 g, 3.0 mmol), initiator AIBN (3.3 mg, 0.02 mmol) and chain transfer agent CPADTBA (27.8 mg, 0.1 mmol) was degassed by three vacuo-nitrogen cycles, followed by the addition of 6.0 mL of toluene which was degassed by nitrogen bubbling for 20 min prior to use. The reaction mixture was further degassed by three freeze-pump-thaw cycles, and then immersed into an oil bath thermostated at 65 °C for 20 h. The cooled down reaction solution was precipitated into anhydrous diethyl ether to obtain pink polymer in yield of 87% (1.1 g). ¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.90 (*m*, 2H, *p*-C₆H₅CS₂), 7.46 (*m*, 3H, C₆H₅CS₂), 7.15-7.26 (*m*, 10nH, 2nC₆H₅CH₂), 4.91-5.14 (*m*, 4nH,

$2n\text{C}_6\text{H}_5\text{CH}_2$), 4.53 (*m*, 1nH, hydrogen on the C-4 of AA), 4.05-4.18 (*m*, 3nH, hydrogen on the C-5 and C-6 of BnAA), 2.40 (*m*, nH, CH on the backbone), 1.67-1.96 (*m*, 2nH, CH_2 on the backbone). n represented repeating unit number.

*Preparation of block polymer with dithiobenzoate ending poly(styrene)- block-poly(benzylascorbyl acrylate) DTB-PS-*b*-PBnAA.* Similar RAFT procedure was conducted in a Schlenk flask containing PBnAA (1.10 g, 0.08 mmol), styrene (0.43 g, 4.0 mmol), AIBN (2.6 mg, 0.016 mmol) and THF (6.0 mL) at 65 °C for 20 h. The pink solid polymer was obtained via precipitating in anhydrous ethyl ether and drying in vacuum in yield of 73% (1.1 g). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.82 (*m*, 2H, $o\text{-C}_6\text{H}_5\text{CS}_2$), 7.42 (*m*, 3nH, $n\text{C}_6\text{H}_5\text{CS}_2$), 6.45-6.58 (*m*, 2mH, $mm\text{-C}_6\text{H}_5$), 4.92-5.15 (*m*, 4nH, $2n\text{C}_6\text{H}_5\text{CH}_2$), 4.53 (*m*, 1nH, hydrogen on the C-4 of AA), 4.05-4.18 (*m*, 3nH, hydrogen on the C-5 and C-6 of AA), 2.41 (*m*, nH, mH, CH on the backbone), 1.45-1.95 (*m*, 2nH, 2mH, CH_2 on the backbone). n, m represented repeating unit number.

*Preparation of PS-*b*-PBnAA.* Into a 25 mL Schlenk flask containing DTB-PS-*b*-PBnAA (1.0 g, 0.06 mmol) was added AIBN (196 mg, 1.2 mmol) and THF (3.0 mL) in N_2 atmosphere. The mixture was maintained at 65 °C for 10 h. The light yellow solid was afforded via precipitating in ethyl ether in yield of 93% (0.92 g). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.15-7.26 (*m*, 13nH, $n\text{C}_6\text{H}_5$, 3mH, $m\text{C}_6\text{H}_5$), 6.49-6.60 (*m*, 2mH, $mm\text{-C}_6\text{H}_5$), 4.91-5.14 (*m*, 4nH, $2n\text{C}_6\text{H}_5\text{CH}_2$), 4.53 (*m*, nH, hydrogen on the C-4 of AA), 4.05-4.17 (*m*, 3nH, hydrogen on the C-5 and C-6 of AA), 2.40 (*m*, nH, mH, CH on the backbone), 1.43-1.95 (*m*, 2nH, 2mH, CH_2 on the backbone).

*Synthesis of amphiphilic copolymers PS-*b*-PAA.* Palladium on carbon (1.2 g) as catalyst was added into the polymer solution comprised of PS-*b*-PBnAA (0.9 g, 59.1mmol), anhydrous THF (40 mL) and methanol (25.0 mL). The benzyl deprotection was performed under normal H_2 pressure at 30 °C for 48 h, and then Pd/C catalyst was filtered off and washed with methanol. After complete evaporation of the combined filtrate, the solid was dissolved in a small amount of THF, and precipitated in cold ether (50.0 mL) to obtain grey white solid in yield of 85% (0.44 g). ^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.12 (*m*, 3mH, $m\text{C}_6\text{H}_5$), 6.57 (*s*,

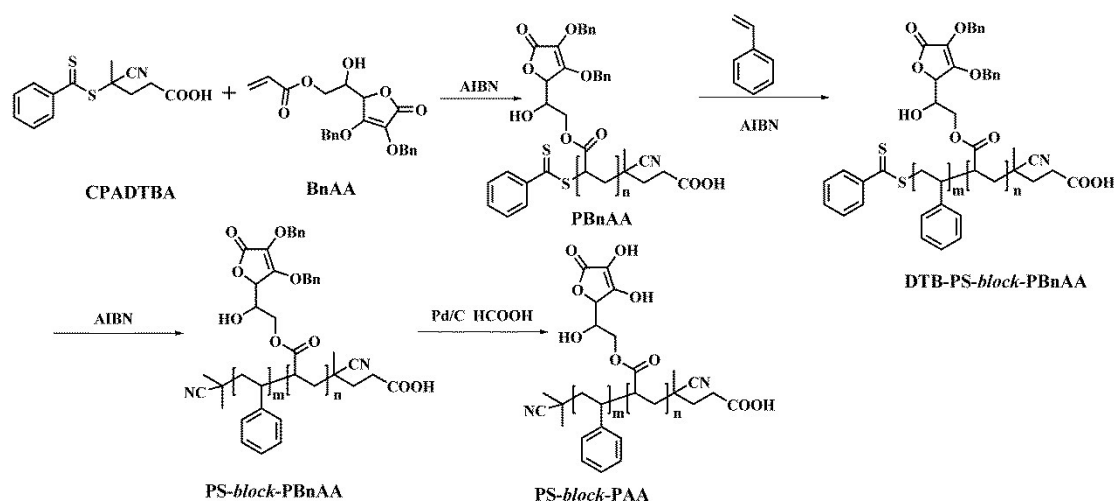
2mH, mC₆H₅), 4.52 (m, nH, hydrogen on the C-4 of AA), 4.01 (s, 3nH, hydrogen on the C-5 and C-6 of AA), 2.28 (m, nH, mH, CH on the backbone), 1.31-2.10 (m, 2nH, 2mH, CH₂ on the backbone).

Synthesis of biotin-PEG-*b*-PS. Three steps were included as followed. *Biotin-labeled polyethylene glycol (biotin-PEG-OH)*. The mixture of HO-PEG-NH₂ (5.2 g, 1.3mmol), biotin-NHS (0.6 mg, 1.8 mmol), Na₂CO₃ (0.1 mg, 1.3 mmol) and 80 mL DMF in 100 mL round-bottom flask was kept stirring at ambient temperature for 48 h. The product was purified by dialysis and freeze-drying. The white solid was obtained in yield of 90% (4.9 g). ¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.8 (s, 1H, CONH), 6.33-6.39 (d, 2H, NHCONH), 4.29 (s, 1H, CHNH), 4.13 (s, 1H, NHCH), 3.44-3.65 (m, 4mH, mOCH₂CH₂O), 3.17 (t, 1H, SCH), 3.09 (s, 1H, SCH), 2.78 (s, 1H, SHCH₂), 2.52 (s, 1H, SHCH₂), 2.06 (t, 2H, CONHCH₂), 1.24-1.60 (m, 6H, CH₂CH₂CH₂).

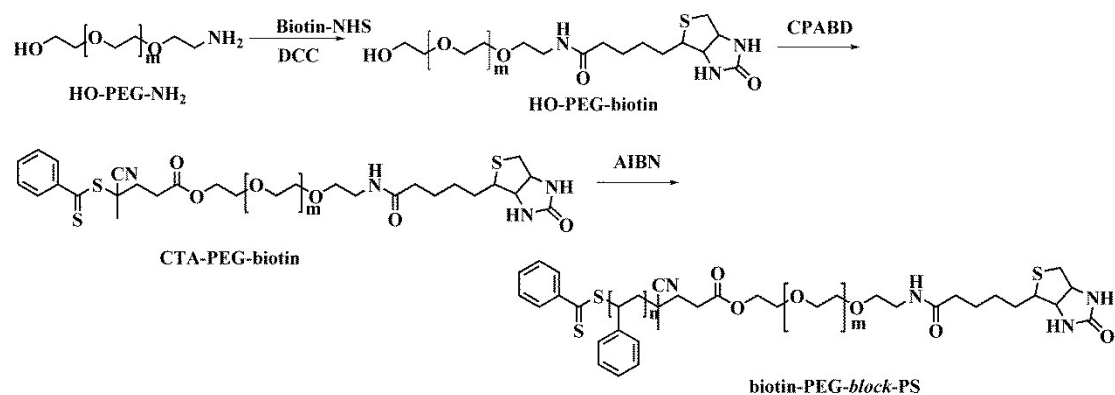
Preparation of biotin-labeled RAFT macroCTA (CTA-PEG-biotin). Into one 100 mL three-neck bottle containing HO-PEG-biotin (4.5 g, 1.1 mmol), DCC (0.9 g, 4.4 mmol), DMAP (0.06 mg, 0.5 mmol) and 80 mL CH₂Cl₂ was droplet added the solution of CPADTBA (0.9 mg, 3.3 mmol) in 20 mL CH₂Cl₂. The reaction was maintained at room temperature for 24 h. Then, the concentrated reaction solution was precipitated in ethyl ether, and the pink solid was obtained after vacuum in a yield of 71% (3.4 g). ¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.93 (s, 2H, *o*-C₆H₅), 7.81 (s, 1H, CONH), 7.68 (m, 1H, *p*-C₆H₅), 7.51 (s, 2H, *m*-C₆H₅), 6.33-6.39 (d, 2H, NHCONH), 4.31 (s, 1H, CHNH), 4.12 (s, 1H, NHCH), 3.39-3.67 (m, 4mH, mOCH₂CH₂O), 3.10 (s, 1H, SCH), 2.81 (s, 1H, SCH₂), 2.56 (s, 1H, SCH₂), 2.06 (t, 2H, CONHCH₂), 1.03-1.26 (m, 6H, CH₂CH₂CH₂). m represented repeating unit number.

*Preparation of biotin-labeled poly(ethylene glycol)-block-polystyrene (biotin-PEG-*b*-PS)*. Similar RAFT polymerization operation was conducted to fabricate biotin-PEG-*b*-PS-DTB except using styrene (1.3 g, 13.0 mmol) as monomer, AIBN (4.3 mg, 0.026 mmol) as initiator, DTB-PEG-*b*-PS (0.6 g, 0.13 mmol) and 3.0 mL toluene at 65 °C for 15 h. The reaction solution was precipitated into ethyl ether to afford pink polymer. Subsequently, the pink polymer dissolved in toluene was reacted with AIBN (0.4 g, 2.6 mmol) under N₂ atmosphere at 65 °C for 10 h. The solution was

precipitated in the ethyl ether for three times, and the slight yellow solid was afforded in yield of 49% (0.9 g). $^1\text{H NMR}$ (400 MHz, CDCl_3), δ (ppm): 7.74 (s, 1H, CONH), 6.85-7.30 (m, 3nH, nC_6H_5), 6.20-6.80 (m, 2nH, nC_6H_5), 3.39-3.67 (m, 4mH, $\text{mOCH}_2\text{CH}_2\text{O}$), 1.80-2.01 (m, nH, CH on the backbone), 1.30-1.50 (m, 2nH, CH_2 on the backbone).



Scheme S1. Synthesis route of PS-*b*-PAA.



Scheme S2. Synthesis route of biotin-PEG-*b*-PS.

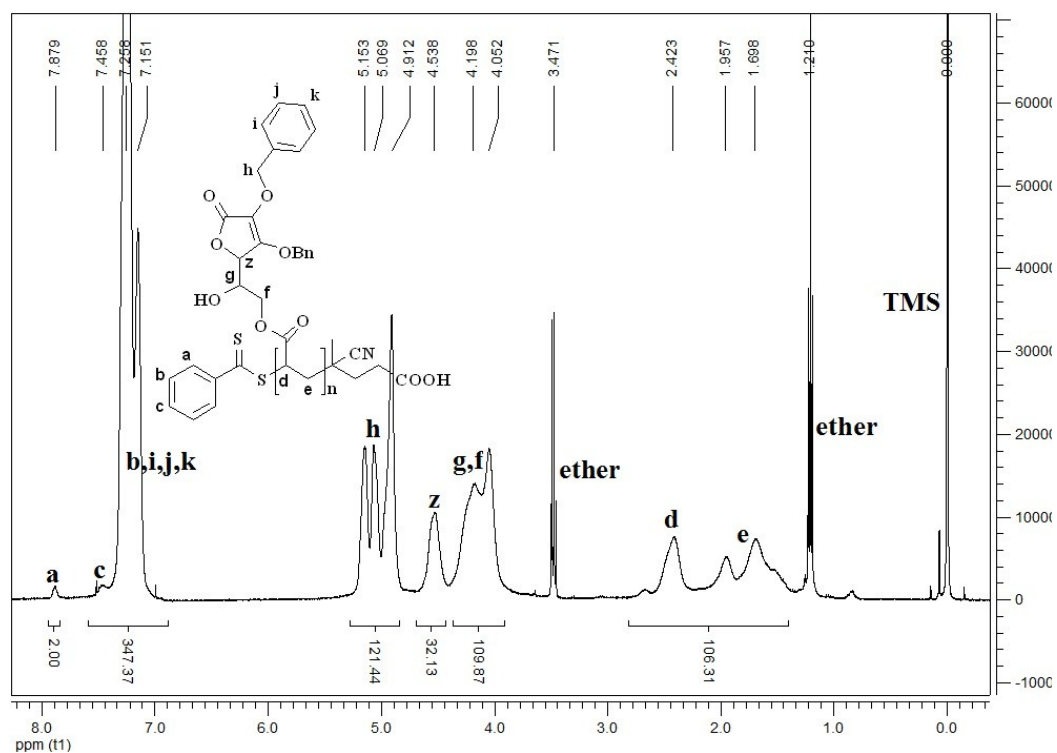


Fig. S1 ¹H NMR spectrum of PBnAA.

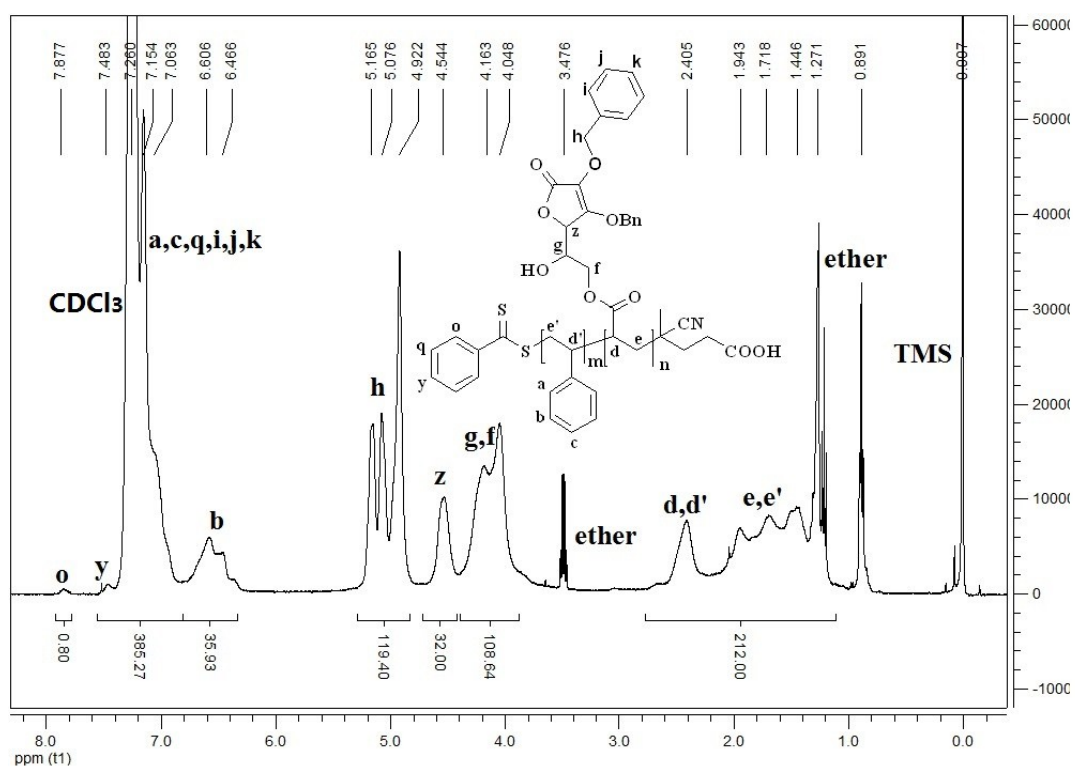


Fig. S2 ¹H NMR spectrum of DTB-PS-*b*-PBnAA.

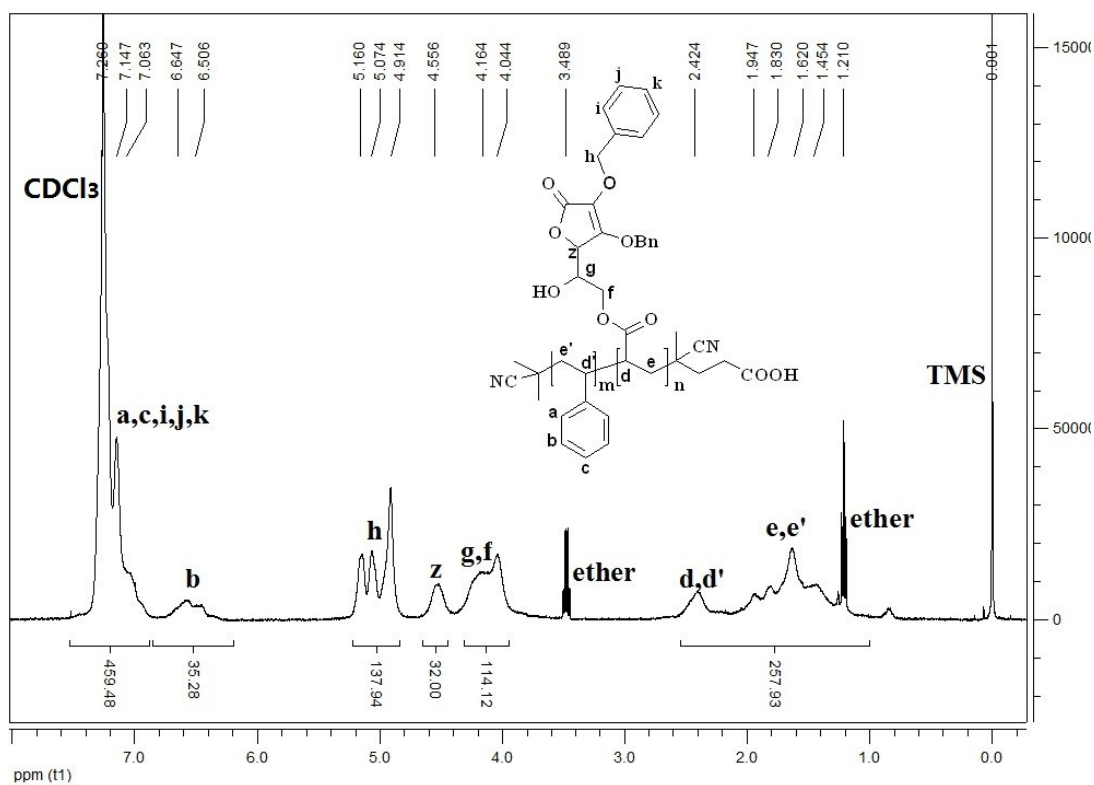


Fig. S3 ¹H NMR spectrum of PS-*b*- PBnAA.

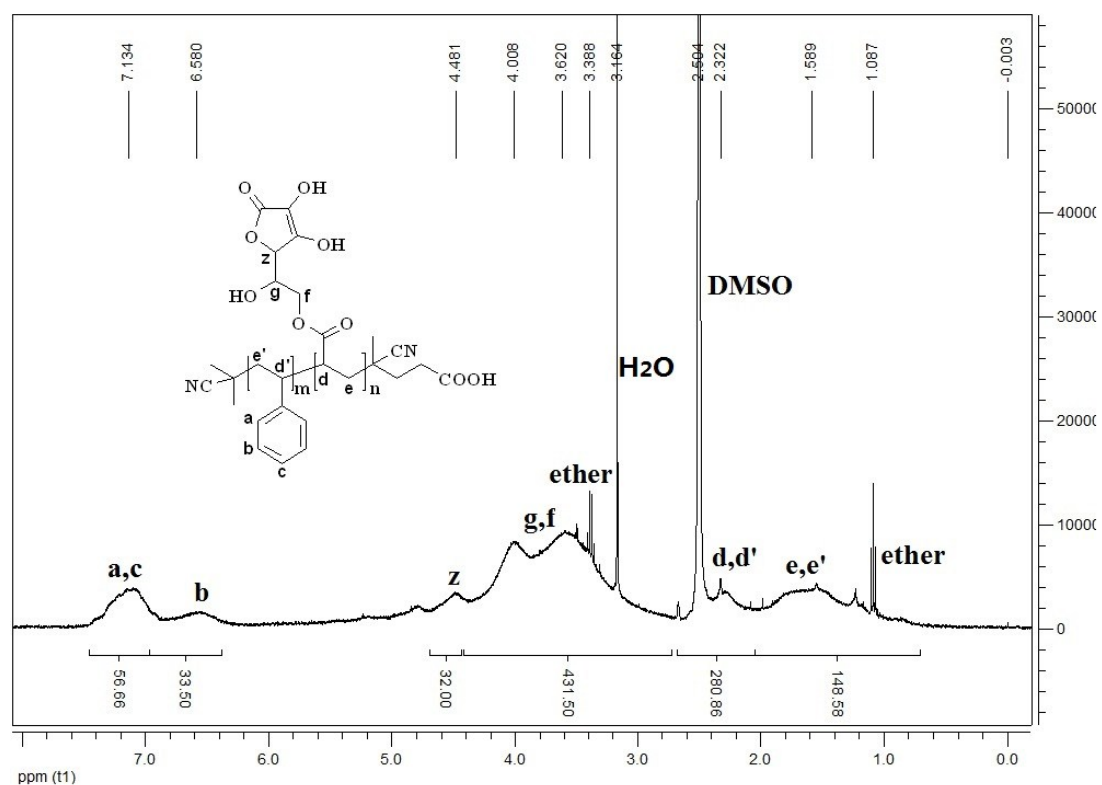


Fig. S4 ¹H NMR spectrum of PS-*b*-PAA.

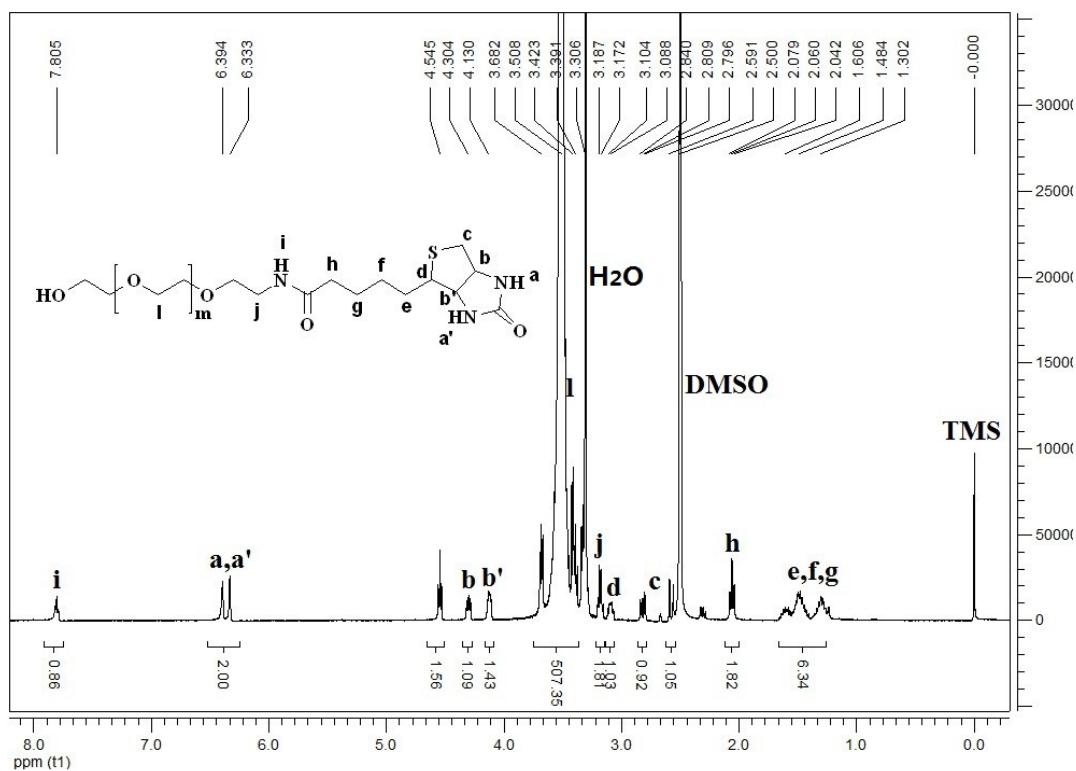


Fig. S5 ¹H NMR spectrum of HO-PEG-biotin.

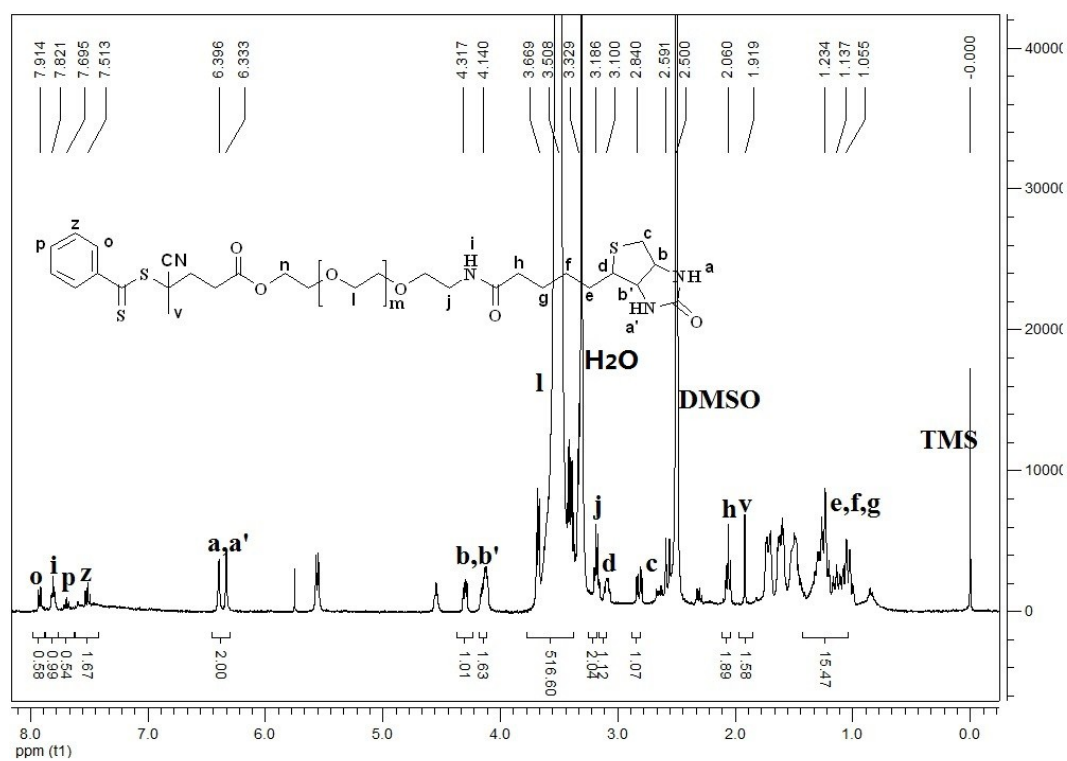


Fig. S6 ¹H NMR spectrum of CTA-PEG-biotin.

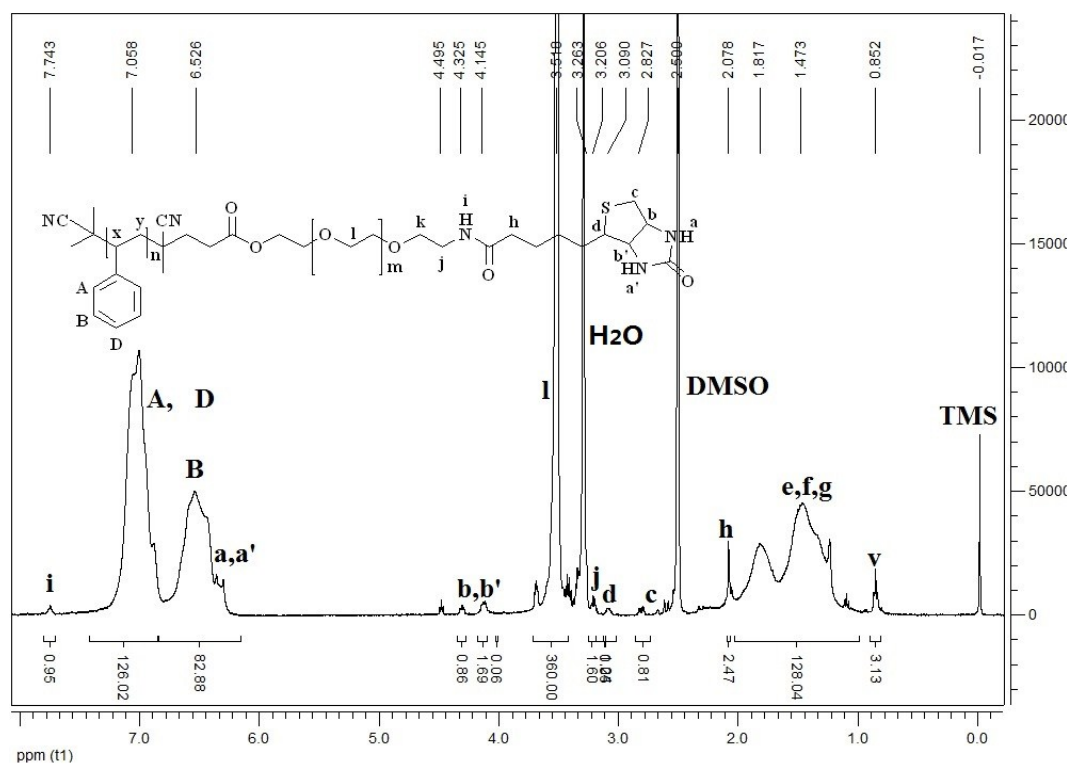


Fig. S7 ^1H NMR spectra of biotin-PEG-*b*-PS.

References:

- (1) Sprong, E.; Wet-Roos, D. D.; Tonge, M. P.; Sanderson, R. D. Characterization and rheological properties of model alkali-soluble rheology modifiers synthesized by reversible addition–fragmentation chain-transfer polymerization (pages 223–235). *J. Polym. Sci. Part A: Polym. Chem.* **2003**, *41*, 223-235.
- (2) Bai, R. K. You, Y. Z. Pan, C. Y. Study on controlled free-radical polymerization in the presence of dithiobenzoic acid (DTBA). *Polym. Int.* **2000**, *49*, 898-902.
- (3) Mark, B.; Ferdinanda C. M.; Jan C. M. Synthesis and self-assembly of well-defined elastin-Like polypeptide–poly(ethylene glycol) conjugates. *Biomacromolecules*, **2014**, *15*, 2751-2759.
- (4) Lee, J. K.; Sikes, H. D. Balancing the Initiation and Molecular Recognition Capabilities of Eosin Macroinitiators of Polymerization-Based Signal Amplification Reactions. *Macromol. Rapid Commun.* **2014**, *35*, 981-986.
- (5) Zhuang, D. Q.; Nie, J.; Yang, J. Poly(ascorbyl acrylate)s: Synthesis and Evaluation of Their Redox Polymerization Ability in the Presence of Hydrogen Peroxide. *J. Polym. Sci. Part A: Polym. Chem.* **2011**, *49*, 1999-2007.
- (6) Wang, Y. Z.; Yang, J. J.; Yang, J. Synthesis and self-Assembly of novel amphiphilic copolymers poly(lactic acid)-*block*-poly(ascorbyl acrylate). *J. Polym. Sci. Part A: Polym. Chem.* **2011**, *49*, 3988-3996.