Supporting Information for
Conventional Fluorophore-free Dual pH- and Thermo- Responsive
Luminescent Alternating Copolymer

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Experimental Section

Materials. 4-(Dimethylamino)pyridine (DMAP, 99%, Aldrich), p-toluenesulfonyl chloride (p-TsCl, 99%, Fluka), triethyleneglycol monomethyl ether (mTEG, 95%, Aldrich), maleimide (MI, 98%, Alfa Aesar), furan (98%,spectrochem), 4-vinylbenzyl chloride (4-VBC, 90%, Fluka), Boc-l-leucine (Boc-l-Leu-OH, 99%, Fluka), trifluoroacetic acid (TFA, 99%, SRL), HPLC water (SRL), triethylamine (TEA, 99%, Merck), sodium carbonate (99%, Merck) were used as received. 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%, Aldrich) was recrystallized from methanol twice and stored in refrigeration. 1,4-Dioxane (99%, Merck) was passed through basic alumina column prior to polymerization. 2-Dodecylsulfanylthiocarbonylsulfanyl-2-methyl-propionic acid (DMP) was synthesized according to the procedure reported previously.1 CDCl3 (99.8% D) and DMSO-d6 (99% D) were purchased from Cambridge Isotope Laboratories, Inc., USA for NMR study. Dry acetonitrile was prepared by following the literature procedure. Toluene, diethylether, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO),
chloroform, N-methyl-2-pyrrolidone (NMP) and 1,4-dioxane were purchased from Merck and used as received without any further purification. All other solvents such as dichloromethane (DCM), methanol, hexanes (mixture of isomers), ethyl acetate (EA) were purified by following standard procedures.

**Instrumentation.** Size exclusion chromatography (SEC) was used to obtain molecular weights and molecular weight distributions (dispersity, $D$) of polymers in THF solvent at 30 °C at 1.0 mL/min flow rate. The SEC instrument contains a Waters 515 HPLC pump, a Waters 2414 refractive index (RI) detector, one PolarGel-M guard column (50 × 7.5 mm) and two PolarGel-M analytical columns (300 × 7.5 mm). The instrument was calibrated by using poly(methyl methacrylate) (PMMA) standards. All the $^1$H, $^{13}$C, DEPT $^{13}$C NMR spectra were acquired in a Bruker Avance$^{III}$ 500 spectrometer operating at 500 MHz. Positive mode electrospray ionization mass spectrometry (ESI-MS) was performed on a Q-Tof Micro YA263 high resolution (Waters Corporation) mass spectrometer. UV-Vis spectroscopic study was monitored by a Perkin-Elmer Lambda 35 spectrophotometer. Fluorescence emission spectra were recorded on a Horiba JobinYvon (Fluoromax-3, Xe-150 W, 250–900 nm) fluorescence spectrometer. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out using dithranol as matrix on a Bruker UltrafleXtreme$^{TM}$ instrument equipped with a smart beam-II laser in the reflector mode and an acceleration voltage of 22 kV.
Scheme S1. Synthetic scheme for the preparation of N-(methoxy diethylene glycol) maleimide (M1).

**Synthesis of compound (2)**

5 g of triethylene glycol monomethylether (30.5 mmol), 10.61 mL of triethylamine (76.1 mmol) and 0.7 g of DMAP (6.09 mmol) were dissolved in 150 mL of DCM in a 250 mL round bottom flask. The system was cooled to 0 °C in an ice bath and 6.6 g of 4-toluenesulfonyl chloride (34.4 mmol) was added to the solution. After few minutes ice bath was removed and the reaction mixture was allowed to stir for 12 h. Then, the reaction mixture was washed with water, 1N HCl (2 × 100 mL), dried over Na$_2$SO$_4$ and the solvent was removed to obtain yellow liquid. Further purification of the yellow product was carried out via column chromatography using 30% EA/hexane mixture as eluent to give compound 2 with 75% yield. $^1$H NMR (CDCl$_3$, δ, ppm, Fig. S1): 2.42 (ArCH$_3$, 3H, s), 3.34 (-OCH$_3$, 3H, s), 3.5-3.7 (-O-CH$_2$-CH$_2^{-}$, 10H, m), 4.13 (-SO$_3$-CH$_2$-H, 2H, m), 7.33 (ArCH, 2H, d), 7.79 (ArCH, 2H, d).
Fig. S1 $^1$H NMR spectrum of 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate in CDCl$_3$.

Synthesis of compound (3)

Compound 3 was synthesized following the previously established procedure.$^2$ Specifically, 1.5 g of maleimide (15.4 mmol) and 2.25 g of furan (33 mmol) were dissolved in 15 mL diethyl ether in a sealed tube. The system was heated to 90-100 °C for 12 h. After completion of the reaction compound 3 was precipitated as white solid, which was collected through filtration. Then, the white solid product was washed with $3 \times 30$ mL diethyl ether to remove excess maleimide, obtaining the pure product as a white solid with a yield of 60%. $^1$H NMR (DMSO-$d_6$, $\delta$, ppm, Fig. S2): 11.1 (-NH, 1H, broad s), 6.53 (-CH=$\equiv$CH-, 2H, d), 5.11 (-COCH$\equiv$CH, 2H, d), 2.85 (-COCH, 2H, s).
Synthesis of compound (4)

1.9 g of compound 2 (5.97 mmol) was dissolved in 50 mL of dry acetonitrile in two-necked round bottom flask. To this solution 1.0 g of compound 3 (6.06 mmol) was added and the reaction mixture was stirred for few minutes until all the components were dissolved under N$_2$ atmosphere. Then, the system was allowed to reflux for 48 h at 50 °C. Completion of the reaction could be monitored by TLC (thin layer chromatography) and the best way to see the product spot in a TLC plate is to use potassium permanganate based stain. After the reaction reached completion solvent acetonitrile was substituted with ethyl acetate and the reaction mixture was washed with water (2 × 50 mL) and brine solution (2 × 50 mL). The yellow organic layer was dried over anhydrous Na$_2$SO$_4$ and the crude yellow product was purified via liquid chromatography using ethyl acetate as eluent. Compound 4 obtained as yellow viscous liquid with 50% yield. $^1$H NMR (CDCl$_3$, δ, ppm, Fig. S3): 6.50 (-CH=CH-, 2H, d), 5.22 (-COCHCH, 2H, d), 3.77 (-NCH$_2$, 2H, m), 3.5-3.65 (-O-CH$_2$-CH$_2$-, 10H, m), 3.33 (-OCH$_3$, 3H, s), 2.83 (-COCH, 2H, s).
Synthesis of compound (5) (M1)

Compound 5 was synthesized via retro-Diels-Alder reaction. 2 g of compound 4 (6.42 mmol) was dissolved in 50 mL of toluene and the reaction mixture was heated to 120 °C for 12 h. After evaporating the solvent, the crude product was purified through liquid chromatography using 75% EA/hexanes mixture as eluent. The pure product, compound 5 obtained as a light yellow liquid with 82% yield. $^1$H NMR (CDCl$_3$, δ, ppm, Fig. S4):6.67 (-C=H=C-, 2H, d), 3.46-3.77 (-NC$_2$H$_4$-O-C$_2$H$_4$-C$_2$H$_3$-, 12H, m), 3.34 (-OC$_3$H$_3$, 3H, s).
**Fig. S4** $^1$H NMR spectrum of compound 5 (M1) in CDCl$_3$.

**Fig. S5** ESI-MS spectrum of compound 5 (M1).

**Scheme S2.** Synthetic scheme for the preparation of Boc-protected leucine appended styrenic monomer (M2) and PP1-PP5.
Synthesis of Boc-protected leucine appended styrenic monomer (M2)

Styrene conjugated leucine was synthesized using previous mentioned procedure. Briefly, to a 250 mL round bottom flask 4.62 g of Boc-L-Leu-OH (20 mmol), 3.05 mL of 4-vinylbenzyl chloride (20 mmol) and 1.05 g of sodium carbonate (10 mmol) were added in 150 mL DMF containing 1% of water. The reaction mixture was allowed to stir for 24 h at 45 °C. Complete consumption of the starting material was monitored by TLC. Then, DMF was evaporated under reduced pressure and 100 mL H₂O was added to the system. Thereafter, organic layer was extracted with ethyl acetate (2 × 50 mL) and dried over anhydrous Na₂SO₄. Further purification of the product was done by column chromatography using 10% EA/hexanes mixture as eluent and product obtained as a colourless liquid with 76% yield. ¹H NMR (CDCl₃, δ, ppm, Figure S6): 7.40 (ArCH, 2H, d), 7.30 (ArCH, 2H, d), 6.71 (-CH=CH₂, 1H, dd), 5.76 (-CH=CH₂, 1H, t), 5.27 (-CH=CH₂, 1H, d), 5.14 (-OCH₂, 2H, AB pattern), 4.88 (-NH, 1H, d), 4.35 (-CH-CH₂-CH(CH₃)₂, 1H, br), 1.33-1.75 (-CH-CH₂-CH(CH₃)₂, 3H, m), 1.43 (-O-C(CH₃)₃, 9H, s), 0.92 (-CH(CH₃)₂, 6H, d).

Fig. S6 ¹H NMR spectrum of M2 in CDCl₃.
Procedure for the synthesis of alternating copolymers, PP1-PP5. Typically, M1, M2, DMP, AIBN and 1, 4-dioxane were sealed in a 20 mL reaction vial equipped with a magnetic stir bar at different feed ratio of 20/40/60/80/100 : 1 : 0.5 ([M1+M2] : DMP : AIBN) and the resulting solutions were purged with dry nitrogen for 20 min. Then, the vials were placed in a preheated polymerization block at 70 °C for 24 h. After 24 h, polymerization reactions were quenched by removing the vials from heating block and placed in an ice bath. Then, the resulting polymers were isolated by precipitation with acetone/hexanes mixture. Molecular weights and dispersity (D) values of all the resulting polymers are summarized in Table 1 in the main manuscript.
Deprotection of alternating copolymers, PP1-PP5 with TFA. To a 20 mL reaction vial, 0.1 g of polymer (PP1-PP5) was dissolved in minimum amount of DCM and 1.0 mL TFA was then added to the polymer solution. The system was allowed to stir for 4 h at room temperature. After 4 h, the resulting reaction mixture was precipitated four times in diethyl ether. Finally, the isolated deprotected polymers (DP1-DP5) were dried under vacuum at 35 °C for 12 h.

Fig. S8 \(^1\)H NMR spectrum of PP4 in CDCl\(_3\).

Fig. S9 \(^1\)H NMR spectra of PP4 in CDCl\(_3\) (A) and DP4 in acetone-\(d_6\) (B).
Fig. S10 UV-Vis spectra of PP2 in THF and DP2 in both THF and water.

Fig. S11 Simulated absorption spectra at UV-Vis region for the PP2 in THF (A) and DP2 in water (B).

Fig. S12 HOMO (A) and LUMO (B) energy levels of PP2 in THF. Green: nitrogen atom; red: oxygen atom; grey: carbon atom; cyan: hydrogen atom.
**Fig. S13** HOMO (A) and LUMO (B) energy levels of DP2 in water. Green: nitrogen atom; red: oxygen atom; grey: carbon atom; cyan: hydrogen atom.

**Fig. S14** Fluorescence spectra of PP2 in different solvents.

**Fig. S15** Variation of transmittance against pH for DP1 at 1.0 mg/mL concentration.

**References**

