Supplementary Information

Dual Responsiveness of a Tunable Thermosensitive Polypeptide

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Experimental

Materials.
l-(+)-Glutamic acid 99% minimum was purchased from EMD Chemicals (Gibbstown, NJ). 2-(2-azidoethoxy)ethanol (EO_2),[1] and *N*-(2-azidoethyl)-*N*-isopropylpropan-2-amine (diisopropylamine)[2] were synthesized as previously reported. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). All materials were used as received.

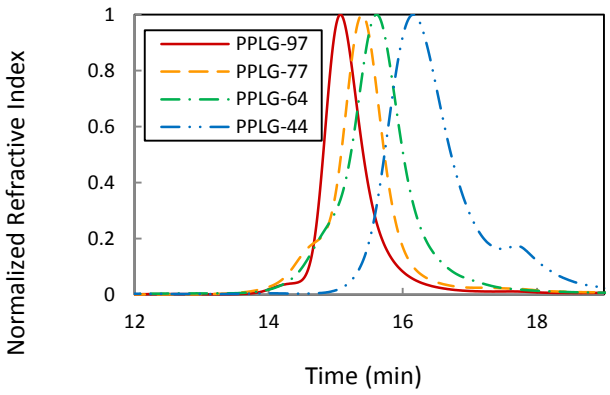


Fig. S1: Normalized GPC traces of PPLG backbones

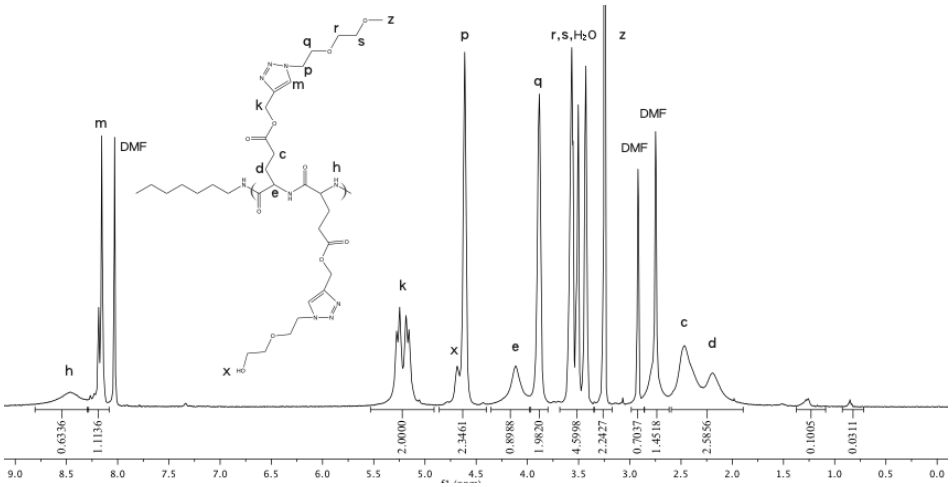
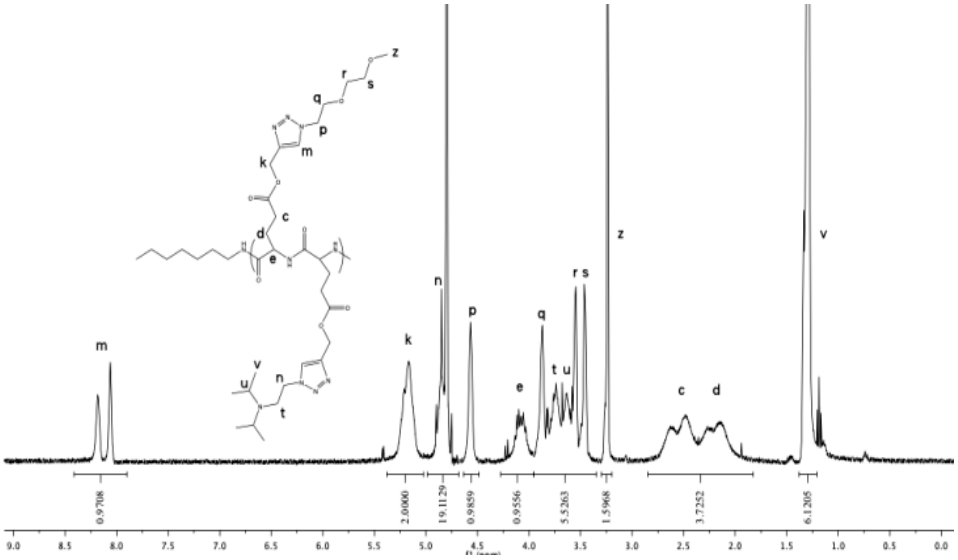


Fig. S2a: ^{1}H-NMR spectrum PPLG backbone substituted with 75% mEO_2 and 25% EO_2 in [d7] DMF.



Feed Ratio Substitution				^{1}H NMR-based Substitution			
Azide 1		Azide 2		Azide 1		Azide 2	
100%	diisopropylamine	-	-	100%	diisopropylamine	-	-
50%	diisopropylamine	50%	mEO_2	50%	diisopropylamine	50%	mEO_2
10%	diisopropylamine	90%	mEO_2	11%	diisopropylamine	89%	mEO_2
50%	diisopropylamine	50%	EO_2	53%	diisopropylamine	47%	EO_2

Fig. S2b: ^{1}H-NMR spectrum PPLG backbone substituted with 50% mEO_2 and 50% diisoproylamine in D_2O and list of diisopropylamine functionalized PPLG-64 polymers synthesized for communication.

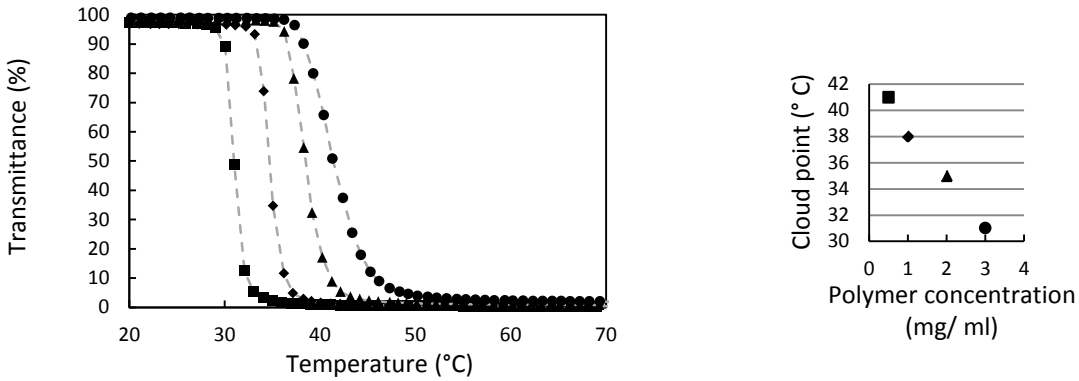


Fig. S3: Influence of temperature on the light transmittance (500 nm, heating 1° C min^{-1}) of PPLG-64 fully substituted with mEO_2 polymers in deionized water at concentrations of ■ 0.5 mg/ ml, ◆ 1 mg/ ml, ▲2 mg/ ml, and ● 3 mg/ ml. (inset: effect of concentration on cloud point)

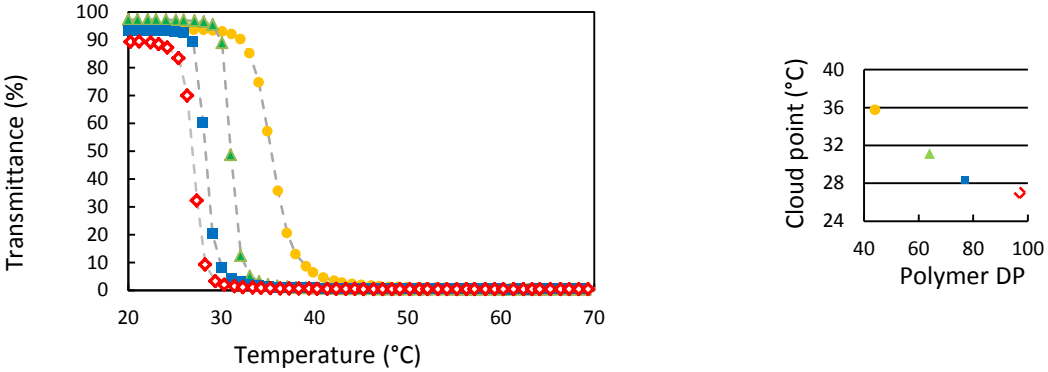


Fig. S4: Influence of temperature on the light transmittance (500 nm, heating 1° C min^{-1}) of PPLG polymers having degree of polymerization of ●44, ▲64, ■77, and ◊97 fully substituted with mEO_2 at 3 mg/ ml in deionised water.

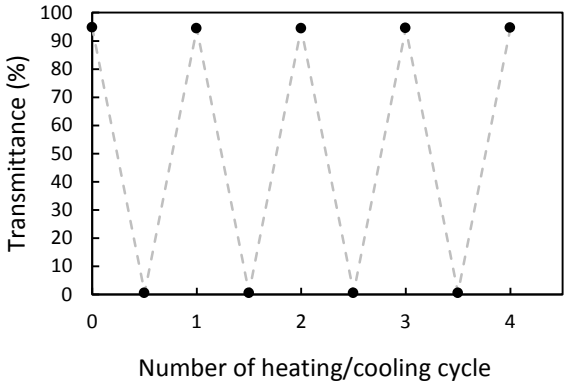


Fig. S5: Influence of cycling temperature across 4 heating and cooling cycles from 20° C to 40° C on the light transmittance of PPLG-64 fully substituted with mEO_2 (500 nm, heating 10° C min^{-1} measurement after 3 min at target temperature) at 3 mg/ ml in deionized water.

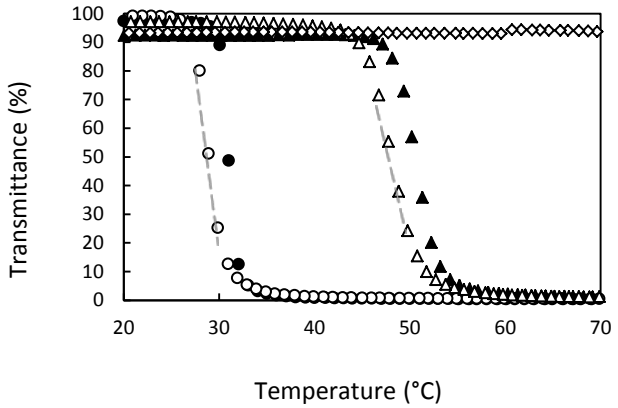


Fig. S6a: Influence of temperature on the light transmittance (500 nm, heating 1° C min^{-1}) of representative PPLG polymers at 3 mg/ ml in deionised water. Figure shows the heating (filled shapes) and cooling (open shapes) traces of four polymers having alkyne groups grafted with the specified percentage EO_2 and the remaining groups with mEO_2: ● 0% EO_2 PPLG-64, ▲39% EO_2 PPLG-64, ■ 100% EO_2 PPLG-97. The reported temperature is that of the UV Vis heating block which, as measured by an external temperature probe, lags the temperature of the polymer solution by 1.6° C ±0.2° C, partially contributing to observed hysteresis. The dotted lines represent the linear fit of the heating traces, as reported in Fig. S6b.

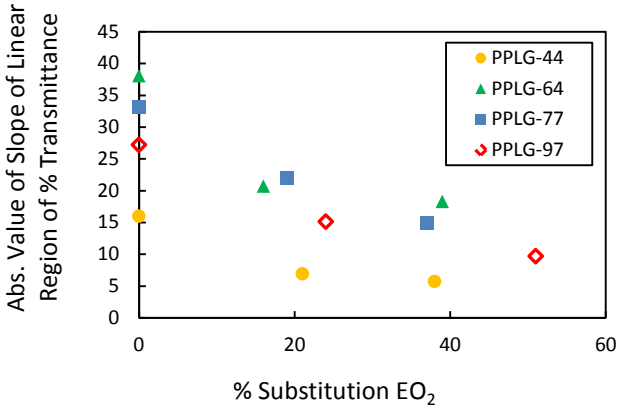


Fig. S6b: Effect of the percent EO_2 groups grafted onto mEO_2 grafted PPLG backbones on the absolute value of the slope of the linear region of the percent transmittance traces used to generate the cloud point values reported in Fig. 1 (500 nm, heating 1° C min^{-1}, 3 mg/ ml in deionized water). A representative linear region is traced with a dotted line for the two thermoresponsive polymers in Fig. S6a. A greater slope indicates more discreet thermosensitivity. All four PPLG backbones show more gradual temperature responsiveness with increasing substitution of EO_2.

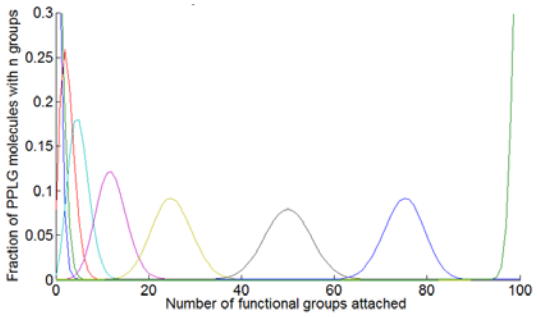
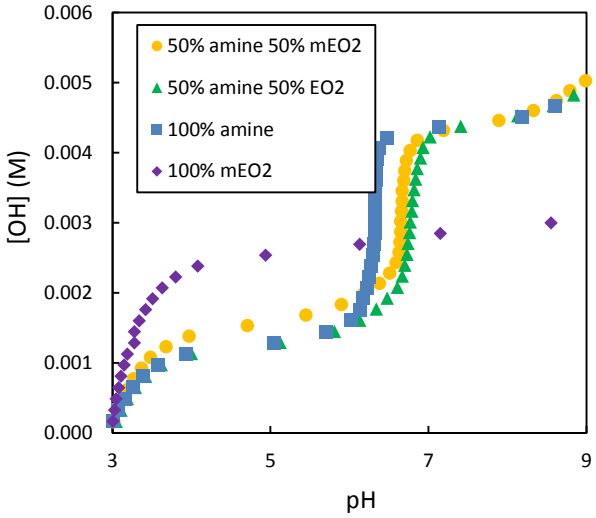


Fig. S6c: Theoretical model of the stochastic functionalization of a monodisperse PPLG backbone, degree of polymerization = 100, with incomplete grafting of a single functional group, where 50 percent substitution shows the greatest variance. In the experimental system of PPLG grafted with mEO_2 and EO_2, this increased polydispersity of functionalized backbones approaching equal substitution of the two grafted groups, results in more gradual temperature responsiveness.

% EO_2 by NMR	Cloud point in water (°C)	Cloud point in PBS (°C)	Difference in cloud point (°C)
0	31	28	3
16	39	34	5
39	51	42	9

Table S1: Comparison of cloud points measured in distilled water and PBS of PPLG-64 at 3 mg/ml substituted with mEO_2 and the indicated percent EO_2 (heating 1° C min^{-1}).

a.



b.

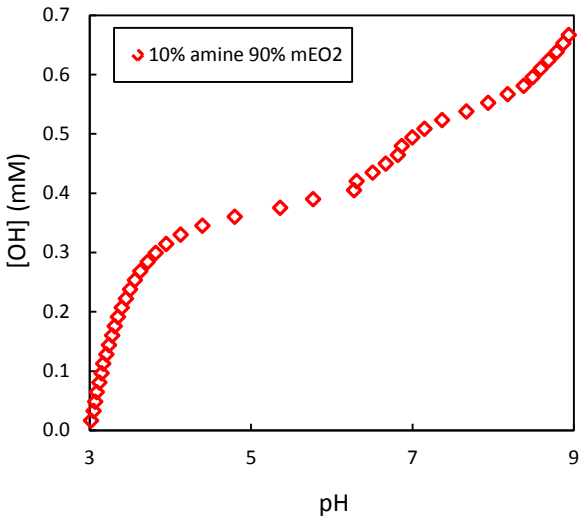


Fig. S7: Titration of grafted PPLG-64 with sodium hydroxide. Legend identifiers correspond to polymers detailed in Table S1. Titration of diisopropylamine mEO_2 graft PPLG was performed on 3 ml solution in 125 mM NaCl adjusted to pH 3. Fig. S7a shows solution of 2.5 mM amine titrated with 5 µL aliquots of 0.1 M NaOH. Fig. S7b shows titration of PPLG graft 11% diisopropylamine performed at 0.25 mM amine with 5 µL aliquots of 0.01 M NaOH.

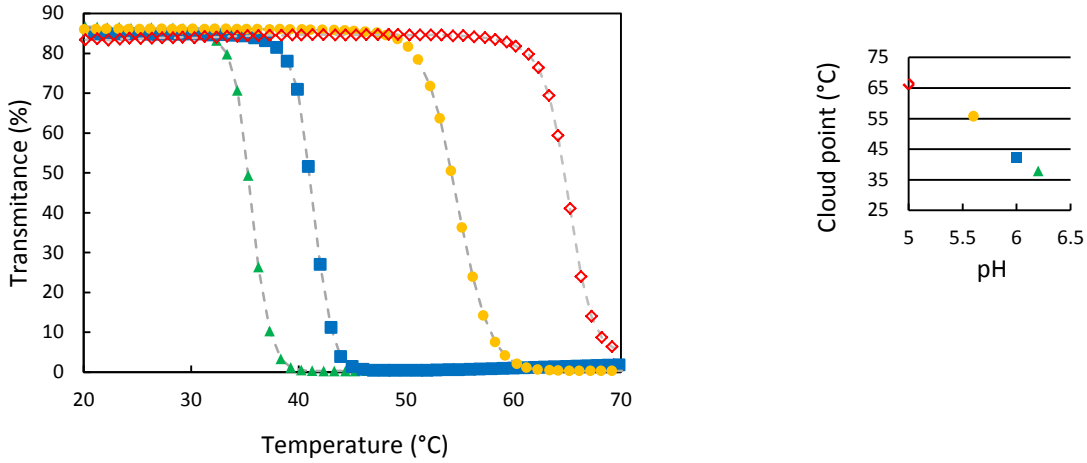
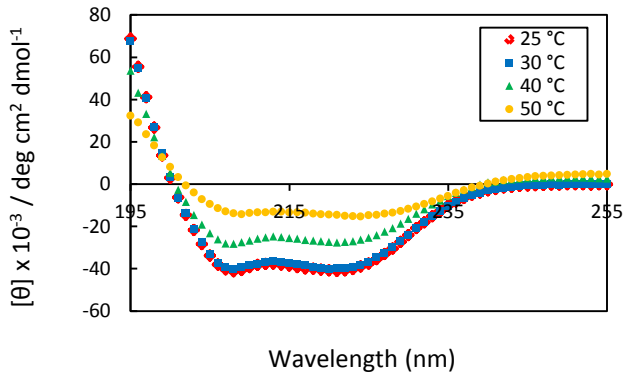


Fig. S8: Influence of pH on the temperature-dependent light transmittance (500 nm, heating 1 °C min^{-1}) of PPLG-64 graft 100% diisopropylamine, 1 mg/ml in 100mM NaCl, 75mM phosphate buffer ◊pH 5.0, ●pH 5.6. ■pH 6.0, and ▲pH6.2.

a.



b.

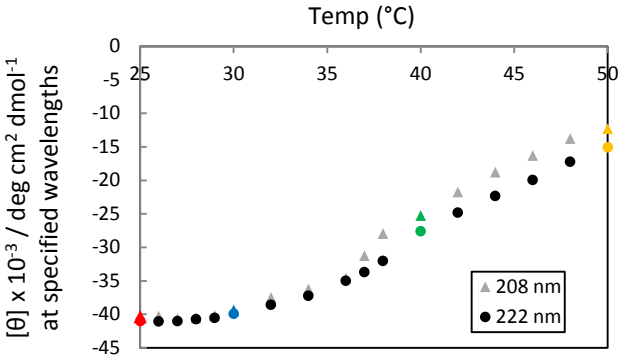
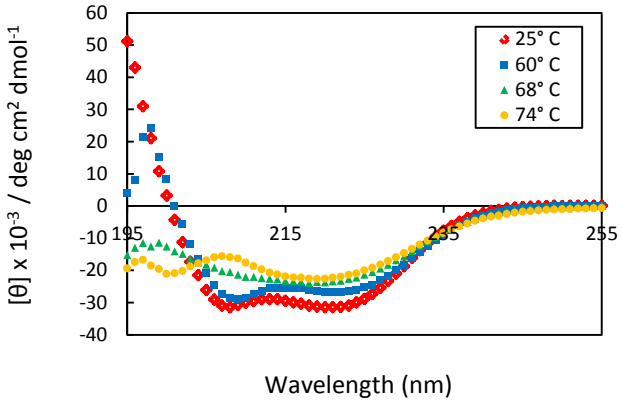


Fig. S9a and Fig. S9b: Representative temperature dependent circular dichroism spectra of PPLG substituted with only mEO_2 or EO_2. This spectrum is of PPLG-97 graft mEO_2 in distilled water at 1 mg/ ml. Fig. S9a shows representative screens of spectra recorded for a manual temperature screen from 25° C to 50° C, allowing the solution to equilibrate at least 2 minutes between measurements. Fig. S9b shows the consistent relative magnitude of α-helical characteristic minima at 208 nm and 222 nm, the absolute magnitude of which begins to decrease around 27 °C, the cloud point reported for this polymer in Fig. 1. The decreased signal intensity is attributed to decreased effective concentration caused by thermo-induced polymers precipitation.

a.



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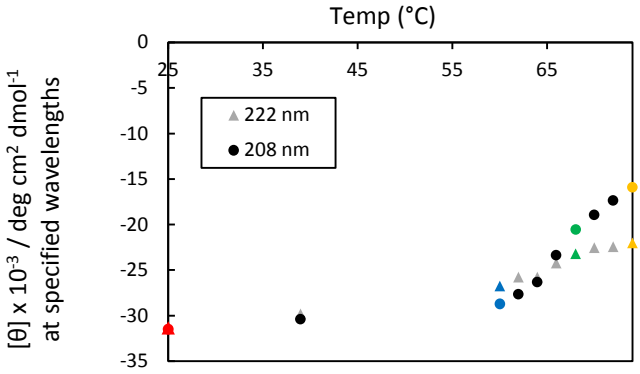

Fig. S10a and Fig. S10b: Representative temperature dependent circular dichroism spectra of PPLG substituted with 100% or 50% diisopropylamine. This spectrum is of PPLG-64 graft 50% diisopropylamine and 50% mEO_2 in 100 mM NaCl, 75mM phosphate buffer pH 5.6 at 1 mg/ ml. Fig. S10a shows representative screens of spectra recorded for a manual temperature screen from 25 °C to 74 °C, allowing the solution to equilibrate at least 2 minutes between measurements. Fig. S10b shows the decrease in magnitude of alpha helical characteristic minima at 222 nm relative to 208 nm, the absolute magnitude of which begins to decrease around 60 °C, the cloud point reported for this polymer in Fig. 4. The decreased signal intensity and associated increase in intensity at 208 nm is attributed to a transition of the polymer from purely α-helical to a mixture of α-helical and random coil secondary structure.

(1) Sinha, J.; Sahoo, R.; Kumar, A. *Macromolecules* **2009**, *42*, 2015-2022.
(2) Engler, A. C.; Bonner, D. K.; Buss, H. G.; Cheung, E. Y.; Hammond, P. T. *Soft Matter* **2011**, *7*, 5627-5637.