

Supporting data

Non monotonous variation of the LCST of light-responsive, amphiphilic poly(NIPAM) derivatives.

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I. Synthesis of the parent polymer (Poly(EGMA_n-co-NAS_m)) by free radical chain transfer polymerization.

Monomers dissolved in THF at ca. 10 wt% were purified by elution through a basic aluminium oxide column and the solvent was removed in a rotatory evaporator. AIBN (38 mg, 0.24 mmol) was added to a 50 ml two-neck round-bottom flask and was degassed by three pump-purge cycle with N₂. NAS (5.0 g, 29 mmol) was dissolved in 20 mL 1,4-dioxane, and then added to EGMA (2.5 g, 5.2 mmol) and 3-MPA (0.15 g, 1.4 mmol). After bubbling with N₂ for 30 min, the mixture was transferred to the two-neck round-bottom flask containing AIBN via a double-ended needle. The reaction mixture was then heated to 60°C under N₂ with gentle stirring. After heating for 6 h, the viscous crude was cooled to room temperature, diluted with THF (20 mL) and precipitated into diethyl ether, and finally dried overnight under vacuum. Dissolving in 1,4-dioxane (20 mL) and precipitating again in diethyl ether yielded the parent polymer Poly(EGMA_n-co-NAS_m) as a white solid after drying overnight under vacuum (6.8 g, 91% yield). The polymer composition was characterized by ¹H NMR in CDCl₃. Its molar mass was obtained from size exclusion chromatography (SEC) in aqueous LiNO₃ 0.5 M at 35°C, after making the polymer water soluble by hydrolysis of NAS monomers in excess ethanolamine (overnight) and dialysis (48h against water, Slide-A-Lyzer MWCO 3500). SEC apparatus was a Viscotek GPCMax VE2001 GPC solvent/sample module equipped with a Viscotek model 2501 UV detector and a Viscotek TDA 302 triple detector array for absolute determination of molar masses.

II. Synthesis of photo-responsive polymers.

The “C6azo” amine was synthesized as described in previous papers (Pouliquen et al., *Macromolecules* (2006) 39, 373-383). “C6azo” under its ammonium chloride form (2.0 mg, 4.9 mg, 14.8 mg, 19.8 mg, 24.7mg for 2%, 5%, 15%, 20% and 25% grafting, respectively.) was dissolved in DMSO (0.5 mL). Then poly(EGMA_n-*co*-NAS_m) (66mg, 290 μmol of monomers) in DMSO (0.5 mL) was added, and the reaction bath was brought to 40°C for 2h. After cooling down to room temperature, an excess isopropylamine (60 - 100 μL) was added to react overnight at room temperature with the remaining NHS functional groups. The crude was mixed with an equal volume of water and dialyzed for 24h against water (3 changes of the bath, in Slide-A-Lyzer MWCO 3500, Pierce) to remove DMSO. The polymers were recovered by freeze-drying as yellow solids and characterized by UV-Visible spectrophotometer, ¹H NMR, dynamic light scattering (DLS) measurements.

III. NMR spectra of the parent chain and azobenzene-modified terpolymers.

¹H NMR spectra of polyNIPAM-modified chains were recorded in MeOD or D₂O on a Bruker DPX-400 operating at 400 MHz. The molar fraction of EGMA and NAS in the parent polymers Poly(EGMA_n-*co*-NAS_m) was determined from its ¹H NMR in CDCl₃ from the overall integration of the methoxy protons of the EGMA (3.4ppm, s, CH₃-O-) to the overall integration of the 4 protons of NAS (2.8ppm, m, -CH₂-CON-).

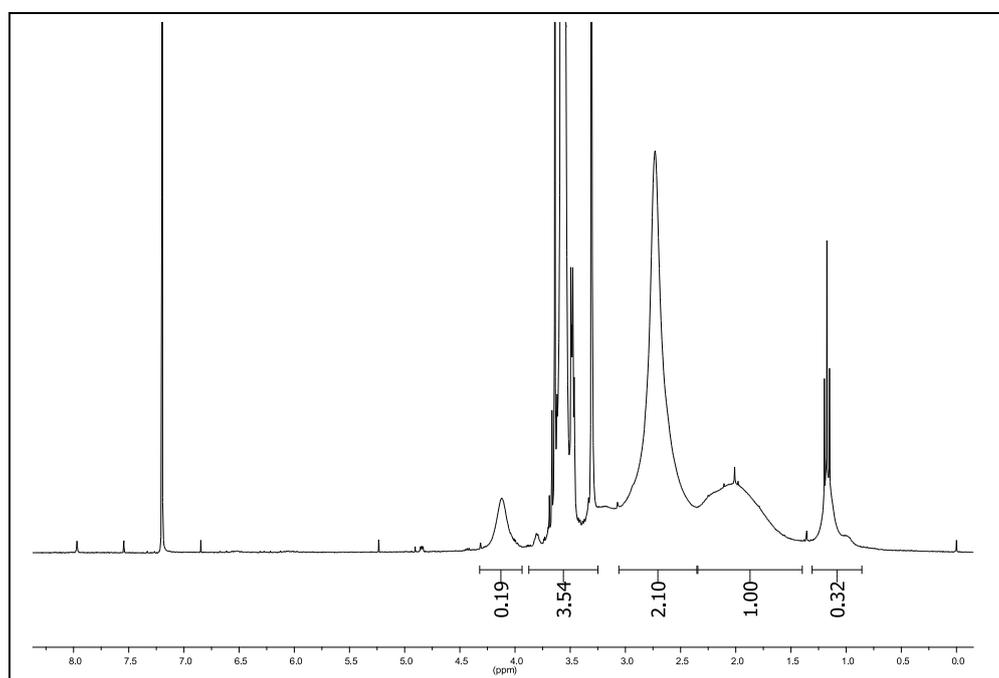


Figure S1: ¹H NMR spectrum in CDCl₃ of the parent copolymer made of *N*-acryloxysuccinimide (NAS) and oligo(ethylene glycol) methyl ether methacrylate (EGMA). The NAS:EGMA ratio was determined from the integration accounting for four protons in the *N*-hydroxysuccinimide group (~2.6-2.8 ppm) and the protons in the ethoxy moieties in EGMA (3.5 ppm, -(CH₂CH₂O)₈₋₉-, and 4.2ppm -CH₂-OCO-), yielding here a molar fraction of 83 ± 2 mol% NAS.

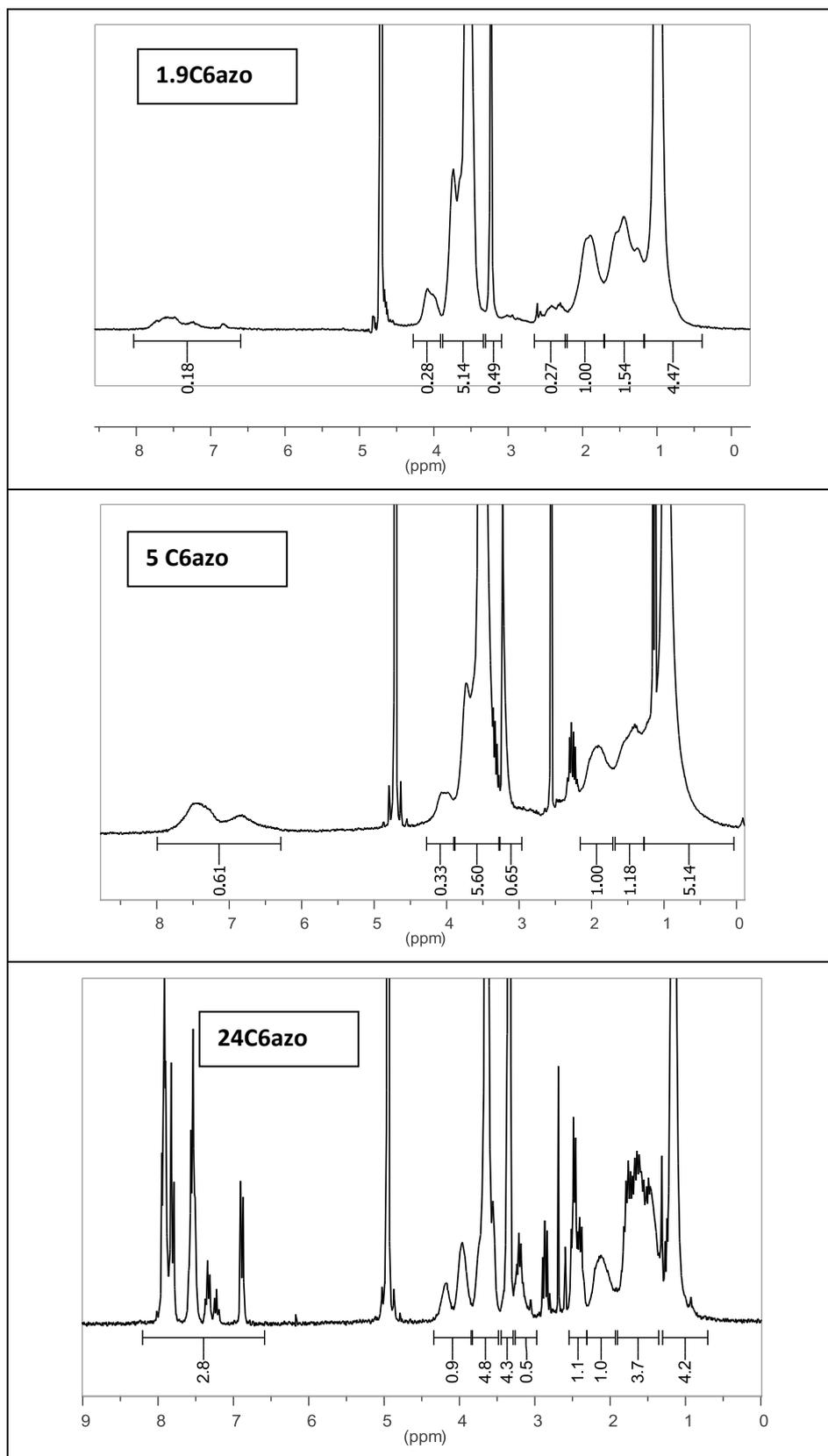


Figure S2: Representative ^1H NMR spectra in MeOD of azobenzene-modified parent copolymer yielding the terpolymers PNIPAM-co-EGMA- τ azo. ^1H NMR (MeOD, δ ppm): 0.9 ($-\text{CH}(\text{CH}_3)_2$), 1.0-1.6 ($-\text{CH}_2-$ backbone and Me- from methacrylate), 1.7-2.1 ($-\text{CHCOR}-\text{CH}_2-$ backbone), 2.1-2.5 ($(-\text{NHCH}_2-(\text{CH}_2)_3-\text{CH}_2\text{CO}-)$), 2.2-2.6 ($(-\text{NHCH}_2-(\text{CH}_2)_3-\text{CH}_2\text{CO}-)$), 3.2 ($(-\text{CH}_2\text{CH}_2\text{O})_{8,9}-\text{CH}_3$), 3.3-4.2 ($\text{COO}-(\text{CH}_2\text{CH}_2\text{O})_{8,9}-\text{CH}_3$), 6.5-8.0 (aromatic protons from azobenzene).

IV. Determination of the fraction of azobenzene in the terpolymers.

Upon increasing the degree of integration of azobenzene, the resolution was gradually lost and peaks overlapped in the window 1.0-3.0 ppm (cf “24C6azo” in Figure S2). NMR estimates of the fraction of azobenzene in the chain were accordingly based on the ratio between aromatic protons and CH₂ from the oligo(ethyleneoxide) moieties.

An additional estimate of the degree of integration of azobenzene was obtained from absorbance measurements using the molar extinction coefficient of $2.32 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 350 nm for the N-(4-phenylazophenyl)hexanamide. Results are summarized in Table S1 below.

Table S1. Composition of the photoresponsive terpolymers (PNIPAM-co-EGMA- τ azo).

τ used in polymer's name	Measured fraction of azobenzene (mol %)	
	DO	NMR
0	-	-
2	1.9	2.2
5	5.4	7
11	11	13
17	17	25
20	20	n.d.
24	24	30

n.d.: not determined

V. Determination of LCST at varying polymer concentrations.

To determine the possible effect of concentration on apparent LCST, UV-Vis. spectra of polymer solutions at increasing concentrations were recorded as a function of temperature in experimental conditions identical to those described in the main text. During the experiments, the samples were vertically and continuously irradiated under blue light (436 ± 10 nm, quoted “blue” in Figure S3) or UV light (365 ± 10 nm, quoted “UV”). The LCST given in Table S2 correspond to linear extrapolations down to the baseline within a 5°C window above the onset of increasing absorbance.

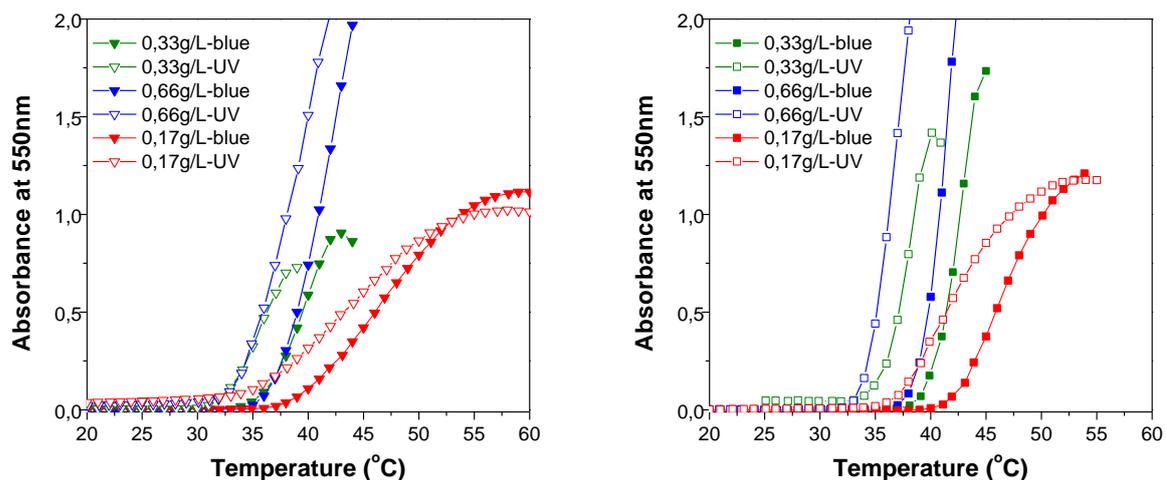


Figure S3. Variation upon temperature upscan (rate $0.2^\circ\text{C}\cdot\text{min}^{-1}$) of absorbance at 550 nm of solutions of azobenzene-modified terpolymers in PBS $1 \times$ buffer; (left) PNIPAM-co-EGMA-11azo and (right) PNIPAM-co-EGMA-17azo. Concentrations are quoted in figures.

Table S2. LCSTs extrapolated from data in Figure S3

Concentration ($\text{g}\cdot\text{L}^{-1}$)	LCST ($^\circ\text{C}$)			
	11azo (Blue)	11azo (UV)	17azo (Blue)	17azo (UV)
0.17	38	33	41	37
0.33	36	32	40	36
0.66	36	32	39	34

VI. AFM imaging of interpolymer clusters.

Solutions of PNIPAM-*co*-EGMA-11azo, or PNIPAM-*co*-EGMA-17azo, in water at 0.0001 g L^{-1} were prepared by dilution in milliQ water of the samples used for LCST measurements. They were immediately used or irradiated under UV light ($365 \pm 10 \text{ nm}$ at a power of 1 mW/cm^2) with the photodiode system CoolLED PE-2 (Roper Scientific France) for 20 min. To adsorb the polymers on mica, aliquots ($3 \mu\text{L}$) of the solution (with or without UV treatment) were deposited on freshly cleaved mica plate (Potassium Aluminosilicate, Muscovite Mica, from Goodfellow) and dried under vacuum for 2 h in the dark. AFM measurements were performed in a 5100 Atomic Force Microscope (Agilent Technologies-Molecular Imaging) operated in the dynamic tip deflection mode (Acoustic Alternating Current mode, AAC at 69 kHz) and fitted with a silicon probe (Applied NanoStructures-FORT) of spring constant of 3 N m^{-1} . Images were scanned in topography mode with a resolution of 512×512 pixels.

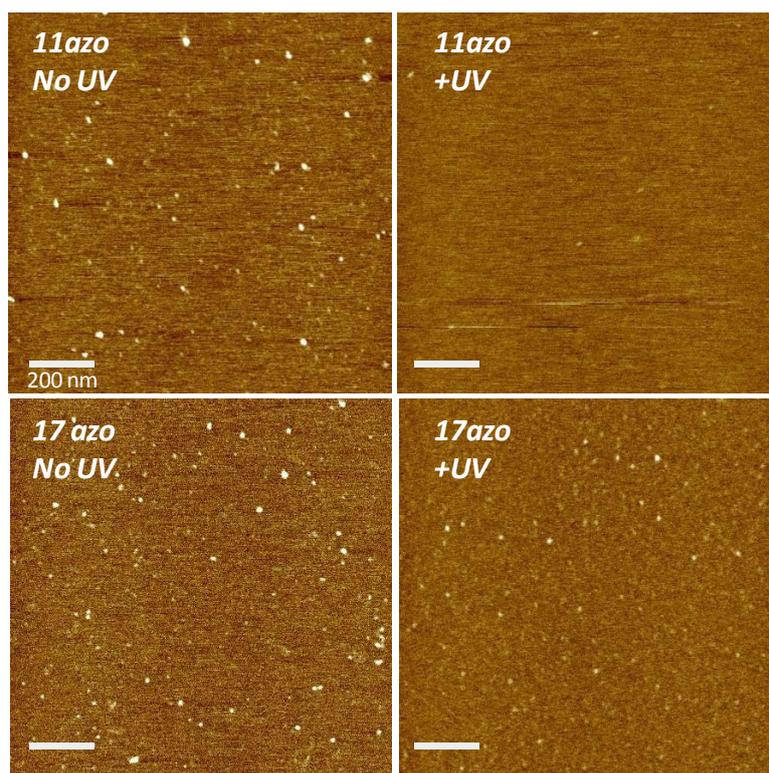


Figure S4. AFM topographic images on mica, representatives of the objects deposited from 0.0001 g.L^{-1} solutions in water of (up) PNIPAM-*co*-EGMA-11azo (bottom) PNIPAM-*co*-EGMA-17azo, under (left) visible light or (right) UV light at 365 nm. Full scale: $1300 \text{ nm} \times 1300 \text{ nm}$; maximum height of the white spots is $< 2 \text{ nm}$.