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PAPER

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

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The response to light of macromolecules in aqueous media is a significant concern for the design of complex fluids, and (bio)materials imparted with stimuli-triggered properties. It is common knowledge that polymer chains containing photochrome groups can be tailored to undergo a transition between poor and good solvent conditions upon exposure to light, thanks to the photoswitch of the polarity/hydrophobicity of their photochromes. In water, marked photo-responses are typically achieved in the vicinity of the low critical solution temperature (LCST) of polymers. To understand and optimize the variation of the LCST of amphiphilic polymers in response to exposure to UV-visible light, we studied the LCST properties of a homologous set of derivatives of poly(*N*-isopropylacrylamide). The chains contained a hydrophilic oligo(ethylene oxide) side group (at 20 mol%) and a light-responsive azobenzene introduced at a varying integration level (ranging from 0% to 24 mol%). Turbidity measurements showed that the LCST passes through a minimum value upon increasing the density of the hydrophobic azobenzene. At a low % of azobenzene, the LCST displayed the conventional decrease with increasing hydrophobicity of the polymers, from *ca.* 56 °C in the absence of azobenzene down to *ca.* 35 °C for the polymer containing 5 mol% azobenzene. Conversely, beyond 11 mol% azobenzene, the LCST increased upon increasing the hydrophobicity of the polymers, and a “reverse” response to exposure to light was observed. Namely the LCST decreased by up to 5 °C upon the photoconversion of azobenzene from the *trans*, apolar isomer, to the *cis*, polar, *i.e.* more water soluble isomer. This counterintuitive behavior was accompanied by the formation of clusters of chains, whose diameter (~20 nm) was measured by dynamic light scattering. AFM and UV-visible spectra confirmed the presence of hydrophobic associates between the azobenzene groups. We ascribed the origin of the “reverse” responses to the presence of these clusters. We proposed accordingly a model of the partitioning of azobenzene between water and the hydrophobic core of the clusters, which allowed us to calculate the LCST as a function of polymer composition. Fitting experimental data to the model quantitatively indicated that self-assemblies scavenge the hydrophobic photochromes and in turn tune their contribution to the LCST.

Introduction

Stimuli-responsive polymer systems are based at the molecular level on marked conformational and/or solubility changes that occur to segments of chains upon receiving an external signal.^{1,2} To achieve the remote and clean control of properties that are for

instance compatible with biomedical applications, it is preferable to use temperature and light as non-invasive triggers.³ Since the seminal work by Irie *et al.*, the general strategy to design light-responsive macromolecules consists of the introduction of chromophore groups into temperature-responsive or solvent-responsive polymer chains.^{4–6} In conditions close to the solubility transition of these chains, the photoswitch of the chromophore's properties could trigger many macroscopic responses, including the swelling/deswelling of polymer chains or gels,^{2,4,7} photo-precipitation/resolubilization,^{4,8–10} delivery of encapsulated drugs,^{11,12} viscosity jumps^{13–15} and destabilisation of emulsions.^{16,17}

The low critical solution temperature, LCST, is sensitive to the presence of chromophores because it is typically shifted to higher temperatures in the presence of hydrophilic comonomers, and in

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contrast drops down in the presence of hydrophobic moieties in the chain.^{4,18} Light-triggered variations of the photochrome's polarity,¹⁹ and/or degree of ionization,²⁰ affect the LCST accordingly. In practice, however, photovariation in the direction opposite to that expected (*i.e.* increasing LCST upon photo-decreasing polarity) has been reported and tentatively ascribed to the competition between the aggregation and formation of clusters of chains.²¹ The architecture of the polymer chains matters in order to control the nature and magnitude of the solubility variation. For instance, diblock copolymers containing a light-responsive solvophobic block may be used to photocontrol self-assemblies.^{22–25} Conversely, when the photochromes are present in the external layer of these assemblies, they control the thickness of their hydrophilic layers²² or shape transition.^{26,27} A few experimental evidences point that the formation of hydrophobic clusters in non-blocky copolymers similarly holds some importance and affects the photo-response. The lack of response and the surprising preservation of the solubility above the expected LCST have been reported in cases of copolymers with azobenzene moieties regularly inserted along the chain.^{28,29} The investigation of the relationships between hydrophobic assembly and molecular photovariations is accordingly in high demand in order to guide the optimization of light-responsive copolymers.³⁰

Here we studied a homologous set of temperature-responsive amphiphilic copolymers that were capable of assembling into clusters of chains depending on the degree of integration of oligo(ethyleneoxide) hydrophilic side groups, and (light-responsive) hydrophobic groups. To fit experimental data, namely the magnitude of the UV-triggered shift of the LCST and the non-monotonous variation with the hydrophobicity of the LCST, we propose a model accounting for the partition of azobenzene chromophores between the aqueous phase and hydrophobic clusters. The popular temperature-responsive chain of poly-*N*-isopropylacrylamide (PNIPAM), which undergoes a temperature-induced transition at ~ 32 °C in water, was chosen as a representative parent polymer. We turned to azobenzene as a model of photochrome side groups, for the following reasons: first, the light-triggered variations of polarity and conformation (*cis*–*trans* isomerization) are readily obtained with excellent reversibility, fast kinetics (<second), in water as well as in the apolar core of micellar assemblies.^{28,30,31} Second, azobenzene dyes are ubiquitous in photo-responsive systems, possibly because their chemistry and grafting on diverse macromolecules is relatively robust.^{6,32} Functional monomers were introduced in the chains by post-polymerization modification, which enabled us to study polymers of varying composition, though with the same chain length and polydispersity. The LCST of these polymers and the formation of hydrophobic clusters were studied by turbidity and by light scattering measurements under exposure to UV or blue light.

Experimental

Materials

N-Acryloxysuccinimide (NAS), oligo(ethylene glycol) methyl ether methacrylate (EGMA), 2,2'-azobis(2-methylpropionitrile) (AIBN), 3-mercaptopropionic acid (3-MPA), isopropylamine, dimethyl sulfoxide (DMSO), deuterated chloroform, phosphate

buffer pH 7.2 with 0.1 M NaCl (1× PBS) and triethylamine (TEA) were purchased from Sigma-Aldrich. Except if specified, reagents and solvents for the synthesis of polymers were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen. Basic activated aluminium oxide (50–200 micron, Al₂O₃) was obtained from Acros and was used as received. Water was deionized with a Millipore Milli-Q Water System.

Methods

The synthesis and characterization of the polymers by ¹H NMR is detailed in the ESI.† The photoresponsive polymers were obtained by grafting a parent chain poly(EGMA-*co*-NAS) with isopropylamine and an amino-derivative of the azobenzene chromophore spaced by an alkyl chain denoted "C6azo" (Fig. 1).

UV-Visible spectrophotometry. UV-Vis spectra were recorded on a diode array UV-visible spectrophotometer (Evolution Array, Thermo Scientific) equipped with a Peltier temperature controller (± 0.1 °C). During the experiments, the samples were vertically and continuously irradiated under blue light (436 ± 10 nm at a power of *ca.* 2 mW cm⁻²) or UV light (365 ± 10 nm at a power of *ca.* 10 mW cm⁻²) with the photodiode system CoolLED PE-2 (Roper Scientific, France) to reach the photo-stationary *trans* : *cis* ratio between the isomers of azobenzene. To determine the lower critical solution temperature (LCST), the absorbance at 550 nm was measured as a function of temperature, *T*. Typically, 3 mL of the polymer solution was filtered through a

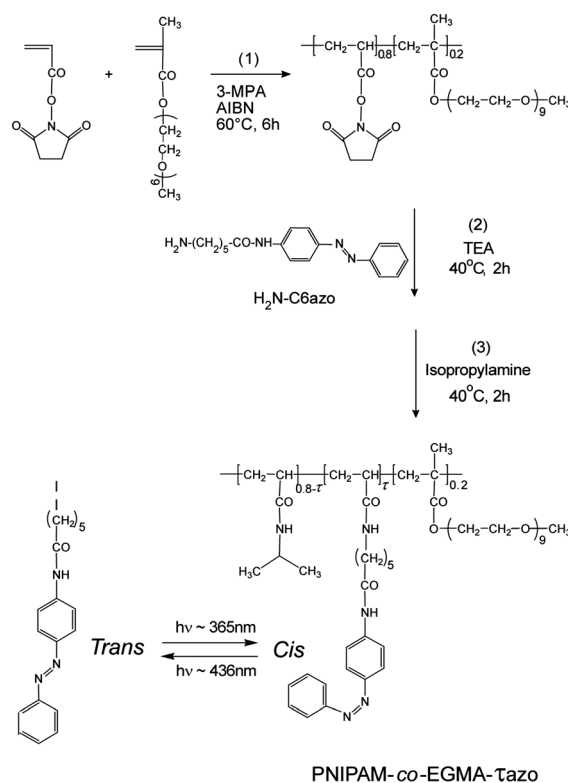


Fig. 1 The structures and scheme of the synthesis of the photoresponsive polymers.

0.2 μm syringe filter (Millex, Millipore) into a quartz cell of one-cm path length and subjected to two cycles of upward and downward temperature sweeps between 15 $^{\circ}\text{C}$ and up to 70 $^{\circ}\text{C}$ at a sweep rate of +0.2 $^{\circ}\text{C min}^{-1}$. The onset of turbidity above a threshold temperature indicates polymer aggregation. The LCST value was defined as the crossover temperature between the baseline and the tangent to a linear segment of the absorbance vs. T curve, just above the onset of the turbidity increase. Measurements were done in duplicate, by repeating the T -sweep on the same sample that was cooled to 10–15 $^{\circ}\text{C}$ for 15 minutes under stirring prior to the second cycle of heating–cooling steps. The LCST determined in this second scan was equal to that obtained in the first scan, within a 1 $^{\circ}\text{C}$ experimental error.

Dynamic light scattering (DLS) measurements. Dynamic light scattering was performed in either a Brookhaven system or ALV/CGS-3 compact goniometer system both equipped with a multiple tau digital correlator and a laser with an output power of 30 mW and 22 mW, respectively (wavelength of 637 nm and 632.8 nm, respectively). The data were collected by monitoring the scattered light intensity at 140 $^{\circ}$ or 90 $^{\circ}$ scattering angles at different temperatures. Temperature control within 0.1 $^{\circ}\text{C}$ was achieved using a thermostated bath circulator. Measurements were performed on polymer solutions that were filtered through membranes of 0.2 μm pore size directly into scintillation vials prior to measurement. Laplace inversion of the correlation function was performed using ALV Software Version 3.0 provided by the manufacturer. Exposure to UV (365 nm) or blue light (436 nm) was performed *in situ* using an optical fiber placed at a distance of *ca.* 1 cm above the top of the cell using a LED illumination system (CoolLED PE-2, Roper Scientific, France).

Sample preparation. Stock solutions of the photoresponsive polymers were prepared by 48 h dialysis against water (see ESI, † Slide-A-Lyzer, MWCO 3500 Da). Aliquots were diluted with 1 \times PBS or DMEM buffers to 0.33 g L $^{-1}$. All solutions were gently mixed at room T , or 8 $^{\circ}\text{C}$ to ensure the complete dissolution of the polymers as well as to avoid foaming and were prepared at least 24 h before use.

Results

LCST properties of the photoresponsive terpolymers

Water-soluble polymers containing a predominant fraction of isopropylacrylamide (NIPAM) units are expected to display LCST. The critical temperature is however shifted away from the 32 $^{\circ}\text{C}$ of pure NIPAM chains, with the magnitude of the shift depending on the hydrophilic–hydrophobic balance involving all the co-monomers. The presence of EGMA is expected to increase the LCST above 32 $^{\circ}\text{C}$, because the homopolymer of EGMA exhibits a LCST of about 90 $^{\circ}\text{C}$ (when its side chains contains 8/9 ethylene oxide units).^{18,33} In contrast, the introduction of the hydrophobic azobenzene group into the polymer chains should decrease the LCST. Irrespective of the chemical nature of the chains, the LCST of the copolymers was shown to decrease linearly with an increasing molar fraction of azobenzene (eqn (1), see ref. 33 for an example with copolymers of EGMA, or ref. 34 for a review on azobenzene-containing copolymers).

$$\text{LCST}(\tau) = \text{LCST}(0) - \alpha\tau \quad (1)$$

With τ being the molar fraction of azobenzene, α a constant depending on the chemical nature of the chromophore. The LCST was determined in dilute polymer solutions (0.33 g L $^{-1}$) in PBS buffer, by the measurement of the absorbance as a function of T . In practice, the absorbance in the range 550 nm–700 nm provided a reliable index of turbidity, since (i) this parameter displays a large increment when polymers form aggregates above the LCST (Fig. 2) and (ii) this window of wavelengths is well above the absorption bands of azobenzene. The LCST value was

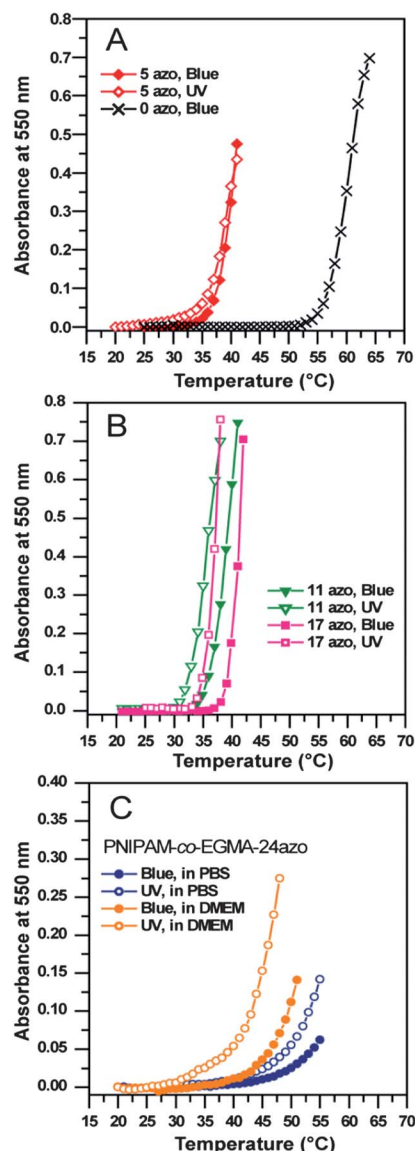


Fig. 2 The absorbance at 550 nm of aqueous solutions of photo-polymers upon a temperature sweep. Solutions of polymers at 0.33 g L $^{-1}$ were irradiated under blue light (436 nm), or UV light (365 nm) prior to and during the measurements. The temperature sweep rate was +0.2 $^{\circ}\text{C min}^{-1}$. (A) PNIPAM-co-EGMA and PNIPAM-co-EGMA-5azo in 1 \times PBS buffer; (B) PNIPAM-co-EGMA-11azo and PNIPAM-co-EGMA-17azo in 1 \times PBS buffer; (C) PNIPAM-co-EGMA-24azo in 1 \times PBS or in DMEM buffer.

defined as the intersection of the base line at low temperatures, with the tangent to absorbance curves above the onset of the turbidity increase. The marked dependence of the LCST on the fraction of azobenzene per chain, τ , is shown in Table 1 for solutions permanently exposed (before and during measurements) to UV (365 nm) or blue (436 nm) light. As expected, the presence of hydrophobic azobenzene in the chain decreased the LCST compared to its value of 56 °C in the case of the chain devoid of chromophore (*i.e.* PNIPAM-*co*-EGMA-0azo comprising 20 mol% EGMA and 80 mol% NIPAM). With only 2 mol% C6azo in the polymer (PNIPAM-*co*-EGMA-2azo), the LCST was decreased to 40 °C (Table 1 and Fig. 3A), which suggests that the order of the magnitude of α in eqn (1) is 8 °C mol%⁻¹. This value of α lies in the range of the usual window of 4 °C mol%⁻¹ to 16 °C mol%⁻¹ found in published data on azo-modified copolymers.³⁴ However, the slope of the LCST vs. τ rapidly vanished with increasing τ (Fig. 3A), both under UV and blue light exposure, and became positive for τ above 17 mol%. Obviously, this set of terpolymers did not obey eqn (1): the variation of the LCST with τ was non-monotonous for both UV and blue-adapted terpolymers. It is also remarkable in Fig. 3A that for $\tau \geq 11$ mol%, the LCST of the UV-adapted polymers is generally below that of the blue-adapted ones. Recalling that the azobenzene side groups are more polar in their *cis*-form (UV-adapted), this result points somewhat surprisingly that the solubility in water of the polymers in their *cis*, polar state is lower than in their predominantly nonpolar *trans* form (see Section "UV-visible spectrophotometry" for data on *cis*-*trans* isomerization). To our knowledge, this "reverse" photo-shift of the LCST has been reported once, though for a single composition of a copolymer of NIPAM and acrylamidoethoxyazobenzene.²¹

It was checked that the LCST did not vary significantly with polymer concentrations (see the ESI, Fig. S3†). The LCSTs were also measured in DMEM buffer. DMEM and PBS are common buffers used in biology and were chosen here as representative experimental conditions for possible biomedical applications of *T*-responsive PEO-containing chains.^{18,35} Here we aimed only at an estimate on whether a more complex composition of buffer may affect the photo-response.³⁵ As shown in Table 1, the photo-shift of the LCST, Δ LCST, does not significantly differ in the DMEM compared to the PBS buffer. Some influence of the buffer composition on the LCST is however observed in the case of PNIPAM-*co*-EGMA-24azo whose LCST (but not Δ LCST) decreased in DMEM as compared to PBS. The amino acids

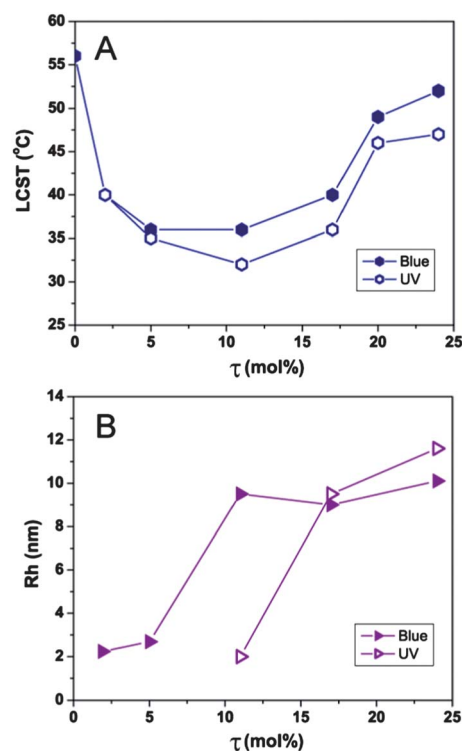


Fig. 3 Characteristic properties in 1× PBS of photopolymers with varying degrees of integration of azobenzene, τ . (A) The LCSTs determined by the extrapolation to the baseline of the variation of the absorbance with temperature, shown in Fig. 2; (B) number averaged hydrodynamic radius, R_h , measured by DLS at 25 °C. The polymers at 0.33 g L⁻¹ were irradiated under blue light (436 nm), or UV light (365 nm) prior to and during the measurements.

present in DMEM can presumably develop interactions with the side groups of the polymer and modulate slightly the solubility.

Photoswitch at fixed temperature

The Δ LCSTs of several degrees in Table 1 suggests that photo-switches of solubility at a fixed temperature could be achieved upon switching between irradiation conditions under different wavelengths. Experimentally, we studied the photoswitch of PNIPAM-*co*-EGMA-24azo in 1× PBS at 50 °C and in DMEM at 43 °C (Fig. 4). These two temperatures were fixed 2 °C below

Table 1 The measured LCST of the photoresponsive polymers

τ (PNIPAM- <i>co</i> -EGMA- τ azo)	LCST ^b (°C) in 1× PBS ^a			LCST ^b (°C) in DMEM ^a		
	Blue	UV	Δ LCST	Blue	UV	Δ LCST
0	56	—	—	—	—	—
2	40	40	0	—	—	—
5	36	35	1	—	—	—
11	36	32	4	35	32	3
17	40	36	4	40	34	6
20	49	46	3	—	—	—
24	52	47	5	45	41	4

^a Phosphate buffered saline (1× PBS) or Dulbecco's Modified Eagle Medium (DMEM). ^b The lower critical solution temperature measured under exposure to light at 436 nm ("Blue") or 365 nm ("UV"), Δ LCST = LCST (Blue) – LCST (UV).

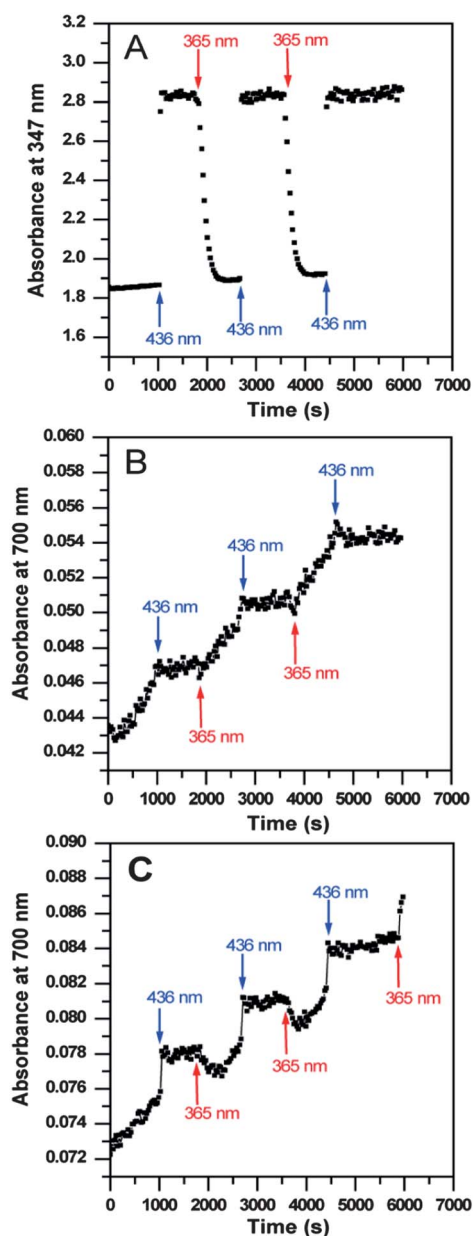


Fig. 4 The effect of alternative exposure to UV and blue light at constant temperature on the turbidity and *cis* : *trans* isomerization in solutions of PNIPAM-*co*-EGMA-24azo. The polymer concentration was 0.33 g L⁻¹. The wavelength of irradiation was switched to UV at times zero and later at times indicated by arrows in the figures. (A) Absorbance measured at 347 nm wavelength (peak of absorption of *trans*-azobenzene), polymer in DMEM, *T* = 43 °C. (B) Absorbance at 700 nm representative of the turbidity index, polymer in 1× PBS, *T* = 50 °C. (C) Absorbance at 700 nm, polymer in DMEM, *T* = 43 °C.

the LCST under blue irradiation and 2–3 °C above the LCST measured under UV light (Table 1). Fig. 4A shows the photo-variation of the absorbance of a solution of PNIPAM-*co*-EGMA-24azo (at 347 nm, *i.e.* near the maximum of the absorption of the *trans*-azobenzene isomer, *cf.* Fig. 7). The rapid variations of the absorbance occurred upon changing the wavelength of irradiation, which confirms that *cis* to *trans*, and *trans* to *cis*, photo-conversions of azobenzene were achieved upon

exposure to blue and UV light, respectively. The typical time lapse needed to reach the stationary state was of the order of 5 min under UV (1 mW cm⁻²), and less than 1 min under blue light.

As shown in Fig. 2, measurements of the absorbance at wavelengths above 550 nm enable one to detect the aggregation of the polymers. Due to the broad absorption band of azobenzene, the absorbance at 550 nm was however slightly sensitive to the *cis* : *trans* transconversion of azobenzene. The influence of absorption compared to scattering vanishes upon increasing the wavelength up to 700 nm. In Fig. 4B and C the index of turbidity was thus measured by absorbance at 700 nm to be insensitive to the *cis*–*trans* state. As shown in Fig. 4B and C, the turbidity increased during the exposure to UV light and was essentially constant under exposure to blue light. Samples were gently agitated (stir bar, 300 rpm) during the experiment to avoid sedimentation. One thus concludes that the aggregation of the polymers was stopped when switching the wavelength from 365 nm to 436 nm, and was triggered by exposure to UV light. Due to the non-ionic nature of the chain, redispersion under blue light could be slow, which may explain the lack of solubilisation. It was however possible to reform fully transparent solutions of PNIPAM-*co*-EGMA-24azo by bringing the aggregated samples down to 20 °C for a few minutes. Altogether, these results show that the polymer was effectively switched from poor solvent to better solvent conditions by exposure to light at constant temperature.

Light-scattering evidence for self-assemblies

Desponds and Freitag²¹ have observed the reverse photo-switch of the LCST with one polymer and tentatively propose that the formation of self-assemblies, such as micelle-like clusters of chains, could “compete” with aggregation and precipitation. To detect the formation of polymer assemblies, we had recourse to dynamic light scattering (DLS) and UV-visible spectroscopy measurements as described in the next sections. DLS was used to measure the hydrodynamic radii of the polymers below the LCST, and to confirm the onset of aggregation at the LCST. In Fig. 5A and B, distributions of the hydrodynamic radii of different polymers at 25 °C, *i.e.* below the LCSTs, in 1× PBS solution display typically the predominant contribution of radii below 5 nm for weakly modified chains (PNIPAM-*co*-EGMA-2azo and PNIPAM-*co*-EGMA-5azo). For the polymers of higher degrees of integration of azobenzene, the radius reached a well defined value (almost a single peak in the distribution) in the range 10 nm to 12 nm. Possible contributions of larger species corresponded to negligible fractions in the number-weighted distribution shown Fig. 5B. In both Fig. 5A and B, radii >10 nm of the main peak in the distribution appear significantly larger than the 4 nm radii of PNIPAM-*co*-EGMA-5azo and PNIPAM-*co*-EGMA-2azo. All polymers were studied at the same concentration and they had the same chain length and polydispersity (same parent chain). This increase in radius thus suggests that polymers with $\tau > 11\%$ self-assembled into clusters of chains. Typical AFM pictures of these clusters are also shown in the ESI (Fig. S4†).

Aggregation above the LCST resulted in the formation of significantly larger and polydisperse objects. Fig. 5C presents a

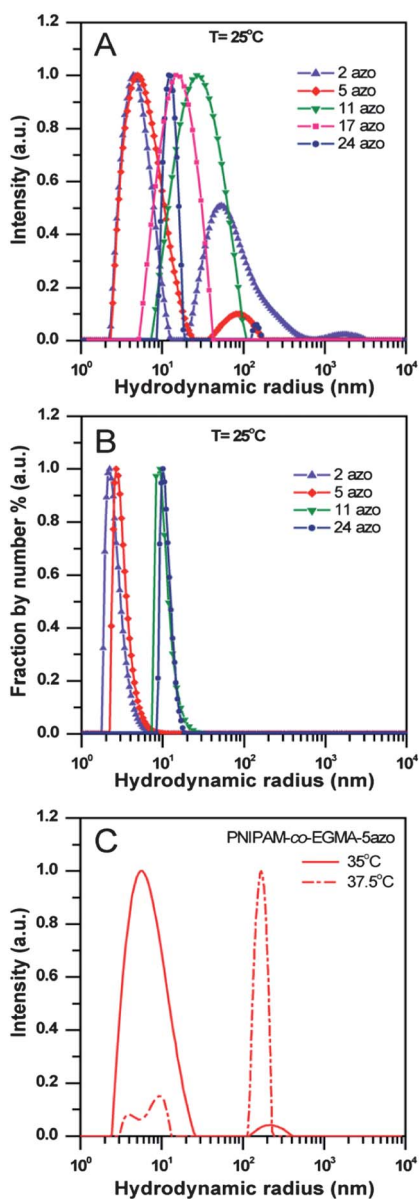


Fig. 5 Distributions of the hydrodynamic radii of photopolymers in 1 × PBS as measured by DLS (A) at 25 °C, intensity weighted size distribution, scattering angle of 140°; (B) 25 °C, number weighted distribution, scattering angle of 140°; (C) $T = 35$ °C or 37.5 °C, intensity weighted distribution, scattering angle of 90°. The names of the polymers are quoted in the figure.

representative example of intensity-weighted distributions of hydrodynamic radii just below and above the LCST: at 35 °C, PNIPAM-*co*-EGMA-5azo was predominantly detected as small objects with a mean Stock's radius of 5.5 nm, similar to its radius at $T = 25$ °C. Upon heating the sample up to 37.5 °C, the radii of the dominant particles increased suddenly from *ca.* 5 nm to >170 nm, which points to the formation of large aggregates in agreement with the LCST of 36 °C determined by turbidity measurements. The presence of a small amount of large objects (~70 nm) at 35 °C was almost unavoidable in practice, and these large species were not removed despite several filtration steps (0.22 μm). The fraction of the scattered intensity and the average

size of these large objects varied however depending on the filtrations and handling of the samples, which suggests that they were not at equilibrium with soluble chains. We believe that it would be hazardous to comment further on the peculiar contribution of these large objects accounting for a minor fraction of the scattered light.

Increases in the hydrodynamic radii of PNIPAM-*co*-EGMA-17azo and PNIPAM-*co*-EGMA-24azo were observed upon crossing the LCST, as illustrated in Fig. 6A and B. PNIPAM-*co*-EGMA-17azo formed large species whose radii increased sharply with increasing temperature, reaching eventually the micrometre range (Fig. 6A). The increase with temperature of the average radius for PNIPAM-*co*-EGMA-24azo was however smoother (Fig. 6B), especially under blue light irradiation, which was consistent with the weaker variation of turbidity, in Fig. 2C, compared to other polymers. The clusters of chains that are present in the solution of PNIPAM-*co*-EGMA-24azo appear accordingly to be less prone to aggregation than the other polymers. A possible origin of the preservation of solubility may be the sequestering of azobenzene into hydrophobic clusters. The stability of the clusters was betrayed by the lack of the variation of the hydrodynamic radii under exposure to UV at temperatures <LCST compared to the case of PNIPAM-*co*-EGMA-11azo. Fig. 3B shows the drop of the hydrodynamic radius from 9 nm under blue light to 2 nm under UV light suggesting that clusters of chains formed by PNIPAM-*co*-EGMA-11azo dissociated upon UV irradiation. The photoconversion into the more polar *cis* form of the azobenzene was in this latter case sufficient to destabilize inter-chain clusters.

Spectrophotometric evidence for clusters of azobenzene

It is known that the absorbance properties of azobenzene can be used as a reporter of the local hydrophilic/phobic environment.^{36,37} To get evidence on the presence of inter-azobenzene associates, we thus carried out absorbance measurements in PBS buffer at $T = 25$ °C. In addition, spectrophotometry enabled us to assess the *cis*-*trans* state of azobenzene under exposure to UV or visible light. Fig. 7 shows the changes in the absorption spectrum that occurred under irradiation. The typical absorption

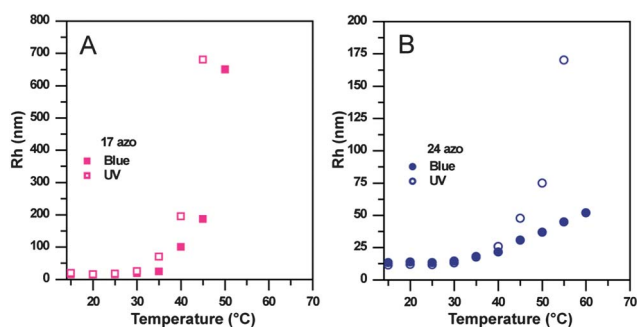


Fig. 6 Variation with temperature of the averaged hydrodynamic radius R_h measured by DLS in solutions of (A) PNIPAM-*co*-EGMA-17azo and (B) PNIPAM-*co*-EGMA-24azo in 1 × PBS. Samples were subjected to stepwise increases of the temperature and pre-incubations for 5 min at each temperature prior to measurement. Scattering angle of 90°; polymer concentration of 0.33 g L⁻¹. R_h was obtained from the second order cumulant analysis of the correlation function.

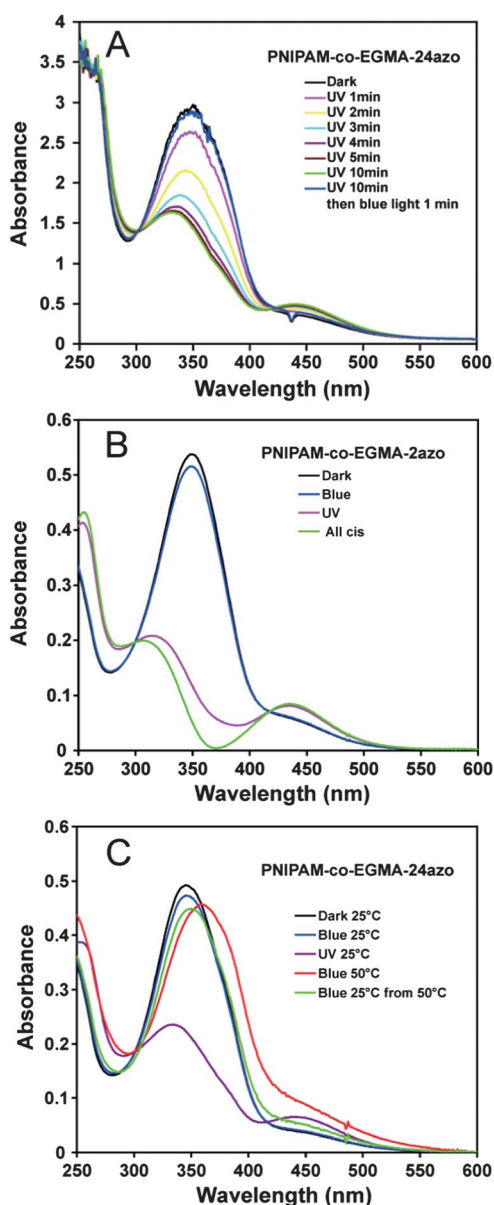


Fig. 7 Variation upon exposure to UV or blue light of the UV-vis absorption spectra of photopolymers in $1 \times$ PBS. (A) 0.2 g L^{-1} PNIPAM-co-EGMA-24azo initially in its all-*trans* isomer form, and exposed to UV light (365 nm , $ca. 1 \text{ mW cm}^{-2}$) for a time quoted in the figure; (B) 0.33 g L^{-1} PNIPAM-co-EGMA-2azo in its all-*trans*, dark-adapted form (*i.e.* incubated overnight in the dark), or UV- or blue-adapted isomer forms (reached upon >10 min exposure under the corresponding wavelength), “all *cis*” quotes for a calculated spectrum of the 100% *cis* isomer form (see text for details); (C) 0.03 g L^{-1} PNIPAM-co-EGMA-24azo under its dark-adapted or light-adapted isomer forms obtained as in (B), at temperatures quoted in the figure; “Blue 25°C from 50°C ” is where the sample whose spectrum at 50°C (“Blue 50°C ”) was then cooled down to 25°C and measured again.

spectrum of the dark-adapted (100% *trans*) polymer, and the blue-adapted one, exhibited a strong absorption peak at 347 nm and a weaker one at 428 nm . Upon irradiation at 365 nm , the major absorption band at 347 nm gradually decreased with increasing time of exposure and shifted to slightly shorter wavelengths

(*ca.* 335 nm), while the band at 428 nm increased in magnitude. These spectroscopic properties confirmed that at room temperature and after incubation in the dark or under blue light, the azobenzene chromophores were predominantly in their *trans* isomer form. Under exposure to UV light, they undergo a conversion from *trans* to *cis* isomers and reach a photo-stationary state with the predominantly *cis* form displaying a markedly lower, often negligible absorbance at 347 nm .³² Under our experimental conditions (irradiance of $ca. 1 \text{ mW cm}^{-2}$), the photo-stationary state was reached in 5 min. Further irradiation did not change the spectrum. Under blue light (436 nm), the band at 347 nm was partially restored, due to the partial photo-reversion toward a predominantly *trans* photostationary state of the chromophores.³² It has been determined in the case of polymers bearing similar azobenzene side groups that at 25°C in water, the stationary *cis* : *trans* ratio reached under UV (365 nm) is $80 : 20 \text{ mol mol}^{-1}$.³⁸ An accurate estimation of the stationary *cis* : *trans* ratio requires one to calculate the UV-vis spectrum of a pure *cis* azobenzene in order to get $\epsilon_{cis}(\lambda)$, the extinction coefficient of the *cis* isomer at wavelength λ . The absorbance of the solution, $A(\lambda)$, depends on the *cis/trans* molar ratio, R , and path length, l , as given in eqn (2):

$$\frac{A(\lambda)}{l\epsilon_{trans}(\lambda)[\text{azo}]} = \frac{1 + R\epsilon_{cis}(\lambda)/\epsilon_{trans}(\lambda)}{1 + R} \quad (2)$$

A first rough estimate of (maximum) residual %*trans* can be obtained by assuming $\epsilon_{cis}/\epsilon_{trans} \ll 1$ at 365 nm (yielding here $R \sim 5.9$, *i.e.* *cis* : *trans* $\sim 85 : 15$ under UV exposure). More accurate determination uses the absorbance measured at three wavelengths, under UV and blue irradiations, to compute six equations with 5 dummy variables (three extinction coefficients of the *cis* form at the corresponding “test” wavelengths, and the two *cis* : *trans* “ R ” ratios of UV-adapted and blue-adapted samples). This procedure enabled us to calculate the spectrum of 100% *cis* isomers shown in Fig. 7B. In polymers with low grafting densities of C6azo (2% and 5%), the *cis* : *trans* composition at photo-stationary states is close to previously estimated values,¹⁵ typically with *cis* : *trans* of the order of $85 : 15 \text{ mol mol}^{-1}$ under UV light and $10 : 90$ under blue light. In contrast, the *trans*-conversions in solutions of PNIPAM-co-EGMA-24azo were clearly of lower magnitude, as indicated by the higher residual absorbance at wavelengths around 350 nm in UV-exposed samples (Fig. 7A and C). We observed this high residual absorbance irrespective of the polymer concentration (0.03 g L^{-1} and 0.2 g L^{-1}), irradiation time (up to 1 hour), and intensities (UV was increased up to 20 mW cm^{-2}). The UV-adapted spectra in Fig. 7A and C correspond to an apparent stationary *cis* : *trans* ratio of $\sim 60 : 40$ ($R \sim 1.5$), as estimated from the approximation of the negligible absorbance of *cis* isomers at 365 nm . In addition, after incubation at 50°C for short times (*e.g.* 5 min in Fig. 7C), the spectrum displayed a red-shift of the band around 350 nm , and a decrease of the apparent extinction coefficient, which are signatures of the aggregation of azobenzenes.^{36,37} Interestingly, relaxation toward the initial spectrum did not occur upon simple incubation below the LCST. In Fig. 7C, the marked difference between the spectra of the blue-adapted samples before and after thermal treatment (both measurements performed at 25°C) shows that the signature of the azobenzene aggregates persisted

even after cooling the sample down to 25 °C, which suggests that the aggregates did not dissolve completely. In practice, it was possible to rapidly obtain a spectrum devoid of a signature of turbidity and aggregation by exposure to UV light, prior to incubation at low temperatures (8 °C) in the dark. Altogether, the results of the UV-visible spectra thus confirm that (i) *cis* : *trans* isomerization occurs in all polymer samples; (ii) for $\tau < 5$ mol% the typical order of magnitude of the *cis* : *trans* ratio reaches the conventional values of 85 : 15 under UV light and 10 : 90 under blue light; (iii) for $\tau > 5$ mol%, azobenzene partitions into hydrophobic clusters that may persist upon cooling.

Qualitative and quantitative models of LCST behavior

Reports of the non-conventional variation of the LCST with increasing hydrophobic–hydrophilic balance of polymers are rare. To our knowledge, the formation of inter-polymer or intra-chain clusters has been reported together with the peculiar LCST properties in three articles: (i) telechelic PNIPAM bearing azobenzene end-groups were shown to become light responsive upon the minimization of their solubility in water–dioxane mixed solvents;³⁹ (ii) one random acrylic terpolymer containing NIPAM and azobenzene was found by Desponds and Freitag²¹ to display a “reverse” response to light, *i.e.* a lower LCST under UV irradiation, and finally (iii) Zhao and coworkers showed a decreasing LCST upon switching from the *trans* to the *cis* form of a PNIPAM-azobenzene copolymer with azobenzenes belonging to the backbone chain and forming hydrophobic clusters.²⁸ Our results by light scattering validate for the first time on a homologous set of chains that the predominance of self-assemblies in solutions and the reverse variation of the LCST with hydrophobicity occur simultaneously. Qualitatively, the link between the “reverse” LCST behavior and the self-assemblies comes from the gathering/scavenging of apolar moieties in the core of these assemblies. The preferred exposure of hydrophilic moieties in the outer corona of inter-chain clusters and the reduced contacts between water and the apolar groups enhance the apparent hydrophilicity of the polymers in this cluster form. Self-assembled chains are accordingly less prone to random aggregation and have an increased solubility. In other terms, the formation of large aggregates above the LCST competes with the formation of intra-cluster hydrophobic associations.

The interpretations of the experimental observations are finally summarized as follows:

(i) When the contribution of the inter-chain-assemblies is not dominant, *i.e.* for low degrees of integration of azobenzene (here $\tau < 5\%$), the polymers show properties similar to those of conventional temperature-responsive macromolecules. It is expected and observed that the LCST decreases with increasing hydrophilicity, *i.e.* with increasing τ . Upon *cis* to *trans* trans-conversion, the LCST should decrease because of the decreasing polarity and increasing hydrophobicity of the azobenzene groups. At low degrees of integration of azobenzene, the effect of light shall however be weak. Here, for $\tau \leq 2$ mol% it was likely below experimental uncertainty.

(ii) For $5\% \leq \tau < 17\%$, an intermediate range of composition exists, though this boundary was not studied in this work. Measurements on PNIPAM-*co*-EGMA-11azo show for instance that the chains self-associate into small clusters under their *trans*

azobenzene form, but these assemblies are dissociated upon exposure to UV light. Upon increasing temperature, the non associated chains would form micrometre large and polydisperse aggregates in the conventional way (as in case (i)). In contrast, aggregation of the self-assembled chains appears more difficult (*e.g.* the diameters of the aggregates formed under blue light remain smaller than those formed under UV light) and occurs at higher temperatures. Altogether, the expected LCST drops upon increasing the hydrophobicity of the chains in this regime (upon increasing τ or exposure to blue light) and is balanced by clustering.

(iii) When $\tau \geq 17\%$, polymers form stable micelle-like clusters that do not vanish under UV irradiation. These globules can aggregate, though at higher temperatures compared to that expected for non-associated polymer chains. They also form smaller aggregates above the LCST. In this regime one observes reverse variations of the LCST (increases upon increasing τ and upon exposure to blue light as compared to UV light). These qualitative features are summarized in Fig. 8.

To go beyond a qualitative interpretation, we propose to modify eqn (1) in order to account for the aggregation between pre-formed clusters of chains. In principle, eqn (1) provides a heuristic relationship between the composition and LCST of any polymer that is well dispersed in water below its LCST. The latter condition is not fulfilled by cluster-forming polymers. The segments of the chain involved in inter-cluster attraction are essentially those comprised in their outer corona. Accordingly, the conditions of aggregation should be better matched with the composition of the segments present in this outer layer. We assume here that a simple equilibrium of transfer provides the correct composition of the outer corona allowing us to predict the LCST. The (average) amount of azobenzene in the corona of a micelle has been shown to depend on the equilibrium of the transfer of azobenzene between water and the micellar core.³⁶ On the basis of recognized approaches developed in theoretical models of polymer micellization, and recently experimentally validated in the case of azobenzene-modified polyacrylates (*cf.* ref. 36 and references therein), the equilibrium transfer of side

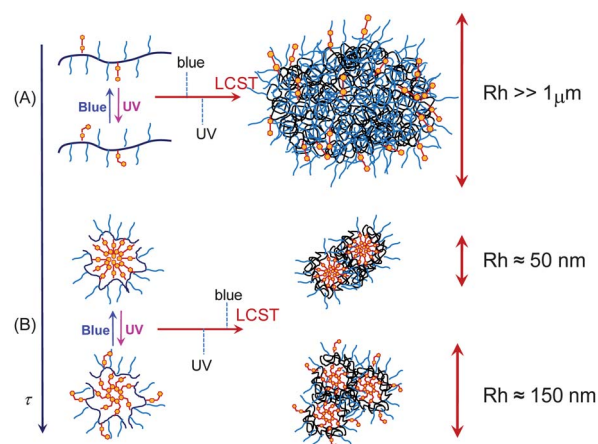


Fig. 8 A schematic representation of the temperature-triggered and light-controlled aggregation of the photopolymers depending on whether they are soluble as isolated chains (A) or self-assemblies (B) at low temperatures.

groups from water to intra-micellar clusters is described by a balance between the free energy gain due to hydrophobic binding and the loss of conformation entropy of the polymer segments (due to folding and attachment to the micelle, *i.e.* formation of a loop). In practice, this balance determines a threshold length for the segments allowed to bind their two (hydrophobic) ends tightly into micellar clusters: longer segments have too high a conformational cost to fold in a loop. In other terms, bound segments must have a density of azobenzene above the threshold molar fraction of hydrophobic groups, called τ^* . In the case of random copolymers bearing azobenzene side groups, these theoretical ideas have been translated into eqn (3) giving the probability P for an azobenzene to partition into the micellar hydrophobic core, as a function of τ , the average degree of integration of the azobenzene in the copolymer (for details, the reader is directed to ref. 36).

$$P = 1 - (1 - \tau)^{2/\tau^*} \quad (3)$$

The apparent (outer) fraction of azobenzene thus writes, $(1 - P) \times \tau$, which when placed into eqn (1) becomes:

$$\text{LCST}(\tau) = \text{LCST}(0) - \alpha\tau(1 - \tau)^{2/\tau^*} \quad (4)$$

Fig. 9A displays the non-monotonous variation of the LCST given by eqn (4), for different values of the parameters τ^* and α . Obviously parameter α governs the slope at the origin, whereas τ^* mostly affects the minimum of the LCST. One calculates easily from eqn (4) that the minimum of the LCST is reached at $\tau = \tau^*/(2 + \tau^*)$ ($\approx \tau^*/2$). Accordingly, the adjustable parameters τ^* and α are essentially fixed by fitting data to eqn (4) with τ between zero and up to $\tau^*/2$ that is reached at the minimum of the LCST. Interestingly in Fig. 9A, the calculated LCST(τ) recovers the value of LCST(0), at $\tau \approx 2 \times \tau^*$, *i.e.* about four times the τ value reached at the minimum LCST. This features a significant distortion of the shape of the curve that cannot be fitted by a parabola, neither by conventional representations of peaks such as the Gaussian shape (Fig. 9A). It is thus remarkable that experimental data reasonably match this peculiar shape as shown in Fig. 9B. In addition, the increase of the polarity of the azobenzene undergoing *trans* to *cis* transconversion shall constraint the variation of the parameters in eqn (4). One expects $\tau_{cis}^* > \tau_{trans}^*$, because the polar *cis* groups must be denser than the apolar *trans* groups to bind tightly to the hydrophobic domains. For similar reasons, $\alpha_{cis} < \alpha_{trans}$ because of the weaker hydrophobicity brought upon by the attachment of polar *cis* groups compared to *trans* ones. In agreement with these expectations, the calculated LCST variation was close to the experimental ones using $\tau_{cis}^* = 1.3 \times \tau_{trans}^*$ and $\alpha_{cis} \approx 0.9 \times \alpha_{trans}$ (Fig. 9B).

The variation of τ^* and α in opposite directions reproduced also the lack of the effect of the UV irradiation observed for polymers of low degrees of integration of azobenzene, a property that was not anticipated. Finally, the full complexity of the experimental responses to light and variation of the LCST with τ is quantitatively captured by eqn (4). In general, the model enables one to distinguish three successive regimes of photo-response. First, at low degrees of modification, τ , the photo-enhanced self-association competes with aggregation resulting in the absence of the variation of solubility upon UV/blue

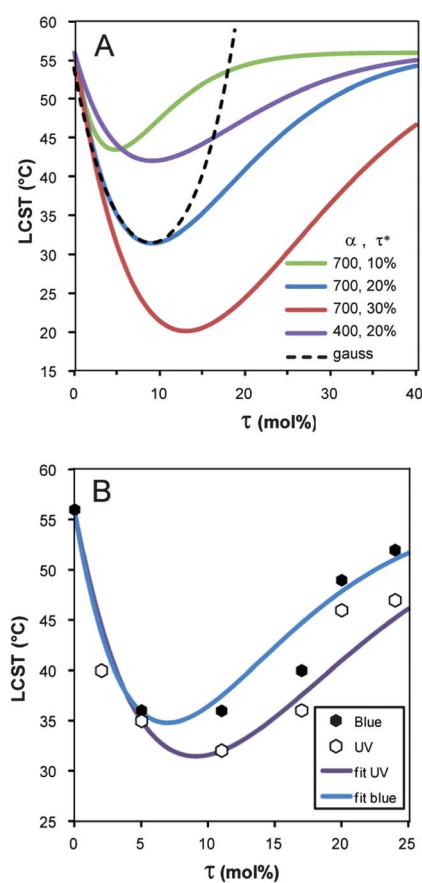


Fig. 9 Variation of the LCST as a function of τ and α , as calculated by eqn (4). (A) Illustrations of the effect of parameters τ^* and α , with LCST(0) = 56 °C; values of parameters are listed in the figure (α is given in °C mol %-azobenzene⁻¹). “Gauss” is the Gaussian variation adjusted to superimpose the curve ($\alpha = 700$, $\tau^* = 20\%$) in the range of τ below 10%. (B) Experimental data as in Fig. 3 superimposed with lines corresponding to fits to eqn (4) with LCST(0) = 56 °C, and parameters (α , τ^*) respectively of (700, 20%) for the curve “fit UV” and (800, 15%) for the curve “fit blue”.

irradiation. Second, above a threshold τ corresponding to the minimum LCST, the *trans* isomer of azobenzene is preferentially bound into the micellar core compared to the *cis* one. This peculiar distribution favors the intra-chain hydrophobic clustering compared to inter-chain aggregation, which explains the “reverse” variation of LCST: an increase of the LCST with increasing degree of integration of hydrophobic azobenzene and with decreasing the polarity upon exposure to blue light. The third regime corresponds to a large excess of azobenzene per chain (beyond four times the τ value at the minimum LCST) affording all the azobenzene to transfer into micellar cores, irrespective of their isomerization state. At such high densities of azobenzene, the model predicts that micelles display mostly hydrophilic monomers in their corona and thus the LCST should reach the value of the purely hydrophilic homopolymer (parent) chain. The effect of UV light is predicted to vanish. This latter regime was not reached in the present work. An experimental illustration of this regime is micelles formed by diblock copolymers bearing a highly hydrophobic, azobenzene-modified block in their core. These assemblies are known to display solubility essentially governed by the outer hydrophilic block.^{24,40–42}

Conclusions

Amphiphilic terpolymers were derived from a parent chain in order to fix the degree of polymerization and control increasing degrees of integration of hydrophobic azobenzene at a fixed density of hydrophilic oligo(oxyethylene) groups. The predominant monomer, *N*-isopropylacrylamide, in the chain made them temperature-responsive in aqueous buffers. The advantage of the present set was the encompassing of polymers that were soluble as isolated chains at low temperature and polymers of higher hydrophobicity that formed self-assemblies. The non-monotonous variation of the LCST with the increasing hydrophobicity of the chains was shown to correlate with the formation of self-assemblies in solutions. In the presence of inter-chain assemblies, a “reverse” variation of the LCST was observed upon exposure to UV light, which corresponds to a decreasing solubility with increasing polarity (upon *trans* to *cis* photoconversion). These results betray the important role of the outer corona of the polymer’s self-assemblies on the aggregation scheme occurring above the LCST. It was possible to predict the variation of the LCST: based on a model that ascribes the onset of aggregation to the loss of the solubility of the polymer segments present in the corona of the polymer’s assemblies, both the magnitude and the direction of the response to light (direct or reverse) were fitted to data. We propose accordingly that the LCST depends on the partition equilibrium of the chromophores between the hydrophobic core of the inter-chain assemblies and the corona.

Here “direct” meant the decrease of the LCST upon increasing the hydrophobicity of the chains; conversely, “reverse” meant an increasing LCST with increasing hydrophobicity, a property that was hardly observed to date in polymer solutions. Other examples of reverse photo-responsiveness reported to date^{43–45} suggest that hydrophobic clustering and/or micellization could be used to tailor original properties in solutions of light-responsive polymers. Of importance to the design of responsive macromolecular systems, we show here that the competition between intra-chain association, inter-chain assemblies, and aggregation offers a general route to cancel, revert or amplify solubility variations with changes of composition and/or polarity of the polymer chains, which finally controls the photo-response. The basic principle illustrated in this work could help to implement in specific polymer architectures the optimized balance between conformation transition and photoisomerization schemes.

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Notes and references

- M. Cohen-Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. M. Winnik, S. Zauscher, I. Iuzinov and S. Minko, *Nat. Mater.*, 2010, **9**, 101–113.
- S. Dai, P. Ravi and K. C. Tam, *Soft Matter*, 2009, **5**, 2513–2533.
- D. Roy, J. N. Cambre and B. S. Sumerlin, *Prog. Polym. Sci.*, 2010, **35**, 278–301.
- M. Irie, Y. Misumi and T. Tanaka, *Polymer*, 1993, **34**, 4531–4535.
- M. Irie, *Adv. Polym. Sci.*, 1993, **110**, 49–65.
- Y. Zhao and T. Ikeda, *Smart Light-Responsive Materials: Azobenzene-Containing Polymers and Liquid Crystals*, John Wiley & Son, Hoboken, NJ, 2009.
- C. Alvarez-Lorenzo, S. Deshmukh, L. Bromberg, T. A. Hatton, I. Sandez-Macho and A. Concheiro, *Langmuir*, 2007, **23**, 11475–11481.
- F. D. Jochum and P. Theato, *Macromolecules*, 2009, **42**, 5941–5945.
- F. D. Jochum, L. zur Borg, P. J. Roth and P. Theato, *Macromolecules*, 2009, **42**, 7854–7862.
- K. Sumaru, M. Kameda, T. Kanamori and T. Shinbo, *Macromolecules*, 2004, **37**, 4949–4955.
- N. Rapoport, *Prog. Polym. Sci.*, 2007, **32**, 962–990.
- J. F. Mano, *Adv. Eng. Mater.*, 2008, **10**, 515–527.
- P. Kujawa, H. Watanabe, F. Tanaka and F. M. Winnik, *Eur. Phys. J. E*, 2005, **17**, 129–137.
- D. Chen, H. Liu, T. Kobayashi and H. F. Yu, *J. Mater. Chem.*, 2010, **20**, 3610–3614.
- G. Pouliquen and C. Tribet, *Macromolecules*, 2006, **39**, 373–383.
- S. Khoukh, R. Oda, T. Labrot, P. Perrin and C. Tribet, *Langmuir*, 2007, **23**, 94–104.
- A. Salonen, D. Langevin and P. Perrin, *Soft Matter*, 2010, **6**, 5308–5311.
- J.-F. Lutz and A. Hoth, *Macromolecules*, 2006, **39**, 893–896.
- F. D. Jochum and P. Theato, *Polymer*, 2009, **50**, 3079–3085.
- M. Emond, J. Sun, J. Gregoire, S. Maurin, C. Tribet and L. Jullien, *Phys. Chem. Chem. Phys.*, 2011, **13**, 6493–6499.
- A. Desponds and R. Freitag, *Langmuir*, 2003, **19**, 6261–6270.
- J.-M. Schumers, C.-A. Fustin and J.-F. Gohy, *Macromol. Rapid Commun.*, 2010, **31**, 1588–1607.
- F. D. Jochuma and P. Theato, *Chem. Commun.*, 2010, **46**, 6717–6719.
- G. Wang, X. Tong and Y. Zhao, *Macromolecules*, 2004, **37**, 8911–8917.
- Q. A. Jin, G. Y. Liu, X. S. Liu and J. A. Ji, *Soft Matter*, 2010, **6**, 5589–5595.
- S. Wu, L. Wang, A. Kroeger, Y. P. Wu, Q. J. Zhang and C. Bubeck, *Soft Matter*, 2011, **7**, 11535–11545.
- Y. Y. Wang, S. L. Lin, M. H. Zang, Y. H. Xing, X. H. He, J. P. Lin and T. Chen, *Soft Matter*, 2012, **8**, 3131–3138.
- O. Boissiere, D. Han, L. Tremblay and Y. Zhao, *Soft Matter*, 2011, **7**, 9410–9415.
- Y. Zhao and L. Tremblay, *Macromolecules*, 2011, **44**, 4007–4011.
- Y. Zhao and J. He, *Soft Matter*, 2009, **5**, 2686–2693.
- J. Ruchmann, S. Fouilloux and C. Tribet, *Soft Matter*, 2008, **4**, 2098–2108.
- H. Rau, in *Photochem. Photophys.*, ed. J. K. Rabek, CRC Press, 1990, vol. II, p. 119.
- J.-F. Lutz, K. Weichenhan, O. Akdemir and A. Hoth, *Macromolecules*, 2007, **40**, 2503–2508.
- C. Tribet, in *Smart Light-Responsive Materials: Azobenzene-Containing Polymers and Liquid Crystals*, ed. Y. Zhao and T. Ikeda, Wiley, 2009, pp. 243–272.
- E. Wischerhoff, K. Uhlig, A. Lanckenau, H. G. Borner, A. Laschewsky, C. Duschl and J.-F. Lutz, *Angew. Chem., Int. Ed.*, 2008, **47**, 5666–5668.
- J. Ruchmann, S. C. Sebai and C. Tribet, *Macromolecules*, 2011, **44**, 604–611.
- S. Wu, L. F. Niu, J. Shen, Q. J. Zhang and C. Bubeck, *Macromolecules*, 2009, **42**, 362–367.
- Y. Morishima, M. Tsuji, M. Kamachi and K. Hatada, *Macromolecules*, 1992, **25**, 4406–4410.
- N. Ishii, R. Obeid, X. P. Qiu, J. Mamiya, T. Ikeda and F. M. Winnik, *Mol. Cryst. Liq. Cryst.*, 2010, **529**, 60–70.
- J. Q. Jiang, X. Tong, D. Morris and Y. Zhao, *Macromolecules*, 2006, **39**, 4633–4640.
- X. D. Tang, L. C. Gao, X. H. Fan, X. C. Liang and Q. F. Zhou, *Macromol. Chem. Phys.*, 2009, **210**, 1556–1562.
- Y. Zhao, *J. Mater. Chem.*, 2009, **19**, 4887–4895.
- C. J. Chen, G. Y. Liu, X. S. Liu, D. D. Li and J. Ji, *New J. Chem.*, 2012, **36**, 694–701.
- J. Zou, B. Guan, X. J. Liao, M. Jiang and F. G. Tao, *Macromolecules*, 2009, **42**, 7465–7473.
- P. J. Zheng, X. Hu, X. Y. Zhao, L. Li, K. C. Tam and L. H. Gan, *Macromol. Rapid Commun.*, 2004, **25**, 678–682.