Reactive thermoresponsive copolymer scaffolds†

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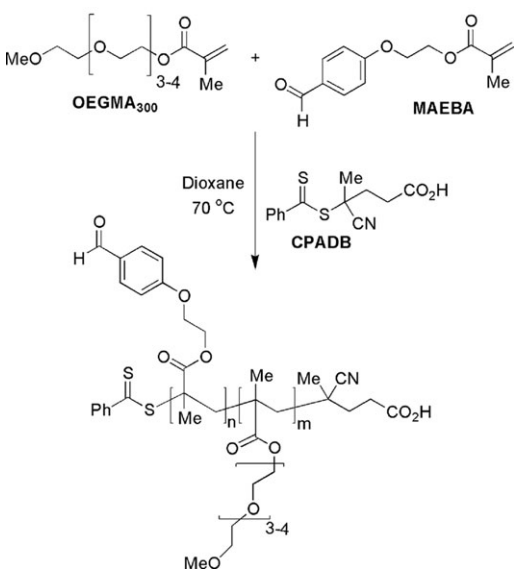
Thermoresponsive copolymer scaffolds containing reactive aldehyde functions were prepared and a selection of organic residues conjugated to these copolymer scaffolds through oxime/hydrazone formation. The conjugation of hydrophobic residues affords copolymers whose lower solution critical temperatures are in most cases higher than that of the parent copolymer scaffold.

Thermoresponsive polymers are an important and extremely useful class of macromolecule which display great promise in numerous nanotechnological and biomedical applications.[1] These polymers undergo a reversible phase transition from soluble to insoluble when their solution temperature is raised above their lower critical solution temperature (LCST), changing from an extended chain conformation below this temperature into a collapsed chain above this temperature.

The utility of thermoresponsive polymers could be increased if they possessed reactive functional groups within their side chains to allow the conjugation of other molecules *e.g.* peptides, carbohydrates, targeting vectors or drugs, onto the polymer scaffold. There are, however, remarkably few examples described in the literature of thermoresponsive polymers featuring reactive functional groups.[2] The groups of Brooks and Kizhakkedathu have prepared[2a] poly(*N*-[(2,2-dimethyl-1,3-dioxolane)methyl]acrylamide), which can be transformed through cleavage of the pendant dioxolane groups followed by oxidation of the resultant diol to afford a thermoresponsive polymer scaffold possessing reactive aldehyde groups. Although this work represents a significant advance, there still remains a need for a thermoresponsive polymer scaffold which can be readily prepared and requires no post-polymerization transformations to activate the reactive functional groups. We report here a thermoresponsive random copolymer scaffold displaying reactive aldehyde groups on its side chains and demonstrate that examples of alkoxyamine and hydrazide residues can be successfully conjugated onto the scaffold, subsequently influencing the copolymers LCST.

We chose to base our copolymer scaffold upon poly-oligo(ethylene glycol) methacrylate polymers, a relatively new class of thermoresponsive polymer possessing a high degree of biocompatibility and tunable LCSTs.[3] These non-linear poly(ethylene glycol) (PEG) analogues are alternative thermoresponsive polymers to poly-*N*-isopropylacrylamide[4]—a well-known thermoresponsive polymer—as there are no toxicity issues yet their thermosensitivity is almost independent of external conditions. In particular, we chose to focus on polymers derived from the $OEGMA_{300}$ monomer which possesses a LCST of ~60 °C.[5] The known aldehyde methacrylate *p*-(methacryloxyethoxy)benzaldehyde[6] (MAEBA) was chosen as a suitable monomer to incorporate reactive aromatic aldehyde functional groups into the polymer chain. Aldehydes are an attractive functional group to facilitate polymer functionalization through hydrolytically stable oxime or hydrazone bond formation with readily available nitrogen nucleophiles.[7] To ensure accurate comparisons between different polymers it is vitally important that they possess uniform molecular weights, and for this reason we have utilized living radical polymerization techniques to prepare our polymer samples. Reversible addition–fragmentation chain transfer (RAFT) polymerization[8] is particularly appealing because of its versatility and experimental simplicity, and has previously demonstrated great utility in polymerizing OEGMA monomers with a high level of control.[9] The RAFT chain transfer agent 4-(4-cyanopentanoic acid)dithiobenzoate (CPADB) was used to mediate the copolymerization of $OEGMA_{300}$ and MAEBA (Scheme 1) in dioxane at 70 °C to produce a series of copolymers **P1–P6** of similar lengths but possessing different ratios of the two monomers. These copolymers, which display excellent water-solubility, were characterized (Table 1) by gel permeation chromatography using THF as eluant, displaying monomodal distributions in all cases and polydispersity



Scheme 1 RAFT polymerization of $OEGMA_{300}$ with methacrylate *p*-(methacryloxyethoxy)benzaldehyde (MAEBA) to form reactive thermoresponsive copolymer scaffolds.

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† Electronic supplementary information (ESI) available: All experimental procedures and characterization of all polymers (**P1–P6**), conjugation procedures and turbimetric analysis of all conjugates. See DOI: 10.1039/c0cc03856c

Table 1 Characterization of polymers **P1–P6**

Polymer	Comonomer ratio $OEGMA_{300}$: MAEBA[a]	Molar wt% of MAEBA	M_n[b]/g mol^{-1}	M_w[b]/g mol^{-1}	PDI	LCST[c]/°C
P1	2 : 1	28.0%	13 700	18 500	1.35	22.0
P2	3 : 1	20.5%	13 000	18 300	1.41	31.0
P3	4 : 1	16.5%	13 800	19 400	1.40	39.0
P4	8 : 1	9.0%	12 200	16 400	1.34	49.0
P5	16 : 1	4.5%	15 800	21 800	1.38	57.5
P6	$OEGMA_{300}$ homopolymer	0%	17 700	25 000	1.41	66.0

[a] As determined by ^{1}H NMR spectroscopy. [b] As determined by gel permeation chromatography in THF. [c] As determined by turbimetric analysis in 0.1 M NaCl solution at pH 7.4. LCST defined as the onset of sharp change in transmission at 550 nm.

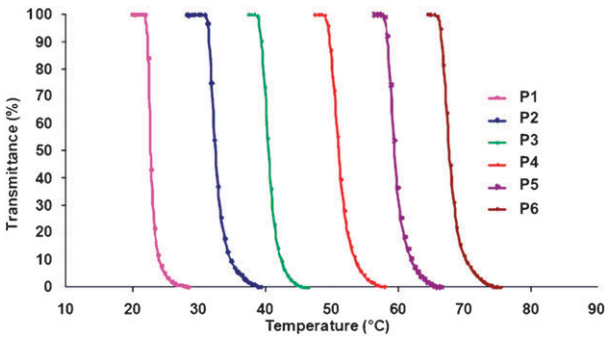


Fig. 1 Temperature–turbidity curves for copolymers/polymers **P1–P6** measured in 0.1 M NaCl solution at pH 7.4.

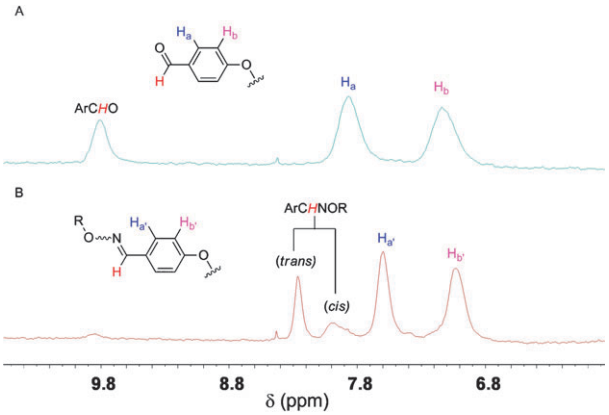


Fig. 2 ^{1}H NMR spectrum (400 MHz, D_2O) before (A) and after (B) conjugation of residue **2** onto copolymer **P3** (R = CH_2CO_2H).

indices 1.34–1.41, and by ^{1}H NMR spectroscopy. The monomer composition of each copolymer was determined by ^{1}H NMR spectroscopy and found to have an excellent match with the theoretical composition as determined by the monomer feed ratios.

The LCSTs of **P1–P6** were measured by turbimetric analysis (Table 1, Fig. 1) in pH controlled aqueous solution, indicating that as expected the LCST decreases linearly as the ratio of the more hydrophobic monomer MAEBA within the copolymer increases (see ESI†, Fig. 1), demonstrating that the LCST of these copolymers can be tuned by varying the comonomer composition. Further studies indicated that the LCSTs of copolymers **P1–P6** was also found to be dependent on salt concentration and pH. The presence of 0.1 M NaCl (pH 7.4) resulted in a reduction in the LCST of ~4 °C compared to identical samples in purite water (pH 7.4) for all polymers at a concentration of 2 mg mL^{-1}. This observation can be explained by the partial dehydration of the PEG side-chains in the presence of NaCl as reported previously.[10] Similarly, a change in pH from 4.0 to 7.4 in a 0.1 M NaCl background resulted in an increase in LCST of ~3–4 °C, observations which can be attributed to the deprotonation of the carboxylic acid end group of the polymers, increasing their hydrophilicities.[11]

To investigate the effectiveness of these copolymers as reactive scaffolds, a selection of small molecule alkoxyamine and hydrazide residues were conjugated (see ESI†) onto the polymer scaffolds through oxime or hydrazone bonds. The selection of residues was chosen to include hydrophilic residues (**1a and b**), an ionic residue (**2**) and hydrophobic residues (**3–8**). For the residues studied, the conjugations were found to be highly efficient in every case. A typical ^{1}H NMR spectrum for the conjugation of *O*-(carboxymethyl)hydroxylamine **2** in D_2O is shown (Fig. 2). As can be observed, the aldehyde signal at δ = 9.8 ppm (Fig. 2A) is greatly decreased in intensity after the conjugation (Fig. 2B) whilst signals for *cis*/*trans*-oxime are observed at δ = 8.0 and 8.3 ppm, confirming efficient conjugation.

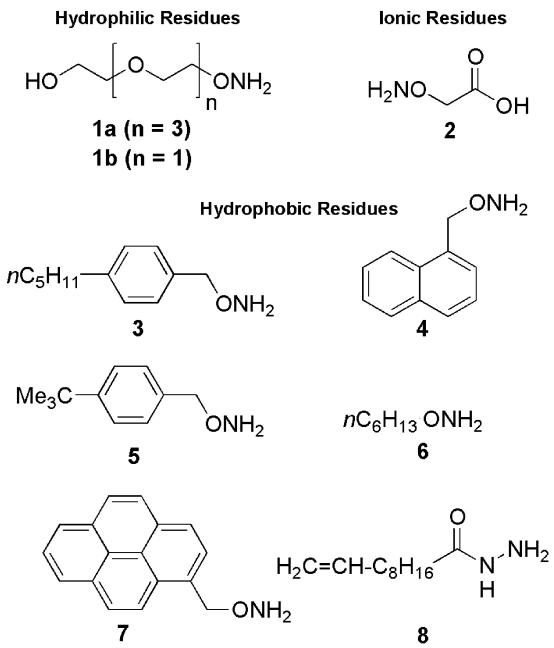
The LCSTs of a selection of conjugated copolymers were measured (Table 2) by turbimetric analysis (see ESI†). Conjugation of the hydrophilic tetraethylene glycol residue **1a** to the copolymer scaffolds **P1**, **P3** and **P4** possessing ratios of monomers $OEGMA_{300}$: MAEBA of 2 : 1, 4 : 1 and 8 : 1, respectively, were found to afford LCSTs corresponding to increases of 20.0 °C, 9.5 °C and 8.5 °C, respectively, relative to the parent copolymer scaffolds. Conjugation of the shorter

Table 2 Measured LCST values for the conjugation residues **1–8** onto copolymer **P3**, and their differences relative to **P3** (final entry) under identical conditions

Residue	LCST[a]/°C	ΔT relative to **P3**/°C	D_h[b]/nm
1a	48.5	9.5	7.5
1b	42.5	3.5	6.7
2	—	—	7.6
3	44.0	5.0	8.8
4	43.0	4.0	8.4
5	43.0	4.0	8.9
6	42.0	3.0	10.0
7	42.0	3.0	9.8
8	38.0	−1.0	8.5
P3	39.0	—	6.1

[a] As determined by turbimetric analysis in 0.1 M NaCl solution at pH 7.4. LCST defined as the onset of sharp change in transmission. [b] As determined by dynamic light scattering.

hydrophilic residue **1b** to the copolymer scaffold **P3** afforded a conjugate whose LCST is only 3.5 °C higher than **P3**. These observations are expected as the conjugation of hydrophilic residues such as **1a–b** should increase the hydrophilicity of the copolymers and consequently raise their LCSTs. The copolymer conjugates derived from conjugation of the hydrophobic residues **3–8** onto copolymer scaffolds **P3** were found to exhibit LCSTs either greater or comparable to that of the parent copolymer, observations which were unexpected.[12] The measured hydrodynamic diameters (Table 2) of these 'hydrophobic' conjugated copolymers below their LCSTs indicate they exist as unimers in solution, suggesting that the unexpected increase in LCST is not as a consequence of the formation of micellar aggregates. The reasons for the unexpected LCSTs of the 'hydrophobic' conjugated copolymers are not clear and an in-depth study beyond the scope of this paper, but may involve the disruption by hydrophobic appendages of the interfacial waters associated with the polymer chain, resulting in an increase in entropy of the polymer (see ESI† for an in-depth discussion). An implication of these observations is that it suggests poly-oligo(ethylene glycol) methacrylate-based polymer scaffolds can be 'loaded' with hydrophobic residues without losing their thermoresponsive properties, a feature which could be important in fields such as drug delivery.



The conjugation of the ionic residue *O*-(carboxymethyl)-hydroxylamine (**2**) to copolymer scaffold **P3** in 0.1 M NaCl solution afforded a copolymer conjugate whose LCST was strongly pH dependent. At pH 3.8, below the pK_a of the conjugated carboxylic acid groups, the LCST was 44.5 °C, an increase of 5.5 °C over the parent copolymer, whilst at pH 7.4, above the pK_a of the carboxylic acid, a LCST was not observed up to 100 °C. These results suggest that copolymers displaying both pH and temperature sensitivity can be accessed with ease through a simple conjugation procedure.[13]

In conclusion, we have described here thermoresponsive copolymer scaffolds bearing reactive aldehyde functionalities which can be conjugated with small molecule residues through oxime formation. Advantages of this copolymer are: (i) it is easy to prepare and does not require any deprotection or post-polymerization modification steps to make the aldehyde function available for reaction, (ii) it is fully water soluble and principally composed of biocompatible oligo(ethylene glycol) segments, (iii) nitrogen nucleophiles such as alkoxyamines and hydrazides can conjugate onto the copolymer with high yields through formation of hydrolytically stable carbon–nitrogen double bonds, and (v) the LCST of the copolymer scaffold can be tuned by changing the composition of the residues conjugated to the scaffold. Furthermore, by using a controlled living radical polymerization method such as RAFT, the structural properties of the polymer *i.e.* the length of the polymer, the density of aldehyde groups, the type of OEGMA monomers used, can easily be tuned thus allowing quick access to a series of polymer scaffolds. The unexpected observation that the hydrophobic residues can, in most cases studied, be conjugated to these copolymer scaffolds whilst maintaining or increasing the LCST of the resulting conjugates may also increase the potential utility of these copolymer scaffolds.

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