Binary mixtures of well-defined, stimuli-responsive elastin-based side-chain polymers show a single transition temperature that depends on blend composition.

Polymers displaying lower critical solution temperature (LCST) behaviour are potentially useful for a variety of biomedically-related applications.\(^1\) By far the most widely studied polymer of this type is poly(N-isopropylacrylamide) (pNiPAm), which displays an LCST in aqueous solution at around 32 °C.\(^2\) This value can be altered, normally within a 10 °C range, by changing the end-group functionality, varying the molecular weight, and by the introduction of co-monomers.\(^3\) An alternative class of thermoresponsive polymers are elastin-like peptides (ELPs), which are bio-inspired polymers composed of repeat units of the amino acids Val–Pro–Gly–Val–Gly (VPGVG). The inherent bio-degradability and (presumed) biocompatibility of ELPs may provide advantages over carbon-backbone polymers (pNiPAm) in certain applications. In addition, the LCST (~35 °C) can be tuned within a wide temperature range by replacing the fourth (Val) residue with any other amino acid (VPGXG) except Pro.\(^6\) Such tuning of the thermal response of pNiPAm, ELPs and other similar polymers is important for applications such as tumour cell targeting, for which the polymers should be soluble at physiological temperature (~37 °C) and have a transition temperature (\(T_t\)) below the temperature for local hyperthermia (~42 °C).\(^7\)

Meyer and Chilkoti demonstrated an inverse dependence between \(T_t\) and both molecular weight and concentration for ELPs synthesised by recombinant DNA techniques.\(^7\)

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Recently, we have described the synthesis of elastin-based side-chain polymers (EBPs),\(^8\)\(^9\) in which a methacrylate derivative of the pentapeptide VPGGVG was polymerised using controlled radical polymerisation (CRP) techniques to give well-defined synthetic polymers with similar thermoresponsive behavior to linear ELPs. These EBPs can be viewed as chemically accessible variants of ELPs (although it should be noted that ELPs are pH- as well as thermally-responsive, as they bear free carboxylic acid groups from the C-terminus of the pentapeptide side-chains).

An investigation of the thermoresponsive behaviour of our EBPs revealed a strong dependence of \(T_t\) on molecular weight,\(^9\) in agreement with the previous findings on ELPs and recent work on narrow polydispersity pNiPAmS prepared by atom transfer radical polymerisation (ATRP).\(^5\) This behaviour is consistent with classical theory on the thermodynamics of polymer solutions, which states that, at the spinodal point, the interaction parameter between polymer and solvent (\(\chi\)) depends on the weight-average molecular weight \(M_w\)

\[ \chi = \frac{1}{2} \left( \frac{1}{\phi_p M_w} + \frac{1}{1 - \phi_p} \right) \]

where \(\phi_p\) is the volume fraction of the polymer in solution. An important observation from eqn. (1) is that \(\chi\) does not depend on polydispersity. Therefore, it should be possible to vary \(M_w\), and thus \(\chi\), by mixing together two narrow polydispersity polymers of different chain length. This should provide a simple method of fine-tuning \(T_t\), since \(\chi \propto 1/T\).

In order to explore this possibility, well-defined EBPs of different chain length are required. Our recent work has employed reversible addition fragmentation chain-transfer (RAFT) polymerisation to prepare well-defined EBPs.\(^9\) RAFT is arguably the most versatile of the CRP techniques, being amenable to the polymerisation of virtually all monomers active in radical polymerisation.\(^1\) Thus, VPGVG-methacrylate derivative \(I\) was polymerised by RAFT using dithioester \(2\) and a commercially available azo initiator at 70 °C to give a series of well-defined, narrow molecular weight distribution EBPs, varying in number average degree of polymerisation (\(\text{DP}_n\)) from 29 to 88 (see Scheme 1 and Table 1). The dithioester end-groups of these EBPs were then removed by treatment with an excess of azo initiator\(^12\) to prevent their interference in turbidimetry experiments.

In a first experiment, polymers A and D (Table 1) were mixed in a 1 : 1 volume ratio in aqueous solution. The
polymers were chosen carefully to give a sufficient difference in their $T_1$ values to be able to determine the effect of mixing on $T_1$ (see Table 1).

In agreement with eqn. (1), only one transition was observed in the turbidimetry profile of the mixture of polymers A and D (Fig. 1). Furthermore, the $T_1$ value of the mixture (38.2 °C) agrees well with the average (40.2 °C) of the individual $T_1$ values of polymers A and D. The $T_1$ values of ELPs and narrow polydispersity pNiPAm have both been shown to be strongly dependent on molecular weight. However, surprisingly, the behaviour of mixtures of such polymers of different chain lengths has, as far as we can determine, not been reported. This is despite the fact that the thermally-induced phase transition has been described as a cooperative process, and that intermolecular aggregation has been described for pNiPAm.

To analyse further the possibility of fine-tuning the transition temperature, two more polymers (B and C, Table 1), with a difference in their $T_1$ values of around 10 °C, were prepared and blended together at a range of compositions. As shown in Fig. 2(a), only one transition could be observed for all mixtures of the two polymers. An important consequence of this cooperative behaviour, as can be seen in Fig. 2(b), is that there is a linear dependence between the fraction of the lower molecular weight polymer in the mixture and $T_1$. This allows $T_1$ to be fine-tuned between the values corresponding to the individual polymers, simply by varying the ratio of the two polymers. This is obviously much more practical than synthesising a range of polymers of different chain lengths.

We believe that these results could have important practical implications, for example regarding the immobilisation of enzymes onto surfaces employing reversible (‘smart’) co-assembly. In previous work on elastin-mediated surface assembly, an ELP immobilised on a gold surface was employed to immobilise the same polymer (as part of a fusion protein) in solution. The ELP in solution underwent self-assembly and simultaneously co-assembly with the polymer attached to the surface. Co-assembly between the molecules (and their aggregates) in solution and hydrophobic ELPs on the surface occurs due to hydrophobic interactions, which could lead to problems with non-specific adsorption onto unfractionised surfaces. Such problems could be overcome by attaching a relatively high molecular weight (low $T_1$) EBP to a surface and using a lower $T_1$ EBP in solution. This should bring about co-assembly between the EBP in solution and that on the surface, instead of self-assembly of the low DP polymer in solution, thus preventing non-specific adsorption.

Table 1 Molecular weight data and thermoresponsive behavior of EBP

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\text{DP}_{n,ab}$</th>
<th>$\text{DP}_a$</th>
<th>$M_w$/[kg mol$^{-1}$]</th>
<th>PDI$^d$</th>
<th>$T_1^e$/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29</td>
<td>43</td>
<td>23.5</td>
<td>1.05</td>
<td>47.9</td>
</tr>
<tr>
<td>B</td>
<td>34</td>
<td>45</td>
<td>24.3</td>
<td>1.05</td>
<td>45.7</td>
</tr>
<tr>
<td>C</td>
<td>76</td>
<td>105</td>
<td>57.0</td>
<td>1.20</td>
<td>36.2</td>
</tr>
<tr>
<td>D</td>
<td>88</td>
<td>113</td>
<td>61.6</td>
<td>1.25</td>
<td>32.4</td>
</tr>
</tbody>
</table>

$^a$ Theoretical number-average degree of polymerisation (see ESI† for calculation formula). $^b$ Number-average degree of polymerisation, determined by aqueous size exclusion chromatography. $^c$ Number average molecular weight, determined by size exclusion chromatography. $^d$ Polydispersity index ($M_w/M_n$), determined by aqueous size exclusion chromatography. $^e$ Transition temperature, calculated from turbidimetry experiments in phosphate buffer (pH adjusted to 3.2; [polymer] = 0.65 mg mL$^{-1}$). Values quoted are the inflection points of the heating curves.

Fig. 1 Turbidimetry profiles of polymers A (▲), D (●) and a 1 : 1 volume ratio of A : D (solid line) measured by UV-Vis spectrometry at pH 3.2 in an aqueous phosphate-buffered solution (0.65 mg mL$^{-1}$). Absorbance values are normalised to 1.

Fig. 2 (a) Turbidimetry profiles for mixtures of polymers B and C of different volume fractions ($x$) of the lower molecular weight polymer (polymer B; ● $x_B = 0.00$; ▲ $x_B = 0.17$; ▼ $x_B = 0.33$; ▣ $x_B = 0.50$; ◼ $x_B = 0.67$; ▲ $x_B = 0.83$; ◼ $x_B = 1.00$). Experiments were carried out in an aqueous phosphate-buffered solution (pH adjusted to 3.2; [polymer] = 0.65 mg mL$^{-1}$). Absorbance values are normalised to 1. (b) Relation between the transition temperature ($T_1$) and the volume fraction of polymer B ($\text{DP}_a = 34$). Values quoted are the inflection points of the heating curves.
In conclusion, we have shown that mixtures of elastin-based side-chain polymers (EBPs) of different molecular weights undergo a simultaneous thermal transition, the temperature of which is linearly dependent on the composition of the mixture. This could lead to novel applications of EBPs that exploit their co-assembly, such as smart surface functionalisation.

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Notes and references


