Fine-tuning the transition temperature of a stimuli-responsive polymer by a simple blending procedure†

Francisco Fernández-Trillo,‡[a] Jan C. M. van Hest,[b] Jens C. Thies,[c]
Thierry Michon,[d] Ralf Weberskirch[e] and Neil R. Cameron*[a]

Received (in Cambridge, UK) 9th January 2008, Accepted 8th February 2008
First published as an Advance Article on the web 5th March 2008
DOI: 10.1039/b800266e

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 bio- and nanotechnological applications.[1,2] 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.[2,3] 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.[3–5] 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 ($\sim$35 °C) can be tuned within a wide temperature range by replacing the fourth (Val) residue with any other amino acid (VPGXG) except Pro.[6] Such tuning of the thermal response of pNiPAm, ELPs and other similar polymers is important for applications such as tumour cell targeting, for which the polymers should be soluble at physiological temperature ($\sim$37 °C) and have a transition temperature (T_t) below the temperature for local hyperthermia ($\sim$42 °C).[3]

Meyer and Chilkoti demonstrated an inverse dependence between T_t, and both molecular weight and concentration for ELPs synthesised by recombinant DNA techniques.[7] Recently, we have described the synthesis of elastin-based side-chain polymers (EBPs),[8,9] in which a methacrylate derivative of the pentapeptide VPGVG 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 EBPs 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 behavior 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 (χ) depends on the weight-average molecular weight (M_w) (eqn. (1))[10]

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

where ϕ_P is the volume fraction of the polymer in solution. An important observation from eqn. (1) is that χ does not depend on polydispersity. Therefore, it should be possible to vary M_w, and thus χ, 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.[11] Thus, VPGVG-methacrylate derivative **1** 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 (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.

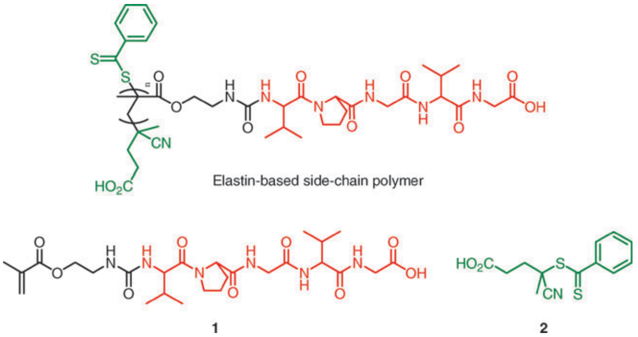
In a first experiment, polymers **A** and **D** (Table 1) were mixed in a 1 : 1 volume ratio in aqueous solution. The

---

[a] *Department of Chemistry and Interdisciplinary Research Centre in Polymer Science and Technology, Durham University, South Road, Durham, UK DH1 3LE*
[b] *Radboud University Nijmegen, Organic Chemistry, Institute for Molecules and Materials, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*
[c] *DSM Research Campus Geleen, Performance Materials—Chemistry & Technology, Urmonderbaan 22, 6167 RD Geleen, The Netherlands*
[d] *INRA, UMR GDPP, IBVM, Interactions Plante Virus, BP 81, 33883 Villenave d'Ornon Cedex, France*
[e] *Technische Universität München, Abteilung Chemie, Lehrstuhl für Makromolekulare Stoffe, Lichtenbergstraße 4, 85747 Garching, Germany*

† Electronic supplementary information (ESI) available: General procedures, calculation of $DP_{n,th}$ and synthetic procedures. See DOI: 10.1039/b800266e

‡ Current address: Departamento de Química Organica, Facultade de Química, Universidade de Santiago de Compostela, Avda. das Ciencias S/N, 15782, Santiago de Compostela, España.



Scheme 1 Structure of elastin-based side-chain polymers (EBPs), elastin-based monomer **1** and RAFT agent **2**.

polymers were chosen carefully to give a sufficient difference in their T_t values to be able to determine the effect of mixing on T_t (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_t value of the mixture (38.2 °C) agrees well with the average (40.2 °C) of the individual T_t values of polymers **A** and **D**. The T_t values of ELPs[7] and narrow polydispersity pNiPAms[5] 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,[13] and that intermolecular aggregation has been described for pNiPAm.[14]

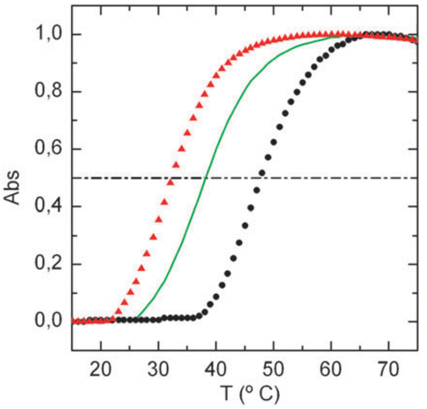


Fig. 1 Turbidimetry profiles of polymers **A** (▲), **D** (●) and a 1 : 1 volume ratio of **A** : **D** (solid line) measured by UV-Vis spectrometry at pH 3.2 in an aqueous phosphate-buffered solution (0.65 mg mL^{-1}). Absorbance values are normalised to 1.

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_t 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_t. This allows T_t 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.[15] 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,[16] which could lead to problems with non-specific adsorption onto unfunctionalised surfaces. Such problems could be overcome by attaching a relatively high molecular weight (low T_t) EBP to a surface and using a lower DP_n (high T_t) 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 EBPs

Polymer	$DP_{n,th}$[a]	DP_n[b]	M_n[c]/kg mol^{-1}	PDI[d]	T_t[e]/°C
A	29	43	23.5	1.05	47.9
B	34	45	24.3	1.05	45.7
C	76	105	57.0	1.20	36.2
D	88	113	61.6	1.25	32.4

[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 aqueous 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.

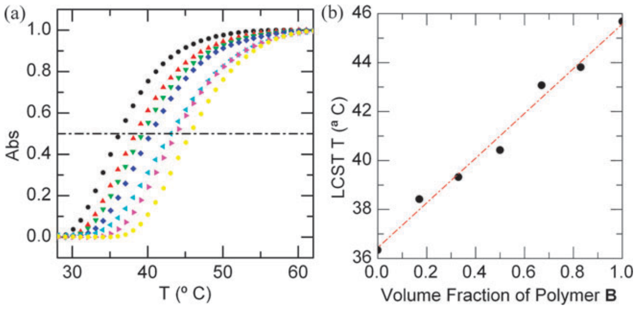


Fig. 2 (a) Turbidimetry profiles for mixtures of polymers **B** and **C** of different volume fractions (x) of the lower molecular weight polymer (polymer **B**; ● x_B = 0.00; ▲ x_B = 0.17; ▼ x_B = 0.33; ■ x_B = 0.50; ◀ x_B = 0.67; ▶ x_B = 0.83; ● x_B = 1.00). Experiments were carried out in an aqueous phosphate-buffered solution (pH adjusted to 3.2; [polymer] = 0.65 mg mL^{-1}). Absorbance values are normalized to 1. (b) Relation between the transition temperature (T_t) and the volume fraction of polymer **B** (DP_n = 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.

This work was performed within the European Research Training Network SMASHYBIO (Smart Assembly of Hybrid Biopolymers) under contract (HPRN-CT-2001-00188). We would also like to thank the rest of the SmashyBio Network for discussion and Dr John Sanderson (Durham University) for his help with peptide synthesis.

Notes and references

1 A. Chilkoti, M. R. Dreher and D. E. Meyer, *Adv. Drug Delivery Rev.*, 2002, **54**, 1093–1111; I. Y. Galaev and B. Mattiasson, *Trends Biotechnol.*, 1999, **17**, 335–340; A. S. Hoffman and P. S. Stayton, *Macromol. Symp.*, 2004, **207**, 139–151.

2 E. S. Gil and S. A. Hudson, *Prog. Polym. Sci.*, 2004, **29**, 1173–1222.

3 D. E. Meyer, B. C. Shin, G. A. Kong, M. W. Dewhirst and A. Chilkoti, *J. Controlled Release*, 2001, **74**, 213–224.

4 S. Furyk, Y. J. Zhang, D. Ortiz-Acosta, P. S. Cremer and D. E. Bergbreiter, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 1492–1501.

5 Y. Xia, N. A. D. Burke and H. D. H. Stover, *Macromolecules*, 2006, **39**, 2275–2283; Y. Xia, X. C. Yin, N. A. D. Burke and H. D. H. Stover, *Macromolecules*, 2005, **38**, 5937–5943.

6 D. W. Urry, C. H. Luan, T. M. Parker, D. C. Gowda, K. U. Prasad, M. C. Reid and A. Safavy, *J. Am. Chem. Soc.*, 1991, **113**, 4346–4348.

7 D. E. Meyer and A. Chilkoti, *Biomacromolecules*, 2004, **5**, 846–851.

8 L. Ayres, K. Koch, P. Adams and J. C. M. van Hest, *Macromolecules*, 2005, **38**, 1699–1704; L. Ayres, M. R. J. Vos, P. Adams, I. O. Shklyarevskiy and J. C. M. van Hest, *Macromolecules*, 2003, **36**, 5967–5973.

9 F. Fernandez-Trillo, A. Dureault, J. P. M. Bayley, J. C. M. Van Hest, J. C. Thies, T. Michon, R. Weberskirch and N. R. Cameron, *Macromolecules*, 2007, **40**, 6094–6099.

10 R. Koningsveld and A. J. Staverman, *J. Polym. Sci., Part A2*, 1968, **6**, 325–347.

11 J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559–5562; G. Moad, Y. K. Chong, A. Postma, E. Rizzardo and S. H. Thang, *Polymer*, 2005, **46**, 8458–8468; G. Moad, E. Rizzardo and S. H. Thang, *Aust. J. Chem.*, 2005, **58**, 379–410; S. Perrier and P. Takolpuckdee, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, **43**, 5347–5393.

12 S. Perrier, P. Takolpuckdee and C. A. Mars, *Macromolecules*, 2005, **38**, 2033–2036.

13 Y. Okada, F. Tanaka, P. Kujawa and F. M. Winnik, *J. Chem. Phys.*, 2006, **125**, 244902-1–244902-11; E. I. Tiktopulo, V. E. Bychkova, J. Ricka and O. B. Ptitsyn, *Macromolecules*, 1994, **27**, 2879–2882; D. W. Urry, *Methods Enzymol.*, 1982, **82**, 673–716.

14 M. Meewes, J. Ricka, M. Desilva, R. Nyffenegger and T. Binkert, *Macromolecules*, 1991, **24**, 5811–5816; J. Ricka, M. Meewes, R. Nyffenegger and T. Binkert, *Phys. Rev. Lett.*, 1990, **65**, 657–660.

15 J. Hyun, W. K. Lee, N. Nath, A. Chilkoti and S. Zauscher, *J. Am. Chem. Soc.*, 2004, **126**, 7330–7335.

16 For an example of the reversible immobilisation of ELPs onto a hydrophobic surface, see: W. Frey, D. E. Meyer and A. Chilkoti, *Adv. Mater.*, 2003, **15**, 248–251.