Molecular recognition in bisurea thermoplastic elastomers studied with pyrene-based fluorescent probes and atomic force microscopy†

Nicole E. Botterhuis, S. Karthikeyan, Dirk Veldman, Stefan C. J. Meskers and Rint P. Sijbesma*

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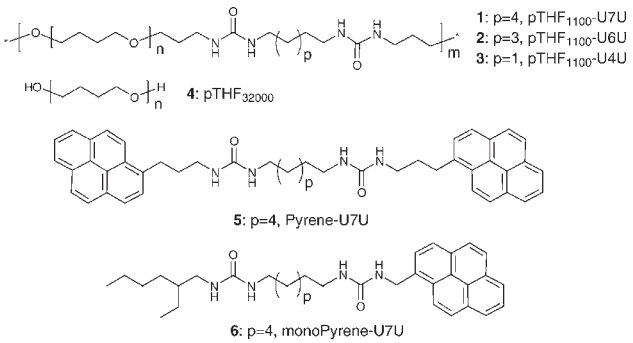
Fluorescence spectroscopy and atomic force microscopy (AFM) measurements using bisurea-pyrene probes show that they are randomly dispersed in the hard blocks of thermoplastic elastomers with matching bisurea groups, whereas they phase separate from polymers with non-matching or no bisurea groups.

Molecular recognition *via* hydrogen bonding is a useful tool to functionalize materials in a noncovalent manner. A wide range of hydrogen bonding motifs has been investigated to perform this function, including the diamidopyridine–thymine couple,[1] ureidopyrimidinone[2] and the bisurea motif. The latter strong and self-complementary motif is based on the formation of bifurcated hydrogen bonds between urea groups,[3] and has been used in organo-gelators,[4] hydrogelators,[5] templates for crystallization,[6] DNA-based coatings,[7] as a patterning tool in self-assembled monolayers[8] and in micelles.[9] Bisurea segments have also been used in thermoplastic elastomers (TPEs).[10–12] These TPEs derive their elastic properties from the microphase separation of the bisurea segments in fibrous hard blocks, consisting of a few layers of polymeric ribbons of linearly aggregated bisureas.[11] The small size of the hard blocks results in highly transparent, elastic materials. When a small amount of a molecule with a matching bisurea motif is mixed into a bisurea TPE, the guest molecules are integrated in the hard block, and increase the Young's modulus of the material without a reduction in tensile strength or strain at break.[12] It has also been shown with extraction experiments that dye molecules with a bisurea unit are strongly bound to the bisurea TPE if the alkyl spacing between the two urea groups is the same (*i.e.* matching) for the TPE and dye, whereas dyes with non-matching bisurea moieties were rapidly released from the matrix.[13] Similar behaviour was observed when a peptide with a bisurea unit was used to functionalize a polycaprolactone bisurea TPE to obtain a biofunctional material.[14]

Because of their role as a molecular reinforcer and their use in noncovalent functionalization of TPEs, the details of guest incorporation in bisurea TPEs deserve detailed investigation with bulk and surface techniques. We therefore decided to use bisurea molecule **5** (Scheme 1) as a guest molecule for characterization with AFM and optical spectroscopy. Bisurea **5** has 2 fluorescent pyrene moieties and was used previously to study guest incorporation in micellar bisurea hosts.[9] Pyrene is known to form excited state dimers (excimers), which fluoresce at a longer wavelength (400–600 nm) than the monomers, which emit between 370 and 450 nm.[15] If guest **5** is randomly incorporated in a host fiber, few excimers will be formed at a low concentration (Fig. 1a). However, at higher concentrations the molecules may form intermolecular excimers as part of the hard block of the host (Fig. 1b). In a non-matching polymer, the probe molecules may form phase separated stacks (Fig. 1c). It has also been shown that freely dissolved **5** forms intramolecular excimers.[9] Therefore, the fluorescence of molecule **5** can be used to probe incorporation of bisurea guests in hard blocks in the bulk of host polymers, while incorporation of the molecules in fibers at the surface can be probed with AFM.



Scheme 1 Set of molecules used for the mixing experiments.

Thin polymer films were prepared containing different amounts of **5** in segmented polytetrahydrofuran (pTHF) block copolymers with matching (**1**) or non-matching (**2**, **3**) bisurea blocks. pTHF without bisurea groups (**4**) with a M_n comparable to **1–3** was used as a reference host. Mixtures of **5** (stock solution of 20 mg ml^{-1} in 15% TFA in $CHCl_3$) with **1–4** (stock solution of 20 mg ml^{-1} in 10% MeOH in $CHCl_3$) with a final polymer concentration of 12 mg ml^{-1} were spincoated on thoroughly cleaned quartz plates‡ at 1500 rpm for 2 min. In the solutions used for spin coating, hydrogen bonding between bisurea compounds is suppressed by the use of TFA and methanol. Investigation of the spin coated films with AFM showed that in annealed films of pristine **1**,[11]

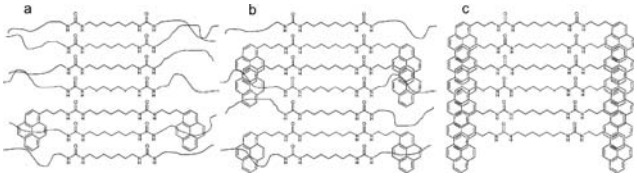


Fig. 1 Bisurea stacking. (a) **5** at low concentration in **1**. (b) **5** at high concentration in **1**. (c) Self-assembled, phase-separated stack of **5**.

Eindhoven University of Technology, Department of Chemical Engineering, Laboratory of Macromolecular and Organic Chemistry, PO Box 513, 5600 MB Eindhoven, The Netherlands. E-mail: r.p.sijbesma@tue.nl; Fax: +31402451036; Tel: +31402473111

† Electronic supplementary information (ESI) available: Synthetic procedures, UV spectra and data for monoPyrene **6**. See DOI: 10.1039/b804457k

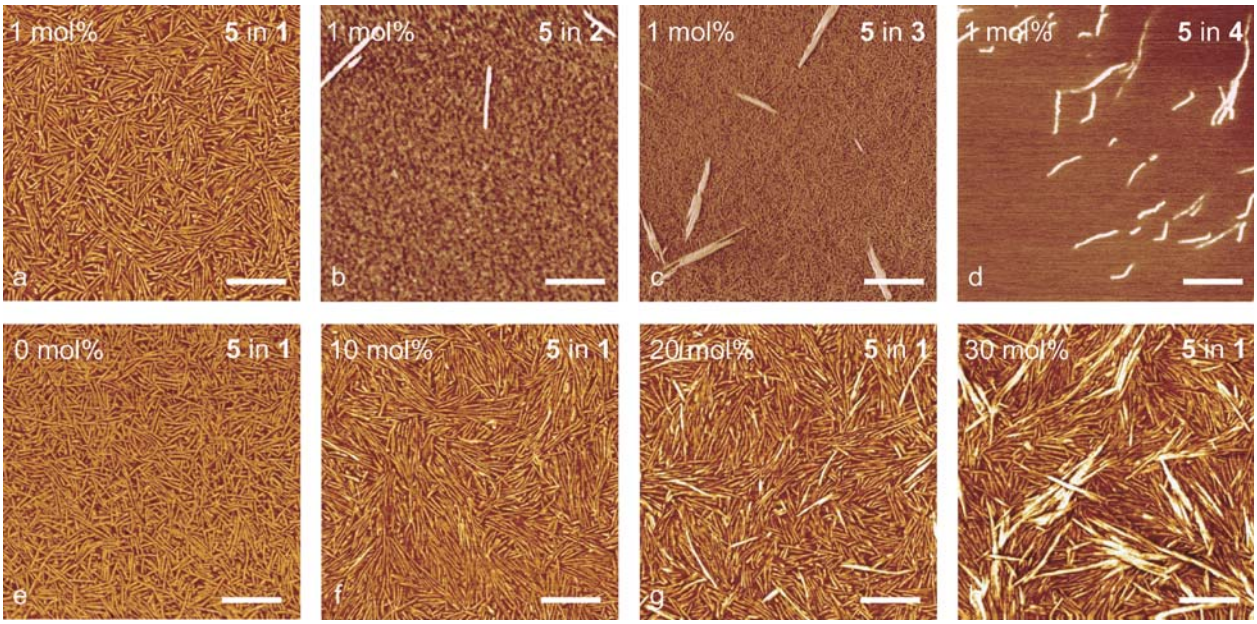


Fig. 2 Intermittent contact mode AFM phase images. Top: thin films of 1 mol% **5** in **1** (a), **2** (b), **3** (c) and **4** (d). Bottom: thin films of 0 (e), 10 (f), 20 (g) and 30 (h) mol% **5** in **1**. Samples in a and e–h were annealed for 30 min at 110 °C prior to AFM imaging. The scale bar is 200 nm in all images.

small, approximately 10 nm wide fibers are present (Fig. 2e). Without annealing, these pTHF-bisurea fibers are less defined (Fig. S1, ESI†). The films of matching polymer **1** with 1 mol% (relative to the bisurea segments in **1**) of **5**, showed no changes in surface morphology (Fig. 2a). However, when 1 mol% of **5** was present in films of polymers with non-matching (**2**–**3**) or no (**4**) bisurea segments,§ hard, needle-like features were observed on the surface of the polymer films, indicative of phase separation of **5** (Fig. 2b–d). These features increased in abundance with decreasing match between guest and host. When higher mole percentages of **5** are mixed in films of matching polymer **1**, phase separation of **5** is observed clearly above 20 mol% (Fig. 2g and h).

The experiments confirm previous results on incorporation of various bisurea guests in polyester based TPEs, which indicated a high specificity for the bisurea molecular recognition and phase separation of a bisurea guest above 23 mol%.[12,14] However, the AFM experiments are not able to probe the behavior of guest molecules below the surface of the film and they cannot shed light on the molecular details of guest incorporation. Therefore, films of the polymers containing increasing amounts of **5** were studied using fluorescence spectroscopy. The fluorescence emission spectrum of a film of **1** containing 3 mol% of **5**, excited at 330 nm, is shown in Fig. 3a, solid line. This spectrum resembles that of molecularly dissolved pyrene and there is only minimal emission between 450 and 600 nm that is characteristic for aggregated pyrene. However, if **5** was incorporated in bisurea polymers with non-matching bisurea groups (**2** or **3**), an excimeric emission band was clearly observed (Fig. 3a), indicating that **5** is not fully molecularly separated by the bisurea segments of these polymers. If no bisurea moiety was present in the polymer (**4**), the excimer band was of even higher intensity relative to the monomeric pyrene emission. Therefore we conclude that the guests are phase-separated in the non-matching hosts, while in the matching polymer (**1**) they are more or less randomly dispersed in the hard segment fibrils. The effect of increasing the guest concentration from 1 to 30 mol% in polymer **1** on the emission spectra is shown in Fig. 3b. Distinct excimer bands were only present in the emission spectra of films with higher (more than 3 mol%) guest concentrations. Excitation spectra of the different films give additional information on the interaction of **5** with the host polymers. Excitation spectra recorded at the monomeric emission wavelength of 377 nm are similar for all samples (Fig. S2, ESI†). However, excitation spectra of the non-matching polymer mixtures recorded at the emission band of the excimer (487 nm, Fig. 3c) are broader and have a higher ratio of intensities I_{330}/I_{347} than the excitation spectrum of 1 mol% of **5** in **1**. This supports the conclusion that the guest molecules are highly aggregated in the non-matching polymers, while in the matching host they are dispersed in the fibers. Interestingly, the excitation spectra of the

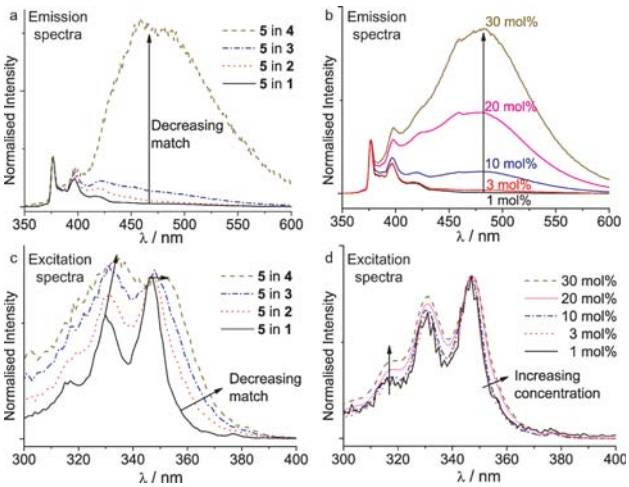


Fig. 3 Fluorescence emission (a–b, λ_{exc} = 330 nm) and excitation (c–d, λ_{em} = 487 nm) spectra of 3 mol% of **5** mixed with different polymers (a and c) or different mol% of **5** in the matching polymer **1** (b and d). The emission (excitation) spectra were normalized to the peak at 377 (347) nm.

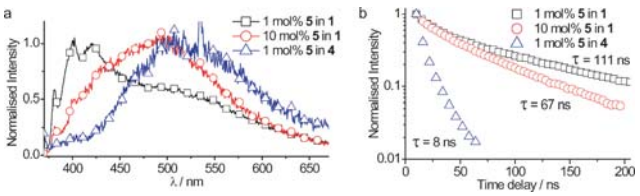


Fig. 4 Fluorescence spectra (λ_{exc} = 358 nm) of thin films containing 1 and 10 mol% of **5** in **1** and 1 mol% of **5** in **4** at 10–30 ns delay time after the excitation pulse (a) and decay of the emission between 400 and 700 nm of these films after the first 10 ns (b).

excimers in the films containing 3 and 10 mol% show almost no broadening compared to the spectrum with 1 mol% of **5**, indicating that most guest molecules are dispersed up to at least 10 mol% (Fig. 3d). At much higher concentrations (20 and 30 mol%), the excitation spectra recorded at the emission wavelength of 487 nm are broadened, however to a much lesser extent than any of the non-matching excitation spectra containing only 1 mol% of **5**. Also with UV/vis spectroscopy, the broadening of spectra was only observed in the non-matching systems and at very high concentrations in the matching system (Fig. S3, ESI†). These observations establish that the needles observed by AFM in these films are not an exclusive surface phenomenon, and that they are in fact the phase separated pyrene molecules. Phase separation of bisurea guests was already studied with DSC measurements by Wisse *et al.*[12]

In order to study the effect of probe solubility on incorporation, a more soluble probe with a single pyrene moiety was synthesized (**6**). When this molecule was mixed with the matching polymer **1**, the excimer band remained low even at 30 mol% incorporation, and no broadening was observed in the excitation spectrum recorded at 487 nm (Fig. S4, ESI†). Furthermore AFM showed that hard needle-like structures were absent, which indicates that **6** is not phase separated from the polymer matrix (Fig. S5, ESI†). These measurements indicate that solubility is an important parameter in the incorporation of probes in bisurea fibers, and that π-stacking is a driving force for phase segregation of probe **5**.

Finally, the emission of the aggregated pyrene moieties was investigated with time-gated fluorescence measurements. Fig. 4a shows the emission spectra acquired in the time interval between 10 and 30 ns after the pulsed excitation of two films of polymer **1** containing 1 and 10 mol% of **5** and one of polymer **4** containing 1 mol% of **5**. The decay of the emission between 400 and 700 nm cannot be described with a single exponential decay (Fig. 4b). Therefore, decay times (τ) were fitted on the part of the curve after 40 ns for the films of polymer **1** and on the part of the curve between 10 and 50 ns for the film of polymer **4**. In the film of **1** with 10 mol% of **5**, the emission decays considerably faster (τ = 67 ns) than that of the sample with only 1 mol% of **5** (τ = 111 ns). The long decay time is typical for pyrene.[16] The shorter fluorescence decay time of the 10 mol% sample could be caused by concentrations quenching with enhanced non-radiative decay. For the sample in polymer **4**, the decay time is an order of magnitude shorter (τ = 8 ns). This reflects the fact that the guest molecules in polymer **4** are phase-separated (Fig. 1c), leading to short decay times, whereas at higher concentration in the matching polymer **1** they are fully dispersed in the bisurea segments of the host and only form excimers with neighboring pyrene guest molecules in the fibers (Fig. 1b).

In conclusion, we have shown that probe **5** is randomly dispersed in the hard blocks of TPEs with matching bisurea groups, but phase separates when the bisurea hard blocks have a different alkyl spacer length between the urea groups. We are currently investigating application of the observed selectivity for colocalization and separation of multiple functional guests in TPEs with more than one type of bisurea hard block.

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Notes and references

‡ The cleaning procedure comprises sonication in acetone for 15 min, rubbing briefly with SDS soap solution, sonication in SDS soap solution for 10 min, rinsing in a stream of demi water for 15 min and finally sonication in isopropanol for 10 min.
§ For polymer **4**, a concentration of 1 mol% relative to the amount of bisurea segments is not possible, therefore the weight concentration of **5** in **4** is kept the same as for **5** in **1**.

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