Supplementary information for:

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

Atomic force microscopy – Effect of annealing

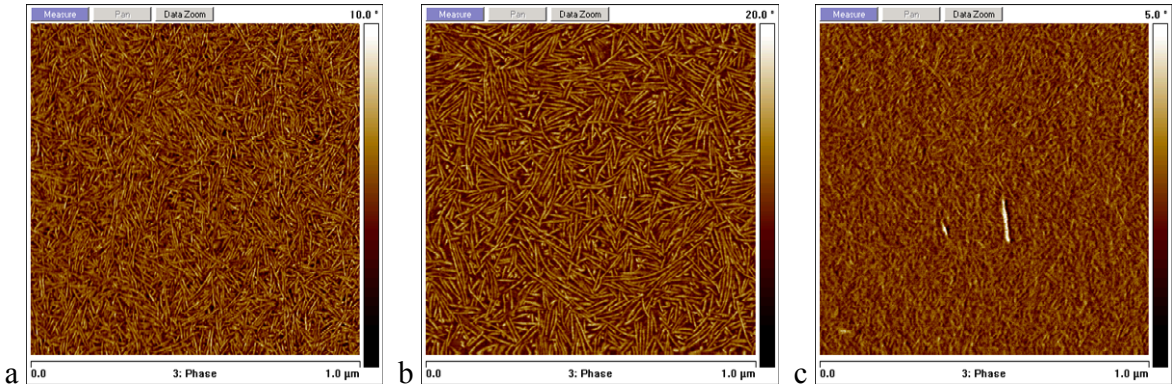


Fig. S1: Intermittent contact mode AFM phase images. a-b) $pTHF_{1100}$-U7U with 1 mol% of **6** before (a) and after (b) annealing for 30 minutes at 110 ºC and slow cooling down (20ºC / h). c) $pTHF_{1100}$-U6U with 1 mol% of **6** without annealing, duplo of Fig. 2b, with fewer hard needles.

Excitation spectra

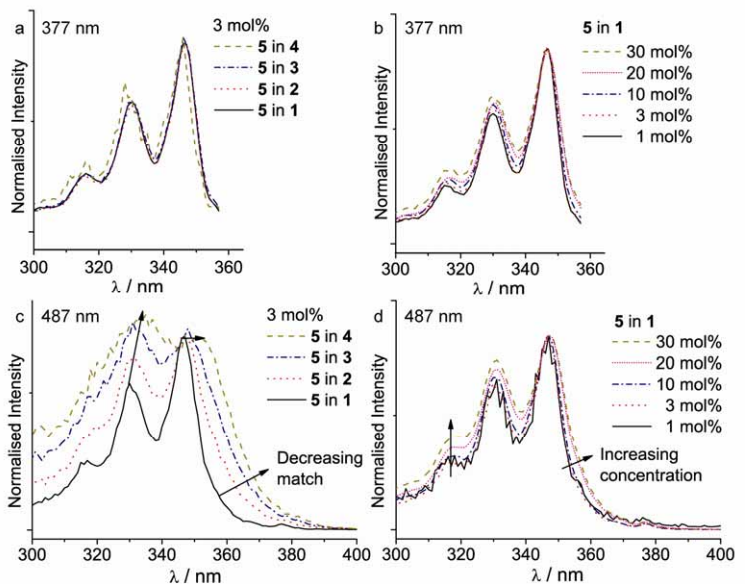


Fig. S2: Excitation spectra recorded at an emission wavelength of 377 nm (a,b) compared to the excitation spectra recorded at an emission wavelength of 487 nm (c,d). No broadening is observed in the spectra in a and b.

UV/Vis absorption spectra

The absorption spectra of the thin films are given in Fig. S2. The absorption spectrum of the film of **5** mixed with **4** could not be obtained due to the large amount of scattering, caused by the crystallinity of **4**.

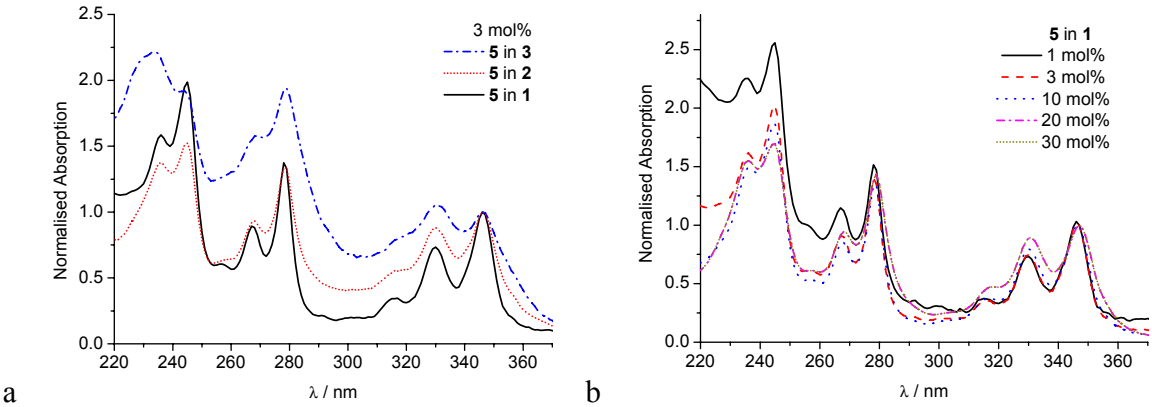


Fig. S3: Absorption spectra of thin films of 3 mol% of **5** mixed with different polymers (a) and different mol% of **5** mixed with matching polymer **1** (b), normalized to the peak at 347 nm.

Monopyrene experiments: Fluorescence and AFM data

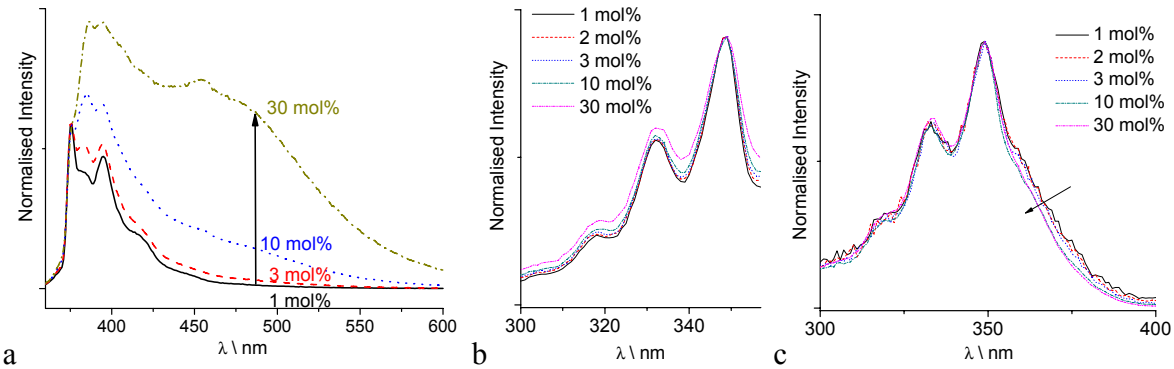


Fig. S4: Fluorescence emission (a, λ_{exc} = 330 nm) and excitation (c, λ_{em} = 377 nm and d, λ_{em} = 487 nm) spectra of different mol% of **6** in the matching polymer **1**. The emission (excitation) spectra were normalized to the peak at 377 (349) nm.

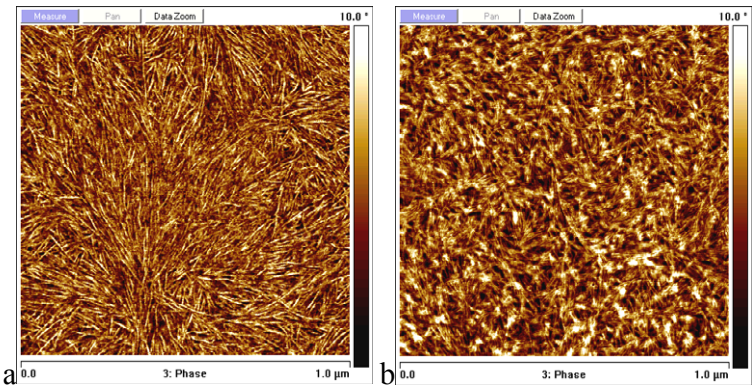


Fig. S5: Intermittent contact mode AFM phase images. Thin films of (a) 3 mol% and (b) 30 mol% of **6** in **1**.

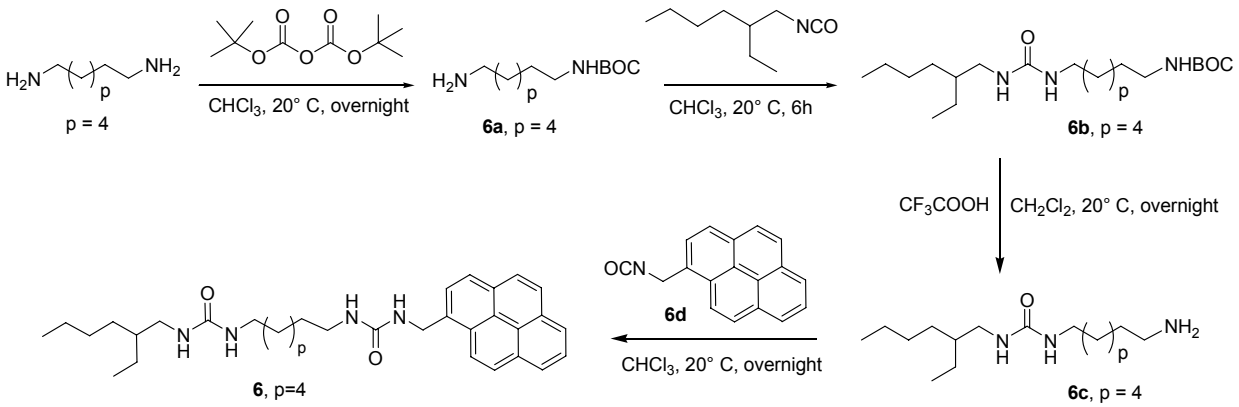
Experimental section

Materials. Solvents used in the synthesis were reagent grade. The reagents 1,7-diaminoheptane, 1-pyrenemethylamine hydrochloride, and 2-ethylhexylisocyanate were purchased from Aldrich and used without additional purification.

Methods. The NMR spectra were acquired on a 400 MHz Varian Mercury Vx (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR), a 400 MHz Bruker (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR) or a 300 MHz Varian Gemini-2000 (300 MHz for ^{1}H-NMR, 75 MHz for ^{13}C-NMR) spectrometer. Proton and Carbon chemical shifts are reported in ppm downfield of tetramethylsilane using the resonance of the deuterated solvent as internal standard. Splitting patterns are designated as singlet (s), doublet (d), triplet (t) and multiplet (m). Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer with a Universal ATR Sampling Accessory. MALDI-TOF was performed on a Perseptive DE PRO Voyager MALDI-TOF mass spectrometer using α-cyano-4-hydroxycinnamic acid as the calibration matrix. UV/vis spectra were recorded on a Perkin Elmer Lambda 900. Fluorescence spectra were recorded on an Edinburgh Instrument FS920 double-monochromator spectrometer with a Peltier-cooled red-sensitive photomultiplier.

Synthesis.
The synthesis of polymers **1-3** was done via a literature procedure.[1, 2]
The synthesis of polymer **4** was done via a literature procedure.[3]
The synthesis of **5** was done via a literature procedure.[4]

Synthesis of **6**, monoPyrene-U7U
The monofunctionalized pyrene guest molecule was synthesized using the synthetic procedure outlined in Scheme S1. First, 1,7-diaminoheptane was monoBoc protected by reaction with di-*tert*-butyl dicarbonate. Secondly, the monoBoc protected diaminoheptane **6a** was coupled to 2-ethylhexylisocyanate, yielding intermediate **6b** with one urea group. In the third step, the Boc groups were removed using trifluoroacetic acid (TFA) to yield the free amine groups (**6c**). In the last step, isocyanate-functionalized pyrene **6d** was coupled to the primary amine, resulting in the formation of mono functionalized bisurea molecule **6**. The compound was purified via colomn chromatography. The isocyanate-functionalized pyrene molecule **6d** was prepared by reacting di-*tert*-butyl tricarbonate[5] with the amino groups of commercially available probe molecules in chloroform. The starting compound for the synthesis of **6d** was obtained from its hydrochloride salts by deprotonation with D*i*PEA (diisopropylethylamine) in $CHCl_3$.



Scheme S1: *Synthesis of monoPyrene bisurea compound* ***6****.*

3-(2-ethylhexyl)-3'-(pyrenylmethyl)-1,7-bisureidoheptane (monoPyrene-U7U, 6): To 1-pyrenylmethylisocyanate (**6d**) (233 mg; 0.906 mmol) in 40 mL of chloroform, 258 mg (1 mmol) of compound **6c** in 5 mL of chloroform was added and the solution was stirred at room temperature overnight. After removal of the solvent, methanol (10 mL) was added and evaporated to obtain a solid precipitate. The product was purified by column chromatography (silica gel, MeOH/$CHCl_3$ (1:9 v/v)). Yield = 80%. The product was characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. ^{1}H-NMR (400 MHz, dmso d_6): δ = 8.0-8.2 (m, 9H, Ar-H), 6.4-6.45 (t, 1H, $CH_2\underline{NH}$, J=5.2 Hz), 5.9-6.0 (t, 1H, $CH_2\underline{NH}$, J=5.2 Hz), 5.6-5.7 (m, 1H, $CH_2\underline{NH}$), 4.9-5.0 (d, 2H, Ar-$\underline{CH_2}$, J=5.6 Hz), 2.8-3.05 (m, 6H, $NH\underline{CH_2}$), 1.01-1.4 (m, 21H, CH_2-$\underline{CH_2}$), 0.7-0.9 (m, 6H, CH_2-$\underline{CH_3}$). ^{13}C-NMR (400 MHz, dmso d_6): δ = 158.6, 158.4, 134.9, 131.2, 130.7, 130.3, 128.4, 127.8, 127.3, 126.7, 126.6, 125.59, 125.52, 125.1, 124.5, 124.4, 123.7, 44.0, 42.2, 41.5, 30.8, 30.4, 29.0, 28.8, 26.8, 24.0, 22.9, 14.4, 11.2. MALDI-TOF-MS: m/z=542.38; calculated for $C_{34}H_{46}N_4O_2$=542.36.

7-(*Tert*-butoxycarbonylamino)-1-heptylamine (6a): The *N*-Boc-1,7-diaminoheptane (7-(*Tert*-butoxycarbonylamino)-1-heptylamine) was synthesized according to the procedure reported in the literature.[6]

***N*-Boc-[*N*-3-(2-ethylhexyl)-ureido]-heptylamine (6b):** A solution 300 mg (1.29 mmol) of 7-(*Tert*-butoxycarbonylamino)-1-heptylamine (**6a**) in chloroform (20 mL) was added to a solution of 201 mg (1.29 mmol) of 2-ethylhexylisocyanate in 10 mL of chloroform. After 6 hours stirring at room temperature, the solvent was removed and the product was analyzed by ^{1}H NMR. The ^{1}H NMR confirmed the formation of the product and the product was used as such for the next step without further purification. ^{1}H-NMR (300 MHz, CDCl3): δ = 4.4-4.6 (m, 3H, -NH), 3.01-3.2 (m, 6H, $C\underline{H_2}$-N), 1.5 (s, 9H, $C(\underline{CH_3})$), 1.2-1.4 (m, 15H, $CH_2\underline{CH_2}$), 0.8-1.0 (t, 6H, CH_2-$\underline{CH_3}$, J=6.5 Hz).

[*N*-3-(2-ethylhexyl)-ureido]-heptylamine (6c): Compound **6b** was dissolved in 30 mL of dichloromethane with 5 mL of trifluoroacetic acid (TFA). After 16 hours stirring at room

temperature, the solvent was removed and fresh dichloromethane was added. The organic layer was then washed with 200 ml of 1M sodium hydroxide solution and the aqueous layer collected. The aqueous sodium hydroxide layer was extracted with dichloromethane (3x20 ml). The combined organic layer was dried under anhydrous sodium sulphate. Solvent was evaporated to obtain the product (340 mg; 91% yield). The ^{1}H and ^{13}C NMR spectroscopic analysis confirmed the formation of the product (3-(2-Ethylhexyl-7-(amino)-ureido-1, 7-heptane)). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.8-4.95 (t, 2H, -NH, J=12 Hz), 3.01-3.2 (m, 4H, $C\underline{H_2}$-N), 2.6-2.7 (m, 2H, $\underline{NH_2}$), 1.1-1.4 (m, 21H, $CH_2\underline{CH_2}$), 0.8-1.0 (t, 6H, CH_2-$\underline{CH_3}$, J=6.5 Hz).
^{13}C-NMR (400 MHz, CDCl3): δ = 158.8, 43.2, 42.1, 40.4, 39.8, 33.7, 30.9, 30.3, 29.2, 28.9, 26.8, 26.7, 24.1, 23.0, 14.0, 10.8.

1-Pyrenylmethylisocyanate (6d): 250 mg (0.9366 mmol) of 1-pyrenylmethylamine hydrochloride was suspended in 30 mL of chloroform. To this 186 mg (260 µl, 1.44 mmol) of D*i*PEA (diisopropylethylamine) was added. After the addition of D*i*PEA, the solution became clear. The solution was stirred for 60 minutes. After stirring, the reaction mixture was washed with distilled water (10x15 ml)), dried under anhydrous sodium sulphate and it was used for further reaction (yield=97%; 210 mg).
210 mg (0.909 mmol) of the 1-pyrenylmethylamine was added to 1.1 eq (262 mg, 1 mmol) of di-*tert*-butyltricarbonate[5] in 15 mL of chloroform and stirred at room temperature for 2 hours. The formation of 1-pyrenylmethylisocyanate (233 mg) was confirmed by IR (NCO peak at 2267 cm^{-1}) and ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.2-8.4 (m, 9H, Ar-H), 5.24 (s, 2H, $\underline{CH_2}$-Ar).

1 R. M. Versteegen, R. P. Sijbesma and E. W. Meijer, *Macromolecules*, 2005, **38**, 3176-3184.
2 R. A. Koevoets, Thesis, Eindhoven University of Technology, 2005.
3 J. M. J. Paulusse, Thesis, Eindhoven University of Technology, 2006.
4 N. Chebotareva, P. H. H. Bomans, P. M. Frederik, N. A. J. M. Sommerdijk and R. P. Sijbesma, *Chem. Commun.*, 2005, 4967-4969.
5 H. W. I. Peerlings and E. W. Meijer, *Tetrahedron Lett.*, 1999, **40**, 1021-1024.
6 J. F. Callahan, D. Ashton-Shue, H. G. Bryan, W. M. Bryan, G. D. Heckman, L. B. Kinter, J. E. McDonald, M. L. Moore, D. B. Schmidt, J. S. Silvestri, F. L. Stassen, L. Sulat, N. C. F. Yim and W. F. Huffman, *J. Med. Chem*, 1989, **32**, 391-396.