Supporting Information to Tuning the Properties of PS-PIAT block copolymers and their assembly into polymersomes

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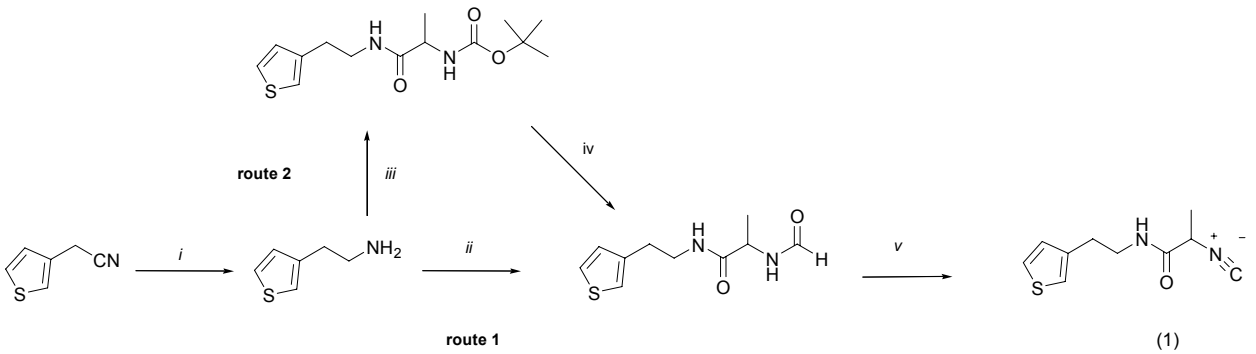
Synthesis routes to 1

Two different routes were followed to obtain the formamides. The first route involved the direct coupling of the *N*-formyl amino acid to the thiophene-amine (Scheme S1 1, route 1). It is known, however, that *N*-acyl amino acids can undergo epimerization via oxazolone formation, if the acid group is activated.[1] A second route preventing racemization therefore was adopted. It involves coupling of the β-3-thienylethylamine to Boc-protected L-alanine (Scheme S1, route 2). The optical rotations of **1** prepared by routes 1 and 2 were $[\alpha]_D^{20} = -40° \cdot cm^2 \cdot g^{-1}$ and $[\alpha]_D^{20} = -54° \cdot cm^2 \cdot g^{-1}$, respectively. Using chiral HPLC the amount of racemization of **1** prepared by route 1was determined to be 22%, whereas **1** obtained via route 2 was optically pure.[2]



Scheme S1 Synthesis of **1** via the two routes; i) $LiAlH_4$, Et_2O; ii) *N*-formyl-L-alanine, DCC, DMAP, CH_2Cl_2; iii) Boc-L-alanine, EDC, HOBt, TEA, NMM, CH_2Cl_2; iv-1) HCl/EtOAc iv-2) HCO_2Et, HCO_2Na; v) $ClCO_2CCl_3$, NMM, CH_2Cl_2.

Synthesis of boc-L-alanine(2-thiophen-3-yl-ethyl)amide: 1

β-3-Thienyl ethylamine (0.30 g, 2.4 mmol) and boc-L-alanine (0.46 g, 2.4 mmol) were dissolved in 55 ml of freshly distilled ethyl acetate, and N-methylmorpholine (NMM; 0.30 g, 2.5 mmol), 1-hydroxybenzotriazole (HOBt; 1.27 g, 9.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC; 0.56g, 2.91 mmol) were added. The suspension was stirred overnight and subsequently washed (3×) with 10% citric acid solution and saturated bicarbonate solution (3×). The mixture was evaporated to dryness and the resulting solid purified by column chromatography (silica gel; CH_2Cl_2/MeOH 99/1 v/v) yielding a white solid. $[\alpha]_D^{20}$ (CH_2Cl_2 *c* 0.5) = -23°. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (dd, 1H, thiophene H-5, J = 1.9 Hz, J = 3.1 Hz), 7.00 (m, 1H, thiophene H-2, J = 2.0 Hz), 6.94 (dd, 1H, thiophene H-4, J = 6.1 Hz, J = 1.2 Hz), 6.12 and 4.89 (br, 1H, N*H*C(O)), 4.08 (m, 1H, C*H*$(CH_3)_3$), 3.52 (m, 2H, CH_2NH), 2.84 (t, 2H, CH_2CH$_2$, J = 6.8 Hz), 1.43 (s, 9H, CH(CH_3)$_3$), 1.32 ppm (d, 3H, C(H)CH_3, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 179.5 and 167.5 (NH*C*(O)), 139.1 (thiophene C-3), 128.2 (thiophene C-4), 126.2 (thiophene C-5), 121.6 (thiophene C-2), 80.5 (*C*$(CH_3)_3$), 48.6 (*C*HCH$_3$), 40.0 (*C*H$_2$NH), 30.3 (*C*H$_2$CH$_2$), 28.5 (C(*C*H$_3$)$_3$), 18.5 ppm (*C*H$_3$). IR (KBr, cm^{-1}) 3342 (NH), 3092, 2979, 2936 and 2864 (CH), 1685 and 1655 (amide I), 1547 and 1522 (amide II). EI-MS: *m/z* = 298 $[M]^+$ (calcd: 298.41). El. anal. calcd. for $C_{10}H_{14}N_2O_3S$ (%): C: 59.54, H: 7.85:, N: 9.91, S: 11.35, found: C: 59.66, H: 7.95, N: 9.87, S: 11.19.

Synthesis of polystyrene-*b*-polyisocyanoalanine(2-thiophene-3-yl-ethyl)amide: 3

The polymerization was carried out as described previously (supproting information).[3] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.1 (br, N*H*C(O)), 7.4-6.2 (br, CH*Ph*, thiophene H-5, thiophene H-4, thiophene H-2), 4.8-3.9 (br, C=NC*H*$(CH_3)_3$), 4.0-3.2 (br, thiophene-CH$_2$CH_2NH), 3.1-2.5 (br, thiophene-CH_2CH$_2$), 2.3-1.7 (br, CH$_2$C*H*Ph), (1.7-0.8 (br, CH_2CHPh), 1.6-1.3 (br, CH(CH_3), 1.3-1.1 (br, C(CH_3)$_3$), 0.8-0.6 ppm (br, *Bu*(CH$_2$CHPh)). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 155 (*C*=N), 147-143 (br, CH*Ph*$_{ipso}$), 140 (br, thiophene C-3), 130-127 (br, CH*Ph*$_{ortho+meta}$), 127-124 (br, thiophene C-1, thiophene C-2 and CH*Ph*$_{para}$), 113 (br, thiophene C-2), 72 (*C*H$_2$OCH$_2$CH$_2$CH$_2$NH), 68 (*C*H$_2$OCH$_2$CH$_2$CH$_2$NH), 63 (C=N*C*$(CH_3)_3$), 42-38 (br, *C*HPh and *C*H$_2$CHPh), 39.8 (br,

$CH_2CH_2NHC(O)$), 37.5-34 (br, $CH_2OCH_2CH_2CH_2NH$), 32-30 (br, C=NC(CH_3)$_3$ and $CH_2CH(CH_3)(CH_2CHPh)_{40}$), 28.7 ($CH_2CH_2NHC(O)$), 25.9 ($CH_2CH(CH_3)(CH_2CHPh)_{40}$), 22.6 (br, $CH_2CH(CH_3)(CH_2CHPh)_{40}$), 21.4 (br, $CH(CH_3)$ 13.4 ppm (br, $CH_3CH_2CH(CH_3)(CH_2CHPh)_{40}$. IR ($CH_2Cl_2$, cm^{-1}) 3282 (NH) 3092, 2979, 2936 and 2864 (CH), 1659 (amide I), 1604 (C=N), 1529 (amide II), 1494, 1453 (Ar C=C). GPC ($CHCl_3$, 30°C): M_n = 11750, PD = 2.1.

Formula used for determination of sliding rate-constant

$$\log\left(\frac{-1}{I_0}\frac{d\ln(M)}{dt}-k_P\right)=\log(k_I-k_P)-\frac{k_I}{2.303}\int_0^t(M)dt \qquad \textbf{(1a)}$$

$$\log\left(\frac{1}{I_0}\frac{d\ln(M)}{dt}+k_P\right)=\log(k_P-k_I)-\frac{k_I}{2.303}\int_0^t(M)dt \qquad \textbf{(1b)}$$

If k_I is greater than k_P **1a** is used; if contrawise **1b**

GPC of polymer (2b) and amino-terminated PS prepared by AP

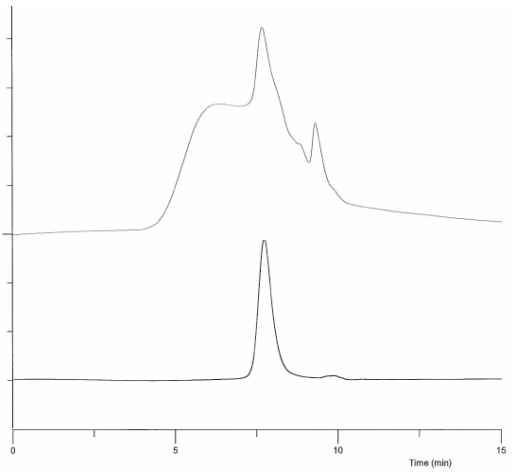


Fig. S1 Elution profiles of polymer 2c (PS-PIAT 75/25) and of amino-terminated polystyrene prepared by AP showing the significant amount of hPS still present in the block copolymer

TEM image of spherical aggregates formed from 2d and 2b

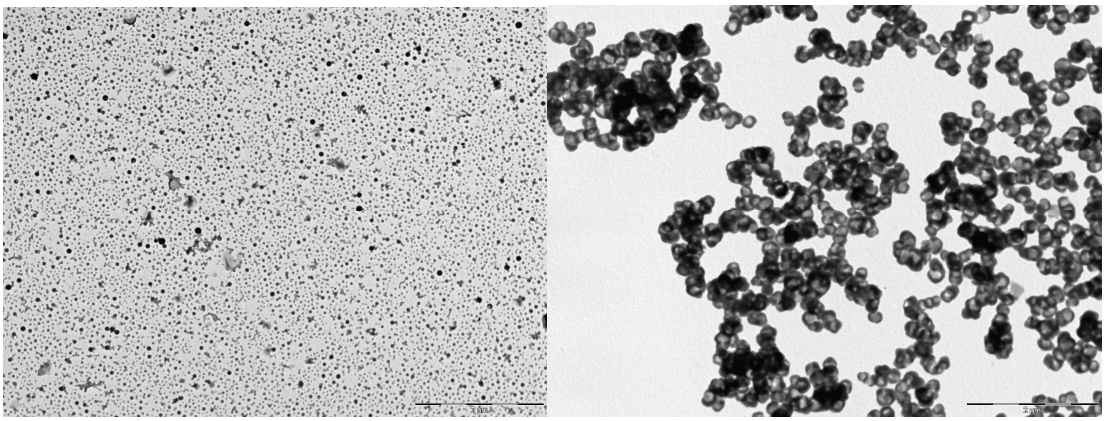


Fig. S2 TEM images of spherical particles formed by PS-PIAT block copolymer of which the excess hPS was removed by repeated precipitation: **2d** (left). In contrast to polymersomes formed by the native block copolymer the spherical aggregates formed by 2d have a reduced uniform density and do not show the typical membrane structure of the native block copolymer **2b (**right).

DLS of spherical aggregates formed from 2d

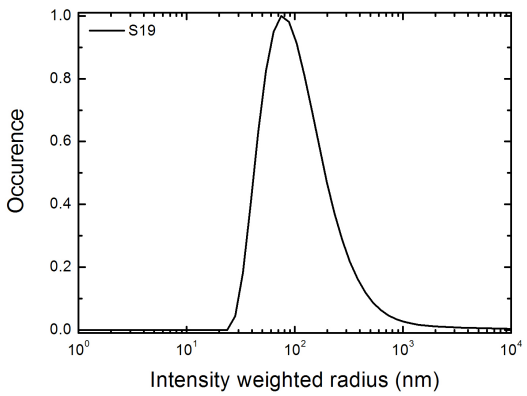


Fig. S3 Size-distribution of aggregates formed by **2d** at approximately 24 hrs**.** Although the radii are larger than observed with electron microscopy the difference with the native polymer **2b** is significant (compare radius of **2b** in Fig. 6 and Fig. S6).

Turbidity measurements as a function of time for polymer 2b and 3

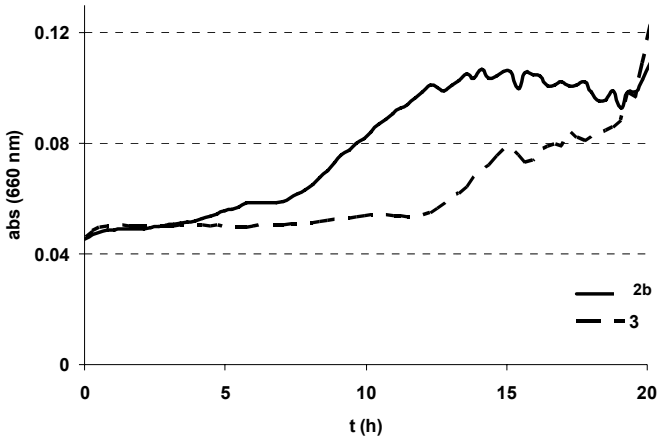


Fig. S4 Mean values for 10 samples of hPS containing **2b** and **3**. For both polymers a 'lag time' during which the absorbance remained constant was observed. The graph clearly shows that the 'lag time' is longer (*i.e.* 12 hrs) for polymer **3**, after which the absorbance seems to increase and then to level off after 15 hrs. This latter point likely corresponds to the situation where the polymersomes have reached their final diameters. Eventually, after 20 hours, the turbidity again increased concomitant with the appearance of a precipitate. This could be the result of the slow evaporation of the solvent, as the samples were left open in air during the experiments and a reduction in volume was observed.

DLS of polymersomes formed from polymer 3 at 0.5, 24 and 48h

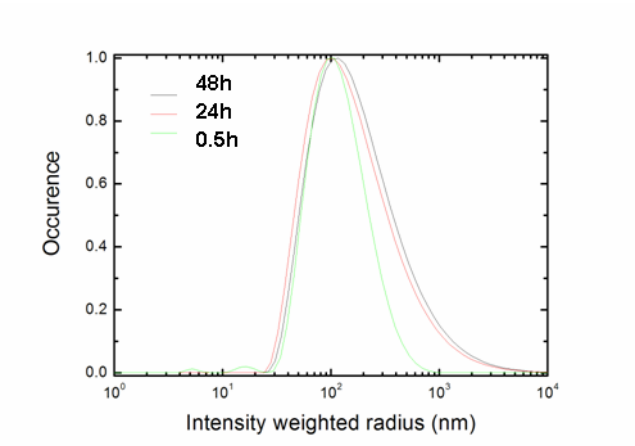


Fig. S5

DLS of polymersomes formed from polymer 2b at 0.5, 24 and 48h

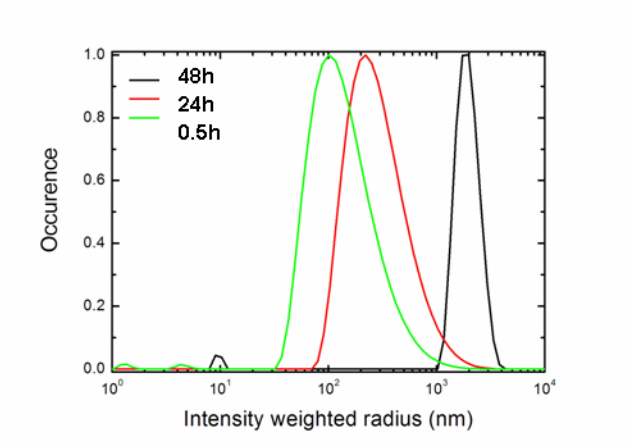


Fig. S6 CONTIN analysis of representative DLS data is shown. Different batches showed comparable trends (i.e. radius after 0.5h ~ 100 nm and final radius after 48h ~ 2000nm), the radius after 24h varied between 150 and 750 nm.

1. N. Sewald and H.-D. Jakubke, *Peptides: Chemistry and Biology*, Wiley-VCH, Weinheim, 2002.
2. D. M. Vriezema, Ph. D. Thesis, Radboud University 2003.
3. D. M. Vriezema, A. Kros, R. de Gelder, J. J. L. M. Cornelissen, A. E. Rowan and R. J. M. Nolte, *Macromolecules*, 2004, **37**, 4736-4739.