Supporting Information

Pathway Dependent Shape-Transformation of Azide-Decorated Polymersomes

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Experimental section

Materials

Poly(ethylene glycol) methyl ether and poly(ethylene glycol) (M_n 2 kg/mol, Sigma, 99%), α -bromoisobutyryl bromide (Sigma, 99%), triethylamine (Sigma, 98%), styrene (Sigma-Aldrich, 98%) N,N,N',N'',N''-pentamethyl-diethylenetriamine (PMDETA) (Sigma, 99%), dibenzocyclooctyne-PEG₄-alcohol (DBCO-PEG4-OH, Sigma) were used as received. Copper (I) bromide (CuBr, Sigma, 99.99%) was purified by stirring in acetic acid, followed by washing with acetone three times.

Instruments

Nuclear Magnetic Resonance Spectroscopy (NMR). ¹H NMR spectra were recorded on a Bruker (400MHz) spectrometer with CDCl₃ as the solvent and TMS as an internal standard.

Gel permeation chromatography (GPC). The molecular weights and dispersities of the polymers were characterized by GPC. GPC measurements were conducted using a Shimadzu Prominence-i SEC system with a PL gel 5 μ m mixed D and mixed C column (Polymer Laboratories) with PS standard and equipped with a Shimadzu RID-20A differential refractive index detector. THF was used as an eluent with a flow rate of 1 mL min⁻¹.

Dynamic Light Scattering (DLS) and Zeta Potential (ζ). A Malvern Z90 Zetasizer equipped with a 633 nm He–Ne laser and an avalanche photodiode detector was used to characterize the hydrodynamic size and surface zeta potential of the particles. The scattering light at a 90° angle was detected and used to analyze the size and distribution.

Multi angle light scattering. Measurements were performed on an ALV Compact Goniometer (CGS-3) Multi-Detector (MD-4) equipped with an ALV-7004 Digital Multiple Tau Real Time Correlator and an Nd-YAG laser operating at a wavelength (λ) of 532 nm. Scattering intensities (I) were recorded at detection angles (θ) ranging from 30° to 150° in increments of 10°, in three runs of 20 s per angle. Scattering intensities were corrected for the sample holder, solvent and scattering volume according to

$$I(\theta) = (I_{sample}(\theta) - I_{solvent}(\theta)) \times \sin(\theta)$$
(1)

where I_{sample} and I_{solvent} are scattering intensities measured for the sample and solvent, respectively. Detection angles were converted to scattering vectors (q) according to

$$q = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right) \tag{2}$$

where $n_0 = 1.332$ is the refractive index of water.^[2]

Transmission Electron Microscopy (TEM). TEM images were recorded by a FEI Tecnai 20 (type Sphera) at 200 kV. 10 μ L of a sample was dropped onto a carbon-coated copper grid. After removing the excess solution by blotting paper, the samples were dried at ambient conditions.

Cryogenic transmission electron microscopy (cryo-TEM). Experiments were performed using a FEI Tecnai G2 Sphera (200 kV electron source) equipped with LaB6 filament utilizing a cryoholder or a FEI Titan (300 kV electron source) equipped with autoloader station. Samples for cryo-TEM were prepared by treating the grids (Lacey carbon coated, R2/2, Cu, 200 mesh, EM sciences) in a Cressington 208 carbon coater for 40 seconds. Then, 3 μ l of the polymersome solution was pipetted on the grid and blotted in a Vitrobot MARK III at 100% humidity. The grid was blotted for 3 seconds (offset -3) and directly plunged and frozen in liquid ethane.

Scanning electron microscopy (SEM). SEM images were obtained on a JEOL 6330 Scanning Electron Microscope. To prepare SEM samples, 10 μ L of the sample was dropped onto a small piece of silicon wafer, and the samples were dried at room temperature.

Synthesis

Synthesis of N_3 -PEG₄₅-OH

N₃-PEG₄₅-OH was synthesized according to a literature procedure (Scheme S1).^[1]



Scheme S1. Synthetic route of N₃-PEG₄₅-OH.

Synthesis of OH-PEG₄₅-OTS. OH-PEG₄₅-OH (2 g, 2 mmol) was dried twice via rotary evaporation in toluene. Then OH-PEG₄₅-OH was dissolved in 10 mL anhydrous dichloromethane (DCM) under an Ar atmosphere. The solution remained at 0 °C, after which silver (I) oxide (Ag₂O, 0.7 g, 3mmol), potassium iodide (KI, 0.24 g, 1.44 mmol), and *p*-toluenesulfonyl chloride (TSCl, 0.4 g, 2.1 mmol) were added to the solution. After two hours, the mixture was allowed to react for 24 hours at room temperature. Then Ag₂O and KI were removed via filtration and most of DCM was removed through rotary evaporation. OH-PEG₄₅-OTS was obtained via precipitation in diethyl ether. From ¹H-NMR (DMSO) (Fig. S1a), tosylation of OH-PEG-OH resulted in OH-PEG₄₅-OTS in 94% yield.

Synthesis of N_3 -PEG₄₅-OH. OH-PEG₄₅-OTS (1 g, 0.5 mmol) and NaN₃ (0.322 g, 5 mmol) were dissolved in 5 mL of anhydrous DMF, and the mixture was stirred 24 hours at 90 °C under Ar atmosphere. After cooling down to room temperature, excess of NaN₃ was removed *via* filtration, and DMF was removed under vacuum. The product was dissolved in 10 mL DCM and washed with brine. The organic layer was collected and dried over anhydrous MgSO₄, after which most of DCM was removed by rotary evaporation, and the product was obtained *via* precipitation in diethyl ether. From ¹H-NMR (DMSO) (Fig. S1b), all OH-PEG₄₅-OTS was converted into N₃-PEG₄₅-OH.

Synthesis of mPEG₄₅-b-PS₂₀₅ and N₃-PEG₄₅-b-PS₂₀₀

The synthesis protocol to PEG-*b*-PS was as follows (Scheme S2):

ATRP macro-initiator mPEG₄₅-Br and N₃-PEG₄₅-Br were prepared according to literature.^[3] mPEG₄₅-*b*-PS and N₃-PEG₄₅-*b*-PS were synthesized by atom transfer radical polymerization (ATRP). Briefly, mPEG₄₅-Br or N₃-PEG₄₅-Br (100 mg, 0.05 mmol), styrene (2.6 g, 25 mmol) and CuBr (7 mg, 0.05 mmol) were added into a 5 mL round bottom flask, followed by degassing with nitrogen for 30 min. Subsequently, PMDETA (10 μ L, 0.05 mmol) dissolved in 0.5 mL toluene was added to the flask, after which the mixture was purged with nitrogen for another 30 min. Then the flask was placed into a 90 °C oil bath. After the desired monomer conversion was reached, the solution was immersed in a liquid nitrogen bath in order to stop the radical polymerization. Then the solution was diluted in 50 mL of THF and passed through a neutral alumina column

twice to remove the copper catalyst. Then the filtrate was concentrated and precipitated into 50 mL methanol twice. The white precipitate was collected and dried in a vacuum oven at room temperature for 24 h. With ¹H NMR (**Fig. S2**), the PS polymerization degrees (DPs) of mPEG₄₅-*b*-PS and N₃-PEG₄₅-*b*-PS were determined, which are 205 and 200, respectively. The molecular weight and polydispersity (Đ) were determined by GPC using THF as the eluent (**Fig. S3**).



Scheme S1. Synthetic route of mPEG₄₅-*b*-PS₂₀₅ and N₃-PEG₄₅-*b*-PS₂₀₀.

Polymer self-assembly

mPEG₄₅–*b*-PS₂₀₅ (10 mg) or N₃-PEG₄₅-*b*-PS₂₀₀ (10 mg) were dissolved in a 1 mL mixture of THF/dioxane (80:20 v/v), followed by addition of 1 mL Milli-Q water via a syringe pump at a rate of 1 mL h⁻¹. A fraction of this solution (10 μ L) was taken from this suspension before dialysis was performed and added at once to 1 mL pure water to rapidly freeze the morphology of the aggregates, which is a common method to prepare spherical polymer vesicles. The assemblies were transferred into a dialysis bag (molecular weight cutoff: 12,000-14,000 Da, flat width 25 mm) and dialyzed against pure water and sodium chloride solution with the dialysis solution changed after one hour. The dialysis process was performed during 24 h. The same procedure was applied to prepare mPEG₄₅–*b*-PS₂₀₅/N₃-PEG₄₅-*b*-PS₂₀₀ blended polymersomes.

Light scattering analysis

Mean decay rates (Γ) were obtained by inverse Laplace transformation of the intensity autocorrelation functions, performed by CONTIN software (AfterALV 1.0e, Dullware). Apparent diffusion coefficients (D) were obtained by linear regression of the mean decay rates according to

$$\Gamma = Dq^2 \tag{3}$$

Diffusion coefficients were converted to apparent hydrodynamic radii (R_h) using the Stokes–Einstein equation,

$$R_h = \frac{k_B T}{6\pi\eta D} \tag{4}$$

where $k_{\rm B}$ is Boltzmann's constant, T = 293 K is the temperature and η = 1.0006 cP is the viscosity of water.

Radii of gyration (R_g) were obtained by linear regression of the scattering intensities using the Guinier approximation,

$$\ln(I) = \ln(I_0) - \frac{R_g^2}{3}q^2$$
(5)

where I_0 is the scattering intensity at zero scattering-angle.

Supplementary figures and tables



Fig. S1 ¹H-NMR spectra of (a) OH-PEG₄₅-OTS and (b) N₃-PEG₄₅-OH in DMSO.





Fig. S3 GPC traces of mPEG₄₅-Br (black), N₃-PEG₄₅-Br (red), mPEG₄₅-*b*-PS₂₀₅ (blue) and N₃-PEG₄₅-*b*-PS₂₀₀ (purple), respectively.



Fig. S4 TEM images of mPEG₄₅-*b*-PS₂₀₅ (**a**) polymersomes and (**b**) stomatocytes. (**c**) cryo-TEM and (**d**) SEM images of mPEG₄₅-*b*-PS₂₀₅ stomatocytes. The stomatocytes were prepared via dialysis of an mPEG₄₅-*b*-PS₂₀₅ suspension against 50 mM NaCl solution. All scale bars are 400 nm.



Fig. S5 TEM image of rapidly quenched (a) $mPEG_{45}$ -*b*- PS_{205} and (b) N_3 - PEG_{45} -*b*- PS_{200} polymersomes.



Fig. S6 DLS analysis of mPEG₄₅–b-PS₂₀₅ after dialysis against pure water, 10 mM NaCl, and 50 mM NaCl, respectively.



Fig. S7 TEM images of mPEG₄₅-*b*-PS₂₀₅ after dialysis against 5 mM, 10 mM, and 20 mM NaCl, respectively.



Fig. S8 A representative TEM image of hexagonally packed hollow hoops which shows that all polymersomes changed to hexosomes after dialysis against 50 mM NaCl.



Fig. S9 Mean decay rate and natural logarithm of the scattering intensity as a function of the squared magnitude of the scattering vector measured for N_3 -PEG-₄₅-*b*-PS₂₀₀ in pure water at (a, b) 2.8, (c, d), 4.2 and (e, f) 5.6 µg/mL. Diffusion coefficients and radii of gyration were obtained by linear regression of the data.

Table. S1.	Hydrodynamic radii (R_h) and radii of gyration (R_g) determined for N ₃ -PEG ₄₅ -
<i>b</i> -PS ₂₀₀ HH	IHs in pure water at various concentrations.

[N ₃ -PEG ₄₅ - <i>b</i> -PS ₂₀₀]/µg/mL	R _h /nm	R _g /nm
2.8	$1.2\pm0.2\times10^{3}$	$9\pm 2\times 10^{1}$
4.2	$1.17 \pm 0.08 \times 10^{3}$	$8 \pm 1 \times 10^{1}$
5.6	$0.8{\pm}0.1{\times}10^3$	$9\pm1\times10^{1}$



Fig. S10 DLS analysis of N_3 -PEG₄₅–*b*-PS₂₀₀ after dialysis against pure water, 10 mM and 50 mM NaCl, respectively.

Table S2. DLS results of mPEG₄₅-b-PS₂₀₅ and N₃-PEG₄₅-b-PS₂₀₀ assemblies in the presence of different concentrations of NaCl.

Samples	[NaCl]/mM	Size/nm	PDI
mPEG ₄₅ - <i>b</i> -PS ₂₀₅	0	403	0.097±0.03
	10	390	0.103±0.04
	50	372	0.104±0.04
N ₃ -PEG ₄₅ - <i>b</i> -PS ₂₀₀	0	425	0.102±0.03
	10	415	0.112±0.04
	50	401	0.127±0.03



Fig. S11 TEM images of N₃-PEG₄₅–*b*-PS₂₀₀ reacted with DBCO-PEG₄-OH followed by dialysis against pure water or 50 mM NaCl. DBCO-PEG₄-OH (1.25 mg, 0.0025 mmol) was added into 1 mL N₃-PEG₄₅–*b*-PS₂₀₀ polymersome aqueous solution (5 mg/mL, 0.25 mM). The dispersion was stirred overnight, after which the polymersomes were transferred to a dialysis membrane (molecular weight cutoff: 12,000-14,000 Da, flat width 25 mm). The polymersomes were dialyzed against Milli-Q for 24 hours to remove the excess of DBCO-PEG4-OH.



Fig. S12 Schematic illustration of the reverse dialysis process of mPEG₄₅-b-PS₂₀₅ stomatocytes. TEM images (a) before and (b) after dialysis against 50 vol % organic solvent. Stomatocytes are reverted to spherical polymersomes. Scale bars are 1 μ m.

References

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- [2] D. Lide, Ed. CRC Handbook of Chemistry and Physics, 82nd ed.; CRC Press: Boca Raton, FL, 2001–2002.
- [3] H. Che, S. Cao, J. C. M. van Hest, J. Am. Chem. Soc. 2018, 140, 5356-5359.