

Supporting Information

Amphiphilic Star-Block Copolymers and Supramolecular Transformation of Nanogel-like Micelles to Nanovesicles

Jing-ling Zhu,^a Kerh Li Liu,^{*b} Zhongxing Zhang,^b Xian-Zheng Zhang^c and Jun Li^{*a,b}

^a Department of Bioengineering, Faculty of Engineering, National University of Singapore, Engineering Drive 1, Singapore 117574, Singapore

^b Institute of Materials Research and Engineering, A*STAR (Agency for Science, Technology and Research), 3 Research Link, Singapore 117602, Singapore

^c Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China

*To whom correspondence should be addressed. Email: bielj@nus.edu.sg; liukl@imre-a-star.edu.sg

1. Experimental

Materials: Hydroxyl-terminated eight-arm poly (ethylene glycol) (sPEG) with M_n of ca. 9.50 and 19.9 kDa were obtained from NOF Corp. (Tokyo, Japan), and dried under high vacuum at 60 °C for 24 h before use. Poly(ethylene glycol) methyl ether (MPEG) with M_n of ca. 2000 Da was obtained from Aldrich, and dried under high vacuum at 60 °C for 24 h before use. (*R,S*)- β -butyrolactone (> 95 %, Tokyo Kasei Inc) was dried over and vacuum distilled from CaH₂ twice before use. Thionyl chloride (SOCl₂, \geq 99 %, Sigma-Aldrich), sodium azide (NaN₃, \geq 99.5%, Sigma-Aldrich), 1-adamantaneacetic acid (98 %, Aldrich), propargyl bromide solution (80 wt. % in toluene, Aldrich), copper sulfate pentahydrate (CuSO₄·5H₂O, 98 %, Sigma-Aldrich), sodium ascorbate (\geq 99%, Aldrich), heptakis(2,6-di-O-methyl)- β -cyclodextrin (DM- β -CD, \geq 98.0 %, Aldrich), dimethylsulfoxide (DMSO, anhydrous, \geq 99.9 %, Aldrich), toluene (99 %, Tedia), chloroform (99.8 %, Tedia), tetrahydrofuran (THF, > 99 %, Tedia), diethyl ether (Et₂O, 99 %, Tedia) and *n*-hexane (95 %, Tedia) were used as received.

Synthesis of α -Adamantyl- ω -alkynyl-poly(3-hydroxybutyrate) (PHB): The heterofunctionalised PHB was synthesized via anionic ring-opening polymerization of (*R,S*)-

β -butyrolactone in DMSO at room temperature under dry N_2 atmosphere, similar to the method reported previously.¹ The initiator sodium adamantaneacetate (Ada- CH_2CO_2Na) was obtained from the neutralization of commercially available adamantaneacetic acid with sodium hydroxide in methanol. In a typical example, the ring opening polymerization was initiated by reacting 0.314 g of dried Ada- CH_2CO_2Na (1.45 mmol) with 1.98 ml of β -butyrolactone monomer (25 mmol) in 80 ml of anhydrous DMSO at room temperature. With the aid of 1H NMR monitoring, the polymerization was quenched by adding an excess of propargyl bromide upon reaching a monomer conversion of >90 %. Solvent DMSO was then removed by rotary evaporation and the crude polymer sample was re-dissolved in chloroform, filtered and precipitated into n-hexane to afford the purified polymer. Yield: 2.05 g (88.3%, based on theoretical yield computed using actual monomer conversion). GPC (THF): M_n = 1.60 kDa, PDI = 1.10. 1H NMR (400 MHz, $CDCl_3$, δ): 1.26 – 1.31 (m, $-OCH(\underline{CH}_3)CH_2CO_2-$ of PHB), 1.59 – 1.71 (m, methylene protons of adamantyl end group), 1.95 (br, methine protons of adamantyl end group), 2.02 (s, $-OCCH_2-$ Ada) 2.43 – 2.62 (m, $-OCH(\underline{CH}_3)CH_2CO_2-$ of PHB and $-OCH_2C\equiv CH$ of alkynyl end group), 4.68 (s, $-OCH_2C\equiv CH$), 5.23 – 5.32 ppm (m, $-OCH(\underline{CH}_3)CH_2CO_2-$ of PHB). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.58 – 172.18 ($-\underline{CO}-$ of PHB and $-\underline{CO}CH_2-$ Ada), 75.53 (alkynyl group), 67.94 ($-\underline{CH}-$ of PHB), 52.42 ($-\underline{CH}_2-$ alkynyl), 49.31 ($-\underline{CH}_2-$ adamantyl), 41.12 ($-\underline{CH}_2-$ of PHB), 42.67, 37.04, 33.10, 28.90 (adamantyl group), 20.07 ppm ($-\underline{CH}_3$ of PHB).

Synthesis of azide-functionalized 8-arm sPEG (sPEG- N_3): sPEG- N_3 was prepared from commercially available hydroxyl-terminated 8-arm PEG via substitution reaction. The 8-arm PEG contains a hexaglycerol core. Typically, 4.75 g of sPEG (M_n 9.50 kDa, 0.5 mmol) and 0.73 mL of $SOCl_2$ (10 mmol) were dissolved in 15 mL of toluene and refluxed overnight under N_2 . Toluene was then removed by distillation. After cooling to room temperature, 1.30

g of NaN₃ (20 mmol) dispersed in 15 mL of DMSO was added to the reaction vessel. The reaction was allowed to stir at 90 °C under N₂ overnight. The reaction mixture was then partitioned between deionised water and chloroform. The aqueous layer was further extracted with chloroform for at least three times and the combined organic fraction was dried over anhydrous magnesium sulphate. After evaporation of solvent, the remaining product was dried under vacuum overnight. Yield: 3.60 g (74.5%, assuming complete azide substitution). ¹H NMR (400 MHz, CDCl₃, δ): 3.30 – 3.41 (m, -CH₂-N₃), 3.41 – 3.88 ppm (m, sPEG backbone). ¹³C NMR (100 MHz, CDCl₃, δ): 74.32 – 68.79 (sPEG backbone), 51.08 ppm (-CH₂-N₃).

Synthesis of sPEG-PHB via alkyne-azide coupling: Four sPEG-PHB star-block copolymers were synthesized via alkyne-azide coupling in DMSO/H₂O system. In a typical example, a bottle with an N₂ inlet was charged with 0.96 g of PHB (*M_n* 1.60 kDa, 0.6 mmol), 0.47 g of sPEG-N₃ (*M_n* 9.50 kDa, 0.05 mmol) and 4 mL DMSO. A freshly prepared 23 wt % aqueous solution of CuSO₄·5H₂O (0.18 mmol) and 33 wt % aqueous solution of sodium ascorbate (0.38 mmol) were then added sequentially. The reaction was allowed to stir at room temperature under N₂ atmosphere for 48 h. The reaction mixture was diluted with THF and passed through alumina column to remove copper residue. Then, the polymer solution was precipitated into Et₂O. The product was collected by filtration and purified twice by dissolution / precipitation with THF / Et₂O, and dried under vacuum overnight. Yield: 0.82 g (73.6 %, assuming all 8 arms of sPEG are conjugated with PHB). ¹H NMR (400 MHz, CDCl₃, δ): 1.14 – 1.36 (m, -OCH(CH₃)CH₂CO₂- of PHB), 1.52 – 1.78 (m, methylene protons of adamantyl end group), 1.96 (br, methine protons of adamantyl end group), 2.02 (s, -OCCH₂-Ada), 2.33 – 2.79 (m, -OCH(CH₃)CH₂CO₂- of PHB), 3.41 – 3.95 (m, sPEG backbone), 4.50 – 4.62 (m, -CH₂-triazole N), 5.12 – 5.36 (m, -OCH(CH₃)CH₂CO₂- of PHB and -CH₂-triazole

C), 7.79 ppm (s, $-\text{C}=\underline{\text{C}}\text{H}-\text{N}-$ of triazole). ^{13}C NMR (100 MHz, CDCl_3 , δ): 168.83 – 171.72 ($-\underline{\text{C}}\text{O}-$ of PHB and $-\underline{\text{C}}\text{OCH}_2\text{-Ada}$), 142.65 ($-\text{CH}=\underline{\text{C}}-$ of triazole), 125.37 ($-\underline{\text{C}}\text{H}=\text{C}-$ of triazole), 73.26 – 68.98 (sPEG backbone), 67.99 ($-\underline{\text{C}}\text{H}-$ of PHB), 58.19 ($-\underline{\text{C}}\text{H}_2\text{-triazole C}$), 50.67 ($-\underline{\text{C}}\text{H}_2\text{-triazole N}$), 49.37 ($-\underline{\text{C}}\text{H}_2\text{-adamantyl}$), 41.18 ($-\underline{\text{C}}\text{H}_2-$ of PHB), 42.73, 37.10, 33.16, 28.96 (adamantyl group), 20.16 ppm ($-\underline{\text{C}}\text{H}_3$ of PHB).

Synthesis of MPEG-PHB via alkyne-azide coupling: Two MPEG-PHB linear copolymers were synthesized using the same route as that for sPEG-PHB star-block copolymers. First, MPEG- N_3 (2.18 kDa) was prepared from commercially available hydroxyl-terminated MPEG via substitution reaction. Then PHBs (M_n 1.60 kDa and 3.07 kDa) were reacted with MPEG- N_3 via alkyne-azide coupling in DMSO/ H_2O system. Yield: 75.2 % and 79.5 %, respectively. ^1H NMR (400 MHz, CDCl_3 , δ): 1.16 – 1.37 (m, $-\text{OCH}(\underline{\text{C}}\text{H}_3)\text{CH}_2\text{CO}_2-$ of PHB), 1.47 – 1.81 (m, methylene protons of adamantyl end group), 1.96 (br, methine protons of adamantyl end group), 2.02 (s, $-\text{OC}\underline{\text{C}}\text{H}_2\text{-Ada}$), 2.32 – 2.72 (m, $-\text{OCH}(\text{CH}_3)\underline{\text{C}}\text{H}_2\text{CO}_2-$ of PHB), 3.38 (s, methyl ether end group), 3.41 – 3.96 (m, MPEG backbone), 4.47 – 4.62 (m, $-\underline{\text{C}}\text{H}_2\text{-triazole N}$), 5.12 – 5.35 (m, $-\text{OCH}(\underline{\text{C}}\text{H}_3)\text{CH}_2\text{CO}_2-$ of PHB and $-\underline{\text{C}}\text{H}_2\text{-triazole C}$), 7.79 ppm (s, $-\text{C}=\underline{\text{C}}\text{H}-\text{N}-$ of triazole).

Preparation of polymeric micelles and inclusion complexes: Dialysis method was used to prepare sPEG-PHB polymeric micelles and their inclusion complexes with DM- β -CD. For micelles preparation, the sPEG-PHB polymers were dissolved in DMSO at concentration of 0.05 mgmL^{-1} , and then dialyzed against distilled water for 48 h using a dialysis membrane (MWCO 1000). The concentration of the micelles obtained from dialysis was determined to be around 0.02 mgmL^{-1} . For inclusion complexes preparation, DM- β -CD and sPEG-PHB polymers were mixed and dissolved in DMSO, then dialyzed against distilled water for 48 h

using a dialysis membrane (MWCO 1000). The initial concentration (before dialysis) of sPEG-PHB is 0.05 mg mL^{-1} and the weight ratio of DM- β -CD to sPEG-PHB is fixed at 20.

Molecular characterizations: Gel permeation chromatography (GPC) measurements were done at $40 \text{ }^\circ\text{C}$ on a Shimadzu SIL-10A and LC-20AD system equipped with two Phenogel 5μ 100 and 10^4 \AA columns (size: $300 \times 4.6 \text{ mm}$) connected in series and a Shimadzu RID-10A refractive index detector. THF was used as the mobile phase at a flow rate of 0.3 mL min^{-1} . Monodispersed PEG standards were used to calibrate the system. The absolute weight-averaged molecular weight (M_w) of sPEG-PHB copolymers was determined by Gel permeation chromatography/Light Scattering (GPC/LS) analysis. GPC was performed using two $10\text{-}\mu\text{m}$ PLgel MIXED-B columns (size: $300 \times 7.5 \text{ mm}$) in series with a THF flow rate of 1 mL min^{-1} . Light scattering and refractive index measurements were acquired using an Alliance Waters 2690 separation module equipped with a Waters 410 differential refractometer and a Wyatt MiniDAWN light scattering detector. The LS detection measures the light-scattering intensity at 90° and the RI detection measures the concentration of the copolymers. The data were processed using Astra 4.50 software. ^1H and ^{13}C NMR spectra were obtained at room temperature on a Bruker Avance DRX 400 MHz NMR spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are reported in ppm with reference to solvent peak (CHCl_3 : $\delta 7.26 \text{ ppm}$ for ^1H NMR and $\delta 77.2 \text{ ppm}$ for ^{13}C NMR). Fourier transform infrared (FTIR) spectra of polymers in KBr were recorded on a Perkin-Elmer FTIR 2000 spectrometer in the region of $4000\text{-}500 \text{ cm}^{-1}$.

Thermal analysis: Thermogravimetric analyses (TGA) were done on a TA Instruments Q500, by heating polymer samples at a rate of $20 \text{ }^\circ\text{C min}^{-1}$ from room temperature to $800 \text{ }^\circ\text{C}$ in a dynamic nitrogen atmosphere (flow rate = 60 mL min^{-1}).

Laser light scattering: Dynamic light scattering (DLS) measurements on polymer and inclusion complex particles were carried out on a Malvern Instrument Zetasizer Nano ZS, with a laser light wavelength of 633 nm at a 173° scattering angle at 25 °C. Z-average hydrodynamic radii (R_h) of the particles were reported. Static light scattering (SLS) was used to study the aggregation behavior of the polymers in aqueous solution. Information on weight-averaged molecular weight ($M_{w,agg}$), z-average radius of gyration (R_g) and second virial coefficient (A_2) of micelles were determined according to the following relation:

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w} \left[1 + \frac{q^2 R_g^2}{3} \right] + 2A_2c \quad (1)$$

where K is the optical constant, which depends on the refractive index increment (dn/dc) of the polymer solution ($K = 4\pi^2 n^2 (dn/dc)^2 / N_A \lambda^4$, where N_A is Avogadro's number, n is the refractive index of the liquid medium, and λ is the wavelength of the laser). ΔR_θ is the excess Rayleigh ratio, and q is the scattering vector determined by the scattering angle θ ($q = 4\pi n / \lambda \sin(\theta/2)$, where n is the refractive index of the liquid medium, and λ is the wavelength of the laser). $M_{w,agg}$ was estimated from the extrapolation of $Kc/\Delta R_\theta$ to zero angle (θ) and zero concentration (c) while R_g and A_2 were estimated from the slope of the angular and concentration dependence of the Zimm plots, respectively. All SLS measurements were carried out at 25 °C within the angular range of 50° to 120° with 5° increments. The refractive index increment (dn/dc) of sPEG-PHB polymers in aqueous solution was measured using a BI-DNDC differential refractometer at a wavelength of 620 nm. The instrument was calibrated with potassium chloride (KCl) in aqueous solution. The dn/dc values of the block copolymers are listed in the following table:

sPEG-PHB polymers	dn/dc [mLg⁻¹]
sPEG-PHB(10-3.1)	0.09618
sPEG-PHB(20-3.1)	0.09461
sPEG-PHB(10-1.6)	0.1537
sPEG-PHB(20-1.6)	0.1063

Microscopy: Transmission electron microscopy (TEM) images were taken on a JEOL JEM-3010 FasTEM field emission transmission electron microscope, operated at 300 kV. TEM sample was prepared by directly depositing a drop of sample solution containing 0.05 wt% of phosphotungstic acid (PTA) onto a 200 mesh carbon coated copper grid. The sample was kept in a dry box for 48 h at room temperature before TEM imaging. Analyses on TEM images were done using ImageJ software. Scanning electron microscopy (SEM) images were obtained at acceleration voltage of 5 kV on a JEOL JSM-6700F microscope. The samples were prepared by casting a drop of sample solution on glass substrate and dried at room temperature for 48 h in a dry box. The samples were sputter-coated with a thin layer of gold for 15 s to make the samples conductive before testing.

2. Molecular characteristics of well-defined heterofunctionalized PHB precursors

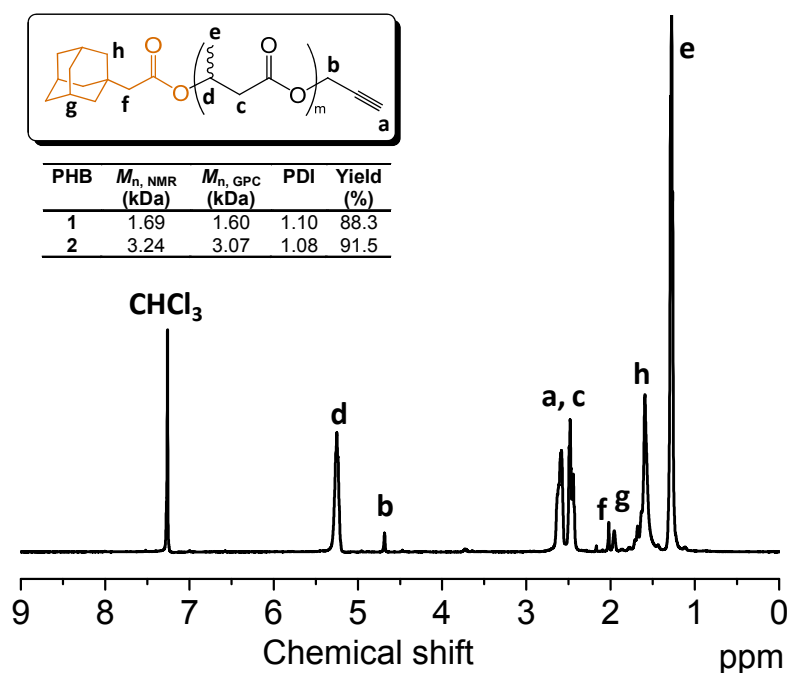


Fig. S1 ¹H NMR spectrum of heterofunctionalized PHB with corresponding peak assignments. Alkynyl and adamantyl end groups can be clearly seen in the spectrum. The close agreement of M_n s as determined from NMR and GPC as well as the narrow PDI of molecular weight confirm the well-defined nature of the two PHB polymers synthesized.

Two PHB polymers with different molecular weight have been synthesized through adjusting the β -butyrolactone monomer to adamantaneacetate initiator ratio. The degree of polymerization (DP) and M_n of PHB polymers were evaluated from ¹H NMR spectra (as shown in Fig. S1) based on the intensity ratio of PHB methine proton at around δ 5.2 ppm to alkynyl end group protons at around δ 4.7 ppm. DPs for the two polymers were estimated to be 17 and 35, and each corresponds to M_n of 1.69 kDa and 3.24 kDa, respectively. The M_n values are in good agreement with GPC measurements. In addition, both alkynyl and adamantyl end groups of the PHB polymers are clearly identified from ¹H NMR spectra. Together with the relatively narrow PDI as determined from GPC, all of the above characterization data attest to the well-defined nature of the two PHB polymers.

3. Synthesis of sPEG-PHB

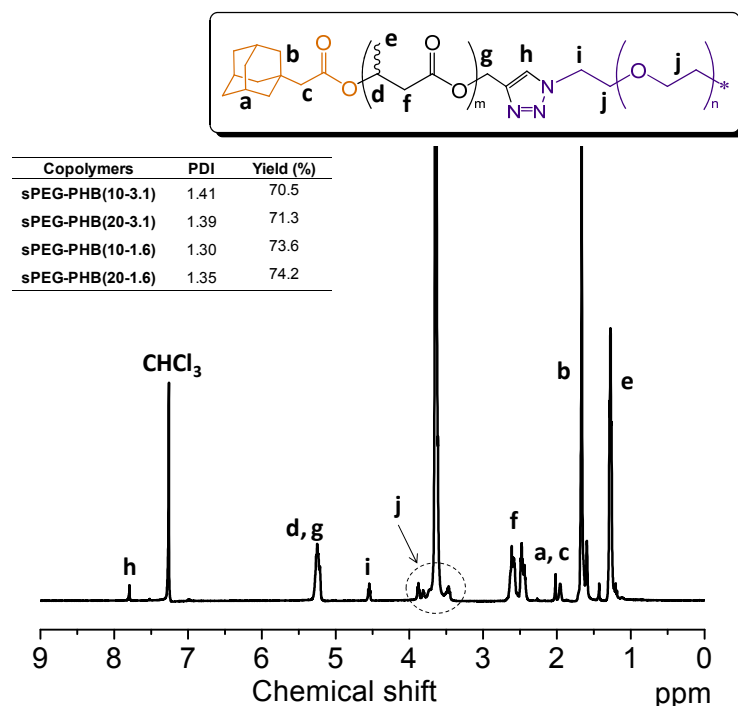


Fig. S2 ¹H NMR spectrum of sPEG-PHB with corresponding peak assignments. Triazole linking group and adamantyl end group can be clearly seen in the spectrum. The star-block copolymers were obtained in moderately good yield with relatively narrow molecular weight distribution.

¹H NMR was used to elucidate the chemical structure of the obtained sPEG-PHB copolymers. Fig. S2 shows the ¹H NMR spectrum of sPEG-PHB(20-1.6), which is typical of all copolymers. All signals in the spectrum can be ascribed to protons belonging to either PHB, sPEG, linkage group or end group. Methylene protons of sPEG contribute to the signals at around δ 3.41 – 3.95 (m, sPEG backbone). Signals of PHB protons and linking triazole group can be found at δ 1.14 – 1.36 (m, -OCH(CH₃)CH₂CO₂- of PHB), 2.33 – 2.79 (m, -OCH(CH₃)CH₂CO₂- of PHB), 4.50 – 4.62 (m, -CH₂-triazole N), 5.12 – 5.36 (m, -OCH(CH₃)CH₂CO₂- of PHB and -CH₂-triazole C), 7.79 (s, -C=CH-N- of triazole). The adamantane end group protons can be seen at δ 1.52 – 1.78 (m, methylene protons of adamantyl end group), 1.96 (br, methine protons of adamantyl end group), 2.02 (s, -OCCH₂-Ada). Conventional GPC measurements on the star-block copolymers gave PDI in the range

of 1.3 – 1.4. This indicates that the polymers have relatively narrow molecular weight distribution. Along with their moderately good yields, the narrow molecular weight distribution of all the star-block copolymers indicate that the alkyne-azide coupling proceeded well to give uniform star-block copolymers.

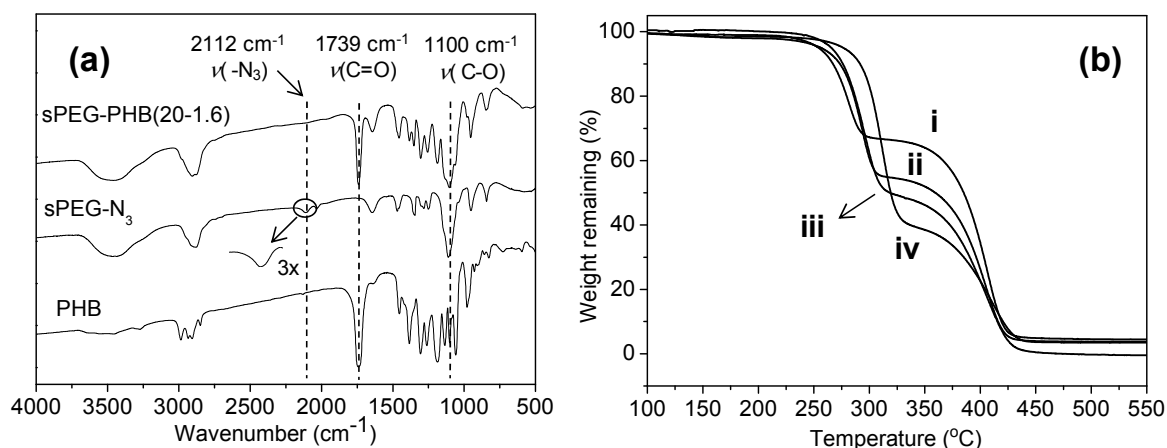


Fig. S3 (a) FTIR spectra of PHB (M_n 1.60 kDa), sPEG-N₃ (M_n 19.9 kDa) and sPEG-PHB(20-1.6) in the form of KBr discs. (b) Thermal degradation profiles of sPEG-PHB copolymers i: sPEG-PHB(20-1.6), ii: sPEG-PHB(20-3.1), iii: sPEG-PHB(10-1.6), iv: sPEG-PHB(10-3.1).

The successful alkyne-azide coupling is also confirmed by comparing the FTIR spectra of PHB, sPEG-N₃ and sPEG-PHB, as shown in Fig. S3(a). The presence of azide end-group of sPEG-N₃ was confirmed by the characteristic azide signal at 2112 cm⁻¹. After coupling reaction, the azide peak was no longer seen from the FTIR spectrum of sPEG-PHB. The molecular characteristics of sPEG-PHB copolymers were further estimated by TGA analyses. Fig. S3(b) shows the thermal degradation profile of sPEG-PHB copolymers. The polymers exhibited a two-step degradation behavior, with the first step occurring between 220 °C and 340 °C followed by the second step from 340 °C to 450 °C. The first and second step of mass reduction can be attributed to the degradation of PHB and sPEG, respectively. The weight percentage of PHB in sPEG-PHB copolymers was estimated from the thermal degradation profile, and the values were further used to calculate the PHB arm numbers of each sPEG

core as listed in Table 1. The results obtained from TGA analyses are in good accordance with those obtained from ^1H NMR estimation.

4. Self-assembly behaviours of sPEG-PHB

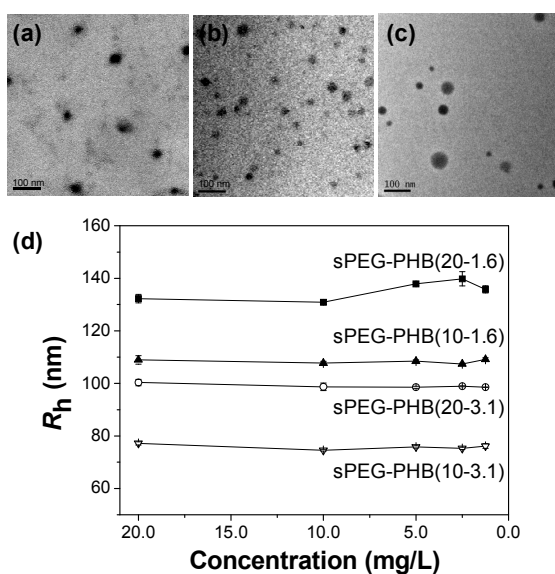


Fig. S4 TEM images of (a) sPEG-PHB(10-1.6) micelles; (b) sPEG-PHB(20-3.1) micelles and (c) sPEG-PHB(20-1.6) micelles (Scale bar = 100 nm); (d) R_h (z-average) stability of sPEG-PHB micelles in aqueous solution against dilution.

Table S1 Particle sizes of sPEG-PHB micelles as determined from DLS at a concentration of 20 mg/L and TEM.

sPEG-PHB micelles	Particle radius (nm)	
	DLS	TEM
sPEG-PHB(10-3.1)	77 ± 1	13 ± 3
sPEG-PHB(20-3.1)	100 ± 1	16 ± 3
sPEG-PHB(10-1.6)	109 ± 2	22 ± 5
sPEG-PHB(20-1.6)	132 ± 2	18 ± 7

5. Self-assembly behaviours of sPEG-PHB/DM- β -CD

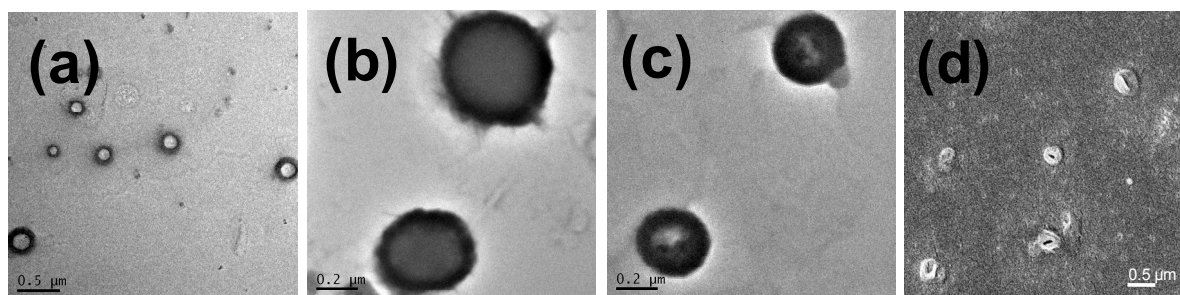


Fig. S5 TEM images of (a) sPEG-PHB(20-1.6)/DM- β -CD vesicles, (b) sPEG-PHB(10-1.6)/DM- β -CD vesicles and (c) sPEG-PHB(20-3.1)/DM- β -CD vesicles; (d) SEM image sPEG-PHB(10-3.1)/DM- β -CD vesicles.

Table S2 Vesicle diameter and wall thickness of sPEG-PHB/DM- β -CD vesicles.

sPEG-PHB/DM- β -CD vesicles	Vesicle diameter (nm)	Wall thickness (nm)
sPEG-PHB(10-3.1)	514 ± 54	155 ± 28
sPEG-PHB(20-3.1)	339 ± 15	106 ± 14
sPEG-PHB(10-1.6)	480 ± 68	57 ± 9
sPEG-PHB(20-1.6)	234 ± 57	46 ± 12

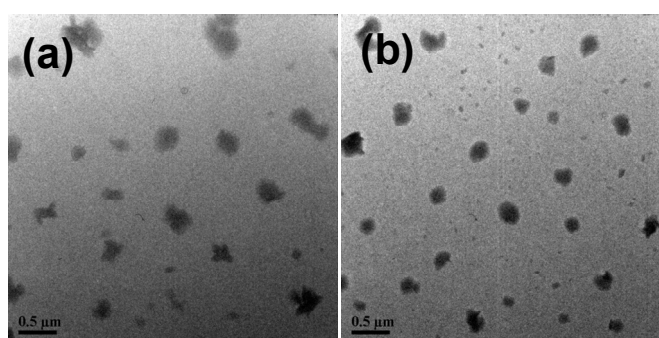


Fig. S6 TEM images (a) MPEG-PHB(2-1.6)/DM- β -CD aggregates, (b) MPEG-PHB(2-3.1)/DM- β -CD aggregates.

Reference

1. K. L. Liu, S. H. Goh and J. Li, *Polymer* 2008, **49**, 732.