Electronic Supplementary Material (ESI) for Polymer Chemistry.
This journal is © The Royal Society of Chemistry 2017

Supporting information

Transition from smectic nanofibers to smectic vesicles in self-assemblies of PEG-*b*-Liquid crystal polycarbonate

Lu Zhou,[a,‡] Dapeng Zhang,[b,‡] Sabrina Hocine,[c,‡] Alessia Pilone,[b] Sylvain Trepout,[d] Sergio Marco,[d] Christophe Thomas,[b,*] Jia Guo[a,*] and Min-Hui Li [a,b,c,*]

a. Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, 15 North Third Ring Road, Chaoyang District, 100029 Beijing, P. R. China.
b. Chimie ParisTech, PSL Research University, CNRS, Institut de Recherche de Chimie Paris, UMR8247, 11 rue Pierre et Marie Curie, 75231 Paris, France.
c. Institut Curie - CNRS - Université Pierre and Marie Curie, Laboratoire Physico-Chimie Curie, UMR168, 26 Rue d'Ulm, 75248 Paris, France.
d. Institut Curie, INSERM U1196 and CNRS UMR9187, 91405 Orsay cedex, France.

Email: min-hui.li@chimie-paristech.fr; guojia@mail.buct.edu.cn; christophe.thomas@chimie-paristech.fr.

‡ These authors contributed equally to this work.

1. Materials and methods

Dichloromethane, 1,4-dioxane, ethyl acetate, hexane, tetrahydrofuran and chloroform d_3 were purchased from Carlo-Erba SDS and used without further purification. Acetonitrile, benzyl bromide, 2,2-bis(hydroxymethyl) propionic acid, triethylamine, cholesteryl hemisuccinate, 1,1,1-tris(hydroxymethyl)ethane, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl), ethyl chloroformate were purchased from Sigma-Aldrich and used as received. 4-(Dimethylamino)pyridine was purchased from Fluka and used as received. Lanthanum tris[bis(trimethylsilyl)amide] was purchased from Sigma-Aldrich and used as received. Calcium bis[bis(trimethylsilyl)amide] was synthesized according to a reported method.[1] Poly(ethylene glycol) methyl ether (mPEG-OH 2000 Da)

was purchased from Fluka and was purified and dried before use. After three cycles of precipitation/filtration from diethyl ether, the solvent residues in mPEG-OH were evaporated under reduced pressure and the resulting solid was dried over P_2O_5 under vacuum for at least 4 days.

2. Instruments and Measurements

Nuclear Magnetic Resonance (NMR)

^1H NMR spectra were recorded on a 300 MHz Bruker Avance spectrometer and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer. Chemical shifts δ are given in ppm and referenced to tetramethylsilane (TMS) in $CDCl_3$ (0 ppm). The degree of polymerization DP and the number-average molecular weight M_n were determined by integration of specific signals in ^1H NMR spectra (see experimental parts for details).

Size Exclusion Chromatography (SEC)

Molecular weight distributions of polymers (M_w/M_n) were evaluated by size exclusion chromatography (SEC), using Waters Styragel HR5E columns and a Waters 410 differential refractometer. Tetrahydrofuran was used as eluent with a flow rate of 1 mL/min at 40°C. Monodisperse poly(styrene) polymers from Polymer Laboratories were used as calibration standards.

Differential Scanning Calorimetry (DSC)

Calorimetric measurements of polymers were performed using a Perkin Elmer DSC7 device. The reference cell was left empty and the sample cell was filled with 5 to 10 mg of polymer sample. Samples were scanned at 10 °C/min rate over a range of temperature between -50°C and 200°C. Transition temperatures were taken at the first heating scan and glass temperatures were measured as the midpoint of the heat capacity jump.

Polarized Optical Microscopy (POM)

The mesomorphic properties of liquid crystalline monomers and polymers were studied

using a Leitz Ortholux polarized optical microscope equipped with a Mettler FP82 hot stage and a digital camera. Samples were scanned at variable speeds from 1 to 10 °C/min and pictures were taken during the cooling cycles.

Small angle X-ray scattering (SAXS)

The SAXS experiments were performed at room temperature on samples in capillary or on fiber samples drawn from melted polymers (in the mesophase at high temperature) and quenched in air. CuKα radiation (λ=1.54 Å) generated from a 1.5 kW rotating anode was used as X-ray beam. The diffraction patterns were recorded on photosensitive image plates

Transmission Electron Microscopy (TEM) and cryo-transmission electron microscopy (cryo-TEM)

Polymer colloids morphologies were studied by classical TEM and cryo-TEM techniques. TEM pictures were taken with a Philips CM120 electron microscope (FEI, Eindhoven, The Netherlands). Images were acquired with a Keenview camera (Olympus Soft Imaging Solutions GMBH, Münster, Germany). Samples were prepared by deposition of a drop of colloidal suspensions on a copper grid coated with a carbon film. The drop was blotted after 45 seconds and a uranyl acetate solution in water (2 wt%) was then used to stain the sample (30 seconds) before washing the grid.

Cryo-TEM images were acquired on a JEOL 2200FS energy-filtered (20 eV slit) field emission gun electron microscope operating at 200 kV using a Gatan US1000 camera . For the sample preparation, a total of 5 μL of samples were deposited onto a 200 mesh holey copper grid (Ted Pella Inc., U.S.A.) and flash-frozen in liquid ethane cooled down at liquid nitrogen temperature using a Leica CPC system.

3. Synthesis of MCC monomer

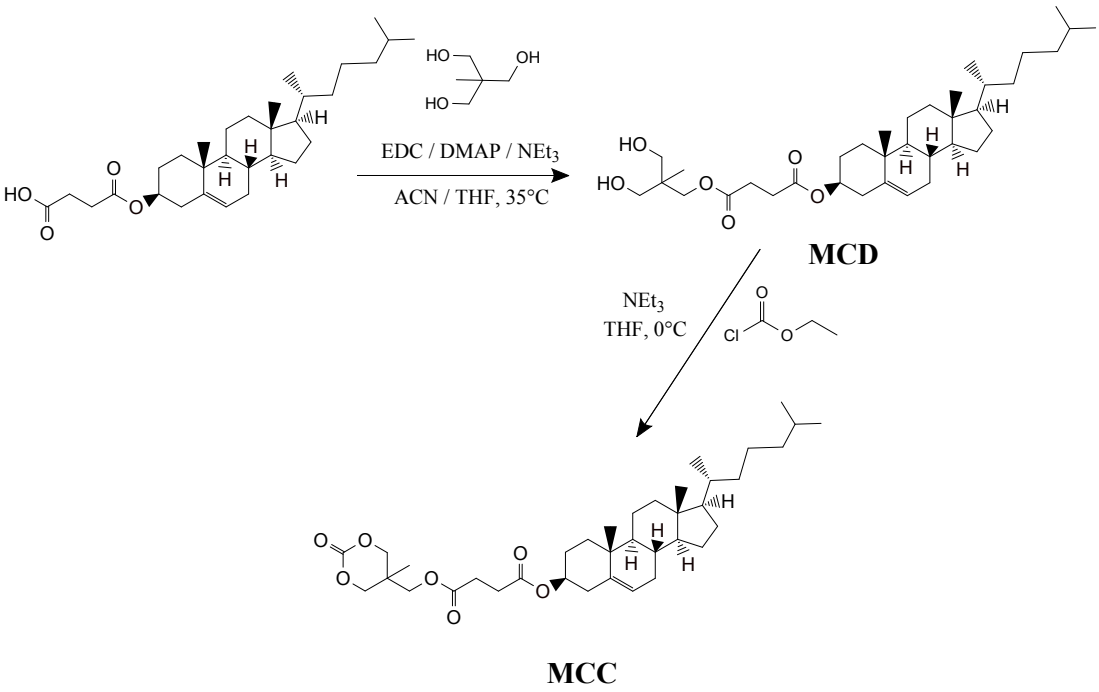

Figure S1. The synthetic scheme of MCC monomer.

a. 1-Cholesteryl 4-(3-hydroxy-2-(hydroxymethyl)-2-methylpropyl) succinate: (MCD)[2]

In a 500 mL flask were loaded 6.2 g (12.8 mmol) of cholesteryl hemisuccinate along with 80 mL THF and 80 mL acetonitrile (ACN). After dissolution, 9.3 g (77.6 mmol) of 1,1,1-tris(hydroxymethyl) ethane were added with additional 20 mL THF and 60 mL acetonitrile. The mixture was stirred at 35 °C until total dissolution. 3.2 g (16.7 mmol) of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) and 120 mg (0.98 mmol) of 4-(dimethylamino)pyridine (DMAP) were then added. After the complete dissolution of the powders, 2.5 mL (18 mmol) of triethylamine (NEt_3) were added via syringe. The reaction was let to proceed for 24 hours. After evaporation of the solvent under reduced pressure, the mixture was resuspended in ethyl acetate to precipitate hydrated EDC salts and the excess of triol. Filtration and solvent evaporation yielded a white powder, which was then dissolved in a minimal volume of THF and poured into 300 mL methanol. This allowed the precipitation of dimers and trimers in white crystals. Filtration of the mixture and evaporation of methanol yielded 8 g of crude product. This crude was finally purified by column chromatography (ethyl acetate/hexanes 6/4 v/v) and 3.6 g of pure product was obtained. Yield: 47 %.

^{1}H NMR ($CDCl_3$, 300 MHz, δ in ppm): 0.84 (s, 3H, -CH_2-C(C**H$_3$**)-CH_2-), 0.62-2.06 (m, 41H cholesterol), 2.27-2.37 (d, 2H, -OCH-C**H$_2$**-C(CH)=CH- cholesterol), 2.57-2.70 (m, 4H, -OC(O)-C**H$_2$**-C**H$_2$**-C(O)O- hemisuccinate), 2.70-2.79 (t, 2H, 2 -C(CH_3)-CH_2-O**H**), 3.47-3.65 (m, 4H H-O-CH_2-C(CH_3)-C**H$_2$**-OH), 4.23 (s, 2H, -C(CH_3)-C**H$_2$**-OC(O)-), 4.54-4.70 (m, 1H,-O-C**H**(CH_2)-CH_2-), 5.34-5.41 (d, 1H, -CH_2-C(CH)=C**H**-CH_2-).

^{13}C NMR (CDCl3, 100 MHz, δ in ppm): 67.8 (HO-**C**H_2-C(CH_3)-**C**H_2-OH), 40.8 (CH_2-**C**(CH_3)-CH_2-), 17 (-CH_2-**C**(CH_3)-CH_2-), 66.9 (-CH_2-C(CH_2)-**C**H_2-OC(O)-), 173.6 (-CH_2-O**C**(O)-CH_2), 29.3, 29.5 (-OC(O)-**C**H_2-**C**H_2-C(O)O-), 171.9 (-CH_2-O**C**(O)-CH(CH_2)-CH_2-), 74.7 (-C(O)O-**C**H(CH_2)-CH_2- cholesterol), 139.6 (-CH_2-**C**(CH)=CH-CH_2- cholesterol), 122.9 (-CH_2-C(CH)=**C**H-CH_2- cholesterol), 11.9, 18.8, 19.4, 21.1, 22.6, 22.9, 23.9, 24.4, 27.8, 28.1, 28.3, 29.3, 29.5, 31.9, 32.0, 35.9, 36.2, 36.6, 37.0, 38.1, 39.6, 39.8, 42.4, 50.1, 56.2, 56.7 (rest of cholesterol ring).

b. 1-Cholesteryl 4-((5-methyl-2-oxo-1,3-dioxan-5-yl)methyl) succinate (MCC):

In a flame dried flask under an argon atmosphere were introduced 3.6 g (6.1 mmol) of MCD molecule and 40 mL of dry tetrahydrofuran. The mixture was stirred for dissolution of the solids and an ice bath was then set. 1.22 mL (12.8 mmol) of ethyl chloroformate were subsequently injected in the flask, followed by 2 mL (14.3 mmol) of triethylamine (NEt_3). The mixture was stirred for 24 hours at room temperature. Tetrahydrofuran was evaporated under reduced pressure and the residue was resuspended in dichloromethane. After washing the organic solution twice with brine and drying over $MgSO_4$, the solvent was then removed. The white residue was then purified by column chromatography (ethyl acetate/hexanes 5/5 v/v) and 2.9 g of pure MCC were obtained. Yield: 80 %. ^{1}H NMR ($CDCl_3$, 300 MHz, δ in ppm): 1.13 (s, 3H, -CH_2-C(C**H$_3$**)-CH_2-), 0.61-2.08 (m, 41H cholesterol), 2.27-2.38 (d, 2H, -OCH-C**H$_2$**-C(CH)=CH- cholesterol), 2.57-2.71 (m, 4H, -OC(O)-C**H$_2$**-C**H$_2$**-C(O)O- hemisuccinate), 4.08-4.37 (2d, 4H -O-C**H$_2$**-C(CH_3)-C**H$_2$**-O- carbonate ring), 4.13 (s, 2H, -C(CH_3)-C**H$_2$**-OC(O)-), 4.54-4.71 (m, 1H, -C(CH_3)-C**H$_2$**-OC(O)-), 5.33-5.41 (d, 1H, -CH_2-C(CH)=C**H**-CH_2-).

^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 147.7 (-O-**C**(O)-O-), 73.7 (-O-**C**H_2-C(CH_3)-**C**H_2-O-), 32.4 (-CH_2-**C**(CH_3)-CH_2-), 17.2 (-CH_2-C(**C**H_3)-CH_2-), 65.3 (-CH_2-C(CH_3)-**C**H_2-OC(O)-), 172

(-CH_2-O**C**(O)-CH_2), 29, 29.3 (-OC(O)-**C**H_2-**C**H_2-C(O)O-), 171.6 (-CH_2-O**C**(O)-CH(CH_2)-CH_2-), 74.7 (-C(O)O-**C**H(CH_2)-CH_2- cholesterol), 139.5 (-CH_2-**C**(CH)=CH-CH_2- cholesterol), 122.9 (-CH_2-C(CH)=**C**H-CH_2- cholesterol), 11.9, 18.8, 19.4, 21.2, 22.7, 22.9, 23.9, 24.3, 28.1, 27.8, 28.3, 31.9, 32, 32.4, 35.9, 36.2, 36.6, 37, 38.1, 39.7, 39.8, 42.4, 50, 56.2, 56.7 (rest of cholesterol ring).

3. Polymerization

All the polymerization was performed under argon in a 15mL flame dried Schlenk tube equipped with a Teflon coated stirring bar. The typical polymerization procedure for PMCC homopolymer was as follows: In the glove box, the Schlenk tube was charged with lanthanum catalyst (C1) (6 mg, 0.007 mmol), MCC monomer (172 mg, 0.280 mmol) and THF (0.75 mL), the mixture was then stirred for 5 min at room temperature. ^{1}H NMR analysis showed that the conversion of monomer was almost 100%. The THF solution was then poured drop-by-drop into cold methanol (25 mL) to precipitate the crude PMCC homopolymer. After re-dissolved in THF and precipitated in methanol twice more, the purified polymer was collected and dried under vacuum at 50 °C for 48 h. For the synthesis of PEG_{45}-*b*-$PMCC_{18}$, all the procedures were the same with that of PMCC homopolymer except that mPEG-OH 2000 Da (43 mg, 0.021 mmol) was added in the beginning to react with the Lanthanum catalyst (C1) to form macro-initiator. The ^{1}H NMR spectra of PMCC hompolymer and PEG_{45}-*b*-$PMCC_{18}$ were shown in Figure S2 and S3.

For the synthesis of PEG_{45}-*b*-$PMCC_{26}$, all the procedures were the same with that of PEG_{45}-*b*-$PMCC_{18}$ except that calcium bis[bis(trimethylsilyl)amide] (C2) was used and the different ratio between monomer and catalyst was different.

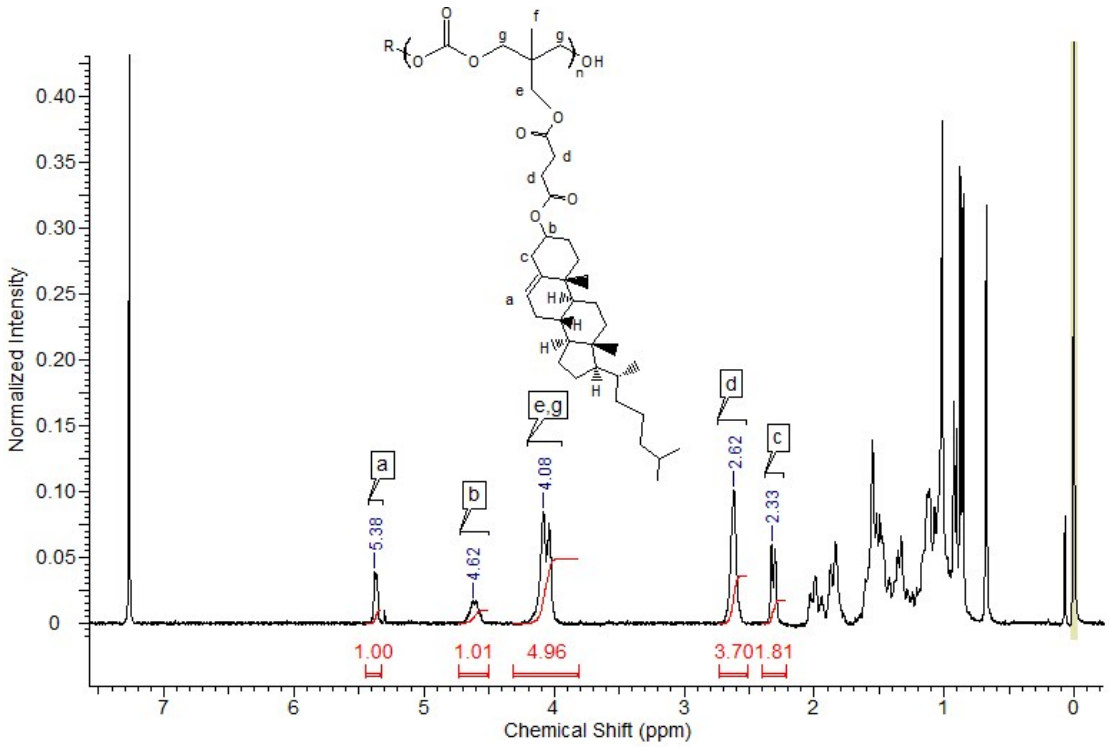

Figure S2. ^{1}H NMR spectrum of PMCC homopolymer. $CDCl_3$, 300 MHz, 25 °C.

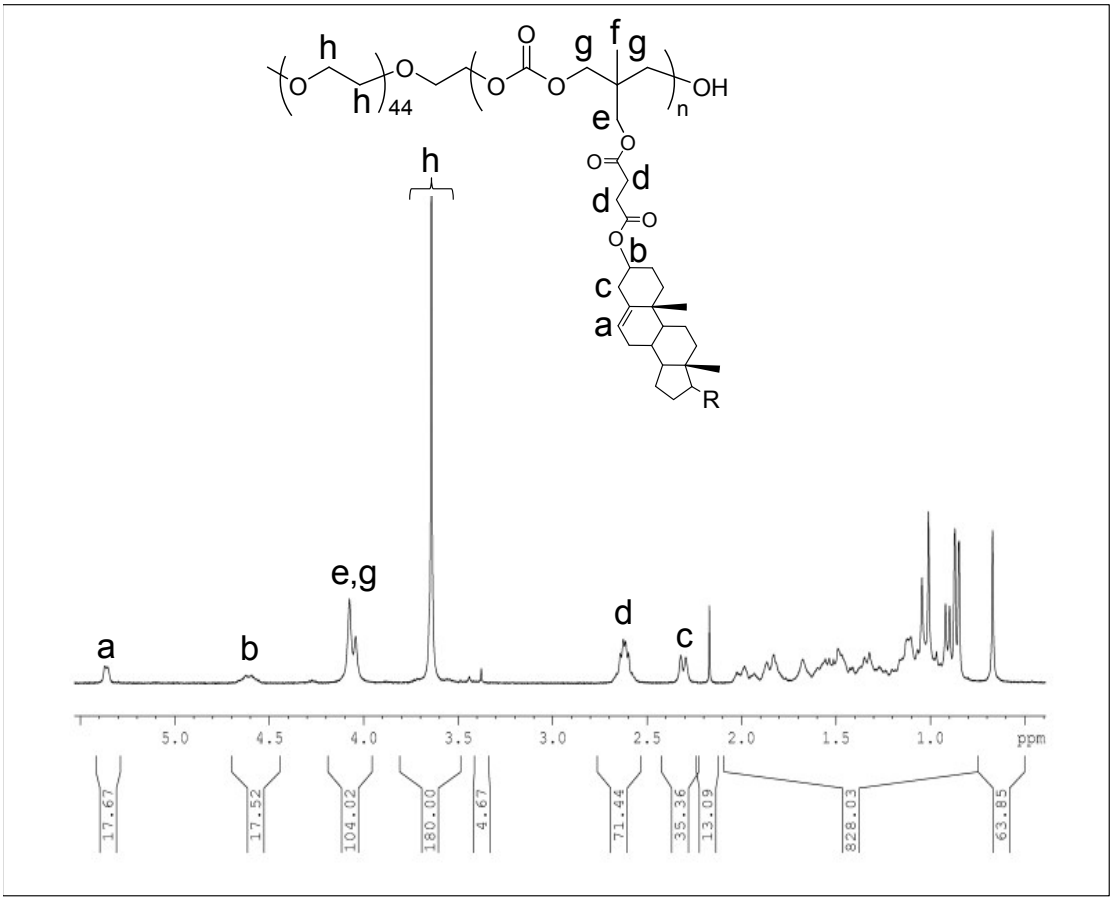

Figure S3. ^{1}H NMR spectrum of PEG_{45}-*b*-$PMCC_{18}$. $CDCl_3$, 300 MHz, 25 °C.

4. SAXS characterization

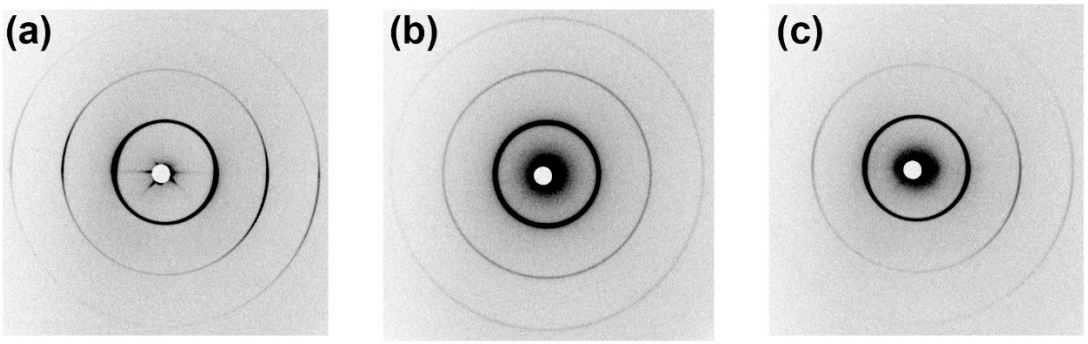

Figure S4. SAXS pattern of (a) MCC monomer, (b) PMCC homopolymer and (c) PEG_{45}-*b*-$PMCC_{26}$ on samples at room temperature after heating to isotropic phase. Three orders of Bragg reflection located at q, $2q$ and $3q$ were observed and a lamellar period of P = 4.90 nm was deduced. $q = 4\pi\sin\alpha/\lambda = 2\pi/P$, is the wave vector, 2α is the diffraction angle.

5. Copolymer self-assembly of PEG-*b*-PMCC

Nanoparticles were prepared from PEG_{45}-*b*-$PMCC_n$ copolymers using a classical nanoprecipitation method. Briefly, the polymer was first dissolved in 1,4-dioxane, which is a good solvent for both polymer blocks. The initial concentration of the polymer in dioxane was ~ 0.5 wt%. Water was then added very slowly to the organic solution (speed of around 2µL/min) until it reached 50 wt% of the whole solution. Colloids were recovered after 3 days dialysis against water in a 6500 Da cut off cellulose bag (dialysis bath refreshed once a day) to remove dioxane.

6. TEM and cryo-TEM images

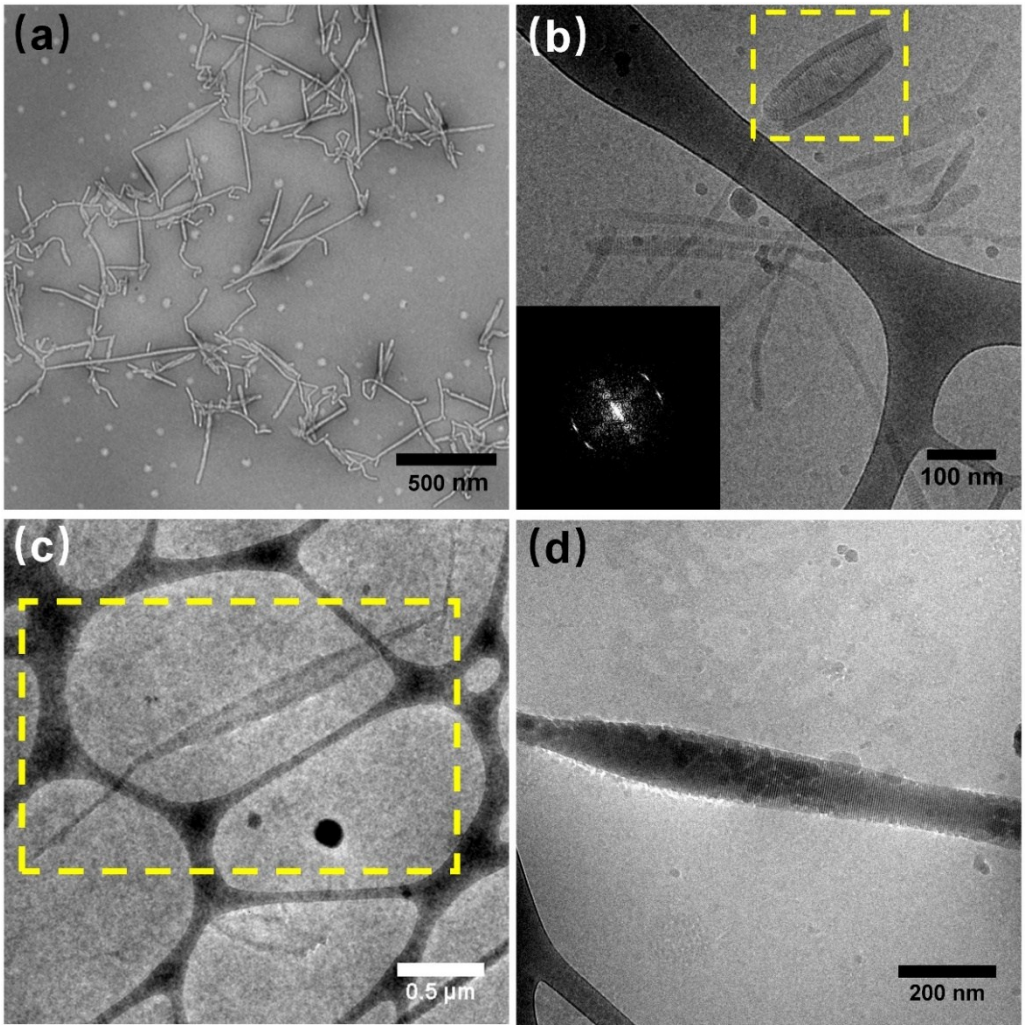

Figure S5. Self-assemblies of PEG_{45}-*b*-$PMCC_{18}$ imaged by TEM (a) and cryo-TEM (b) and PEG_{45}-*b*-$PMCC_{26}$ imaged by cryo-TEM (c, d). The inset in (b) is the fast Fourier transform (FFT) of the image part in the yellow rectangle showing two series of Bragg diffractions, which indicate the smectic layers in the membranes of upper side and lower side of the vesicle are not parallel , but oriented with an angle of 31.5° between them.

Notes and references

1 X. He, B. C. Noll, A. Beatty, R. E. Mulvey and K. W. Henderson, *J. Am. Chem. Soc.*, 2004, **126**, 7444-7445.

2 R. A. Earl, M. Ezawa, X. Fang, D. S. Garvey, R. D. Gaston, S. P. Khanapure, L. G. Letts, C.-E. Lin, R. R. Ranatunga, S. K. Richardson, J. D. Schroeder, C. A. Stevenson, and S.-J. Wey, *US2005022243A1*, 2005.