

Supporting Information

One-pot Synthesis of Amphiphilic ABC Triblock Copolymer PEO-*b*-PEHOx-*b*-PEtOz and its Self-Assembly into Nanoscopic Asymmetric Polymersomes

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1. Synthesis of PEO-*b*-PEHOx-*b*-PEtOz polymers

a. Calculation of EHOx and EtOz block length by ¹H NMR

The block ratio of ABC PEO-*b*-PEHOx-*b*-PEtOz was determined by integrating the PEO backbone peak at 3.60 ppm as a reference (always 45 units for 2000 Da PEO used). Firstly, the peaks of PEHOx side chain at 0.86 ppm are integrated, integral "a" to calculate the PEHOx block length. The PEtOz block length is then calculated by integrating the peaks of the backbone of PEHOx and PEtOz at 3.43 ppm, integral "b", to which we subtract the number of H coming from PEHOx as summarized in the following Table S1.

Table S1 - Calculations of EHOx block length for 5 different ABC triblock. ^a Integral "a" (m, 6H, CH₃) 0.86 ppm. ^bIntegral "b" (m, 4H, N(COCH₂CH₂)-CH₂CH₂) 3.43 ppm. ^c Calculated via N(PEHOx length)= a/6. ^dCalculated via N(PEtOz length)= (b-3-(N(PEHOx length)*4))/4. We subtract 3H from the integral b because of the methyl end-group of PEO which overlap with the peaks of backbone of PEHOx and PEtOz

Triblock terpolymers	Integral "a" ^a	Integral "b" ^b	PEHOx length ^c	PEtOz length ^d
PEO ₄₅ - <i>b</i> -PEHOX ₃₀ - <i>b</i> -PEtOz ₁₄	182	179	30	14
PEO ₄₅ - <i>b</i> -PEHOX ₆₅ - <i>b</i> -PEtOz ₁₉	392	341	65	19
PEO ₄₅ - <i>b</i> -PEHOX ₅₃ - <i>b</i> -PEtOz ₅₆	319	440	53	56
PEO ₄₅ - <i>b</i> -PEHOX ₈₇ - <i>b</i> -PEtOz ₁₀	520	390	87	10
PEO ₄₅ - <i>b</i> -PEHOX ₁₃₈ - <i>b</i> -PEtOz ₁₄	827	610	138	14

b. Remaining ABC triblock synthesized

Table S2 – Remaining characterization of PEO-*b*-PEHOx-*b*-PEtOz triblock terpolymers using ¹H NMR, GPC (CHCl₃) and hydrophilic weight fraction, *f*. ^aObtained from ¹H NMR. ^bObtained by GPC.

^cCalculated by the equation $f = (M_n(\text{PEO}) + M_n(\text{PEtOz})) / (M_n(\text{PEO}) + M_n(\text{PEHOx}) + M_n(\text{PEtOz}))$. ^dRatio Monomer (EHOx) to Initiator (PEO-Nos).

PEO _t - <i>b</i> -PEHOx _i - <i>b</i> -PEtOz _g	M _n [Da] ^a	\bar{D}_M ^b	<i>f</i> [%] ^c	Ratio (Mono/Ini) ^d	R _h [nm]	R _g [nm]	$\rho = R_g / R_h$
A ₄₅ B ₄₉ C ₉	12600	1.35	23	50	103 ± 9	100	0.97
A ₄₅ B ₅₁ C ₁₁	13200	1.32	24	50	107 ± 12	103	0.96
A ₄₅ B ₄₅ C ₁₁	12000	1.36	26	50	102 ± 13	103	1.01
A ₄₅ B ₄₃ C ₁₁	11600	1.34	27	50	98 ± 9	98	1.00
A ₄₅ B ₅₇ C ₁₂	14400	1.35	22	60	105 ± 15	106	1.01
A ₄₅ B ₅₃ C ₁₆	14000	1.40	25	50	105 ± 16	105	0.98

2. Length of the polymer segments and their conformation

a. Stretched conformation of PEO and PEHOx (contour length)

Although the average bond length of PEHOx is not reported, the chemical structure is similar to PEO (however, Nitrogen instead of Oxygen). Since any changes of the average bond length are not likely to be substantial, we rounded the above distance to 145 pm as bond length and 109.5 as angle for a perfect tetrahedron. The repeating unit has three atoms (l), which has to be multiplied with the number repeating units (n).

$$R_{contour} = l * n * \sin\left(\frac{\theta}{2}\right)$$

$$R_{contour} = 3 * 10 * 145nm * \sin\left(\frac{109.5}{2}\right) = 3.5nm \text{ for PEtOz-10}$$

$$R_{contour} = 3 * 45 * 145nm * \sin\left(\frac{109.5}{2}\right) = 16.0nm \text{ for PEO-45}$$

$$R_{contour} = 3 * 48 * 145nm * \sin\left(\frac{109.5}{2}\right) = 17.0nm \text{ for PEHOx-48}$$

$$R_{contour} = 3 * 65 * 145nm * \sin\left(\frac{109.5}{2}\right) = 23.1 nm \text{ for PEHOx-65}$$

$$R_{contour} = 3 * 87 * 145nm * \sin\left(\frac{109.5}{2}\right) = 30.9nm \text{ for PEHOx-87}$$

$$R_{contour} = 3 * 139 * 145nm * \sin\left(\frac{109.5}{2}\right) = 49.3nm \text{ for PEHOx-139}$$

b. Random conformation of PEO and PEHOx (ideal coil)

The average bond length and effective bond length can be taken from the equation above (d = 145 pm and the tetrahedral bond angle (109.5 degrees)). Similar to the equation above, the repeating unit has three atoms (l), which has to be multiplied with the number repeating units (n).

$$R_{coil} = \frac{1+\cos\theta}{1-\cos\theta} * \sqrt{l * n} * d * \sin\left(\frac{109.5}{2}\right)$$

$$R_{coil} = \frac{1.33}{0.67} * \sqrt{3 * 45} * 145pm * \sin\left(\frac{109.5}{2}\right) = 2.7nm \text{ for PEO-45}$$

$$R_{coil} = \frac{1.33}{0.67} * \sqrt{3 * 48} * 145pm * \sin\left(\frac{109.5}{2}\right) = 2.8nm \text{ for PEHOx-48}$$

$$R_{coil} = \frac{1.33}{0.67} * \sqrt{3 * 65} * 145pm * \sin\left(\frac{109.5}{2}\right) = 3.3nm \text{ for PEHOx-65}$$

$$R_{coil} = \frac{1.33}{0.67} * \sqrt{3 * 87} * 145pm * \sin\left(\frac{109.5}{2}\right) = 3.8nm \text{ for PEHOx-87}$$

$$R_{coil} = \frac{1.33}{0.67} * \sqrt{3 * 139} * 145pm * \sin\left(\frac{109.5}{2}\right) = 4.8nm \text{ for PEHOx-139}$$

c. Effective conformation of PEO and PEHOx – Mix of random coil and stretched

Since PEHOx is a polymer with a branched side chain, the ideal random coil cannot be formed. It also not completely stretched, but has a mixed conformation of both components. The amount of stretching (x) can be calculated, since the real length is known from Cryo-TEM. Since it is an ABC triblock this length can be taken as it has been measured as R_{eff} .

$R_{eff} = x * R_{contour} + (1 - x) * R_{coil}$ reforming the equation towards x yields

$x = \frac{R_{eff} - R_{coil}}{R_{contour} - R_{coil}}$ for the specific polymers, this yields x as dimensionless number:

$$x_{PEO,45} = \frac{12.6 - 2.7}{16.0 - 2.8} = 75\%$$

PEO-45 is 74% stretched within the cavities of the tubes

$$x_{PEHOx,48} = \frac{6.3 - 2.8}{17.0 - 2.8} = 25\%$$

PEHOx-48 is 25% stretched within the polymersome membrane

$$x_{PEHOx,65} = \frac{7.8 - 3.3}{23.1 - 3.3} = 23\%$$

PEHOx-65 is 23% stretched within the polymersome membrane

$$x_{PEHOx,87} = \frac{9.9 - 3.8}{30.9 - 4.0} = 22\%$$

PEHOx-87 is 22% stretched within the MCV membrane

$$x_{PEHOx,138} = \frac{12.6 - 4.8}{49.3 - 4.8} = 18\%$$

PEHOx-138 is 18% stretched within the membrane of the polymersomes with a thicker membrane

3. Self-assembly of PEO-*b*-PEHOx-*b*-PEtOz

a. Characterisation of solvent switch of PEO-*b*-PEHOx-*b*-PEtOz

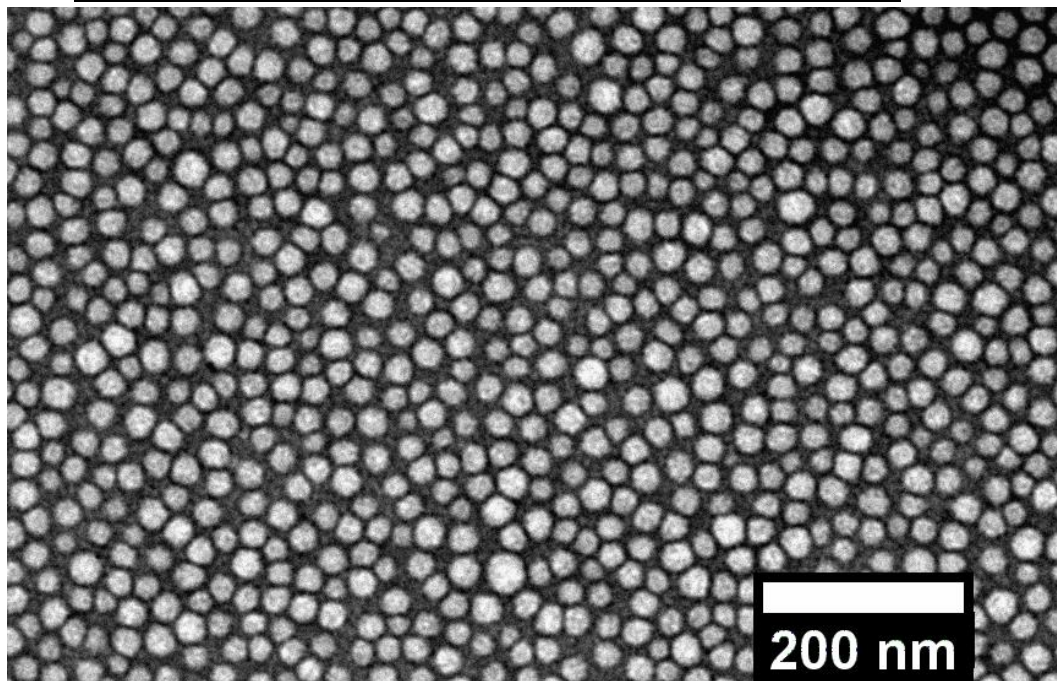


Figure S1 – Representative TEM image of micelles formed by solvent switch of PEO₄₅-*b*-PEHOx₆₅-*b*-PEtOz₁₉.

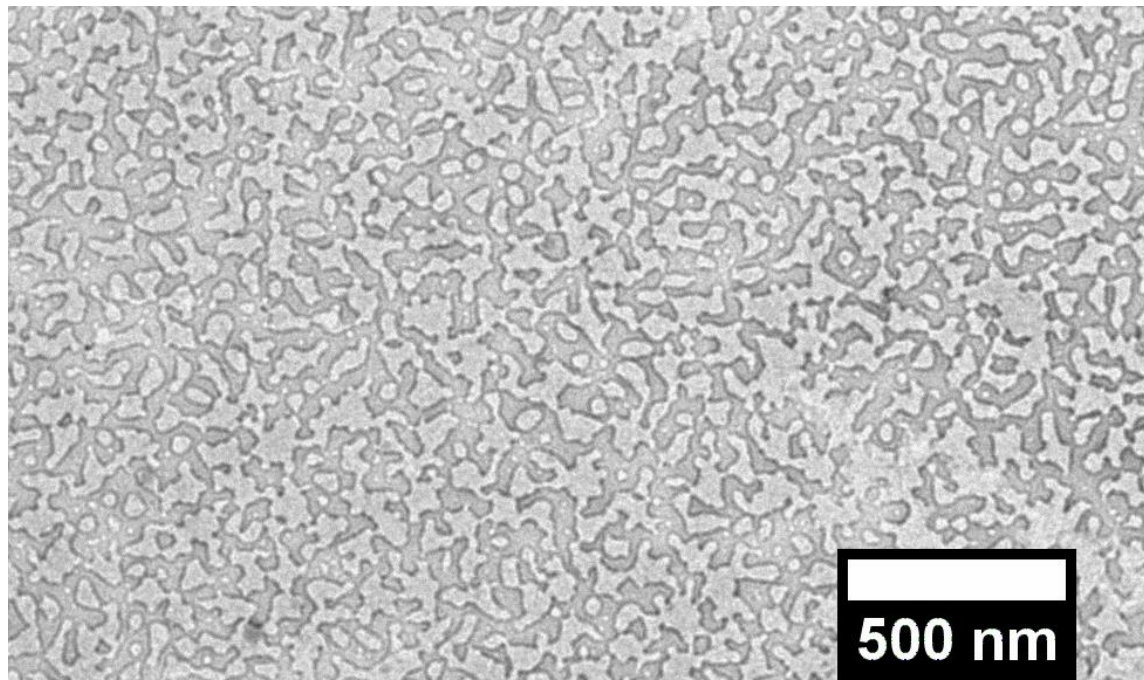


Figure S2 – Representative TEM image of polymer films observable after solvent switch of PEO₄₅-*b*-PEHOx₁₃₈-*b*-PEtOz₁₄.

b. Characterisation of film rehydration of PEO-*b*-PEHO_x-*b*-PEtOz

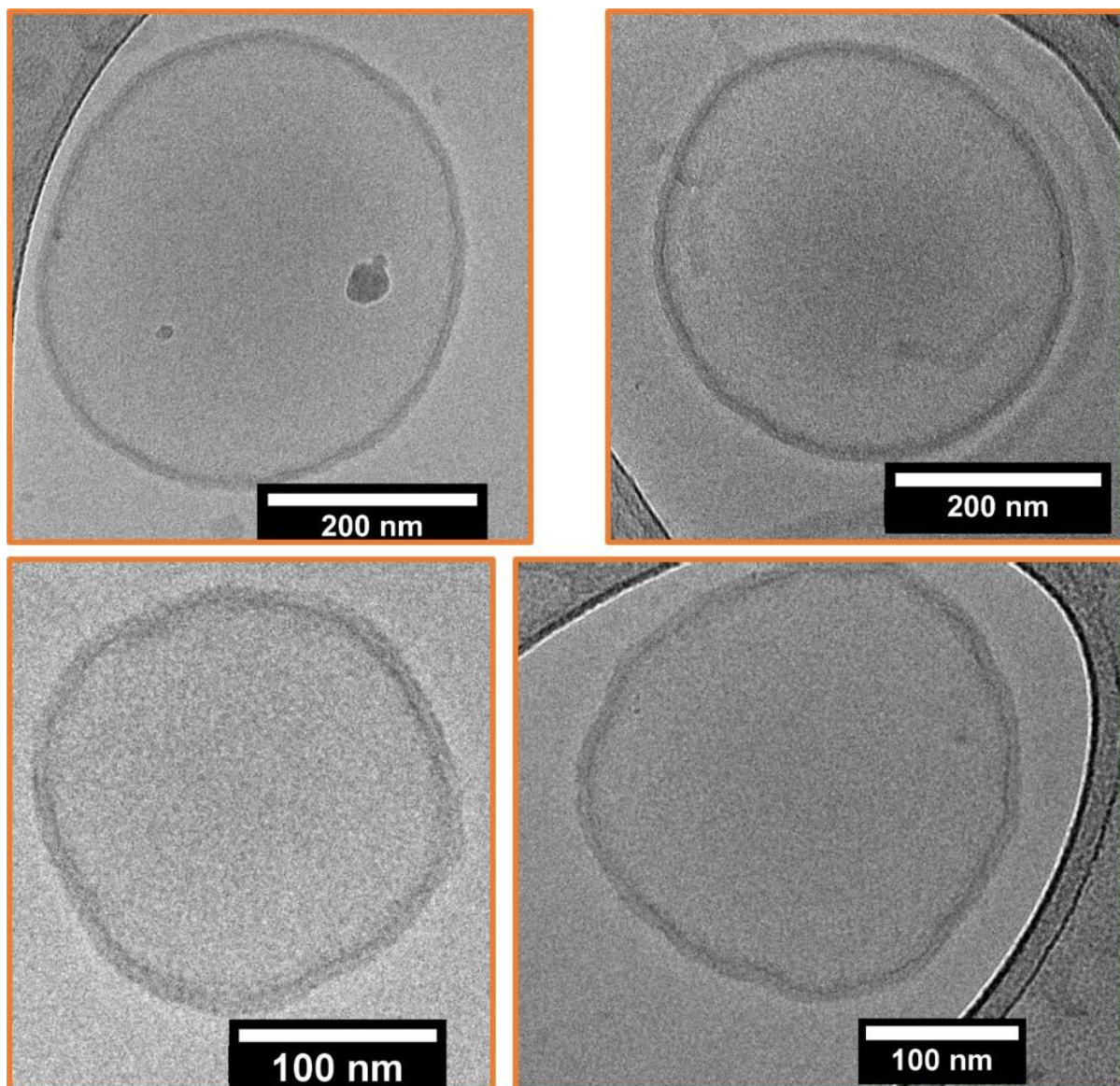


Figure S3 – Supplementary Cryo-TEM images of polymersomes formed by film rehydration of PEO₄₅-*b*-PEHO_{x48-65}-*b*-PEtOz₈₋₃₅.

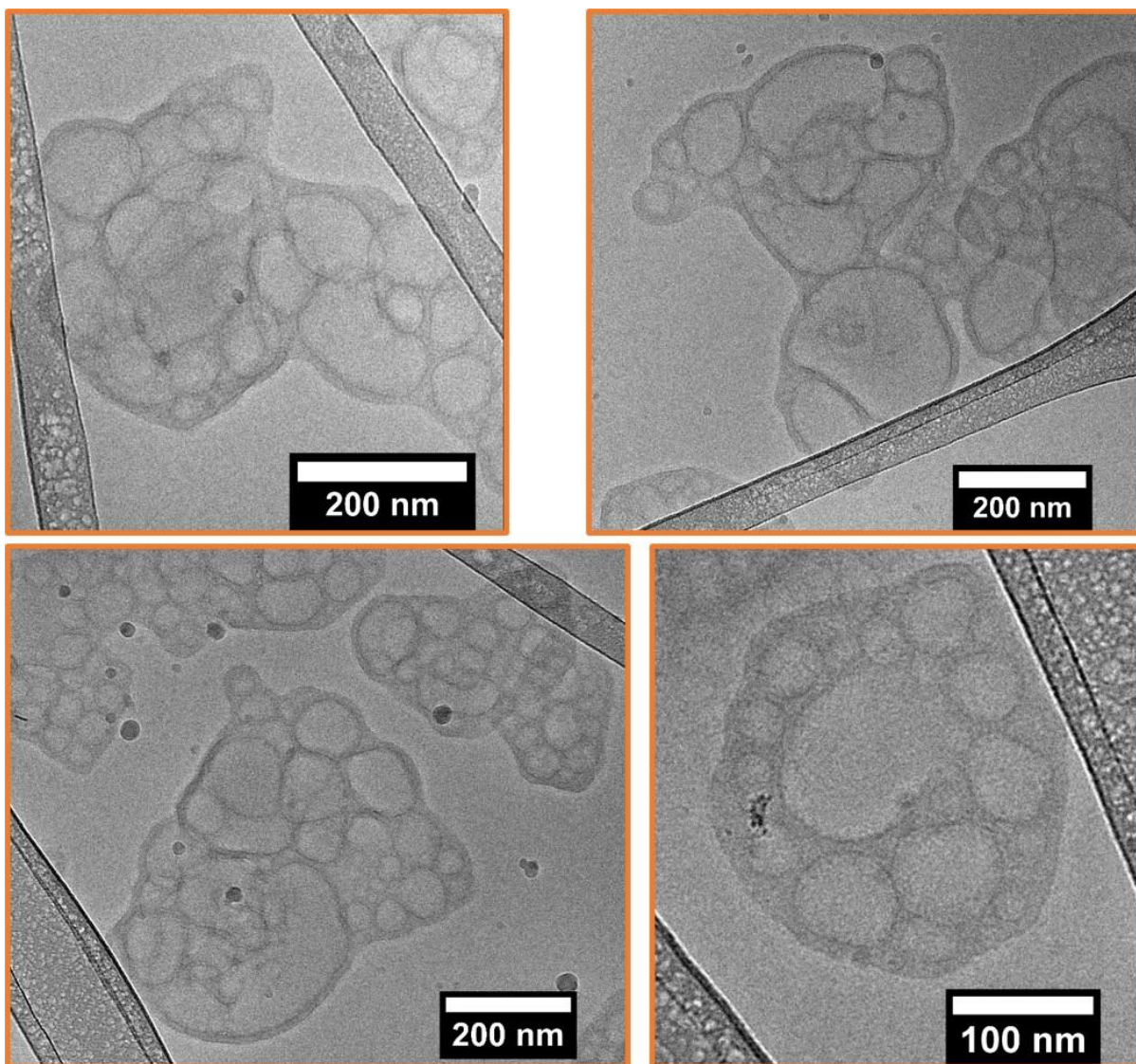


Figure S4 - Supplementary Cryo-TEM images of multicompartiment vesicles formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOx}_{87\text{-}96}\text{-}b\text{-PEtOz}_{10\text{-}11}$.

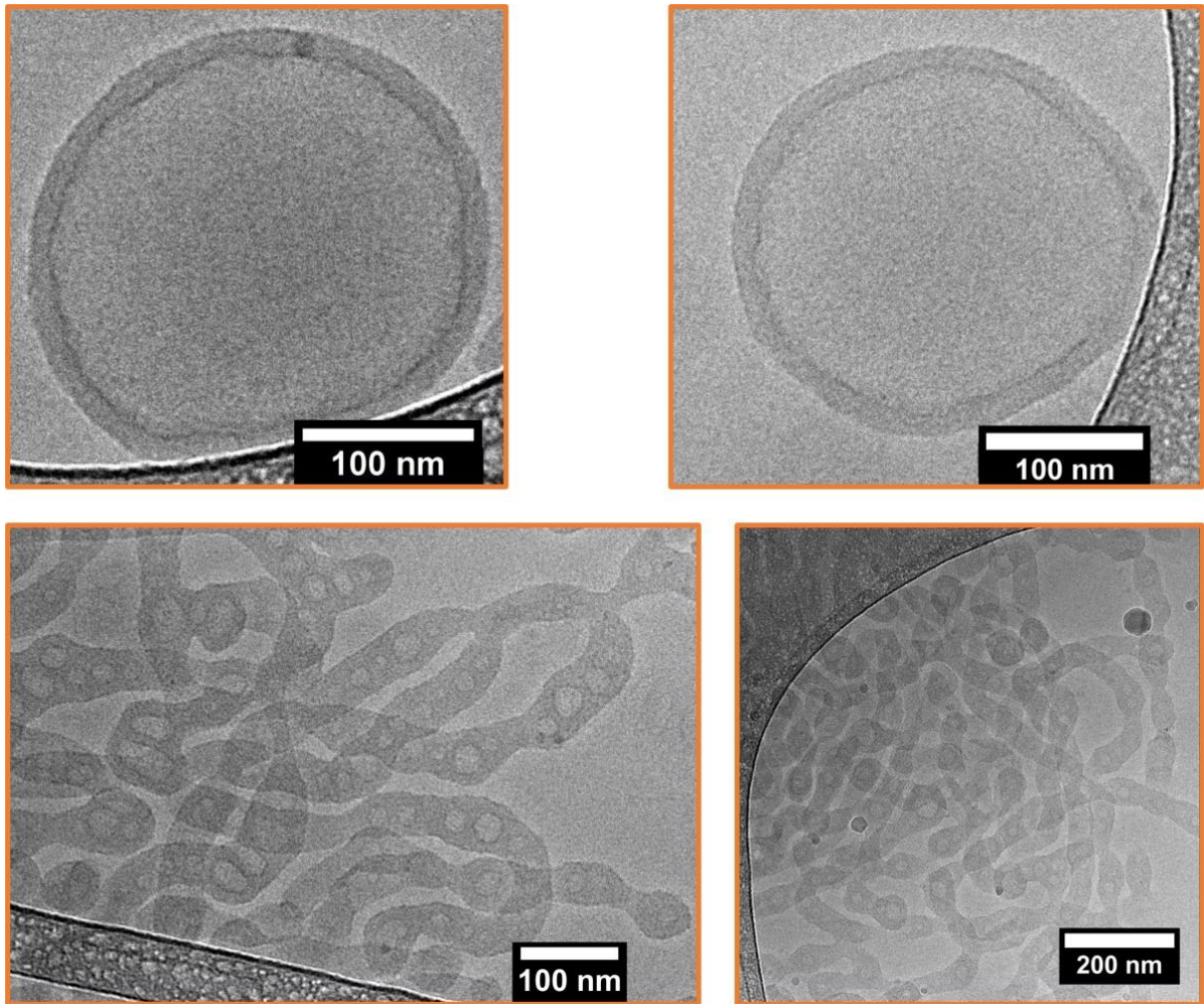


Figure S5 - Supplementary Cryo-TEM images of tubes and polymersomes formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOX}_{138\text{-}139}\text{-}b\text{-PEtOz}_{10\text{-}11}$.

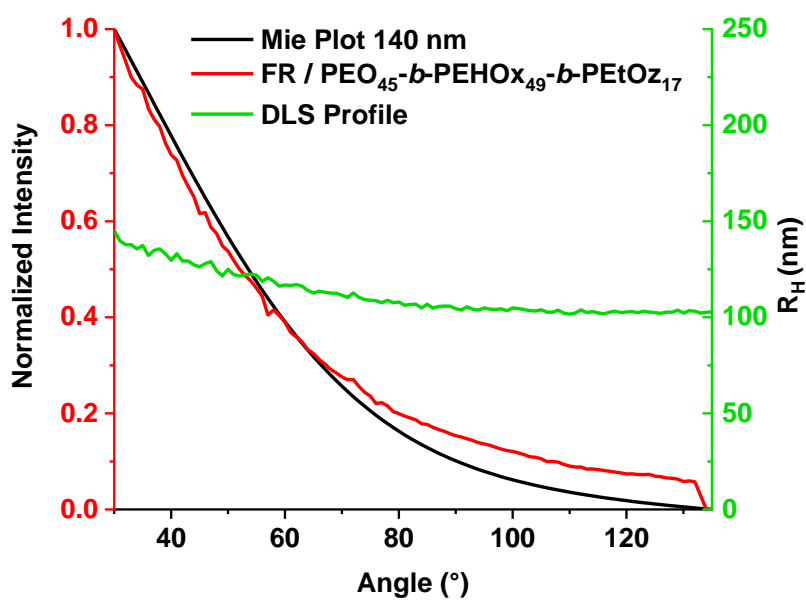


Figure S6 – Representative MIE plot and DLS profile of ABC triblock, here $\text{PEO}_{45}\text{-}b\text{-PEHOX}_{49}\text{-}b\text{-PEtOz}_{17}$. R_g of 108 nm was calculated from the MIE Plot fit at 140 nm.

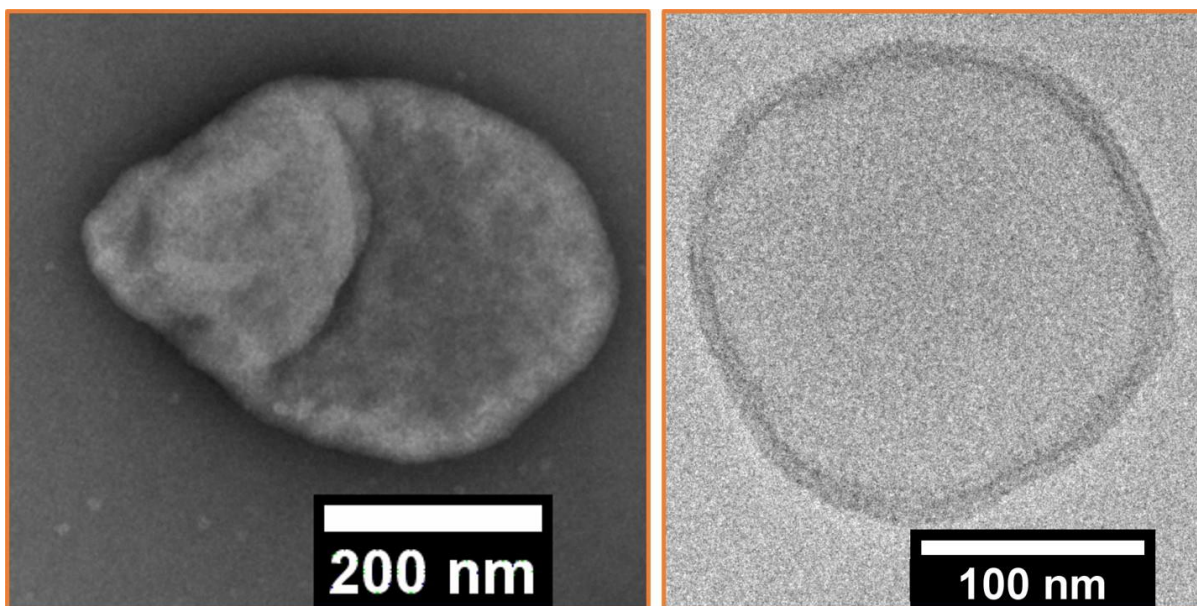


Figure S7- Representative TEM and Cryo-TEM images of polymersomes formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOX}_{48\text{-}65}\text{-}b\text{-PEtOz}_{8\text{-}35}$.

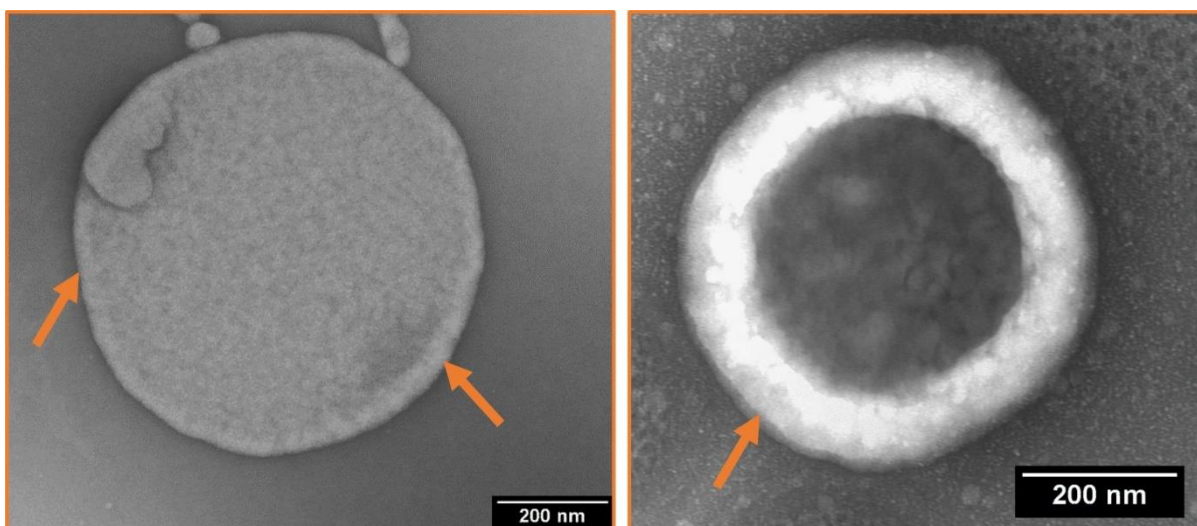


Figure S8 – Representative TEM images of polymersomes formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOX}_{48\text{-}65}\text{-}b\text{-PEtOz}_{8\text{-}35}$ (left) and $\text{PEO}_{45}\text{-}b\text{-PEHOX}_{138\text{-}139}\text{-}b\text{-PEtOz}_{10\text{-}14}$ (right). The orange arrows highlight the white halo of contrast that suggests the presence of a membrane.

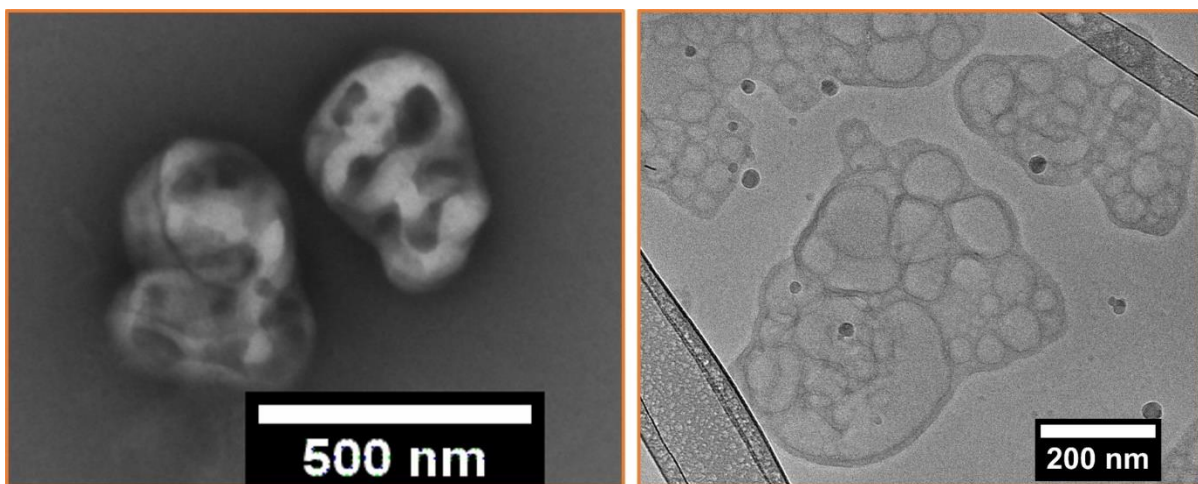


Figure S9- Representative TEM and Cryo-TEM images of multicompartiment vesicles formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOx}_{87\text{-}96}\text{-}b\text{-PEtOz}_{10\text{-}11}$.

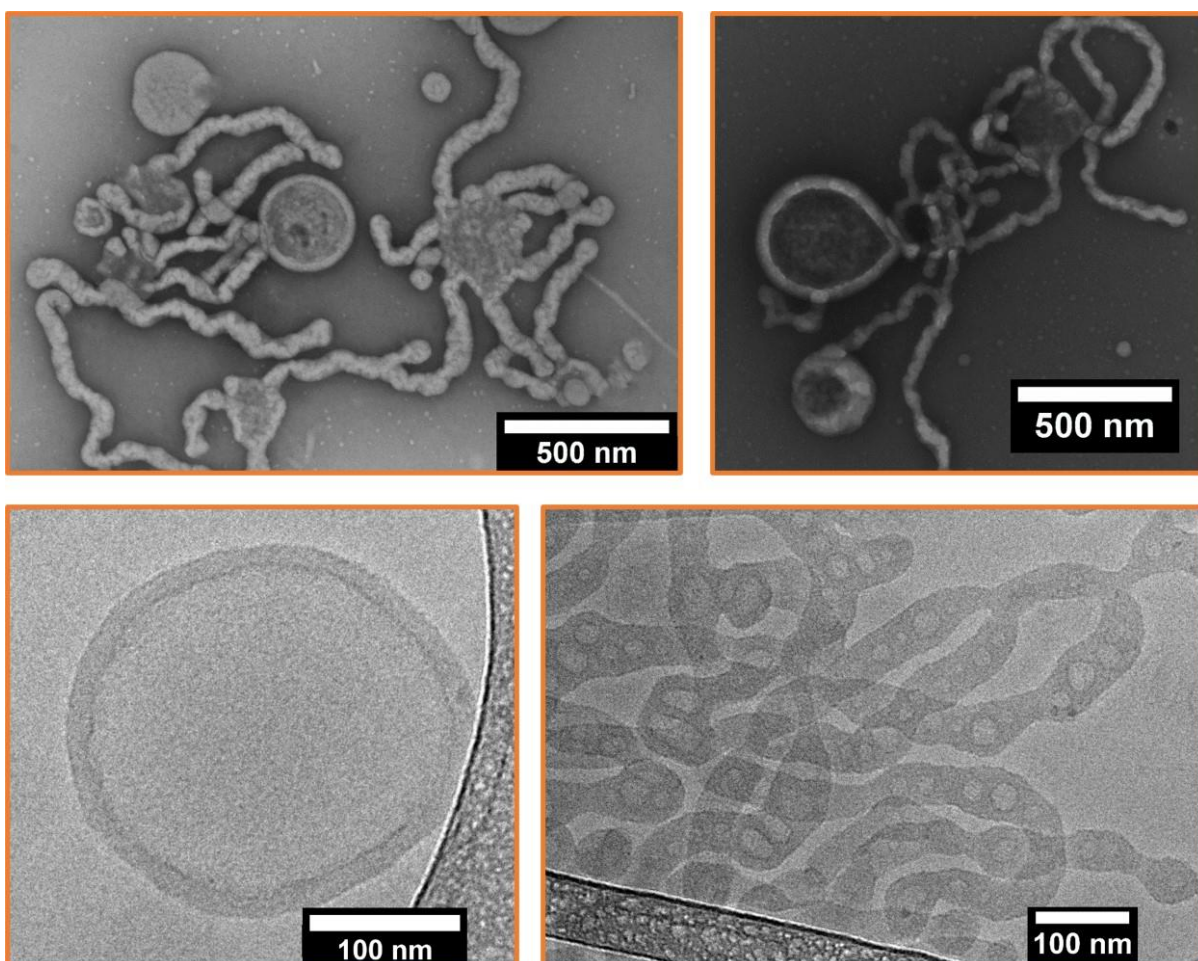


Figure S10- Representative TEM and Cryo-TEM images of tubes and vesicles formed by film rehydration of $\text{PEO}_{45}\text{-}b\text{-PEHOx}_{138\text{-}139}\text{-}b\text{-PEtOz}_{10\text{-}14}$.

c. Orientation of PEO-*b*-PEHOx-*b*-PEtOz chains in the membrane of polymersomes

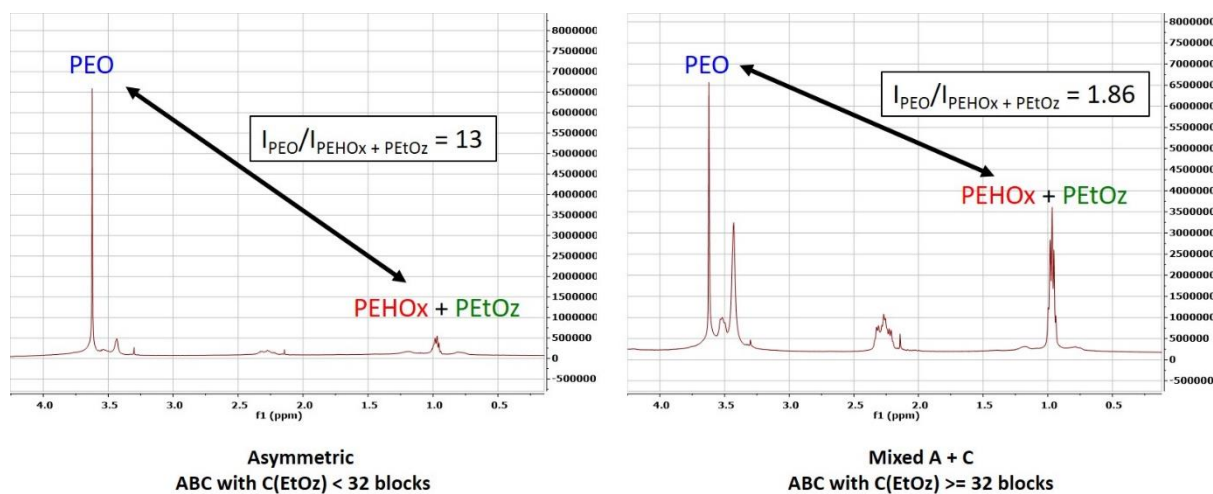


Figure S11 – Representative ^1H NMR Spectra in D_2O of polymersomes formed by film rehydration with ABC triblock with C(EtOz) inferior to 32 blocks, here $\text{PEO}_{45}\text{-}b\text{-PEHOx}_{54}\text{-}b\text{-PEtOz}_{24}$ (left) and with ABC triblock with C(EtOz) superior or equal to 32 blocks, here $\text{PEO}_{45}\text{-}b\text{-PEHOx}_{54}\text{-}b\text{-PEtOz}_{32}$ (right). The ratio of the intensity of the peak at 3.60 ppm of the backbone of PEO (3.60 ppm) and the intensity of the overlapping peaks at 0.96 ppm of the methyl group of the side chain of PEtOz (0.96 ppm) and the protons of the side chain of PEHOx (0.96 ppm) is calculated and compared.