

Electronic Supplementary Information

for

Complex domain architecture of multicompartment micelles from a linear ABC triblock copolymer revealed by cryogenic electron tomography

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Methods

Cryogenic transmission electron microscopy (cryo-TEM)

The samples for cryogenic transmission electron microscopy (cryo-TEM) were prepared at room temperature by placing a droplet (6 μ L) of the solution on a hydrophilized perforated carbon filmed Quantifoil grid (60 s Plasma treatment at 8 W using a BALTEC MED 020 device). The excess fluid was blotted off to create an ultra thin layer (typical thickness of 100 nm) of the solution spanning the holes of the carbon film. The grids were immediately vitrified in liquid ethane at its freezing point (-184°C) using a standard plunging device. Ultra-fast cooling is necessary for an artifact-free thermal fixation (vitrification) of the aqueous solution avoiding crystallization of the solvent or rearrangement of the assemblies. The vitrified samples were transferred under liquid nitrogen into a Philips CM12 transmission electron microscope using the Gatan cryoholder and -stage (Model 626). Microscopy was carried out at -175°C sample temperature using the microscopes low dose mode at a primary magnification of 58300 \times . Accelerating voltage was 100 kV and the defocus was chosen to be 1.5 μ m.

Cryogenic electron tomography (cryo-ET)

Sample preparation for tomography was performed in the same way as described for conventional cryo-TEM described above. The vitrified grids were transferred by the use of a Gatan tomography cryo-holder (Model CT3500) into a Tecnai F20 TEM equipped with a field emission gun operating at 160 kV. Images were recorded using a 2k-Eagle CCD camera at

full resolution (2048×2048 pixel). Tilt series were recorded under low-dose conditions collecting images between -22.5° and $+22.5^\circ$ (45° segment) in 2.5° tilt increments accumulating a total electron dose of $< 100 \text{ e}\text{\AA}^{-2}$. The primary magnification was 19k corresponding to a pixel size of 1.12 nm in the images. The defocus was chosen at a high value of 15μ in order to create sufficient phase contrast in the images of the relatively low-contrast aggregates. The three-dimensional reconstruction based on the tomographic series was determined by the use of the Inspect 3D software by employing the SIRT projection algorithm. All together five tomographic series were performed collecting the volume information of several tens of aggregates. Due to the limitations in the three-dimensional volume information caused by the missing information of higher tilt-angles (“missing wedge”) we performed all measurements in selected cross sectional slices of the reconstructed z-stack. For visualization of the volume data we used Amira version 4.1.0.

Materials

Chain transfer agent 2-(butylsulfanylthiocarbonyl)-2-methylpropionic acid was prepared by reaction of 1-butanethiol with 50 wt % aqueous NaOH, CS_2 , acetone and CHCl_3 under phase transfer catalysis with Aliquat 336, adapting a procedure from Lai et al.^{S1} The triblock copolymer was synthesized by three consecutive RAFT polymerizations at 65°C , using AIBN as initiator, and toluene, or α,α,α -trifluorotoluene in the last step, respectively, as solvent. Starting with 2-ethylhexylacrylate **EHA** and chain transfer agent 2-(butylsulfanylthiocarbonyl)-2-methylpropionic acid, the obtained polymer was engaged as macro RAFT agent in the polymerization of (oligo(ethyleneglycol)monomethylether) acrylate **OEGA** ($M_r = 454$). Subsequently, the resulting block copolymer was used as macro RAFT agent in the polymerization of *1H,1H,2H,2H*-tetrahydroperfluorodecyl acrylate **PFDA**. The molar ratio of RAFT agent to initiator was set as 10:1 in the first and second, and 5:1 in the last polymerization step. Yields were kept below 65% for all reactions. The polymers were purified by precipitation and dialysis. The nominal polydispersity indices of the homopolymer, the diblock and the triblock copolymer measured by size exclusion chromatography SEC were 1.27, 1.31 and 1.33. Analysis of the molar masses of the block copolymers by SEC analysis were not conclusive, as - apart from using polystyrene standards -, the block copolymer samples apparently adsorb partially to the column material and/or tend to form aggregates in the eluent. Though therefore the data obtained by SEC must be taken with care, the low PDI values suggest that the amount of contaminating homo- and diblock copolymers is low in the final triblock copolymer, in agreement with the use of high ratios RAFT

agent/initiator, the limited monomer conversions, and the elaborate purification procedures applied.

Table S1: Molecular characterization of the ternary block copolymer and its precursors

polymer ^a	SEC ^b			NMR ^c	UV ^d
	$M_n \times 10^{-3}$ [g/mol]	$M_w \times 10^{-3}$ [g/mol]	PDI	$M_n \times 10^{-3}$ [g/mol]	$M_n \times 10^{-3}$ [g/mol]
poly(EHA)₁₂₀	15	19	1.27	-	22
poly(EHA)₁₂₀-b-(OEGA)₅₀	13	17	1.31	45	47
poly(EHA)₁₂₀-b-(OEGA)₅₀-b-(PFDA)₄₀	14	19	1.33	66	73

^a DP_n according to combined UV and ¹H NMR spectroscopy
^b eluent: THF at 25°C, molar mass and PDI according to calibration by polystyrene
^c M_n of di- and triblock copolymer determined by ¹H NMR spectroscopy from averaged compositional data assuming that M_n(UV) of the first block is preserved
^d calculated by UV spectroscopic end-group analysis, based on the thiocarbonyl absorption band at λ_{max} = 307 nm, using ε = 14,400 L • mol⁻¹ • cm⁻¹, as determined by 2-(butylsulfanylthiocarbonyl)-propionic acid butyl ester as low molar mass reference compound

Additional cryo-TEM micrographs

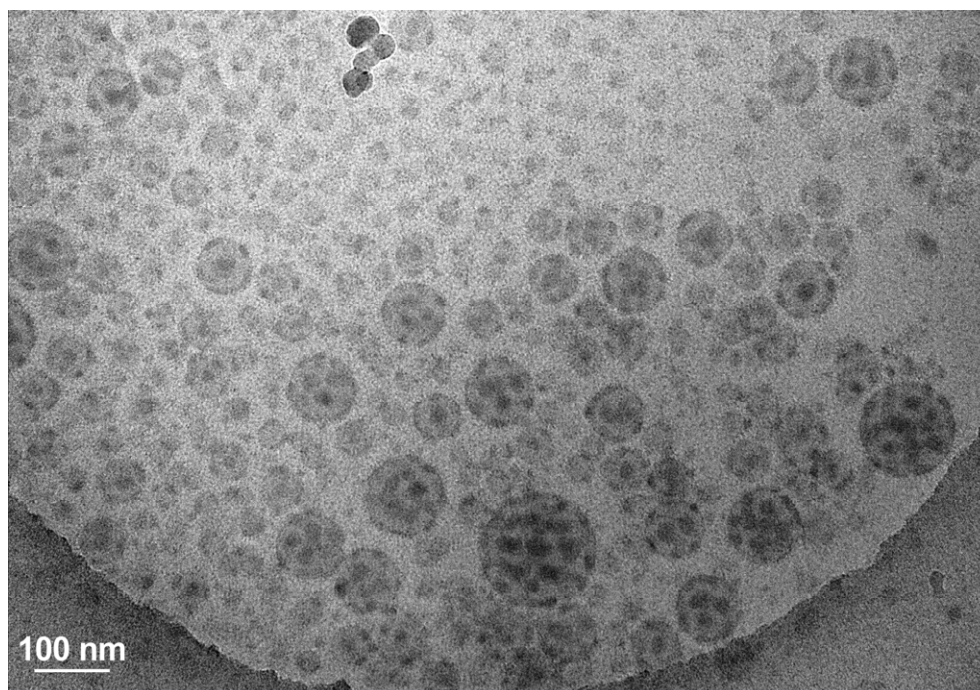


Figure S1: Cryo-TEM image of a 0.5 wt% aqueous solution of triblock copolymer (EHA)₁₂₀-(OEGA)₅₀-(FDA)₄₀ dispersed at 25 °C.

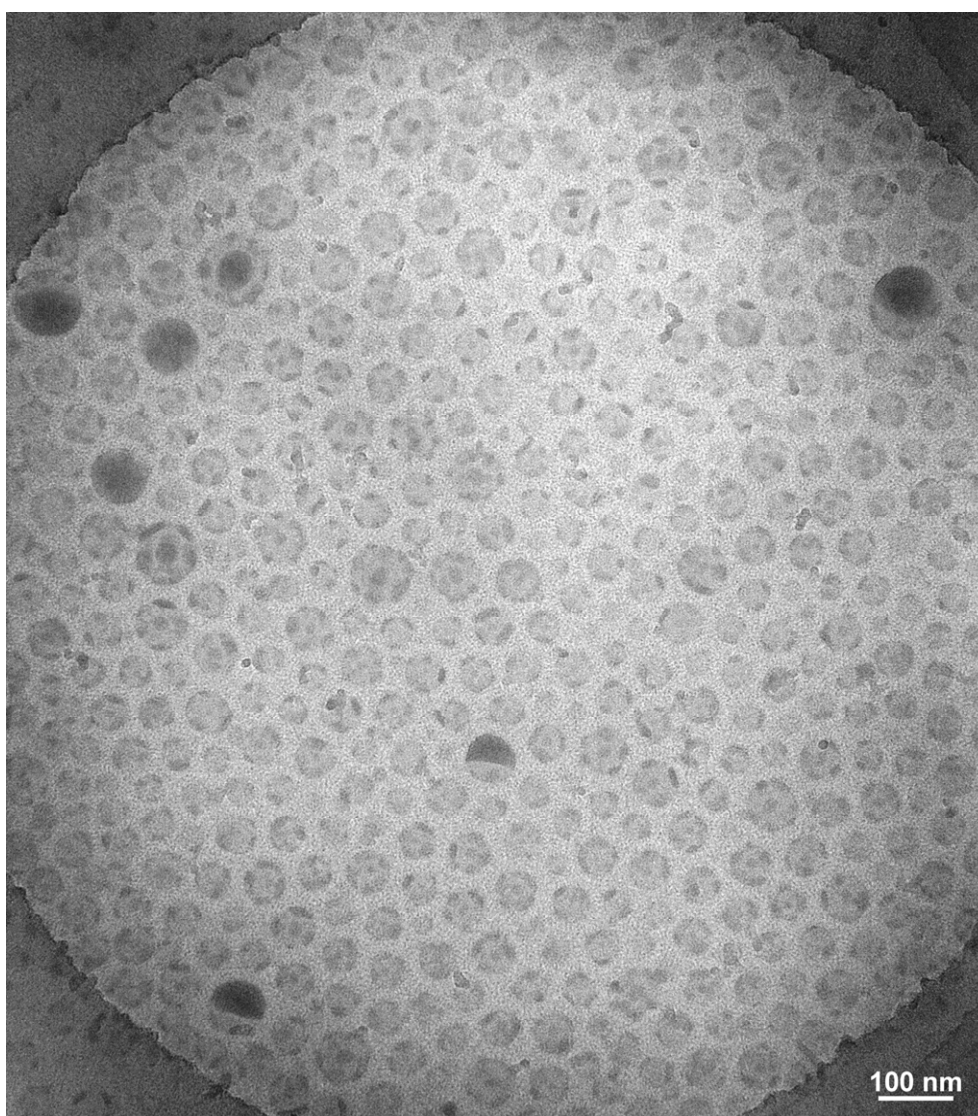


Figure S2: Cryo-TEM image of a 0.5 wt% aqueous solution of triblock copolymer (EHA)₁₂₀-(OEGA)₅₀-(FDA)₄₀ dispersed at 70 °C.

References:

S1) J. T. Lai, D. Filla and R. Shea, *Macromolecules* 2002, **35**, 6754.