CHEMISTRY
A European Journal

Supporting Information

In Situ Visualization of Block Copolymer Self-Assembly in Organic Media by Super-Resolution Fluorescence Microscopy

Charlotte E. Boott,[a] Romain F. Laine,[b] Pierre Mahou,[b] John R. Finnegan,[a] Erin M. Leitao,[a] Stephen E. D. Webb,*[c] Clemens F. Kaminski,*[b] and Ian Manners*[a]

chem_201504100_sm_miscellaneous_information.pdf

Supporting Information

General Experimental Considerations

Anionic polymerizations were carried out in an argon atmosphere glovebox. All other manipulations were carried out under an open atmosphere unless otherwise stated. All reagents were purchased from Sigma-Aldrich unless otherwise stated. Monomer purifications were performed under an atmosphere of purified N_2. THF was distilled from Na/benzophenone immediately before use. The dyes STAR635 NHS ester, CAGE552 NHS ester and CAGE635 NHS ester were purchased from Abberior GmbH. Photoirradiation experiments were carried out with Pyrex-glass filtered emission from a 125 W medium-pressure mercury lamp (Photochemical Reactors Ltd.). An ethylene glycol/water bath in conjunction with a thermostat was used to maintain constant temperatures of 20 °C during the photoirradiation experiments. ^{1}H and ^{13}C NMR spectra were recorded using Jeol Eclipse 400 MHz or Varian VNMR 400 MHz spectrometers.

Polymer Characterization

Gel permeation chromatography was carried out on PFS_{56}-b-$PDMS_{775}$/$PMVS_{20}$ and an aliquot of PFS_{56} using a Viscotek VE 2001 Triple-Detector Gel Permeation Chromatograph equipped with an automatic sampler, a pump, an injector, an inline degasser, and a column oven (30 °C). The elution columns consisted of styrene/divinylbenzene gels with pore sizes between 500 Å and 100,000 Å. Detection was conducted by means of a VE 3580 refractometer, a four-capillary differential viscometer, and 90° and low angle (7°) laser light (λ_0= 670 nm) scattering detectors, VE 3210 & VE 270. THF (Fisher) was used as the eluent, with a flow rate of 1.0 mL/min. Samples were dissolved in the eluent (2 mg/mL) and filtered with a Ministart SRP 15 filter (polytetrafluoroethylene membrane of 0.45 μm pore size) before analysis. The calibration was conducted using a PolyCAL™ polystyrene standard (PS115K) from Viscotek. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry measurements were performed using a Bruker Ultraflextreme running in linear mode. Samples were prepared using a trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile matrix (20 mg/mL in THF) and the polymer sample (2 mg/mL in THF), mixed in a 10:1 (v/v) ratio. Approximately 1 μL of the mixed solution was deposited onto a MALDI sample plate and allowed to

dry in air. The molecular weights of the diblock copolymers were then determined by combining the molecular weight M_n of the first block from MALDI-TOF measurements with the block ratio of the diblock copolymer obtained by integrating the ^{1}H NMR spectroscopic signal intensities of the respective blocks.

Transmission electron microscopy (TEM)

Copper grids from Agar Scientific, mesh 400, were coated with a carbon film. Carbon coating was done using an Agar TEM Turbo Carbon Coater where carbon was sputtered onto mica sheets before deposition on the copper grids via flotation on water. Bright field TEM micrographs were obtained on a JEOL1200EX II microscope operating at 120 kV and equipped with an SIS MegaViewIII digital camera.

Stimulated emission depletion (STED) microscopy

STED imaging was performed on a home-built pulsed STED microscope described in detail in the following reference.[1] Briefly, the excitation and STED beam are obtained from a single titanium-sapphire oscillator centered at λ_{STED} = 765 nm (Ti:S, Mai Tai HP, Spectraphysics). The excitation beam, centered at λ_{Exc} = 640 nm, was extracted from a supercontinuum source (FemtoWhite, NKT Photonics) by a bandpass filter (637/7 BrightLine HC, Semrock) and coupled into a polarization maintaining single-mode fiber (PM630-HP, Thorlabs). The pulse duration of the STED beam was stretched to approximately 100-200 ps thanks to a 50 cm glass block of SF66 (IC Optical Systems, United Kingdom) and 100 m long polarization maintaining single-mode fiber (PM-S630-HP, Thorlabs). Additionally the STED beam was shaped into a donut beam by a spatial light modulator (X10468−02, Hamamatsu). The excitation and STED beam were recombined with a dichroic mirror (T735spxr, Chroma) and sent to a commercial point-scanning microscope (Abberior Instruments) comprising: a microscope frame (IX83, Olympus), a set of galvanometer mirrors (Quad scanner, Abberior Instruments) and a detection unit.

The beams were focused onto the sample by a 100x/1.4 NA oil immersion objective lens (UPLSAPO 100XO, Olympus) and images were acquired by raster scanning the beams across the sample using the Imspector Image Acquisition software (Andreas Schönle, Abberior Instruments GmbH, Göttingen, Germany). Typically for the dyes STAR635 and CAGE635, a field of view of 80×80 μm^2 was used with a pixel size of 20×20 nm^2, a pixel dwell times of 30 µs and 50 µs respectively and a time gated detection of 1.5-9 ns for the dye STAR635 only. Moreover, for the dye CAGE635, uncaging was performed directly by two-photon excitation from the STED beam and each line was scanned successively five times with a pixel dwell time of 10 µs per line-scanning. This resulted in a single scanning mode where dyes were successively uncaged and excited during each line scan. Fluorescence photons emerging from the sample were collected by the microscope objective lens, de-scanned by the galvanometer mirrors, focused onto a pinhole and sent to an avalanche photodiode (SPCM-AQRH, Excelitas Technologies). Laser powers, measured at the objective back aperture, were ~20-30 µW for the excitation beam and ~100-150 mW for the STED beam.

Single molecule localization microscopy (SMLM)

Imaging was performed with a 100x/1.49 NA objective on an inverted TIRF microscopes (Nikon TE-300) custom-built for SMLM acquisition, as previously described,[2–4] using highly inclined illumination.[5] The microscope was equipped with an iXon3 897 EM-CCD camera (Andor, UK). The following laser and filter combinations were used: 640 nm diode laser (iBeam Smart, Toptica, Germany) and filter 676/37 (Semrock, USA) for CAGE635, 561 nm laser (DPSS laser, Oxxius, France) and 607/70 (Semrock, USA) for CAGE552, 491 nm (DPSS laser, Cobolt, Sweden) and 530/55 (Semrock, USA) for CAGE500, with radiation intensities of 1-5 kW/cm². A UV laser diode (405 nm) was used to control the rate of uncaging. For each SMLM acquisition, 10,000-20,000 frames (256x256 pixels, corresponding to ~41x41 µm field of view) were acquired at a frame rate of ~65 Hz (15 ms exposure). For the two-colour SMLM acquisitions, CAGE635 images were acquired first, followed by CAGE500.

All SMLM dataset were analyzed using rapidSTORM 3.3,[6] and images with a final pixel size of 20 nm were generated. The wide-field fluorescence images were obtained from summing all frames of the stack. Further analysis was performed using the ImageJ software package developed at the US National Institute of Health. The continuous background of the wide-field fluorescence images was removed by applying a rolling-ball algorithm.[7] Noise was removed from SMLM images by applying a despeckling noise filter.

Sample preparation for TEM

The samples for electron microscopy were prepared by drop casting one drop (*ca.* 10 µL) of the micelle colloidal solution onto a carbon coated copper grid.

Sample preparations for STED and SMLM

Samples were prepared by placing ca. 8 µL of diluted micelle solution on a glass slide and a cover slip was placed on top of solution and sealed in place with nail polish. For STED and SMLM dilution ranging from 0.01-0.05 mg/mL were used for imaging, with the addition of 5% isopropanol for the CAGE dyes.

Manual tracing of the micelle images

Micelle contour lengths were estimated from the TEM, STED and SMLM images manually using the ImageJ software package developed at the US National Institute of Health. For the statistical length analyses, 55 to 300 objects were processed to determine the contour length depending on the data set. Each TEM/STED/SMLM image was analyzed completely, *i.e.* every micelle in each image was counted in order to reduce subjectivity. From this data, histogram were constructed and values of the mean contour length L, weighted contour length L_w, standard deviation σ and polydispersity index (*PDI*) were estimated using the following equations where N is the sample size:

$$(1) \qquad L_n = \frac{\sum_{i=1}^{n} N_i L_i}{\sum_{i=1}^{n} N_i} \qquad (2) \qquad L_w = \frac{\sum_{i=1}^{n} N_i L_i^2}{\sum_{i=1}^{n} N_i L_i}$$

$$(3) \qquad \sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{n}(x_i - \mu)^2} \qquad (4) \qquad PDI = \frac{L_w}{L_n}$$

Automated tracing of the micelle images

Micelle contour lengths were estimated from the TEM, STED and SMLM images automatically by using a tracing algorithm previously described in the following reference.[8] The method comprises three distinctive steps (Figure S1): (i) segmentation and clustering of the micelles (ii) removing of the overlapping micelles based and the segmented area and (iii) tracing of the micelles based on a stepping algorithm using the segmented and intensity images. For step (i), we used a plugin from ImageJ (Find Connected Regions, from Mark Longair) and for the steps (ii) and (iii), we used custom written MATLAB routines (Mathworks). To construct histograms of the contour length 150 to 450 objects were processed depending on the data set (See Table S1 and S2). From these data, we determined the mean contour length, the weighted average contour length, the standard deviation and the polydispersity index using equations (1-4).

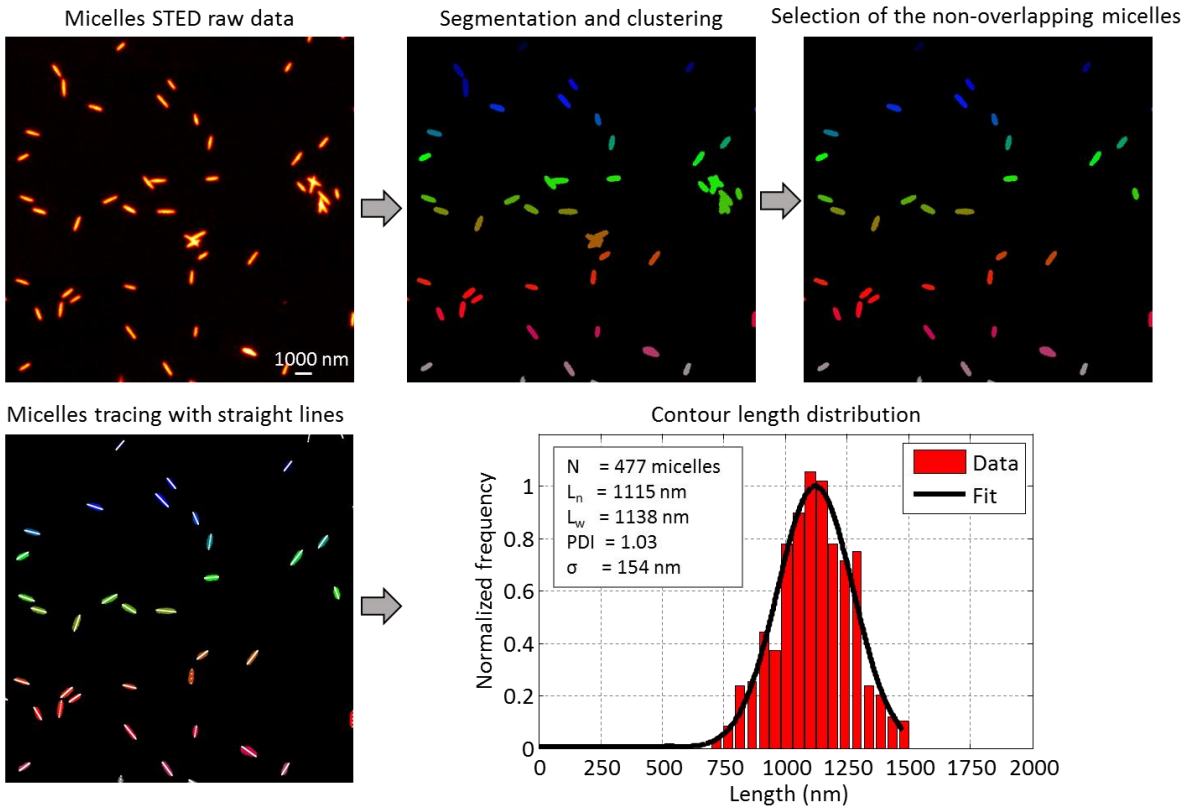


Figure S1: Automated tracing of micelles labelled with STAR635 and imaged by STED. This automated method can also be used for SMLM and TEM images equivalently. See Table S1 and S2 for an overview of the extracted parameters.

Comparison between manual and automatic tracing

Table S1 and Table S2 show the comparison of the micelle tracing between the manual and automatic method from STED and SMLM images respectively. We observe an excellent agreement between the two methods, therefore validating the automatic tracing as an efficient method for length measurement which allows much faster analysis of our data than the manual method.

Table S1: Analysis of the contour length distributions of the micelles labelled with STAR635 and imaged by STED and TEM. Parameters characterizing the distribution were extracted using either manual or automated tracing.

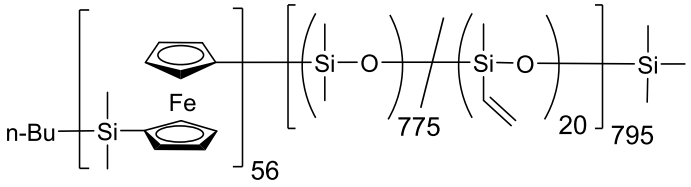
	TEM		STED	
	Manual	Automatic	Manual	Automatic
L_n (nm)	1121	1121	1153	1115
L_w (nm)	1146	1145	1172	1138
PDI	1.02	1.02	1.02	1.03
N	310	318	312	477
σ (nm)	167	163	148	154

Table S2: Analysis of the contour length distributions of the micelles labelled with CAGE552 and imaged by SMLM and TEM. Parameters characterizing the distribution were extracted based on hand and automated tracing.

	TEM		SMLM	
	Manual	Automatic	Manual	Automatic
L_n (nm)	1752	1779	1724	1706
L_w (nm)	1784	1820	1742	1750
PDI	1.02	1.02	1.01	1.03
N	235	218	206	174
σ (nm)	236	271	175	273

Polymer Synthesis

Polyferrocenyldimethylsilane$_{56}$-*b*-[(polydimethylsiloxane)$_{775}$/(polymethylvinylsiloxane$_{20}$), (PFS$_{56}$-*b*-(PDMS$_{775}$/PMVS$_{20}$))



In a glove box under an argon atmosphere 1.6 M *n*-butyllithium (24 µL, 0.04 mmol) was added in one portion to a vigorously stirring solution of dimethylsila[1]ferrocenophane (484 mg, 1.65 mmol) in dry THF (10 mL) in a greaseless Schlenk flask. The reaction mixture was stirred for 1 h over which time the colour changed from red to orange. An aliquot (0.2 mL) for later analysis was removed, diluted with THF (1 mL) and quenched with 3,5-di-*tert*-butyl-4-hydroxytoluene. To the remaining reaction mixture, a solution of 1,3,3,5,5-pentamethyl-1-vinylcyclotrisiloxane (169 mg, 0.72 mmol) and 1,1,3,3,5,5-hexamethylcyclotrisiloxane (1.62 g, 7.28 mmol) in dry THF (2 mL) was added in one portion. After 2 h the flask was removed from the glove box, set up under a nitrogen atmosphere and the reaction quenched with a few drops of chlorotrimethylsilane. The product was precipitated once in methanol with 10% triethylamine and twice more in methanol. To remove the homopolymer the crude product was dissolved in a minimum amount of THF and ethyl acetate was added with stirring until the solution became cloudy. The suspension was centrifuged at 6000 rpm for 15 minutes and the supernatant concentrated *in vacuo*. This process was repeated twice more, then the resulting solid was dried *in vacuo* at 40 °C to afford pure block copolymer (1.34 g, 59%, PDI = 1.09) as an orange solid. (See Table S1).

^{1}H NMR (400 MHz; CD_2Cl_2): δ_H 6.07-5.90 (m, 40H, CHC*H*$_2$), 5.86-5.77 (m, 20H, SiC*H*CH$_2$), 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.03 (t, J = 1.7 Hz, 224H, Cp*H*), 0.48 (s, 336H, FcSi(C*H*$_3$)$_2$), 0.15 (s, 60H, Si(C*H*$_3$), 0.08 (s, 4650H, Si(CH$_3$)$_2$) ppm.

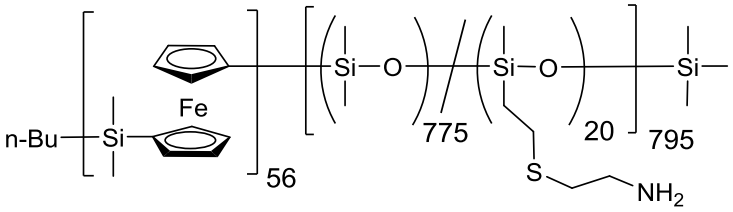
^{13}C NMR (101 MHz; CD_2Cl_2): δ_C 137.7 (CH*C*H$_2$), 133.3 (*C*HCH$_2$), 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H$_3$)$_2$), -0.3 (Si(*C*H$_3$)CHCH$_2$) -0.8 (Si*C*H$_3$)$_2$ ppm.

Table S3: Polymer Characterisation

Polymer	DP_n[a)]	M_n ($gmol^{-1}$)	PDI[b)]
PFS Aliquot	56	13611[a)]	1.05
$PFDMS_{56}$-b-($PDMS_{775}$/$PMVS_{20}$)	-	74300	1.09

[a)]Determined by MALDI-TOF; [b)]Determined by GPC with multi-detector (aliquot) or conventional calibration (block copolymer) using polystyrene standards; [c)]Determined by ^{1}H NMR spectroscopy integration of the vinyl protons (3H) of PMVS and the methyl protons (6H) of PFS and PDMS.

Hydrothiolation of PFS_{56}-*b*-($PDMS_{775}$/$PMVS_{20}$) with 2-aminoethanethiol



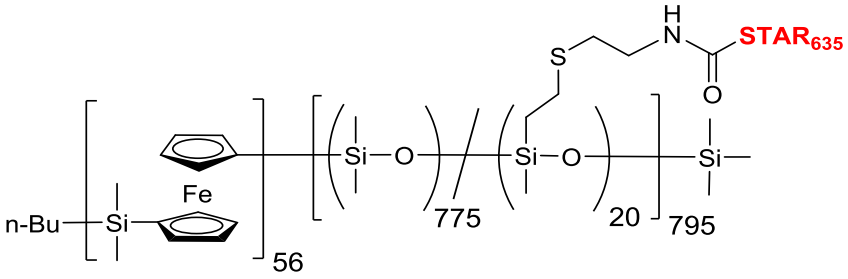
To a solution of PFS_{56}-*b*-($PDMS_{775}$/$PMVS_{20}$) (75 mg, 1.0 x10^{-3} mmol, 0.021 mmol vinyl groups) in dry THF (2 mL) was added 2-mercaptoethylamine hydrochloride (24 mg, 0.21 mmol, 10 equiv.) and the photo-initiator 2,2-dimethoxy-2-phenylacetophenone (5 mg, 0.02 mmol). The orange solution was sealed under an argon atmosphere and irradiated 3 cm away from a mercury lamp for 4 h. The mixture was then precipitated once in methanol with 10% triethylamine and twice more in methanol, then dried *in vacuo* to afford pure block copolymer (76 mg, 98%). ^{1}H NMR analysis showed > 95% functionalization of the siloxane vinyl groups with 2-aminoethanethiol.

^{1}H NMR (400 MHz; CD_2Cl_2): δ_H 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.02 (t, J = 1.7 Hz, 224H, Cp*H*), 2.81 (t, J = 6.4 Hz, 40H, C$H_2$$NH_2$), 2.68-2.53 (m, 80H, C$H_2SCH_2$), 0.93-0.83 (m, 40H, SiC$H_2$$CH_2$S), 0.48 (s, 336H, FcSi(CH_3)$_2$), 0.18- -0.02 (m, 4710H, Si(CH_3) and Si(CH_3)$_2$) ppm.

^{13}C NMR (101 MHz; CD_2Cl_2): δ_C (100 MHz; CD_2Cl_2) 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H_3)$_2$), -0.8 (SiCH_3)$_2$ ppm.

This polymer interacts with the stationary phase in the above-described GPC equipment, and was used without further characterization.

Synthesis of PFS$_{56}$-*b*-(PDMS$_{775}$/STAR635$_{20}$)



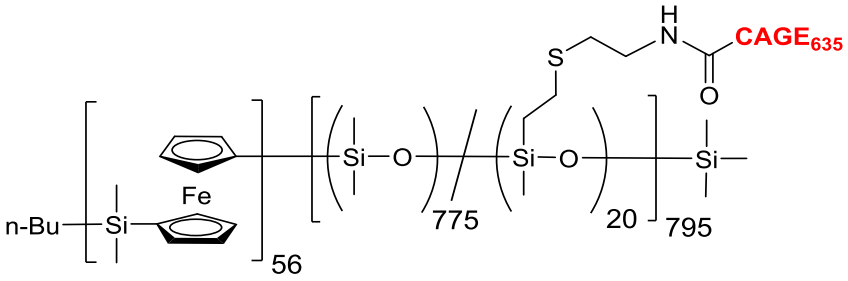
To a 7 mL screw-cap vial with stir bar was added amine-functionalized PFS$_{56}$-*b*-(PDMS$_{775}$/PMVS$_{20}$) (30 mg, 4.1 x 10^{-4} mmol, 8.1 x 10^{-3} mmol amine groups), STAR635 succinimidyl ester (STAR635, 15 mg, 0.016 mmol, 2 equiv.) and 4 mL dry THF. The reaction was stirred under an argon atmosphere at room temperature for 72 h, then precipitated five times into methanol and dried *in vacuo* to afford the pure block copolymer (30 mg, 81%, PDI = 1.16). ^{1}H NMR analysis showed quantitative functionalization of the amine functional groups with STAR635 dye.

^{1}H NMR (400 MHz; CD_2Cl_2, ***mixture of two amide rotamers*****):** δ_H 7.13 (s, 10H, C*H*), 6.96 (s, 20H, C*H*), 6.84 (s, 10H, C*H*), 5.86-5.65 (m, 40H, 2×C*H*), 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.03 (t, J = 1.7 Hz, 224H, Cp*H*), 3.85-3.40 (m, 240H, 6×CH_2), 3.04-2.93 (m, 120H, 3×CH_2) 2.72-2.47 (m, 150H, CH_2SCH_2CH_2NH and NCH_3), 2.37-1.95 (m, 120H, *C*H_2CO, 2×*C*H_2), 1.73-1.44 (m, 320H, 4×CH_3, 2×CH_2), 0.96-0.80 (m, 40H, SiCH_2CH$_2$S), 0.48 (s, 336H, FcSi(CH_3)$_2$), 0.18--0.01 (m, 4710H, Si(CH_3) and Si(CH_3)$_2$) ppm.

^{13}C NMR (101 MHz; CD_2Cl_2): δ_C (100 MHz; CD_2Cl_2) 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H$_3$)$_2$), -0.8 (SiCH$_3$)$_2$ ppm.

NOTE: Structures of the dyes STAR635, CAGE635, CAGE552 and CAGE500 are currently not shown due to confidentiality.

Synthesis of PFS$_{56}$-*b*-(PDMS$_{775}$/CAGE635$_{20}$)

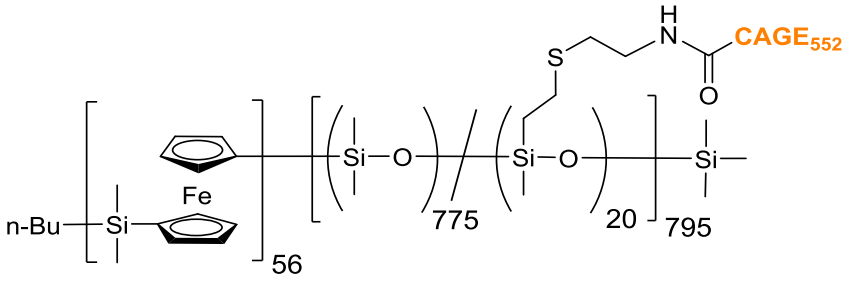


To a 7 mL screw-cap vial with stir bar was added amine-functionalized PFS$_{56}$-*b*-(PDMS$_{775}$/PMVS$_{20}$) (35 mg, 4.7 x 10^{-4} mmol, 9.4 x 10^{-3} mmol amine groups), CAGE635 succinimidyl ester (CAGE635, 20 mg, 0.018 mmol, 2 equiv.) and 4 mL dry THF. The reaction was stirred under an argon atmosphere at room temperature for 72 h, then precipitated five times into methanol and dried *in vacuo* to afford the pure block copolymer (14 mg, 33%, PDI = 1.14). ^{1}H NMR analysis showed quantitative functionalization of the amine functional groups with CAGE635 dye.

^{1}H NMR (500 MHz; CD_2Cl_2): δ_H 7.78 (d, J = 8.0 Hz, 20H, Ar*H*), 7.52-7.34 (m, 40H, 2Ar*H*), 6.85 (d, J = 8.0 Hz, 20H, Ar*H*), 6.77-6.69 (m, 40H, 2Ar*H*), 6.35 (d, J = 8.0 Hz, 40H, 2Ar*H*), 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.03 (t, J = 1.7 Hz, 224H, Cp*H*), 3.83-3.19 (m, 420H, 9×CH_2 and OCH_3), 2.72-2.26 (m, 180H, CH_2SCH_2CH_2NH and 3×CH_2), 1.81 (s, 120H, 2×CH_3), 0.96-0.80 (m, 40H, SiCH_2CH_2S), 0.48 (s, 336H, FcSi(CH_3)$_2$), 0.18--0.01 (m, 4710H, Si(CH_3) and Si(CH_3)$_2$) ppm.

^{13}C NMR (101 MHz; CD_2Cl_2): δ_C (100 MHz; CD_2Cl_2) 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H$_3$)$_2$), -0.8 (SiCH$_3$)$_2$ ppm.

Synthesis of PFS$_{56}$-*b*-(PDMS$_{775}$/CAGE552$_{20}$)

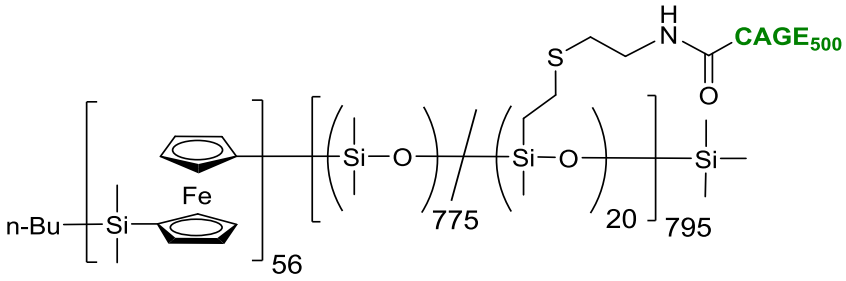


To a 7 mL screw-cap vial with stir bar was added amine-functionalized PFS$_{56}$-*b*-(PDMS$_{775}$/PMVS$_{20}$) (50 mg, 6.7 x 10^{-4} mmol, 1.3 x 10^{-3} mmol amine groups), CAGE552 succinimidyl ester (CAGE552, 15 mg, 0.027 mmol, 2 equiv.) and 4 mL dry THF. The reaction was stirred under an argon atmosphere at room temperature for 72 h, then precipitated five times into methanol and dried *in vacuo* to afford pure block copolymer (54 mg, 96%, PDI = 1.12). ^{1}H NMR analysis showed quantitative functionalization of the amine functional groups with CAGE552 dye.

^{1}H NMR (400 MHz; CD_2Cl_2, ***mixture of two isomers*****):** δ_H 8.11 (app. s, 10H, Ar*H*), 7.95 (app. s, 10H, Ar*H*), 7.08 (br s, 40H, 2Ar*H*), 6.84-6.36 (m, 120H, 6Ar*H*), 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.02 (t, J = 1.7 Hz, 224H, Cp*H*), 2.96 (s, 240H, 4NCH_3), 2.84-2.52 (m, 120H, CH_2SCH_2CH_2NH), 0.95-0.78 (m, 40H, SiCH_2CH$_2$S), 0.48 (s, 336H, FcSi(CH_3)$_2$), 0.17- -0.01 (m, 4710H, Si(CH_3) and Si(CH_3)$_2$) ppm.

^{13}C NMR (101 MHz; CD_2Cl_2): δ_C (100 MHz; CD_2Cl_2) 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H$_3$)$_2$), -0.8 (SiCH$_3$)$_2$ ppm.

Synthesis of PFS$_{56}$-*b*-(PDMS$_{775}$/CAGE500$_{20}$)



To a 7 mL screw-cap vial with stir bar was added amine-functionalized PFS$_{56}$-*b*-(PDMS$_{775}$/PMVS$_{20}$) (42 mg, 5.7 x 10^{-4} mmol, 1.1 x 10^{-2} mmol amine groups), CAGE500 succinimidyl ester (CAGE500, 15 mg, 0.023 mmol, 2 equiv.) and 4 mL dry THF. The reaction was stirred under an argon atmosphere at room temperature for 72 h, then precipitated five times into methanol and dried *in vacuo* to afford pure block copolymer (36 mg, 75%, PDI = 1.14). ^{1}H NMR analysis showed quantitative functionalization of the amine functional groups with CAGE500 dye.

^{1}H NMR (400 MHz; CD_2Cl_2, *mixture of two isomers*): δ_H 7.89-7.72 (m, 40H, 2Ar*H*), 7.43 (s, 20H, Ar*H*), 6.77-6.19 (m, 120H, 6Ar*H*), 4.23 (t, J = 1.7 Hz, 224H, Cp*H*), 4.02 (t, J = 1.7 Hz, 224H, Cp*H*), 3.78 (s, 80H, 2CH_2CF$_3$), 2.73-2.45 (m, 120H, CH_2SCH_2CH_2NH), 0.91-0.78 (m, 40H, SiCH_2CH$_2$S), 0.48 (s, 336H, FcSi(CH_3)$_2$), 0.18- -0.02 (m, 4710H, Si(CH_3) and Si(CH_3)$_2$) ppm.

^{13}C NMR (101 MHz; CD_2Cl_2): δ_C (100 MHz; CD_2Cl_2) 73.7 (Cp*C*), 72.2 (Cp*C*Si), 71.8 (Cp*C*), 1.3 (OSi(*C*H$_3$)$_2$), -0.8 (SiCH$_3$)$_2$ ppm.

Preparation of Crystallite Seed Micelles in EtOAc:

A sample of long PFS_{63}-*b*-$PDMS_{513}$ cylinders (> 10 μm) were prepared by heating a 1 mg/mL solution in EtOAc for 1 h at 75 °C, then allowing the sample to cool to room temperature and stand for 7 days. PFS_{63}-*b*-$PDMS_{513}$ crystallite seeds were then prepared by sonication of 4 mL of this solution for 4 h at 0 ˚C using a 50 W sonication processor equipped with a titanium sonotrode at 50% power. A stock solution (0.1 mg/mL, used in subsequent experiments) was prepared by diluting 1 mL of seeds in 9 mL of EtOAc.

L_n = 34 nm; L_w = 39 nm; PDI = 1.15; N = 253, σ = 13.1 nm.

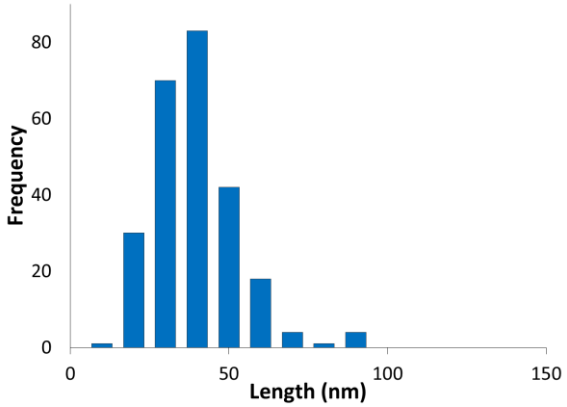
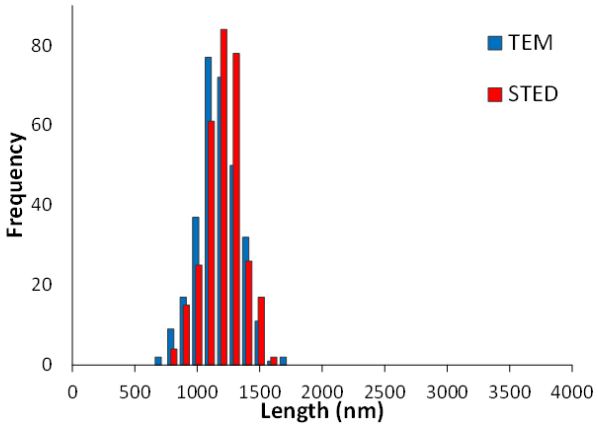


Figure S2: Analysis of PFS_{63}-*b*-$PDMS_{513}$ crystallite seed micelles based on hand-tracing. Histograms of micelle length (L_n) determined by TEM.

Preparation of STAR635 Labelled Cylindrical Micelles Analyzed by STED/TEM:

To a 7 mL screw-cap vial was added 1.8 mL EtOAc, 200 μL of a 0.1 mg/mL solution of seed micelles in EtOAc. To this mixture was then added 48 μL of PFS_{56}-*b*-$PDMS_{775}$/$STAR635_{20}$ stock unimer solution (10 mg/mL in THF) with shaking and the sample was left to grow 3 weeks.

a)



b)

	STED	TEM
L_n (nm)	1153	1121
L_w (nm)	1172	1146
PDI	1.02	1.02
N	312	310
σ (nm)	148	167

Figure S3: Analysis of STAR635 labelled micelles based on hand-tracing. a) Histogram of micelle length (L_n) determined by STED and TEM b) Table comparing micelle length data from STED and TEM.

Preparation of CAGE635 Labelled Cylindrical Micelles Analyzed by STED/TEM and SMLM /TEM:

To a 7 mL screw-cap vial was added 0.9 mL hexane and 100 µL of a 0.1 mg/mL solution of seed micelles in EtOAc. To this mixture was then added 12.8 µL of PFS_{56}-*b*-$PDMS_{775}$/$CAGE635_{20}$ stock unimer solution (10 mg/mL in THF) with shaking and the sample was aged for 3 weeks.

Preparation of CAGE552 Labelled Cylindrical Micelles Analyzed by SMLM /TEM:

To a 7 mL screw-cap vial was added 0.9 mL hexane and 100 µL of a 0.1 mg/mL solution of seed micelles in EtOAc. To this mixture was then added 11.4 µL of PFS_{56}-*b*-$PDMS_{775}$/$CAGE552_{20}$ stock unimer solution (10 mg/mL in THF) with shaking and the sample was aged for 3 weeks.

Preparation of CAGE500 Labelled Cylindrical Micelles Analyzed by SMLM/TEM:

To a 7 mL screw-cap vial was added 0.9 mL hexane and 100 µL of a 0.1 mg/mL solution of seed micelles in EtOAc. To this mixture was then added 7.8 µL of PFS_{56}-*b*-$PDMS_{775}$/$CAGE500_{20}$ stock unimer solution (10 mg/mL in THF) with shaking and the sample was aged for 3 weeks.

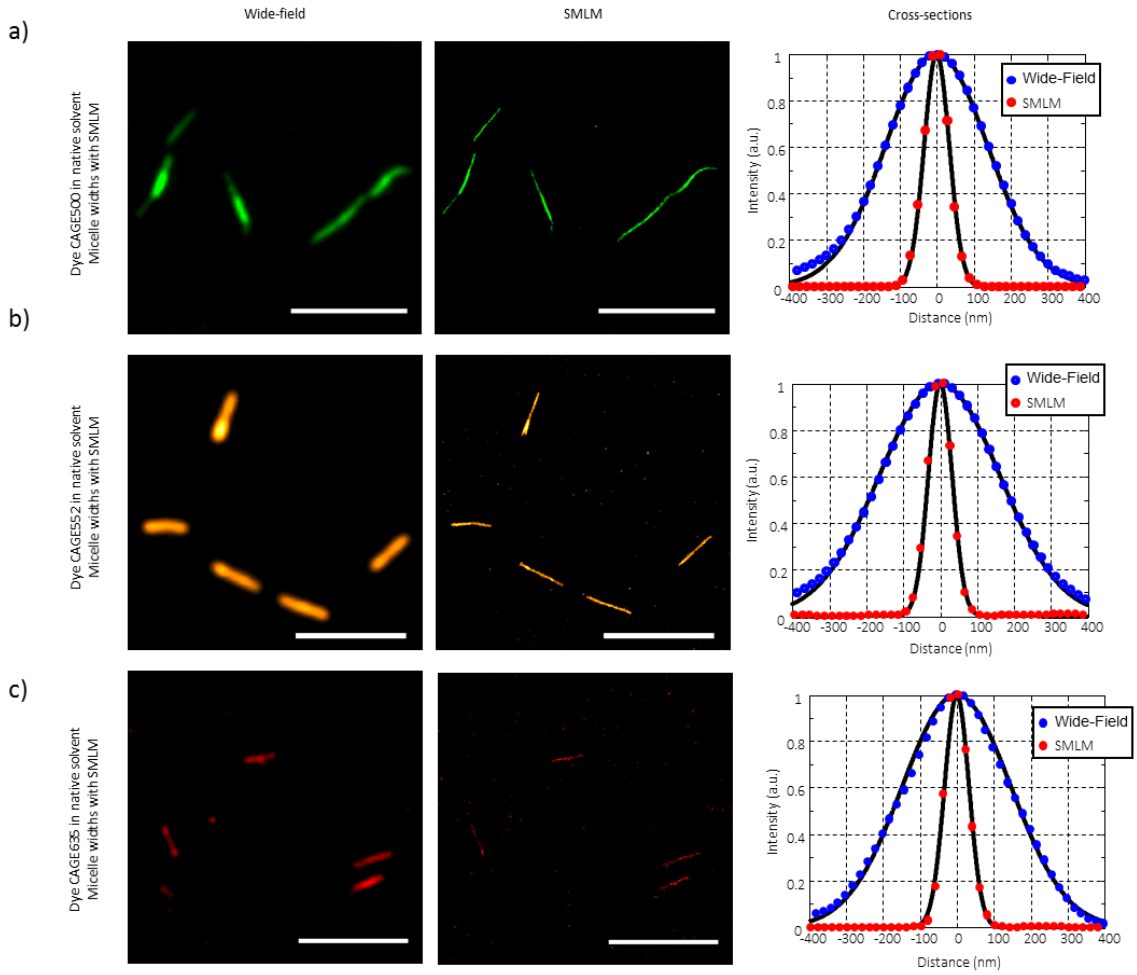
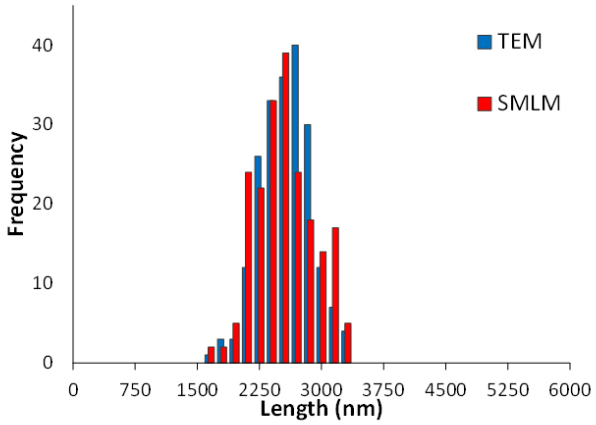


Figure S4: Wide field and SMLM images. a) CAGE500 labelled cylindrical micelles. FWHM: Wide-field: 326 nm and SMLM: 80 nm. b) CAGE552 labelled cylindrical micelles. FWHM: Wide-field: 383 nm and SMLM: 76 nm. c) CAGE635 labelled cylindrical micelles. FWHM: Wide-field: 347 nm and SMLM: 78 nm. Scale bars = 5000 nm.

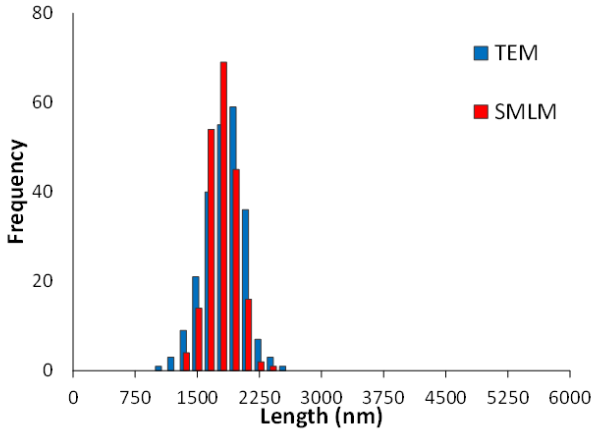
a)



b)

CAGE500	SMLM	TEM
L_n (nm)	2476	2500
L_w (nm)	2526	2538
PDI	1.02	1.02
N	217	207
σ (nm)	354	308

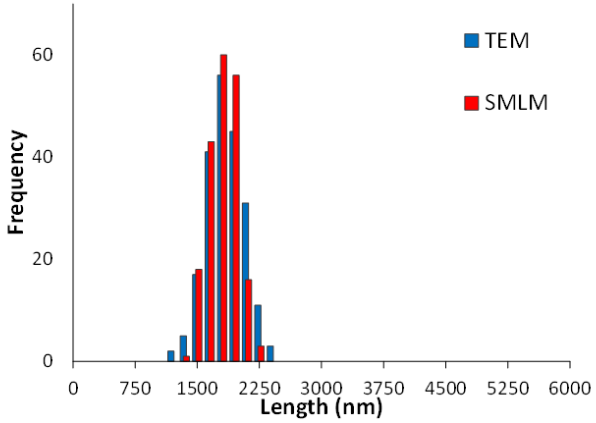
c)



d)

CAGE552	SMLM	TEM
L_n (nm)	1724	1752
L_w (nm)	1742	1784
PDI	1.01	1.02
N	206	235
σ (nm)	175	236

e)



f)

CAGE635	SMLM	TEM
L_n (nm)	1733	1765
L_w (nm)	1751	1792
PDI	1.01	1.02
N	197	212
σ (nm)	178	218

Figure S5: Analysis of CAGE labelled micelles based on hand-tracing. Histograms of micelle length (L_n) determined by SMLM and TEM a) CAGE500, c) CAGE552, e) CAGE635 Table comparing micelle length data from SMLM and TEM. b) CAGE500, d) CAGE552, f) CAGE635.

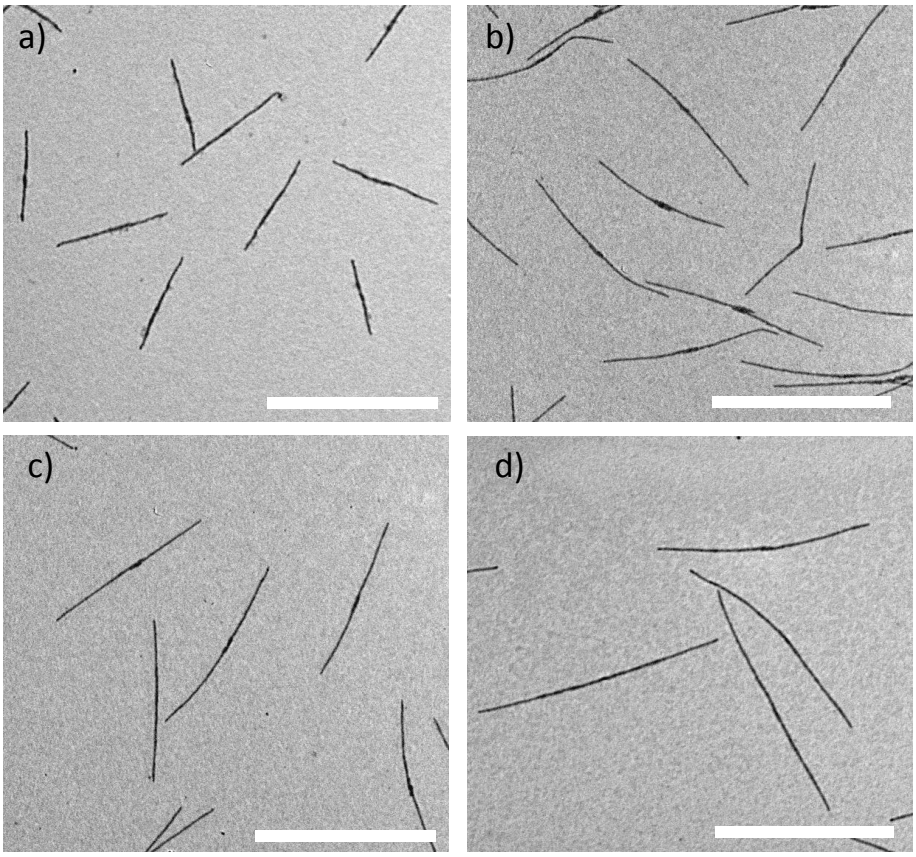
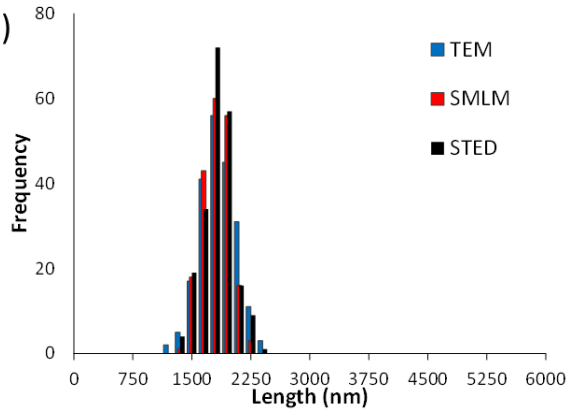


Figure S6: Representative TEM images of cylindrical micelles labelled with the dye a) STAR635, b) CAGE635, c) CAGE552, d) CAGE500. Scale bars = 2000 nm.

Comparison of CAGE635 Labelled Cylindrical Micelles Analyzed by STED/SMLM/TEM

a)



b)

CAGE635	SMLM	TEM	STED
L_n (nm)	1733	1765	1745
L_w (nm)	1751	1792	1773
PDI	1.01	1.02	1.02
N	197	212	213
σ (nm)	178	218	222

Figure S7: Analysis of CAGE635 labelled micelles based on hand-tracing. a) Histogram of micelle length (L_n) determined by STED, SMLM and TEM b) Table comparing micelle length data from STED, SMLM and TEM.

Preparation of RGR Triblock Co-micelles Analyzed by SMLM /TEM:

To a 1.75 mL screw-cap vial was added 200 μL of CAGE500 micelles prepared as above. To this mixture was then added 7.7 μL of PFS_{56}-*b*-$PDMS_{775}$/$CAGE635_{20}$ stock unimer solution (2.5 mg/mL in THF) with shaking and the sample was aged for 3 weeks.

Table S4: Micelle length data of RGR triblock co-micelles obtained by hand-tracing using SMLM and TEM

	Total Length		**Green Segment**		**Red Segments (SMLM)**	
	SMLM	TEM	SMLM	TEM	SHORT	LONG
L_n (nm)	3892	3858	2359	2500	708	766
L_w (nm)	3925	3899	2395	2538	724	782
PDI	1.01	1.01	1.02	1.02	1.02	1.02
N	66	130	87	207	55	55
σ (nm)	361	400	290	308	107	108

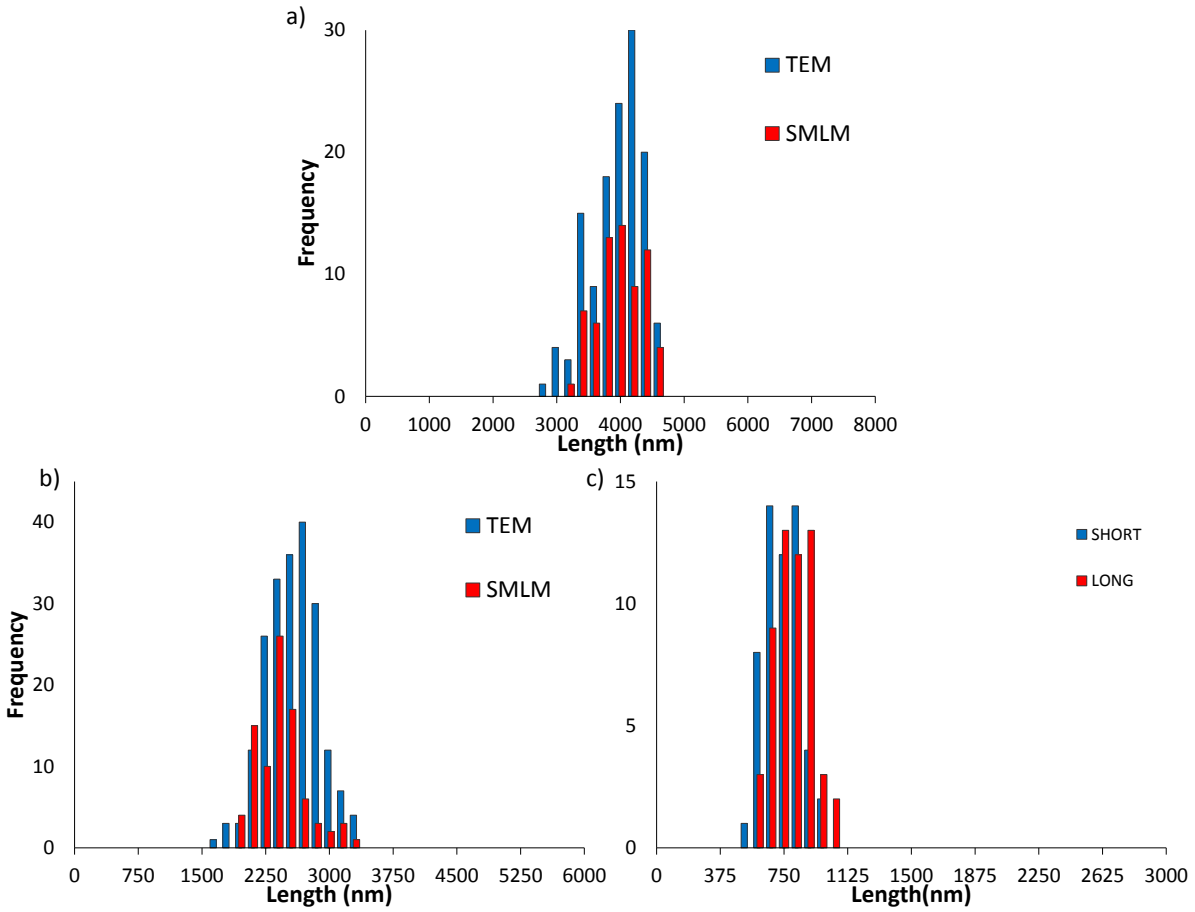


Figure S8: Histogram of lengths of RGR triblock co-micelles determined by hand-tracing of the SMLM and TEM images. a) Full micelle length, b) length of green segment, c) lengths of the two red segments.

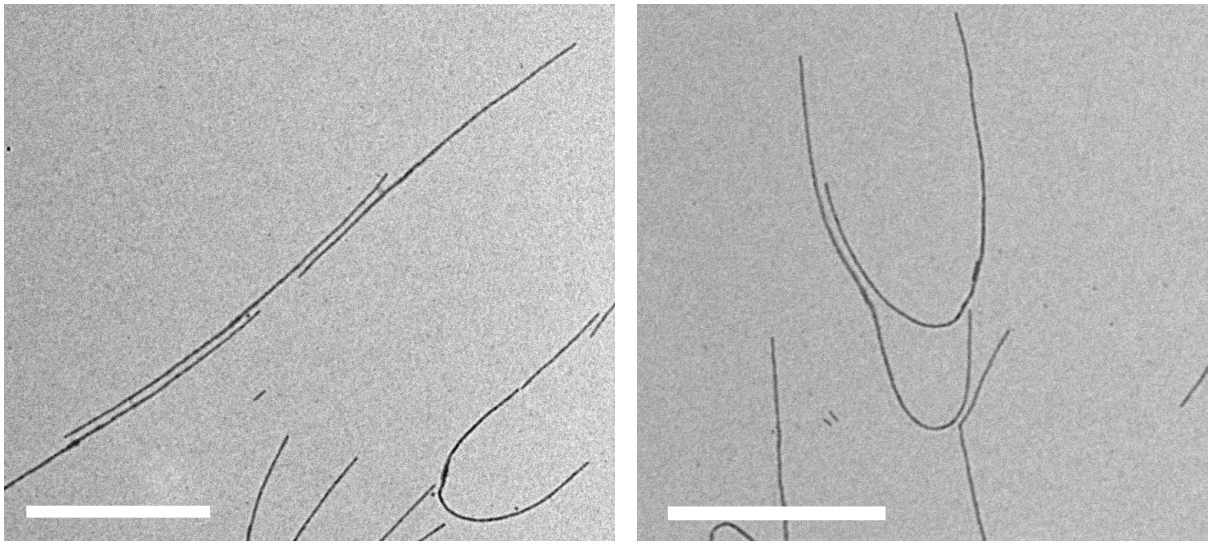


Figure S9: Representative TEM images of RGR triblock cylindrical micelles.

References

(1) Mahou, P.; Curry, N.; Pinotsi, D.; Kaminski Schierle, G.; Kaminski, C. In *SPIE BiOS*; 2015; p 93310U.

(2) Erdelyi, M.; Rees, E.; Metcalf, D.; Schierle, G. S. K.; Dudas, L.; Sinko, J.; Knight, A. E.; Kaminski, C. F. *Opt. Express* **2013**, *21*, 10978.

(3) Laine, R. F.; Albecka, A.; van de Linde, S.; Rees, E. J.; Crump, C. M.; Kaminski, C. F. *Nat. Commun.* **2015**, *6*, 5980.

(4) Kaminski Schierle, G. S.; Van De Linde, S.; Erdelyi, M.; Esbjörner, E. K.; Klein, T.; Rees, E.; Bertoncini, C. W.; Dobson, C. M.; Sauer, M.; Kaminski, C. F. *J. Am. Chem. Soc.* **2011**, *133*, 12902-12905.

(5) Tokunaga, M.; Imamoto, N.; Sakata-Sogawa, K. *Nat. Methods* **2008**, *5*, 159.

(6) Wolter, S.; Löschberger, A.; Holm, T.; Aufmkolk, S.; Dabauvalle, M.-C.; van de Linde, S.; Sauer, M. *Nat. Methods* **2012**, *9*, 1040.

(7) Sternberg. *Computer* **1983**, *16*, 22.

(8) Albertazzi, L.; van der Zwaag, D.; Leenders, C. M. A.; Fitzner, R.; van der Hofstad, R. W.; Meijer, E. W. *Science* **2014**, *344*, 491.