

Supporting Information

Synthesis and self-assembly of carborane-containing ABC triblock terpolymer: morphology control on a dual-stimuli responsive system

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1- Materials and Methods

Materials

Tert-butyl acrylate (*t*BA, 98%) and 4-Acetoxystyrene (AOS, 96%) were obtained from Sigma-Aldrich and purified by distillation under reduced pressure from CaH_2 followed by filtration over a column with silica gel and were subsequently stored at -20°C under N_2 in dark. 2,2'-Azobis(2-methylpropionitrile) (98%, AIBN) was recrystallized from ethanol. Toluene (p.a., Lachner) was dried by distillation under reduced pressure from P_2O_5 . Trifluoroacetic acid (TFA, 99%, Sigma-Aldrich), 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (CTA, 98%, Sigma-Aldrich), dichloromethane (HPLC grade, Lachner), CsF (Sigma-Aldrich) and methanol (HPLC grade, Lachner) were used as received. Styrene with attached carborane cage ("Styrene carborane", or "SC") was delivered by Núñez group and reported in article [1]. Dialysis tubes used in this work (supplied by Spectrum Laboratories, Inc., CA USA) had a nominal molar mass cutoff of 3.5 kDa.

Syntheses

Synthesis of Poly(*tert*-butyl acrylate), PtBA, **P1**.

A 50 ml Schlenk flask, equipped with a Teflon-coated stir bar was filled with 5 g (39.01 mmol, 200 eq.) of *t*BA, 71.1 mg (0.195 mmol, 1.00 eq.) of CTA, 6.4 mg (39 μmol , 0.20 eq.) of AIBN and 1 g of toluene, in this order of sequence. The mixture was deoxygenated by purging with Ar for 30 min under intense stirring, after which the flask with the yellow solution was capped by a rubber septum and degassed by 3 – 5 cycles of freezing / evacuation / thawing. The Schlenk flask was subsequently filled with Ar. A small aliquot of the well-mixed starting mixture was removed for conversion analysis (using a syringe with a long needle piercing the rubber septum). Thereafter, the Schlenk flask was immersed in an oil bath pre-heated to 70°C . After 3.5 h, another small sample was taken for conversion determination and the reaction was stopped by quenching it with a liquid nitrogen bath. Subsequently, the mixture was allowed to reach room temperature. Most of the residual monomer was removed by vacuum distillation. Next, the crude polymer was dissolved in a small amount of dichloromethane and precipitated by adding its solution to an ice-cold 1:1 water/methanol mixture. The pale yellow (solid) precipitate was separated by decantation and dissolved again in dichloromethane. This solution was evaporated and dried overnight under vacuum at RT. The final product was obtained as a yellow sticky rubber.

Yield: 3.2 g, monomer (*t*BA) conversion 76%.

Characterization: SEC: $M_n=18001$ Da, $M_w=20548$ Da, $\bar{D}=1.11$; $^1\text{H-NMR}$ (CDCl_3): $M_n=11259$ Da; $\delta=0.86$ (trip, CH_3CH_2-), 1.20-1.98 (br, $-\text{CH}_2\text{CH}-$ methylene backbone, alkyl chain and $\text{HOCC}(\text{CH}_3)_2-$ from CTA), 1.25-1.65 (br, $(\text{CH}_3)_3\text{C}-$), 2.05-2.50 (br, $-\text{CHCH}_2-$ methine backbone), 3.10–3.25 (br, $-\text{SCSCH}_2-$), 4.62–4.72 (br, $-\text{CH}_2\text{CHS}$) ppm.

Synthesis of Poly(*tert*-butyl acrylate)-*block*-Poly(4-acetoxystyrene), PtBA-*b*-PAOS, **P2**.

A 50 ml Schlenk flask, equipped with a Teflon-coated stir bar was filled with 2g (0.17 mmol, 1 eq.) of the macrotransfer agent (**P1**), 3g (18.49 mmol, 100 eq.) of AOS, 9.11 mg (56.16 μmol , 0.30 eq.) of AIBN and 1 g of toluene. The mixture was deoxygenated by purging with Ar for 30 min under simultaneous intense

stirring (important also for the dissolution of (**P1**)), after which the flask with the transparent yellow solution was capped by a rubber septum and degassed by 3 – 5 cycles of freezing / evacuation / thawing. The Schlenk flask was subsequently filled with Ar. A small aliquot of the well-mixed starting mixture was removed for conversion analysis (using a syringe with a long needle piercing the rubber septum). Thereafter, the Schlenk flask was immersed in an oil bath pre-heated to 70°C. After 16 h, another small sample was taken for conversion determination and thereafter the reaction was stopped by quenching it with a liquid nitrogen bath. Subsequently, the mixture was allowed to reach room temperature. Most of the residual monomer was removed by vacuum distillation. Next, the crude polymer was dissolved in a small amount of dichloromethane and precipitated by adding its solution to an ice-cold 1:1 water/methanol mixture. The pale yellow (solid) precipitate was separated by decantation and dissolved again in dichloromethane. This solution was evaporated and dried overnight under vacuum at RT. The final product was obtained as a light-yellow solid.

Yield: 2.23 g, second-block-monomer (AOS) conversion 26%.

Characterization: SEC: $M_n=22177$ Da, $M_w=28711$ Da, $\bar{D}=1.29$; $^1\text{H-NMR}$ (CDCl_3): $M_n=14016$ Da; $\delta=0.86$ (trip, CH_3CH_2-), 1.20-1.98 (br, $-\text{CH}_2\text{CH}-$ methylene backbone, alkyl chain and $\text{HOCC}(\text{CH}_3)_2-$ from CTA), 1.25-1.65 (br, $(\text{CH}_3)_3\text{C}-$), 2.05-2.50 (br, $-\text{CHCH}_2-$ methine backbone and $-\text{OCOCH}_3$), 3.10–3.25 (br, $-\text{SCSCH}_2-$), 4.62–4.72 (br, $-\text{CH}_2\text{CHS}$), 6.2-7 (br, Ar-H) ppm.

Synthesis of Poly(*tert*-butyl acrylate)-*block*-Poly(4-acetoxystyrene)-*block*- poly{1-[4-(1-methyl-1,2-dicarba-*closo*-dodecaborane-2-yl methyl)-phenyl] ethylene}}, PtBA-*b*-PAOS-*b*-PSC, **P3**.

A 50 ml Schlenk flask, equipped with a Teflon-coated stir bar was filled with 1.0 g (71.35 μmol , 1 eq.) of macrotransfer agent (**P2**), 1.0 g (3.63 mmol, 50 eq.) of SC, 1.7 mg (0.29 μmol , 0.50 eq.) of AIBN and 1.5 g of toluene. The mixture was deoxygenated by purging with Ar for 30 min under simultaneous intense stirring (important also for the dissolution of (**P2**)), after which the flask with the transparent yellow solution was capped by a rubber septum and degassed by 3 – 5 cycles of freezing / evacuation / thawing. The Schlenk flask was subsequently filled with Ar. A small aliquot of the well-mixed starting mixture was removed for conversion analysis (using a syringe with a long needle piercing the rubber septum). Thereafter, the Schlenk flask was immersed in an oil bath pre-heated to 70°C. After 27 h, another small sample was taken for conversion determination and thereafter the reaction was stopped by quenching it with a liquid nitrogen bath. Subsequently, the mixture was allowed to reach room temperature. Most of the residual monomer was removed by vacuum distillation. Next, the crude polymer was dissolved in a small amount of dichloromethane and precipitated by adding its solution to an ice-cold 1:1 water/methanol mixture. The amber-colored solid precipitate was separated by decantation and dissolved again in dichloromethane. This solution was evaporated and dried overnight under vacuum at RT. The final product was obtained as an amber solid.

Yield: 1.11g, third-block-monomer (SC). corresponding to 27% conversion of the

Characterization: SEC: $M_n=22177$ Da, $M_w=28711$ Da, $\bar{D}=1.29$; $^1\text{H-NMR}$ (CDCl_3): $M_n=14016$ Da; $\delta=0.86$ (trip, CH_3CH_2-), 1.20-1.98 (br, $-\text{CH}_2\text{CH}-$ methylene backbone, alkyl chain and $\text{HOCC}(\text{CH}_3)_2-$ from CTA), 1.25-1.65 (br, $(\text{CH}_3)_3\text{C}-$), 2.05-2.50 (br, $-\text{CHCH}_2-$ methine backbone, $-\text{OCOCH}_3$ and $-\text{CCH}_3$), 3.10–3.25 (br, $-\text{SCSCH}_2-$), 3.27-3.50 (br, $-\text{CCH}_2\text{C}-$), 4.62–4.72 (br, $-\text{CH}_2\text{CHS}$), 6.20-7.00 (br, Ar-H) ppm.

Preparation of amphiphilic block copolymers Poly(acrylic acid)-*block*-Poly(4-hydroxystyrene), PAA-*b*-PHS, **P4** and Poly(acrylic acid)-*block*-Poly(4-hydroxystyrene)-*block*- poly{1-[4-(1-methyl-1,2-dicarba-closo-dodecaborane-2-yl methyl)-phenyl] ethylene}}, PAA-*b*-PHS-*b*-PSC, **P5**.

The simultaneous acidolysis of the ester pendant groups in the poly(*t*BA) and poly(AOS) blocks similar to [2,3], which yielded poly(Acrylic acid) and poly(4-Hydroxystyrene) blocks in the final product, was carried out in a 100 ml one-neck round bottom flask equipped with a Teflon-coated stir bar. Diblock and triblock copolymers (which both contained poly(*t*BA) as well as poly(AOS) blocks) were processed in an identical way: **P2** (0.7 g, 50 μ mol) or **P3** (0.7 g, 45 μ mol) was put into the reaction flask together with 15 ml of dichloromethane and the flask was capped by a rubber septum. The respective copolymer was dissolved by 30 min of intense stirring. Thereafter, 5 ml of 99% TFA were injected through the septum and the reaction mixture was stirred for 24 h, at room temperature. In the next step, the solvent, TFA, as well as the volatile by-products were removed under vacuum. The crude product was dissolved in 15 ml of THF and transferred into a dialysis tube with nominal molar mass cutoff of 3.5 kDa. It was dialyzed against water for 3 days to remove remaining low-molecular impurities including TFA. The purified solution of the acidolysis product was evaporated and dried overnight under vacuum at RT. **P4** was obtained as a light-yellow solid, and **P5** as an amber solid.

Yield: **P4**: 0.377 g (92%); **P5**: 0.411 g (93%).

Self-assembly

General procedure for preparation of core-shell micelles from **P4** and **P5**

Each polymer was initially dissolved in a mixture of THF/H₂O (ranging from 10 to 90% v/v THF) to a final concentration of 2mg/ml in a total volume of 2 ml. The mixtures that resulted soluble were then added dropwise into 2 ml of deionized water under vigorous agitation. After 30 min of agitation the samples were moved to dialysis membranes (MWCO= 3.5 kDa) and dialyzed against deionized water for 1 day with several changes of outer water bath to ensure complete removal of organic solvent. One (90% THF, henceforth **P4-90**) and three (10, 50 and 90% v/v THF-water; **P5-10**, **P5-50**, **P5-90**) solvent mixtures were chosen to further study **P4** and **P5** self-assemblies in water, respectively.

General procedure for deboronation of Carborane in **P5** micelles

An aqueous solution of **P5** micelles (1 mg/ml) and CsF (0.2 mg/ml) were heated to 80°C in a closed vial for 24h. Blank solutions were prepared following an identical procedure with the exception of CsF addition, this was added after the solution was cooled down to room temperature.

Characterization Techniques

NMR spectroscopy

¹H, ¹¹B and ¹⁹F-NMR spectra were recorded on a Bruker Avance 400 spectrometer at 25 °C. Solid state ¹¹B and ¹³C NMR spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C.

Size Exclusion Chromatography (SEC)

The used SEC system consisted of a Waters 1515 pump, three μ -styragel separation columns with a continuous porosity of 102–105 Å, and a Waters 2414 differential refractive index detector. The solvent

was tetrahydrofuran and the flow rate was 1 mL/min. The calibration of the instrument was performed using narrow polystyrene standards (M_w varying from 2500 to 900000).

Dynamic Light Scattering

For most samples the light scattering setup (ALV, Langen, Germany) consisted of a 22 mW He-Ne laser ($\lambda = 632.8$ nm), an ALV-CGS/8F goniometer, an ALV High QE APD detector, and an ALV 50004, multibit, multitau autocorrelator. The measurements were carried out at 296 K for scattering angles ranging from 30° to 150° corresponding in aqueous solutions to scattering vector magnitudes ranging from 6.8 to 25.6 μm^{-1} . The normalized time autocorrelation function of the scattered light intensity was fitted by the constrained regularization algorithm (CONTIN), which provides the distribution of relaxation times τ . Effective angle dependent hydrodynamic radii, $R_H(q)$, were obtained from the mean values of relaxation times, $\tau(q)$, of individual diffusive modes using the Stokes-Einstein equation. R_H values correspond to apparent hydrodynamic radii.

Electrophoretic Light Scattering

The measurements were carried out with Nano-ZS Zetasizer (Malvern Instruments, UK). Zeta-Potential was obtained from electrophoretic mobility values using the Henri equation in the Smoluchowski approximation. Values presented are averaged from 45 measurements. For comparison of zeta potential values samples were measured at matching ionic strength. NaCl was used to increase ionic strength when required.

Fluorescence

All steady-state fluorescence spectra were acquired at a polymer concentration of 0.5 mg ml^{-1} using a Fluorolog FL-3-22 (Horiba Jobin Yvon, France) equipped with an excitation and emission double monochromators and a 450 W xenon lamp. For both **P4** and **P5** micelles $\lambda_{\text{ex}} = 274$ nm, $\lambda_{\text{em}} = 350$ nm. After changes in pH samples were allowed to equilibrate for 30 min before measuring.

Cryo -Transmission Electron Microscopy (Cryo-TEM)

Cryo-Tem measurements were carried out using a Tecnai G² Sphera 20 electron microscope (FEI Company, Hillsboro, OR, USA) equipped with a Gatan 626 cryo-specimen holder (Gatan, Pleasanton, CA, USA) and a LaB₆ gun. The samples for cryo-TEM were prepared by plunge-freezing [4]. Briefly, 3 μL of the sample solution was applied to a copper electron microscopy grid covered with a perforated carbon film forming woven-mesh-like openings of different sizes and shapes (the lacey carbon grids #LC-200 Cu, Electron Microscopy Sciences, Hatfield, PA, USA) and then glow discharged for 40 s with 5 mA current. Most of the sample was removed by blotting (Whatman No. 1 filter paper) for approximately 1 s, and the grid was immediately plunged in liquid ethane held at -183 °C. The grid was then transferred without rewarming to the microscope. In both cases, images were recorded at an accelerating voltage of 120 kV and magnifications ranging from 11 500× to 50 000× using a Gatan UltraScan 1000 slow scan CCD camera in the low-dose imaging mode, with an electron dose not exceeding 1500 electrons per nm^2 . The magnifications resulted in final pixel sizes ranging from 1 to 0.2 nm, and the typical value of the applied under focus ranged from 0.5 to 2.5 μm . The applied blotting conditions resulted in specimen

thicknesses varying between 100 and ca. 300 nm. Brightness and contrast corrections of the acquired images were performed using the ImageJ software.

Negative staining techniques in TEM

-Uranyl acetate [$\text{UO}_2(\text{CH}_3\text{COO})_2$]: 5 μL of the sample solutions were applied to a glow discharged carbon-coated copper grids. The excess of solution were blotted, samples were stained with 2% uranyl acetate.

Positive staining techniques in TEM

-Osmium tetroxide [OsO_4]: 5 μL of the sample solutions were applied to a glow discharged carbon-coated copper grids. The excess of solution were blotted and the grids were put in a close container along with OsO_4 crystals for 2 hours.

-Ruthenium tetroxide [RuO_4]: Grids were prepared identically as for Osmium tetroxide and were put in a close container with a fresh solution of RuO_4 for 5 min. The solution was prepared according to procedure by Li et al. [5].

Small Angle X-ray Scattering (SAXS)

SAXS experiments were carried out on the P12 BioSAXS beamline (PETRA III storage ring, EMBL/DESY, Hamburg, Germany), equipped with a Pilatus 2M detector. The measurement was carried out at 20 °C and the sample-detector distance 3 m, allowing for covering the q-range interval from 0.07 to 4.4 nm^{-1} for the X-ray wavelength $\lambda = 0.1 \text{ nm}$. The q range was calibrated using the diffraction patterns of silver behenate. The experimental data were normalized to the transmitted beam intensity and corrected for nonhomogeneous detector response, and the background scattering of the solvent was subtracted. The solvent scattering was measured before and after the sample scattering to control for possible sample holder contamination. 20 consecutive frames with 0.05 s exposures comprising the measurement of the solvent, sample, and solvent were performed. Data have been checked for radiation damage. The final scattering curves were processed by automated data acquisition software and recalculated to absolute scattering intensities. The data were fitted to different models as described in the Supporting Information using the SASfit 0.94.7 software.

^{19}F -NMR for TFA quantification

A known amount of **P5** was dissolved in d_6 -DMSO. Inside the NMR cuvette a smaller capillary NMR cuvette was inserted. The later, contained a known amount of hexafluoro benzene which was used as a reference for the quantification. The same reference was used for all three measurements and all measurements were performed the same day. The acquisition of signal was done with a 40 s relaxation time and a total of 12 acquisitions. To perform the quantification, various samples with known amount of TFA were prepared, the ratio of the integration of the signals (always using the hexafluorobenzene signal as common reference for integration) was determined to be proportional to the ratio of concentrations. Then the ratio of any of the signals was used to determine the concentration of TFA in the P5 sample. For example, the integration of “High TFA” in arbitrary units is 4.94 and the one for “Low TFA” is 3.44. The ratio of such signals equals the ratio of concentrations (0.403 and 0.28 mol l^{-1} respectively).

2- Characterization of polymer samples by ^1H -NMR, ^{13}C -NMR, ^{11}B -NMR, ^{19}F -NMR spectroscopy and SEC, and further details on polymer synthesis

Table S1. Molecular weight and dispersity ($\bar{D}$) of the prepared polymers, as determined by SEC and ^1H NMR spectroscopy; ratios monomer/chain-transfer-agent/initiator are also listed.

Polymer ^c	[M]/[CTA]/[I] ^a	M_n (SEC) ^b (Da)	M_w (SEC) ^b (Da)	$\bar{D}$ (SEC) ^b	M_n (^1H NMR) ^c (Da)
P1 (tBA) ₈₅	200/1/0.2	18 001	20 548	1.11	11 259
P2 (tBA) ₈₅ -b-(AOS) ₁₇	100/1/0.3	22 177	28 711	1.29	14 016
P3 (tBA) ₈₅ -b-(AOS) ₁₇ -b-(SC) ₅	50/1/0.5	21 173	31 066	1.46	15 383
P4 (AA) ₈₅ -b-(HS) ₁₇	-	-	-	-	8 532
P5 (AA) ₈₅ -b-(HS) ₁₇ -b-(SC) ₅	-	-	-	-	9 899

^a[M]/[CTA]/[I] eq. of monomer, chain transfer agent or macrotransfer agent and initiator.

^bMeasured by SEC calibrated with PS standards in THF as eluent.

^cDetermined by ^1H NMR spectroscopy.

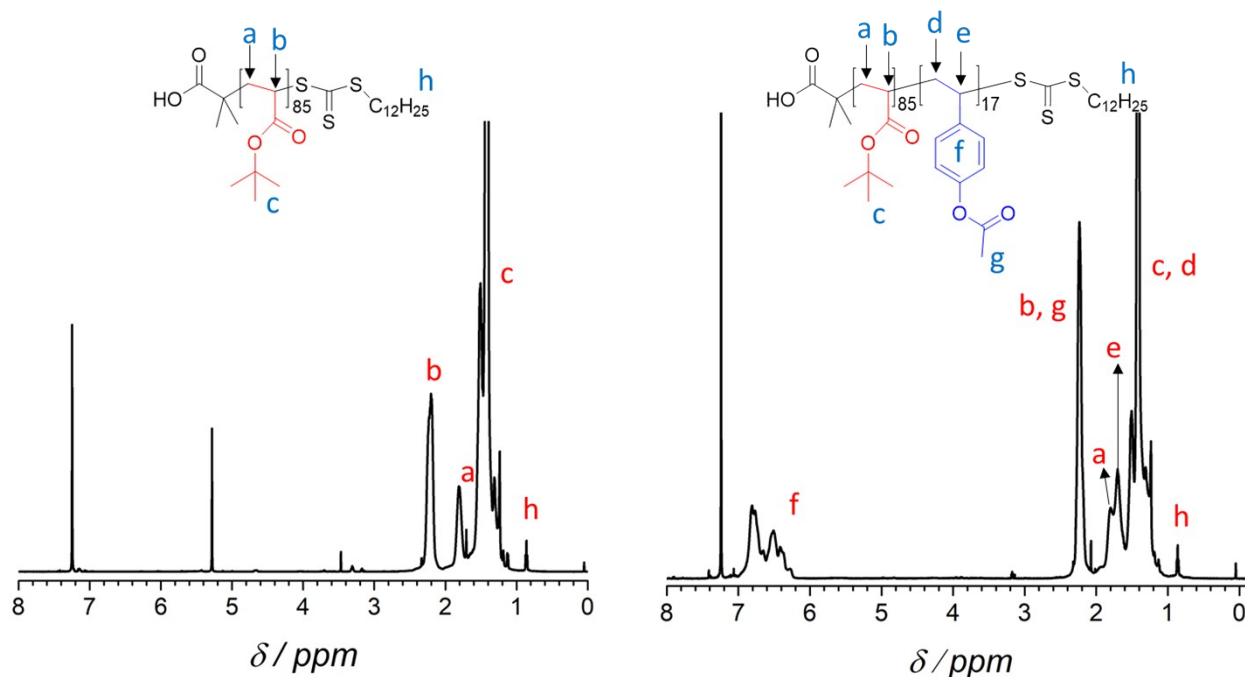


Figure S1. ^1H -NMR spectra of homopolymer (**P1**) and diblock copolymer (**P2**) with detailed signal assignment before deprotection.

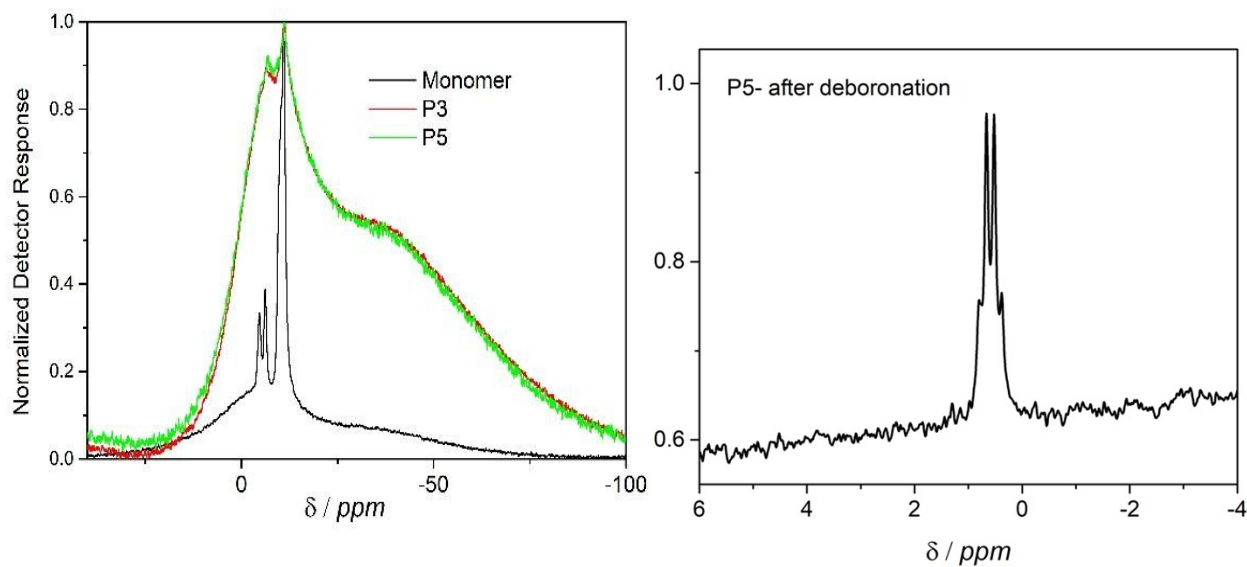


Figure S2. (left) ^{11}B -NMR spectra of monomer before polymerization, triblock terpolymer before deprotection (**P3**) and final triblock terpolymer (**P5**). (right) ^{11}B -NMR spectrum of triblock terpolymer **P5** after deboronation; spectrum is zoomed to peak at 0.6 ppm.

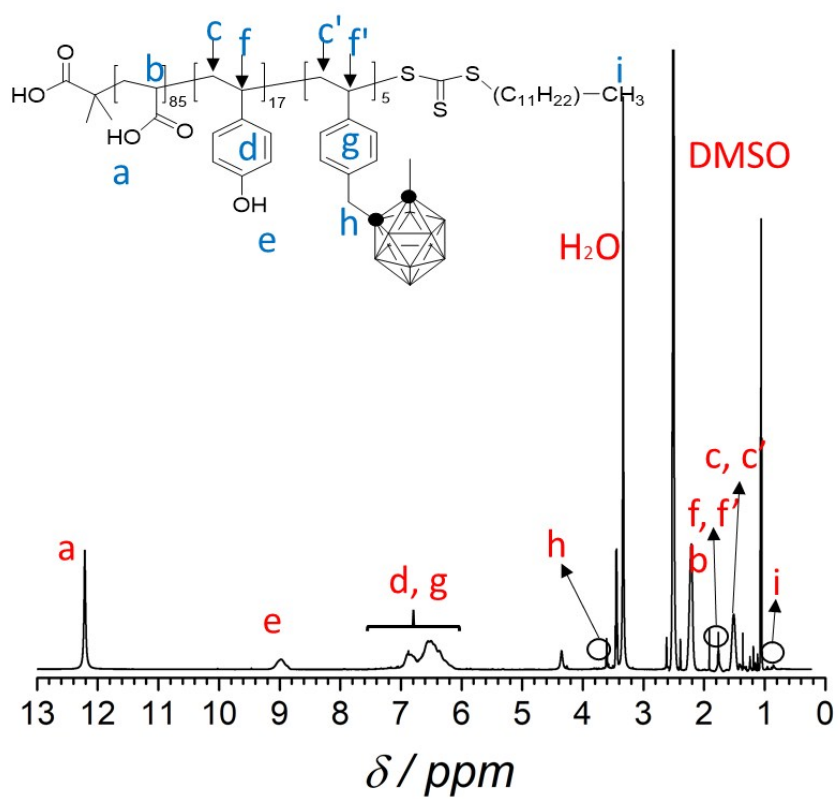


Figure S3. ^1H -NMR spectrum of triblock copolymer (**P5**) after deprotection step with TFA.

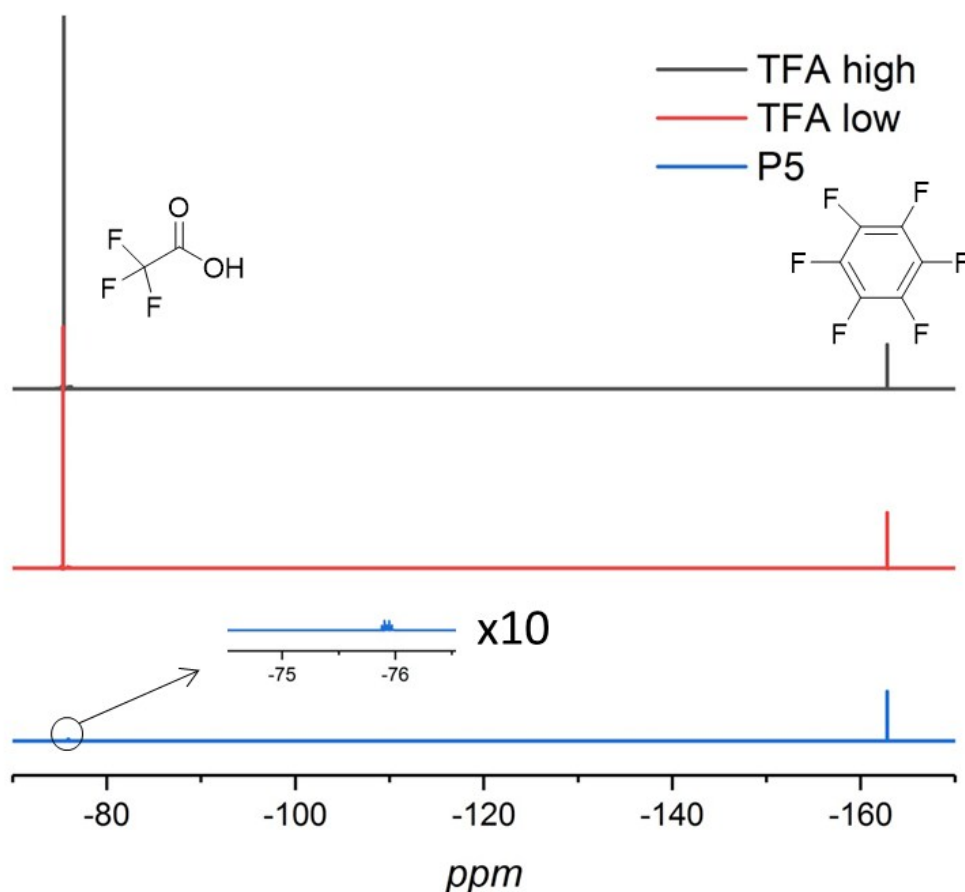


Figure S4. ^{19}F -NMR spectra of **P5** (blue) after deprotection with TFA. For comparison, ^{19}F -NMR spectra of TFA in concentrations corresponding to 100% of ionizable polymer segments (**TFA high, black**) and 80% the polymer segments (**TFA low, red**) to polymer chain are shown. Hexafluorobenzene was used as internal standard for quantification purposes. It proves that only traces of TFA remained in the sample. TFA signal is still visible at -77 ppm even after purification. Nonetheless, after quantification by NMR we determined the concentration is equivalent to 6.2 molecules of TFA per polymer chain, therefore the effect on polymer solubility is negligible and self-assembly is not influenced by the presence of TFA traces.

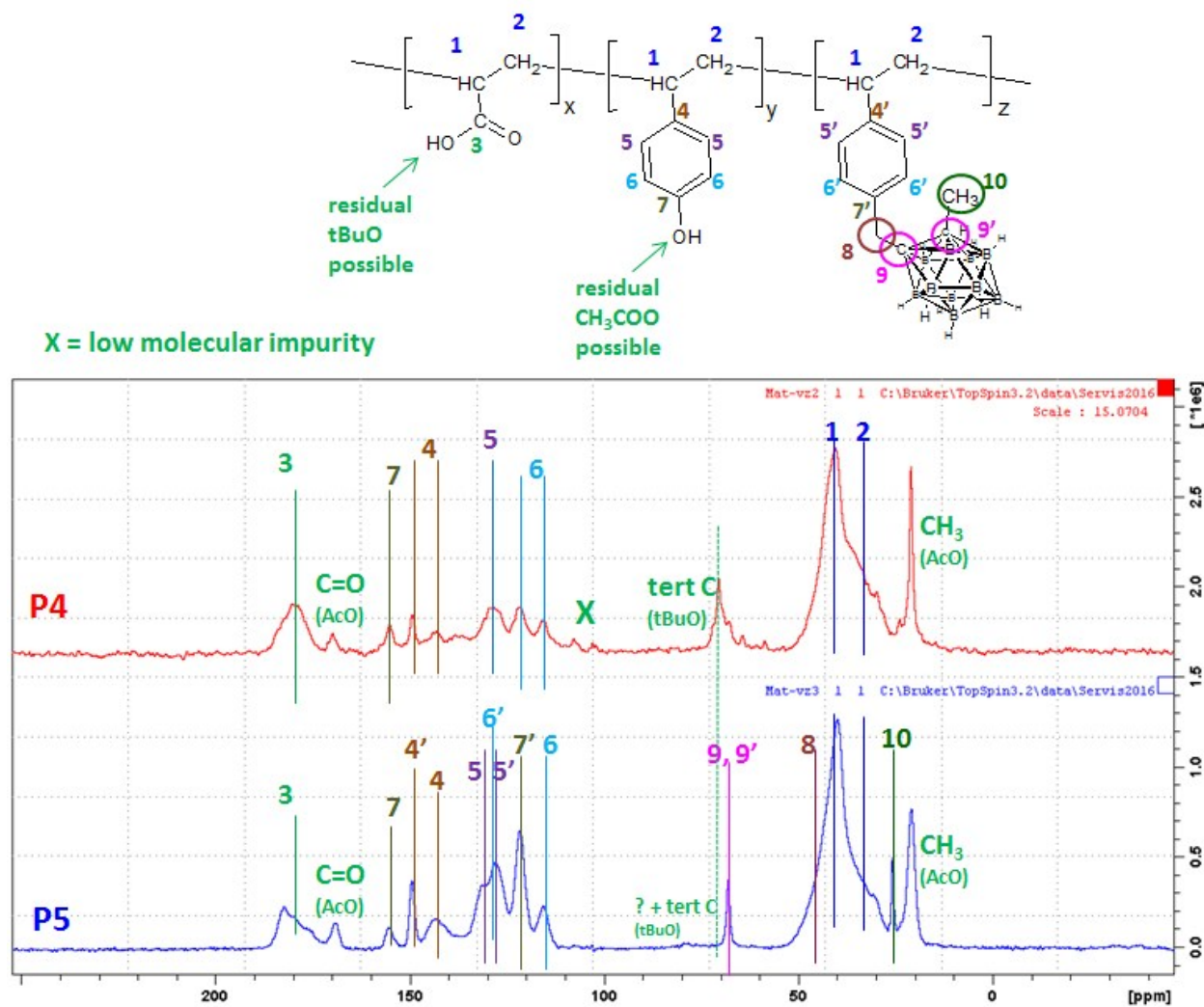


Figure S5. Solid State ¹³C MAS NMR spectra for diblock (P4) and triblock (P5) copolymers after deprotection step with TFA.

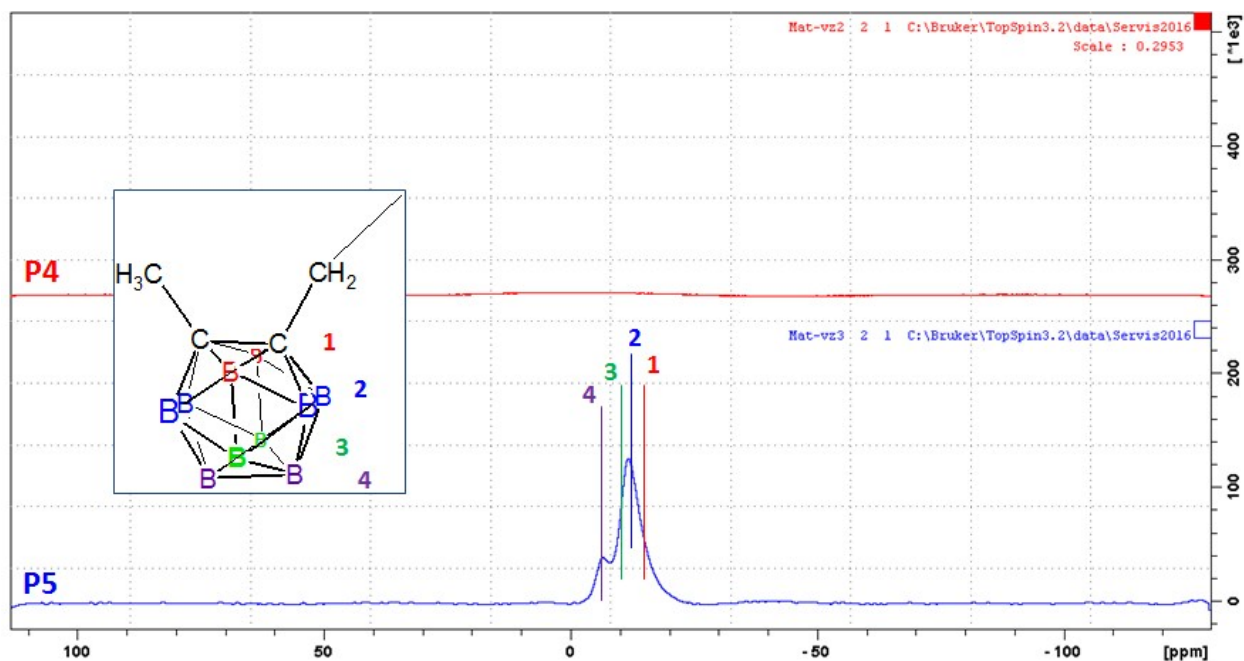
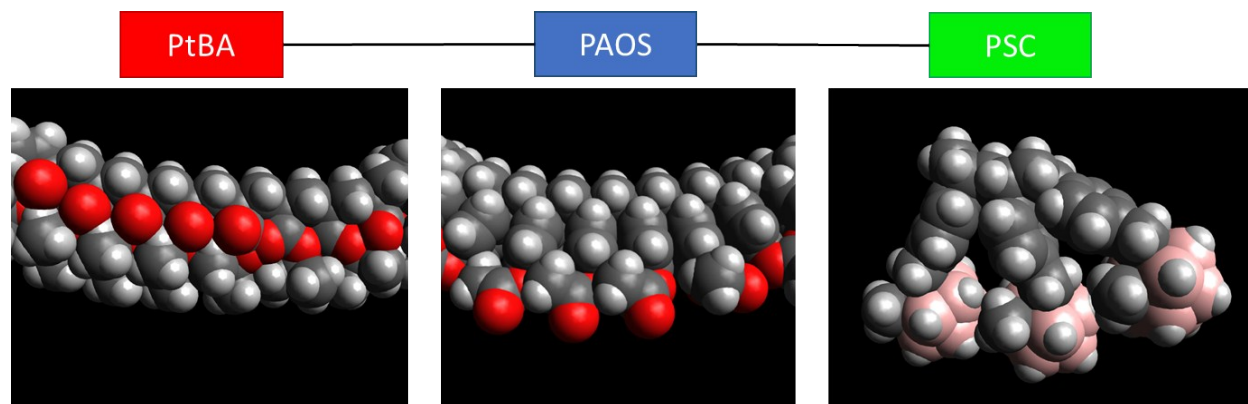


Figure S6. Solid State ^{11}B MAS NMR spectra of **P4** and **P5** after deprotection step.



Figures S7. Simplistic model of repetitive units of different monomers used for synthesis of **P3**. Poly(tert-butylacrylate), poly(4-acetoxystyrene) and poly{1-[4-(1-methyl-1,2-dicarba-closo-dodecaborane-2-yl methyl)-phenyl] ethylene (from left to right).

It illustrates the increased strain on the main chain of the polymer with successive addition of repetitive units. Notably, with only 3 repetitive units the bulky carborane increases the accessibility to the active site. The problem relies in the rigid spacer between the main chain and the carborane moiety.

3- Evaluation of SAXS data for P4, P5-10, P5-50 and P5-90 micelles in water

a) The SAXS curves of **P5-10** and **P5-50** were fitted by the Fisher-Burford model.

$$I(q, D, R_g) = \left(1 + \frac{2}{3D} q^2 R_g^2\right)^{-\frac{D}{2}} \quad (\text{S1})$$

Where D is the fractal dimension and R_g is the radius of gyration of the aggregate. Results obtained from the fitting process are presented in Table S2.

Table S2. Forward scattering (I_0), Radius of gyration (R_g) and Power law exponent obtained from fit of Equation S1 to SAXS curves, and characteristics of micelles from other techniques (DLS and TEM) for **P5-10** and **P5-50**.

<i>Sample</i>	<i>R_g (nm)</i>	<i>R_{core} (nm) (TEM)</i>	<i>R_H (nm) (DLS)</i>	<i>I₀</i>	<i>Power law exponent</i>
P5-10	29.2	18	58	3.3 x 10 ⁵	3.0
P5-50	57.3	9	101	2.6 x 10 ⁶	3.1

Both **P5-10** and **P5-50** were also fitted with a form factor of simple sphere to simulate small scattering units and the adhesion between scatterers was simulated by a mass fractal structure factor.

(b) The SAXS curves from **P5-90** were fitted by a combination of a sphere and a long cylinder form factor.

$$I(R, q, r, \Delta\eta) = (\Delta\eta\pi R^2 L)^2 \frac{2q}{L} \left\{ Si_{\frac{\pi}{2}}(qL) \Lambda_1^2(qR) - \frac{\omega(2qR)}{qL} - \frac{\sin_{\text{foi}}(qL)}{(qL)^2} \right\} + \left(\frac{4}{3} \pi r^3 \Delta\eta^3 \frac{\sin\sin(qr) - qr * \cos_{\text{foi}}(qr)}{(qr)^3} \right)^2 \quad (\text{S2})$$

Where $\Delta\eta$ is the scattering length density difference between particle and matrix, R is the radius of cylinder, L is the length of the cylinder, and r is the radius of sphere.

The functions Si and Λ_1 are defined as

$$Si_{\frac{\pi}{2}}(x) = \left(Si(x) + \frac{coscos x}{x} + \frac{sinsin x}{x} \right) \quad (S3)$$

$$\Lambda_1 = \frac{2}{x} J_1(x) \quad (S4)$$

Where J_1 is the regular cylindrical Bessel function of order 1. Porod's approximations for long cylinders was taken because previous analysis of cryo-TEM images confirmed the condition of $L > 2R$ was satisfied.

Table S3. Parameters obtained from fit of Equation S2 to SAXS curves and characteristics of micelles from TEM for **P5-90**.

$R_{cylinder} (nm)$	$R_{cylinder} (nm) (TEM)$	$L_{cylinder} (nm)$	$R_{sphere} (nm)$	$R_{sphere} (nm) (TEM)$
20	18	215	17.3	15

(c) Second fitting of **P5-10** by sphere model and mass fractal structure factor. The Sphere model was described above (right part of equation **S2**).

$$I(\xi, q, r, \Delta\eta, D, r_0) = I_0 \left\{ 1 + \frac{D\Gamma(D-1)sin[(D-1)arctanarctan(q\xi)]}{(qr_0)^D(1+q^{-2}\xi^{-2})} \right\} x \left(\frac{4}{3} \pi r^3 \Delta\eta^3 \frac{sinsin(qr) - qr * cos[(qr)]}{(qr)^3} \right)^2 \quad (S5)$$

Where ξ is the correlation length of the fractal cluster, I_0 is the forward scattering, D is the mass fractal dimension and r_0 is the characteristic dimension of a particle forming part of the cluster. The gamma function Γ is defined as

$$\Gamma(a, x) = \int_x^{\infty} t^{-a} expexp(-t) dt \quad (S6)$$

Table S4. Results obtained from best fit of Equation S5 to SAXS curves of **P5-10**

$r_0 (nm)$	$\xi (nm)$	D
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0.4

13.4

2.6

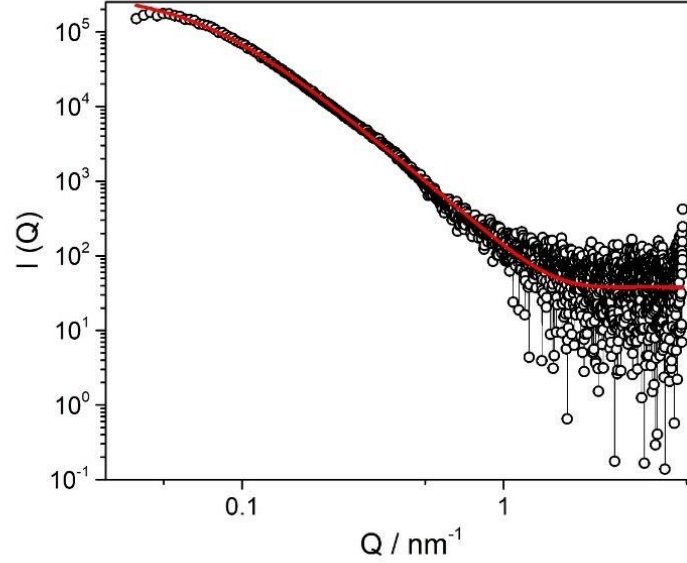


Figure S8. SAXS curve of **P5-10** fitted with a sphere model form factor and a mass fractal structure factor.

(d) The SAXS curves for **P4-90** were fitted to the Pedersen-Gertenberg form factor. To account for the dispersity of the sample a Gaussian distribution model was used.

$$I(q, r_0, k, \rho_s, \rho_c, N_{agg}) = \int_0^\infty f(R, r_0, k) P_{mic}(q, R, R_g, \rho_s, \rho_c, N_{agg}, d) dR \quad (S7)$$

Where r_0 is the radius of the core, r_g is the radius of gyration of the chains, ρ_s is the excess scattering of the length of a block in the core, ρ_c is the excess scattering length of a block in the chains, N_{agg} is the aggregation number, and d is a parameter describing the penetration of chains into the core (no penetration is expressed as $d \approx 1$). Parameter d was not fixed due to the possibility of PAA chains penetrating the PHS core via hydrogen bond interactions. The form factor consists of a hard sphere with the scattering length of $\rho_s N_{agg}$ surrounded by N_{agg} Gaussian chains with an R_g . And it takes the following form

$$P_{mic}(q, R, r_g, \rho_s, \rho_c, N_{agg}, d) = N_{agg}^2 \rho_s^2 F_s(q, R) + N_{agg} \rho_c^2 F_c(q, r_g) + N_{agg}(N_{agg} - 1) \rho_c^2 S_{cc}(q, R, r_g, d) + 2 N_{agg}^2 \rho_s \rho_c S_{sc}(q, R, r_g, d) \quad (S8)$$

Functions stated in the form factor stand for self-correlation of the sphere ($F_s(q, R)$), self-correlation of the chains ($F_c(q, r_g)$), cross-correlation between the chains ($S_{cc}(q, R, r_g, d)$), and cross correlation between the sphere and the chains ($S_{sc}(q, R, r_g, d)$). They are given by the following equations

$$F_s(q, R) = 9 \left[\frac{\sin(qR) - qR \cos(qR)}{(qR)^3} \right]^2 \quad (\text{S9})$$

$$F_c(q, r_g) = 2 \frac{\exp(-r_g^2 q^2) - 1 + r_g^2 q^2}{r_g^4 q^4} \quad (\text{S10})$$

$$S_{cc}(q, R, R_g, d) = \left[\frac{1 - \exp(-r_g^2 q^2)}{r_g^2 q^2} \right]^2 \left[\frac{\sin(qR + qdr_g)}{qR + qdr_g} \right]^2 \quad (\text{S11})$$

$$S_{sc}(q, R, R_g, d) = 3 \left[\frac{1 - \exp(-r_g^2 q^2)}{r_g^2 q^2} \right] \left[\frac{\sin(qR) - qR \cos(qR)}{(qR)^3} \right] \left[\frac{\sin(qR + qdr_g)}{qR + qdr_g} \right] \quad (\text{S12})$$

And the Gaussian distribution is given by

$$f(R, N, \sigma, R_0) = \frac{N}{c_{Gauss}} e^{-\frac{(R - R_0)^2}{2\sigma^2}} \quad (\text{S13})$$

$$c_{Gauss} = \sqrt{\frac{\pi}{2}} \sigma \left(1 + \operatorname{erf} \left(\frac{R_0}{\sqrt{2}\sigma} \right) \right) \quad (\text{S14})$$

Table S5. Results obtained from fit of Equation S5 to SAXS curves characteristics from TEM for **P5-90**

N_{agg}	R_{core} (nm)	R_{core} (nm) (TEM)	R_g (nm)	d	σ
504	7.8	7	27	0.95	10.5

4- Dynamic light scattering characterization of micelles

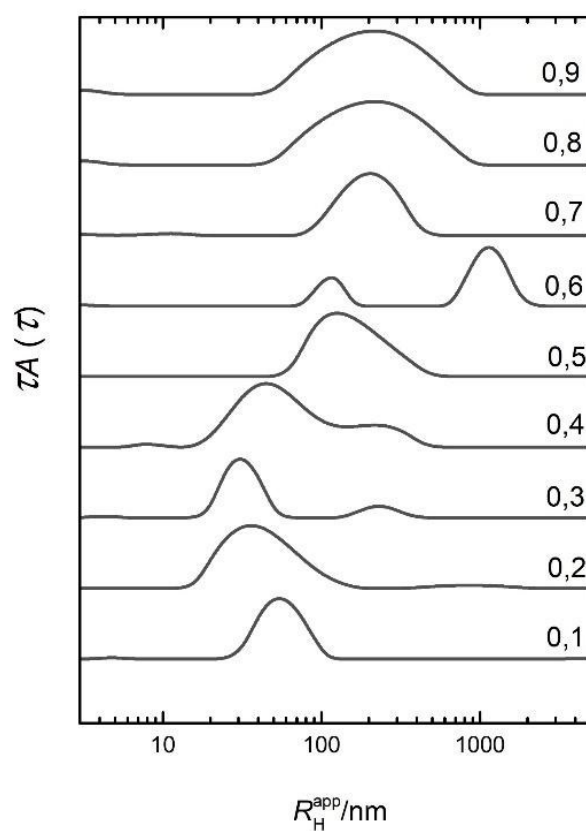


Figure S9. Hydrodynamic radii distribution of **P5** polymer in THF-H₂O mixtures. Numbers to the right indicate the volumetric fractions of THF.

Table S6. Data obtained from DLS for polymer samples in aqueous solution.

Sample	R_H (nm)	PDI
PAA-b-PHS-b-PSC P5-10	58	0.110
PAA-b-PHS-b-PSC P5-50	101	0.113
PAA-b-PHS-b-PSC P5-90	73	0.207
PAA-b-PHS P4-90	116	0.120

Table S7. Zeta potential values for micellar solutions at 1 mg/ml in water

Sample	Zeta potential (ζ) (mV)
PAA-b-PHS P4-90	-40.2 $\pm$ 2
PAA-b-PHS-b-PSC P5-10	-44 $\pm$ 2
PAA-b-PHS-b-PSC P5-10*	-33 $\pm$ 4

*P5-10 after deboronation with the aid of THF.

5- Fluorescence spectroscopy

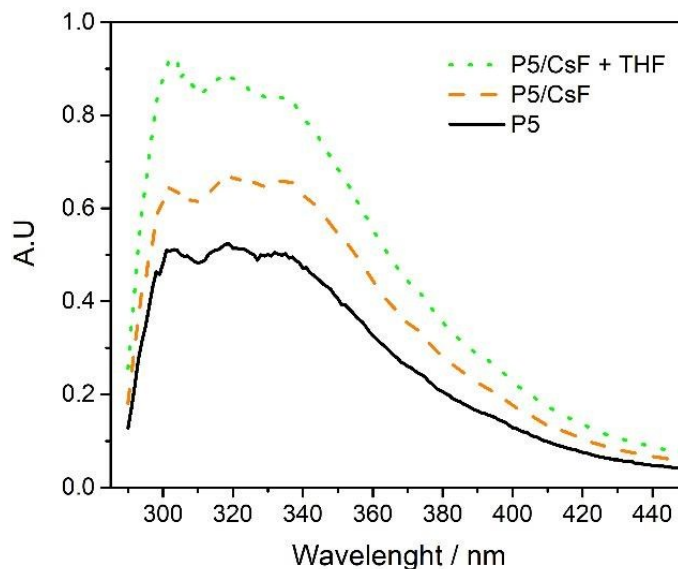


Figure S10. Comparison of fluorescence spectra of **P5** micelles under different stimuli.

Cs^+ is an effective fluorescence quencher due to the heavy atom effect. Nonetheless it is necessary for it to come in close contact with the fluorescent compound, in our specific case the PHS block. Dynamic quenching experiments with heavy ions have shown that a polyelectrolyte shell around the micelles makes it difficult for the quenching ions to access the core [6,7].

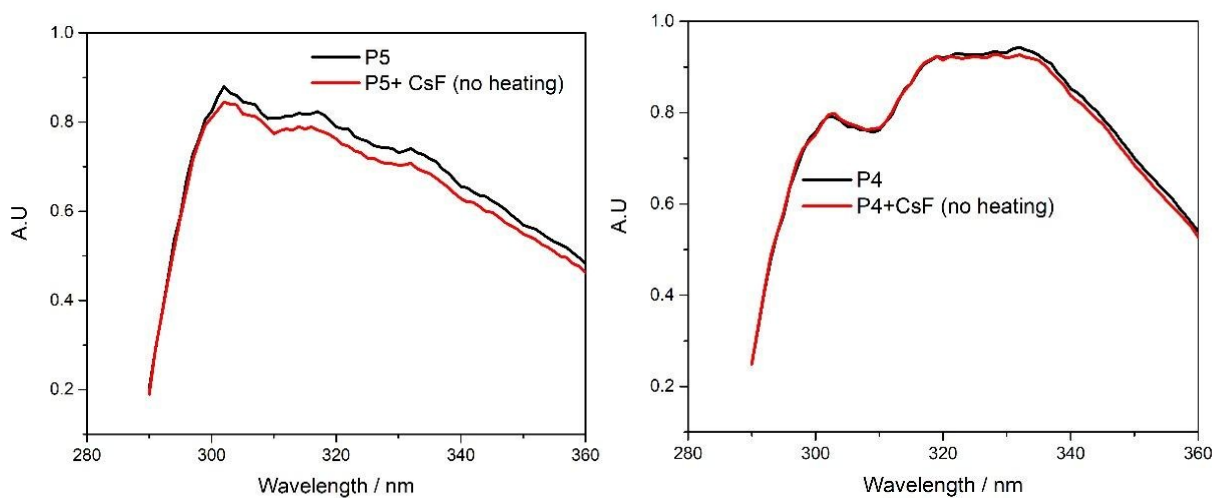


Figure S11. Fluorescence spectra of **P5** micelles after exposure to CsF without heating (control experiments).

6- Additional Cryo-TEM micrographs

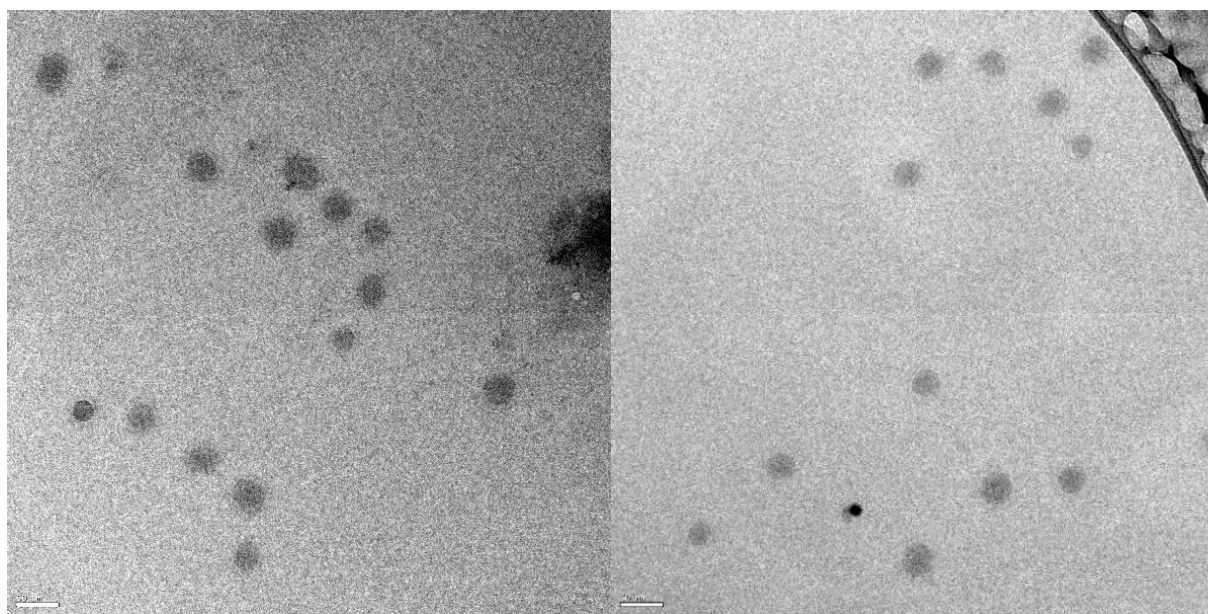


Figure S12. Cryo-TEM images of **P5-10** micelles. Scale bar 100 nm.

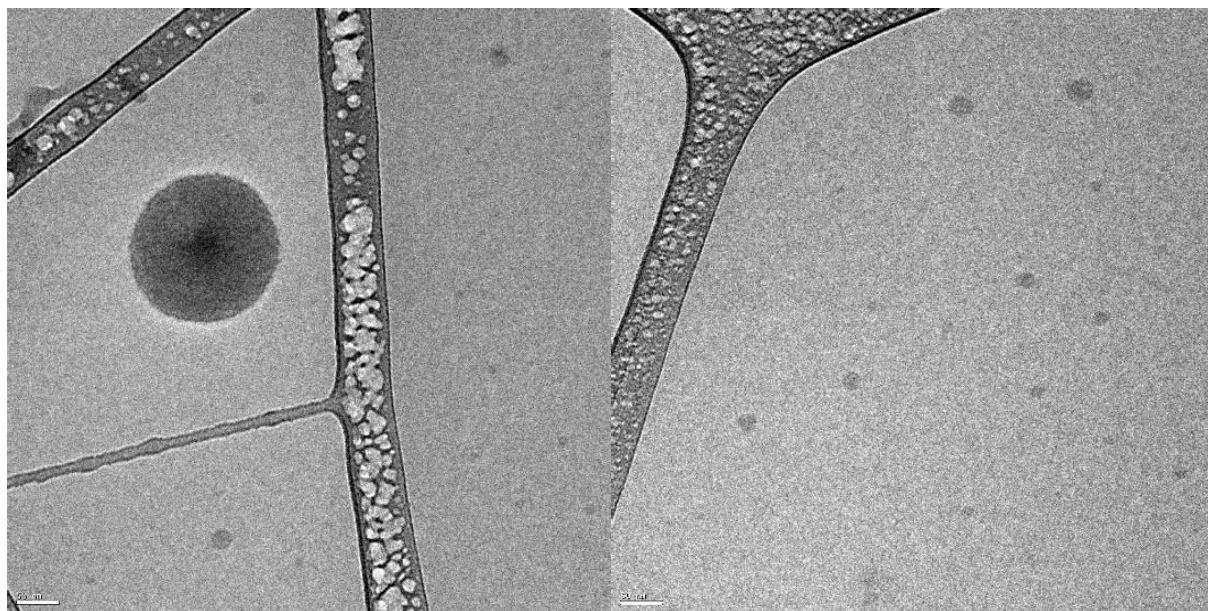


Figure S13. Cryo-TEM images of **P5-50** micelles. Scale bar 100 nm.

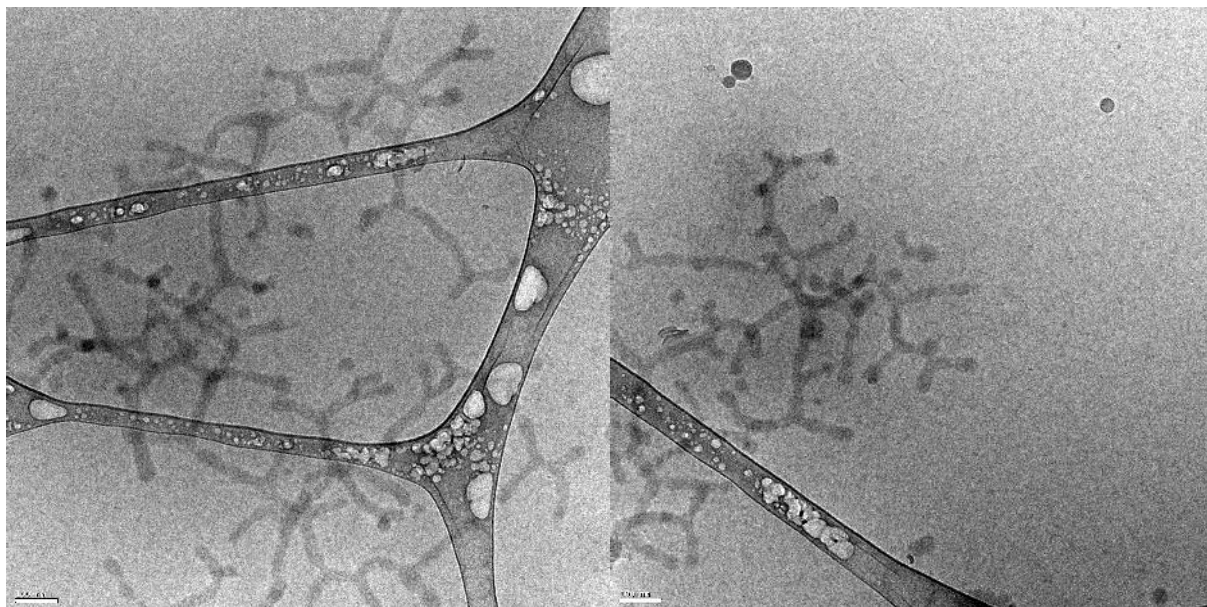


Figure S14. Cryo-TEM images of **P5-90** micelles. Scale bar 100 nm.

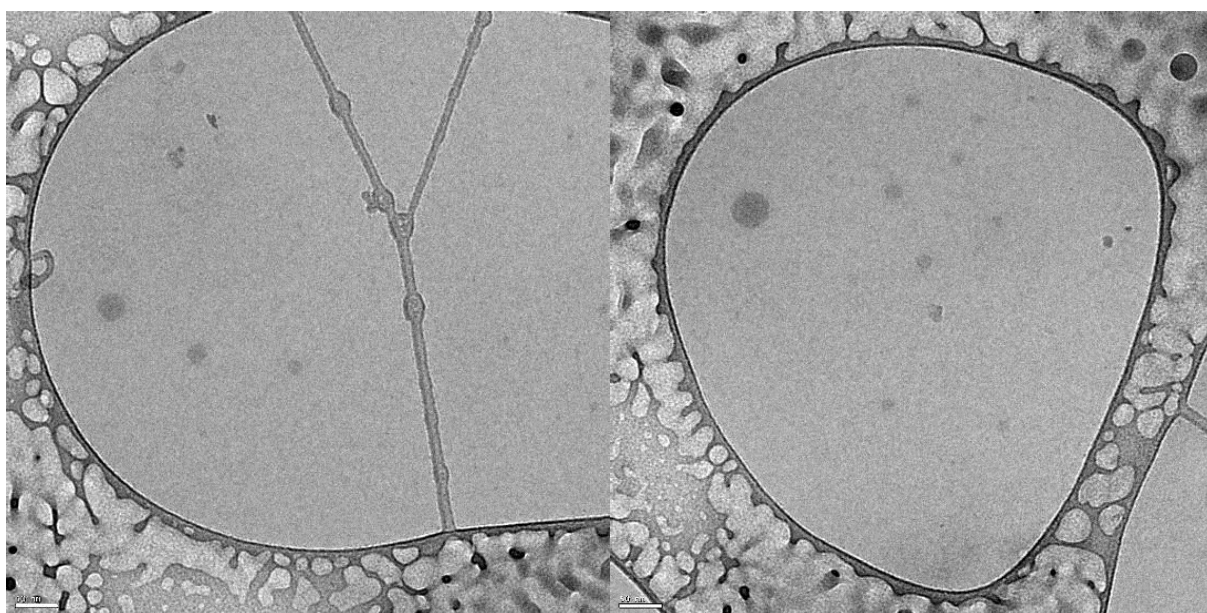


Figure S15. Cryo-TEM images of **P4-90** micelles. Scale bar 100 nm.

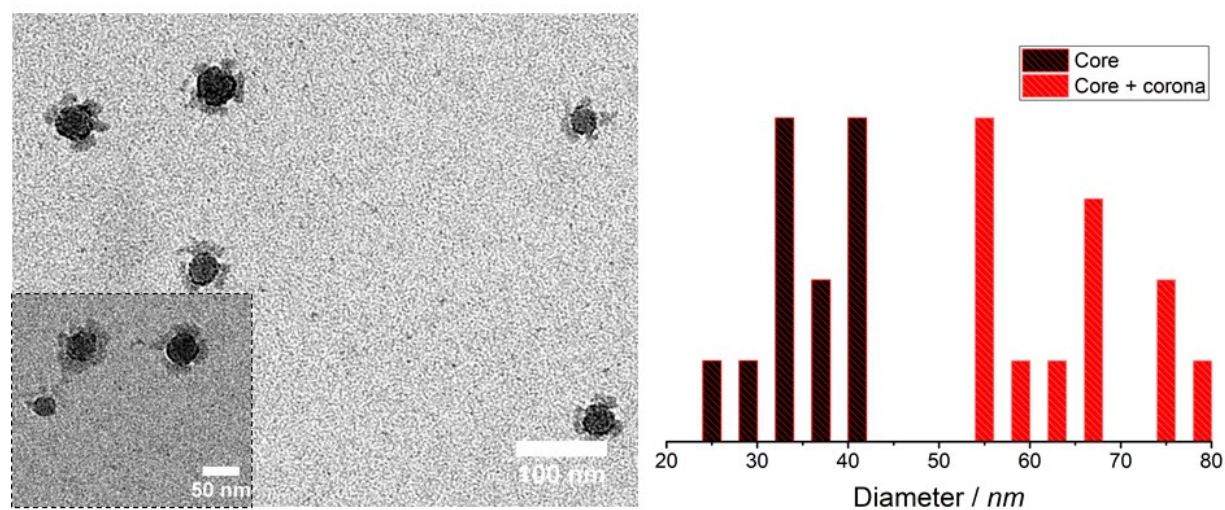


Figure S16. TEM images of **P5-10** micelles and size distribution after OsO₄ staining.

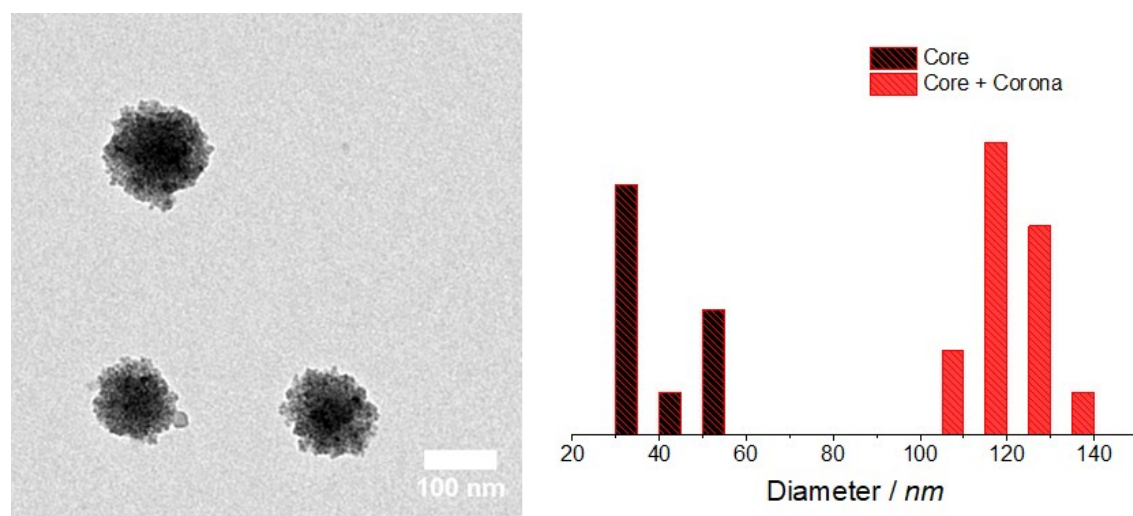


Figure S17. TEM images of **P5-10** micelles and size distribution after RuO₄ staining.

Additional staining agents were used in order to selectively visualize different polymer blocks and their location in the micelle. OsO₄ and RuO₄ are typically used to selectively stain double bonds and aromatic rings, respectively [8]. After staining with OsO₄ clear images showing core-corona division were obtained. In the case of RuO₄ the corona-core division is not as evident. Moreover the micellar size agrees with that one obtained by light scattering. This indicates the corona is more extensively stained with this agent.

7- References

- [1] A. Ferrer-Ugalde, E. J. Juárez-Pérez, F. Teixidor, C. Viñas, R. Sillanpää, E. Pérez-Inestrosa and R. Núñez, *Chem. Eur. J.*, 2012, **18**, 544-553.
- [2] A. M. Nyström and K. L. Wooley, *Tetrahedron*, 2008, **64**, 8543-8552.
- [3] A. M. Nyström, J. W. Bartels, W. Du and K. L. Wooley, *Journal of Polymer Science: Part A: Polymer Chemistry*, 2009, **47**, 1023-1037.
- [4] J. Dubochet, M. Adrian, J.-J. Chang, J.-C. Homo, J. Lepault, A. W. McDowell and P. Schultz, *Q. Rev. Biophys.*, 1988, **21**, 129–228.
- [5] J. X. Li, J. N. Ness, W. L. Cheung. *Journal of Applied Polymer Science*, 1996, **59**, 1733-1740.
- [6] M. Štěpánek, K. Procházka. *Langmuir* 1999, **15**, 8800-8806.
- [7] K. Krijtová, M. Štěpánek, K. Procházka, S. E. Webber. *J Fluoresc.* 1998, **8**, 21-25.
- [8] A. H. Gröschel, F. H. Schacher, H. Schmalz, O. V. Borisov, E. B. Zhulina, A. Walther, A. H.E. Müller. *Nat. Commun.* 2012, **3**, 1-10