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Perfluorocarbon-loaded Shell Crosslinked Knedel-like Nanoparticles: Lessons regarding polymer mobility and self assembly

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Abstract

Reversible addition-fragmentation chain transfer polymerization was employed to synthesize a set of copolymers of styrene (PS) and 2,3,4,5,6-pentafluorostyrene (PPFS), as well as block copolymers with *tert*-butyl acrylate (PtBA)-*b*-PS-*co*-PPFS, with control over molecular weight and polydispersity. It was found that the copolymerization of styrene and PFS allowed for the preparation of gradient copolymers with opposite levels of monomer consumption, depending on the feed ratio. Conversion to amphiphilic block copolymers, PAA-*b*-(PS-*co*-PPFS), by removing the protecting groups was followed by fitting with monomethoxy poly(ethylene glycol) chains. Solution-state assembly and intramolecular crosslinking afforded shell crosslinked (SCK) block copolymer nanoparticles. These fluorinated nanoparticles (*ca.* 20 nm diameters) were studied as potential magnetic resonance imaging (MRI) contrast agents based on the ¹⁹F-nuclei, however, it was found that packaging of the hydrophobic fluorinated polymers into the core domain restricted the mobility of the chains and prohibited ¹⁹F-NMR spectroscopy when the particles were dispersed in water without an organic cosolvent. Packing of perfluoro-15-crown-5-ether (PFCE) into the polymer micelle was demonstrated with good uptake efficiency, however, it was necessary to swell the core with a good solvent (DMSO) to increase the mobility and observe the ¹⁹F-NMR signal of the PFCE.

Keywords

Block copolymers; copolymerization; fluoropolymers; amphiphilic polymers; micelles; nanoparticles; reversible addition-fragmentation chain transfer (RAFT) polymerization; NMR

Introduction

Nanoparticle-based *in vivo* diagnostic imaging is one part of the broad research field of nanomedicine that is currently the focus of numerous interdisciplinary research groups worldwide.^{1–6} Nanoscale diagnostic constructs are being developed for a range of imaging modalities such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI), among others.^{1, 4, 5, 7–10} Apart from the size, the advantages of using nanoparticles for such applications include their surface multi-functionality and internal capacity, which provide the possibility to redirect the biodistribution of the particle by attaching tissue-specific ligands to the surface of the

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Supporting information available: ¹H-NMR spectra for the polymers, DLS of the micelles and SCKs, DSC traces of the polymers.

particle, and also to pack therapeutic cargos within the confined volume of the nanoparticle.
2, 3, 6, 11-15

Although MRI can suffer from sensitivity issues, MRI-based diagnostics have the benefit of both not relying on ionizing radiation and displaying larger contrast between different types of biological soft-tissues, which is important in cancer diagnostics and imaging of the brain, muscle, and cardiovascularity.⁴ Clinical MRI is typically based on ^1H nuclei and is sometimes also used in conjunction with paramagnetic contrast agents such as Gd^{III} (which increases contrast in T_1 weighted imaging, where T_1 is the spin-lattice relaxation time) or superparamagnetic iron oxide nanoparticles (which provide contrast in T_2 weighted imaging, where T_2 is the spin-spin relaxation time).¹⁶⁻²⁰ Other contrast agents that are under development are based on other nuclei, for instance ^{19}F , which is thought to be the most useful in functional MRI applications and for applications involving *in vivo* drug monitoring.^{1, 4, 21-23} Fluorinated contrast agents have the advantage of having little or no background signal in tissue, a high gyromagnetic ratio, good biocompatibility, and a NMR-shift that is highly sensitive to changes in local microenvironment.^{24, 25} As ^{19}F contrast agents, fluorinated compounds, such as small molecule perfluorocarbons (PFCs) with high fluorine content and a single fluorine resonance, have been utilized. Examples of such small molecules are perfluoro-15-crown-5-ether (PFCE)²⁶⁻²⁸ and perfluorobenzene (PFB),²⁹ among others.^{27, 29, 30} For MRI applications a high fluorine concentration is advantageous since it enhances the signal to noise ratio (SNR) and decreases the scanning time necessary for high resolution image acquisition. For *in vivo* applications, these PFCs have been previously emulsified into lipid particle formulations with sizes in the range of several hundred nm.^{27, 31, 32} The large size of these lipids restricts these contrast agents from imaging smaller features than the vasculature with *intravenous* administration. In comparison, systems based on polymer- and protein-based fluorinated materials suffer from low elemental concentration of fluorine, due to dilution by other atoms, limiting the fluorine concentration attainable and the applicability as ^{19}F -MRI contrast agents.³³⁻³⁹ These polymers (self assembled into polymer micelles or as free polymers) are, therefore, less suitable as blood pool contrast agents, and must either be concentrated by cellular uptake (for cell tracking *in vivo*) or fitted with targeting ligands for tissue specific uptake and local concentration enrichment.^{22, 35, 40-42}

Another unique possibility to create small nanoscale (<30 nm) MRI contrast agents is to sequester highly fluorinated small molecules within the core domain of a polymeric nanoparticle. Shell crosslinked knedel-like (SCK) nanoparticles are one such construct, in which hydrophobic therapeutics can be loaded within the core domain.⁴³⁻⁴⁶ The sequestering of small molecules within the nanoparticle framework may be achieved by either selective swelling of the core with organic solvent or by performing the self assembly in the presence of the PFC, after which the establishment of hydrophobic interactions between the core and the small molecule entities stabilizes the host-guest system.⁴⁴ SCK nanoparticles are derived from polymeric micelles assembled in aqueous solution from amphiphilic block copolymers; these micelles are then crosslinked throughout the shell domain.^{47, 48} The introduction of covalent crosslinks within the nanostructures provides stabilization of the structure, allowing for higher loading capacity, and also circumventing limitations that the critical micelle concentration (CMC) imposes on polymeric micelle assemblies for *in vivo* applications.⁴⁵⁻⁴⁹ SCKs can be prepared from a range of amphiphilic block copolymers that are easily accessible by controlled radical polymerization techniques.^{43, 45, 46, 50, 51} This versatility allows for the preparation of a wide range of nanostructures, for which the hydrophilic/hydrophobic block ratio, degree of polymerization, and functionality of the core and shell domains can each be tuned.^{43, 45, 50, 51} In addition, these robust structures can be fitted with multiple detection elements and/or targeting moieties for *in vivo* diagnostic applications.^{9, 10, 25, 52-55}

In this current work, we were interested in creating SCK nanoparticles for which the core domains are optimized to passively harbor a high loading of PFCs. Such nanoparticle constructs may then further serve as ^{19}F -MRI contrast agents for targeted *in vivo* applications, after the attachment of tissue specific targeting groups, such as peptides and/or antibodies, to enhance local concentration. We hypothesized that the loading capacity of these nanoparticles for PFCs would be enhanced if the core domain consisted of amorphous fluorinated hydrophobic polymer. Therefore, the core domain of these nanoparticles was constructed as a block copolymer of PFS and styrene. As coincidence, it was found that the unique reactivity ratios of these monomers gave tapered/gradient block copolymers, for which the corresponding nanoparticle construct would either have the fluorinated polymer segments topologically placed closer to the core-shell interface, or buried within the core domain, allowing for further insight into how to optimize nanoparticles for cargo carrying applications and the effects on ^{19}F -NMR properties.

Experimental

Instrumental

^1H -NMR, ^{13}C -NMR, and ^{19}F -NMR spectra were recorded as solutions in CDCl_3 , CD_2Cl_2 , CD_2Cl_2 -TFA-*d*, or $\text{DMSO}-d_6$ on a Varian Unity-plus 300 or Varian Inova 500 spectrometer, with the solvent proton signal as internal standard.

Gel permeation chromatography (GPC) was conducted on a Waters 1515 HPLC (Waters Chromatography, Inc.) equipped with a Waters 2414 differential refractometer, and a three-column series PL_{gel} 5 μm Mixed C, 500 Å, and 10^4 Å, 300×7.5 mm columns (Polymer Laboratories, Inc.). The system was equilibrated at 35 °C in THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL/min. Polymer solutions were prepared at a known concentration (*ca.* 1–3 mg/mL) and an injection volume of 200 μL was used. Data collection and analysis were performed, respectively, with Precision Acquire software and Discovery 32 software (Precision Detectors, Inc.).

The height measurements (H_{av}) and distributions for the SCKs were determined by tapping-mode atomic force microscopy (AFM) under ambient conditions in air. The AFM instrumentation consisted of a Nanoscope III BioScope system (Digital Instruments, Veeco Metrology Group; Santa Barbara, CA) and standard silicon tips (type, OTESPA-70; L, 160 μm ; normal spring constant, 50 N/m; resonance frequency, 246–282 kHz). The sample solutions were either spin cast from native concentration, or drop (50–100 μL) deposited onto freshly-cleaved mica and allowed to settle freely for 30 seconds, after which the excess solution was removed by a filter paper and the mica surface was allowed to dry in air.

Hydrodynamic diameters (D_h) and distributions for the SCKs in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Brookhaven Instruments Limited (Worcestershire, U.K.) system, including a model BI-200SM goniometer, a model BI-9000AT digital correlator, a model EMI-9865 photomultiplier, and a model 95–2 Ar ion laser (Lexel, Corp.; Farmindale, NY) operated at 514.5 nm. Measurements were made at 25 ± 1 °C. Prior to analysis, solutions were filtered through a 0.45 μm Nylon filter, and centrifuged in a model 5414 microfuge (Brinkman Instruments, Inc.; Westbury, NY) for 4 min to remove dust particles. Scattered light was collected at a fixed angle of 90 °. The digital correlator was operated with 522 ratio spaced channels and initial delay of 0.5 μs , a final delay of 800 ms, and a duration of 10 min. A photomultiplier aperture of 400 μm was used, and the incident laser intensity was adjusted to obtain a photon counting of 300 kcps. Only measurements in which the measured and calculated baselines of the intensity autocorrelation function agreed to within 0.1% were used to calculate particle size. Particle size distributions were performed with the ISDA

software package (Brookhaven Instruments Company), which employed CONTIN particle size distribution analysis routine. The data presented is the average of 3 or 4 runs and the standard deviation is the variation between these runs and not the error in each.

The ^{19}F relaxation parameters experiments, such as the T_1/T_2 measurements, of the micelles were conducted on a Varian Inova 500 MHz spectrometer using inversion recovery spectroscopy with 10 inversion times for T_1 , and multi-echo spin echo spectroscopy with 10 different echo times for T_2 . For T_1 measurements, $sw = 20000$ Hz, $pw = 13.75$ μs , $nt = 32$. For T_2 measurements, $sw = 20000$ Hz, $pw = 13.75$ μs , $nt = 32$.

Differential scanning calorimetric (DSC) studies were performed on a Mettler Toledo DSC822 (Mettler Toledo, GmbH.), calibrated according to standard procedures. The heating and cooling rates were 10 $^{\circ}\text{C min}^{-1}$ with a temperature range of -20 $^{\circ}\text{C}$ to 150 $^{\circ}\text{C}$. The glass transition temperatures (T_g) were determined as the midpoint of the tangent to the inflection, during the second heating run.

Materials

Polymerizations were performed on a double manifold (0.1 mm Hg vacuum, 99.99% N_2), with glassware and syringes that were dried in an oven (100 $^{\circ}\text{C}$) for at least 1 h, and with syringes that were washed with N_2 (3 x), prior to use. Styrene (Sty, 99%), Pentafluorostyrene (PFS, 99%) and *tert*-butyl acrylate (*t*-BA, 99%) were received from Sigma-Aldrich Company (St. Louis, MO) and distilled from calcium hydride, and stored under N_2 prior to use. Trifluoroacetic acid (TFA; 95%; Aldrich), 2,2'-(ethylenedioxy)-bis(ethylamine) (97%; Aldrich), and 1-(3'-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI; 98%; Aldrich) were used as received. MeO-PEG_{2kDa}-NH₂ was obtained from Intezyme Technologies. Perfluoro-15-crown-5-ether (PFCE) was obtained from Oakwood Chemicals. *S*-1-dodecyl-*S'*-(α,α' -dimethyl- α'' -acetic acid)trithiocarbonate (DDMAT) (**1**) was synthesized as reported previously.⁵⁶ Spectra/Por membrane tubes were purchased from Spectrum Medical Industries, Inc., and were used for dialysis. Nanopure water (18 $\text{M}\Omega\cdot\text{cm}$) was acquired by means of a Milli-Q water filtration system (Millipore Corp.). All other reagents were obtained from Sigma-Aldrich and used as received.

Synthesis

Preparation of PS-co-PPFS (**2**) 75:25; general copolymerization procedure

A flame-dried 25 mL Schlenk flask equipped with a magnetic stir bar was charged with DDMAT **1** (93.5 mg, 0.256 mmol), styrene (Sty, 2.00 g, 19.2 mmol), pentafluorostyrene (PFS, 1.24 g, 6.41 mmol), AIBN (4.2 mg, 26 μmol), and 1,4-dioxane (2.26 g, 25.6 mmol). The flask was sealed with a rubber septum and stirred for 10 min at room temperature to ensure a homogeneous mixing. The reaction mixture was degassed by several freeze-pump-thaw cycles (> 3), after which the flask was allowed to return to room temperature and was stirred for an additional 10 min. The flask was then immersed into a pre-heated oil bath at 60 $^{\circ}\text{C}$ to start the polymerization. The polymerization was monitored by analyzing aliquots collected at pre-determined times by ^1H -NMR spectroscopy. As the expected monomer conversion was reached, after *ca.* 19 h, the polymerization was quenched by quick immersion of the reaction flask into liquid N_2 . THF (15 mL) was added to the reaction flask and the polymer was purified by precipitation into methanol (3×500 mL). The precipitants were collected and dried under vacuum overnight to afford **2** (1.1 g, yield 92 %, conversion Sty 30 %, conversion PFS 49 %) as a pale yellow powder. ^1H -NMR: $M_n = 5100$ Da. GPC RI: $M_n = 5500$ Da, $M_w = 6100$ Da, PDI = 1.10. GPC LS: $M_n = 4800$ Da, $M_w = 4900$ Da, PDI = 1.02. ^1H -NMR (CD_2Cl_2): δ 0.85–0.91 (m, CH_3CH_2 -), 0.97–1.34 (br, -alkyl chain of initiator, and $\text{HOCC}(\text{CH}_3)_2$ -), 1.35–2.82 (br, CHCH_2 - and $-\text{CHCH}_2$ - of the polymer backbone), 3.18–3.36 (br, $-\text{SCSCH}_2$ -), 4.62–4.80 (br, $-\text{CH}_2\text{CHS}$), 6.25–7.25 (br,

Ar-*H*) ppm. ^{19}F -NMR (CD_2Cl_2 relative to 1,1,2-trichloro-1,1,2-trifluoroethane): δ Ar*F-ortho* (−74.4–(−)75.4), Ar*F-para* (−87.4–(−)92.0), Ar*F-meta* (−94.2–(−)96.8), ppm. IR (KBr): 3100–3000, 3000–2800, 2070, 1951, 1877, 1700, 1652, 1522, 1496, 1455, 1418, 1365, 1302, 1215, 1128, 1091, 962, 865, 761, 702, 549 cm^{-1} .

Preparation of PS-co-PPFS (3) 25:75

DDMAT **1** (93.5 mg, 0.256 mmol), styrene (Sty, 0.66 g, 6.4 mmol), pentafluorostyrene (PFS, 3.73 g, 19.2 mmol), AIBN (4.2 mg, 26 μmol), and 1,4-dioxane (2.26 g, 25.6 mmol) were allowed to react according to the general polymerization procedure outlined for **2**. The polymer was purified by precipitation into methanol ($3 \times 500 \text{ mL}$). The precipitants were collected and dried under vacuum overnight to afford **3** (1.4 g, yield 91 %, conversion Sty 58 %, conversion PFS 35 %) as a pale yellow powder. ^1H -NMR: $M_n = 7000 \text{ Da}$. GPC RI: $M_n = 6800 \text{ Da}$, $M_w = 7400 \text{ Da}$, PDI = 1.09. GPC LS: $M_n = 8500 \text{ Da}$, $M_w = 8600 \text{ Da}$, PDI = 1.02. ^1H -NMR (CD_2Cl_2): δ 0.91–0.96 (m, CH_3CH_2 -), 1.03–1.41 (br, - alkyl chain of initiator, and $\text{HOCC}(\text{CH}_3)_2$ -), 1.35–2.82 (br, CHCH_2 - and $-\text{CHCH}_2$ - of the polymer backbone), 3.22–3.41 (br, $-\text{SCSCH}_2$ -), 4.64–4.81 (br, $-\text{CH}_2\text{CHS}$), 6.38–7.25 (br, Ar-*H*) ppm. ^{19}F -NMR (CD_2Cl_2 relative to 1,1,2-trichloro-1,1,2-trifluoroethane): δ Ar*F-ortho* (−74.4–(−)75.4), Ar*F-para* (−87.4–(−)92.0), Ar*F-meta* (−94.2–(−)96.8), ppm. IR (KBr): 3100–3000, 3000–2800, 1945, 1870, 1807, 1743, 1699, 1656, 1598, 1521, 1498, 1453, 1368, 1298, 1202, 1091, 1029, 961, 869, 759, 700, 545 cm^{-1} .

Preparation of Pt BA (4)

A flame-dried 50 mL Schlenk flask equipped with a magnetic stir bar was charged with DDMAT **1** (406 mg, 1.12 mmol), *t*-BA (20.0 g, 156 mmol), AIBN (9.2 mg, 56 μmol), and 2-butanone (11.3 g, 156 mmol). The flask was sealed with a rubber septum and stirred for 10 min at room temperature to ensure a homogeneous mixing. The reaction mixture was degassed by several freeze-pump-thaw cycles (> 3), after which the flask was allowed to return to room temperature and was stirred for an additional 10 min. The flask was then immersed into a pre-heated oil bath at 60 $^\circ\text{C}$ to start the polymerization. The polymerization was monitored by analyzing aliquots collected at pre-determined times by ^1H -NMR spectroscopy. As the expected monomer conversion was reached, after *ca.* 4.5 h, the polymerization was quenched by quick immersion of the reaction flask into liquid N_2 . THF (20 mL) was added to the reaction flask and the polymer was purified by precipitation into 2 L of a methanol/ice mixture thrice. The precipitants were collected and dried under vacuum overnight to afford **4** (12.6 g, yield 74 %, conversion 68 %) as a yellow powder. ^1H -NMR: $M_n = 13200 \text{ Da}$. GPC RI: $M_n = 11900 \text{ Da}$, $M_w = 12800 \text{ Da}$, PDI = 1.07. GPC LS: $M_n = 20300 \text{ Da}$, $M_w = 21300 \text{ Da}$, PDI = 1.05. ^1H -NMR (CDCl_3): δ 0.86 (m, CH_3CH_2 -), 1.14–1.85 (br, $-\text{CHCH}_2$ - of the polymer backbone, alkyl chain of initiator, and $\text{HOCC}(\text{CH}_3)_2$ -), 1.36–1.56 (br, CH_3C), 2.14–2.37 (br, $-\text{CHCH}_2$ - of the polymer backbone), 3.25–3.38 (br, $-\text{SCSCH}_2$ -), 4.62–4.72 (br, $-\text{CH}_2\text{CHS}$) ppm. IR (KBr): 3000–2800, 1734, 1457, 1391, 1367, 1257, 1148, 1035, 905, 845 cm^{-1} .

Preparation of Pt BA-*b*-(PS-co-PPFS) 75:25, general procedure for the block extension (5)

A flame-dried 25 mL Schlenk flask equipped with a magnetic stir bar was charged with PtBA **4** (2.0 g, 0.16 mmol), Sty (1.2 g, 12 mmol), PFS (0.75 g, 3.9 mmol), AIBN (2.5 mg, 15 μmol), and 1,4-dioxane (1.36 g, 15.4 mmol). The flask was sealed with a rubber septum and stirred for 10 min at room temperature to ensure a homogeneous mixing. The reaction mixture was degassed by several freeze-pump-thaw cycles (> 3), after which the flask was allowed to return to room temperature and was stirred for an additional 10 min. The flask was then immersed into a pre-heated oil bath at 70 $^\circ\text{C}$ to start the polymerization. The polymerization was monitored by analyzing aliquots collected at pre-determined times by ^1H -NMR spectroscopy. As the expected monomer conversion was reached, after *ca.* 8.5

h, the polymerization was quenched by quick immersion of the reaction flask into liquid N₂. THF (10 mL) was added to the reaction flask and the polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford **5** (1.7 g, yield 66 %, conversion Sty 25 %, conversion PPFS 39 %) as a pale yellow powder. ¹H-NMR: M_n = 19000 Da. GPC RI: M_n = 22100 Da, M_w = 26200 Da, PDI = 1.18. GPC LS: M_n = 14700 Da, M_w = 16500 Da, PDI = 1.13. ¹H-NMR (CD₂Cl₂): δ 0.92 (m, CH₃CH₂-), 1.02–2.45, 2.47–2.80 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, -CH₃C, alkyl chain of initiator, and HOCC(CH₃)₂-), 3.27–3.40 (br, -SCSCH₂-), 4.64–4.78 (br, -CH₂CHS), 6.32–7.31 (br, *Ar-H*) ppm. ¹⁹F-NMR (CD₂Cl₂ relative to 1,1,2-trichloro-1,1,2-trifluoroethane): δ *ArF-ortho* (−74.4–(−)75.4), *ArF-meta* (−87.4–(−)92.0), *ArF-meta* (−94.2–(−)96.8) ppm. IR (KBr): 3100–3000, 3000–2800, 1734, 1654, 1602, 1522, 1499, 1457, 1368, 1257, 1148, 1033, 962, 908, 846, 758, 700, 545, 471, 428 cm^{−1}.

Preparation of **PtBA-*b*-(PS-co-PPFS) 25:75 (6)**

PtBA **4** (2.0 g, 0.15 mmol), Sty (0.40 g, 3.9 mmol), PFS (2.24 g, 11.5 mmol), AIBN (2.5 mg, 15 μmol), and 1,4-dioxane (1.36 g, 15.4 mmol) were reacted according to the general block extension procedure outlined in **5**. The polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford **6** (2.2 g, yield 81 %, conversion Sty 46 %, conversion PFS 25 %) as a yellow powder. ¹H-NMR: M_n = 21000 Da. GPC RI: M_n = 21100 Da, M_w = 23300 Da, PDI = 1.16. GPC LS: M_n = 18600 Da, M_w = 21800 Da, PDI = 1.17. ¹H-NMR (CD₂Cl₂): δ 0.91 (m, CH₃CH₂-), 1.03–2.58, 2.59–2.92 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, -CH₃C, alkyl chain of initiator, and HOCC(CH₃)₂-), 3.25–3.39 (br, -SCSCH₂-), 4.61–4.74 (br, -CH₂CHS), 6.37–7.38 (br, *Ar-H*) ppm. ¹⁹F-NMR (CD₂Cl₂ relative to 1,1,2-trichloro-1,1,2-trifluoroethane): δ *ArF-ortho* (−74.4–(−)75.4), *ArF-para* (−87.4–(−)92.0), *ArF-meta* (−94.2–(−)96.8) ppm. IR (KBr): 3100–3000, 3000–2800, 1732, 1652, 1524, 1504, 1456, 1394, 1368, 1257, 1149, 962, 846, 761, 703, 550, 472, 431 cm^{−1}.

Preparation of **PAA-*b*-(PS-co-PPFS) 75:25; general procedure for TFA deprotection of the polymers (7)**

A flame-dried 50 mL round bottom flask equipped with a magnetic stir bar was charged with PtBA-*b*-(PS-co-PPFS) **5** (1.3 g, 68 μmol), and 5 mL of dichloromethane. TFA (5 mL, 70 mmol) was added to the stirred solution and the reaction was stirred for 20 h at room temperature, after which the solvent was removed under vacuum. The crude product was resuspended in 15 mL of THF and transferred to a presoaked dialysis tubing (MWCO *ca.* 6,000–8,000 Da), and dialyzed against nanopure H₂O for 4 days, to remove all of the impurities, after which the solution was lyophilized to yield polymer **7** as a yellowish solid (0.86 g, 94 %). ¹H-NMR: M_n = 13800 Da. ¹H-NMR (DMSO-*d*₆): δ 0.94 (m, CH₃CH₂-), 1.10–2.05, 2.10–2.38 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, alkyl chain of initiator, and HOCC(CH₃)₂-), 6.21–7.43 (br, *Ar-H*), 12.10–12.37 (br, -COOH) ppm. ¹⁹F-NMR (DMSO-*d*₆ relative to trifluoroacetic acid): δ *ArF-ortho* (−65.2–(−)70.4), *ArF-para* (−82.4–(−)87.0), *ArF-meta* (−87.4–(−)92.1) ppm. IR (KBr): 3500–2500, 1718, 1522, 1499, 1453, 1416, 1243, 962, 801, 760, 700, 520 cm^{−1}.

Preparation of **PAA-*b*-(PS-co-PPFS) 25:75 (8)**

PtBA-*b*-(PS-co-PPFS) **6** (2.0 g, 95 μmol), 5 mL dichloromethane, and TFA (5 mL, 70 mmol) were allowed to react according to the general procedure outlined in **7** to afford polymer **8** as a white solid after lyophilization (1.35 g, 92 %). ¹H-NMR: M_n = 15400 Da. ¹H-NMR (DMSO-*d*₆): δ 0.93 (m, CH₃CH₂-), 1.03–2.01, 2.05–2.41 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, alkyl chain of initiator, and HOCC(CH₃)₂-), 6.18–7.27 (br, *Ar-H*), 11.90–12.42 (br, -COOH) ppm. ¹⁹F-NMR (DMSO-*d*₆ relative to trifluoroacetic acid): δ *ArF-ortho* (−64.6–(−)70.5), *ArF-para* (−77.8–(−)87.1), *ArF-meta* (−87.2–

(−)92.0) ppm. IR (KBr): 3500–2500, 1713, 1523, 1499, 1455, 1366, 1243, 1173, 1090, 1057, 961, 866, 801, 702, 601, 523 cm^{−1}.

Preparation of PAA-*g*-(mPEG)-*b*-(PS-*co*-PPFS) 75:25; general procedure for amidation of the polymers (9)

A flame-dried 25 mL round bottom flask equipped with a magnetic stir bar was charged with polymer **7** (600 mg, 44.8 μmol) and 10 mL of dry DMF. To the stirred solution HOBt (61 mg, 450 μmol) and EDCI (86 mg, 450 μmol) were added and the reaction was left to proceed for 1 h, after which a solution of MeO-PEG_{2kDa}-NH₂ (448 mg, 224 μmol, 5 eq.) dissolved in 2 mL of DMF was added. The reaction mixture was further stirred for 20 h at room temperature before being transferred to presoaked dialysis tubing (MWCO *ca.* 6,000–8,000 Da), and dialyzed against nanopure H₂O for 4 days, to remove all of the impurities and afford polymer **9** as a white solid after lyophilization (728 mg, 93 %, 2 mPEG/polymer chain, 40 % coupling efficiency). ¹H-NMR: M_n = 17800 Da. ¹H-NMR (DMSO-*d*₆): δ 0.92 (br, CH₃CH₂-), 1.03–2.01, 2.05–2.37 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, alkyl chain of initiator, and HOCC(CH₃)₂-), 3.46–3.54 (br, -OCH₂CH₂O-), 6.22–7.28 (br, *Ar-H*), 11.88–12.45 (br, -COOH) ppm. ¹⁹F-NMR (DMSO-*d*₆ relative to trifluoroacetic acid): δ *ArF-ortho* (−65.0–(−)68.0), *ArF-para* (−82.0–(−)85.0), *ArF-meta* (−87.1–(−)90.2) ppm. IR (KBr): 3500–2500, 1732, 1504, 1455, 1351, 1294, 1162, 961, 843, 757, 700, 667, 536 cm^{−1}.

Preparation of PAA-*g*-(mPEG)-*b*-(PS-*co*-PPFS) 25:75 (10)

8 (600 mg, 38.9 μmol), 10 mL DMF, HOBt (53 mg, 200 μmol), EDCI (75 mg, 200 μmol) and MeO-PEG_{2kDa}-NH₂ (389 mg, 195 μmol, 5 eq.) were allowed to react according to the general procedure outlined in **9** to afford polymer **10** as a white solid after lyophilization (730 mg, 88 %, 3 mPEG/polymer chain, 60 % coupling efficiency). ¹H-NMR (DMSO-*d*₆): δ 0.90 (br, CH₃CH₂-), 1.03–2.03, 2.07–2.40 (br, -CHCH₂- and -CHCH₂- of the polymer backbone, alkyl chain of initiator, and HOCC(CH₃)₂-), 3.44–3.58 (br, -OCH₂CH₂O-), 6.30–7.32 (br, *Ar-H*), 11.90–12.56 (br, -COOH) ppm. ¹⁹F-NMR (DMSO-*d*₆ relative to trifluoroacetic acid): δ *ArF-ortho* (−64.2–(−)70.4), *ArF-para* (−79.2–(−)87.1), *ArF-meta* (−87.2–(−)92.4) ppm. IR (KBr): 3500–2500, 1732, 1651, 1520, 1503, 1455, 1353, 1167, 961, 866, 803, 761, 702, 652, 536 cm^{−1}.

General procedure for the preparation of the micelles (11)

To a solution of diblock copolymer **9**, in DMF (50 mg, 1.0 mg mL^{−1}), an equal volume of nanopure H₂O was added dropwise *via* a syringe pump over the course of 5 h. The reaction mixture was further stirred *ca.* 16 h at room temperature before being transferred to presoaked dialysis tubing (MWCO *ca.* 6,000–8,000 Da), and dialyzed against nanopure H₂O for 4 days, to afford a micelle solution **11**, with the final polymer concentration of 0.255 mg/mL. (D_h)_n (DLS) = 18 ± 2 nm; (D_h)_{vol} (DLS) = 23 ± 3 nm; (D_h)_{int} (DLS) = 51 ± 6 nm; H_{av} (AFM) = 5 ± 1 nm. ¹⁹F-NMR (D₂O relative to trifluoroacetic acid + 10 % (vol) DMSO): δ No signal detected.

Preparation of micelle (12)

Polymer **10** was assembled into micelle **12** according to the general procedure outlined in **11**, to give a micelle with a final concentration of 0.240 mg mL^{−1}. (D_h)_n (DLS) = 17 ± 2 nm; (D_h)_{vol} (DLS) = 28 ± 4 nm; (D_h)_{int} (DLS) = 116 ± 10 nm; H_{av} (AFM) = 6 ± 1 nm. ¹⁹F-NMR (D₂O relative to trifluoroacetic acid + 10 % (vol) DMSO): δ No signal detected.

General procedure for the preparation of the SCKs (13)

A solution of 2,2'-(ethylenedioxy)bis(ethylamine) in nanopure H₂O (*ca.* 1.0 mg/mL, nominal 50 % crosslinking, 19.2 mg, 130 μ mol) was added dropwise over 15 min to a solution of micelle **11** in nanopure H₂O (150 mL, 0.255 mg mL⁻¹ polymer concentration). The reaction mixture was stirred for *ca.* 1 h at room temperature. To this solution, a solution of 1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide in nanopure H₂O (32 mg, 140 μ mol) was added dropwise *via* syringe pump over 15 min. The reaction mixture was further stirred 20 h at room temperature before being transferred to presoaked dialysis tubing (MWCO *ca.* 6,000–8,000 Da), and dialyzed against nanopure water for 4 days, to remove all of the impurities and afford the SCK solution **13** with a final concentration of 0.25 mg mL⁻¹. (D_h)_n (DLS) = 26 $\pm$ 2 nm; (D_h)_{vol} (DLS) = 48 $\pm$ 5 nm; (D_h)_{int} (DLS) = 136 $\pm$ 4 nm; H_{av} (AFM) = 32 $\pm$ 4 nm. ¹⁹F-NMR (D₂O relative to trifluoroacetic acid + 10 % (vol) DMSO): δ No signal detected.

Preparation of the SCKs (14)

Micelle **12** was crosslinked into SCK **14** according to the general procedure outlined in **13**, to give a SCK with a final concentration of 0.238 mg mL⁻¹. (D_h)_n (DLS) = 22 $\pm$ 3 nm; (D_h)_{vol} (DLS) = 32 $\pm$ 3 nm; (D_h)_{int} (DLS) = 85 $\pm$ 10 nm; H_{av} (AFM) = 20 $\pm$ 3 nm. ¹⁹F-NMR (D₂O relative to trifluoroacetic acid + 10 % (vol) DMSO): δ No signal detected.

General procedure for the loading of perfluoro-15-crown-5-ether into the SCKs (15–20)

A solution of perfluoro-15-crown-5-ether (PFCE, 13.5 mg mL⁻¹ in CH₂Cl₂), 50% w/w with respect to the SCK) was added to a vial containing a magnetic stir bar and the SCK solution (4 mL, polymer concentration $\sim$ 0.25 mg mL⁻¹). The solution was protected from light and stirred without a cap in a well-ventilated fume hood for 16 h in order to evaporate the CH₂Cl₂. The solution was then transferred to a centrifugal filter device (Amicon Ultra 4, 30 kDa MWCO, Millipore Corp., Billerica MA, USA) and washed extensively with 5 mM PBS pH 7.4 buffer at 37 °C to remove unincorporated PFCE. The PFCE-nanoparticle solution was then reconstituted to a final volume of 4 mL. ¹⁹F-NMR (D₂O relative to trifluoroacetic acid): δ No signal detected.

General procedure for the micellization in the presence of PFCs (21)

To a solution of diblock copolymer **9**, in DMF (30 mg, 1.0 mg mL⁻¹) and perfluoro-15-crown-5-ether (15 mg), an equal volume of nanopure H₂O was added dropwise *via* a syringe pump over the course of 4 h. The reaction mixture was further stirred *ca.* 16 h at room temperature before being transferred to presoaked dialysis tubing (MWCO *ca.* 6,000–8,000 Da), and dialyzed against nanopure H₂O for 4 days, to afford a micelle solution **21**, with the final polymer concentration of 0.261 mg/mL. (D_h)_n (DLS) = 20 $\pm$ 3 nm; (D_h)_{vol} (DLS) = 31 $\pm$ 6 nm; (D_h)_{int} (DLS) = 158 $\pm$ 12 nm; ¹⁹F-NMR (D₂O relative to trifluoroacetic acid + 10 % (vol) DMSO): δ PFCE 6.2 ppm.

Results and Discussion

Preparation of the copolymers

As a first step, two copolymers of styrene (Sty) and 2,3,4,5,6-pentafluorostyrene (PFS) were prepared in order to investigate the kinetics of the copolymerization of these monomers. The copolymers were prepared according to Scheme 1, by RAFT copolymerization in 1,4-dioxane solution using two different feed ratios of Sty and PFS with AIBN as radical initiator and *S*-1-dodecyl-*S'*-(α,α' -dimethyl- α'' -acetic acid)trithiocarbonate (DDMAT) as chain transfer agent (CTA).⁵⁶ As depicted in Figure 1 (top) and summarized in Table 1 (entries 1–2), polymers **2** and **3** showed unimodal and narrow molecular weight

distributions (polydispersity indices (*PDI*) < 1.2), as measured by GPC, indicating good control over the copolymerization process. There was agreement between the molecular weight values obtained by GPC and those determined by ¹H-NMR spectroscopy. ¹H-NMR spectroscopy also allowed for monitoring of the consumption of the two monomers independently, due to the unique shifts of their vinyl protons. A unique and opposite preferential incorporation of Sty vs. PFS monomers was observed for the two polymerizations that employed a majority of PFS vs. Sty monomers, respectively, in the feed. For the copolymerization with a monomer feed of 75:25 mol % Sty:PFS, it was found that the conversion of Sty was only 30 %, whereas 49% of PFS had undergone polymerization, after 19 h. The much faster propagation of the PFS monomer indicates that the polymer forms a tapered copolymer (PPFS-*tap*-PS), with the majority of the PFS consumed early during the polymerization. Still, the resulting polymer was comprised predominantly of Sty repeat units. In the case of the reversed monomer feed (25:75 Sty:PFS), the conversion was 58 % for Sty and only 35 % for PFS, suggesting that the polymer obtained is a tapered copolymer with the opposite connectivity (PS-*tap*-PPFS), again, though having an overall dominance of PFS in the polymer composition.

Synthesis of the block copolymers

The amphiphilic block copolymers in this study were prepared in a four-step fashion outlined in Scheme 1, by the copolymerization of Sty and PFS from a macro chain transfer agent (macroCTA). We hypothesized that having a high *T_g* and fluorine-rich hydrophobic chain segment would help to increase the loading capacity of small molecule fluorinated components in the corresponding shell crosslinked nanoparticle core domain. The macroCTA of *tert*-butyl acrylate (*t*BA) was prepared by RAFT polymerization of *t*BA in 2-butanone with DDMAT as the CTA, followed by chain extension with different ratios of Sty and PFS to yield well-defined block copolymers **5** and **6**. As in the case of the copolymers described in the previous section, the copolymerization of Sty and PFS resulted in similarly tapered monomer consumptions, with higher PFS conversion for the copolymer with low PFS feed. GPC characterization of the block copolymers indicated a clear molecular weight increase from the macroCTA after chain extension with Sty and PFS (Figure 1 (bottom)). The *PDI*'s of the block copolymers were low (<1.2), however, a small tail towards lower molecular weight can also be seen for polymer **6**. Both block copolymers had molecular weights in fair agreement with the theoretical values (Table 1) indicating good control over the polymerization. The amphiphilic block copolymers were prepared by the removal of the *tert*-butyl group of the *Pt*BA by acidolysis with TFA in CH₂Cl₂ to yield the PAA-*b*-(PS-*tap*-PPFS) **7** and PAA-*b*-(PPFS-*tap*-PS) **8** block copolymers.

Because of our interest, ultimately, in the application of the final materials in medical applications and since systemically administered nanoparticles tend to be sequestered by the mononuclear phagocyte system (MPS, also referred to as the reticuloendothelial system (RES)), and rapidly accumulate mainly in the liver and spleen, it is necessary to increase the blood circulation time of these materials if they are to be used *in vivo*.⁵⁴ The biodistribution can be altered by attaching poly(ethylene glycol) chains to the diblock copolymer. For this purpose, 2 kDa monomethoxy-terminated poly(ethylene glycol) (mPEG) chains were grafted by amidation onto the acrylic acid segments of the block copolymer backbones of **7** and **8**, respectively, to yield polymer **9** with approximately two mPEG grafts per polymer chain and polymer **10** with three mPEG grafts per polymer chain.⁵⁴ These polymers were then further self assembled into micelles and SCKs.

Preparation of the SCKs

As depicted in Scheme 2, micelles from the PAA-*g*-PEG-*b*-(PS-*co*-PPFS) block copolymers **9** and **10** were prepared by following the protocol described in the experimental section to

result in polymeric micelles **11** and **12**. The SCKs were obtained by intramolecular crosslinking of their hydrophilic shell layers through condensation reactions, nominally, upon 50% of the carboxylic acid functionalities of the PAA segments with a diamino crosslinker (2,2'-(ethylenedioxy) bis-(ethylamine)) to yield SCKs **13** and **14**. The crosslinking reactions were followed by extensive dialysis against nanopure water to remove the small molecule by-products.[†]

Characterization of the SCKs

The shapes and sizes of micelles **11** and **12** and SCKs **13** and **14** were investigated by atomic force microscopy (AFM) and dynamic light scattering (DLS). The results of these characterizations are summarized in Table 2. AFM imaging on mica was employed to characterize the micelles and SCKs. The micelles **11** and **12** were observed to flatten on the mica substrate and were found to have average heights of *ca.* 8 and 7 nm, respectively. Crosslinking to afford SCKs **13** and **14** resulted in more shape-persistent nanoparticles that flattened less on the substrate, giving an average height of *ca.* 32 nm for SCK **13** and *ca.* 20 nm for SCK **14**.

The micelles and SCKs were also analyzed by DLS in order to study the size of the particles in solution (Table 2). The micelles ranged in size between 17 and 18 nm (hydrodynamic diameter by number), and the SCKs between 22 and 26 nm. Micelle **12**, and SCKs **13** and **14** had larger intensity-average hydrodynamic diameter values, in comparison to the number- and volume-based averages, indicating the presence of a small portion of aggregated micelles of larger size (see DLS histograms in the Supporting Information).

¹⁹F-NMR characterization of the polymers, micelles and SCKs

Figure 3 depicts the ¹⁹F-NMR spectra of the polymers prepared, in which it can be seen that the resonances corresponding to the *ortho*, *meta* and *para* aromatic fluorines of the pentafluorostyrene are broad signals with line widths exceeding 300 Hz. When the *tert*-butyl groups of the polymer are removed (polymer **7**) and poly(ethylene glycol) chains are grafted (polymer **9**), the line width increases further, and the signals shift by *ca.* 15 ppm, due to the change from CD₂Cl₂ to DMSO as NMR solvent. When these polymers were assembled into polymeric micelles (micelle **21**, Figure 3) the signal corresponding to the fluorinated backbone completely disappeared, indicating a significant reduction of mobility in the core domain, which has been seen for other fluorinated polymer assemblies.³⁹ To ensure that the signal from the polymer did not shift beyond the expected shift region, these NMR experiments were run with very broad spectral width (1200 ppm) and over extended periods of time (6000 scans) with long delay times.

Loading of perfluoro-15-crown-5-ether

To utilize these nanoparticle constructs for MRI applications, higher fluorine concentrations are necessary than those provided by the PPFS units and, therefore, a procedure similar to the one developed for incorporating doxorubicin into SCKs (Scheme 2 middle) was explored for incorporating PFCE into the SCK cores.⁴⁴ We selected PFCE because it is a small molecule with high fluorine ratio and twenty NMR-equivalent ¹⁹F atoms. A dilute PFCE solution in dichloromethane (DCM) was added to a stirred SCK solution at nominal loading levels of 50, 100, and 200 % of the polymer mass. DCM acted as a selective solvent for the hydrophobic core, facilitating the incorporation of PFCE in the core domain. After the solvent was evaporated, the nanoparticle solutions were purified by several washings

[†]The reported percentages of crosslinking are the stoichiometries of amine crosslinker added to the carboxylic acid groups in the polymer. These values are, therefore, the maximum amounts of crosslinking that could have occurred, rather than the true extents of crosslinking.

using a centrifugal filter (MWCO 30 kDa), to yield transparent nanoparticle solutions. The filtrate was analyzed for non-encapsulated PFCE by ^{19}F -NMR, which was not detected. However, no PFCE was detectable in any of the SCKs either, regardless of the relative loading percentages, even after extended ^{19}F -NMR experiments (5000 scans). To test whether the restricted mobility provided by the glassy PPFS/PS hydrophobic core was limiting the possibility to see the signals arising from PFCE, the samples were loaded with 10 % DMSO (by volume), which acted as a solvent for both core and shell of the nanoparticle. Unfortunately, no signals from either the PPFS core or the PFCE were detectable by ^{19}F -NMR spectroscopy. From this result, one can draw two conclusions: 1) that the loading procedure was not suitable for high surface tension liquids, such as PFCE, and/or 2) that the mobility of the fluorinated polymer in the SCK core was highly restricted and, thereby, prohibiting ^{19}F -NMR spectroscopy. A simple mixing of PFCE with the SCK without the aid of an organic solvent did not result in any PFCE incorporation, most likely due to the PFCE phase separating from the SCK solution, increasing the surface tension to the extent that a small magnetic stir bar would float in the PFCE-SCK interface. The restricted mobility is also apparent from the DSC runs on the lyophilized SCKs, for which the T_g increases from *ca.* 75 °C for the starting copolymer **9** (PS-*co*-PPFS) to *ca.* 95 °C for the assembled and crosslinked SCK **13**. A similar trend was observed for SCK **14**, which derived from **10** (See Supporting Information).

Instead, a co-micellization procedure was elaborated, in which polymer **9** and PFCE (50 % by weight) were mixed in DMF prior to the slow addition of nanopure water (Scheme 2) to induce self assembly to polymer micelles. The micelles were subsequently dialyzed for 4 days to remove the unincorporated PFCE, and were found to have a hydrodynamic diameter of 20 ± 3 nm, similar in size to the neat polymer micelle based on polymer **9**. The micelle was further analyzed by ^{19}F -NMR (Figure 3), showing no signals except for the TFA reference. This sample was then also loaded with DMSO and, as seen in Figure 3, a signal corresponding to PFCE at 6 ppm could then be detected. This peak is shifted somewhat compared to PFCE in D_2O DMSO that resonates at *ca.* 13 ppm, relative to TFA (image not shown). This shift is indicative that the local environment affects the PFCE, even though the polymer core has been swollen with DMSO. In all, this study suggests further that the fluorinated core restricts the mobility of small molecule guests confined therein, but that addition of a core selective solvent allows for the detection of the PFCE guests by ^{19}F -NMR. However, importantly, confirmation was made that this loading process during micellization was effective. The loading of PFCE was then calculated by ^{19}F -NMR spectroscopy, integrating the peak corresponding to PFCE, and comparing it to the TFA that was used as internal standard (Figure 3). The loading percentage was 9 % (mass % polymer) for the PFCE-micelle based on polymer **9**, corresponding to a fluorine concentration of 0.82 mM and a loading effectiveness of 8 %.

Conclusions

Reversible addition-fragmentation chain transfer (RAFT) polymerization was employed to prepare copolymers of PS and PPFS, as well as block copolymers of *PtBA-b*-PS-*co*-PPFS with good control over molecular weight and polydispersity. It was found that the copolymerization of Sty and PFS with a 75:25 mol % feed ratio results in a greater proportion of consumption of the minor PFS monomer, to afford copolymers of a gradient sequence, PPFS-*grad*-PS, having decreasing relative PFS along the backbone as the conversion proceeded, but with a majority of Sty units overall. An opposite trend was observed when the feed ratio was reversed. This interesting copolymerization behaviour was observed for both neat copolymerization of Sty and PFS, and also for the block extensions from a *PtBA* macro chain transfer agent.

The block copolymers were converted into amphiphilic PAA-*b*-(PS-*co*-PPFS), fitted with mPEG grafts and, subsequently, assembled into polymeric micelles and SCK nanoparticles of small size, *ca.* 25 nm, in aqueous solution. It was found that the assembly of the polymers into core-shell micelles and SCKs restricted the mobility of the fluorinated core domain, which then limited their ^{19}F -NMR signal and their use as MRI contrast agents. Softening of the core of the SCK by the addition of DMSO did not improve the mobility to the extent that the polymer signals could be observed, suggesting that the glassy amorphous core of the SCK (T_g of 97 °C) restricted mobility. Additionally, loading of PFCE as a small molecule ^{19}F label within the SCKs in aqueous solution in a post-preparation loading process gave either insufficient loading or insufficient mobility to allow for the PFCE to be observed by ^{19}F NMR spectroscopy. In contrast, by a co-micellization procedure beginning from neat DMF as an organic cosolvent, PFCE could be loaded into the polymer micelle with good loading capacity and the ^{19}F NMR signal could be observed after swelling with DMSO. In order to solve the problem of the restricted mobility in the core, which prohibits the use of these nanoparticles as MRI agents, polymers with low T_g and high fluorine content must be developed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Preparation of the copolymers and block copolymers employed in this study.

Scheme 2.

Preparation of the polymeric micelles, SCKs, and perfluoro-15-crown-5-ether loaded SCKs and micelles.

Figure 1. GPC chromatograms of the copolymers **2–3** (upper panel), and the block copolymers **4–6** (lower panel).

Figure 2.
AFM images of micelles **11** and SCKs **13**.

Figure 3.

^{19}F -NMR spectra of the copolymer **2** in CD_2Cl_2 , block copolymer **5** in CD_2Cl_2 , amphiphilic block copolymer **7** in $\text{DMSO}-d_6$, PEG grafted amphiphilic block copolymer **9** in $\text{DMSO}-d_6$, PCFE loaded micelle **21** in D_2O , PCFE loaded micelle **21** in D_2O with 10 % DMSO.

Summary of data for the polymers prepared.

Table 1

Entry	Sample	M _n theo [Da] ^a	M _n [Da] ^b	M _w [Da] ^b	PD ^b	M _n [Da] ^c	M _w [Da] ^c	PD ^c	DP Sty ^d	DP PFS ^d	No. mPEG ^e	T _g [°C] ^g
2	PS- <i>co</i> -PPFS	5100	5500	6100	1.10	4800	4900	1.02	23	12	-	87
3	PS- <i>co</i> -PPFS	7000	6800	7400	1.09	8500	8600	1.02	15	26	-	89
4	PtBA ₁₀₀	13200	11900	12800	1.07	20300	21300	1.05	-	-	-	41
5	PtBA- <i>b</i> -(PS- <i>co</i> -PPFS)	19000	22100	26200	1.18	14700	16500	1.18	30	14	-	52, 82
6	PtBA- <i>b</i> -(PS- <i>co</i> -PPFS)	21000	21100	23300	1.16	18600	21800	1.17	19	30	-	56, 88
7	PAA- <i>b</i> -(PS- <i>co</i> -PPFS)	13800	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	30	14	-	87
8	PAA- <i>b</i> -(PS- <i>co</i> -PPFS)	15400	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	19	30	-	79
9	PAA- <i>g</i> -PEG- <i>b</i> -(PS- <i>co</i> -PPFS)	17800	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	30	14	2	75
10	PAA- <i>g</i> -PEG- <i>b</i> -(PS- <i>co</i> -PPFS)	21400	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	19	30	3	85

^aCalculated from ¹H-NMR spectroscopy

^bRI GPC

^cLS GPC

^dCalculated from ¹H-NMR spectroscopy

^eCalculated from ¹H-NMR spectroscopy

^fUnsuitable for GPC analysis with THF as the eluent

^gDetermined from DSC (see Supporting Information).

Table 2

Summary of data for micelles and SCKs prepared.

Sample	Conc. [mg mL ⁻¹]	(D _h) _n [nm] ^a	(D _h) _{val} [nm] ^a	(D _h) _{int} [nm] ^a	H _{av} [nm] ^b	PFCE conc. [% of poly]	Theoretical ¹⁹ F conc. [mM]	Experimental ¹⁹ F conc. [mM]	[°C] ^g
11	0.255	18±2	23±3	51±6	8±1	0 %	1 ^c	Not detected	-
12	0.240	17±2	28±4	116±10	7±1	0 %	1.7 ^c	Not detected	-
13	0.25	26±2	48±5	136±4	32±4	0 %	0.98 ^c	Not detected	98
14	0.238	22±3	32±3	84±10	20±3	0 %	1.7 ^c	Not detected	97
15 ^e	0.25	31±3	87±10	202±11	-	50 %	6.5 ^d	Not detected	
16 ^e	0.24	25±5	39±9	178±10	-	100 %	12.9 ^d	Not detected	
17 ^e	0.25	24±3	51±10	189±12	-	200 %	25.9 ^d	Not detected	
18 ^f	0.24	37±4	155±20	236±10	-	50 %	6.2 ^d	Not detected	
19 ^f	0.25	44±4	188±22	227±17	-	100 %	12.3 ^d	Not detected	
20 ^f	0.24	54±4	270±13	245±10	-	200 %	24.6 ^d	Not detected	
21	0.26	20±3	31±6	158±12	-	50 %	5.1 ^d	0.82	

^aFrom DLS

^bFrom AFM

^c[¹⁹F] originating from PPFS

^d[¹⁹F] originating from PFCE

^eFrom SCK 13

^fFrom SCK 14

^gDetermined from DSC (see Supporting Information).