ADVANCED FUNCTIONAL MATERIALS

Supporting Information

Surface-Reactive Patchy Nanoparticles and Nanodiscs Prepared by Tandem Nanoprecipitation and Internal Phase Separation

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Supporting Information

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Table of Contents

Materials

Styrene (99.5%, Acros), 4-vinylbenzyl chloride (VBC; 97%, Sigma Aldrich), and pentafluorostyrene (PFS; 98%, ABCR) were passed through a plug of basic alumina (Roth) in order to remove the inhibitor prior to use. Isoprene (99%, ABCR) was distilled at 40 °C before use. Sodium azide (NaN$_3$, ≥ 99%, Roth), polystyrene-*b*-polyisoprene **P5** (Polymer Source Inc., Ltd., Canada; M_n = 78 kg mol^{-1}, *Đ* = 1.05, f_{PS} = 0.57), osmium tetroxide (OsO$_4$, 2 vol% solution for electron microscopy), fluorescein-PEG-thiol (FITC-PEG-SH, MW = 2000 Da, NANOCS), *L*-Cysteine (97%, Aldrich), and Tween®20 (Sigma) were used as received. 1,4-dioxane (Roth) and tetrahydrofuran (THF, VWR) were used as received.

N-tert-Butyl-*N*-[1-diethylphosphono-(2,2-dimethylpropyl)] nitroxide (SG1)[1] and 2-methyl-2-[*N-tert*-butyl-*N*-(1-diethoxyphos-phoryl-2,   2-dimethylpropyl)aminoxy]propionic   acid (MAMA-SG1)[2] were synthesized according to the previously reported procedures.

The pH 13 buffer solution was prepared by mixing 0.4 M NaOH (90 mL), a 7.52 g L^{-1} solution of glycine (140 mL) and a 5.86 g L^{-1} NaCl solution (140 mL).[3]


Characterization

Nuclear magnetic resonance (NMR) spectroscopy Measurements were performed on a Bruker Avance III HD nano spectrometer at 400 MHz. The analytes were dissolved in CDCl$_3$ and the residual solvent signals were employed for shift correction. For the ^{19}F-CPD NMR spectra, baseline correction with the method Bernstein polynomial fit (with polynomial order of 14), manual correction, or multipoint baseline correction was performed using MestreNova 9.0.

Size-exclusion chromatography (SEC) Measurements for both polystyrene (PS) and polystyrene-b-polyisoprene (PS-b-PI) were performed on a TOSOH Eco-SEC HLC-8320 GPC system, comprised of an autosampler, a SDV 5 μm bead size guard column (50 × 8 mm, PSS) followed by three SDV 5 μm columns (300 × 7.5 mm, subsequently 100, 1000, and 10^5

Å pore size, PSS), and a differential refractive index (DRI) detector with THF as the eluent at 30 °C with a flow rate of 1 mL min^{-1}. The SEC system was calibrated by using linear polystyrene standards ranging from 266 to 2.52×10^{-6} g mol^{-1}. Calculation of the molecular weight proceeded by using the Mark–Houwink–Sakurada (MHS) parameters for polystyrene in THF at 30 °C, that is, $K = 14.1 \times 10^{-5}$ dL g^{-1}, $\alpha = 0.70$.

Synthesis of functional PS-*b*-PI block copolymers by nitroxide-mediated polymerization (NMP)
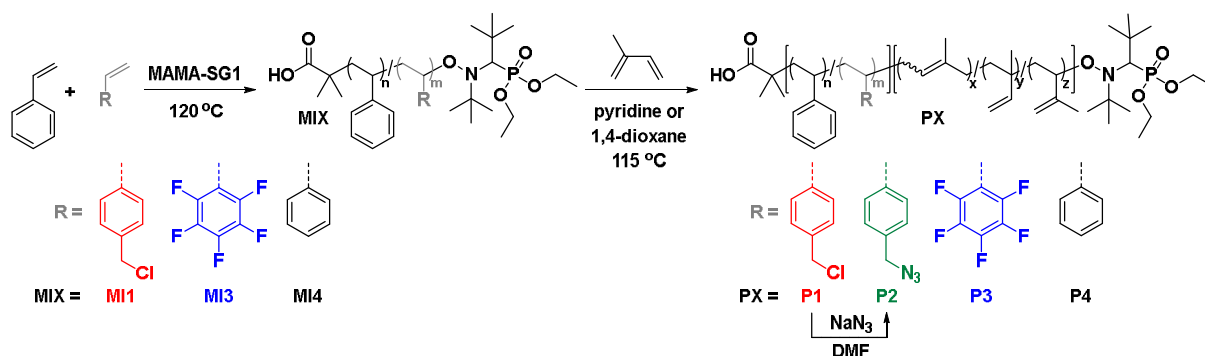
Scheme S1. Synthetic route towards the synthesis of macroinitiators **M1**, **M2**, **M4** and **P1–4** by NMP.

The synthesis of analogues of **P1–4** was described in a recent contribution (Scheme S1).[3] Briefly, styrene was copolymerized with either vinylbenzylchloride (VBC) or pentafluorostyrene (PFS) in bulk at 120 °C for 130 min, using MAMA-SG1 as an initiator, with [monomers]/[MAMA-SG1] ≈ 390. A similar procedure was followed for MI4 (pure PS) by omitting the addition of VBC or PFS. After two-fold precipitation in cold methanol, the macroinitiators were obtained as white powders and characterized by SEC in THF (polystyrene calibration) and ^{1}H NMR spectroscopy to obtain their macromolecular characteristics (see Table S1).

Table S1. Characteristics of the functional PS macroinitiators **MI1**, **MI3**, and **MI4**.

Macro initiator	Comonomer in PS block	%F[a]	$M_{n,SEC}$ g mol^{-1}	Đ
MI1	VBC	6.0	26,500	1.17
MI3	PFS	4.0	25,300	1.10
MI4	-	-	22,300	1.09

[a]%F: percentage of functional monomer introduced in the PS block.

The macroinitiators **MI1**, **MI3**, and **MI4** were then chain-extended with isoprene by further NMP at 115 °C in the presence of free SG1 ([SG1]/[macroinitiator] = 0.1), The polymerizations were carried out in solution (1,4-dioxane when using **MI1** and pyridine for **MI3** and **MI4**) in pressure tubes. Polymerization time was typically 13.5–15 h in order to reach a conversion of about 15 % in isoprene, yet not higher in order to limit side-reactions leading to a broadening of the molar mass distribution. After polymerization, residual isoprene and solvent were removed on a rotary evaporator. The BCPs were recovered by dilution in a minimal amount of THF and precipitation in a methanol/isopropanol mixture (3:1 v/v). BCPs **P1**, **P3**, and **P4** (from **MI1**, **MI3**, and **MI4**, respectively) were thus obtained as white powders and characterized by NMR spectroscopy and SEC to determine molar masses, dispersities, and volume fractions (Table S2, Figure S1 and Figure S2).

BCP **P2** was obtained by nucleophilic substitution of chloride moieties in **P1** with sodium azide: **P1** (100 mg) was dissolved in THF (3 mL) and mixed with NaN$_3$ (10 mg) in DMF (50 mL) and stirred at room temperature for 4 days. After solvent evaporation and redissolution in a small amount of THF, **P2** was recovered as a white powder by precipitation in a methanol/isopropanol mixture (3:1 v/v) and characterized as the other BCPs (Table S2, Figure S1 and Figure S2).

Table S2. Characteristics of the PS-*b*-PI block copolymers **P1–4**.

Functional BCP	%F^a	$M_{n,NMR}$ g mol^{-1}	$M_{n,SEC}$ g mol^{-1}	Đ	f_{PS}:f_{PI}
P1	6.0	48,900	62,800	1.32	0.47:0.53
P2	6.0	48,800	64,300	1.35	0.47:0.53
P3	4.0	47,300	61,900	1.32	0.50:0.50
P4	-	43,000	56,400	1.35	0.48:0.52

^{a}%F: percentage of functional monomer introduced in the PS block.
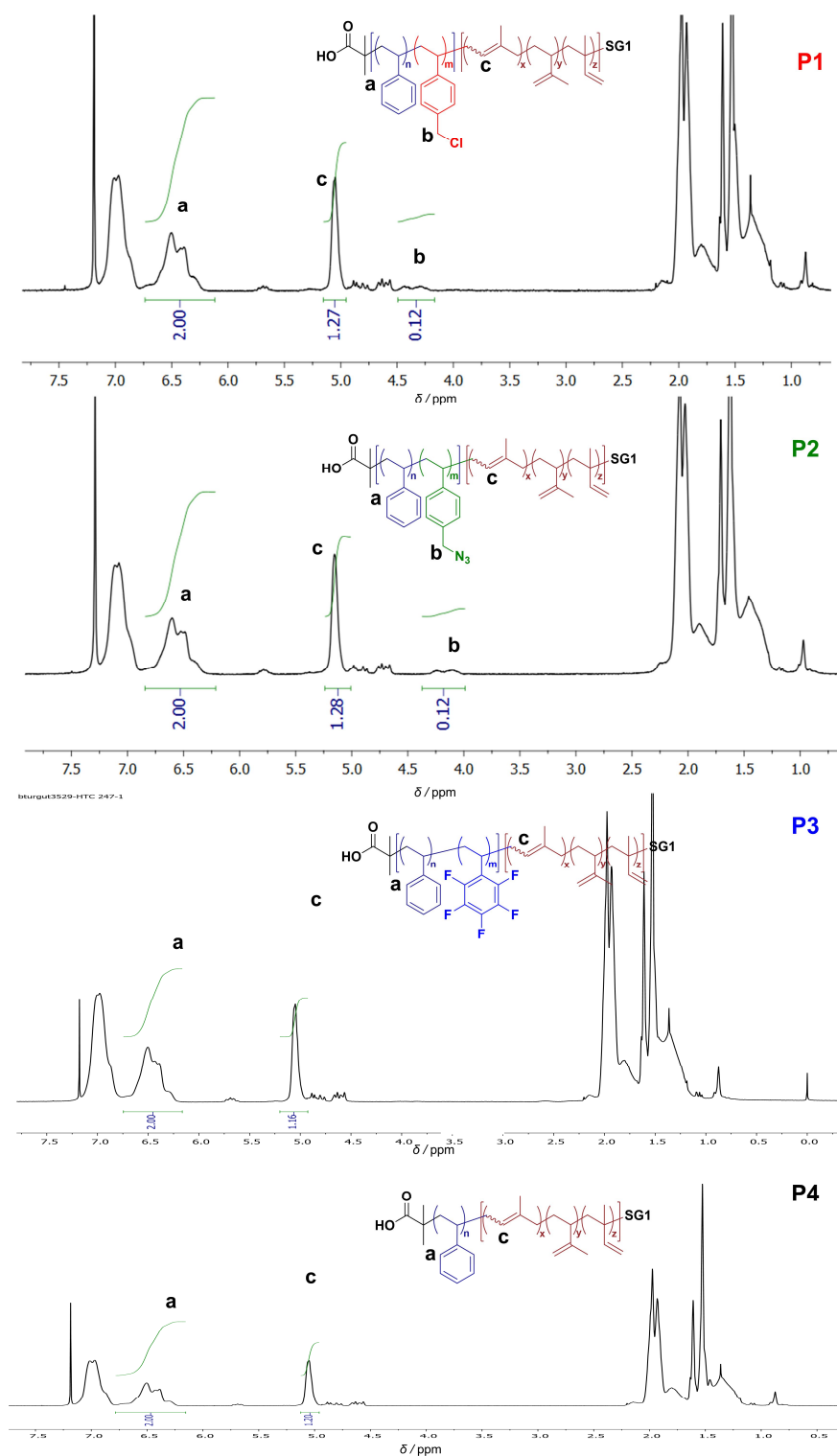
Figure S1. ^{1}H NMR spectra of block copolymers **P1–4**.
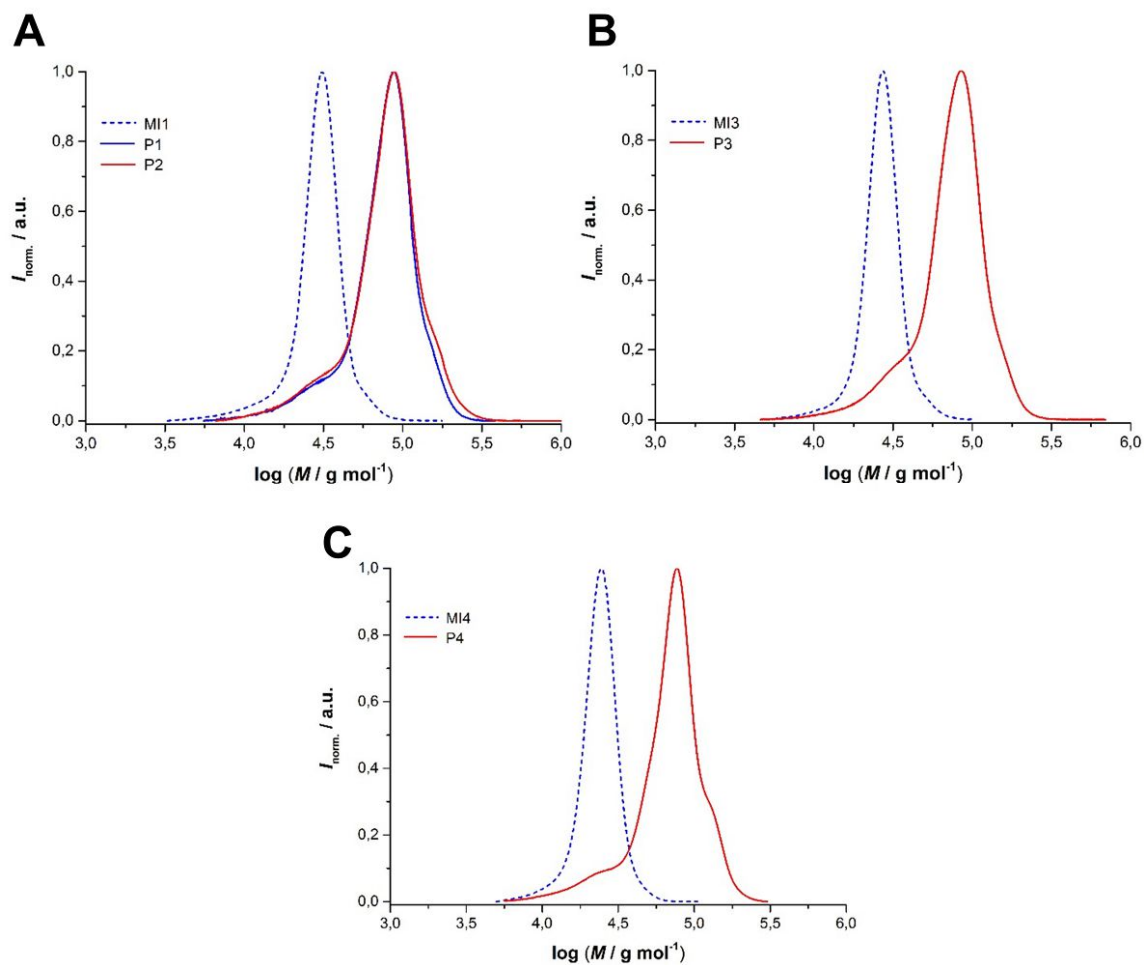
A

B

C

Figure S2. SEC traces of macroinitiator **MI1** (A), **MI3** (B), **MI4** (C) (blue dotted lines) and BCPs **P1** and **P2** (A), **P3** (B), and **P4** (C) (red solid lines).

Additional Details for the Preparation of Nanoparticles by Nanoprecipitation and Corresponding Data

Table S3. Summary of different conditions used to prepare the functional nanoparticles, their diameter determined by statistical analysis of electron micrographs, and their intensity-based hydrodynamic diameters (Z-average) as measured by DLS.

Polymer	Polymer conc. $mg\ mL^{-1}$	Entry	% THF	$D_{h,DLS}{}^a$ nm	PDI_{DLS}	D_{TEM} nm	PDI_{TEM}
P1	1	1	25	150	0.232	417	0.452
		2	50	223	0.219	362	0.317
		3	75	234	0.173	436	0.427
	0.1	4	25	183	0.080	170	0.099
		5	50	168	0.214	210	0.057
		6	75	256	0.102	237	0.04
P2	1	7	25	203	0.175	390	0.302
		8	50	1676	0.667	1066	0.082
		9	75	2885	0.764	1360	0.077
	0.1	10	25	350	0.309	217	0.126
		11	50	1074	0.429	1125	0.073
		12	75	2968	0.803	2010	0.129
P3	1	13	25	227	0.106	199	0.148
		14	50	293	0.120	147	0.045
		15	75	207	0.054	200	0.066
	0.1	16	25	136	0.022	125	0.034
		17	50	359	0.110	295	0.017
		18	75	187	0.019	215	0.037

[a]Note that DLS measurements were performed after full evaporation of THF.
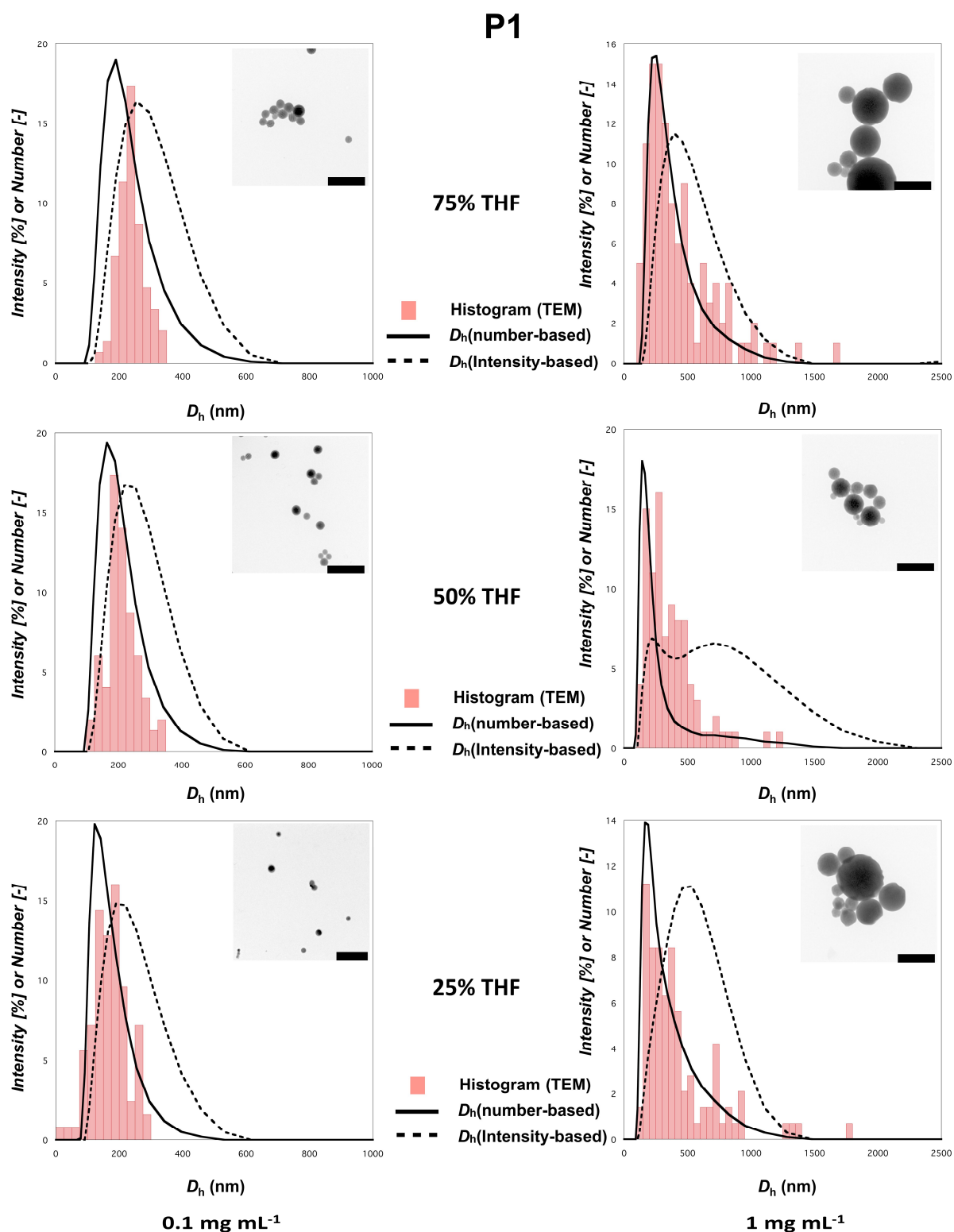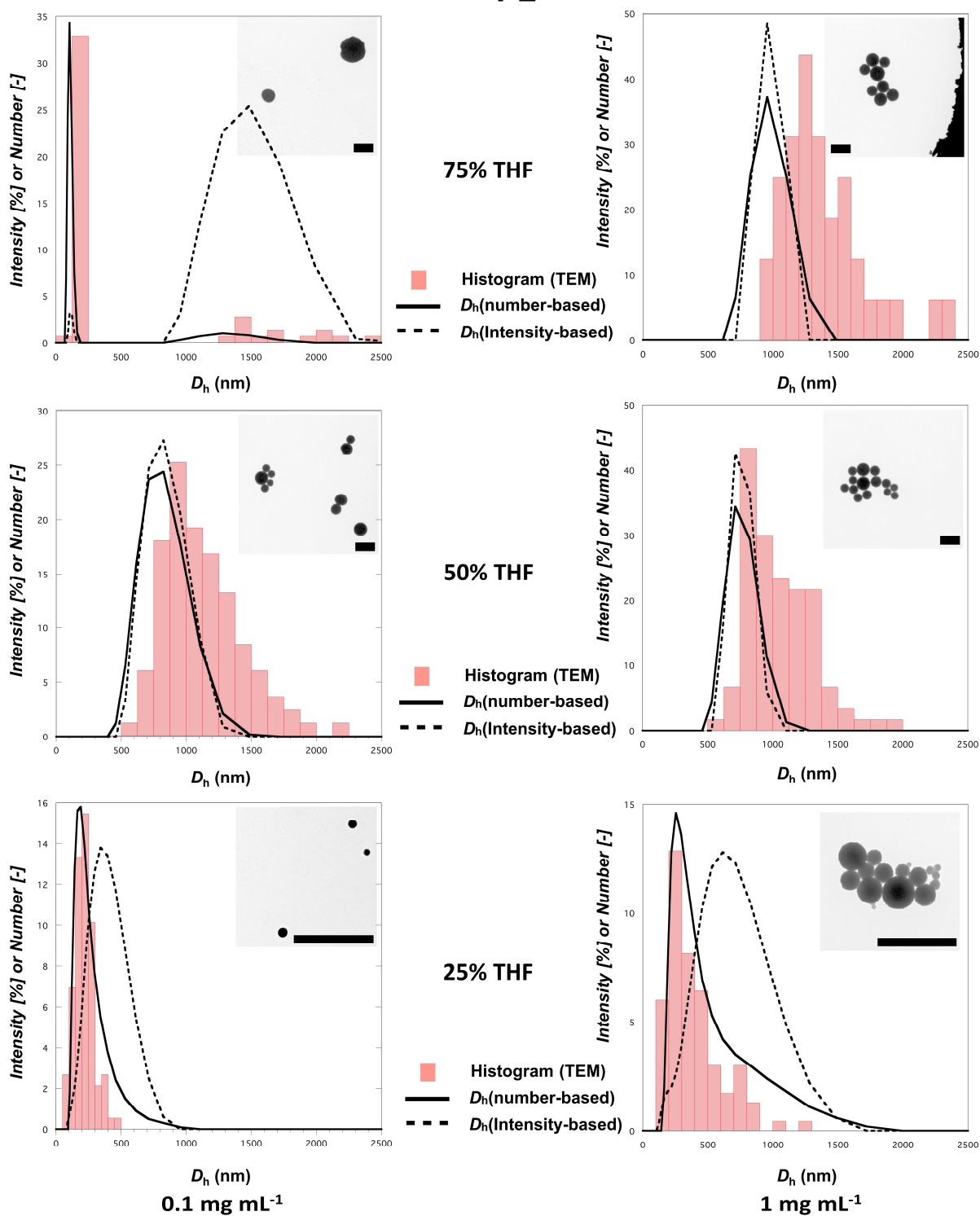
P1

Figure S3. Particle size distribution of particles prepared from BCP **P1** using different conditions (mixing ratio of THF/water and polymer concentration, see Table S3) obtained from statistical analysis of TEM images (histogram), DLS (number-based, black solid line), and DLS (intensity-based, black dotted line). Insets represent an exemplary TEM overview image used for statistical analysis. All scale bars represent 1 μm. Note that DLS measurements were performed after full evaporation of THF.
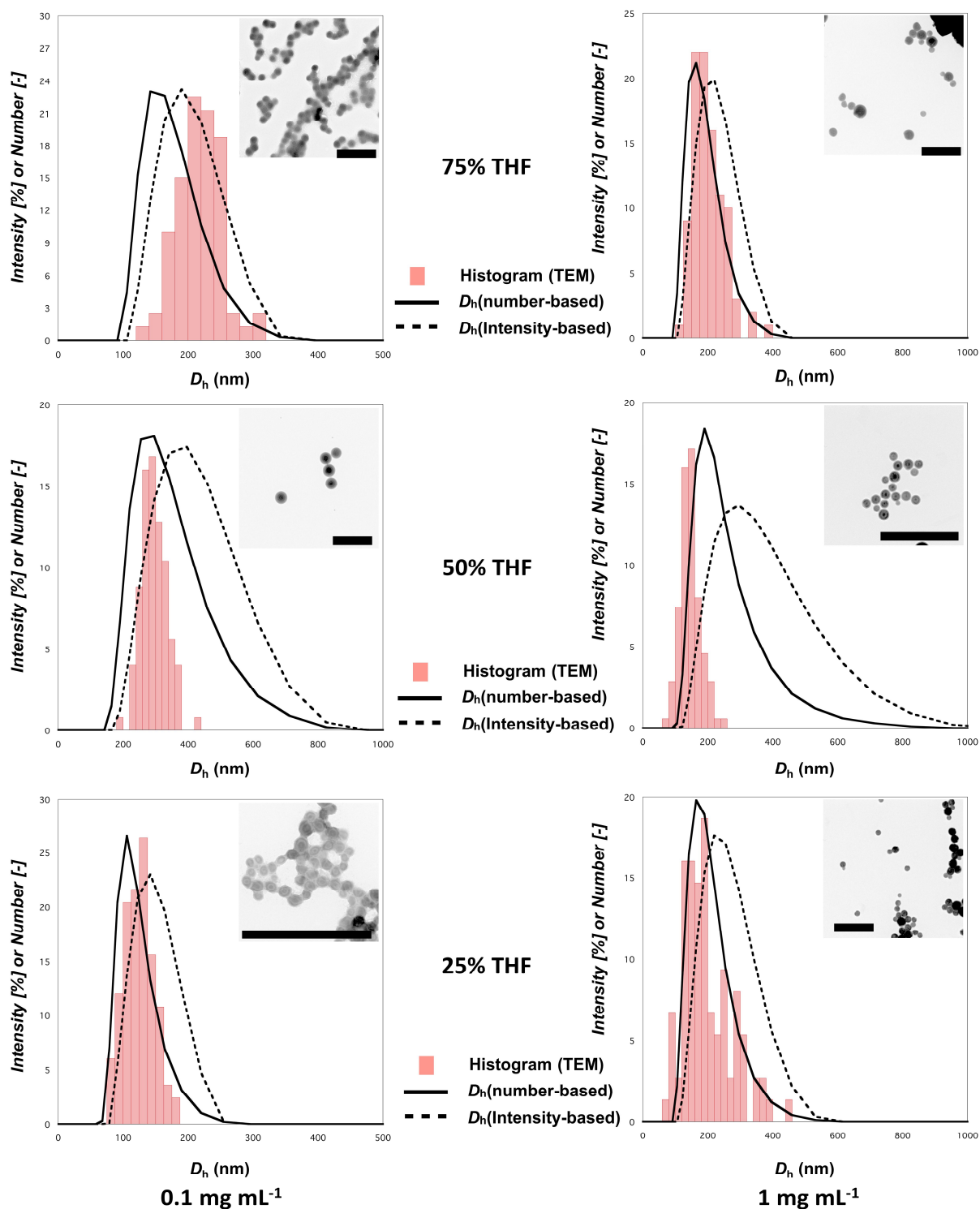
Figure S4. Particle size distribution of particles prepared from BCP **P2** using different conditions (mixing ratio of THF/water and polymer concentration, see Table S3) obtained from statistical analysis of TEM images (histogram), DLS (number-based, black solid line), and DLS (intensity-based, black dotted line). Insets represent an exemplary TEM overview image used for statistical analysis. All scale bars represent 2 μm. Note that DLS measurements were performed after full evaporation of THF.

P3

Figure S5. Particle size distribution of particles prepared from BCP **P3** using different conditions (mixing ratio of THF/water and polymer concentration, see Table S3) obtained from statistical analysis of TEM images (histogram), DLS (number-based, black solid line), and DLS (intensity-based, black dotted line). Insets represent an exemplary TEM overview image used for statistical analysis. All scale bars represent 1 µm. Note that DLS measurements were performed after full evaporation of THF.
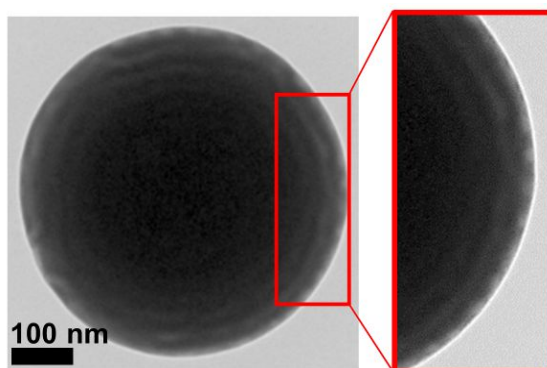
Figure S6. An enlarged view of Figure 2 B3 showing the outermost layer of the nanoparticles obtained from **P2** at 0.1 mg mL^{-1} and 50 vol% of THF.
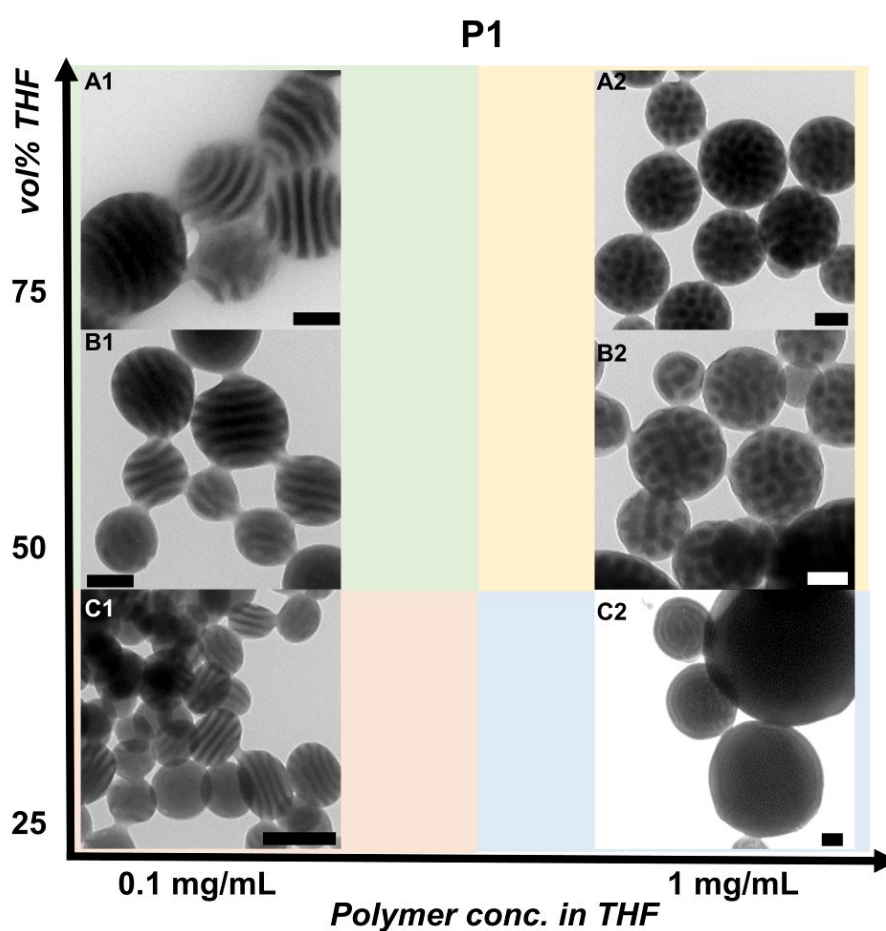

Figure S7. Bright field transmission electron microscopy images for nanoparticles obtained by nanoprecipitation with **P1** in various conditions, presented here at a lower magnification than in Figure 2 (codes match those of Figure 2). All scale bars represent 100 nm.
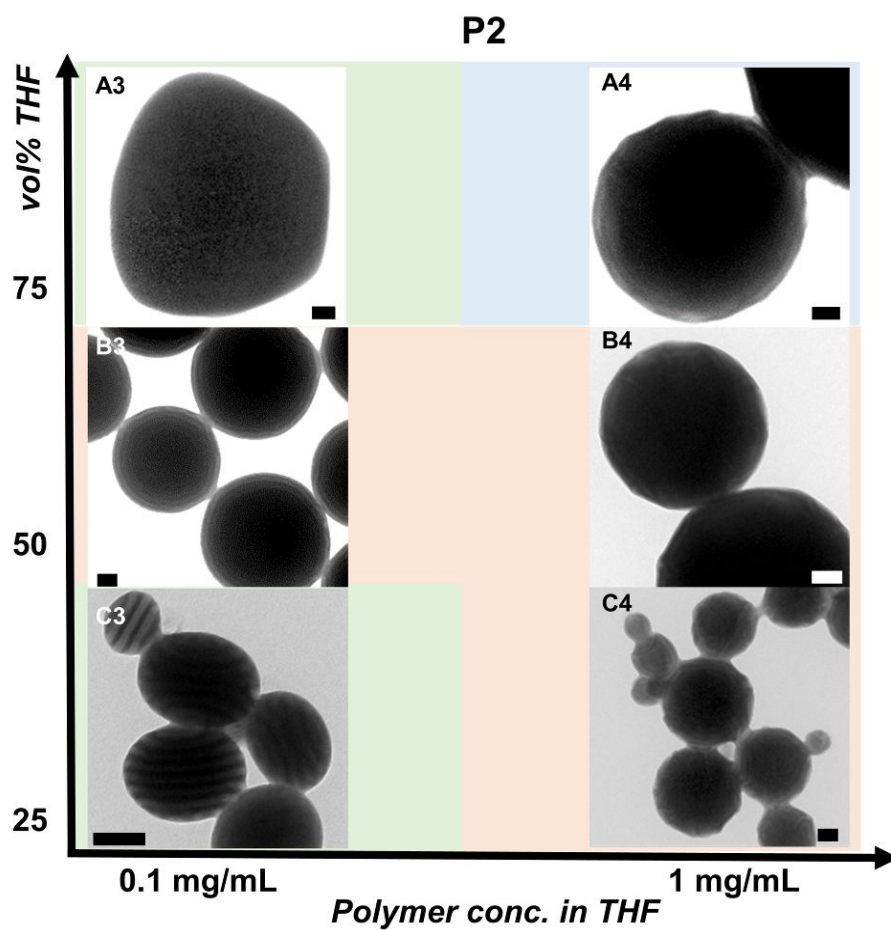
Figure S8. Bright field transmission electron microscopy images for nanoparticles obtained by nanoprecipitation with **P2** in various conditions, presented here at a lower magnification than in Figure 2 (codes match those of Figure 2). All scale bars represent 100 nm.
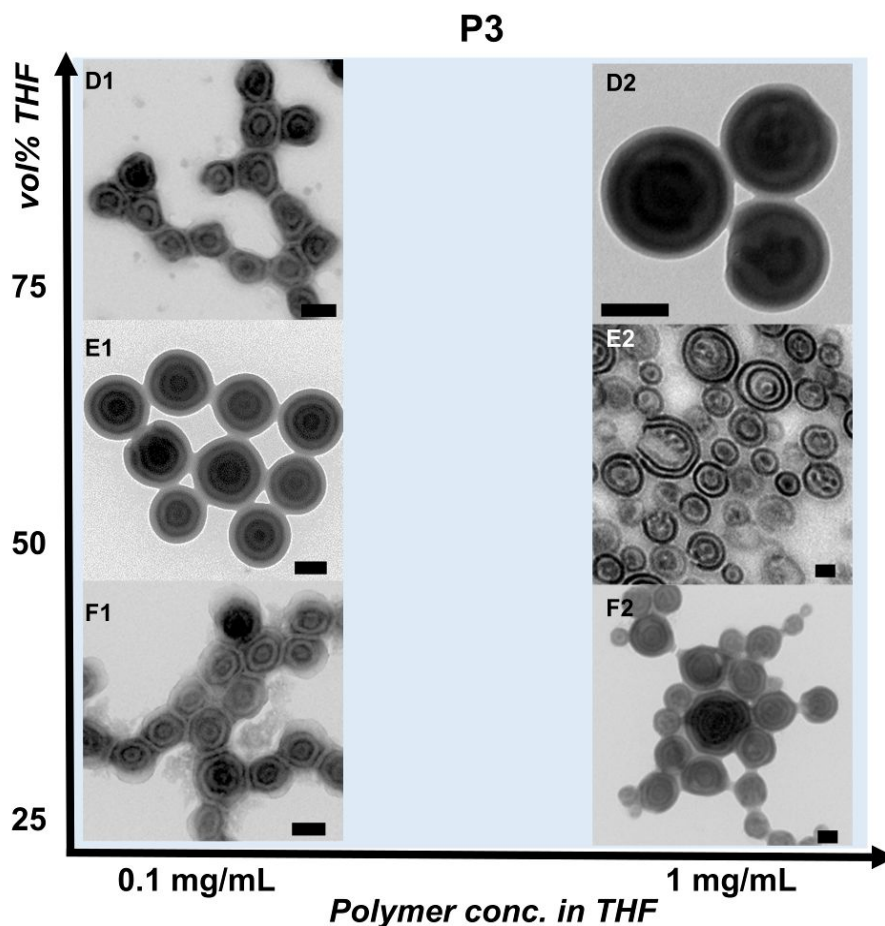
Figure S9. Bright field transmission electron microscopy images for nanoparticles obtained by nanoprecipitation with **P3** in various conditions, presented here at a lower magnification than in Figure 2 (codes match those of Figure 2). All scale bars represent 100 nm.

Table S4. Composition of block copolymer blends of **P1–3** and non-functional PS-*b*-PI **P5**.

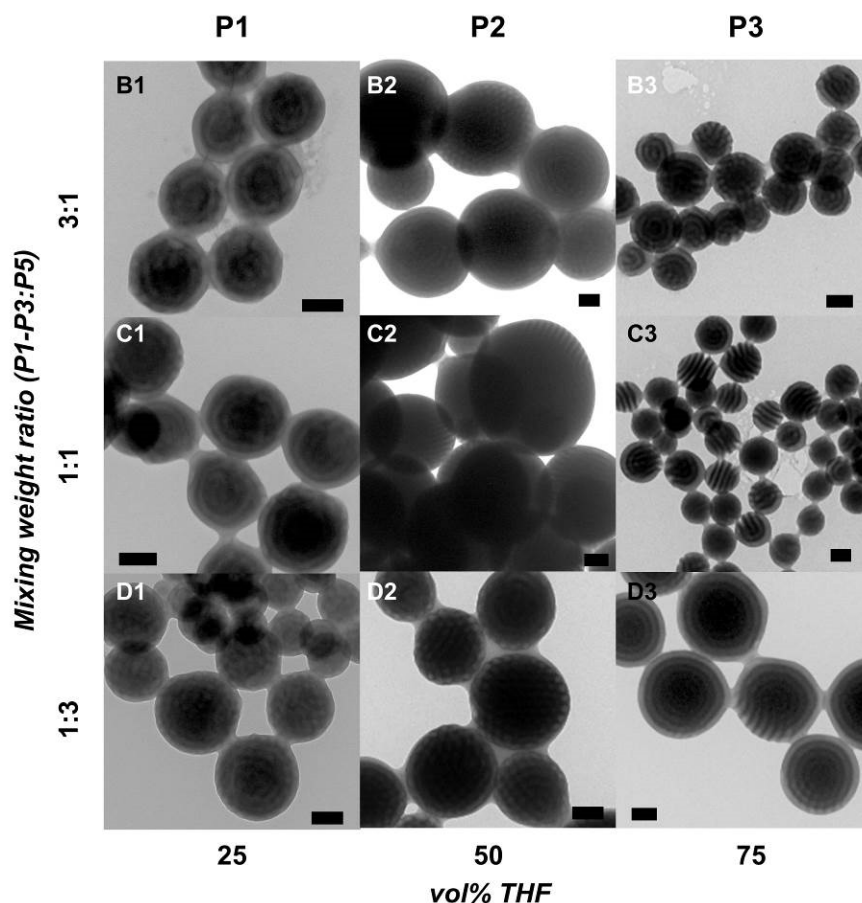| Polymer mixtures	% THF for nanoprecipitation	Blend number	Mixing ratio (weight)
P5:P1	25	**1**	3:1
		2	1:1
		3	1:3
P5:P2	50	**4**	3:1
		5	1:1
		6	1:3
P5:P3	75	**7**	3:1
		8	1:1
		9	1:3

Figure S10. Bright field transmission electron microscopy images for nanoparticles obtained by nanoprecipitation with mixtures of custom-made BCPs **P1–3** and commercial BCP **P5**, at various weight ratios, presented here at a lower magnification than in Figure 2 (codes match those of Figure 2). All scale bars represent 100 nm.
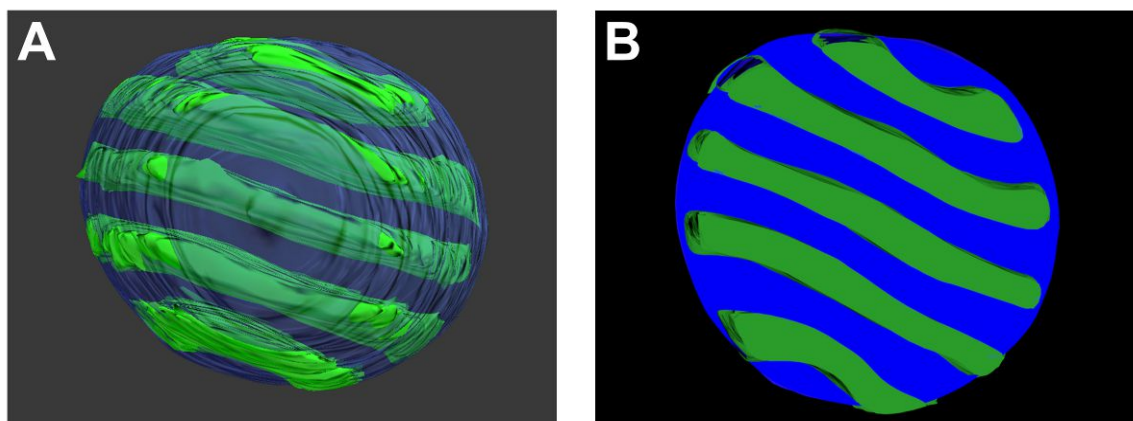
Electron Tomography Data for NP3

Figure S11. Composite images of PS and PI phases, where PS phases are transparent and the PI phases are represented in green (A); 3D reconstructed cross-sectional half cut image of the PS and PI phases in **NP3** nanoparticles (B).

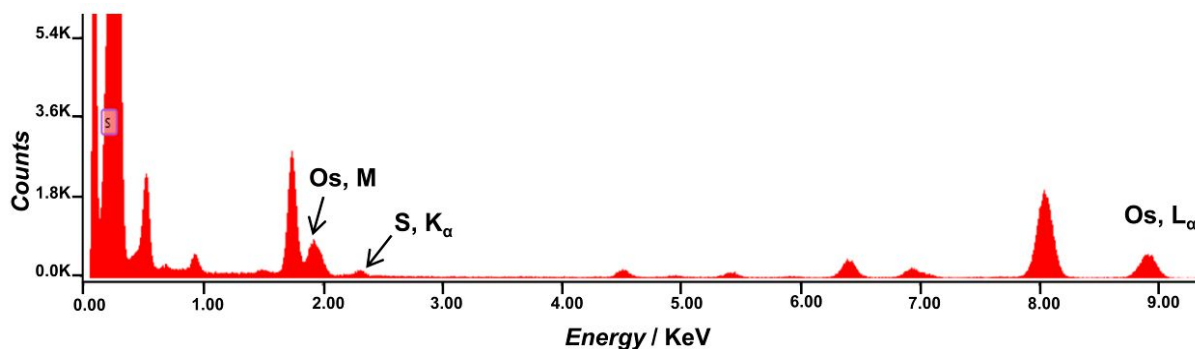# Additional Data on the PFTR-Based Surface Functionalization of Nanoparticles and Nanodiscs

Figure S12. EDX spectrum of the nanoparticles **NP3** after reaction with cysteine showing the presence of Os-M, Lα and S-Kα lines in coherence with Figure 5A–E.
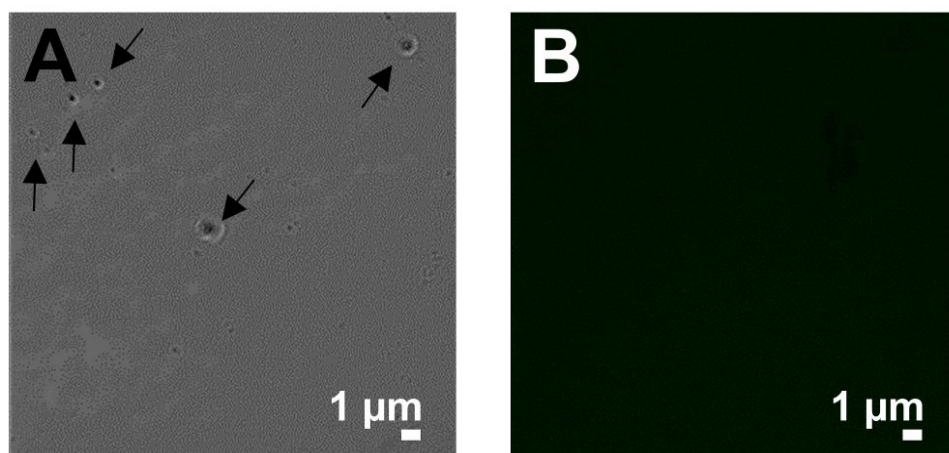

Figure S13. Micrographs of the control reaction between **ND3** and FITC-PEG-SH in bright field mode (A) and STED mode (B). The black arrows in A mark the particles. No fluorescence signal is detected in STED mode. Note that the functional nanodiscs **ND3** appear larger in size in STED and bright field images as compared to STEM images due to the limitations in resolving sub-100 nm by optical microscopy.

References

[1] L. Hlalele, B. Klumperman, *Macromolecules* **2011**, *44*, 6683.
[2] S. Harrisson, P. Couvreur, J. Nicolas, *Polym. Chem.* **2011**, *2*, 1859.
[3] H. Turgut, A. C. Schmidt, P. Wadhwani, A. Welle, R. Müller, G. Delaittre, *Polym. Chem.* **2017**, *8*, 1288.