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Supporting Information

Tuning the nanoparticles internal structure: fluorinated single-chain nanoparticles (SCNPs) generated by chain collapse of random copolymers

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I. Experimental Procedures

i. Chemical materials

Poly(ethylene glycol) methyl ether methacrylate (PEGMA, M_n= 300 Da, contains 300 ppm BHT as inhibitor, 100 ppm MEHQ as inhibitor, purified by passing through a basic alumina column, ≤100%, Sigma-Aldrich), 2,2,3,3,4,4,4-Heptafluorobutyl methacrylate (HFBMA, contains MEHQ as inhibitor, purified by passing through basic alumina column, 97%, Sigma-Aldrich), 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDPA, 97%, abcr), 2,2′-Azobis(2-methylpropionitrile) (AIBN, recrystallized with methanol, 98%, Sigma-Aldrich), Butyl methacrylate (BuMa, 99%, contains monomethyl ether hydroquinone as inhibitor, Purified by passing through basic alumina column, Sigma-Aldrich), 3-Chloro-1-propanol (98%, Sigma-Aldrich), Sodium azide (abcr), Tetrabutylammonium bromide (> 99%, TCI), Triethylamine (99%, Alfa Aesar), Methacryloyl chloride (97%, 200 ppm monomethyl ether hydroquinone as stabilizer, Sigma-Aldrich), Hydroquinone (99%, Sigma-Aldrich), Sodium sulphate (Roth), Sodium bicarbonate (Sigma Aldrich), Copper(II) sulphate pentahydrate (≥99%, VEB), Sodium chloride (Roth), Chloroform-d ($CDCl_3$, stabilized with Ag, ≤100%, ARMAR), deuterium oxide (D_2O, 99.9%, Sigma Aldrich), dimethylsulfoxide-d6 (DMSO, 100%, ARMAR), N,N,N′,N′′,N′′-Pentamethyl diethylenetriamine (PMDTA, 99%, Sigma Aldrich), 1,7-Octadiyne (98%, Sigma Aldrich), (+)-Sodium L-ascorbate (≥98%, Sigma-Aldrich), 2,2′-Bipyridine (≥99%, Sigma-Aldrich), Copper(I) bromide (98%, Sigma-Aldrich), 1-bromodecane (98%, Lancaster), 4-methylpyridine (98%, Sigma-Aldrich), 4-hydroxybenzaldehyde (98%, Sigma-Aldrich), Piperidine (98%, Sigma-Aldrich), 1-Iodo-1*H*,1*H*,2*H*,2*H*-perfluorodecane (97%, BLD Pharmatec), 5-DOXYL stearic acid, ammonium salt 5-DSA (>99%, Avanti polar lipids). All monomers were dried using activated molecular sieves (2 Å).

ii. Instrumentation

^1H, ^{13}C and ^{19}F-NMR and DOSY spectra were measured with Agilent Technologies 400MHz VNMRS (400 MHz) in deuterated solvents like $CDCl_3$ and D_2O at 27°C and analyzed with MestReNova 14.2.1-27684 from Mestrelab Research S.L. Chemical shifts (δ) are reported in ppm and referred to the solvent residual signal ($CDCl_3$ 7.26 ppm for ^1H, D_2O 4.66 ppm for ^1H).

DOSY measurements were done on an Agilent VNMR DD2 500 MHz (sfrq = 499.727 MHz). The experiment was performed under OpenVnmrJ 1.1 and equipped with a 5 mm PFG One NMR probe, z-gradient and temperature unit (27 °C and 45 °C). Diffusion ordered NMR data were acquired by means of the Agilent pulse program DgcsteSL cc using a stimulated echo with self-compensating gradient schemes and conventional compensation. The length of the gradient pulse was set to 3.0 ms for ^1H in combination with a diffusion period of 300 ms (D_2O). Data were systematically accumulated by linearly varying the diffusion encoding gradients over a range from 2% to 95% for 64 gradient increment values.

DLS measurements were done using Litesizer 500 from Anton Paar with 10 mm quarts cuvette. The irradiation wavelength was 658 nm with detection angles at 90° (side scattering) or at 175° (back scattering). The temperature was set to 25°C with sample concentration between 0.5 – 1.0 mg/mL in water.
AFM measurements were done using nanosurf CoreAFM with Tap190AI-G Cantilevers in the Phase-contrast mode. Sample concentrations of 500 µg/mL in deionized filtered water. The solution was deposited on the mica surface and stored in dust-free place until the water has evaporated before measurements. Data analysis was done using Gwyddion 2.53 (http://gwyddion.net/).

ATR-IR spectra were measured on a Bruker Tensor Vertex 70 equipped with a Golden Gate Heated Diamond ATR Top-plate with the software Opus 8.2 for analyzing data.

THF-based SEC measurements were performed at 30 °C on a Viscotek GPCmax VE 2001 from Malvern applying a CLM3008 precolumn and a CLM3008 main column. Sample concentration was 3 – 4 mg/mL in THF with flow rate of 1 mL/min. The refractive index detection was performed with a VE 3580 RI detector of ViscotekTM. The UV absorption detection was performed with UV detector 2600 of ViscotekTM. External calibration was done using poly styrene (PS) standards (form Malvern) with a molecular weight range from 1.050 to 115.000 g/mol or poly isobutylene (PIB) standards (form PSS) with a molecular weight range from 750 to 134.000 g/mol. OmniSEC software (V 5.12.) was used for evaluation.

H_2O-based SEC measurements with 0.05 % NaN_3 were performed at 25 °C on a Viscotek GPCmax VE 2001 from Malvern applying a PSS SUPREMA 5 µm, 8x50 mm precolumn and a PSS SUPREMA analytical Linear M 5 µm main column. Water with 0.05 % NaN_3 as solvent was used and the sample concentration was between 3 – 4 mg/mL with flow rate of 1 mL/min. The refractive index detection was performed with a TDA 302 Detector of Viscotek^{TM}. The UV absorption detection was performed with a Knauer Azura UVD 2.19. OmniSEC software (Version 5.12.) was used for data evaluation.

ESI-TOF-MS measurements were done with Bruker Daltonics microTOF via direct injection at a flow rate of 180 µL/hr in positive mode with an acceleration voltage of 4.5 kV. Samples were prepared by dissolving in methanol (0.1 – 0.3 mg/mL). The instrument was calibrated using the ESI-L low concentration tuning mix from Agilent Technologies (product no. G1969-85000). The software Data Analysis (version 4.0) was used for evaluation.

All CW-EPR spectra were measured using the Miniscope MS 5000 (Magnettech GmbH, Berlin, and Freiberg Instruments, Freiberg, Germany), the MS 5000 temperature controller (Magnettech GmbH, Berlin, Germany) and Freiberg Instruments software. The temperature was set to 25 °C with an accuracy of 0.2 °C. For all X-band measurements a magnetic field sweep of 10 mT centred around 337.5 mT with a scan time of 60 s, a modulation amplitude of 0.05 mT and a microwave power of 4.79 mW were used. Each spectrum is an accumulation of 10 scans. EPR spectra were simulated using Matlab (The Mathworks, Inc.) in combination with the EasySpin program. The 'chili' function was utilized to compute fast- and slow-motional regimes of 5-DSA. Each simulated component and their associated the rotation correlation times as well as g- and A-tensors is shown in the SI. Samples were prepared in deionized water with NP concentration of 0.5 µM. The ratio between 5-DSA and NPs is 1:2. Molar concentration of NPs in water is 1 mol%.

UV/VIS-absorption measurements were done with Perkin Elmer LAMBDA 365 UV/Vis Spectrophotometer using Helma analytics quartz glass cuvettes with diameter 10 mm and NPs concentration of 0.4 µM and NP to dye ratio equals 2:1

Turbidimetry measurements were done with JASCO J-1500 and PTC-510 cell holder. The samples were measured in a Helma analytics quartz glass cuvettes with diameter 10.0 mm) at concentration of 1 mg/mL in deionized water. The samples were heated from 30 °C to 90°C at a rate of 1 °C/min. The transmittance was measured at a wavelength of 500 nm. The temperature at 90% of the normalized transmittance was taken as cloud point temperature T_{cp}.

Decay associated spectra were measured with Hamamatsu R5900 16-channel multi-anode photomultiplier tube (PMT) with 16 separate output (anode) elements and a common cathode and dynode system (PML-16C, Becker&Hickl, Berlin, Germany) as described in Schmitt et al. 2020. A 632 nm pulsed laser diode (PDL-600, Becker&Hickl, Berlin) delivering 80 ps FWHM pulses at a repetition rate of 20 MHz was used for excitation. The fluorescence was observed via a 633 nm longpass filter (F76-631, AHF Analysentechnik, Tübingen, Germany). NPs concentration in deionized water was 37.5 µM with NP to dye ratio equals 3:2

Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrometer of Agilent using Helma analytics quartz glass cuvettes with diameter 10 mm and NPs concentration in deionized water was 0.4 µM and NP to dye ratio equals 2:1

The fluorescence quantum yields of modified Brooker's merocyanine dyes (ϕ_f) were determined after excitation of aqueous solutions of all samples at a final concentration of 25 µM in deionized water (molar ratio of SCNP: Dye molecule is 1: 0.7, respectively. Excitation was performed with 405 nm picosecond laser diode driven at 20 MHz (LDH-405, Picoquant, Berlin). The pulse train was focused on the back focal plane of the objective to achieve a uniform illumination of the whole field of view. An emission filter (405 nm Notch, AHF Analysentechnik, Tübingen) were used to remove the stray light of the laser from the fluorescence signal. The spectra were recorded with a commercial USB-connected fluorometer system with CCD array (EPP2000, Scientific Instruments, Berlin, Germany) and evaluated with Origin®. ϕ_f was determined in respect to the known $\phi_{f,ref}$ of 4-[(1-methyl-4(1H)-pyridinylidene)-ethylidene]-2,5-cyclohexadien-1-one, better known as Brooker's merocyanine, in water ($\phi_{f,ref}$ = 0.007)[1] as an internal standard, according to the formula $\phi_f = \phi_{f,ref}$ (A_{ref} /A) (F/F_{ref}), A being the absorbance of the sample, A_{ref} the absorbance of the internal standard, and F and F_{ref} the total emitted fluorescence (area under the spectrum) of the sample and the internal standard, respectively. Decay associated spectra (DAS) were determined from time- and wavelength- correlated fluorescence data performed with a setup as described in the reference [2] employing a Hamamatsu R5900 16-channel multi-anode photomultiplier tube (PMT) with 16 separate output (anode) elements and a common cathode and dynode system (PML-16C, Becker&Hickl, Berlin, Germany). The polychromator was equipped with a 300 grooves/mm grating resulting in a spectral bandwidth of the PML-16C of about 12.5 nm /channel. A 405 nm pulsed laser diode (LDH-405, Picoquant, Berlin) was used for excitation. The fluorescence decay was analyzed employing a Levenberg-Marquardt algorithm for the minimization of the reduced χ_r^2 after iterative reconvolution with the instrumental response function (IRF). The fit function was chosen as a multiexponential decay with three components, common time constants for all decay curves and wavelength-dependent pre-exponential factors. The result of this analysis is usually plotted as a graph of for all wavelength independent lifetimes representing so-called decay-associated spectra (DAS) thus revealing the energetic position of individual decay components. The quality of the fit was judged by the value of χ_r^2 For further details see the reference. [3]

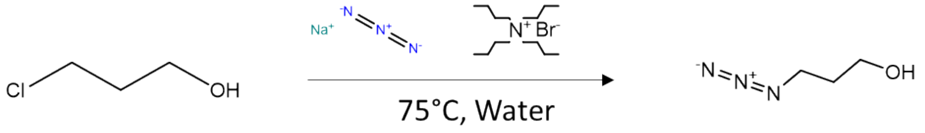
DLS measurements were done with Litesizer 500 from Anton Paar using 1.5 ml quartz low-volume cuvette with irradiation wavelength at 658 nm at room temperature (25°C). The detection angle was maintained at 90° (side scattering). The sample concentration was 0.5 – 1.0 mg/mL in deionized water filtered more than once with PTTE (d= 0.2 µm) filters.

iii. Synthesis

a. Synthesis of 3-azido-1-propanol

Sodium azide (192 mmol, 12.5 g) and tetrabutylammonium bromide (0.8 mmol, 0.26 g) were dissolved in 150 ml of deionized water and 3-chloropropanol (94 mmol, 8.9 g) was added dropwise to the reaction solution afterwards the reaction was heated to 75°C and left to stir. After 3 days the reaction was let to cool down to room temperature. The aqueous phase was washed 3 times with DCM (300 mL) and the organic phase was washed 3 times with water (300 mL). The organic phase was dried over Na_2SO_4. Afterwards, DCM was removed using vacuum. The product is a colourless liquid. Yield: 80%

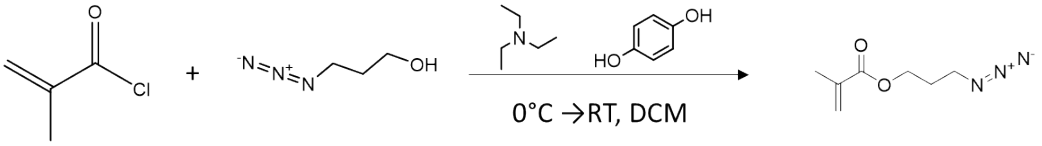
^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 3.63 (HO-CH_2-, 2H), 3.36 (-CH_2-N_3, 2H), 2.99 (HO-, 1H), 1.75 (-CH_2-CH_2-CH_2-, 2H).



b. Synthesis of 3-azidopropyl methacrylate (APMA)

3-azido-1-propanol (66.3 mmol, 6.7 g) and triethylamine (43 mmol, 4.36 g) were dissolved in dry DCM (90 mL) and hydroquinone (<2 mg) was added. The solution was stirred at 0°C. methacryloyl chloride (43 mmol, 4.5 g) was dissolved in a separate flask in dry DCM (15 ml) and added slowly and dropwise to the main reaction flask. The reaction was maintained at 0°C for an hour and then overnight at room temperature. Afterwards, the organic phase was washed 3 times with water (300 mL), 3 times with sodium bicarbonate solution (300 mL) and 3 times with sodium chloride solution (300 mL). The organic yellow phase was dried over sodium sulphate and DCM was removed by vacuum. Column chromatography was used to purify the product using a solution of hexane: DCM (1:1). R_f= 0.24 and yield after column= 60%

^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 6.07 (=CH-, 1H), 5.53 (=CH, 1H), 4.2 (-O-CH_2-), 3.37 (-CH_2-N_3, 2H), 1.92 (-CH_2-CH_2-CH_2-, 2H), 1.90 (-CH_3).



c. Synthesis of A_x-Poly and F_x-Poly

RAFT polymerizations were performed in dry DMSO (c = 3.3 M), using AIBN (≈ 1 mmol equivalent) and the CTA CDPA (≈ 5 mmol equivalent). The co-monomers PEGMA (1 mol equivalent), BuMA (or HFBMA) and azido APMA were added to the reaction solution (for exact amounts see Table S1). The mixture of the co-monomers, CTA and initiator with the solvent was purified from oxygen via 5 cycles of freeze-pump-thaw using liquid nitrogen under vacuum (p = 0 - 5 mbar). Subsequently, the reaction flask was placed in a preheated oil bath and the solution polymerization was carried out for 3 hours at 70° C. The reaction was quenched by using an ice bath. The polymer solution was transferred to dialysis in water for three days using dialysis tubes with 5 kDa molecular weight cut-off, followed by additional 3 days of dialysis in THF. The copolymers were characterized using SEC in THF and ^{1}H- and ^{19}F-NMR in $CDCl_3$. See Scheme 1 and Table 1 in the main script.

^{1}H NMR for A_x-Poly (400 MHz, $CDCl_3$, δ in ppm): 0.84 - 0.99 (-CH_3 protons of the backbone, 3H), 1.21 - 1.85 (-CH_2- protons of the backbone, 2H), 3.51 - 3.62 (-O-CH_2-CH_2-O- protons of PEGMA, 4H), 3.34 (-O-CH_3 protons of PEGMA, 3H), 3.38 (-COO-CH_2- protons of APMA, 2H), 3.90 (-COO-CH_2- protons of BuMA, 2H) and 4.04 (-COO-CH_2- protons of PEGMA, 2H).

^{1}H NMR for F_x-Poly (400 MHz, $CDCl_3$, δ in ppm): 0.82 - 1.03 (-CH_3 protons of the backbone, 3H), 1.21 - 1.89 (-CH_2- protons of the backbone, 2H), 3.50 - 3.65 (-O-CH_2-CH_2-O- protons of PEGMA, 4H), 3.33 - 3.37 (-O-CH_3 protons of PEGMA, 3H), 3.38 - 3.42 (-COO-CH_2- protons of APMA, 2H), 4.05 - 4.08 (-COO-CH_2- protons of PEGMA, 2H) and 4.38 - 4.42 (-COO-CH_2- protons of HFBMA, 2H).

d. Synthesis of A_x-NP_{Water} and F_x-NP_{Water}

Crosslinking reactions were conducted in oxygen-free environment using copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) click reaction with high dilution (≈ µM). The precursor polymer (200 mg) and octadiyne (see Table S1) as crosslinker were dissolved in 20-25 mL dry THF. This solution was slowly added to the reaction solution via a syringe pump (1 mL/h). The reaction solution contained copper (II) sulphate pentahydrate (CuSO4.5H2O, 0.5 mmol), PMDTA (2.0 mmol), and sodium ascorbate (7.6 mmol) in deionized water (250 - 300 mL). After complete addition of the polymer solution (>25 hr), the product (A_x-NP_{water} or F_x-NP_{water}) was extracted 3 times with DCM (300 ml). Afterwards, THF is added to the organic phase and DCM was completely removed by vacuum. The nanoparticle's solution in THF was purified by dialysis in water (+ 1 mL of PDTMA) for 3 days using 5 kDa molecular weight cut-off and additional 3 days in THF: water (1: 1) solution. The solvent was removed by vacuum. The nanoparticles were characterized using SEC in THF and ^{1}H- and ^{19}F-NMR in $CDCl_3$. See scheme 1 and table 1 in the main script.

1H NMR (400 MHz, CDCl3, δ in ppm): 0.85 - 1.04 (-CH3 protons of the backbone, 3H), 1.24 - 1.85 (-CH2- protons of the backbone, 2H), 3.33 - 3.39 (-O-CH3 protons of PEGMA, 3H), 3.54 - 3.67 (-O-CH2-CH2-O- protons of PEGMA, 4H), 3.94 (-COO-CH2- protons of BuMA, 2H), 4.05 - 4.08 (-COO-CH2- protons of PEGMA, 2H) and 4.38 - 4.42 (-COO-CH2- protons of HFBMA, 2H).

e. Synthesis of A_x-NP_{THF} and F_x-NP_{THF}

Crosslinking reactions were conducted in oxygen-free and water-free environment using Copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) click reaction with high dilution (≈ µM). The precursor polymer (200 mg) and octadiyne (see Table S1) as crosslinker were dissolved in 20-25 mL dry THF. This solution was slowly added to the reaction solution *via* a syringe pump (1 mL/h). The reaction mixture contained copper (I) bromide (CuBr, 0.4 - 0.5 mmol) and 2,2'-bipyridine (1.2 - 1.3 mmol) dissolved in dry THF (250 - 300 mL). After complete addition of the polymer solution (>25 hr), water (≈ 20 mL) was added and THF amount was reduced using vacuum. Water (50 mL) was added and the product (A_x-NP_{water} or F_x-NP_{water}) was extracted 3 times with DCM (200 mL). Afterwards, THF is added to the organic phase and DCM was removed by vacuum. The nanoparticles solution in THF was purified by dialysis in water for 3 days using 5 kDa molecular weight cut-off and additional 3 days in THF. The solvent was removed by vacuum. The nanoparticles were characterized using SEC in THF and ^{1}H- and ^{19}F-NMR in $CDCl_3$. See Scheme 1 and Table 1 in the main script.

^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 0.82 – 1.05 (-CH_3 protons of the backbone, 3H), 1.24 – 2.17 (-CH_2- protons of the backbone, 2H), 3.33 – 3.39 (-O-CH_3 protons of PEGMA, 3H), 3.53 – 3.66 (-O-CH_2-CH_2-O- protons of PEGMA, 4H), 3.94 (-COO-CH_2- protons of BuMA, 2H), 4.05 – 4.09 (-COO-CH_2- protons of PEGMA, 2H) and 4.42 (-COO-CH_2- protons of HFBMA, 2H).

f. Synthesis of 1-decyl-4-methylpyridinium bromide

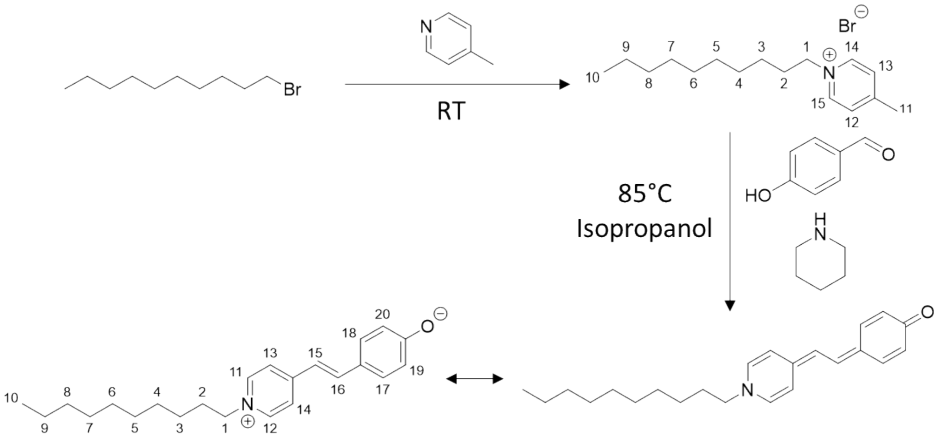
1-Bromodecane (5 mmol, 1.12 g) and 4-methylpyridine (25 mmol, 2.33 g) were stirred at room temperature overnight to give the ionic liquid product. Afterwards, the ionic liquid was dissolved in water and washed 3 times with diethyl ether (50 mL). Water was removed by vacuum. Product is yellowish and very viscous. Yield= 38%.

^{1}H NMR (400 MHz, DMSO, δ in ppm): 8.88 (H^{14}, H^{15}, 2H), 7.94 (H^{13}, H^{12}, 2H), 4.48 (H^1, 2H), 2.57 (H^{11}, 3H), 1.84 (H^2, 2H), 1.2 (H^{3-9}, 14H), 0.82 (H^{11}, 3H).

g. Synthesis of decyl-Brooker's Merocyanine (A-BM)

To synthesize (1-decylpyridin-1-ium-4-yl)vinyl phenolate (A-BM); 1-decyl-4-methylpyridinium bromide (1.95 mmol, 0.612 g) was dissolved with 4-hydroxybenzaldehyde in isopropanol (15 mL) and then piperidine (5 mmol, 0.43 g) was added. After leaving the reaction overnight at 85°C, isopropanol was removed by vacuum and the product was dissolved in DCM (saturated solution). The concentrated solution was precipitated twice in diethyl ether (400 mL) and the product was collected using centrifugation. The product was placed in a vacuum oven at 0 mbar and 80°C overnight. The product is solid red powder. Structure was confirmed using ESI-ToF and ^{1}H-NMR in $CDCl_3$. (see Figure S17). Yield=84%.

^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 8.61 ($H^{11, 12}$,2H), 7.7 ($H^{13, 14}$, 2H), 7.34 – 7.28 ($H^{15,17,18}$, 3H), 6.86 ($H^{19, 20}$, 2H), 6.65 (H^{16}, 1H), 4.57 (H^1, 2H), 1.74 (H^2, 2H), 1.31 – 1.14 (H^{3-9}, 14H) 0.85 (H^{10}, 3H).



h. Synthesis of heptadecafluorodecyl-4-methylpyridinium iodide

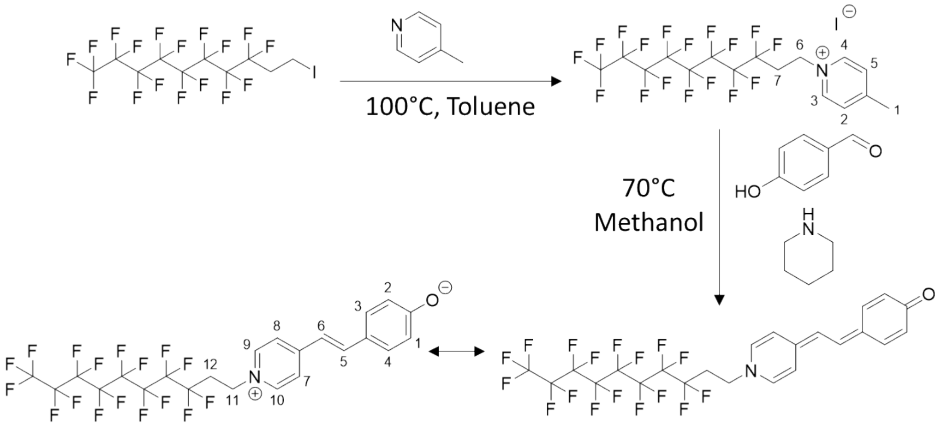
Heptadecafluoroiodo decane (5 mmol, 2.87 g) and 4-methylpyridine (25 mmol, 2.33 g) were stirred at 100°C in toluene (15 mL) overnight. The produced ionic compound precipitated in the reaction flask and the solvent was removed using vacuum at 50°C for 1 hour. Afterwards, the product was washed with THF (100 mL) and dried in vacuum oven at 50°C. The product is brown powder with strong odor. Yield= 33%

^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 9.33 (H^3, H^4, 2H), 7.86 (H^2, H^5, 2H), 5.43 (H^6, 2H), 3.08 (H^{10}, 2H), 2.72 (H^1, 2H).

i. Synthesis of heptadecafluorodecyl Brooker's Merocyanine (F-BM)

To synthesize (3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl)pyridin-1-ium-4-yl)vinyl)phenolate (F-BM); heptadecafluorodecyl-4-methylpyridinium iodide (1.65 mmol, 1.1 g), 4-hydroxybenzaldehyde (8.25 mmol, 1.0 g) and piperidine (8.25 mmol, 0.70 g) were dissolved in methanol (20 mL) at 70°C overnight. The reaction product was left to cool down and the product precipitated. The precipitate was filtered, washed with THF (100 mL) and dried in vacuum oven at 45°C. Structure was confirmed using ESI-ToF and ^{1}H-NMR in DMSO (see Figure S17). Yield= 25%

^{1}H NMR (400 MHz, DMSO, δ in ppm): 8.56 (H^9, H^{10}, 2H), 7.80 – 7.81 (H^6, H^7, H^8, 3H), 7.44 (H^3, H^4, 2H), 6.83 (H^5, 1H), 6.47 (H^1, H^2, 2H), 4.65 (H^{11}, 2H), 3.08 (H^{12}, 2H).



j. Encapsulating A-BM/F-BM in A_{30}-NP_{Water} and F_{30}-NP_{Water}

To prepare the stock solutions of the nanoparticles; A_{30}-NP_{Water} and F_{30}-NP_{Water} were dissolved in THF (0.27 mM), separately. A-BM (1.55 mM) and F-BM (3 mM) were dissolved in Methanol. 111 µL (30 nmol) of the nanoparticle's solution was added to a vial followed by adding the dye (20 nmol). 250 µL of Methanol was added to the vial and stirred for a short time. Afterwards, the solvents in the vial were removed slowly using a vacuum pump at slow rotation speed. The product is a thin film of the nanoparticles with the dye encapsulated in the core. 800 µL of deionized water was added and the solution was stirred at high speed for 3 hours.

II. Supporting Tables and Figures

i. Table S1: Synthesis

Table S1: Amounts of reactants used in the synthesis reactions.

Polymer	PEGMA	BuMA/HFBMA	APMA	CDPA	AIBN	Octadiyne A/F		Yield%$_{Poly}$	Yield%$_{SCNP}$
A/F$_{30}$-Poly	10mmol, 3.0g	4.3mmol, 0.61g/1.2g	2.14mmol, 0.36g	82µmol, 33mg	16µmol, 2.7mg	4.4mg, 42µmol	3.0mg, 27µmol	33 - 57	80 - 39
A/F$_{20}$-Poly	10mmol, 3.0g	2.5mmol, 0.36g/0.70g	1.90mmol, 0.32g	72µmol, 29mg	14µmol, 2.4mg	4.2mg, 40µmol	3.5mg, 33µmol	57 - 59	93 - 40
A/F$_{10}$-Poly	10mmol, 3.0g	1.1mmol, 0.16g/0.3g	1.7mmol, 0.28g	64µmol, 26mg	13µmol, 2.1mg	4.0mg, 37µmol	4.5mg, 43µmol	49 - 38	9 - 56

ii. ATR-FTIR

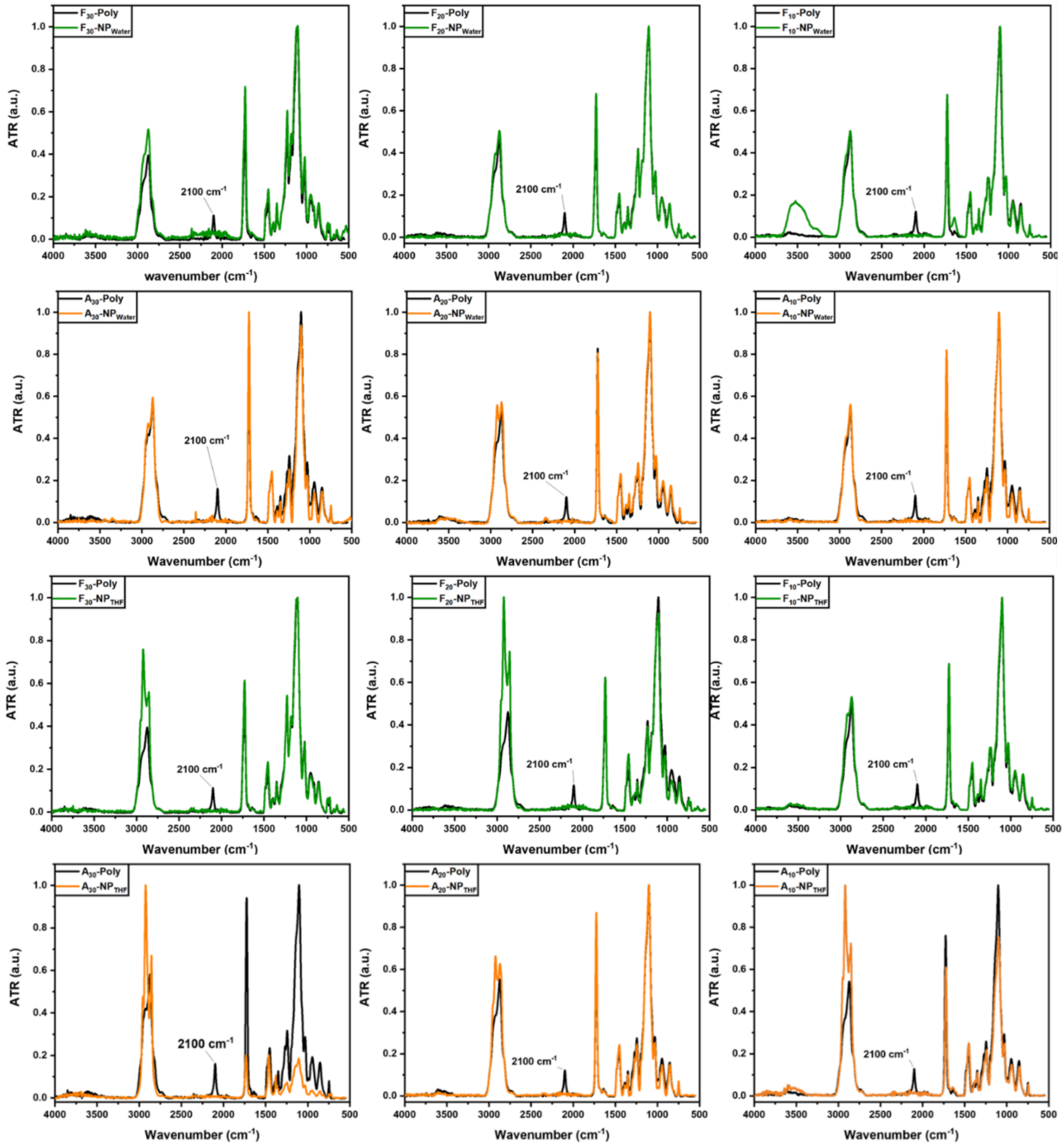


Figure S1: ATR-IR absorption spectra for all copolymers and their corresponding SCNPs

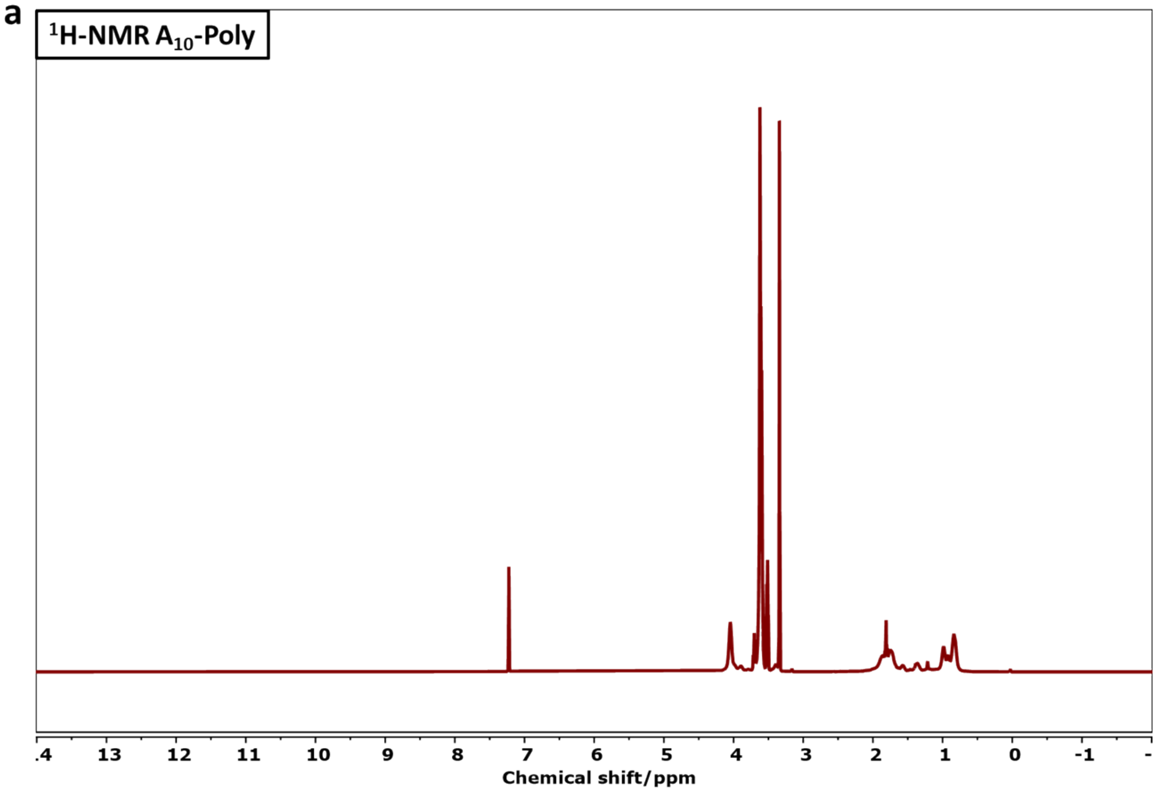
iii. NMR spectra

a. FWHM analysis:

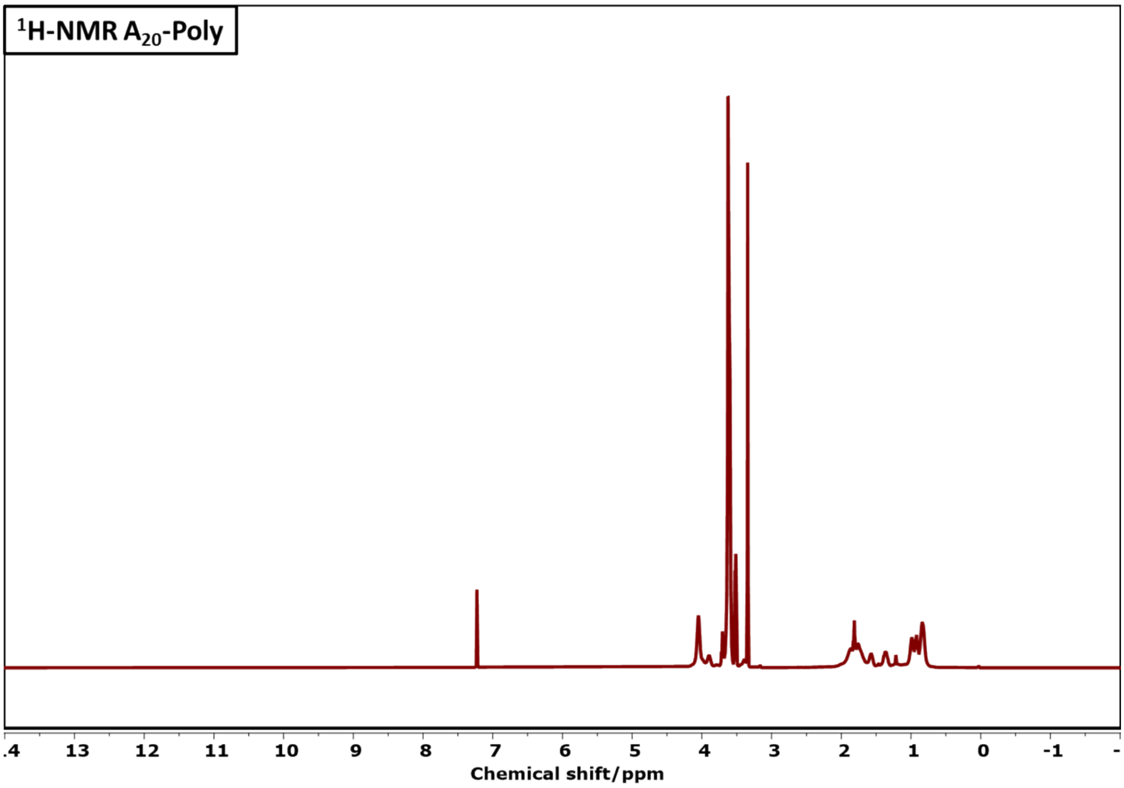
Table S2: FWHM from ^{1}H-NMR spectra (units in mHz). a: -O-CH$_3$ (PEGMA), b: -COO-CH$_2$- (BuMA/HFBMA)

FWHM*	A_{10}-Poly		A_{20}-Poly		A_{30}-Poly		F_{10}-Poly		F_{20}-Poly		F_{30}-Poly	
	a	b	a	b	a	b	a	b	a	b	a	b
	6.1	43	7.3	60	7.3	55	6.0	44	6.1	90	9.0	93
	A_{10}-NP$_{Water}$		A_{20}-NP$_{Water}$		A_{30}-NP$_{Water}$		F_{10}-NP$_{Water}$		F_{20}-NP$_{Water}$		F_{30}-NP$_{Water}$	
	9.0	50	20	110	14	120	8.1	55	15	110	12	110
	A_{10}-NP$_{THF}$		A_{20}-NP$_{THF}$		A_{30}-NP$_{THF}$		F_{10}-NP$_{THF}$		F_{20}-NP$_{THF}$		F_{30}-NP$_{THF}$	
	8.0	80	7.4	64	10	93	8.0	40	14	130	11	110

b. H-NMR spectra:

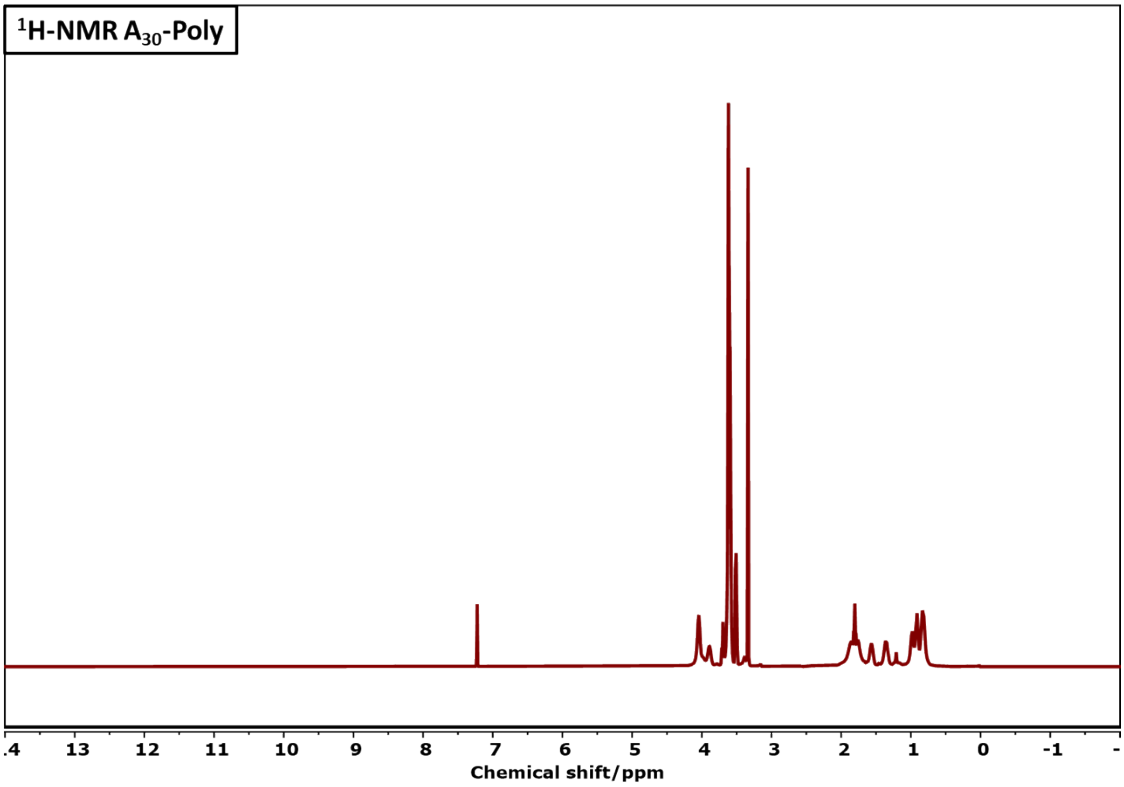


b

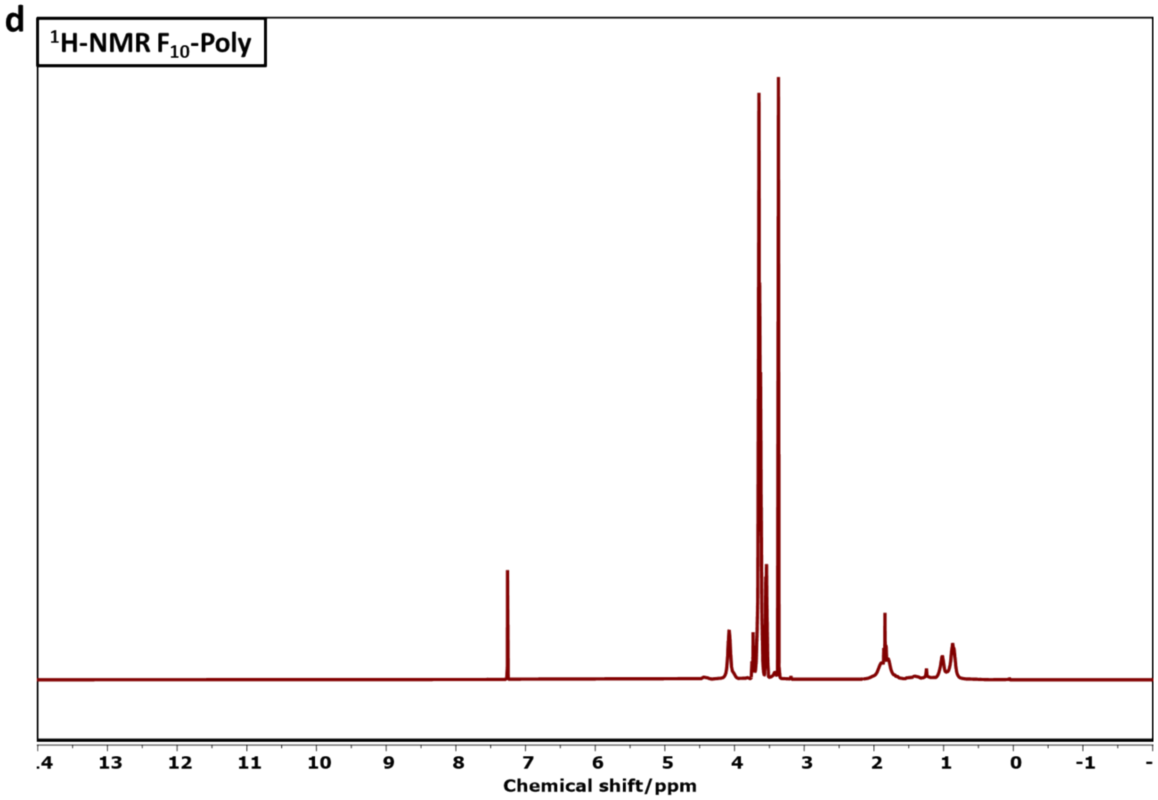


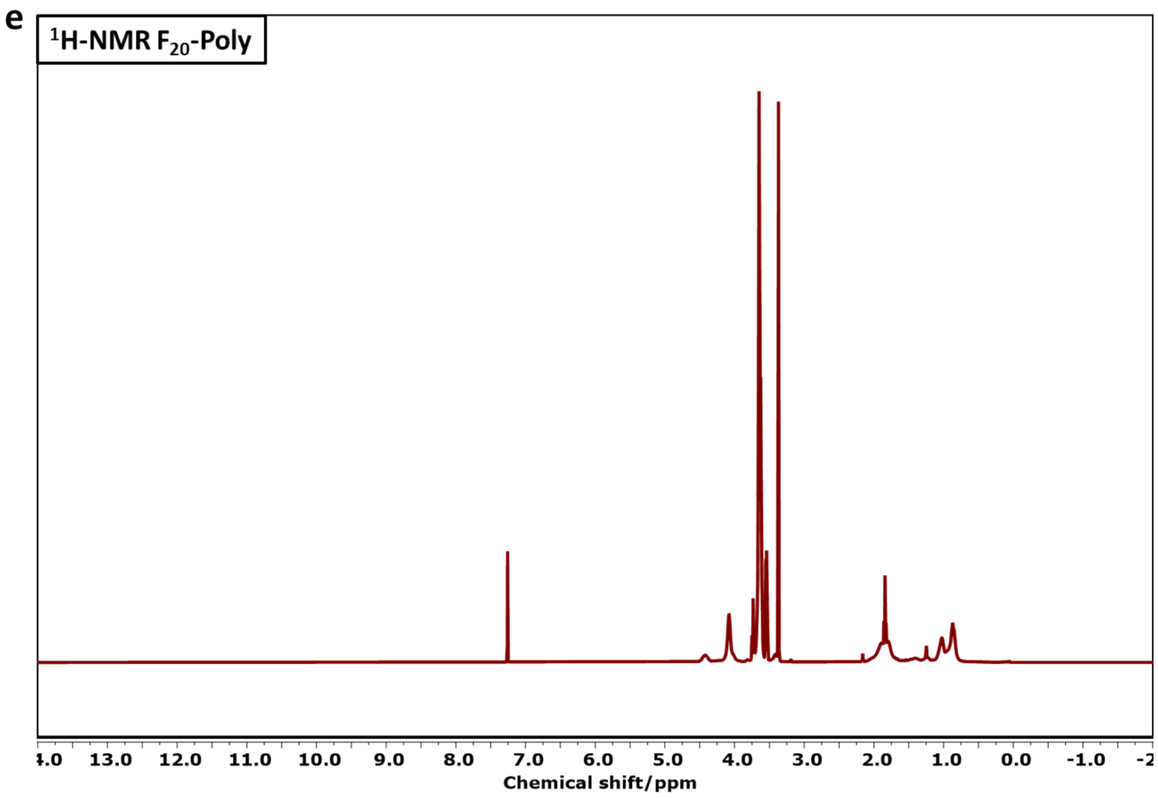
^{1}H-NMR A_{20}-Poly
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm

c



^{1}H-NMR A_{30}-Poly
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm


d
^{1}H-NMR F_{10}-Poly
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm


e
^{1}H-NMR F_{20}-Poly
4.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2
Chemical shift/ppm

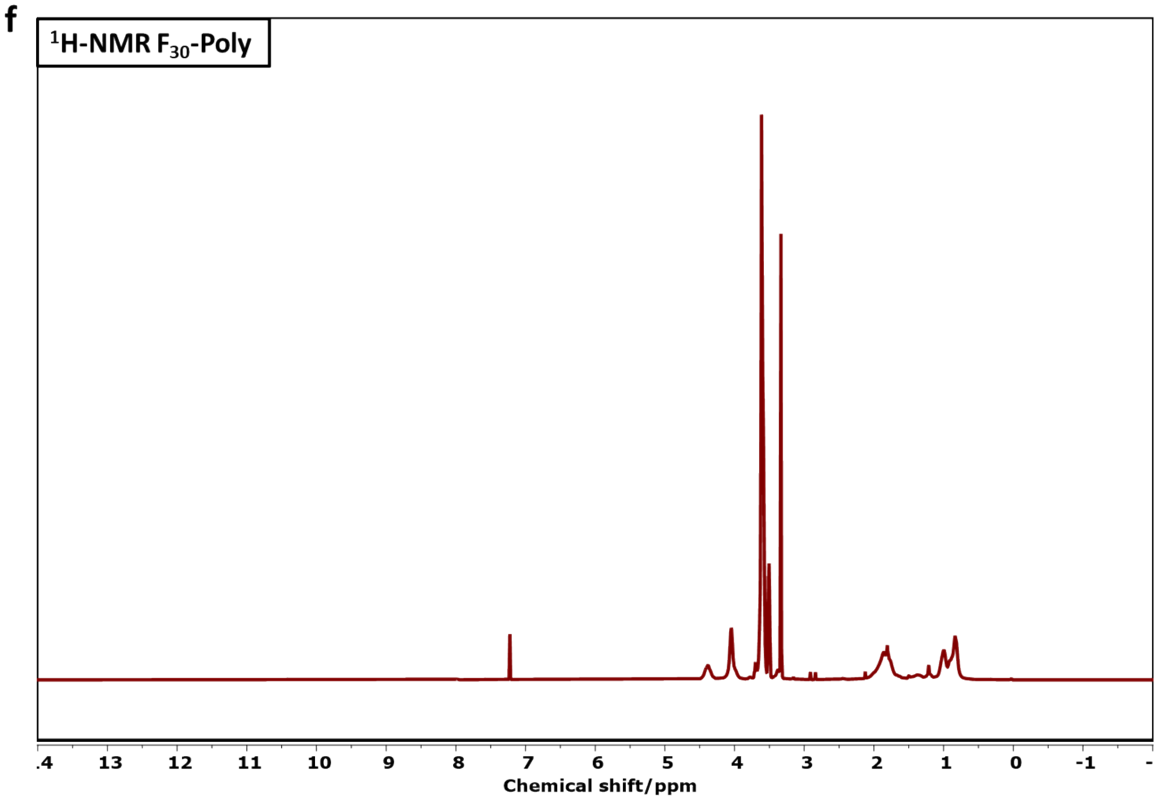


Figure S2: ^{1}H-NMR spectra in $CDCl_3$ of copolymer species a) A_{10}-Poly b) A_{20}-Poly c) A_{30}-Poly d) F_{10}-Poly e) F_{20}-Poly f) F_{30}-Poly

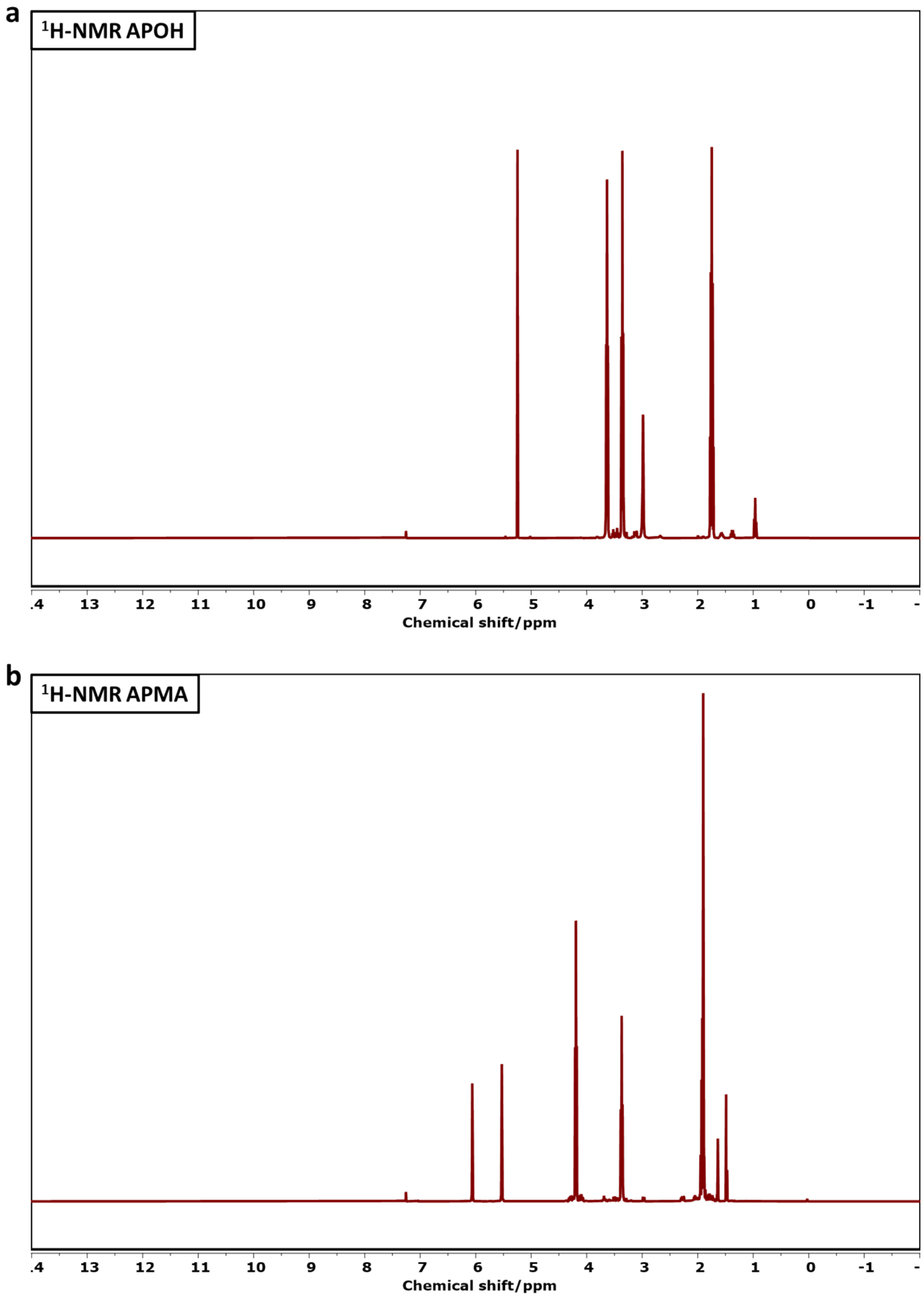


Figure S3: ^{1}H-NMR spectra in $CDCl_3$ of a) APOH and b) APMA

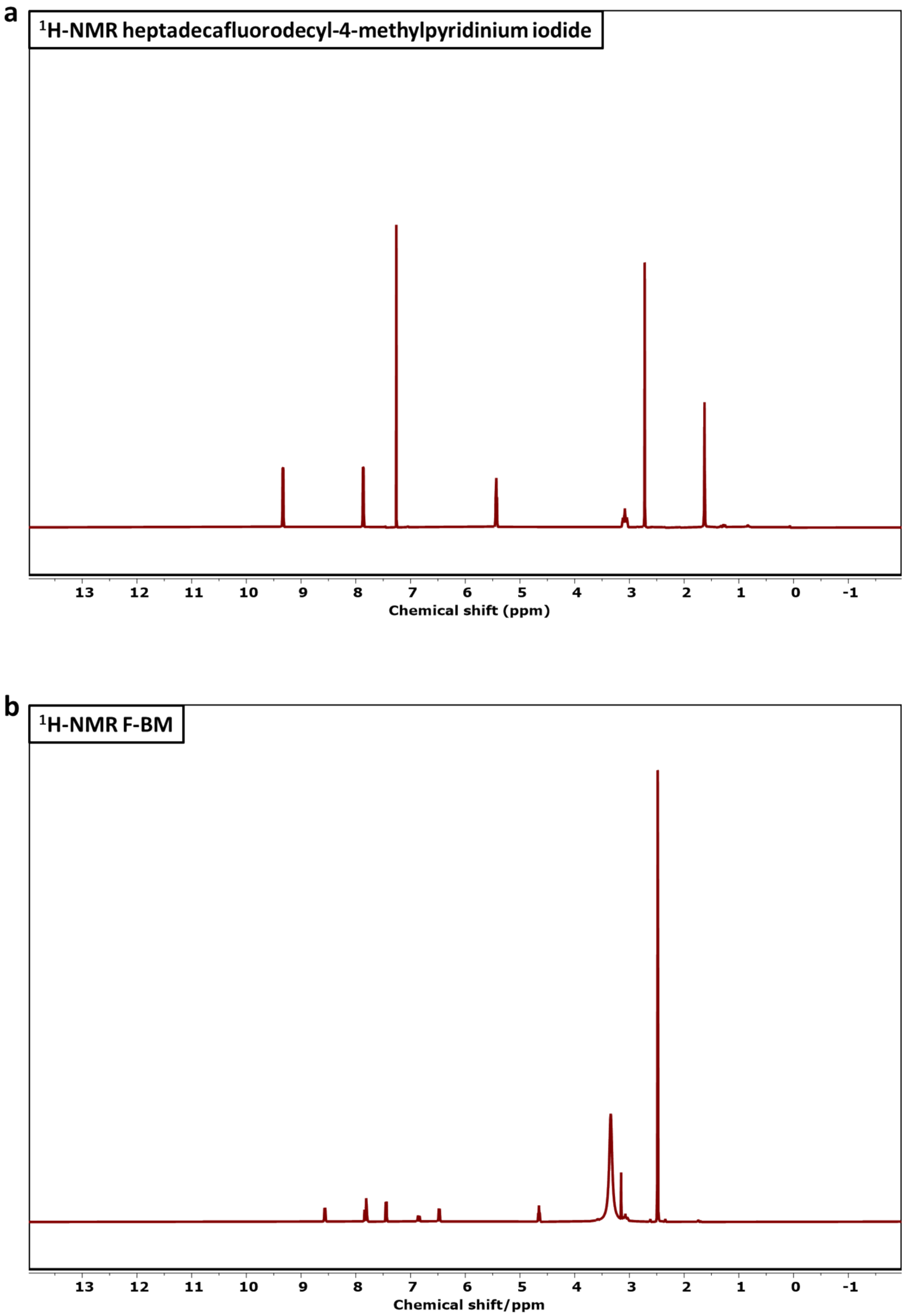


Figure S4: ^{1}H-NMR spectra of a) heptadecafluorodecyl-4-methylpyridinium iodide in $CDCl_3$ and b) F-BM in DMSO

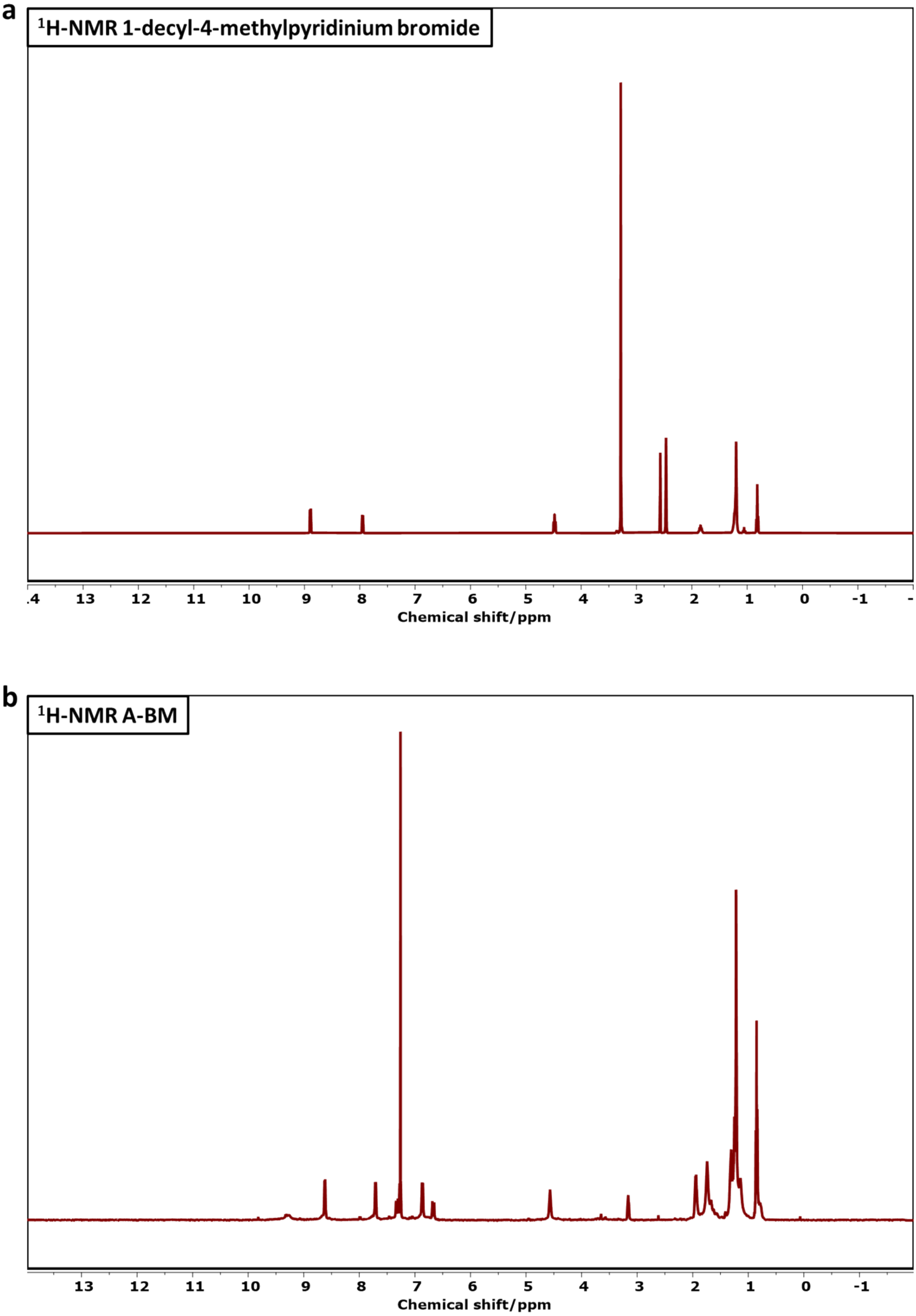
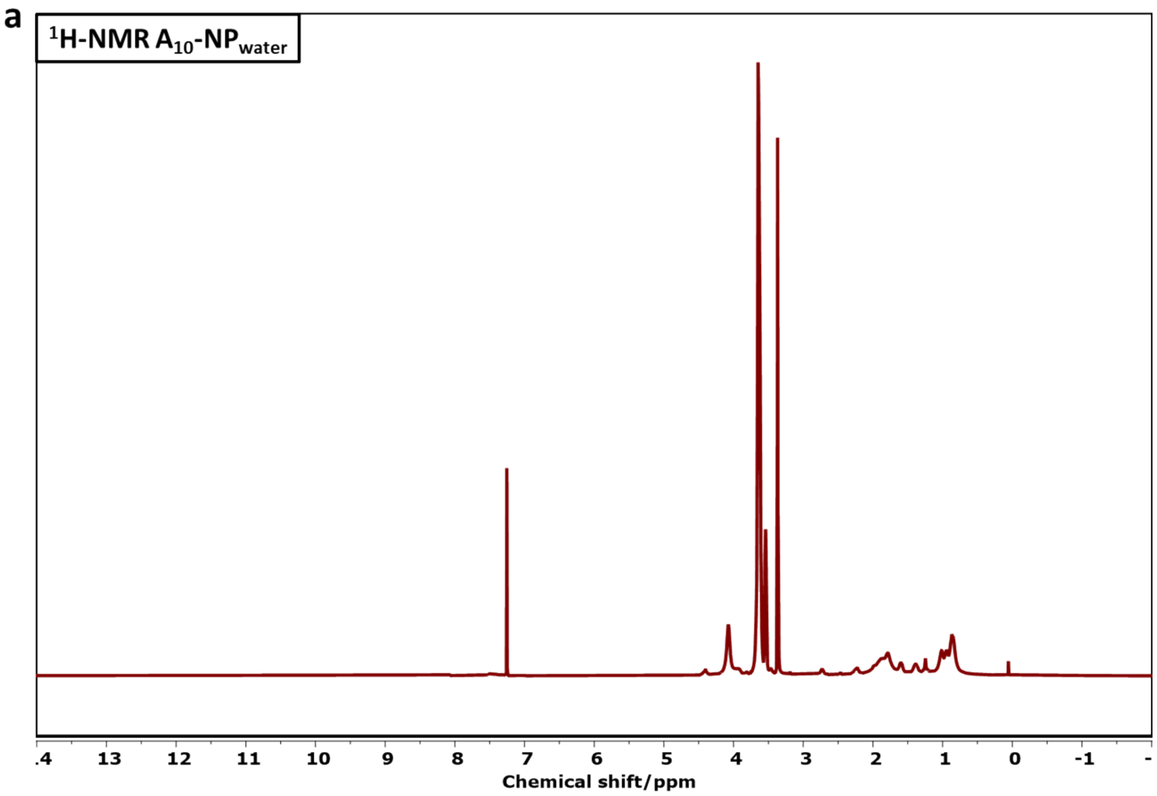
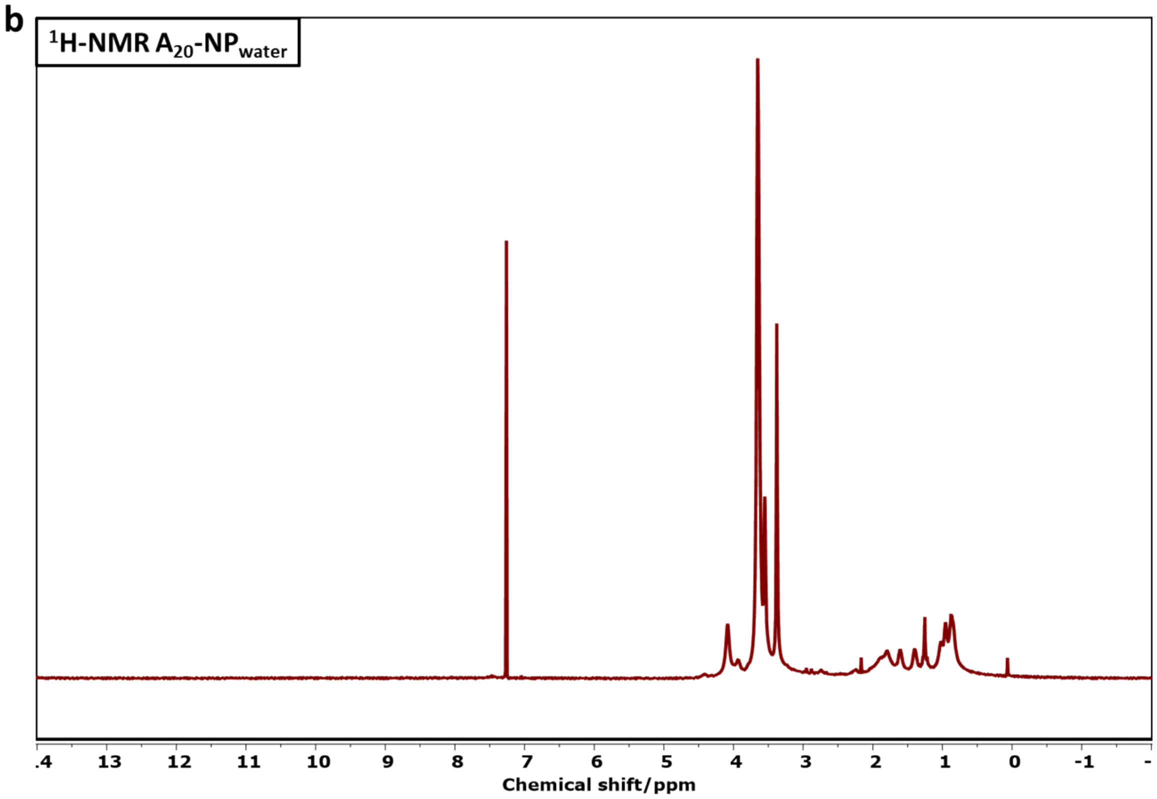
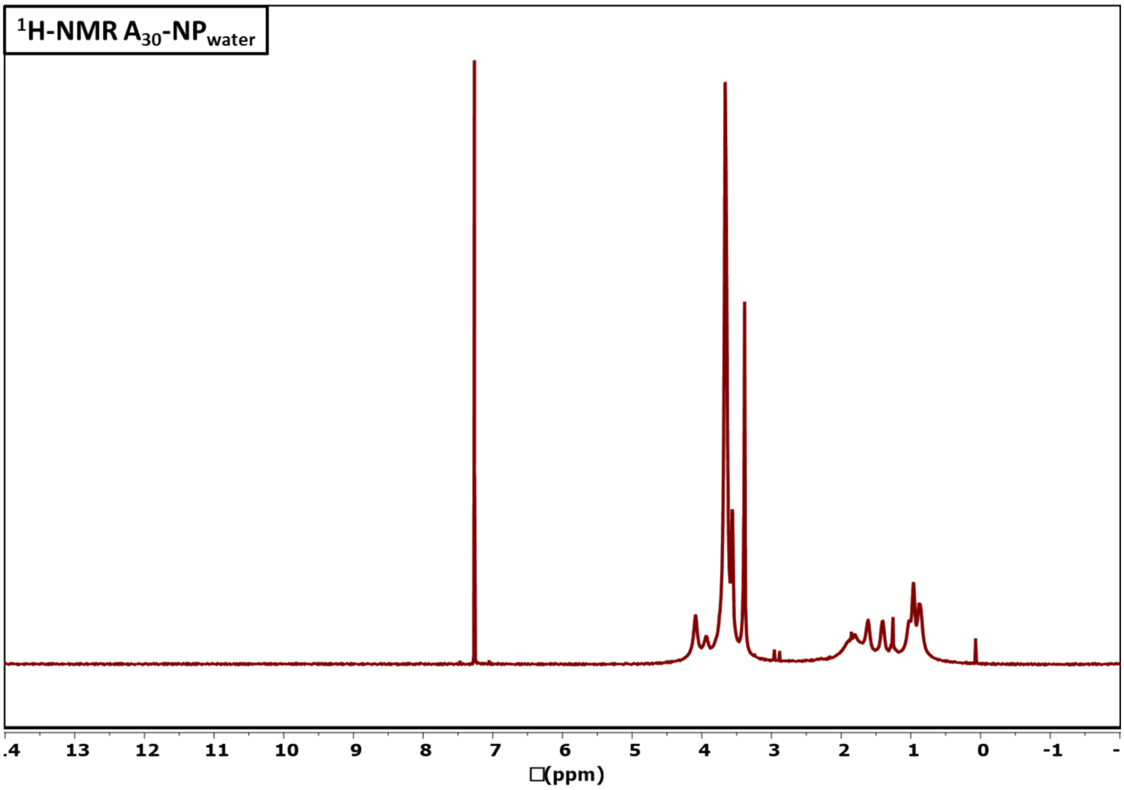


Figure S5: ^{1}H-NMR spectra of a) 1-decyl-4-methylpyridinium bromide in DMSO and b) A-BM in $CDCl_3$

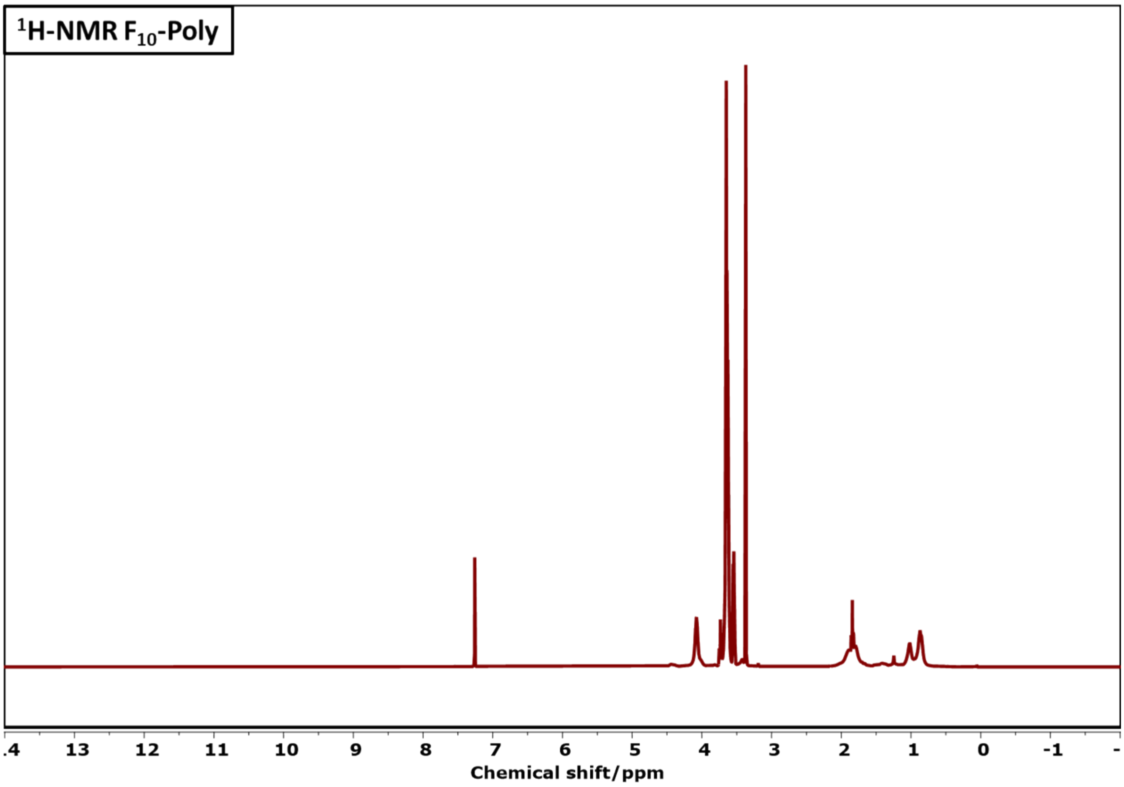

a
^{1}H-NMR A_{10}-NP_{water}
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm

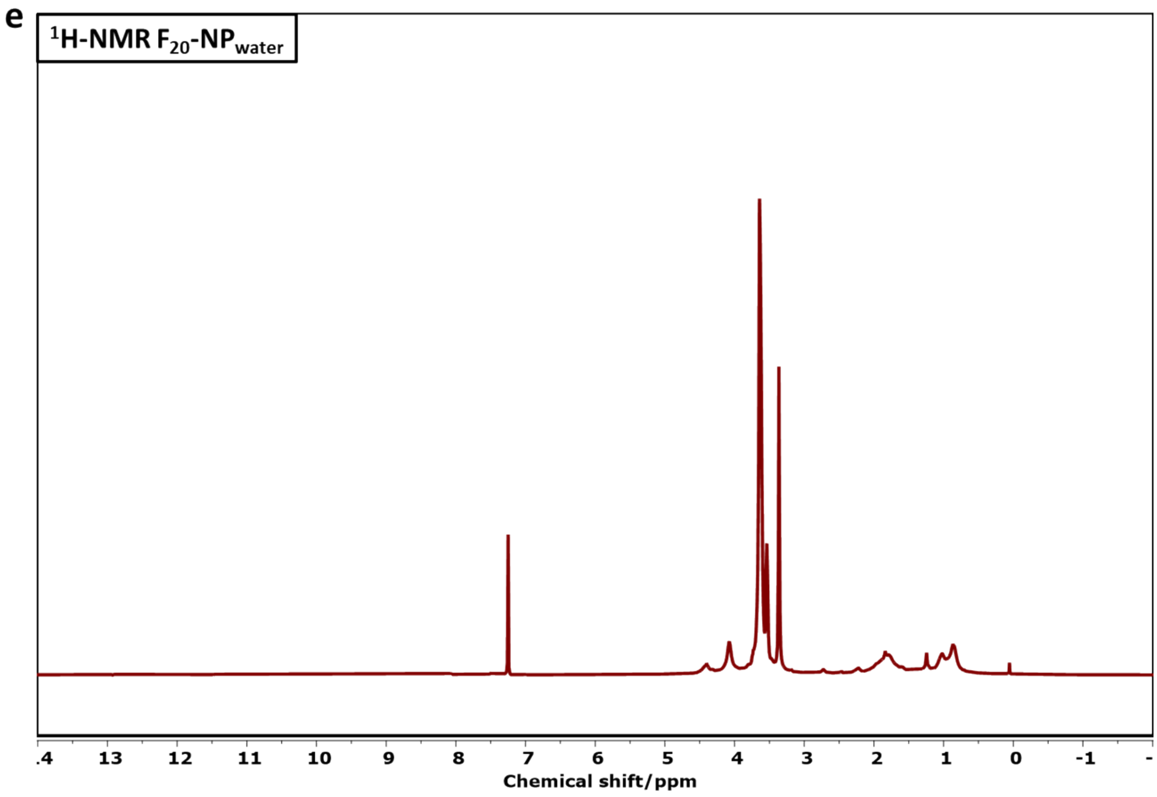

b
^{1}H-NMR A_{20}-NP_{water}
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm

c


^{1}H-NMR A_{30}-NP_{water}
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
□(ppm)

d


^{1}H-NMR F_{10}-Poly
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm



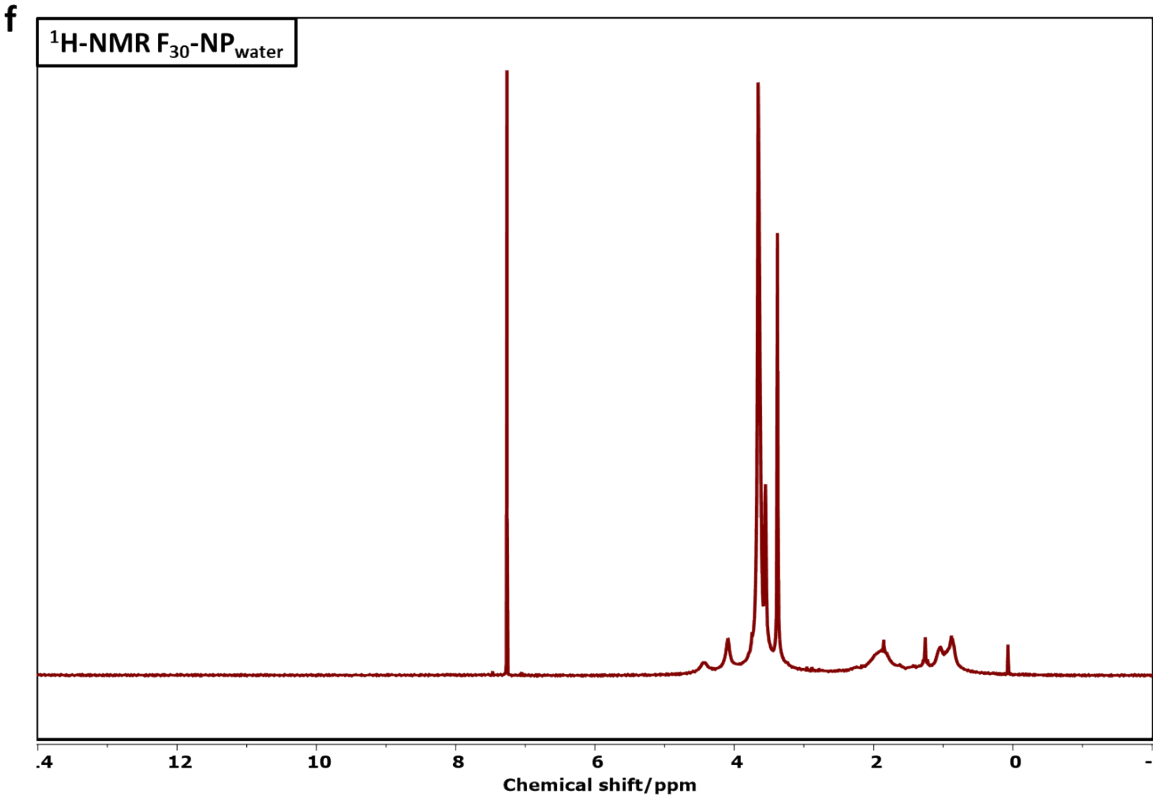
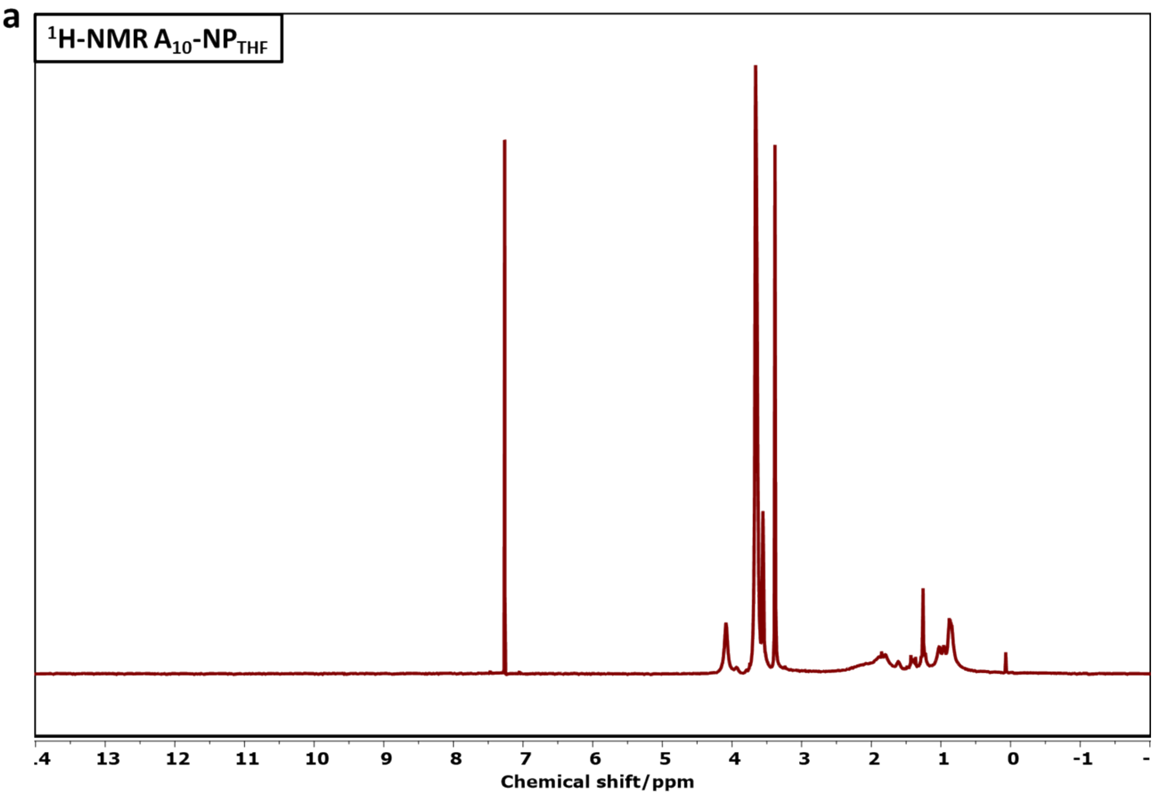
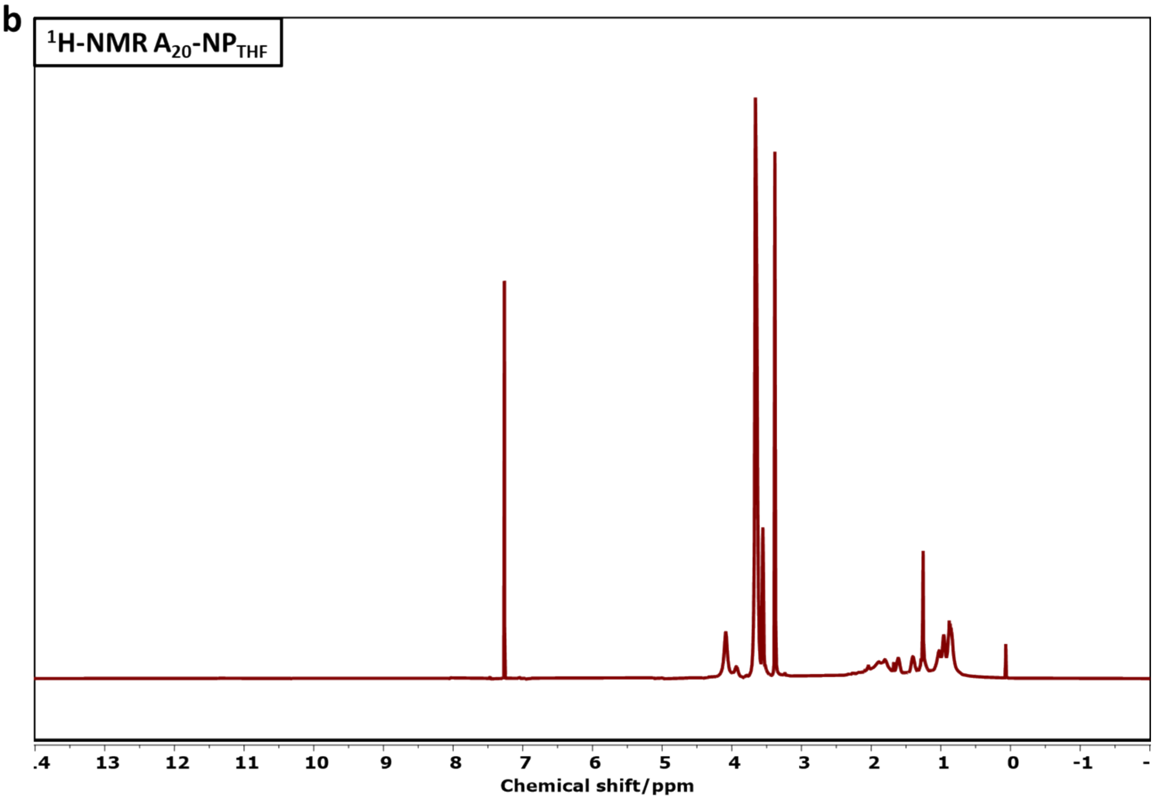
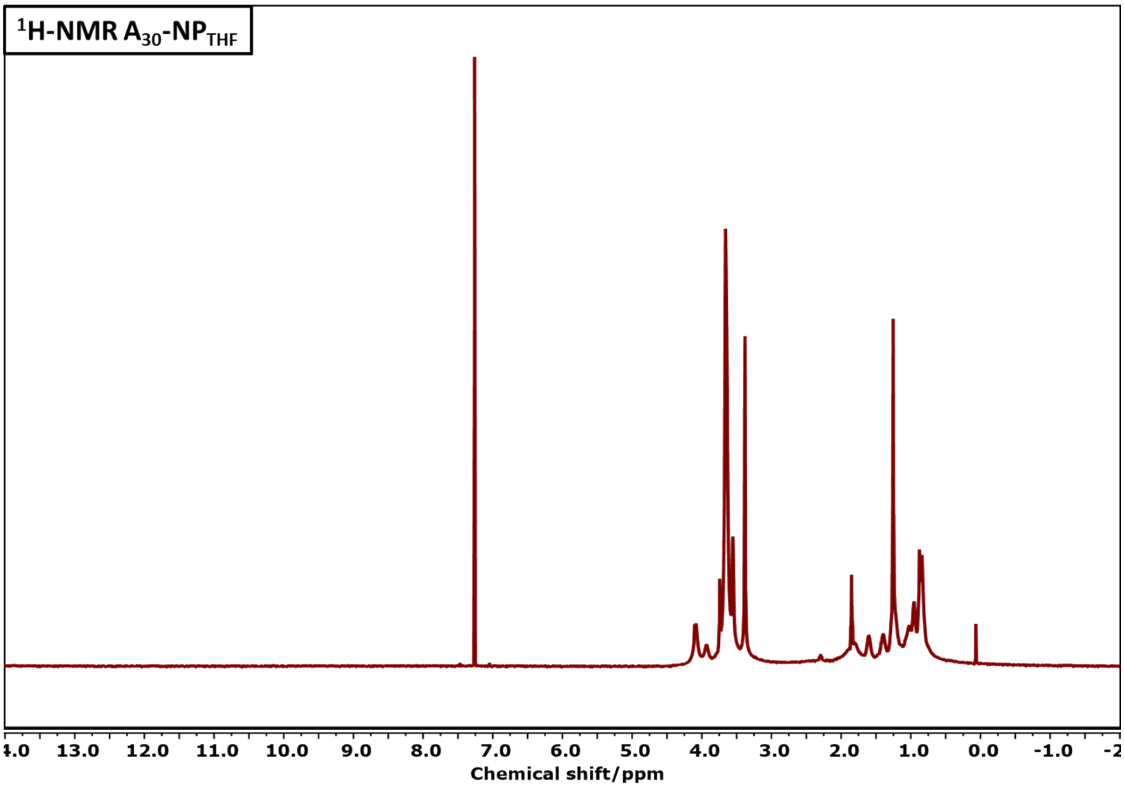


Figure S6: ^{1}H-NMR spectra in $CDCl_3$ of a) A_{10}-NP_{water} b) A_{20}-NP_{water} c) A_{30}-NP_{water} d) F_{10}-NP_{water} e) F_{20}-NP_{water} f) F_{30}-NP_{water}

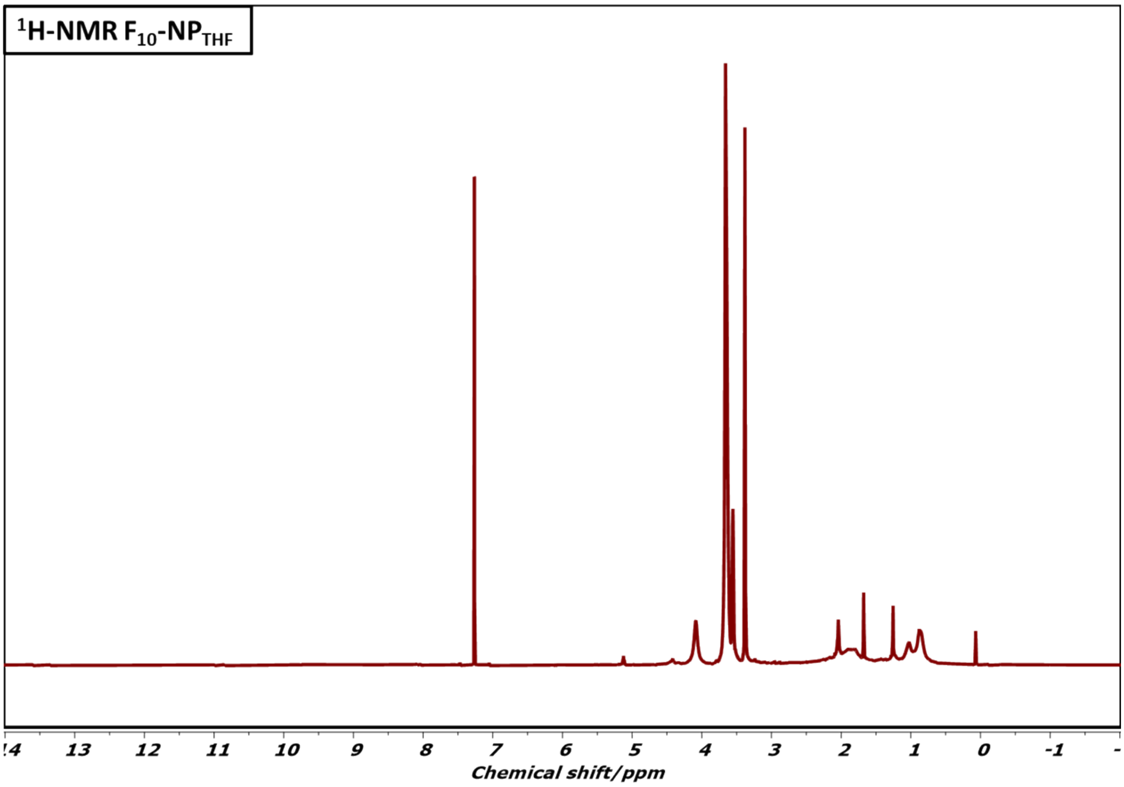

a
^{1}H-NMR A_{10}-NP_{THF}
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm


b
^{1}H-NMR A_{20}-NP_{THF}
.4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-
Chemical shift/ppm

c


^{1}H-NMR A_{30}-NP_{THF}
4.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2
Chemical shift/ppm

d


^{1}H-NMR F_{10}-NP_{THF}
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
Chemical shift/ppm

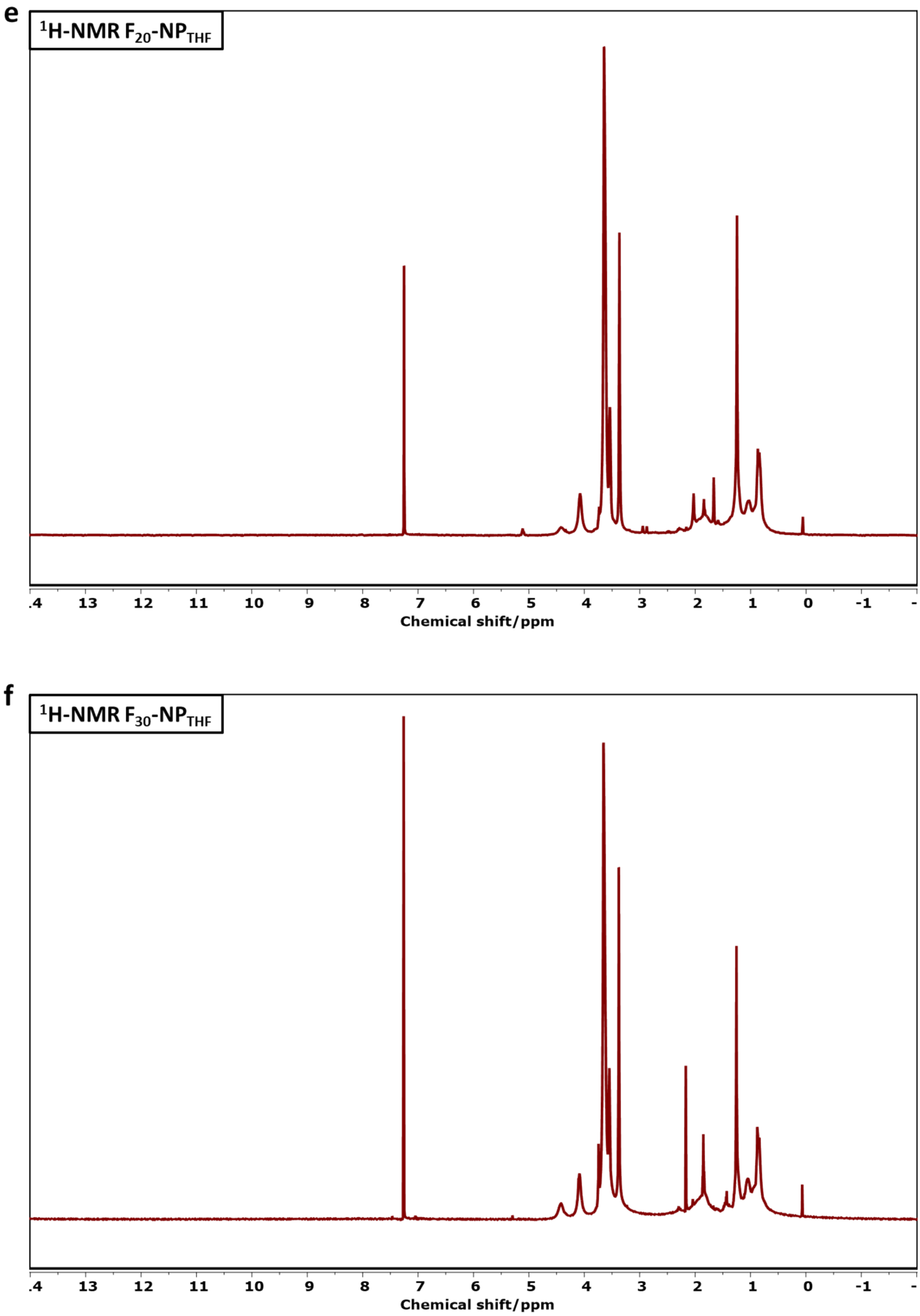
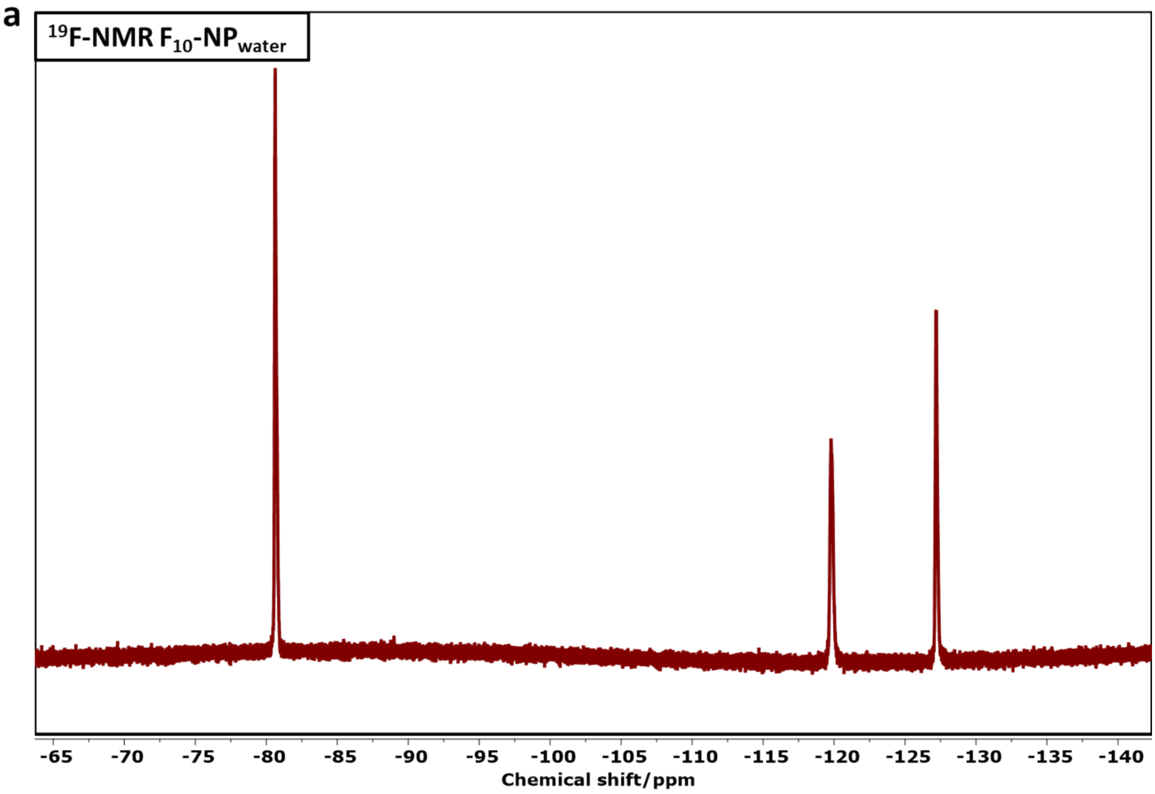
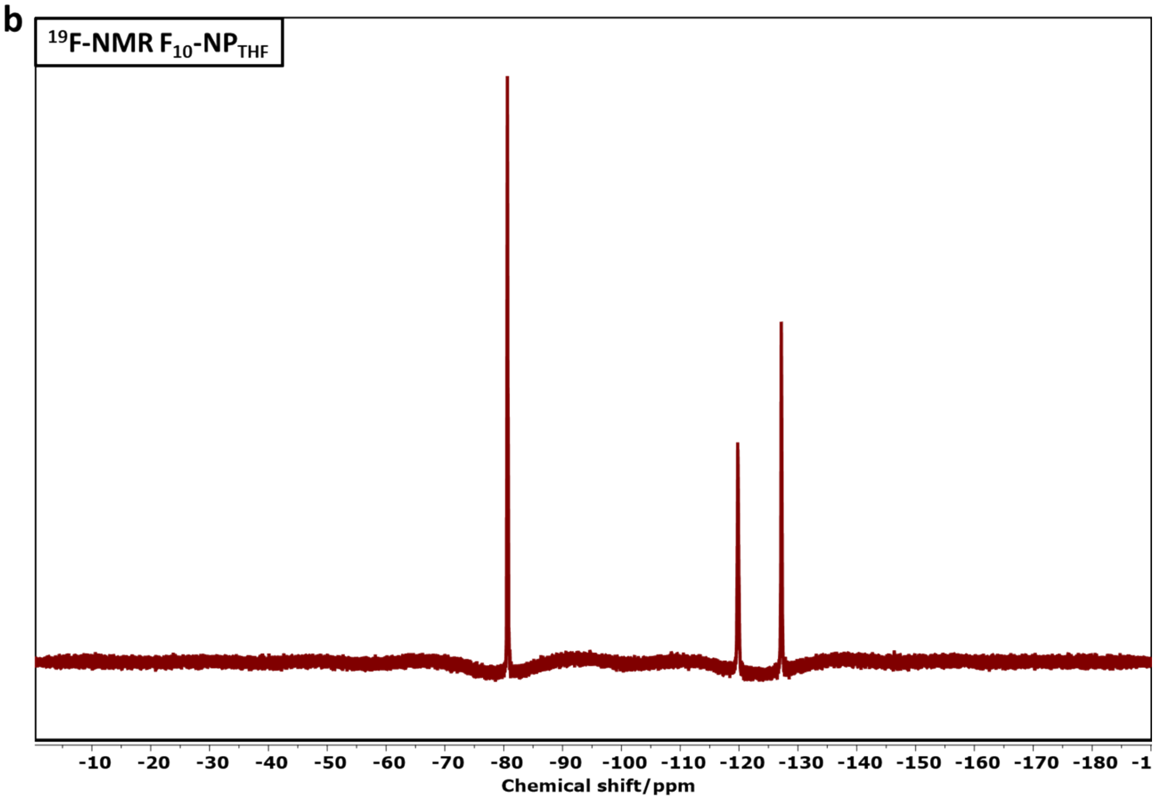


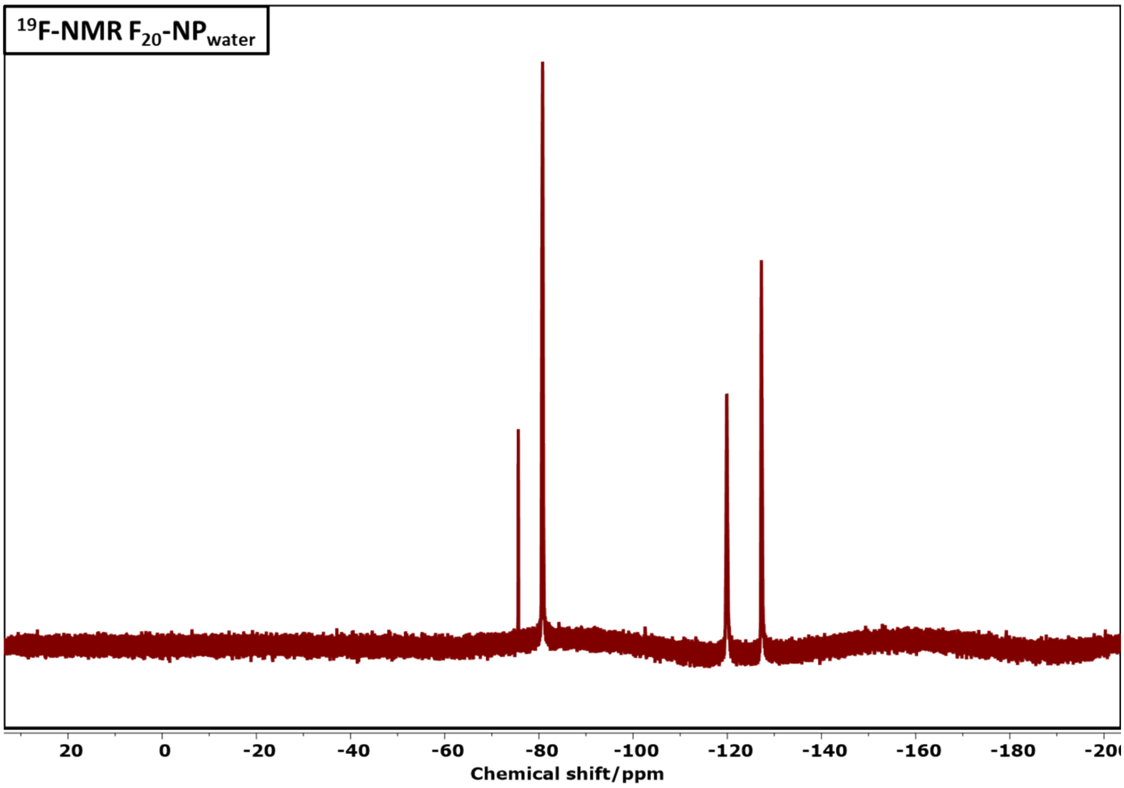
Figure S7: ^{1}H-NMR spectra in $CDCl_3$ of a) A_{10}-NP_{THF} b) A_{20}-NP_{THF} c) A_{30}-NP_{THF} d) F_{10}-NP_{THF} e) F_{20}-NP_{THF} f) F_{30}-NP_{THF}



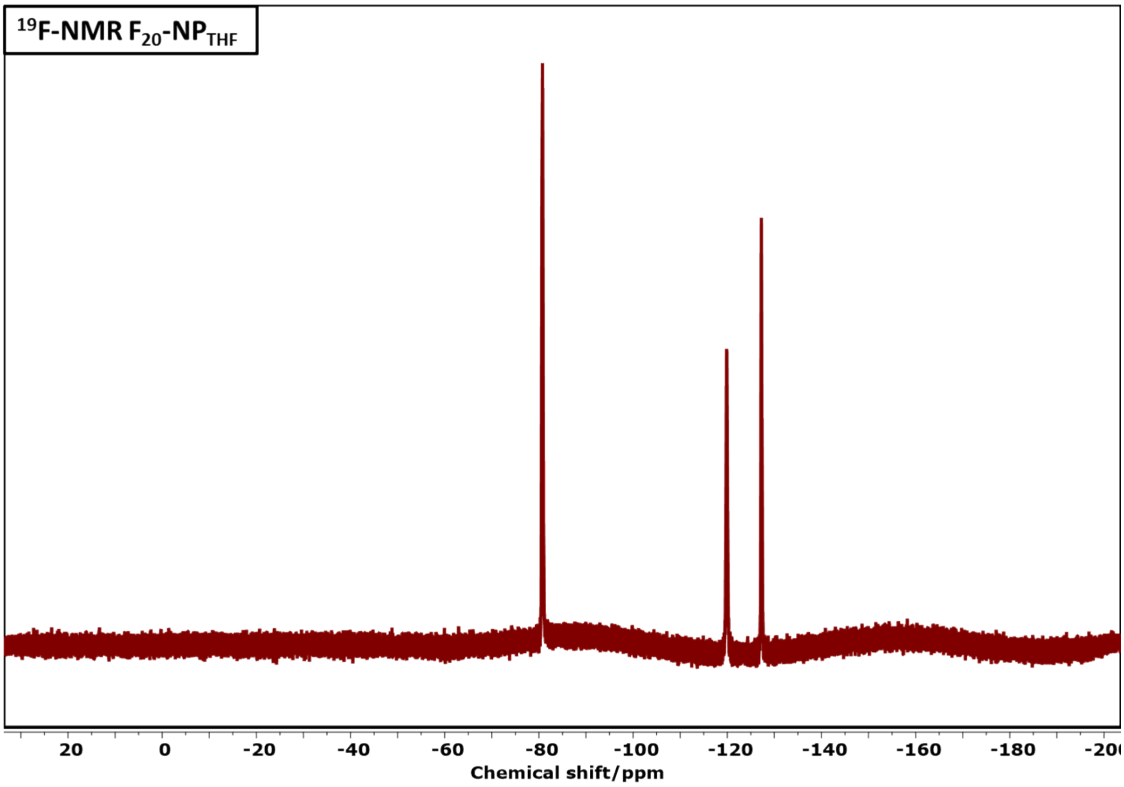
c. ^{19}F-NMR spectra:

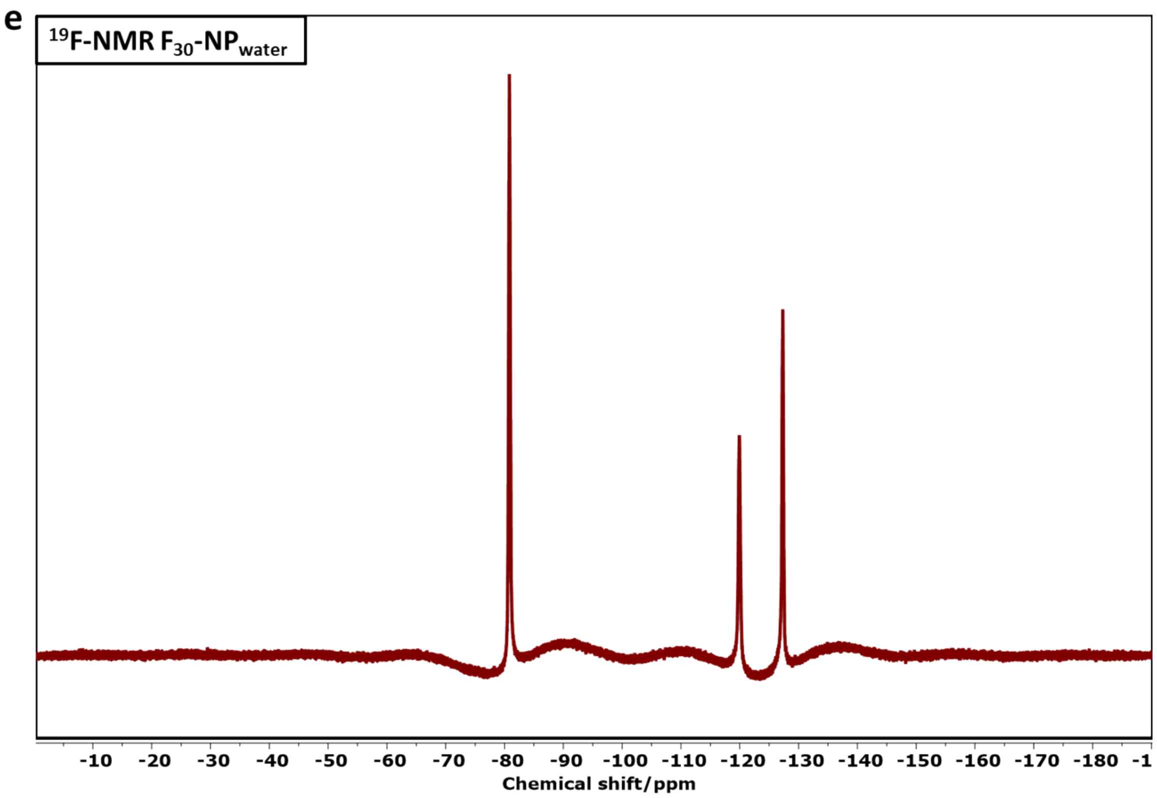


c


^{19}F-NMR F_{20}-NP_{water}
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-20
Chemical shift/ppm

d


^{19}F-NMR F_{20}-NP_{THF}
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-20
Chemical shift/ppm



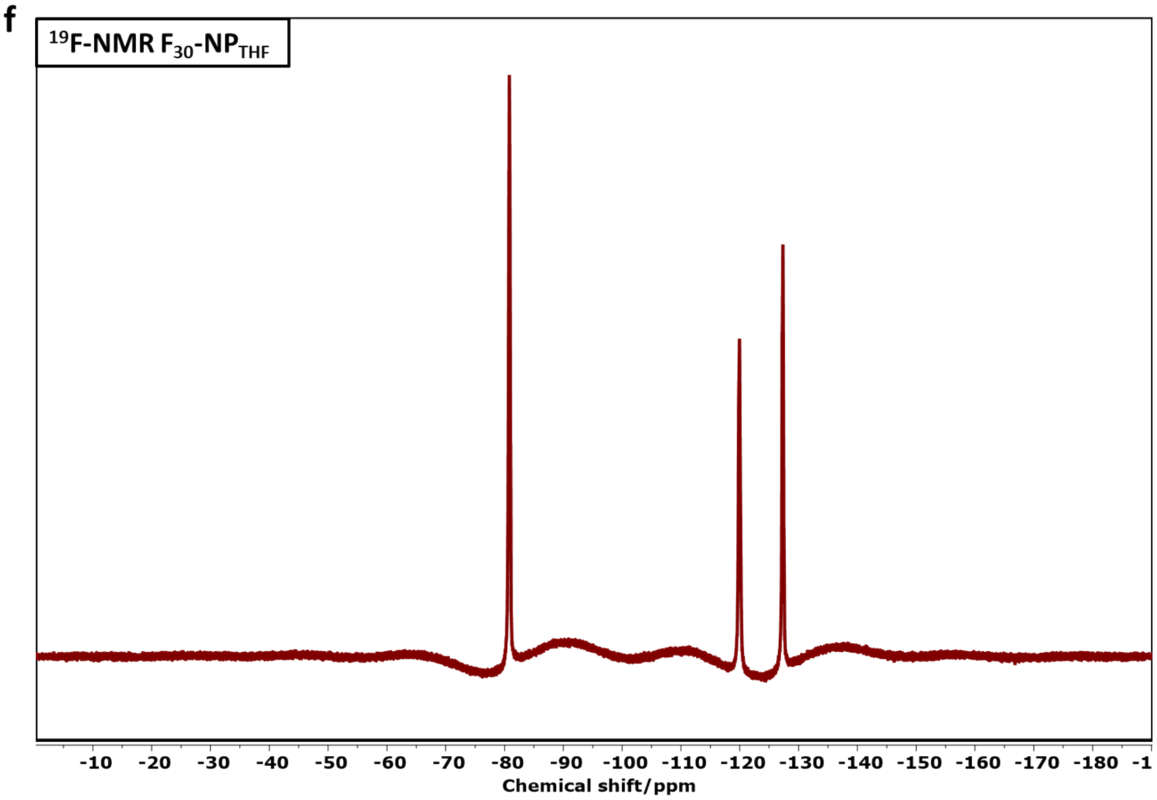
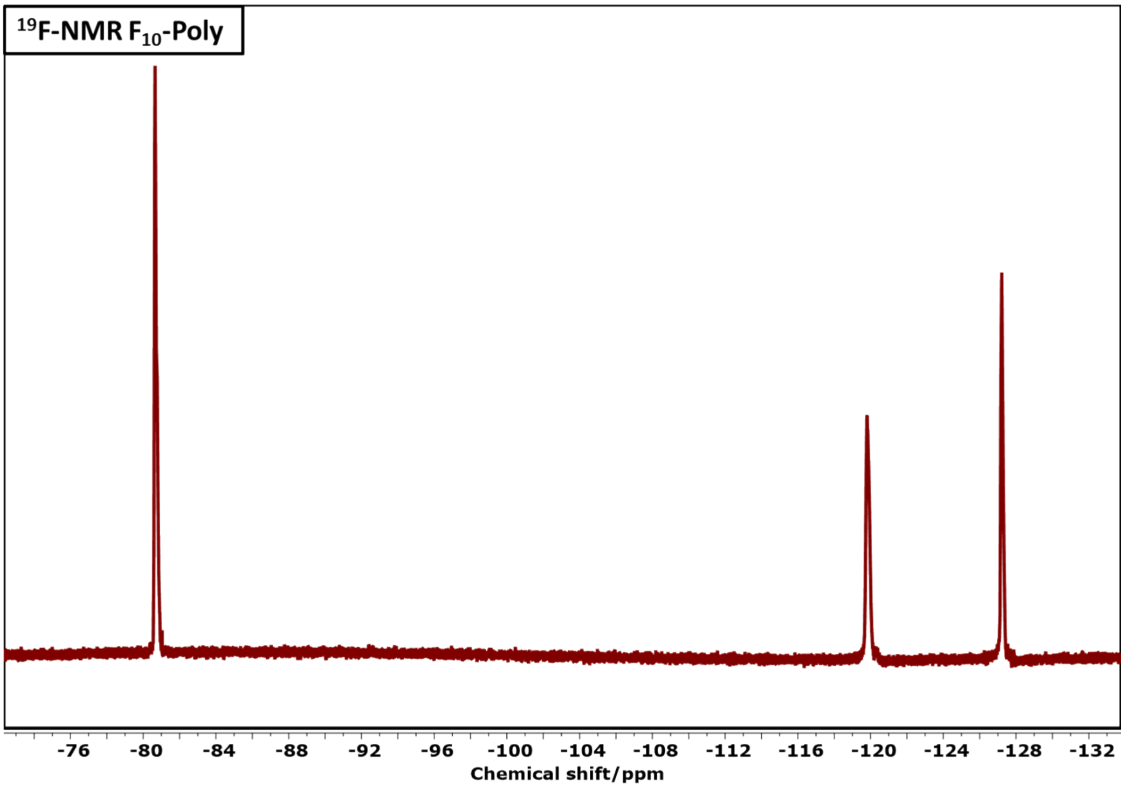
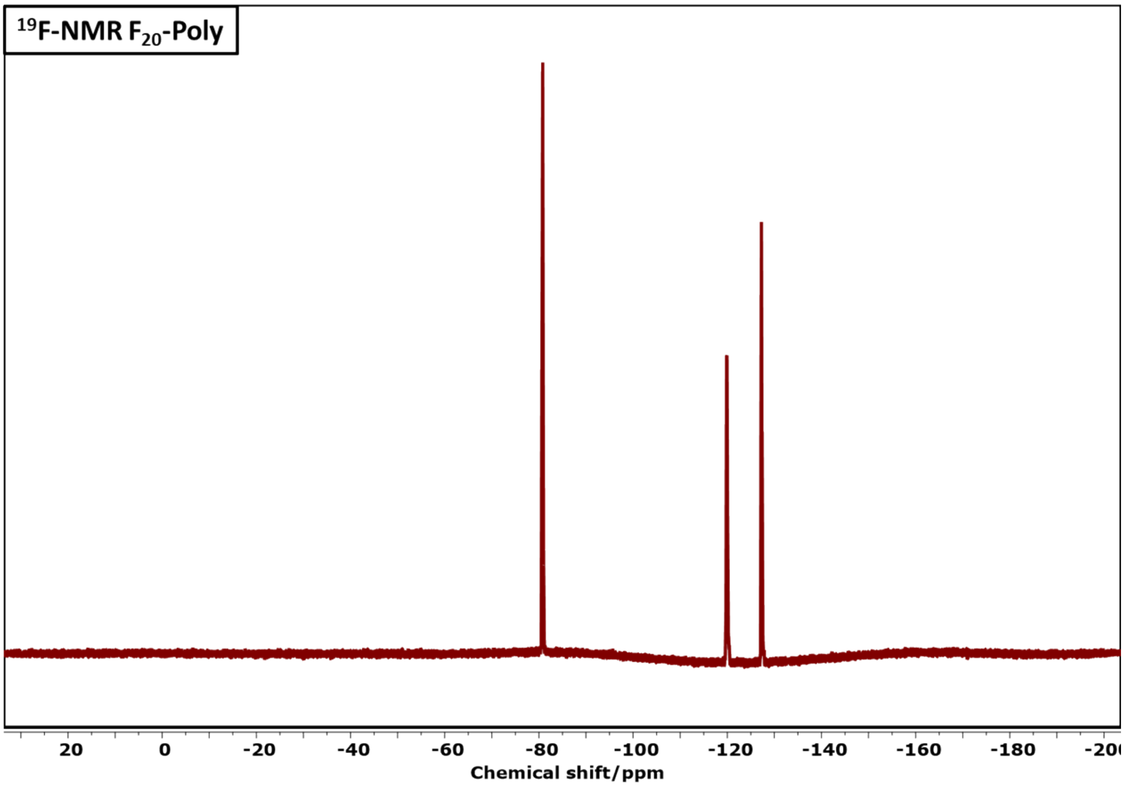


Figure S8: ^{19}F-NMR spectra in $CDCl_3$ of a) F_{10}-NP_{water} b) F_{10}-NP_{THF} c) F_{20}-NP_{water} d) F_{20}-NP_{THF} e) F_{30}-NP_{water} f) F_{30}-NP_{THF}

a


^{19}F-NMR F_{10}-Poly
-76
-80
-84
-88
-92
-96
-100
-104
-108
-112
-116
-120
-124
-128
-132
Chemical shift/ppm

b


^{19}F-NMR F_{20}-Poly
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-20
Chemical shift/ppm

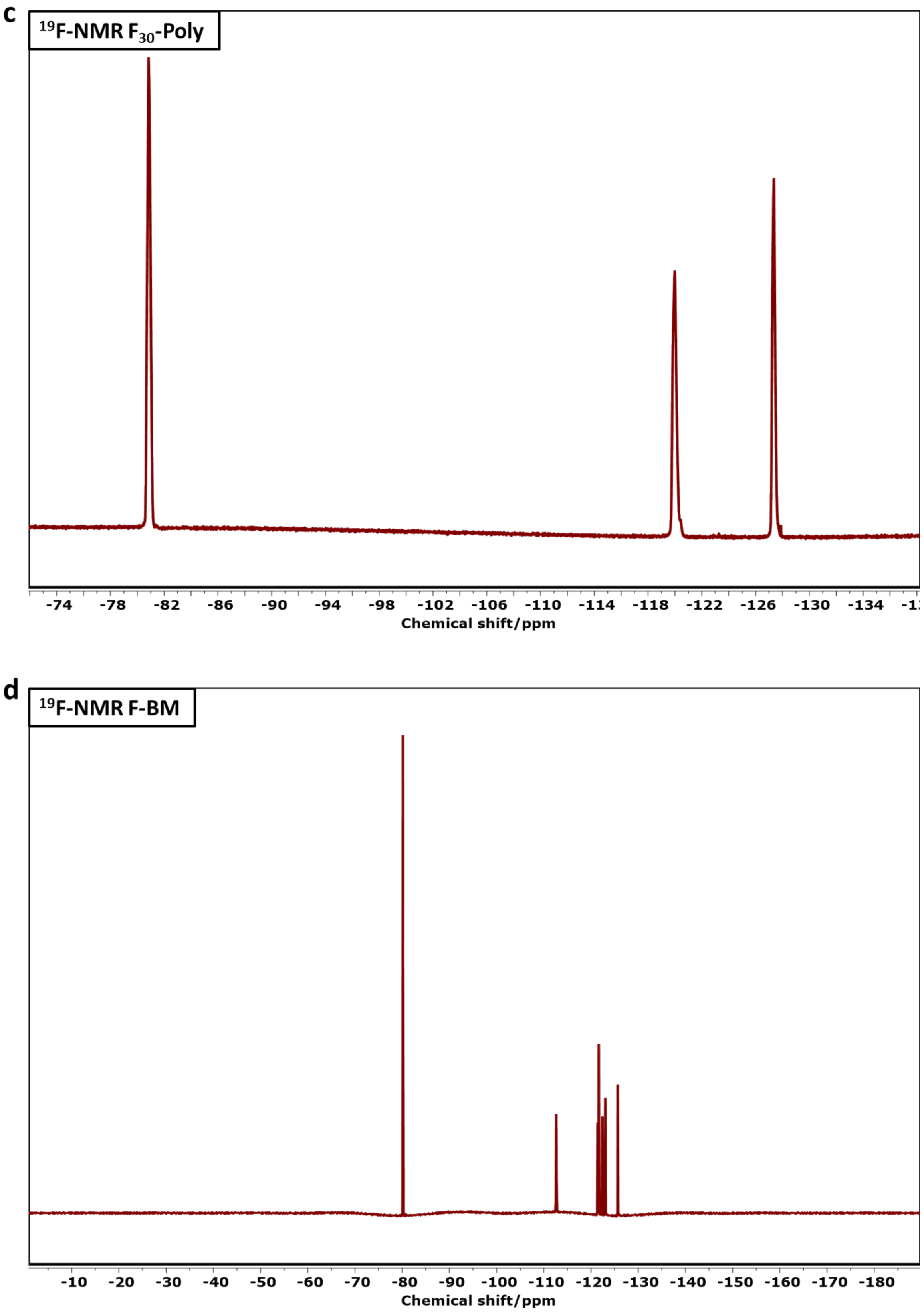


Figure S9: ^{19}F-NMR spectra in $CDCl_3$ of a) F_{10}-Poly b) F_{20}-Poly c) F_{30}-Poly d) F-BM

iv. SEC/GPC

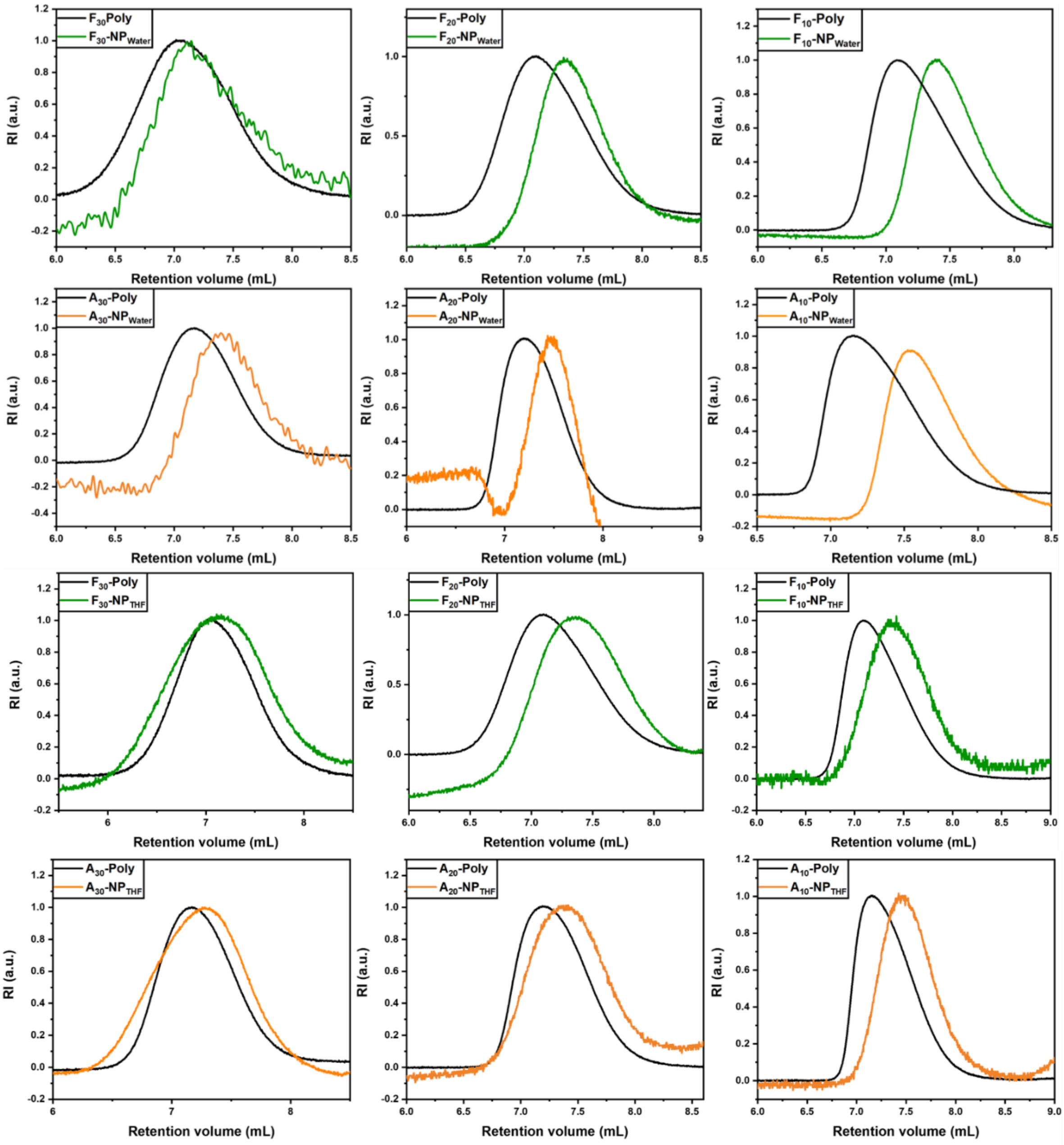


Figure S10: Retention curves of all SCNPs and their corresponding precursor polymers in THF

v. AFM

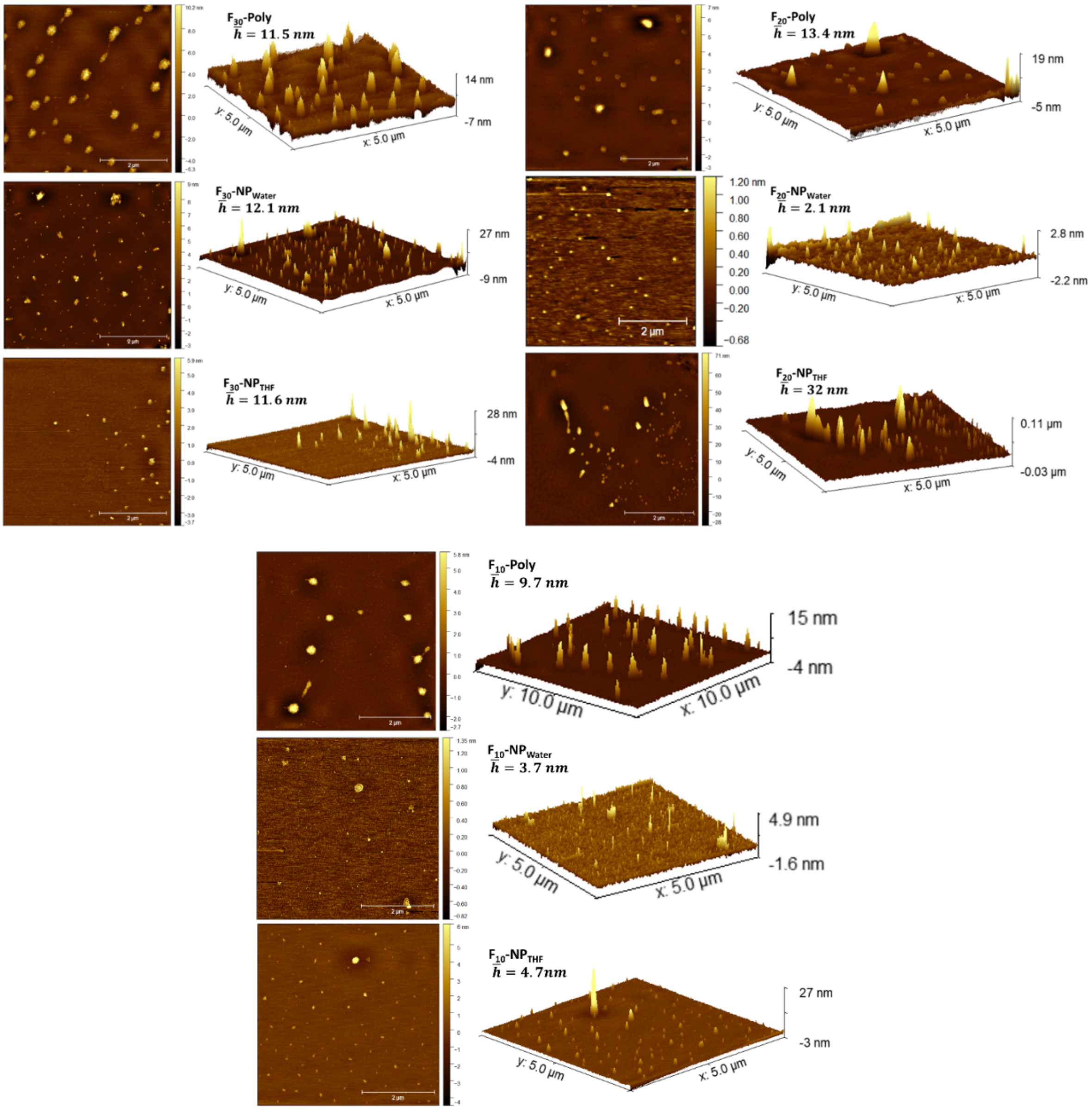

F_{30}-Poly
$\bar{h} = 11.5\ nm$
14 nm
-7 nm
y: 5.0 μm
x: 5.0 μm
F_{20}-Poly
$\bar{h} = 13.4\ nm$
19 nm
-5 nm
y: 5.0 μm
x: 5.0 μm
F_{30}-NP_{Water}
$\bar{h} = 12.1\ nm$
27 nm
-9 nm
y: 5.0 μm
x: 5.0 μm
1.20 nm
1.00
0.80
0.60
0.40
0.20
0.00
−0.20
−0.68
2 μm
F_{20}-NP_{Water}
$\bar{h} = 2.1\ nm$
2.8 nm
-2.2 nm
y: 5.0 μm
x: 5.0 μm
F_{30}-NP_{THF}
$\bar{h} = 11.6\ nm$
28 nm
-4 nm
y: 5.0 μm
x: 5.0 μm
F_{20}-NP_{THF}
$\bar{h} = 32\ nm$
0.11 μm
-0.03 μm
y: 5.0 μm
x: 5.0 μm
F_{10}-Poly
$\bar{h} = 9.7\ nm$
15 nm
-4 nm
y: 10.0 μm
x: 10.0 μm
F_{10}-NP_{Water}
$\bar{h} = 3.7\ nm$
4.9 nm
-1.6 nm
y: 5.0 μm
x: 5.0 μm
F_{10}-NP_{THF}
$\bar{h} = 4.7 nm$
27 nm
-3 nm
y: 5.0 μm
x: 5.0 μm
2 μm

Figure S11: AFM images of F_x-Poly and their corresponding SCNPs.

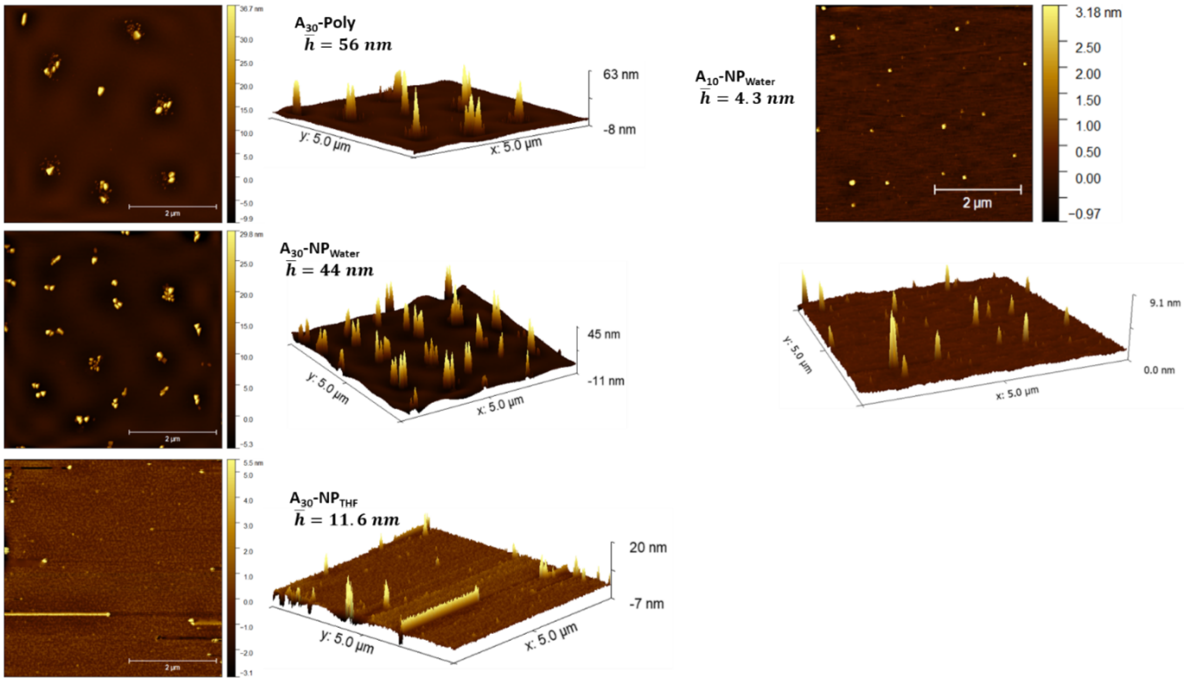


Figure S12: AFM images of A$_x$-Poly and their corresponding SCNPs.

vi. CW-EPR

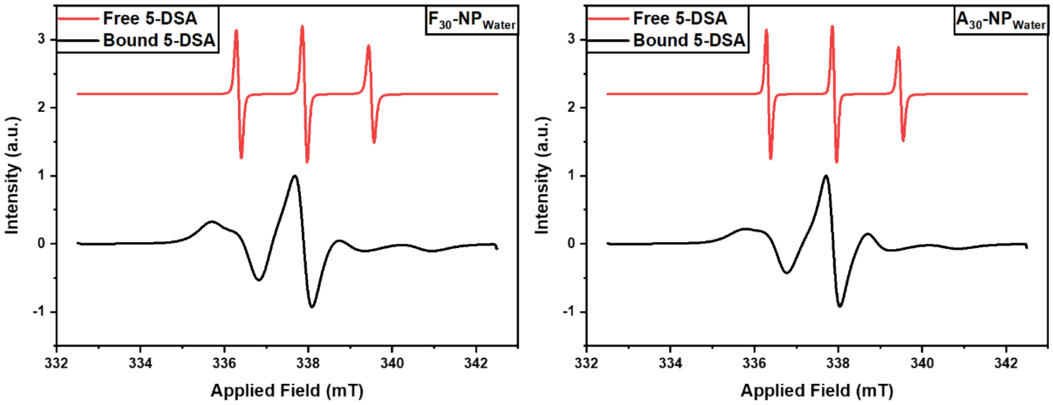
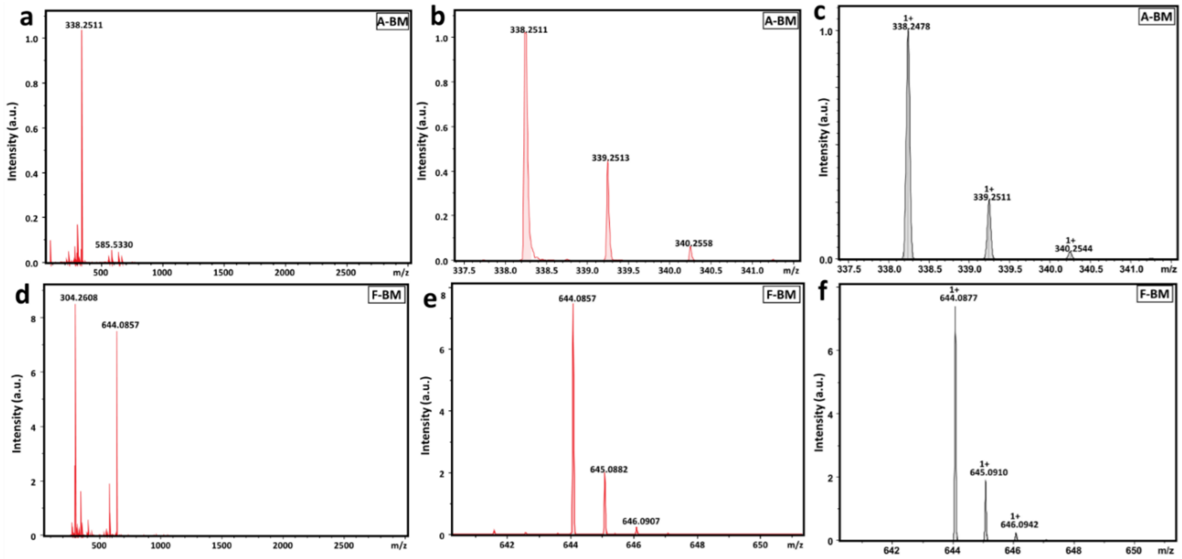


Figure S13: The two simulated components combined to yield the optimal simulations for the measured spetra. The freely rotating 5-DSA and the SCNP-bound '2 are depicted in red and blue, respectively.

Table S3: Simulation parameters of CW-EPR spectra of F_{30}-NP$_{Water}$ and A_{30}-NP$_{Water}$

	F_{30}-NP$_{Water}$		A_{30}-NP$_{Water}$	
	Free 5-DSA	Bound 5-DSA	Free 5-DSA	Bound 5-DSA
g_{xx}	1.9962567	2.0074281	2.0094929	2.0067197
g_{yy}	2.0135246	2.0049001	2.0021913	2.0053832
g_{zz}	2.0036917	2.0015733	2.0021577	2.0020345
A_{xx}	24.7734956	9.1496265	12.9680748	15.6047771
A_{yy}	6.9195420	27.1443999	19.3527560	23.8674000
A_{zz}	100.6686946	87.2809999	99.8334766	87.1368015
*logtcorr**	-9.99	-8.12	-9.75	-8.12



vii. ESI-ToF

Figure S14: ESI-ToF mass spectra of A-BM and F-BM. Full spectra: a) A-BM and d) F-BM. Detected spectra: b) A-BM and e) F-BM. Simulated spectra: c) A-BM and f) F-BM

viii. Absorption and Emission Spectra

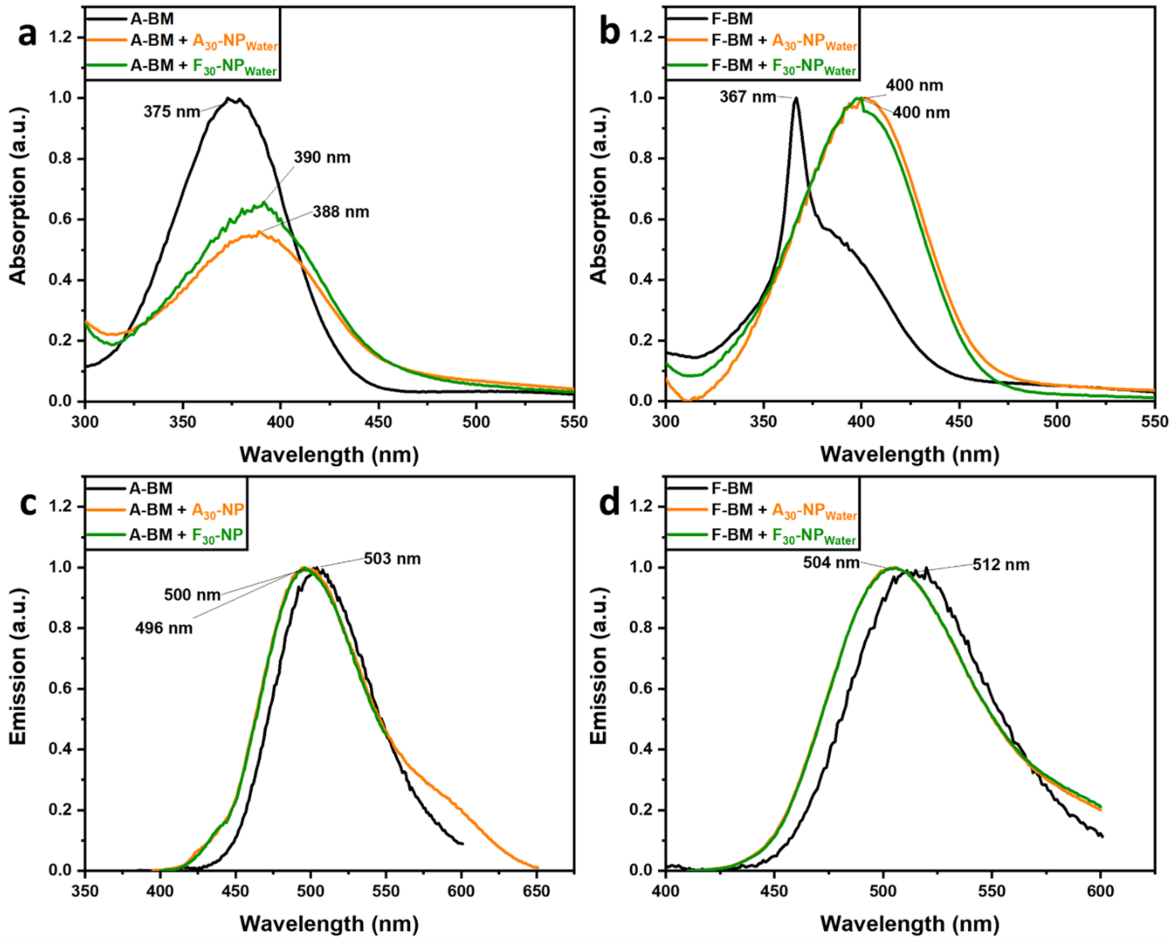


Figure S15: Absorption spectra of a) A-BM and b) F-BM in A/F_{30}*-*NP_{Water}*. Emission spectra of c) A-BM and d) F-BM in A/*F_{30}*-*NP_{Water}

ix. DLS size measurements:

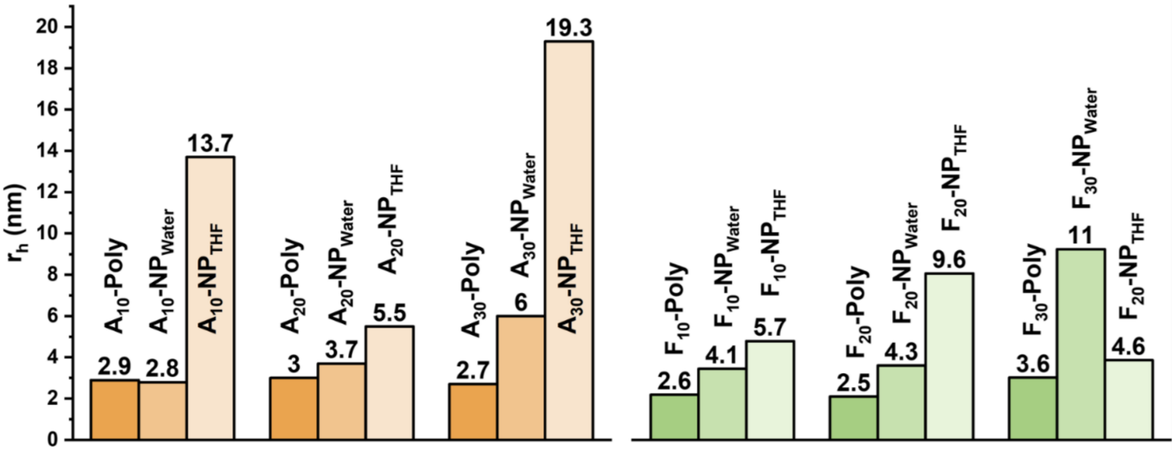


Figure S16: r_h from DLS measurements for all copolymer species and their corresponding SCNPs

Table S4: r_h and their standard deviations (σ) from DLS measurements for all copolymer species and their corresponding SCNPs

Type		Aliphatic				Fluorinated		
Species	Size/nm	10	20	30	Size/nm	10	20	30
Poly	r_h	2.9	3.0	2.7	r_h	2.6	2.5	3.6
	σ	0.5	0.4	0.4	σ	1.1	0.4	0.6
NP_{Water}	r_h	2.8	3.7	6.0	r_h	4.1	4.3	11
	σ	0.1	0.6	0.8	σ	0.5	0.7	0.5
NP_{THF}	r_h	13.7	5.5	19.3	r_h	5.7	9.6	4.6
	σ	1.0	0.3	3.7	σ	1.0	1.1	0.7

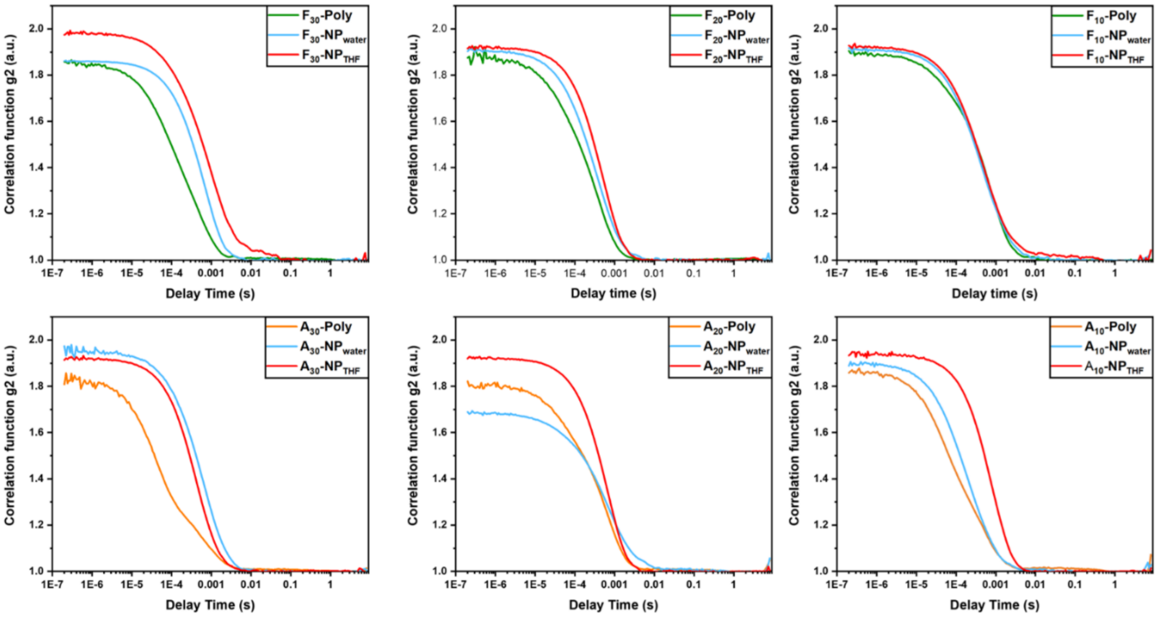


Figure S17: Correlation function g^2 of all DLS measurements for the precursor copolymers and their corresponding SCNP

x. T_{cp}- LCST behavior:

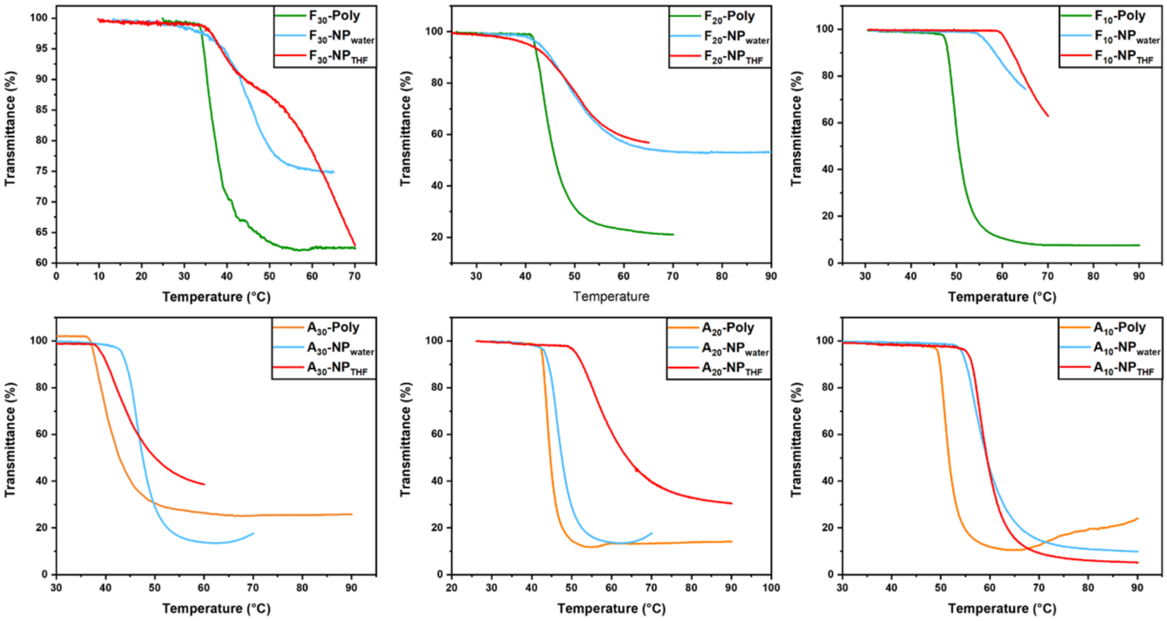


Figure S18: Transmittance of the precursor copolymer aqueous solutions and their corresponding SCNP as a function of temperature

III. References:

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