Supplementary Information

C_2-symmetric H-Bonding Benzene Core Motif Promotes Rapid Growth of Long Supramolecular Fibers from Amphiphilic Polymers

Sebastian Städter[1], Hesam Makki[2], Ulrich Mansfeld[3], Stephanie Hoeppener[4,5], Albena Lederer[6,7], Johannes C. Brendel[1,3]*

1 Macromolecular Chemistry, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany
2 Department of Chemical Engineering, University of Bath, Bath, BA2 7AY, United Kingdom
3 Institute of Macromolecular Research (BIMF) and Bavarian Polymer Institute (BPI), University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany
4 Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstraße 10, 07743 Jena, Germany
5 Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7, 07743 Jena, Germany
6 Leibniz-Institut für Polymerforschung, Department Advanced Macromolecular Structure Analysis, Hohe Str. 6, 01069 Dresden, Germany
7 Department of Chemistry and Polymer Science, Stellenbosch University, Private Bag X1, Matieland 7599, South Africa

*Corresponding author: johannes.brendel@uni-bayreuth.de

Contents:

1. Synthesis

1.1 Materials and methods

All reagents and solvents were commercial products purchased from Sigma-Aldrich, TCI, Carl Roth, abcr, Deutero, Thermo Fisher Scientific or Alfa Aesar, and were used without further purification. PEO-NHS esters were purchased from RAPP and also used without further purification.

NMR. ^{1}H NMR in solution were measured with a Bruker Avance Ultrashield 300 (300 MHz) and Bruker Avance III HD 500 (500 MHz) at room temperature. The chemical shifts of the peaks were determined by using the residual solvent signal as reference and are given in ppm in comparison to TMS. Coupling constants were reported whenever their determination was possible.

SEC. Size-exclusion chromatography (SEC) of polymers was performed on an Agilent system (series 1200) equipped with a PSS degasser, a G1310A pump, a G1362A refractive index detector and a PSS GRAM 30 and 1000 column with DMAc (+ 0.21 wt.% LiCl) as eluent and a flow rate of 1 mL min^{-1}. The column oven was set to 40°C and poly(ethylene oxide) (PEO) standards were used for calibration.

DLS. Dynamic light scattering measurements were carried out on two ZetaSizer Nano ZS (Malvern, Germany or UK) equipped with a He–Ne laser with a wavelength of λ = 633 nm at a scattering angle of 173°. All measurements were conducted in triplicate at 25 °C after an equilibration time of 60 s and an acquisition time of 18x10 s. The apparent distribution of intensity-weighted hydrodynamic radii Stokes-Einstein Equation (1):

$$R\mathrm{h} = \frac{kT}{6\pi\eta D} \qquad (1)$$

R_h = hydrodynamic radius k = Boltzmann constant T = absolute temperature,
η = viscosity of the solvent D = apparent translational diffusion coefficient.

All solutions were measured in disposable UV-Cuvettes micro from Brand. The hydrodynamic radius R_h was calculated as half of the measured z-average diameter.

cryo-TEM. *(1)* The measurements were performed on a FEI Tecnai G^2 20 equipped with a LaB6 filament with an acceleration voltage of 200 kV. Samples were prepared on Quantifoil grids (R2/2) which were treated with Ar plasma prior to use for hydrophilization and cleaning. 8.5 µL of the solution were vitrified on Quantifoil grids using a Vitrobot Mark IV system. Liquid ethane was used as a cryogen. Samples were transferred to a Gatan 626 cryo holder and were maintained at a temperature <175°C during the entire process. All images were acquired with a Mega View (OSIS, Olympus Soft ImagingSystems) or an Eagle 4k CCD camera, respectively.

(2) The measurements were performed on a JEOL JEM-2200FS operating at 200 kV and equipped with a field emission gun and an in-column energy omega filter. The zero-loss energy-filtered, bright field micrographs were recorded at cryo conditions (Holder T = -174 °C) with a bottom-mounted CMOS 4K camera (OneView, Gatan). The images were processed with digital processing software (Digital Micrograph 3.5, Gatan). For cryo preparation, 4 µL of the aqueous sample solution were deposited on a Cu grid with a holey carbon film (Quantifoil, Germany) that was glow-discharged before. The grid was then blotted at 20 °C and 90% humidity with filter paper and plunged into liquid ethane using a Leica EM GP plunge freezer (Leica, Germany). After preparation, the grids were stored and transferred to the TEM under liquid nitrogen.

AF4. Measurements were performed on an AF2000 MT System from Postnova Analytics GmbH Landsberg, Germany. The channel was coupled to a multiangle laser light scattering (MALLS) detector equipped with a 532 nm laser and measuring 21 angles, a refractive index (RI) detector and a UV-detector set to 280 nm. The channel (300x60x40 mm) had a trapezoidal geometry with a nominal height of 350 µm. Regenerated cellulose (RC) membrane from Postnova Analytics GmbH (10 kDa RC membrane) with a molar mass cutoff of 10 kDa was used as accumulation wall. As the mobile phase aqueous solution with 0.02 *w*% of NaN_3 was used. Samples were measured at a final concentration of 1 mg mL^{-1}. Before the start of the next measurement, a rinsing step was performed. After each sample measurement, a blank measurement was run which was subtracted from the data of the sample measurement for analysis. The MALLS data of the scattering angles from 20°-124° was analyzed via ZIMM plot to obtain the radius of gyration (R_g, z-averaged) at the specified elution times. The crossflow during the run was set accordingly: for 8.2 min at 1 mL min^{-1} (constant), for 20 min from 1 mL min^{-1} to 0 mL min^{-1} and for 25 min at 0 mL min^{-1} (constant). The lengths of the species were calculated according to rigid rod model with $L = \sqrt{12} \cdot R_g$.

FCS. A commercial confocal microscope MicroTime 200 (PicoQuant) was used for fluorescence correlation spectroscopy. For excitation of the Alexa AF488 molecules (in solution and attached to the BDUA) we used a laser diode with a wavelength of 485 nm (LDH-D-C-485, PicoQuant) (operated in pulsed mode with a repetition rate of 20 MHz). The excitation intensities were adjusted to approximately to 130 W/cm^2 and 1300 W/cm^2 depending on the brightness of the sample. Radiation from the laser was directed through a single-mode optical fibre into the main optical unit, reflected by a dichroic mirror (ZT405/488rpc, AHF/Croma) into an inverted confocal optical microscope (Olympus), and focused into the sample using a super-apochromatic objective (UPLSAPO60XW, NA = 1.2, Olympus). The signal from the sample was transmitted to the dichroic mirror and passed through a long pass filter (488 LP Edge Basic, Semrock). The signal was detected by a single-photon counting avalanche diode (SPCM-AQRH-14-TR, Excelitas) and a time-correlated single-photon counting unit (TCSPC TimeHarp 260 PICO Dual, PicoQuant, temporal resolution of 250 ps) was used to collect fluorescence data. The microscope was calibrated with an Atto488 solution excited at 485 nm, yielding lateral radius ω_r of 250±20 nm of the detection volume. The after-pulsing effects of the avalanche photodiode were removed by applying fluorescence lifetime correlation spectroscopy (FLCS).[1, 2] The fluorescence data was corrected and autocorrelated using the commercial software SymPhoTime 64 (Picoquant).

1.2 Synthetic route

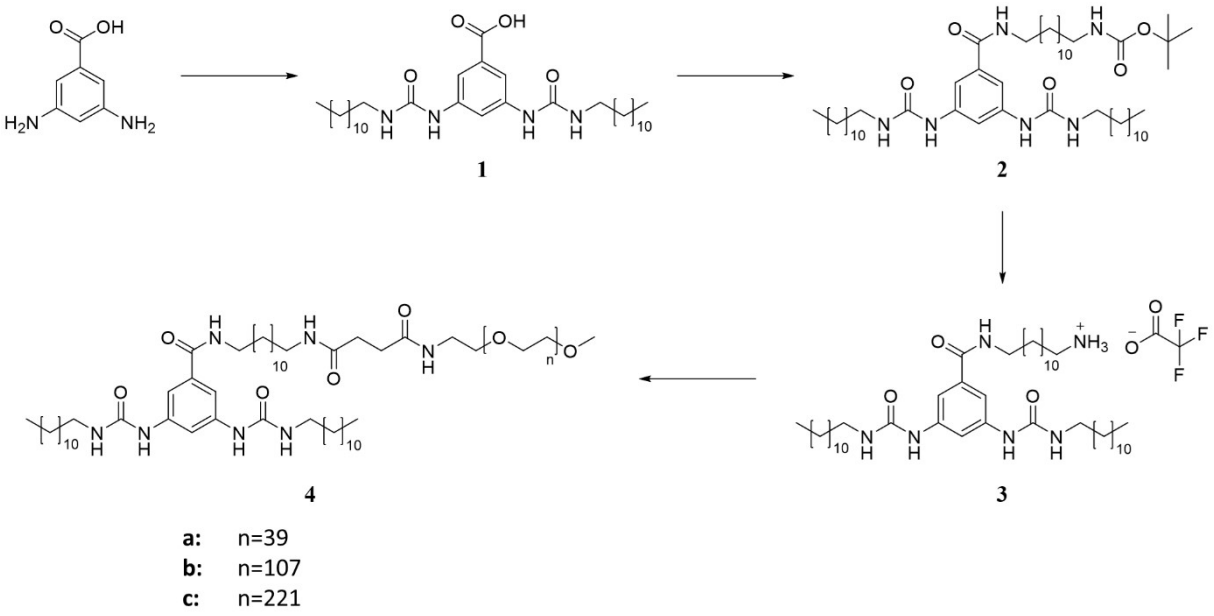


Figure S1. Synthetic procedure for BDUAs.

The synthesis of tert-butyl (12-aminododecyl)carbamate was previously published by Klein *et al.*.[3]

3,5-bis(3-dodecylureido)benzoic acid (1)

3,5-diaminobenzoic acid (10.00 g, 65.41 mmol) was suspended in THF. Afterwards, dodecyl isocyanate (30.56 g, 144.59 mmol) was added portionwise. The suspension was stirred for 24 h at 25°C. The reaction mixture was filtrated and the solid dried *in vacuo*. After evaporation a greyish powder was obtained.

Yield: 32.13 g, 55.89 mmol (85%) (lab scale: 691.8 mg, 1.20 mmol (73%))

^{1}H-NMR (500 MHz, d_6-DMSO, 298 K): δ [ppm] = 12.70 (s, 1H, COOH), 8.55 (s, 2H, -**NH**-CO-NH-CH_2-R), 7.67 (s, 1H, CH_{aromat}), 7.58 (s, 2H, CH_{aromat}), 6.05 (t, *J* = 5.7 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.05 (m, *J* = 6.5 Hz, 4H, -NH-CO-NH-**CH_2**-R), 1.51-1.05 (m, 40H, CH_2), 0.84 (t, *J* = 6.8 Hz, 6H, CH_3).

tert-butyl (12-(3,5-bis(3-dodecylureido)benzamido)dodecyl)carbamate (2)

[1] (32.13 g, 55.89 mmol) and DMAP (0.68 g, 5.58 mmol) were suspended in 300 mL DMF. tert-butyl (12-aminododecyl)carbamate (20.15 g, 67.07 mmol) and HBTU (25.44 g, 67.07 mmol) were suspended in 500 mL DCM and both suspensions were combined. Afterwards, DIPEA (36.12 g, 279.45 mmol) was added, resulting in a clear solution. The reaction mixture was stirred overnight at 25°C. The precipitation was filtered off and dried *in vacuo*. A brownish, solid product was obtained.

Yield: 46.30 g, 54.01 mmol (97%) (lab scale: 1.41 g, 1.65 mmol (51%))

^{1}H-NMR (500 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.45 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.20 (t, 1H, -CO-**NH**-CH_2-R), 7.62 (s, 1H, CH_{aromat}), 7.29 (s, 2H, CH_{aromat}), 6.75 (t, 1H, NH-Boc), 6.06 (t, 2H, -NH-CO-**NH**-CH_2-R), 3.17 (m, 2H, -CO-NH-**CH_2**-R), 3.05 (m, 4H, -NH-CO-NH-**CH_2**-R), 2.87 (m, 2H, -CH_2-**CH_2**-NH-Boc), 1.60-1.03 (m, 69H, CH_2+Boc), 0.85 (t, 6H, CH_3).

12-(3,5-bis(3-dodecylureido)benzamido)dodecan-1-aminium (TFA salt) (3)

[2] (46.3 g, 54.00 mmol) was dissolved in 1.1 L DCM. Afterwards, TIPS (51.3 g, 323.95 mmol) was added. The solution was cooled down to 0°C and TFA (221.68 g, 1.94 mol) was added dropwise. The reaction mixture was stirred overnight at 25°C and then reduced *in vacuo*. The residue was re-dissolved in DCM and again reduced *in vacuo*. The procedure was repeated five times to remove all TFA. The concentrated solution was precipitated in 10x excess of DEE. The solid was filtered off, dissolved in 200 mL DMF and precipitated in 10x excess of ice water with NaCl. The product was filtered off and dried *in vacuo*. After evaporation a brownish powder was obtained.

Figure S2. Upscaling of **(3)**

Yield: 44.80 g, 51.42 mmol (95%)
(lab scale: 470.0 mg, 0.54 mmol (92%))

^{1}H-NMR (500 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.53 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.21 (t, *J* = 5.7 Hz, 1H, -CO-**NH**-CH_2-R), 7.70 (t, 3H, NH_3^+), 7.57 (s, 1H, CH_{aromat}), 7.33 (s, 2H, CH_{aromat}), 6.17 (t, *J* = 5.7 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.18 (m, *J* = 6.6 Hz, 2H, -CO-NH-**CH_2**-R), 3.06 (m, *J* = 6.5 Hz, 4H, -NH-CO-NH-**CH_2**-R), 2.76 (m, *J* = 13.9, 2H, -CH_2-**CH_2**-NH_3^+), 1.57-1.13 (m, 60H, CH_2), 0.85 (t, *J* = 6.7 Hz, 6H, CH_3).

[C_{12}-ureido]$_2$[B][NH][C_{12}][NH][PEO_{2k}] (4a)

[3] (462 mg, 0.53 mmol) and α-methoxy-ω-succinamic acid NHS ester PEO_{2k} (1.50 g, 0.75 mmol) were dissolved in 14 mL DMF. Afterwards, triethylamine (TEA, 1.07 g, 10.61 mmol) was added and the solution stirred for 24 h at 25°C. The reaction mixture was precipitated in 10x excess of DEE, centrifuged and then redissolved in DMF/Water (1:1; solvent switch from DMF to water). The solution was dialyzed (15 kDa MWCO) against water for 3 days and then freeze-dried. After evaporation a white-greyish powder was obtained.

Yield: 1.40 g, 0.53 mmol (quant.)

^{1}H-NMR (300 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.44 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.20 (t, *J* = 5.5 Hz, 1H, -CO-**NH**-CH_2-R), 7.86 (t, 1H, -NH-CO-C_2H_4-CO-**NH**-PEO), 7.75 (t, *J* = 5.7 Hz, 1H, -**NH**-CO-C_2H_4-CO-NH-PEO), 7.64 (s, 1H, CH_{aromat}), 7.30 (s, 2H, CH_{aromat}), 6.06 (t, *J* = 5.6 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.51 (m, 158H, -**CH_2**-O-**CH_2-CH_2**-), 3.24 (s, 3H, -O-**CH_3**) 3.20-2.93 (m, 10H, -NH-**CH_2**-R), 2.27 (t, 4H, NH-CO-**C_2H_4**-CO-NH-PEO), 1.56-1.04 (m, 60H, CH_2), 0.85 (t, *J* = 6.7 Hz, 6H, CH_3).

[C_{12}-ureido]$_2$[B][NH][C_{12}][NH][PEO_{5k}] (4b)

[3] (191 mg, 0.22 mmol) and α-methoxy-ω-succinamic acid NHS ester PEO_{5k} (1.59 g, 0.32 mmol) were dissolved in 10 mL DMF. Afterwards, triethylamine (TEA, 444 mg, 4.39 mmol) was added and the solution stirred for 24 h at 25°C. The reaction mixture was precipitated in 10x excess of DEE, centrifuged and then redissolved in DMF/Water (1:1; solvent switch from DMF to water). The solution was dialyzed (100 kDa MWCO) against water for 3 days and then freeze-dried. After evaporation a white-greyish powder was obtained, containing less than 5% of (3).

Yield: 942.0 mg, 0.17 mmol (76%)

^{1}H-NMR (500 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.44 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.20 (t, *J* = 5.7 Hz, 1H, -CO-**NH**-CH_2-R), 7.87 (t, *J* = 5.7 Hz, 1H, -NH-CO-C_2H_4-CO-**NH**-PEO), 7.75 (t, *J* = 5.6 Hz, 1H, -**NH**-CO-C_2H_4-CO-NH-PEO), 7.63 (s, 1H, CH_{aromat}), 7.29 (s, 2H, CH_{aromat}), 6.06 (t, *J* = 5.7 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.51 (m, 430H, -**CH_2**-O-**CH_2-CH_2**-), 3.24 (s, 3H, -O-**CH_3**) 3.21-2.95 (m, 10H, -NH-**CH_2**-R), 2.27 (t, 4H, NH-CO-**C_2H_4**-CO-NH-PEO), 1.53-1.14 (m, 60H, CH_2), 0.85 (t, 6H, CH_3).

[C_{12}-ureido]$_2$[B][NH][C_{12}][NH][PEO_{10k}] (4c)

[3] (81 mg, 0.09 mmol) and α-methoxy-ω-succinamic acid NHS ester PEO_{10k} (1.30 g, 0.13 mmol) were dissolved in 14 mL DMF. Afterwards, triethylamine (TEA, 188 mg, 1.86 mmol) was added and the solution stirred for 24 h at 25°C. The reaction mixture was precipitated in 10x excess of DEE, centrifuged and then redissolved in DMF/Water (1:1; solvent switch from DMF to water). The solution was dialyzed (300 kDa MWCO) against water for 3 days and then freeze-dried. After evaporation a white-greyish powder was obtained, containing less than 5% of (3).

Yield: 966.0 g, 0.09 mmol (98%)

^{1}H-NMR (500 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.43 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.20 (t, *J* = 5.7 Hz, 1H, -CO-**NH**-CH_2-R), 7.86 (t, *J* = 5.7 Hz, 1H, -NH-CO-C_2H_4-CO-**NH**-PEO), 7.75 (t, *J* = 5.6 Hz, 1H, -**NH**-CO-C_2H_4-CO-NH-PEO), 7.63 (s, 1H, CH_{aromat}), 7.29 (s, 2H, CH_{aromat}), 6.06 (t, *J* = 5.7 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.51 (m, 430H, -**CH_2**-O-**CH_2-CH_2**-), 3.24 (s, 3H, -O-**CH_3**) 3.21-2.95 (m, 10H, -NH-**CH_2**-R), 2.27 (t, 4H, NH-CO-**C_2H_4**-CO-NH-PEO), 1.52-1.16 (m, 60H, CH_2), 0.85 (t, 6H, CH_3).

1.3 SEC

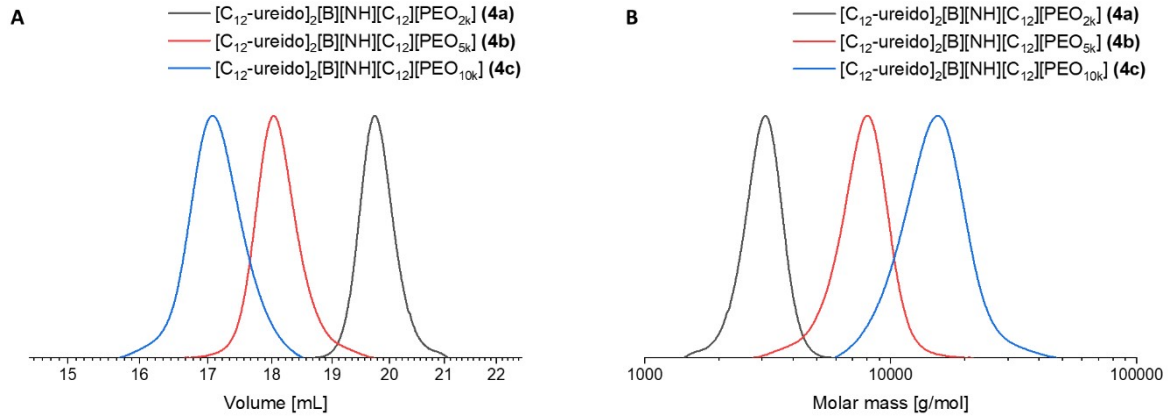


Figure S3. SEC (DMAc/LiCl, 25°C, RI, PEO calibration): SEC curves (A) and molar mass distribution (B) of compounds **4a-c**.

Table S1. Molar masses of compounds **4a-c** (PEO calibration).

samples	**4a**	**4b**	**4c**
M_n [g/mol]	2900	7400	14300
M_w [g/mol]	3100	7900	15800

Đ	1.04	1.07	1.10

2. Self-Assembly sample preparation

Solvent switch:

Samples of the supramolecular motifs were dissolved in THF supported by short time heating (40°C) or/and sonication. Syringes containing water were prepared air free and placed in the syringe pump. Needle tips were immersed in the THF solution. Under intense stirring water was added to the organic solution at a specific rate. Afterwards, THF was evaporated at air over one or two days. Samples were always freshly prepared for all characterization studies and shortly stored at room temperature.

Quenching:

Samples of the supramolecular motifs were dissolved in THF supported by short time heating (40°C) or/and sonication. The organic solution was transferred into a syringe, air was removed and the syringe placed in the syringe pump. Water was placed in a vial and the needle immersed inside the solution. Under intense stirring the organic solution was added to water at a specific rate. Afterwards, THF was evaporated at air over one or two days. Samples were always freshly prepared for all characterization studies and shortly stored at room temperature.

3. ^{1}H-NMR investigation of solvent-ratio-dependent assembly behavior

Deuterated solvent mixtures were individually prepared by dissolving 9 mg of **4a** in d_8-THF, followed by dropwise addition of 50 µL of a 6 mg mL^{-1} trioxane stock solution (internal standard) in D_2O and subsequent dropwise addition of D_2O to the desired ratio yielding 720µL of total volume. All spectra were referenced to trioxane at 5.1 ppm, with the integral normalized to six protons.

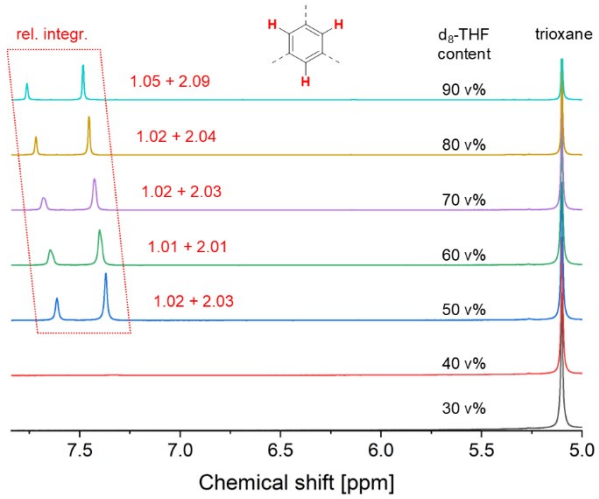
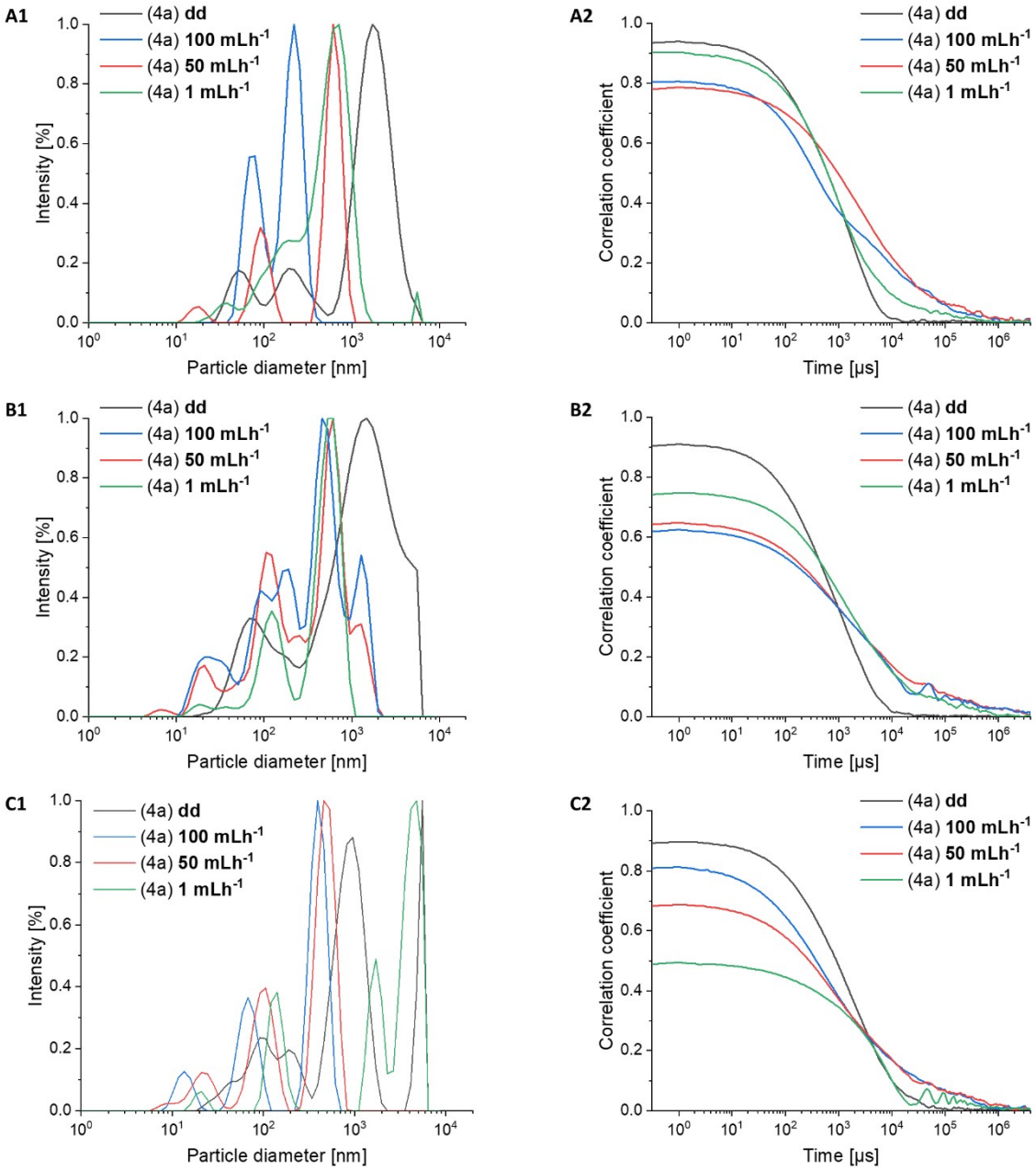


Figure S4. ^{1}H-NMR spectra of **4a** in d_8-THF:D_2O with decreasing d_8-THF content, showing aggregation onset via disappearance of aromatic signals from the benzene core.

4. Dynamic light scattering (DLS)

Each measurement consists of 3 individual runs, each run consists of 18 scans with an individual scanning time of 10s. Size distributions are intensity-based.



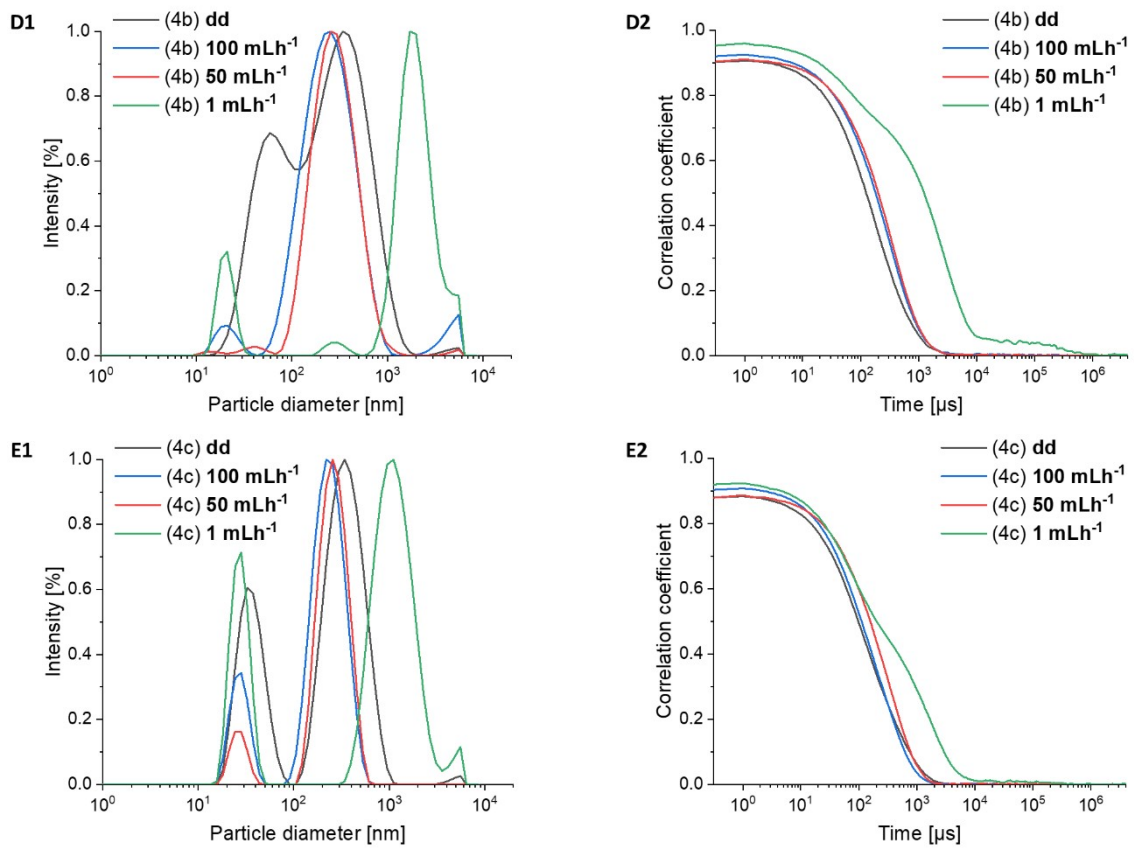
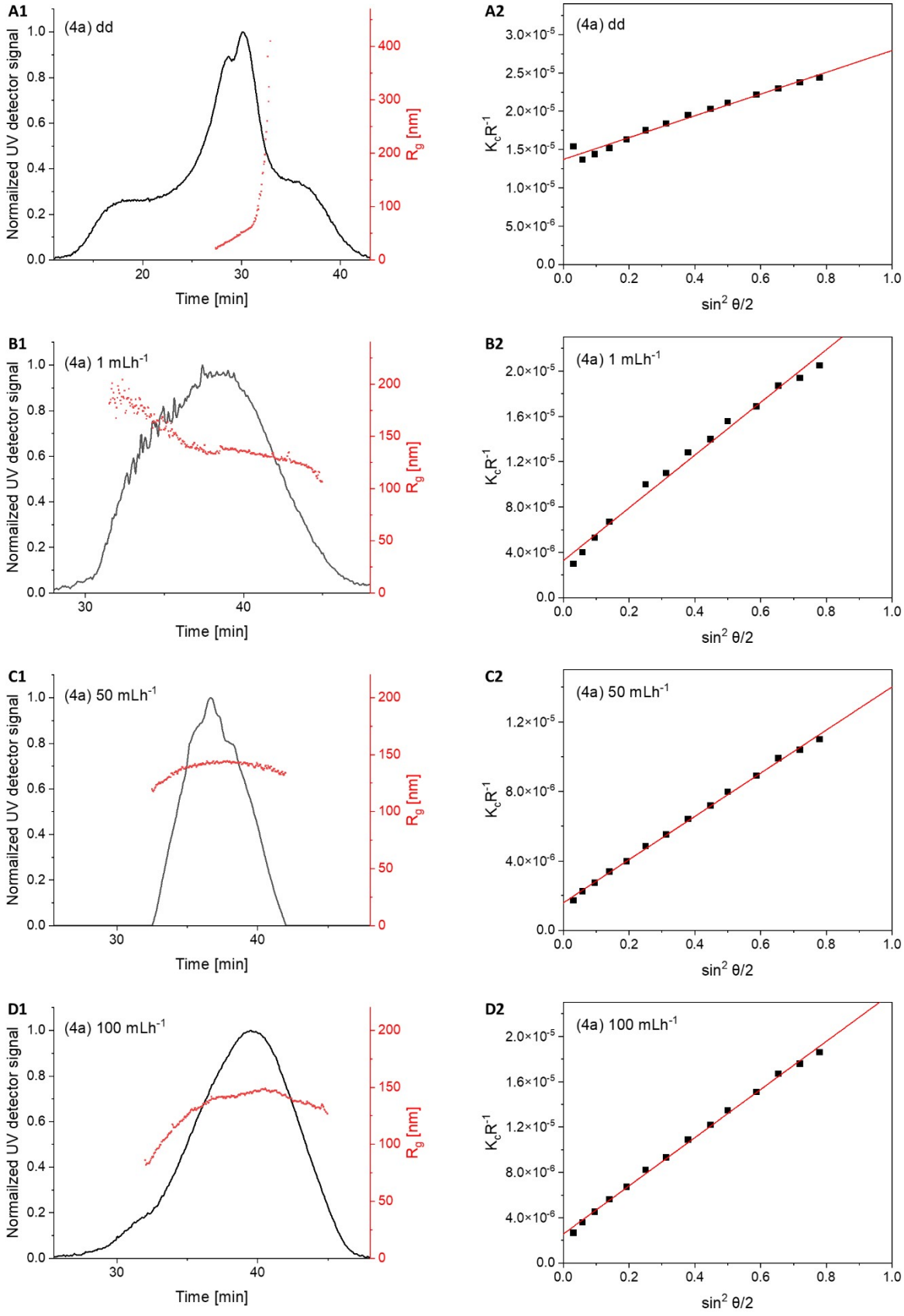


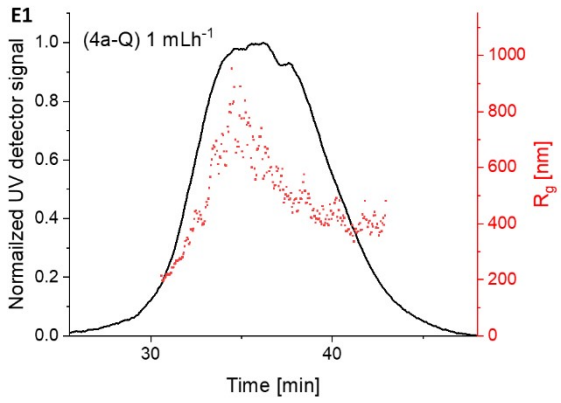
Figure S5. Intensity-based size distribution curves (1) and correlograms (2) of compounds **4a** (A-C), **4b** (D), **4c** (E). Concentration: 5 mg mL^{-1} (except A: 1 mg mL^{-1} and B: 3 mg mL^{-1}).

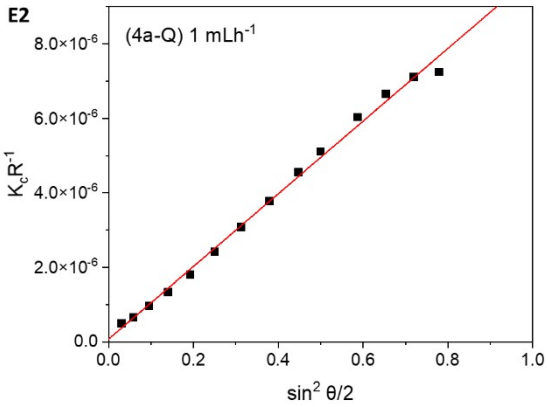
Table S2. Summary of intensity-based size distribution data

Parameter		**4a**		**4b**		**4c**	
c [mg mL^{-1}]	*rate [mL h^{-1}]*	*Size [nm]*	*PDI*	*Size [nm]*	*PDI*	*Size [nm]*	*PDI*
1	dd	475	0.93				
	100	602	0.71				
	50	1072	1.00				
	1	607	0.75				
3	dd	397	1.00				
	100	782	0.88				
	50	806	0.85				
	1	1106	0.88				
5	dd	796	1.00	130	0.52	108	0.66
	100	682	0.76	183	0.45	115	0.61
	50	747	0.85	216	0.30	181	0.34
	1	1176	1.00	712	0.87	141	1

5. Asymmetric flow field flow fractionation




E1
(4a-Q) 1 mLh-1
Normalized UV detector signal
Rg [nm]
Time [min]


E2
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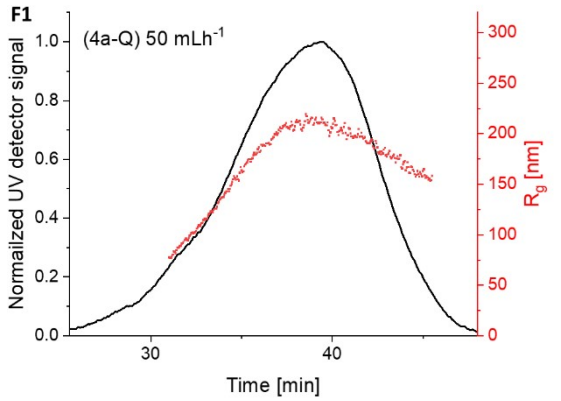
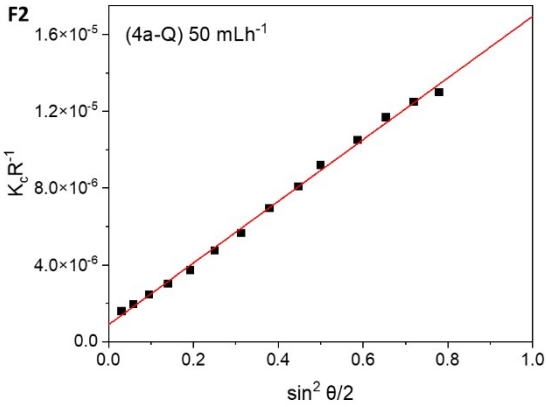
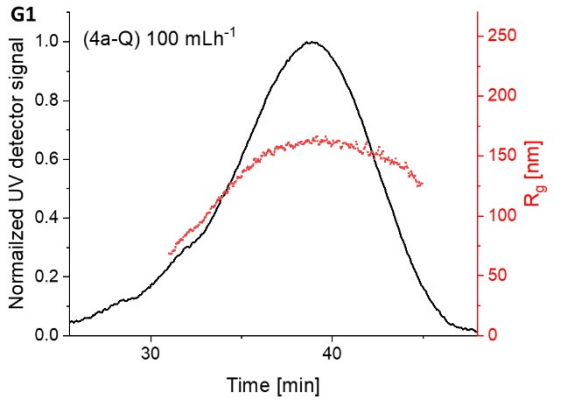
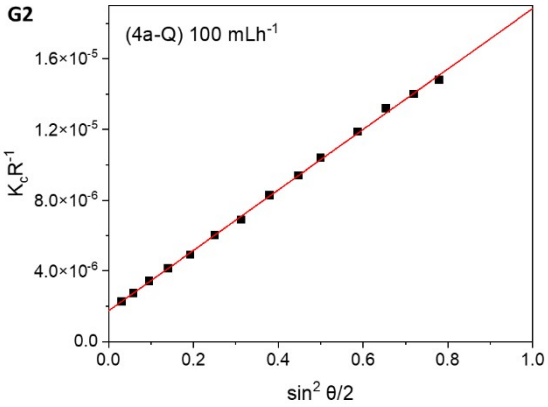
KcR-1
sin2 θ/2


F1
(4a-Q) 50 mLh-1
Normalized UV detector signal
Rg [nm]
Time [min]


F2
(4a-Q) 50 mLh-1
KcR-1
sin2 θ/2


G1
(4a-Q) 100 mLh-1
Normalized UV detector signal
Rg [nm]
Time [min]


G2
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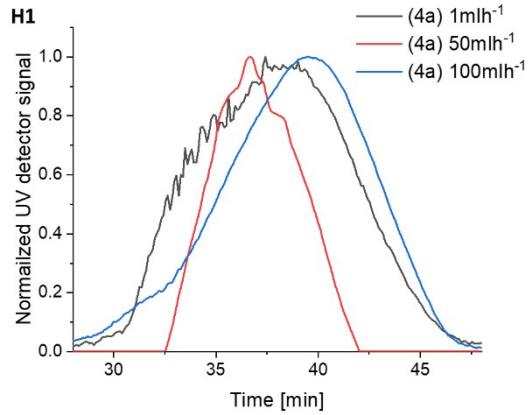
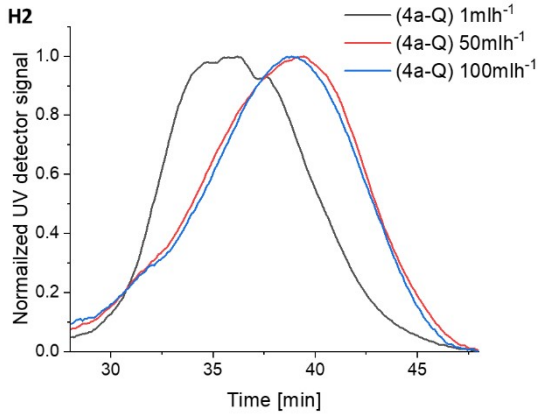
KcR-1
sin2 θ/2


H1
(4a) 1mlh-1
(4a) 50mlh-1
(4a) 100mlh-1
Normalized UV detector signal
Time [min]


H2
(4a-Q) 1mlh-1
(4a-Q) 50mlh-1
(4a-Q) 100mlh-1
Normalized UV detector signal
Time [min]

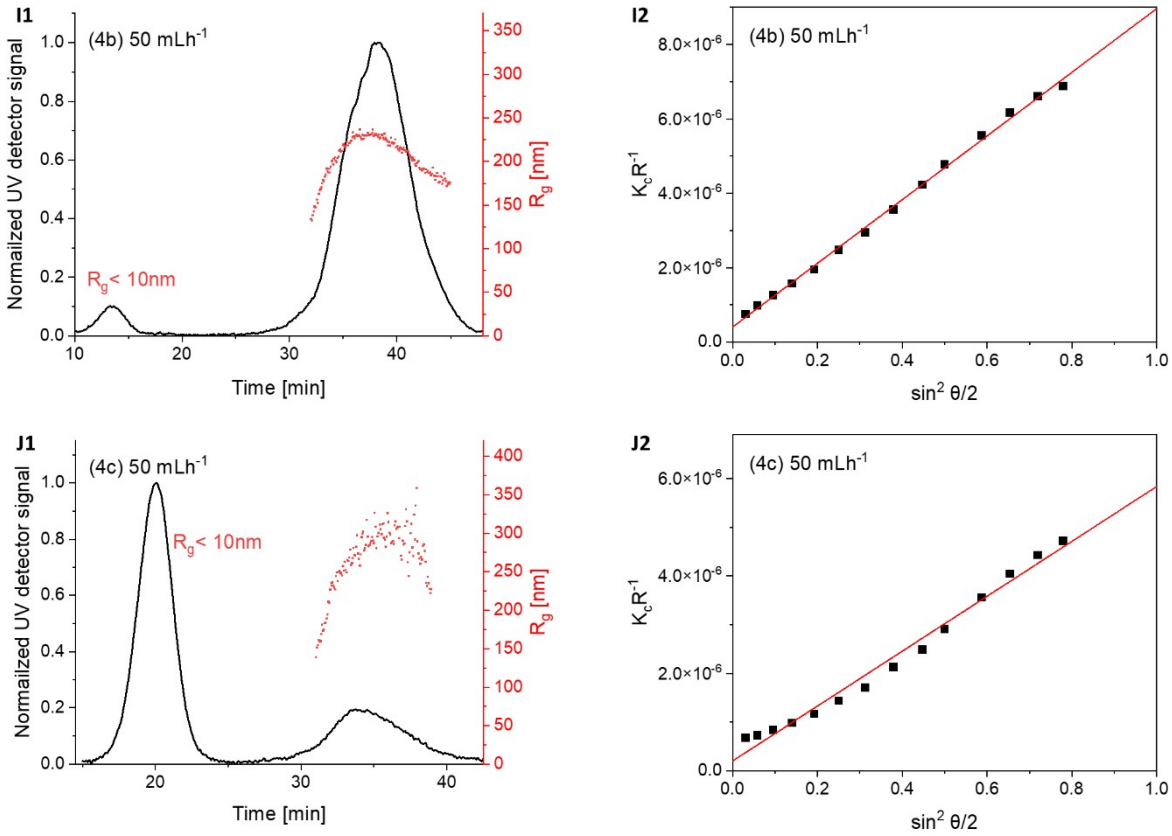


Figure S6. AF4: Normalized UV traces and corresponding R_g values (1) and Zimm-Plots (2) of compounds **4a** (A-H; A-D: solvent switch method; E-G: quenching method; H: comparison of both methods, H1: solvent switch method, H2: quenching method), **4b** (I) and **4c** (J). Concentration: 1 mg mL^{-1}. The Zimm plots derive from the maximum of the UV traces. For **4b** and **4c**, only the right-hand peak was selected.

Table S3. Summary of AF4-MALS (c = 1 mg mL^{-1}) and intensity-based DLS data for comparison (c = 5 mg mL^{-1}).

Parameter		**R_g [nm]**	**L [nm]**	**R_h [nm]**	**R_g/R_h**
Molecule	*rate [mL h^{-1}]*				
4a	dd	52	180	398	0.1
	100	135	468	588	0.2
	50	143	495	373	0.4
	1	146	506	341	0.4
4b	50	230	797	108	2.1
4c	50	271	939	90	3.0

6. Cryo-TEM

Samples were measured at a concentration of 1 mg mL^{-1}. Here, the original sized images after contrast enhancement are shown.

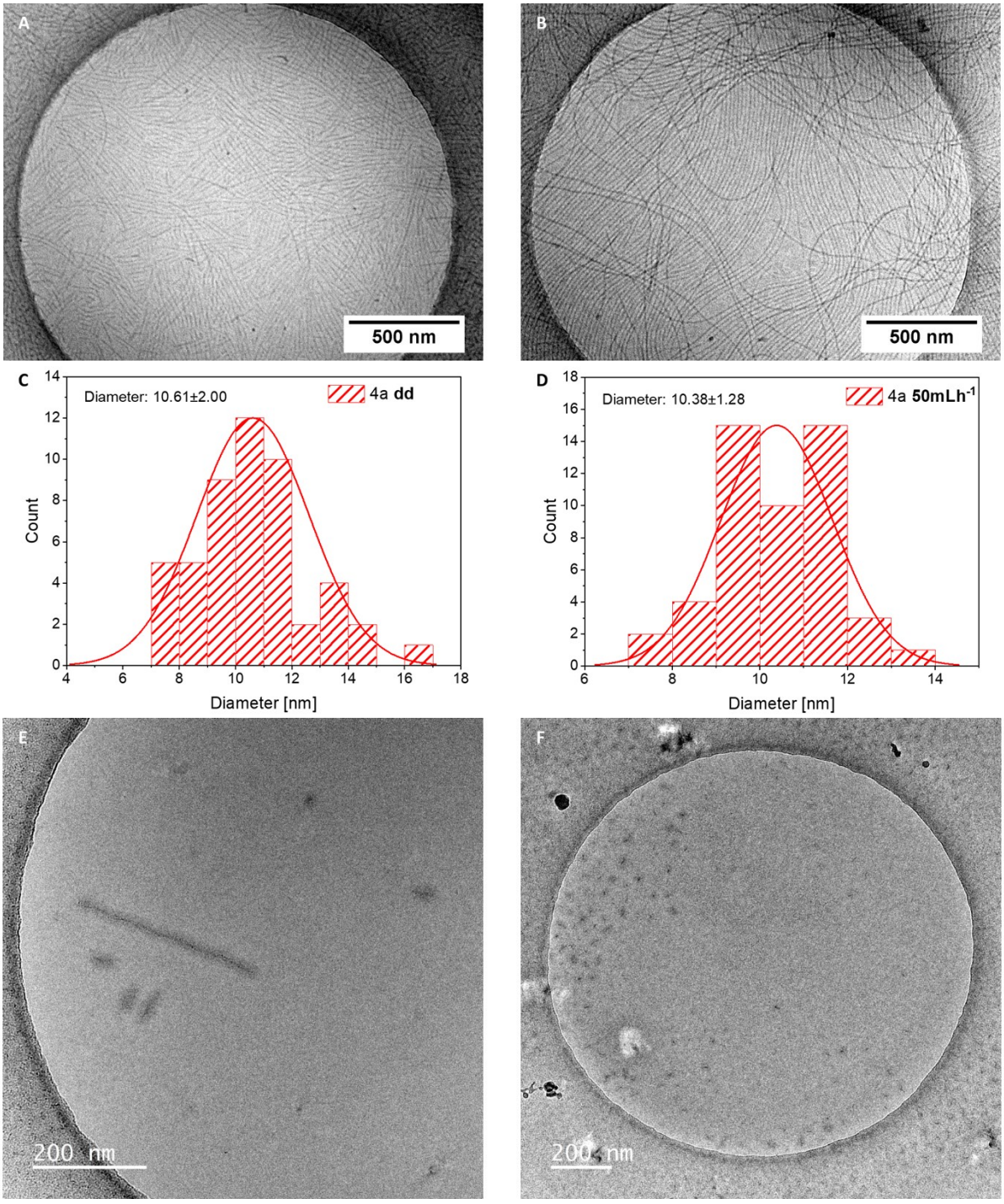


Figure S7. cryo-TEM images of (A) **4a** at dd, (B) **4a** at 50 mL h^{-1}, (E) **4b** at 50 mL h^{-1} and (F) **4c** at 50 mL h^{-1} together with the size distribution histograms of fiber diameters of (C) **4a** at dd and (D) **4a** at 50 mL h^{-1}.

7. Fluorescence correlation spectroscopy

The molecule **[3]** was used further for labeling experiments according to the following protocol:

Step 1: Attachment of NHS-PEO-NH-Boc (3kDa) **[5]**

[3] (69.5 mg, 79.77 µmol) and α-butyric acid NHS ester-ω-Boc amino PEO_{3k} (299.2 mg, 0.10 µmol) were dissolved in 4 mL DMF. Afterwards, triethylamine (TEA, 80.7 mg, 0.80 mmol) was added and the solution stirred for 24 h at 25°C. The reaction mixture was precipitated in 10x excess of DEE, centrifuged and then redissolved in THF/Water (1:4; solvent switch from THF to water). The solution was dialyzed (15 kDa MWCO) against water for 3 days and then freeze-dried. After evaporation a white-greyish powder was obtained.

Yield: 247.40 mg, 68.16 µmol (85%)

^{1}H-NMR (300 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.47 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.22 (t, *J* = 5.7 Hz, 1H, -CO-**NH**-CH_2-R), 7.73 (t, *J* = 5.5 Hz, 1H, -**NH**-CO-C_3H_6-PEO), 7.63 (s, 1H, CH_{aromat}), 7.30 (s, 2H, CH_{aromat}), 6.77 (t, *J* = 5.8 Hz, 1H, -PEO-**NH**-Boc), 6.09 (t, *J* = 5.6 Hz, 2H, -NH-CO-**NH**-CH_2-R), 3.51 (m, 244H, -**CH_2**-O-**CH_2-CH_2**-), 3.27 (t, 2H, -CO-CH_2-CH_2-**CH_2**-PEO), 3.19-2.96 (m, 10H, -NH-**CH_2**-R), 2.23 (t, 2H, -CO-**CH_2**-CH_2-CH_2-PEO), 2.07 (t, 2H, -CO-CH_2-**CH_2**-CH_2-PEO), 1.50-1.01 (m, 69H, CH_2 and Boc), 0.85 (t, 6H, CH_3).

Step 2: Boc-Deprotection **[6]**

[5] (234,1 mg, 64.50 µmol) was dissolved in 4 mL DCM. Afterwards, TIPS (61.3 g, 0.39 mmol) and TFA (529.6 g, 4.64 mmol) were added. Then the solution was stirred overnight at 25°C. The reaction mixture was precipitated in 10x excess of DEE, stored overnight at -20°C and then centrifuged and dried at air. After evaporation a white-greyish powder was obtained.

Yield: 250.90 mg, 68.86 µmol (quant.)

^{1}H-NMR (300 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.46 (s, 2H, -**NH**-CO-NH-CH_2-R), 8.21 (t, *J* = 5.6 Hz, 1H, -CO-**NH**-CH_2-R), 7.73 (t, 4H, -**NH**-CO-C_3H_6-PEO and -NH_3^+), 7.62 (s, 1H, CH_{aromat}), 7.30 (s, 2H, CH_{aromat}), 6.08 (t, 2H, -NH-CO-**NH**-CH_2-R), 3.51 (m, 244H, -**CH_2**-O-**CH_2-CH_2**-), 3.27 (t, 2H, -CO-CH_2-CH_2-**CH_2**-PEO), 3.19-2.92 (m, 10H, -NH-**CH_2**-R), 2.24 (t, 2H, -CO-**CH_2**-CH_2-CH_2-PEO), 2.08 (t, 2H, -CO-CH_2-**CH_2**-CH_2-PEO), 1.50-1.01 (m, 60H, CH_2 and Boc), 0.85 (t, 6H, CH_3).

Step 3: Labeling with NHS-AF488 **[7]**

[6] (15.0 mg, 4.12 µmol) was dissolved in 50 µL DMF. Afterwards, 190 µL of a 42 mg mL^{-1} DIPEA stock solution in DMF were added and the solution stirred for 15 min at rt. To this, 328.8 µL of a 12.5 mg mL^{-1} NHS-AF488 stock solution in DMF was added and the reaction mixture stirred for 3 d at rt. Afterwards, the reaction mixture was dialyzed against MeOH (MWCO 500-100Da, Float-A-Lyzer) for nine days and then 2 days against water. The aqueous solution was then freeze-dried and a bright red powder was obtained. Purity was confirmed by SEC analysis.

Yield: 12.60 mg, 2.97 µmol (quant.)

Dilution series: For FCS measurements a 1 mg mL^{-1} solution of **[7]** was prepared (dd) and diluted to 103 µmol/L. Then the samples were further diluted down to 0.103 nmol L^{-1}, adhering to a dilution of factor 10 per each sample.

Table S4. Summary of characteristic FCS data.

Sample	Solvent	c in nmol L^{-1}	τ_D in ms	D in $\mu m^2 s^{-1}$	N
AF488-NHS	water	1.03	0.030±0.001	408±7	0.529±0.005
BDUA-AF488	water	1030.00	1.80±0.19	7±1	30±2.200
		103.00	3.20±0.74	4±1	27±0.910
		10.30	6.40±1.80	2±1	38±25.000
	DMF	1030.00	0.053±0.001	232±6	27 ±0.027
		1030.00	0.037±0.001	334±8	14±0.230
		103.00	0.049±0.001	253±2	0.005±0.001
		103.00	0.142±0.001	385±10	1.2±0.016

τ_D characteristic correlation time due to translational diffusion

D translational diffusion coefficient

N fit parameter for the number of fluorescent molecules within the observation volume, which may deviate from the true physical value due to fitting inaccuracies

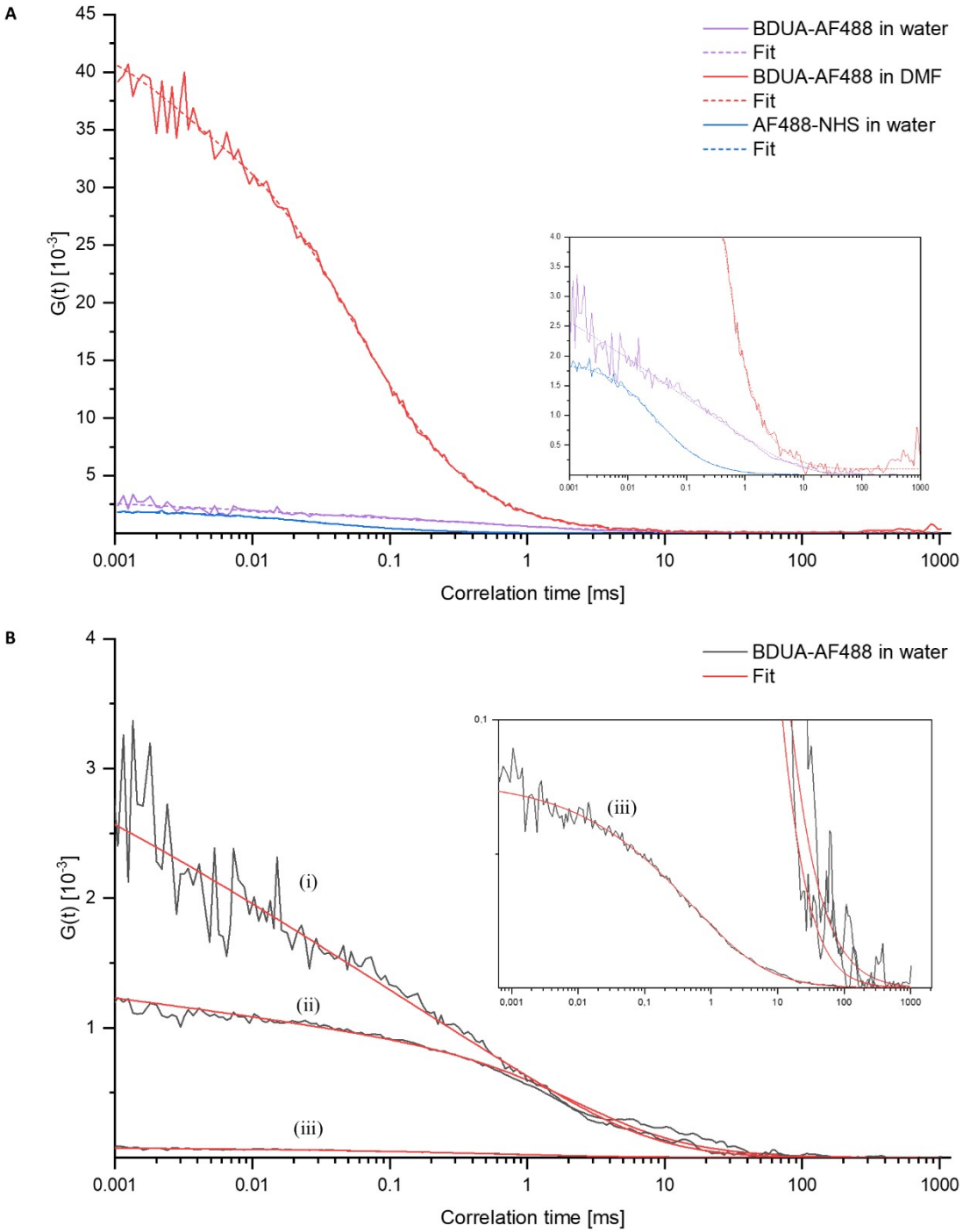


Figure S8. FCS measurements of (A) DMF or aqueous solutions of BDUA-AF488 and reference dye. The following concentrations were selected: 10.3 nmol L^{-1} BDUA-AF488 in water; 1030 nmol L^{-1} BDUA-AF488 in DMF; 1.03 nmol L^{-1} reference dye in water. (B) BDUA-AF488 in water at 10.3 nmol L^{-1} (i), 103 nmol L^{-1} (ii) and 1030 nmol L^{-1} (iii).

Table S5. Determination of fiber length via extended Stokes–Einstein equation (Tirado & García de la Torre Model).[4] Temperature was set to T = 20°C and the diameter was set to d = 10.61 nm, corresponding to the diameter of the directly dispersed sample as determined by cryo-TEM analysis. The equation was solved numerically using an iterative approach, with the length varying in 1 nm steps until the model value D_{model} showed minimal deviation from the experimental value.

Sample	Solvent	c in nmol L^{-1}	D in $\mu m^2 s^{-1}$	D_{model} in $\mu m^2 s^{-1}$	L [nm]*
BDUA-AF488	water	1030.00	7±1	7.003	201
		103.00	4±1	4.0005	432
		10.30	2±1	2.0006	1053

*The calculated values are subject to significant error potential due to inaccuracies in the determination of the diameter and diffusion coefficients, as well as the mathematical model used, and should therefore be considered as approximate estimates.

8. References

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[4] Appel, R.; Fuchs, J.; Tyrrell, S. M.*; et al.*, *Chemistry – A European Journal* **2015**, *21* (52), 19257-19264.