# Supporting information

# Fluorescent conjugated block copolymer nanoparticles by controlled mixing

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***General methods and materials.*** Particle sizes were determined by dynamic light scattering with a Zetasizer Nano ZS (Malvern Instruments; 173° back scattering). The autocorrelation function was analyzed using the Malvern dispersion technology software 5.1 algorithm to obtain volume weighted particle size distributions, and polydispersities. Transmission electron microscopy was performed with a Zeiss Libra 120 EF-TEM instrument. Samples were prepared by application of a drop of aqueous particle dispersion to a carbon-coated grid and evaporation of water. Absorption spectra were acquired on a Varian Cary 100 scan UV/VIS-spectrometer. Fluorescence spectra and quantum yield measurements were performed on a Hamamatsu Absolute PL Quantum Yield Measurement System C9920-02. Real-color fluorescence images were taken with a commercially available digital camera.

NMR spectra were recorded on a Varian Unity INOVA 400. $^{1}$H and $^{13}$C NMR chemical shifts were referenced to the solvent signal. Multiplicities in $^{1}$H NMR spectra are given as follows: s, singlet; m, multiplet. Gel permeation chromatography (GPC) was carried out on a Polymer Laboratories PL-GPC 50 with two PLgel 5 µm MIXED-C columns in THF at 40 °C against polystyrene standards with refractive index detection.

$MeO(CH_2CH_2O)_nH$ ($M_n$ = 2000 g/mol), DIPEA (99 %), thionyl bromide (97 %) and piperidine were purchased from Aldrich. Triethylamine (99 %) and diisopropylamine (99 %) were purchased from Acros. 4-Bromophenol (98 %) was supplied by Fluka, and $[Pd(PPh_3)_4]$

$[Pd(PPh_3)_2Cl_2]$ by MCAT, Konstanz. All substances were used without further purification. Toluene was destilled from sodium, THF from sodium/benzophenone, and DMF from $CaH_2$. All dispersions were prepared with Milli-Q-Water. Monomers and perylene diimide dye **2** were synthesized according to reported procedures.[1,2]

***Spatially resolved fluorescence measurements.*** Spectra were recorded with a Leica fluorescence microscope. The sample was illuminated from the top using a mercury lamp as a light source, with a UG11 filter to block emission of the lamp at wavelength longer than 400 nm. Fluorescence of the sample was collected through an air objective. For achieving spatial resolution a 100 µm pinhole was placed in the back focal plane of the microscope. The picture of the pinhole was imaged on the slit of a spectrometer (Shamrock_303i), equipped with a 300 lines/mm grating and the spectrum imaged on an Andor Newton EMCD-camera.

***CARS measurements.*** A custom made CARS microscope was used for quantification of the microfluidic mixing behaviour of THF in water. The CARS setup is based on a multiphoton microscope (Leica TCS SP5) and an Er:fiber laser source (based on a Toptica FemtoFiber pro) at a repetition rate of 40 MHz. The experiments were performed at 2860 $cm^{-1}$ resonance frequency using the Stokes beam tuned to 999.5 nm with a power of 6.5 mW and the pump laser tuned to 777.3 nm with a power of 62 mW. A Leica 0.7 NA, 20× air objective was used for focusing the excitation beams. Data were recorded in transmission type geometry and were collected by a Leica 0.55 NA air condenser and transmitted through suitable emission filters (641/75 and 680/SP, Semrock).

To obtain a calibration curve premixed concentrations of THF in water were measured. For normalization the intensities were divided by the non-resonant signal of the surrounding glass. The measurements were performed by stitching the maximum projection of 26 z-stacks (each with 19 layers, 400×400 pixels, 2.5 Hz and a frame average of 10) covering the complete height of the channel. The maximum value at each distance was taken and divided by the corresponding non-resonant signal of the surrounding glass. For illustration 50 consecutive values were averaged and plotted against the propagation distance.

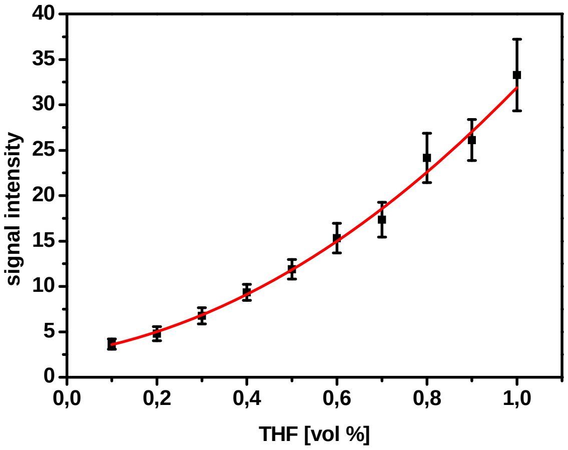

**Figure S1.** Calibration curve of CARS signal intensity vs. vol. % THF and parabolic fit (red line).

***Nanoparticle synthesis.*** Focused-flow droplet generator chips and chip holders were supplied by Micronit Microfluidics. The mixing channel used was 100 µm wide, 20 µm high and 50 mm long. Water and THF were injected using 500 µL and 25 µL, respectively, gastight syringes (Hamilton) with Luer tips and PTFE tubing. Fluidic connections were made by inserting fused silica capillaries (375 µm O.D., 150 µm I.D.) into the PTFE tubings (300 µm I.D.). The fused silica capillaries were connected to the chip by ferrules (N-123-03, IDEX) and nuts (F-123H, IDEX). The THF syringe was driven by a PHD 22/2000 syringe pump (Harvard Apparatus) and the water syringe was driven by an Orion M 362 syringe pump (Thermo Scientific).

In all experiments the water flow rate was kept at 10 µL/min, while the flow rate of the THF stream was varied between 0.5 µL/min and 1.2 µL/min. The block copolymers **1** and **3** were dissolved in THF and diluted to a concentration of 0.05 wt % or 0.03 wt %, respectively. For encapsulation studies 20 µg perylene diimide dye **2** were dissolved in 1 mL of the 0.05 wt % polymer solution. These polymer solutions were filtrated through a 0.45 µm filter before further use. Syringes and tubings were rinsed with Milli-Q-water or THF, respectively, before loading with the liquids.

After starting the syringe pumps, the channels were rinsed with the liquids for several minutes in order to overcome the dead volume of the channels and the outlet tubing and to provide a stable flow of the liquids. The outlet stream was collected in disposable low volume cuvettes (Sarstedt) or glass vials.

### *Synthesis of α-(4-bromophenoxy)-ω-methoxy poly(ethylene glycol.)*

#### α-bromo-ω-methoxy poly(ethylene glycol)[3]

25 g (12.5 mmol) poly(ethylene glycol) monomethyl ether ($M_n = 2000$ g/mol) were dissolved in 150 mL of toluene under a nitrogen atmosphere with mild heating. 3.5 mL (25 mmol) of triethylamine were added, followed by dropwise addition of 1.55 mL (20 mmol) thionyl bromide. The colourless solution turned dark brown immediately and a black solid precipitated. The mixture was refluxed for 30 minutes, and filtered after cooling to room temperature. The filtrate was stirred over night. Toluene and thionyl bromide were removed under vacuum at 80 °C. The brown residue was dissolved in toluene at 70 °C, precipitated in diethyl ether and the product was filtered off. 19.5 g (81 % yield) of a white solid were obtained.

$^1$H-NMR ($CDCl_3$, 25 °C): δ (ppm) = 3.63 (m, PEG-backbone), 3.37 (s, 3H, $OCH_3$).

$^{13}$C-NMR ($CDCl_3$, 25 °C): δ (ppm) = 72.1 ($CH_2O$), 71.4 ($CH_2O$), 70.7 (PEG-backbone), 59.1 ($CH_3O$), 30.4 ($CH_2Br$).

#### α-(4-bromophenoxy)-ω-methoxy poly(ethylene glycol)

18 g (9 mmol) α-bromo-ω-methoxy poly(ethylene glycol) ($M_n = 2000$ g/mol) and 2.8 g (16.2 mmol) 4-bromophenol were dissolved in 100 mL of dry DMF. 1.9 g (18 mmol) of sodium carbonate were added. The reaction mixture was refluxed over night and filtered over

celite after cooling to room temperature. DMF was removed under reduced pressure. The residue was dissolved in toluene and precipitated in diethyl ether. 17.3 g (95 % yield) of a white solid were obtained.

$^1$H-NMR: ($CDCl_3$, 25 °C) δ (ppm) = 7.38 – 7.32 (m, 2H, $H_{aryl}$), 6.82 – 6.76 (m, 2H, $H_{aryl}$), 4.11 – 4.06 (m, 2H, PhOC$H_2$), 3.86 – 3.43 (m, PEG-backbone), 3.37 (s, 3H, OC$H_3$).

$^{13}$C-NMR: ($CDCl_3$, 25 °C) δ (ppm) = 158.1 (O$C_{quaternary}$CH), 132.3 (Br$C_{quaternary}$*C*H), 116.6 (O$C_{quaternary}$*C*H), 113.1 (Br$C_{quaternary}$CH), 72.1 (*C*$H_2$OC), 71.0 (*C*$H_2$OC), 70.7 (PEG-backbone), 70.5 (*C*$H_2$OC), 69.8 (*C*$H_2$OC), 67.9 (PhO*C*$H_2$), 59.2 (*C*$H_3$O).

***Synthesis of α-azido-ω-methoxy poly(ethylene glycol)*[4]**

5 g (2.5 mmol) poly(ethylene glycol) monomethyl ether ($M_n$ = 2000 g $mol^{-1}$) and 0.066 g (2.75 mmol) NaH were mixed, and 100 mL of THF were added at 0 °C. Gas evolution was observed immediately. When the gas evolution stopped, the mixture was heated to 80 °C for 5 h. After cooling to room temperature 0.62 g (3.25 mmol) TsCl were added and stirred for 4 days at room temperature. The resulting solution was added to a mixture of 150 mL of HCl (conc.) and 150 g ice, extracted 3 times with 100 mL of $CH_2Cl_2$ each, dried over $MgSO_4$ washed with diethyl ether and recrystallized from toluene to yield 4.8 g (96 %) MeO-PEG-OTs ($M_n$ = 2000 g $mol^{-1}$) as a white solid.

$^1$H-NMR: ($CDCl_3$, 25 °C) δ (ppm) = 7.74 (d, $^3J_{HH}$ = 8.3 Hz, 2H, ortho-$H_{aryl}$), 7.30 (d, $^3J_{HH}$ = 8.0 Hz, 2H, meta-$H_{aryl}$), 4.13 - 4.07 (m, 2H, C$H_2$OTs), 3.59 (m, 190 H, PEG-backbone), 3.33 (s, 3H, C$H_3$O), 2.40 (s, 3H, para aryl-C$H_3$).

4.8 g (2.4 mmol) MeO-PEG-OTs ($M_n$ = 2000 g $mol^{-1}$) was dissolved in 85 mL of water and 35 mL of methanol. Addition of 1.87 g $NaN_3$ (28.8 mmol) led to a red colored solution, that was heated to reflux for 3 h. After stirring for additional 3 days, the reaction mixture was extracted 3 times with 100 mL of $CH_2Cl_2$. The organic layer dried over $MgSO_4$ and removed, yielding 4.2 g (87.5 %) MeO-PEG-$N_3$ ($M_n$ = 2000 g $mol^{-1}$) as a white solid.

$^1$H-NMR: ($CDCl_3$, 25 °C) δ (ppm) = 3.60 (m, 190H, PEG-backbone), 3.33 (m, 5H, $CH_3O$ and $CH_2N_3$).

### *Synthesis of polymer 1.*

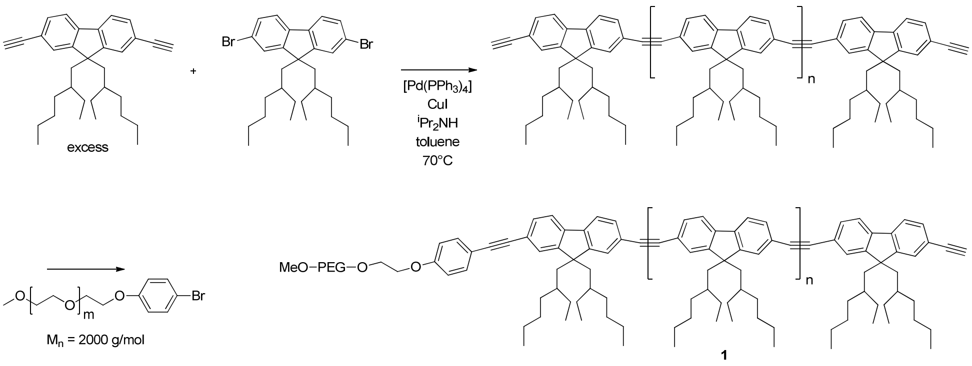

**Figure S2.** Synthesis of polymer **1**.

In a screw cap vial, 439 mg (1 mmol) 2,7-diethynyl-9,9’-bis(2-ethylhexyl)fluorene, 480 mg (0.87 mmol) 2,7-dibromo-9,9’-bis(2-ethylhexyl)fluorene, 0.1 mg CuI and 20 mg $[Pd(PPh_3)_4]$ were dissolved in 3 mL of toluene and 1 mL of $^iPr_2NH$ under an argon atmosphere. The solution was added to a mixture of 80 mL of toluene and 4 mL of $^iPr_2NH$, and was stirred at 70 °C for 3 days. The reaction was quenched by addition of 800 mg (0.4 mmol) α-(4-bromophenoxy)-ω-methoxy poly(ethylene glycol) and stirred over night at 70 °C. The solvent volume was reduced to approximately 50 mL by rotary evaporation. The polymer was precipitated in methanol and isolated by centrifugation. Unsubstituted poly(fluorene ethynylene) starting material was removed by dissolving the polymer in toluene and precipitation in pentane. Polymer **1** was obtained as a yellow solid.

$^1$H NMR ($CDCl_3$, 25 °C) δ (ppm) = 7.80 – 7.40 (m, PFE-backbone), 6.94 – 6.88 (m, 2H, $H_{phenyl}$ ortho to $OCH_2$), 4.16 (s, 2H, $PhOCH_2$), 3.78 – 3.41 (m, PEG-backbone), 3.38 (s, 3H, $OCH_3$), 3.12 (s, 1H, CCH), 2.19 – 1.88 (m, PFE-backbone), 1.06 – 0.48 (m, PFE-backbone).

### *Synthesis of polymer 3*

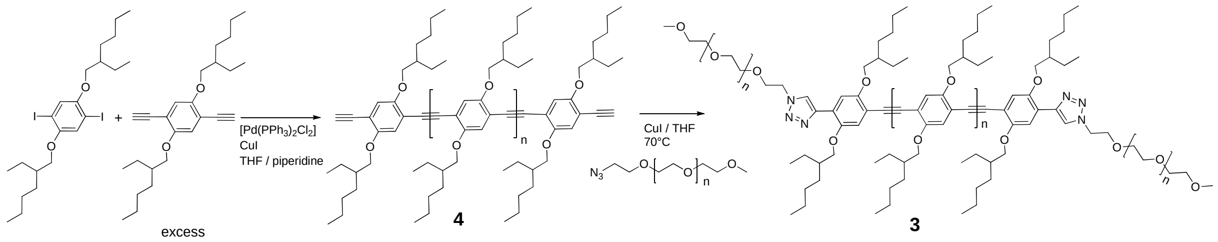

**Figure S3.** Synthesis of polymer **3**.

200 mg (523 µmol) of 1,4-bis((2-ethylhexyl)oxy)-2,5-diethynylbenzene and 246 mg (418 µmol) of 1,4-bis((2-ethylhexyl)oxy)-2,5-diiodobenzene were dissolved in 1.5 mL of THF and 0.7 mL of piperidine. The reaction mixture was degassed with three freeze-pump-cycles. CuI (0.2 mol%) and $[Pd(PPh_3)_2Cl_2]$ (0.2 mol%) were added and the mixture was degassed again with two freeze-pump-cycles. After stirred for 24 h at 40 °C, the mixture was poured into methanol upon which a yellow to orange solid precipitated. Polymer **4** was filtered off from the suspension, washed with methanol and dried under vacuo.

$^1$H NMR ($CDCl_3$, 25 °C) δ (ppm) = 7.04 – 6.98 (m, PPE-backbone), 6.98 – 6.95 (m, 2H), 4.02 – 3.80 (m, PPE-backbone), 3.32 (s, 1H), 1.88 – 1.73 (m, PPE-backbone), 1.72 – 1.21 (m, PPE-backbone), 1.08 – 0.79 (m, PPE-backbone).

80 mg (9.7 µmol) of polymer **4** and 40 mg (19.5 µmol) of $N_3$-PEG-OMe ($M_n$ = 2000 g/mol) were dissolved in 1 mL THF and 0.5 mL DIPEA. The mixture was degassed three times before 0.4 mg CuI were added. After stirring for 4 days at 70 °C the reaction mixture was diluted with toluene and washed with water. Precipitation in pentane gave a yellow solid.

$^1$H NMR ($CDCl_3$, 25 °C) δ (ppm) = 8.18 (s, 2H), 7.92 (s, 2H), 7.07 (s, 2H), 7.04 – 6.86 (m, PPE-backbone), 4.70 – 4.52 (m, 2H), 4.05 – 3.79 (m, PPE-backbone), 3.75 – 3.48 (m, PEG-backbone), 3.43 – 3.33 (m, 5H), 1.93 – 1.76 (m, PPE-backbone), 1.69 – 1.11 (m, PPE-backbone), 1.09 – 0.66 (m, PPE-backbone).

### *Spatially resolved fluorescence spectra of polymer 3.*

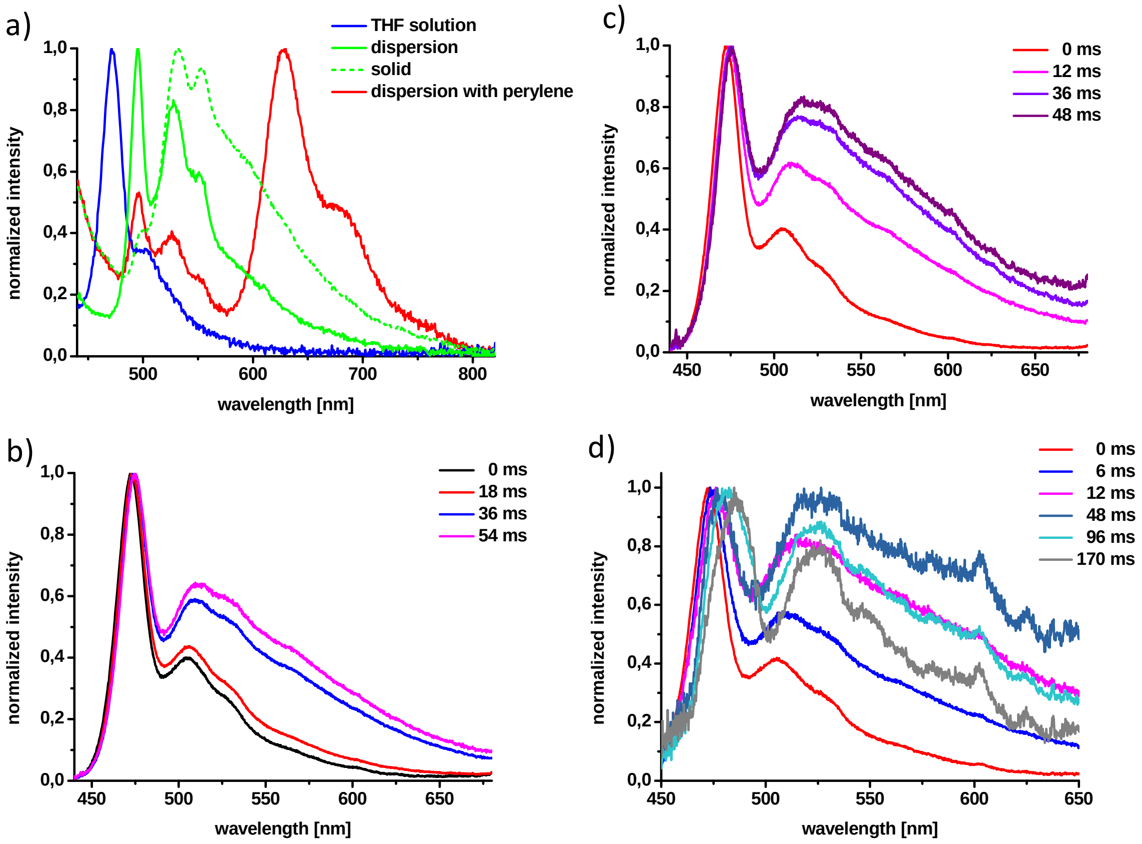

**Figure S4.** a) fluorescence spectra of **3** in THF (blue), dispersion (green), solid (dashed green) and dispersion with perylene (red) ($\lambda_{exc.}$ = 380 nm). Spatially resolved fluorescence spectra of **3** at a THF flow rate of b) 1 µL/min c) 0.6 µL/min d) 0.4 µL/min (water flow rate = 10 µL/min; $\lambda_{exc.}$ = 350-400 nm).

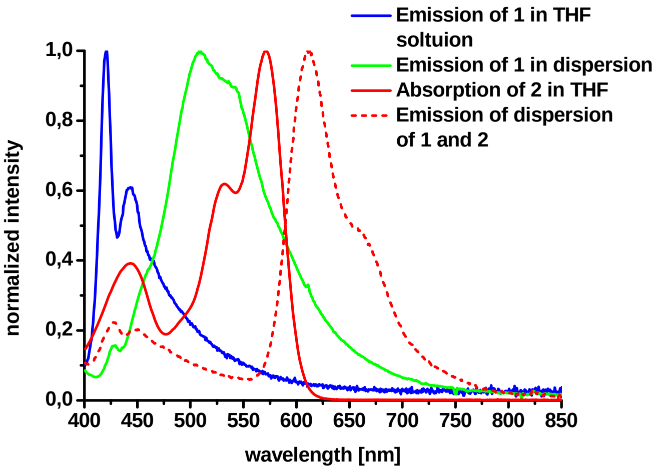

**Figure S5.** Emission spectra of **1** in THF solution (blue) and in dispersion (green). Absorption spectra of **2** in THF (red). Emission spectra of a mixture of **1** and **2** in dispersion (dashed red).

**Table S1**. Particle preparation from polymer **1**.[a]

| entry | conc. **1** wt.-%[b] | flow ratio THF / $H_2O$ | particle size[c] | PDI[d] |
|---|---|---|---|---|
| 1 | 0.05 | 0.05 | 53 | 0.15 |
| 2 | 0.05 | 0.05 | 58 | 0.04 |
| 3 | 0.05 | 0.06 | 56 | 0.09 |
| 4 | 0.05 | 0.08 | 69 | 0.08 |
| 5 | 0.03 | 0.08 | 58 | 0.08 |
| 6 | 0.03 | 0.10 | 63 | 0.09 |
| 7 | 0.03 | 0.12 | 80 | 0.07 |

[a] Flow rate of water stream: 10 µL $min^{-1}$. [b] concentration of **1** in THF. [c] volume average particle size. [d] Polydispersity index (from dynamic light scattering).

**Table S2.** Particle preparation from polymer **3**.[a]

| entry | conc. **3** wt.-%[b] | flow ratio THF / $H_2O$ | particle size[c] | PdI[d] |
|---|---|---|---|---|
| 1 | 0.05 | 0.10 | 39 | 0.16 |
| 2 | 0.05 | 0.08 | 37 | 0.19 |
| 3 | 0.05 | 0.06 | 29 | 0.23 |
| 4 | 0.01 | 0.10 | 57 | 0.15 |
| 5 | 0.01 | 0.08 | 48 | 0.17 |
| 6 | 0.01 | 0.04 | 38 | 0.11 |

[a] Flow rate of water stream: 10 µL $min^{-1}$. [b] concentration of **3** in THF. [c] volume average particle size. [d] Polydispersity index (from dynamic light scattering).

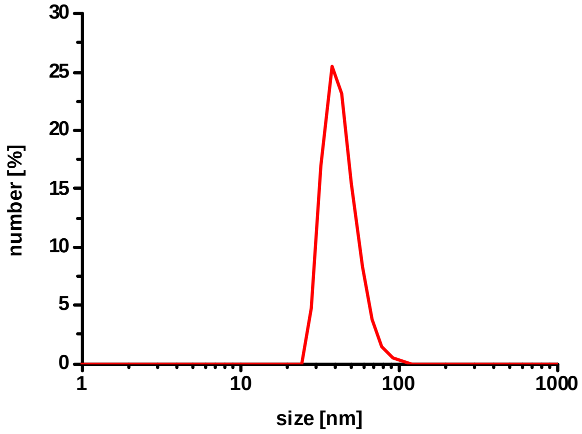

**Figure S6**: DLS trace of an aqueous dispersion of **1**.

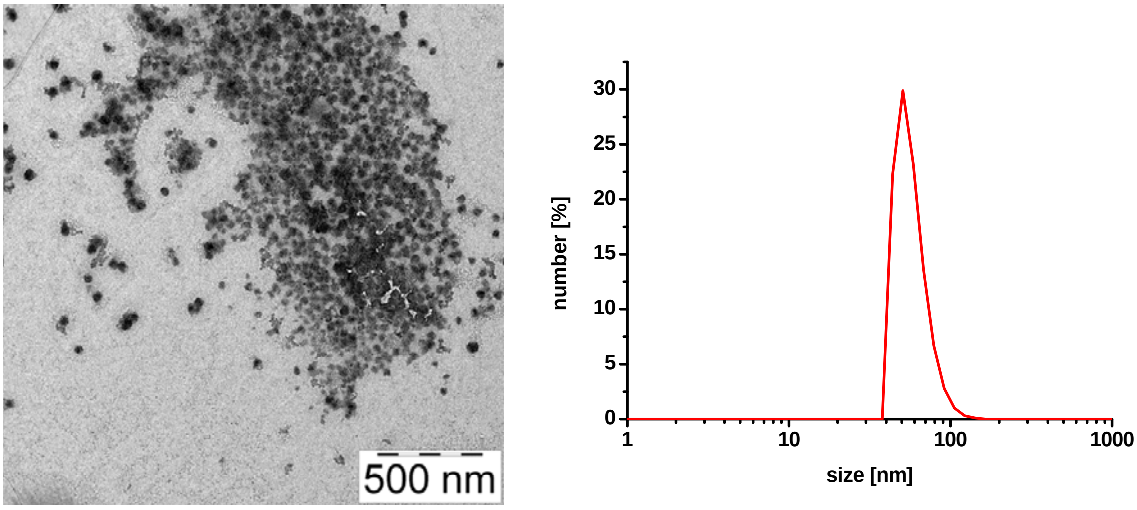

**Figure S7.** TEM image of nanoparticles of **1** with incorporated dye **2** (left) and DLS trace (right).

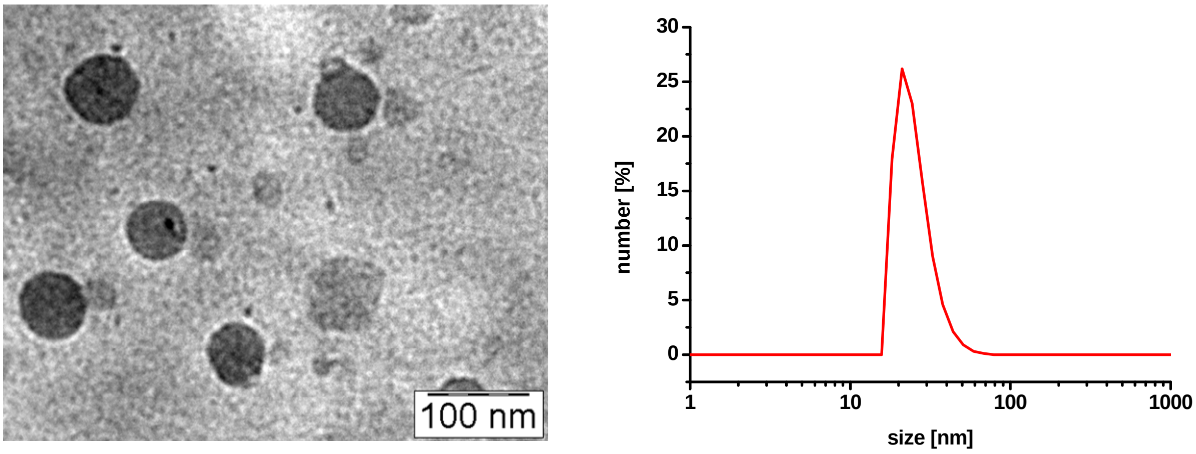

**Figure S8.** TEM image of nanoparticles from **3** (left) and DLS trace (right).

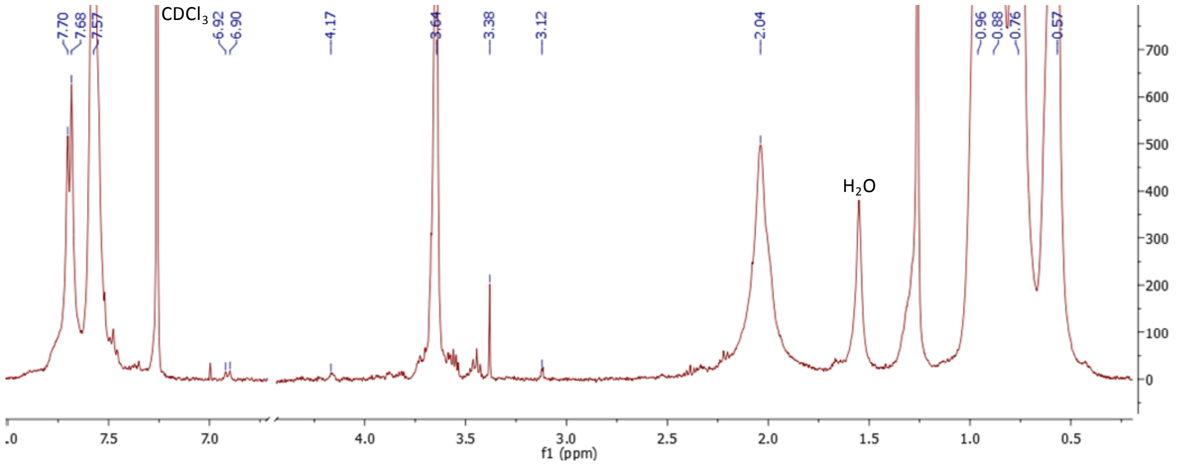

**Figure S9.** $^1$H NMR spectrum of **1** (400 MHz, 25 °C, CDCl$_3$).

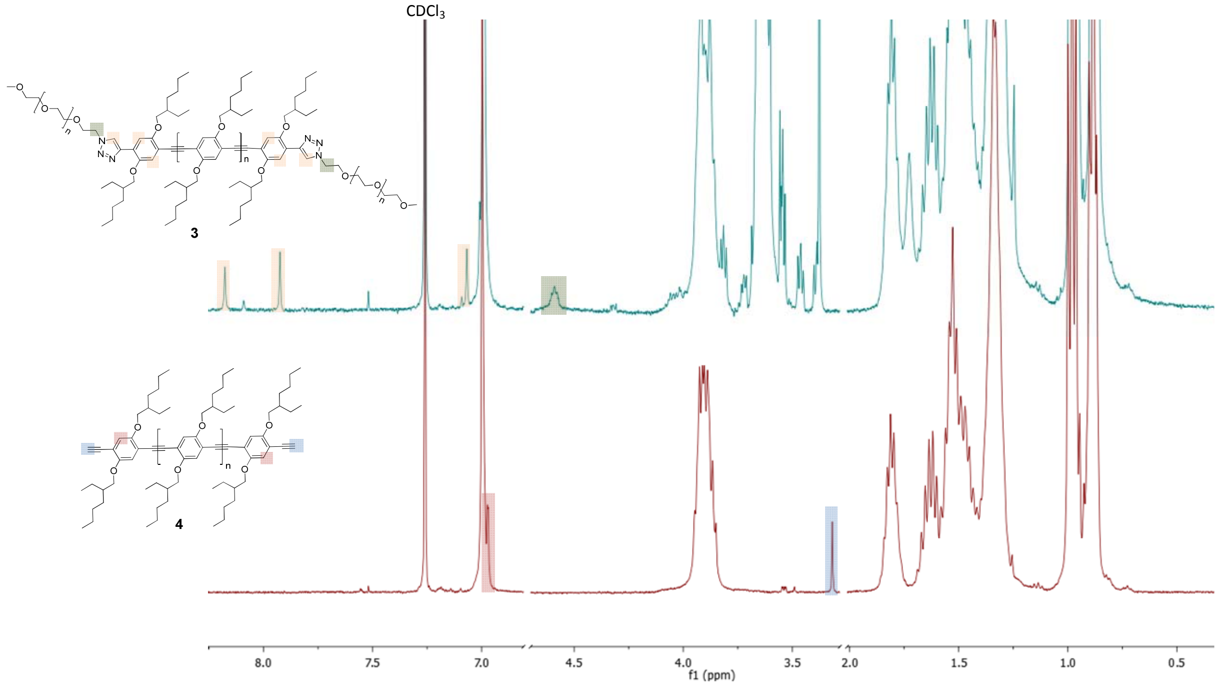

**Figure S10.** $^1$H NMR spectra of polymers **3** and **4** (400 MHz, 25 °C, CDCl$_3$).

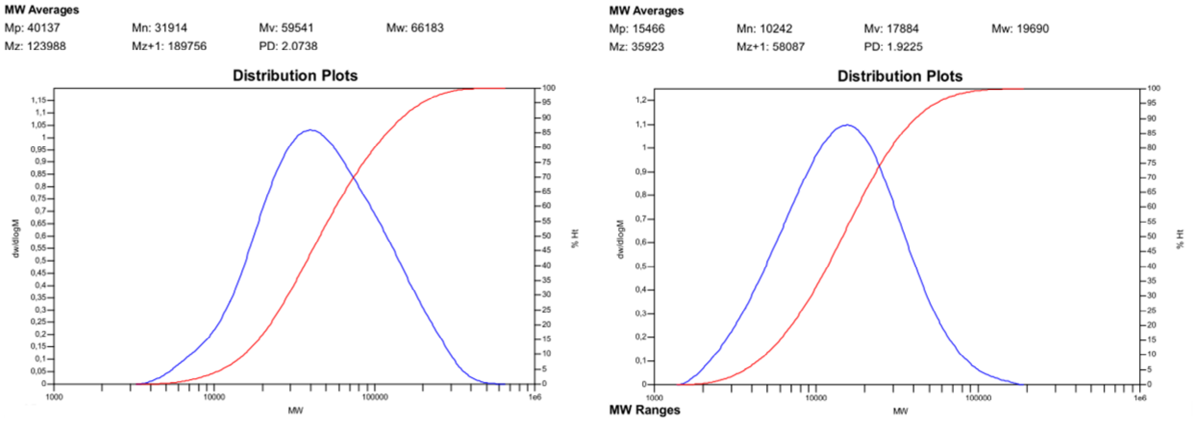

**Figure S11.** GPC trace of diblock copolymer **1** (left) and triblock copolymer **3** (right).

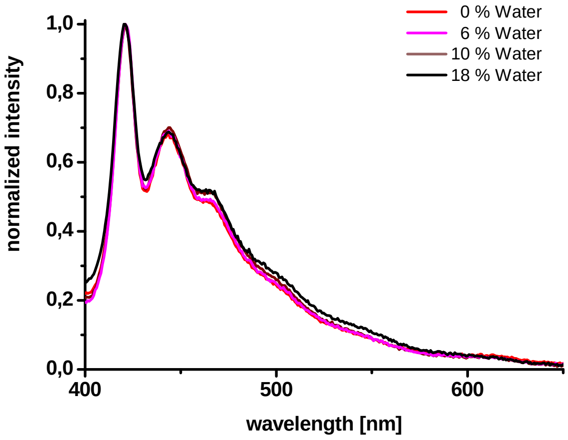

**Figure S12.** Emission spectra of **1** in THF/water mixtures. Normalized on emission maximum at 424 nm ($\lambda_{exc.}$ = 380 nm).

## References

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[2] C. Ego, D. Marsitzky, S. Becker, J. Zhang, A. C. Grimsdale, K. Müllen, J. D. MacKenzie, C. Silva, and R. H. Friend, *J. Am. Chem. Soc.*, 2003, **125**, 437 - 443.

[3] U. Rauwald, and O. A. Schermann, *Angew. Chem. Int. Ed.*, 2008, **47**, 3950-3953.

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