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Thermoresponsive Swelling of Fluorescent Single-Chain Nanoparticles

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Experimental Procedures

Chemicals

All chemicals were purchased from Sigma Aldrich except tetrabutylammonium bromide (TCI), sodium hydroxide (Grüssing), triethylamine (TCI), 3-chloro-1-propanol (TCI), *p*-hydroxychalcone (TCI), diethylamine (Fluka), propargyltosylate (Fluka), nitromethane (Alfa Aesar) and $CuSO_4$ x 5 H_2O (VEB Laborchemikalien). Before use, azobisisobutyronitrile (AIBN) was freshly recrystallized from methanol. Oligo(ethylene glycol) methyl ether methacrylate (M_n=300) was passed through a basic AlO_x-coloumn to remove the stabilizer.

Instrumentation and Analysis

ESI-TOF-MS measurements were performed on a Bruker Daltonics microTOF via direct injection at a flow rate of 180 µL h^{-1} in positive mode with an acceleration voltage of 4.5 kV. Samples were prepared by dissolving in a mixture of LC-MS grade methanol and LC-MS grade tetrahydrofuran in a ratio of 1 to 8. 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 data evaluation.

NMR spectra were measured on an Agilent Technologies 400 MHz VNMRS and 500 MHz DD2 at 27°C. Chemical shifts (δ) are reported in ppm and referred to the solvent residual signal (CDCl3 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C, methanol-d4 3.31 ppm for ^{1}H and 49.0 ppm for ^{13}C, D_2O 4.66 ppm for ^{1}H).

DOSY-NMR 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. 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 ^{1}H 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.
The hydrodynamic diameter D_h were calculated using the Stokes-Einstein equation

$$D_h = \frac{k_B \cdot T}{3 \cdot \pi \cdot \eta_{D2O}(T) \cdot D} \quad \text{(S1)}$$

with the Boltzmann constant k_B, temperature *T*, diffusion coefficient *D*, and the temperature dependent viscosity of the solvent η_{D2O}*(T).The viscosity* η_{D2O} was calculated with

$$\eta_{D2O}[mPa \cdot s] = 0.313 + 38112{,}5 * e^{-0{,}036*T[K]} \quad \text{(S2)}$$

plotting data were taken from Millero et al. (1971).[1]

ATR-IR spectra were measured on a Bruker Tensor Vertex 70 equipped with a Golden Gate Heated Diamond ATR Top-plate.

THF-based SEC measurements were performed at 30 °C on a Viscotek GPCmax VE 2001 from Viscotek™ applying a CLM3008 precolumn and a CLM3008 main column. As solvent THF was used and the sample concentration was adjusted to 3 mg·mL^{-1} while applying a flow rate of 1 ml·min^{-1}. For determination of the molecular weights the refractive index of the investigated sample was detected with a VE 3580 RI detector of Viscotek™. External calibration was done using poly(styrene) (PS) standards (purchased form PSS) with a molecular weight range from 1050 to 115000 g mol^{-1}.

Cell toxicity of the SCNP formulations was tested using a Resazurin reduction 96 well assay on NHDF cells. Two time points were analyzed. NHDF were cultured in DMEM (high glucose) + 10% FCS + 1% penicillin-streptomycin @ 37 °C under 5% CO_2 in a standard cell culture incubator. For 24 h 20,000 cells/100 µL were seeded per 96 well, for 96 h 5,000 cells/100 µL were used. On the 2nd day after seeding, SCNP formulations were added in increasing concentrations from 0.00001 mg/ml to 0.5 mg/mL. Pure cell culture medium served as negative control (= 100% viability) and 0.025% Triton X100 was used as positive control (= 0% viability). All varying concentrations of the assay were performed as octuplicates (n = 8). After incubating for 24 or 96 h, resp., 20 µL Resazurin stock solution (440 µM in PBS, f.c. 44 µM) was added to each well and the mixture was incubated for 2 h @ 37 °C under 5% CO_2. Then, fluorescence intensity was determined with the CytationTM 5 imaging reader (BioTek Instruments) using for excitation the BP 531(20) and for emission BP 593(20). Cell viability was expressed as a percentage of the negative controls (untreated cells) after subtraction of the blank. The assay was performed three times and then, mean and S.D. were calculated and plotted for data evaluation (n = 3).

Turbidimetry measurements were performed on a JASCO J-1500 with a PTC-510 cell holder. The samples were measured in Helma analytics quartz glass cuvettes (d = 1 mm) at concentrations of 1 mg/mL in water. The samples were heated from 30 °C to 90°C with a heating rate of 1 K/min. The transmittance was measured at a wavelength of 500 nm. The temperature at 50% of the normalized transmittance was taken as cloud point temperature T_{cp}.

UV/VIS/NIR-absorption measurements were performed on a Perkin Elmer LAMBDA 365 UV/Vis Spectrophotometer using Helma analytics quartz glass cuvettes (d = 10 mm). Temperature control was achieved using the Perkin Elmer Peltier System L365. The measurements were each repeated three times.

Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrometer of Agilent using Helma analytics quartz glass cuvettes (d = 10 mm). Temperature control was achieved using the Agilent Cary Single Cell Peltier Accessory Type SPVF-1x0. The measurements were each repeated three times.

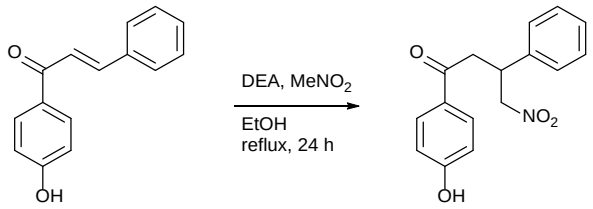
Decay associated spectra were recorded employing a Hamamatsu R5900 16-channel multi-anode photomultiplier tube (PMT) with 16 separate output (anode) elements and a common cathode and dynode system (PML-16C, Becker&Hickl, Berlin, Germany) as described in Schmitt et al. 2020. A 632 nm pulsed laser diode (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). The determination of the DAS is described in detail in Schmitt et al. 2019.
The sample was placed in a standard 1x1 cm Quartz cuvette and cuvette holder (CHV100, Thorlabs, Newton, USA). The temperature was adjusted by a Peltier element at the surface of the cuvette holder with the warm side attached with thermally conductive paste. Temperature was then monitored with a standard thermoelement with an accuracy of about +/- 1°C. The measurements were each repeated three times.

Photoacoustic (PA) pump-probe spectroscopy: A wavelength tuneable dual-OPO laser system (SpitLight EVO III Dual OPO, Innolas Laser GmbH, Germany) provided pump and probe excitation pulses of 5 ns duration at a repetition frequency of 100 Hz. The outputs of the OPO laser system were coupled into a bifurcated fiber bundle (8 mm core diameter) (Laser Components GmbH, Germany) for the measurement. A custom-made cuvette (path length = 6 mm, volume = 2 mL) was placed in a water bath filled in with distilled water and illuminated with the output of the fiber bundle (10 mm beam diameter at the cuvette). Neutral density filters were used to control the optical fluence. The PA signal was detected using a polyvinylidene fluoride

transducer (PA1483, Precision Acoustics Ltd., UK), amplified with a 20 dB voltage preamplifier (HVA-200M-40-F, FEMTO Messtechnik GmbH, Germany) and recorded using a digitizer card (National Instruments, USA). The efficiency of the PA signal generation in fluorophores was varied by exploiting the effects of stimulated emission, i.e., the SCNP solutions were illuminated with a pump pulse that coincides with the absorption spectrum while the probe pulse coincided with the maximum of the fluorescence spectrum. The time-course of the PA signal amplitude was generated using simultaneous pump-probe excitation. PA signals were measured at room temperature in aqueous SCNP solutions with a concentration of 1 $mgmL^{-1}$ and in pure aBOD dye solutions with a concentration of 10^{-4} M using pump and probe wavelengths that coincided with the wavelengths of their peak absorption and fluorescence (λ_{pump} = 704 nm and λ_{probe} = 720 nm for SCNP solutions, λ_{pump} = 700 nm and λ_{probe} = 720 nm for pure dye solutions).

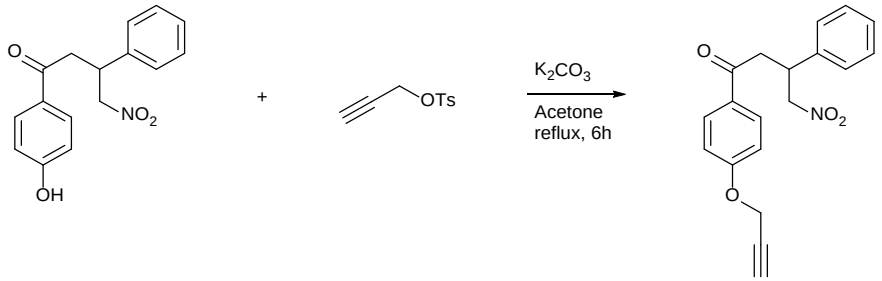
Synthesis and Sample Preparation

Synthesis of 1-(4-hydroxyphenyl)-4-nitro-3-phenylbutan-1-one ***1***



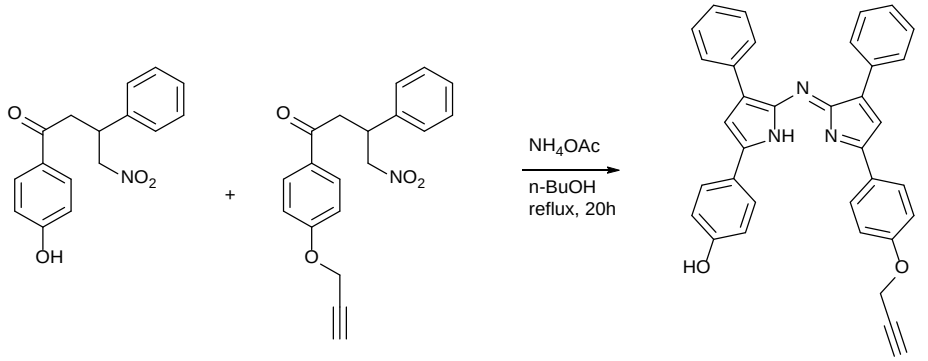
p-Hydroxychalcone (14.7 mmol, 3g) was dissolved in ethanol (20 mL). The solution was heated to reflux (90°C oil bath temperature). Diethylamine (66.75 mmol, 4.88 g, 6.9 mL) and nitromethane (147 mmol, 8.15 g, 7.2 mL) were added dropwise and the reaction solution was stirred under reflux for 18 h. The solution was poured in 100 mL 1 M HCl and the product extracted with ethyl acetate. The crude product was purified by column chromatography (methanol:DCM 1:10, R_f = 0.47). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.85 (2H, m, H_{ar}), 7.33 (2H, m, edge://settings/profiles
H_{Ar}), 7.27 (3H, m, H_{Ar}), 6.86 (2H, m, H_{Ar}), 5.74 (1H, s (broad), O*H*), 4.88-4-64 (2H, m, NO_2-CH_2), 4.21 (1H, m, Ph-C*H*), 3.39 (2H, m, O=C-CH_2).

Synthesis of 4-nitro-3-phenyl-1-(4-(prop-2-yn-1-yloxy)phenyl)butan-1-one ***2***



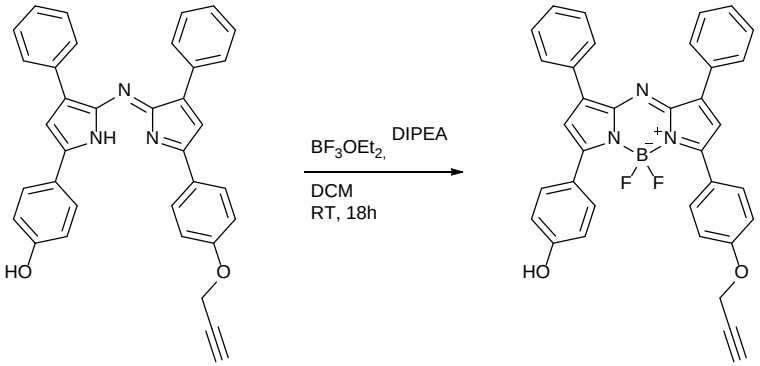
1 (5 mmol, 1.43g) was dissolved in acetone (30 mL). K_2CO_3 (15 mmol, 2.07 g) was added and the dispersion was heated to 70°C (reflux). After 1 h propargyl tosylate (7.5 mmol, 1.58 g, 1.28 mL) was dissolved in 5 mL acetone and added to the reaction mixture. After 6 h it was cooled to room temperature, filtrated and the acetone was removed under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water. The organic phase was dried with Na_2SO_4 and removed under reduced pressure. The product was purified by column chromatography (ethyl acetate:hexane 1:3, R_f = 0.25). Yield: 55%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.91 (2H, m, H_{Ar}), 7.33 (2H, m, H_{Ar}), 7.27 (3H, m, H_{Ar}), 7.01 (2H, m, H_{Ar}), 5.74 (1H, s (broad), O*H*), 4.86-4-65 (2H, m, NO_2-CH_2), 4.75 (2H, d, J = 2.4 Hz, ≡C-CH_2), 4.21 (1H, m, Ph-C*H*), 3.40 (2H, m, O=C-CH_2), 2.55 (1H, t, J = 2.4 Hz, ≡C*H*).

Synthesis of [5-(4-Hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[4-prop-2-yn-1-yloxyphenyl)-3-phenylpyrrol-2-ylidene]amine ***3***



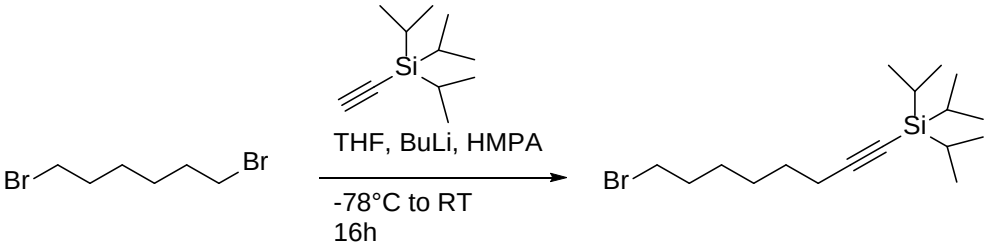
1 (2.16 mmol, 618 mg), **2** (2.16 mmol, 700 mg) and ammonium acetate (150 mmol, 11.6 g) in *n*-BuOH (50 mL) were heated under reflux (130°C oil bath) for 20 h. The reaction mixture was cooled in an ice bath and filtrated. The isolated solid was washed with cold *n*-BuOH and dried under vacuum. The product was purified by column chromatography ($CHCl_3$:methanol 20:1, R_f = 0.51). Yield: 7%. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.05 (4H, m, H_{Ar}), 7.93 – 7.82 (4H, m, H_{Ar}), 7.42 (4H, m, H_{Ar}), 7.35 (2H, m, H_{Ar}), 7.13 (4H, m, H_{Ar}), 6.99 (2, m, H_{Ar}), 4.80 (2H, d, *J* = 2.3 Hz, ≡C-CH_2), 2.59 (1H, t, *J* = 2.3 Hz, ≡C*H*).

*Synthesis of BF_2-chelate of [5-(4-Hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[4-prop-2-yn-1-yloxyphenyl)-3-phenylpyrrol-2-ylidene]amine **4 (aBOD)***



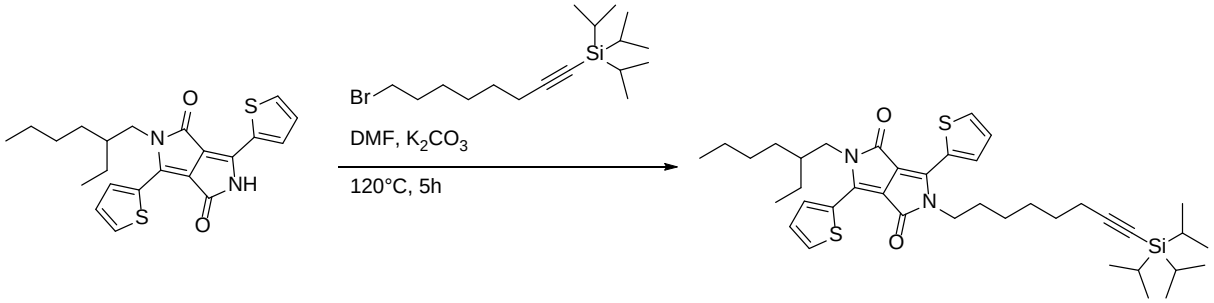
3 (0.15 mmol, 80 mg) was dissolved in dry DCM (20 mL). DIPEA (1.8 mmol, 233 mg, 306 µL) was added. The solution was stirred for 10 min at room temperature before being cooled to 0°C. BF_3OEt_2 (2.7 mmol, 383 mg, 333 µL) was added. The resulting solution was stirred at 0 °C for 30 min and then at room temperature for 18 h. The product solution was diluted with ethyl acetate, washed with NH_4Cl-solution, brine, and water, dried with Na_2SO_4, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (ethyl acetate:hexane 1:1, R_f = 0.38) to get the product as a red solid. Yield: 91%. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 8.13 - 8.02 (8H, m, H_{Ar}), 7.50 – 7.40 (6H, m, H_{Ar}), 7.13 – 7.06 (2H, m, H_{Ar}), 7.04 (2H, m, H_{Ar}), 6.96 - 6.92 (2H, m, H_{Ar}), 4.78 (2H, d, *J* = 2.4 Hz, ≡C-CH_2), 2.58 (1H, t, *J* = 2.4 Hz, ≡C*H*).

*Synthesis of (8-bromooct-1-yn-1-yl)triisopropylsilane **5***



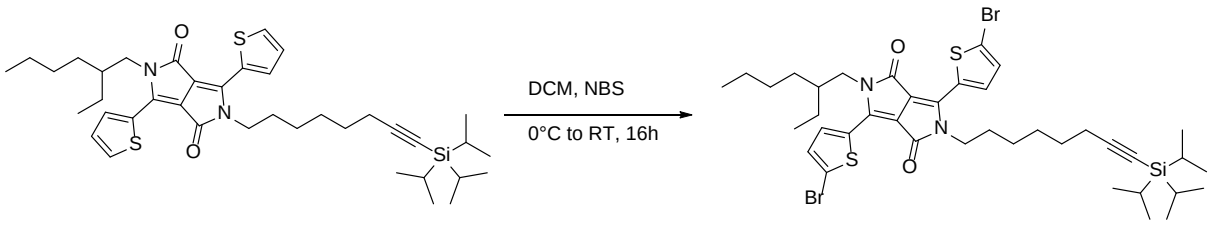
A flame dried flask flushed with argon was cooled to −78 °C and charged with a solution of ethynyltriisopropylsilane (2.0 g, 10.98 mmol) in dry THF (15 mL). After stirring the mixture at −78°C for 5 min, n-BuLi was added dropwise (2.5 M in hexane, 4.39 ml, 10.98 mmol). After stirring at −78°C for 1 h, HMPA (5.43 ml, 31.22 mmol) was added. To this resulting solution a solution of 1,5-dibromohexane (3.20 g, 13.11 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at −78°C for 5 min and then at room temperature for 16 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (10 mL) and volatiles were removed under reduced pressure. The aqueous phase was extracted with DCM (3 × 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified using column chromatography (hexane) to get **5** as a colourless oil. Yield: 88%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 3.41 (m, 2H), 2.26 (t, 2H), 1.56 (q, 3H), 1.46 (m, 4H), 1.27 (m, 3H), 1.05 (m, 16H), 0.80 (m, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ in ppm): 108.79, 80.24, 77.31, 76.99, 76.87, 34.65, 33.57, 33.42, 32.69, 32.51, 31.58, 29.05, 28.72, 28.56, 27.74, 27.61, 27.29, 26.90, 22.64, 22.57, 20.65, 19.60, 18.59, 18.50, 14.06, 11.37, 11.29. 11.14.

*Synthesis of 2-(2-ethylhexyl)-3,6-di(thiophen-2-yl)-5-(8-(triisopropylsilyl)oct-7-yn-1-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione **6***



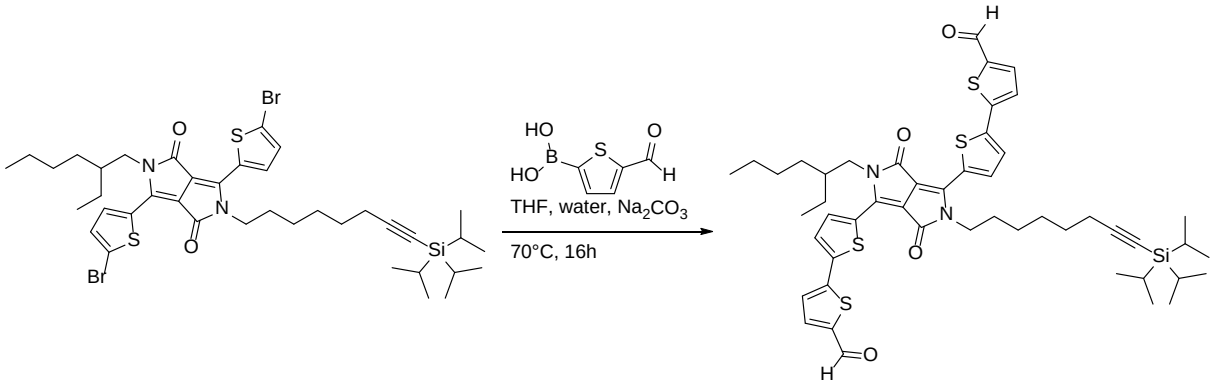
In 50 ml anhydrous dimethylformamide 2-(2-methylheptyl)-3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (1.0 g, 2.42 mmol) and 18-crown-6 (0.032 g, 0.12 mmol) were dissolved at 120°C. Anhydrous K_2CO_3 (0.502 g, 3.63 mmol) was added. After 1 h **5** (1.08 g, 3.13 mmol) was added dropwise over 1 h and stirring was maintained for 20 h. The cooled reaction mixture was diluted with 20 ml water and 15 ml ethyl acetate, concentrated under reduced pressure, dissolved in ethyl acetate, washed with water and dried using $MgSO_4$. After removing solvents under reduced pressure, the residue was precipitated from chloroform into methanol and the collected precipitates were further purified via column chromatography using 0 - 10 % diethyl ether in hexane (best separation effect at 5 %) to get **6** as a dark red solid. Yield: 73%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.93 (d, J = 2Hz, 2H) 7.63 (d, 2H), 7.26 (t, 2H), 4.07 (t, 4H), 2.24 (t, 2H), 1.75 (m, 4 H), 1.52 (m, 10 H), 1.04 (m, 30H); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ in ppm): 161.72, 161.32, 140.36, 139.95, 135.34, 135.25, 133.42, 132.26, 130.62, 129.71, 128.59, 128.36, 108.96, 107.68, 80.17, 52.11, 45.83, 42.09, 39.07, 30.21, 29.88, 28.27, 26.36, 23.51, 23.05, 19.72, 18.63, 14.00, 11.56, 11.26, 10.48 ppm; MALDI-TOF: m/z calculated for $C_{39}H_{56}N_2O_2S_2Si$, 676. 355 [M] found 676.304.

*Synthesis of 3,6-bis(5-bromothiophen-2-yl)-2-(2-ethylhexyl)-5-(8-(triisopropylsilyl)oct-7-yn-1-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione **7***



To a solution of compound **6** (1.0 g, 1.47 mmol) in 35 mL chloroform at 0 °C N-bromosuccinimide (0.579 g, 3.25 mmol) was added in portions. After stirring for 16 h protected from light the reaction solution was washed with water and brine, and dried over $MgSO_4$. Upon removing solvents under reduced pressure, the target material was isolated by column chromatography, using 8 - 10 % diethyl ether in hexane to get **7** as purple solid. Yield: 48%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.69 (d, J = 2Hz, 2H) 7.26 (d, 2H), 4.00 (t, 4H), 2.26 (t, 2H), 1.75 (m, 4 H), 1.35 (m, 10 H), 1.04 (m, 30H); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ in ppm): 160.96, 135.35, 131.64, 131.03, 108.90, 42.15, 29.94, 28.65, 28.26, 28.33, 23.02, 19.72, 18.62, 11.26, 10.45 ppm; MALDI-TOF: m/z calculated for $C_{39}H_{54}Br_2N_2O_2S_2Si$, 832. 176[M] found 832.056.

Synthesis of 5',5'''-(2-(2-ethylhexyl)-3,6-dioxo-5-(8-(triisopropylsilyl)oct-7-yn-1-yl)-2,3,5,6-tetrahydropyrrolo[3,4-c]pyrrole-1,4-diyl)bis(([2,2'-bithiophene]-5-carbaldehyde)) ***8 (DPP)***



7 (0.2 g, 0.24 mmol), Pd(PPh3)4 (0.013 g, 0.01 mmol), and Na_2CO_3 (0.254 g, 2.39 mmol) were dissolved in degassed THF (19 mL) and degassed water (5 mL) under argon atmosphere. The mixture was heated to 45°C and stirred for 30 min. A solution of 5–formylthiophen–2–yl boronic acid (0.082 g, 0.52 mmol) in degassed THF (25 mL) was added slowly, and the mixture was refluxed for 12 h until a blue coloration was observed. After cooling to room temperature, the mixture was extracted with DCM and dried over Na_2SO_4. Upon removing solvents under reduced pressure, the crude product was purified by column chromatography using 28% DCM in hexane to get **8** as a dark purple solid. Yield: 46%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.90 (s, 2H), 8.93 (d, J = 2 Hz, 2H), 7.72 (d, 2H), 4.30 (t, 4H), 2.26 (t, 2H), 1.75 (m, 4 H), 1.35 (m, 10 H), 1.03 (m, 30H); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ in ppm): 182.29, 161.03, 144.96, 143.15, 140.95, 138.95, 136.95, 136.50, 130.34, 131.64, 125.59, 109.07, 108.86, 80.27, 42.21, 29.99, 29.67, 28.30, 26.37, 19.74, 18.60, 11.27, 10.55 ppm; ESI-TOF: m/z calculated for $C_{49}H_{60}N_2O_4S_4Si$, 896. 320 [M] found 895.964.

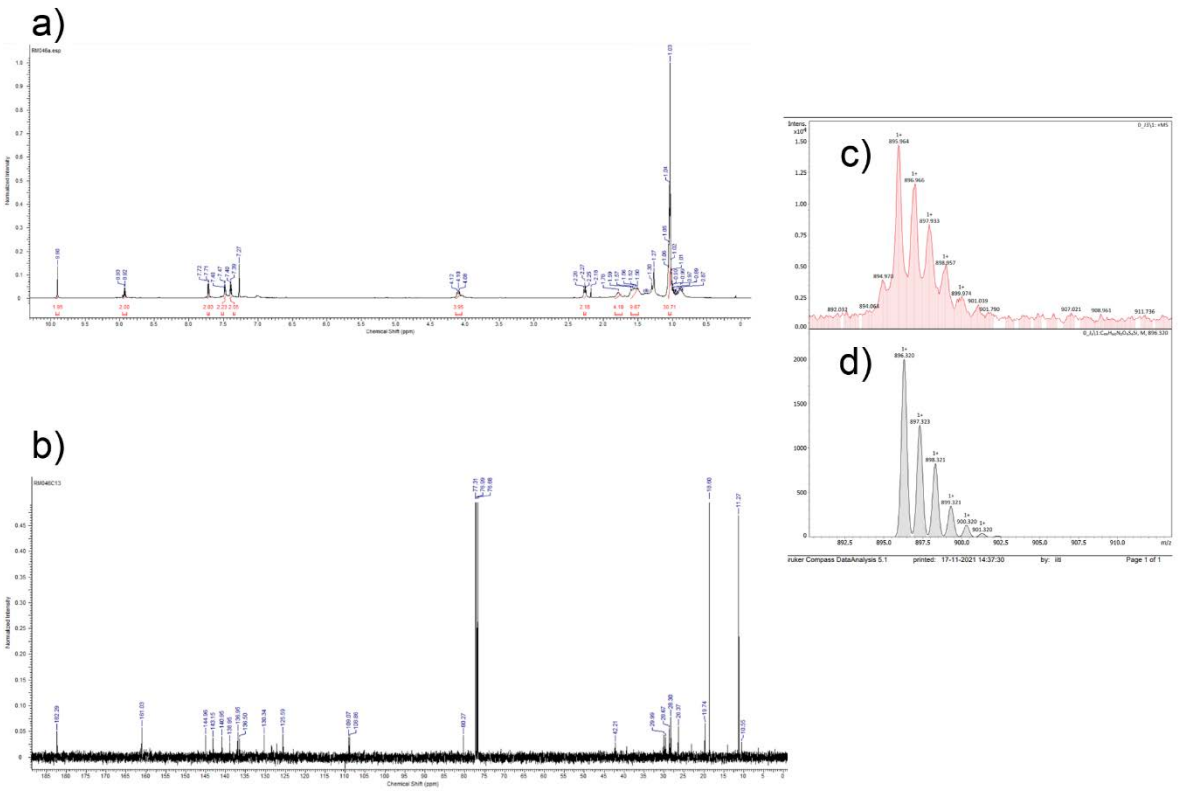
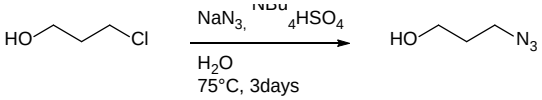


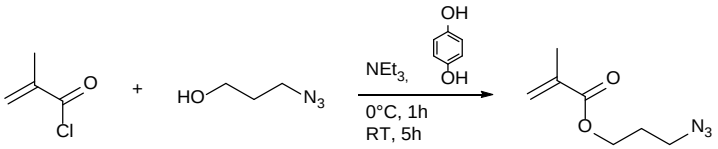
Figure S1. a) ^{1}H, and b) ^{13}C NMR spectrum of DPP. c) Measured, d) simulated ESI-TOF MS of DPP.

Synthesis of 3-Azido-1-propanol ***9***



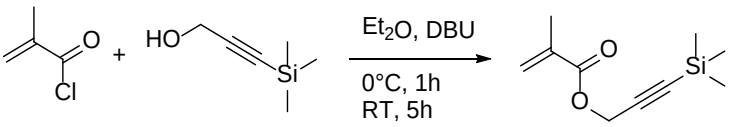
3-Chloro-1-propanol (23.5 mmol, 2.2 g, 2 ml) was added dropwise to a solution of sodium azide (48 mmol, 3.13 g) and tetrabutylammonium hydrogen sulphate (0.2 mmol, 70 mg) in 50 ml water at room temperature. The resulting solution was heated to 75°C. After stirring for three days the product was extracted with DCM. The combined organic phases were dried with Na_2SO_4 and the solvent evaporated under vacuum to get the product as a colorless liquid. Yield: 85%. %. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 3.71 (2H, t, *J*= 6.0 Hz, HO-C*H*$_2$), 3.42 (2H, t, *J* = 6.6 Hz, C*H*$_2$-N$_3$), 2.21 (1H, s, O*H*), 1.80 (2H, tt, *J* = 6.6, 6.0 Hz, $CH_2CH_2CH_2$).

Synthesis of 3-Azidopropyl methacrylate ***10 (APMA)***



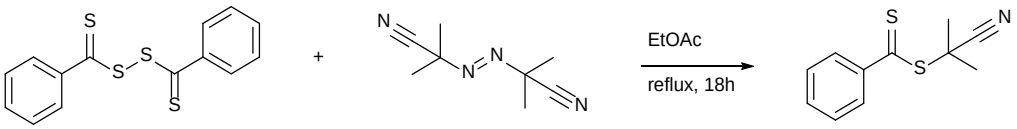
9 (19.3 mmol, 1.95 g, 1.8 ml), triethylamine (28.7 mmol, 2.9 g, 4 ml) and hydroquinone (10 µmol, 1 mg) were dissolved in 25 ml of dry DCM and placed in an ice bath. Methacryloyl chloride (28.7 mmol,3 g, 2.8 ml) in 5 ml of dry DCM was added dropwise. The resulting solution was stirred at 0°C for 1 h and at room temperature for additional 5 h. The white precipitate was removed by filtration and the product solution washed with water, saturated sodium bicarbonate solution and brine. The organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum. The product was purified by column chromatography (hexane:diethyl ether 2:1, R_f = 0.46) to get the product as a pale-yellow liquid. The product was stored with hydroquinone as stabilizer. Yield: 25%. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 6.11 (1H, dq, *J* = 2.0, 1.0, =C*H*), 5.58 (1H, dq, *J* = 2.0, 1.6 Hz, =C*H*), 4.24 (2H, t, *J* = 6.2 Hz, COO-C*H*$_2$), 3.42 (2H, t, *J* = 6.7 Hz, C*H*$_2$-N$_3$), 1.96 (2H, tt, *J* = 6.7, 6.2 Hz, $CH_2CH_2CH_2$), 1.95 (3H, dd, *J* = 1.6, 1.0 Hz, C*H*$_3$).

Synthesis of 3-(Trimethylsilyl)propargyl methacrylate ***11 (TMSPMA)***



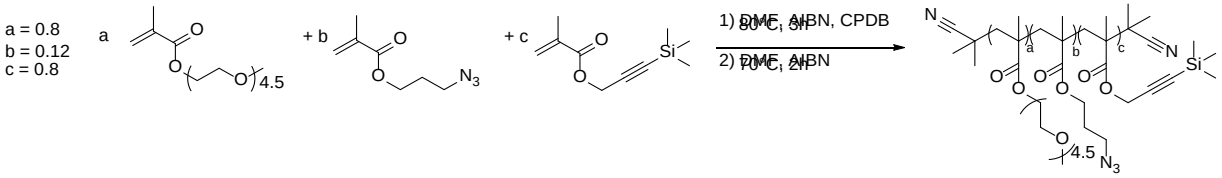
3-(Trimethylsilyl)propargyl alcohol (15.58 mmol, 2.0 g, 2.15 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (18.7 mmol, 1.42 g, 1.4 ml) were solved in 20 ml of dry diethyl ether and cooled to 0°C. Methacryloyl chloride (18.7 mmol, 1.95 g, 1.8 ml) in 10 ml of dry diethyl ether was added dropwise. The resulting solution was stirred at 0°C for 1 h and at room temperature for additional 5 h. The yellow precipitate was removed by filtration and the product solution washed with water and brine. The organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum. The product was purified by column chromatography (hexane:diethyl ether 20:1, R_f = 0.19) to get the product as colourless liquid. Yield: 30%. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 6.17 (1H, dq, *J* = 1.0, 2.2 Hz, =C*H*), 5.61 (1H, dq, *J* = 1.6, 2.2 Hz, =C*H*), 4.76 (2H, s, OC*H*$_2$), 1.96 (3H, dd, *J* = 1.6, 1.0 Hz, C*H*$_3$), 0.18 (9H, s, Si(C*H*$_3$)$_3$).

Synthesis of Cyanoisopropyl dithiobenzoate ***12 (CPDB)***



Bis(thiobenzoyl) disulfide (0.8 mmol, 245.2 mg) and AIBN (1.2 mmol, 197.1 mg) were dissolved in 10 ml ethyl acetate. The solution was degassed by five freeze-pump-thaw cycles and stirred under reflux for 18 h. The crude product was dried under vacuum and purified by column chromatography (hexane:ethyl acetate 10:1, R_f = 0.27) to get the product as a red oil. Yield: 56%. %. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.92 (2H, m, *o*-H_{Ar}), 7.56 (1H, m, *p*-H_{Ar}), 7.39 (2H, m, *m*-H_{Ar}), 1.94 (6H, s, CH_3).

Synthesis of Poly[(oligo(ethylene glycol) methyl ether methacrylate)-co-(3-azidopropyl methacrylate)-co-(3-(trimethylsilyl)propargyl methacrylate)] ***(PEATMA)***



Oligo(ethylene glycol) methyl ether methacrylate (Mn=300) (11.52 mmol, 3.46 g), APMA (1.73 mmol, 292 mg, 273 µL) and TMSPMA (1.15 mmol, 226 mg, 243 µL) were dissolved in 2.8 ml dry DMF in a Schlenk tube. 2 mL of a stock solution of AIBN (4.8 mM, 9.6 µmol) and CPDB (24 mM, 48 µmol). The resulting mixture was degassed by five freeze-pump-thaw cycles and stirred at 80°C for 3 h. The product was precipitated in cold hexane:diethyl ether (2:1) as a pink polymer. To remove the CTA-endgroup, the polymer was dissolved in 20 mL DMF, AIBN (152 µmol, 25 mg) was added and the solution degassed by bubbling with N_2. The solution was stirred at 70°C for 18 h. The solvent was removed under reduced pressure. The resulting yellow polymer was purified by dialysis in THF. The highly viscous product was stored in THF at 5°C to prevent auto-crosslinking. Yield: 54%. GPC (THF): M_n = 33.6 kDa, $Đ$ = 1.9. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 4.66-4.55 (CH_2-≡-Si(CH_3)$_3$) 4.25-3.90 (COOCH_2), 3.82-3.49 (OCH$_2$CH_2O), 3.48-3.43 (N$_3$CH_2), 3.39 (OCH_3), 2.07-1.72 (CH_2), 1.12-0.77 (CH_3), 0.20 (Si(CH_3)$_3$). IR (KBr): 2178 cm^{-1} (ν_{alkyne}), 2100 cm^{-1} (ν_{N3}).

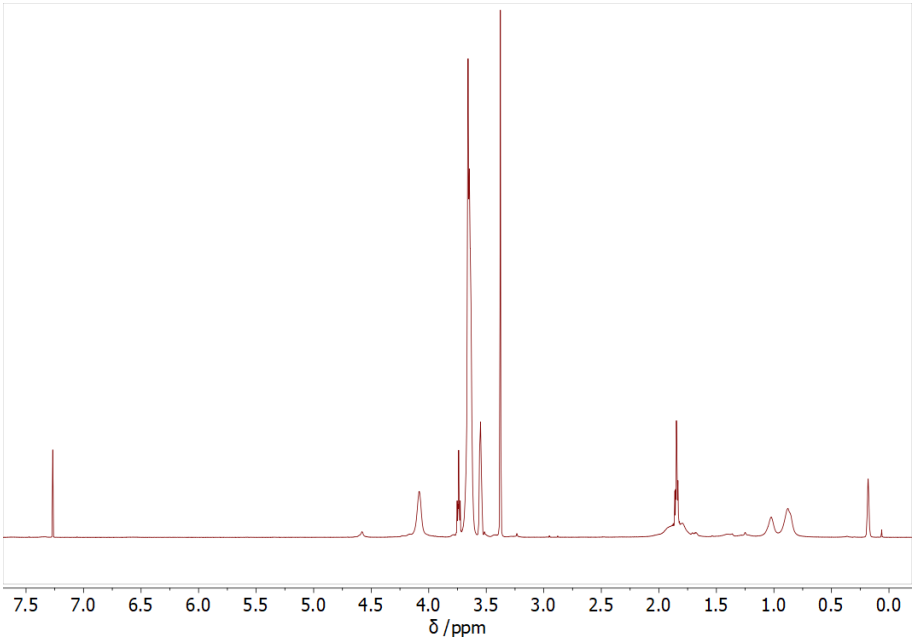
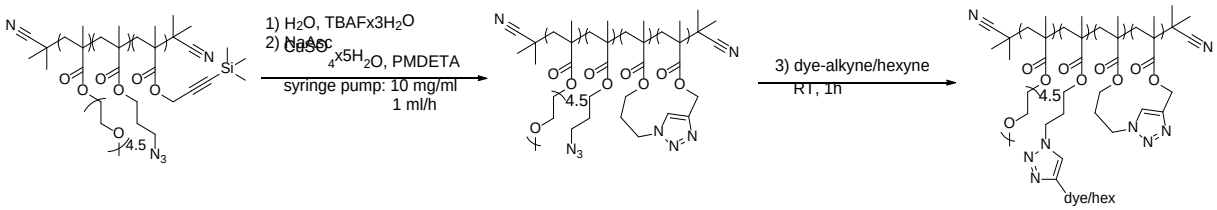


Figure S2. NMR spectrum of the precursor polymer PEATMA in $CDCl_3$ (residual THF signals at 3.74 ppm and 1.85 ppm).

General procedure for the single chain collapse ***(SCNP_[dye]_[dye content per SCNP])***



PEATMA (100 mg, 4.35x10^{-5} mol N_3, 2.90 x 10^{-5} mol alkyne) was dissolved in 9 ml deionized water. A solution of TBAF x $3H_2O$ (1.2 eq. 3.48 x 10^{-5} mol 11 mg) in 1 mL deionized water was added. The solution was put into a syringe pump (0.5 mL/h) and added to a solution of sodium ascorbate (7.5 mmol, 1.5 g), $CuSO_4$ x 5 H_2O (0.5 mmol, 125 mg) and PMDTA (1 mmol, 173 mg, 209 µl) in 100 ml degassed water. After 20 h the solution was stirred for one additional hour. For labelling, a solution of the corresponding, deprotected (for DPP) dye in 5 mL THF followed by a solution of hexyne in 5 mL THF were added, with 30 min stirring after each addition. For exact amounts of dye and hexyne for each synthesis see Table S1 below. For the purification it is important to keep the product wet since the redissolution of dried samples is difficult. The product (and THF) was extracted with DCM. The DCM was reduced under vacuum to get a solution in THF. This solution was passed over a silica column to remove remaining copper impurities. The resulting solution was purified by dialysis going from THF with 1 mL PMDTA to a mixture of water/THF (1:1) and in the end to pure THF. The product was dried under vacuum. Yield: 50%.

Table S1. Amount of the corresponding dyes and hexyne, that were used for the labelling reaction.

Sample name	dye	dye molecules per SCNP	amount of dye (n / 10^{-5} mol; m / mg)	amount of hexyne (n / 10^{-5} mol; m / mg)
SCNP_Hex	-	(hexyne only)	-	1.74; 1.44
SCNP_aBOD_1	aBOD	1	0.29; 1.65	1.74; 1.44
SCNP_aBOD_5	aBOD	5 (aBOD only)	1.74; 9.90	-
SCNP_DPP_1	DPP	1	0.29; 2.60	1.74; 1.44
SCNP_DPP_5	DPP	5 (DPP only)	1.74; 15.60	-

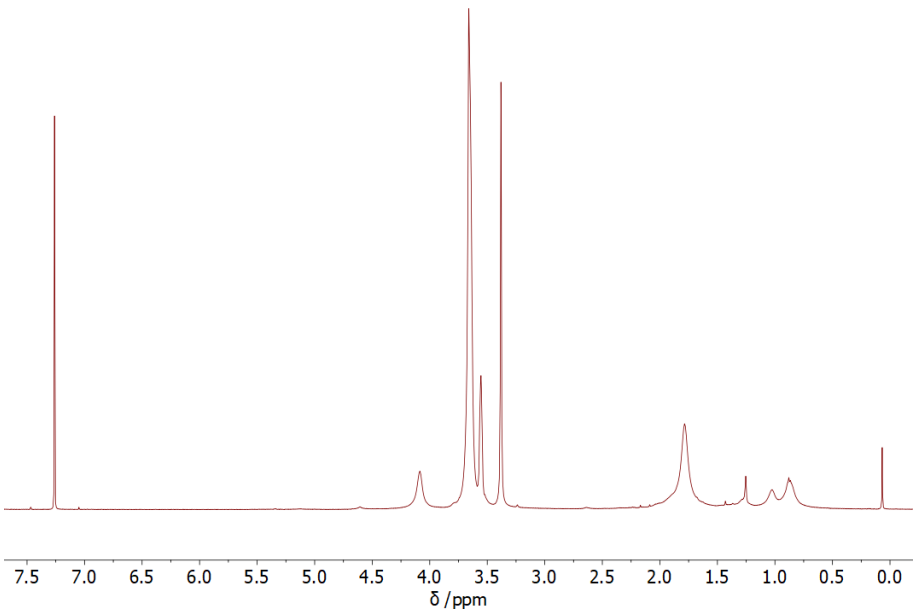


Figure S3. Exemplary NMR spectrum of the SCNP_Hex in $CDCl_3$, showing the complete consumption of the TMS-protected alkyne (removal of TMS at 0.20 ppm).

Dispersing of SCNPs in water

The solid (agglomerated) SCNP were placed in a vial and water was added. The suspension was stirred for 18 h until a milky dispersion was formed. The resulting dispersion was sonicated for several hours until it was clear. It is important for the temperature not to rise higher than 50°C. The SCNPs show LCST-behaviour in water and will not solubilize then.

Solving aza-BODIPY in water ($c = 5x10^{-5}$ M)

aza-BODIPY (5 µmol, 3.03 mg) was dissolved in dry THF (2 ml) and Kolliphor EL (KolEl, 0.2 ml) was added. The resulting solution was sonicated for 30 minutes. The THF was removed under vacuum and the residue was dissolved in 100 ml water to get a dark green solution.

Preparation of mixtures with complex biological media

A dispersion of SCNP_aBOD_1 at a concentration of 1 mg/mL was prepared as described above. 200 µL of this dispersion were added to 1.8 mL water, phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), or DMEM with 10% bovine serum albumin (BSA) to reach concentrations of 0.1 mg/mL. The mixtures were vortexed and equilibrated for 24 h.

Supporting Figures

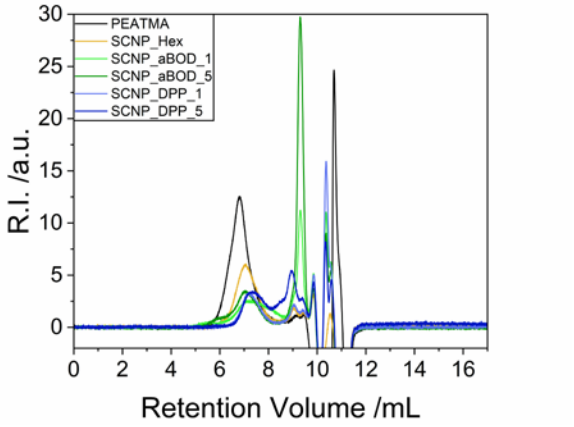


Figure S4. Baseline corrected R.I. traces of THF-based GPC measurements of PEATMA and the SCNPs. System peaks occur starting from ~8.5 mL.

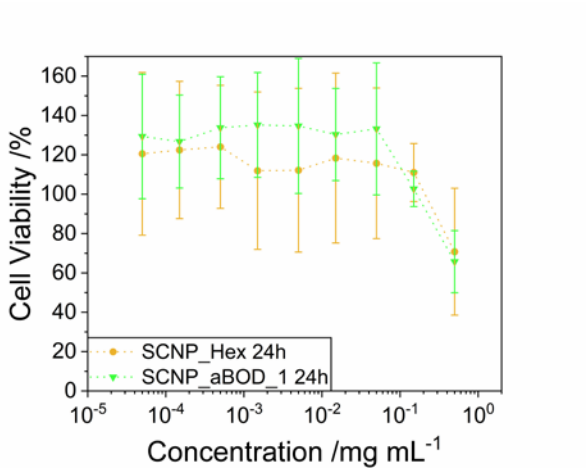


Figure S5. 24h Cell viability assay on NHDF cells of SCNP_Hex and SCNP_aBOD_1.

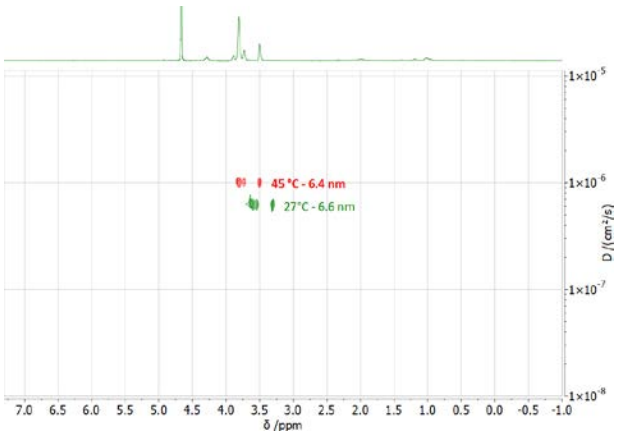


Figure S6. DOSY-NMR spectra of SCNP_Hex in D_2O, measured at 27°C (green) and 45°C (red), showing a reduction in hydrodynamic diameter at high temperatures.

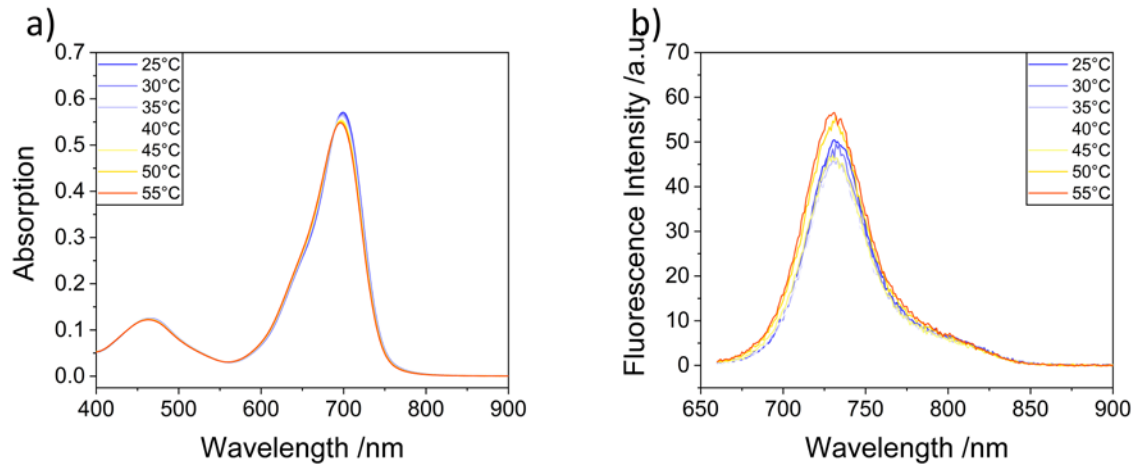


Figure S7. Temperature dependent a) Absorption and b) fluorescence spectra of aBOD in water (KolEI) at concentrations of 1 x 10^{-5} M, λ_{ex} = 650 nm.

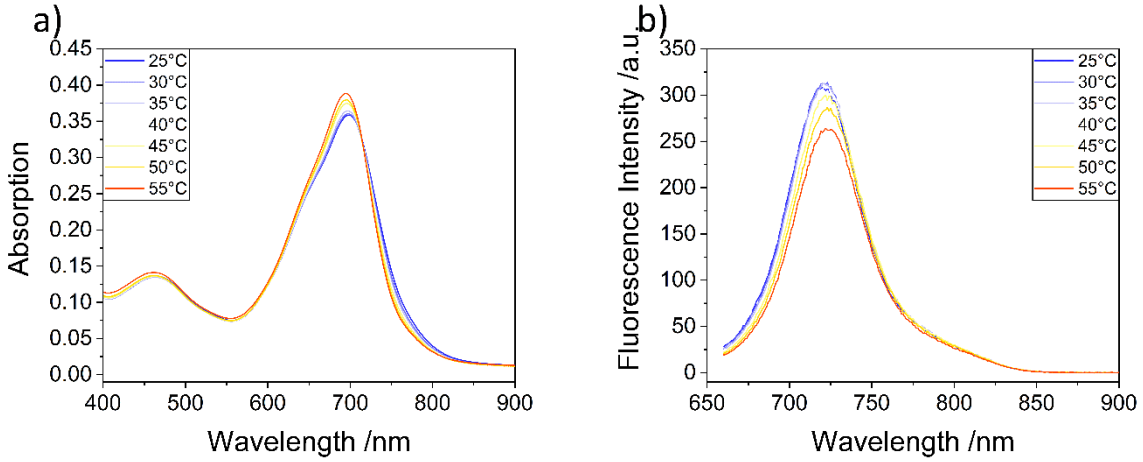


Figure S8. Temperature dependent a) absorption and b) fluorescence spectra of SCNP_aBOD_1 in water at a dye concentration of 5 x 10^{-5} M, λ_{ex} = 650 nm.

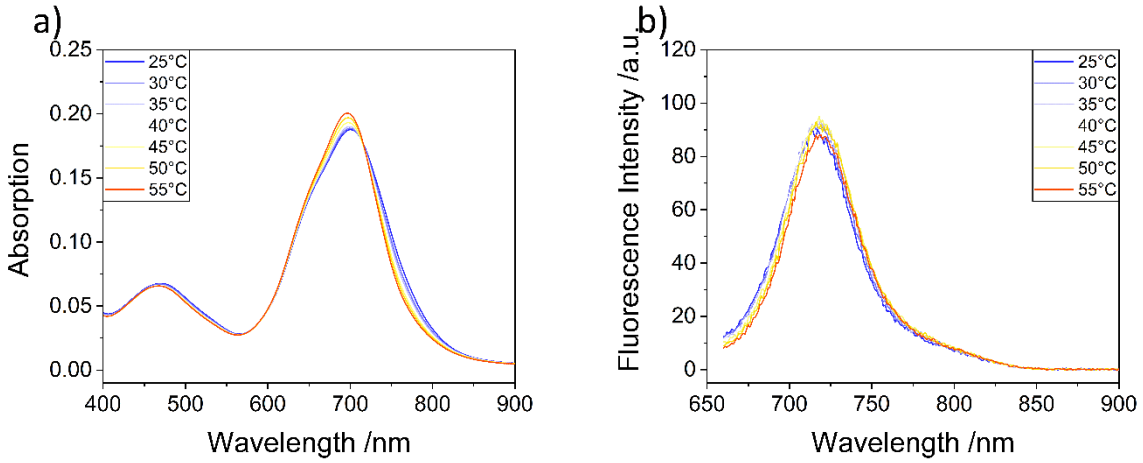


Figure S9. Temperature dependent a) absorption and b) fluorescence spectra of SCNP_aBOD_5 in water at a dye concentration of 5 x 10^{-5} M, λ_{ex} = 650 nm.

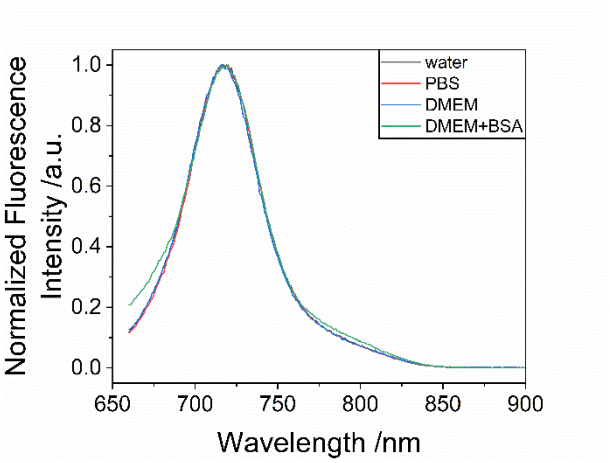


Figure S10. Fluorescence spectra of SCNP_aBOD_1 in water, phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), and DMEM with 10% bovine serum albumin (BSA) at concentrations of 0.1 mg/mL, λ_{ex} = 650 nm. The spectra were normalized due to differences in the pH values of the solutions which are close to the pK_a value of the aBOD labeled SCNPs and trigger major differences in fluorescence intensities.[2]

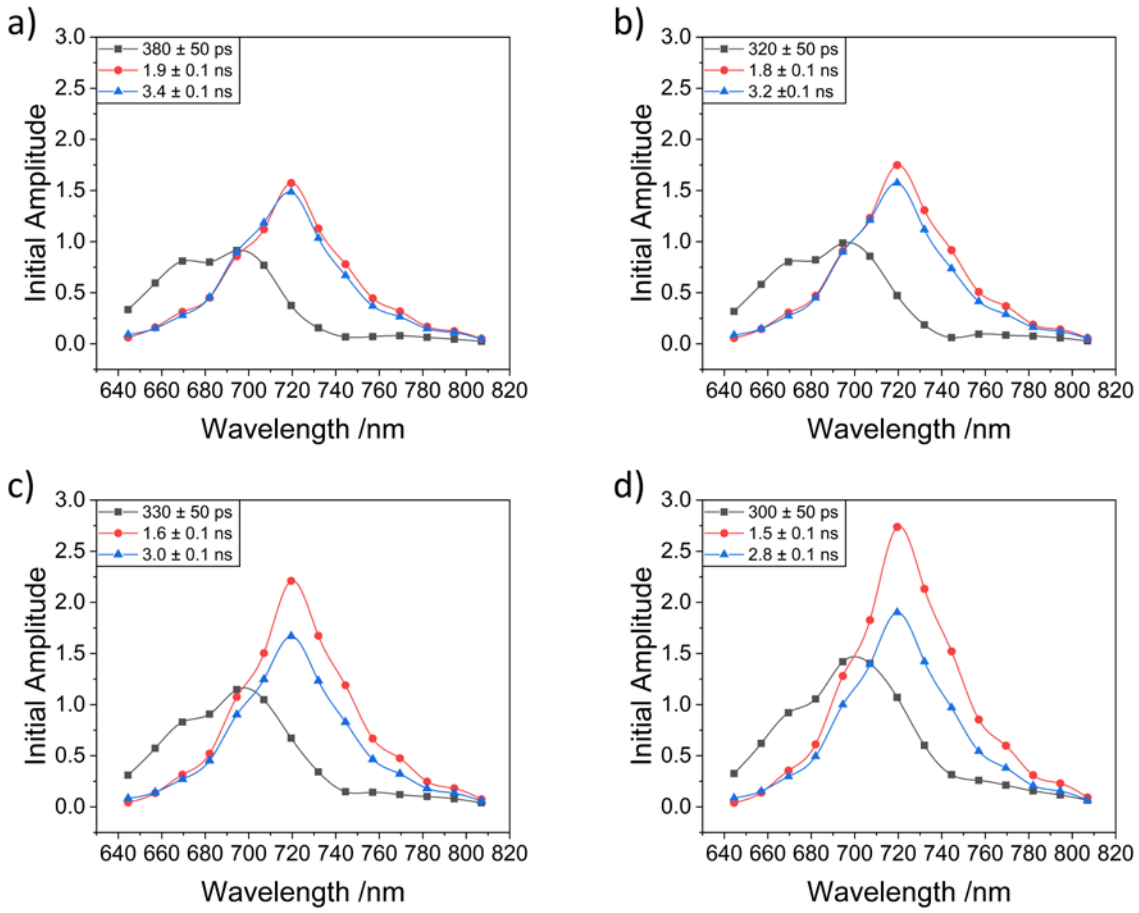


Figure S11. Decay associated spectra of SCNP_aBOD_1 at a) 20 °C, b) 30 °C, c) 40 °C, and d) 50 °C.

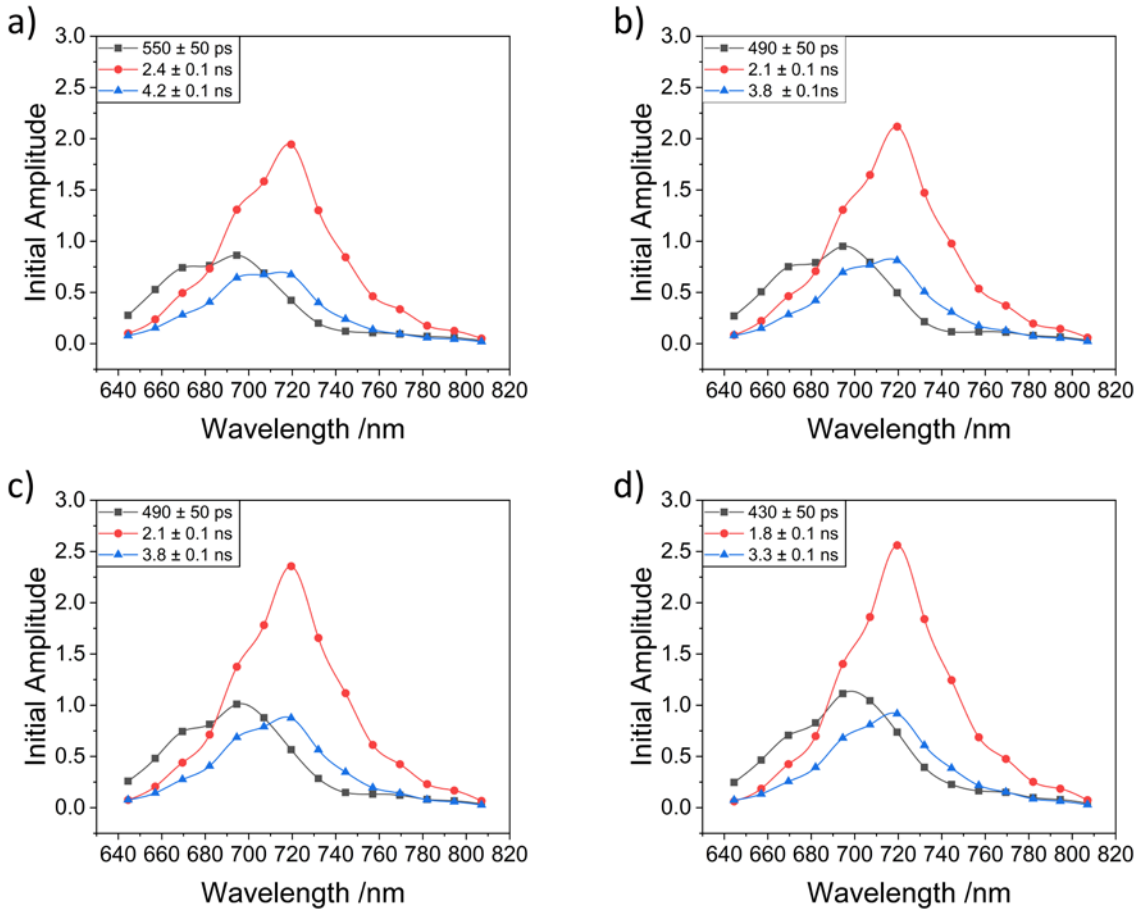


Figure S12. Decay associated spectra of SCNP_aBOD_5 at a) 20 °C, b) 30 °C, c) 40 °C, and d) 50 °C.

1 F. J. Millero, R. Dexter and E. Hoff, *Journal of Chemical Engineering Data*, 1971, **16**, 85-87.

2 J. F. Hoffmann, A. H. Roos, F. J. Schmitt, D. Hinderberger and W. H. Binder, *Angew. Chem. Int. Ed.*, 2021, **60**, 7820-7827.