Electronic Supplementary Information (ESI)

Controlling and tuning the dynamic nature of supramolecular polymers in aqueous solutions

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Instrumentation

Characterization of prepared compounds and materials were performed by NMR spectroscopy using a 400 MHz NMR spectrometer at 298 K, with the chemical shifts being reported in ppm downfield from tetramethylsilane (TMS), and using the following abbreviations for splitting patterns in ^{1}H NMR: s = singlet, t = triplet, q = quartet, m = multiplet or multiple signals, dd = double doublet. Reversed phase high performance liquid chromatography–mass spectrometry (RP-HPLC-MS) was performed using a Shimadzu LC-10 AD VP series HPLC coupled to a diode array detector (Finnigan Surveyor PDA Plus detector, Thermo Electron Corporation) and an ion-trap (LCQ Fleet, Thermo Scientific), and applying an Alltech Alltima HP C18 3µ column using an injection volume of 1-4 µL, a flow rate of 0.2 mL/min and a water to acetonitrile gradient with eluents containing 0.1% formic acid. HRMS (high resolution mass spectrometry) was performed on a Waters Acquity UPLC system equipped with a Xevo G2 Qtof detector, and applying Zspray lockspray ionisation. Preparative reversed phase high performance liquid chromatography (prep-RP-HPLC) was performed on a system consisting of the following components: Shimadzu LC-8A preparative liquid chromatography pumps (with an Phenomenex Jupiter C18 5u (125 x 20 mm) preparative reversed phase column and gradients of water-acetonitrile, supplemented with 0.1% trifluoroacetic acid), a Shimadzu CBM-20A prominence communications bus module and Shimadzu DGU 20A3 prominence degasser, Thermo Finnigan Surveyor PDA detector, Finigan LCQ Deca XP and Thermo Finnigan surveyor auto sampler.

For cryogenic transmission electron microscopy, vitrified films were prepared in a 'Vitrobot' instrument (PC controlled vitrification robot, patent applied, Frederik et al 2002, patent licensed to FEI) at 22°C and at a relative humidity of 100%. In the preparation chamber of the 'Vitrobot', a 3µl sample was applied on a Quantifoil grid (R 2/2, Quantifoil Micro Tools GmbH), which was surface plasma treated just prior to use (Cressington 208 carbon coater operating at 5 mA for 40 s). Excess sample was removed by blotting using filter paper for 3 s at –3 mm, and the thin film thus formed was plunged (acceleration about 3 g) into liquid ethane just above its freezing point. The vitrified film was transferred to a cryoholder (Gatan 626) and observed at temperatures below -170 °C in a Tecnai Sphera microscope operating at 200 kV. Micrographs were taken at low dose conditions, using a defocus setting of 10 µm at 25000 magnification (detail pictures), or defocus setting of 40 µm at 6500 magnification (overview pictures).

STORM and TIRF images were acquired with a Nikon N-STORM system. Cy3 and Cy5 were excited using a 561 and 647 nm laser respectively. Fluorescence was collected by means of a Nikon 100x, 1.4NA oil immersion objective and passed through a quad-band pass dichroic filter (97335 Nikon). Images were recorded with an EMCCD camera (ixon3, Andor, pixel size 0.17µm). The movies were subsequently analyzed with the STORM module of the NIS element Nikon software

Fluorescence emission scans were recorded on a Varian Fluorescence spectrophotometer with an excitation at 540 nm. Emission scans were recorded from 550 to 750 nm, the excitation and emission slits were fixed at 5 nm, and the scan rate used was 600 nm/min. PMT detector voltage was set to 1000V and temperature was fixed at 20 °C.

Materials

All compounds were used as received, unless stated otherwise. Dichloromethane, Heptane, N,N-Dimethylformamide, Tetrahydrofuran, and HPLC pure Trifluoroacetic acid were purchased from Biosolve. Copper sulfate, sodium ascorbate and PBS tablets were obtained from Sigma. Triethylamine was purchased from Acros and Acetonitrile from Actu-All Chemicals and Biosolve. Water was purified on an EMD Milipore Mili-Q Integral Water Purification System. Cyanine3-NHS, Cyanine3

alkyne, Cyanine5-NHS and Cyanine5 alkyne were purchased from lumiprobe. Bim(py)2 was synthesized as previously reported in [1].

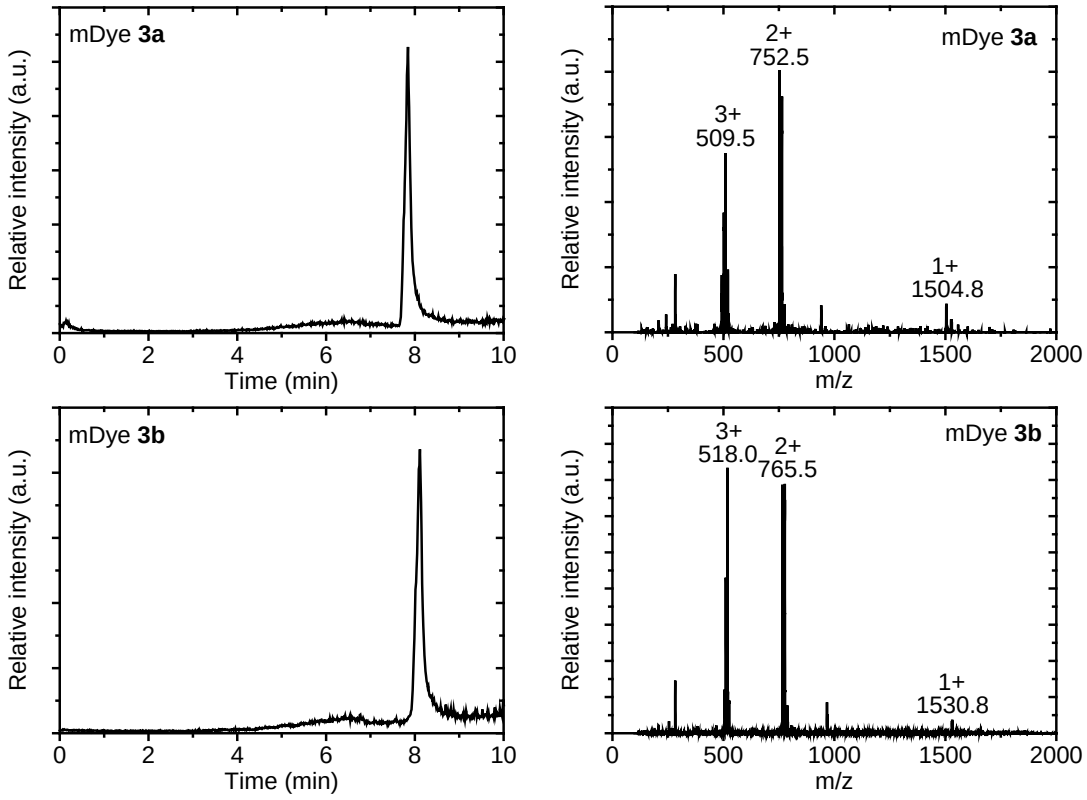
Synthetic procedures

*Synthesis of the UPy scaffolds mUPy **1** and bUPy **2**.* The UPy scaffold molecules, monovalent UPy **1** and bivalent UPy **2** were synthesized as previously described in [2] and [3].

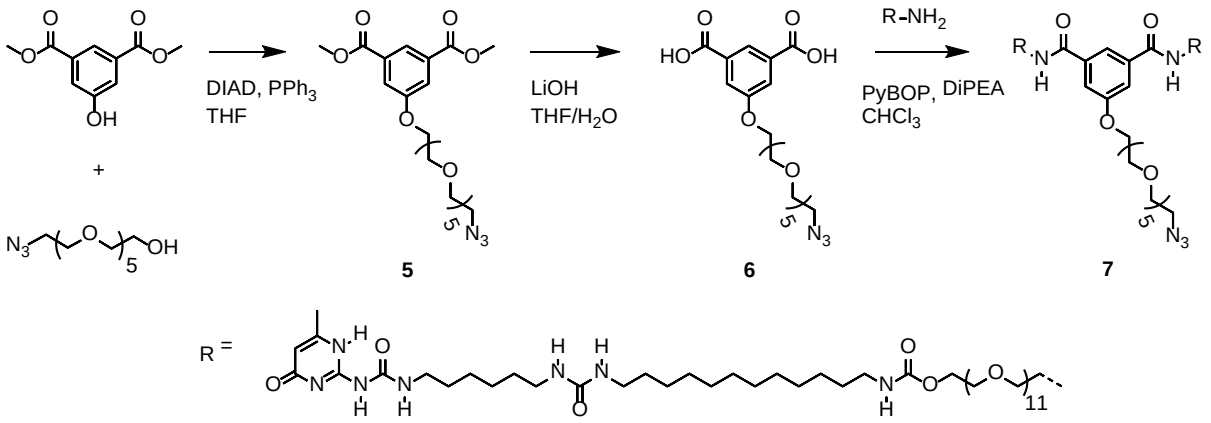
*Synthesis of monovalent UPy-dyes mDye **3a** and mDye **3b***

UPy-NH_2 was synthesized as previously reported [4]. UPy-NH_2 (6.1 µmole), Cy3-NHS or Cy5-NHS (8.5 or 8.1 µmole, 1.4 eq) and trimethylamine (24.0 µmole, 4 eq) were dissolved in DCM and agitated overnight at room temperature. The reaction mixtures were precipitated in heptane, and after centrifugation (10 min at 2k RPM) the heptane layer was removed. The pellet was dissolved in DCM again and precipitated to remove all remaining trimethylamine. Remaining free Cy3 and Cy5 were removed by purification with a preparative RP-HPLC using a gradient of 64-71% or 68% acetonitrile (ACN) in water resulting in ultra-pure mDye **3a** (53.8% yield, 4.9 mg) and mDye **3b** respectively (39.3% yield, 3.5 mg). LC-MS: mDye **3a**; Mw_{calc} = 1503.97 g/mol, m/z_{obs} = 1504.8 $[M+H]^+$, 752.5 $[M+2H]^{2+}$, 509.5 $[M+2H+Na]^{3+}$. mDye **3b**; Mw_{calc} = 1531.02 g/mol, m/z_{obs} = 1530.8 $[M+H]^+$, 765.5 $[M+2H]^{2+}$, 518.0 $[M+2H+Na]^{3+}$.

*LCMS spectra of the monovalent UPy dyes **3a** and **3b**. Left: Total ion current (TIC) chromatogram of analytical reversed phase-high performance liquid chromatography (RP-HPLC) with a gradient of 5-100% acetonitrile over a course of 10 minutes, Right: Electrospray ionization-mass spectrometry (ESI-MS) m/z spectrum.*



*Synthesis of bivalent UPy-dyes bDye **4a** and bDye **4b***
Synthesis of UPy-PEG$_{11}$-N$_3$-PEG$_{11}$-UPy



*5-dPEG6-azide-dimethylisophthalate (**5**)*

Compound 5 was synthesized as described in [5] using mono-azide hexaethylene glycol as prepared by [6]. Briefly, to a cooled (0 °C) solution of 5-hydroxy-dimethylisophthalate (163 mg; 0.78 mmol) and the mono-azide hexaethylene glycol (0.29 g; 0.93 mmol, 1.2 eq.) in tetrahydrofuran (THF; 1.5 mL) was added triphenylphosphine (PPh_3; 214 mg; 0.814 mmol, 1.05 eq.) and, finally, di-isopropyl-aza-dicarboxylate (DIAD; 204 mg; 197 µL; 1 mmol, 1.3 eq.). After stirring at room temperature for 16 hours some extra PPh_3 (62 mg; 0.23 mmol, 0.3 eq.) was added and the mixture was stirred at room temperature for another 24 hours. The solvent was removed *in vacuo* and the remaining viscous yellow oil was purified by column chromatography using dichloromethane/ethyl acetate (DCM/EtOAc) 4:1 (v/v) to remove impurities and DCM/1,2-dimethoxyethane/methanol 78:20:2 (v/v/v) to collect and isolate **5** as a waxy solid (225 mg; 58%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.77 (s, 2H), 4.22 (m, 2H), 3.94 (s, 6H), 3.89 (t, *J* = 4.0 Hz, 2H), 3.75 (m, 2H), 3.72 – 3.60 (m, 16H), 3.39 (t, *J* = 5.1 Hz, 2H). LC-MS: a single dominant peak is observed, indicating that the purity of the material is >95%. Mw_{calc} ($C_{22}H_{33}N_3O_{10}$) = 499.51 g/mol, m/z_{obs} = 500.0 $[M+H]^+$.

*5-PEG6-azide isophthalic acid (**6**)*

The dimethylisophthalate **5** (225 mg; 0.45 mmol) was dissolved in a 1:1 mixture of THF and water (5 mL). Lithium hydroxide (76 mg; 1.8 mmol) was added and the solution was stirred at room temperature for 16 hours. The reaction mixture was diluted with water (ca. 25 mL), was transferred to an extraction funnel and was extracted with $CHCl_3$ (3 x 10 mL). The water layer was then acidified with a 1M HCl-solution, and was extracted with $CHCl_3$ (3 x 10 mL), now to collect the product. The combined $CHCl_3$ layers from the second (acidic) extraction were dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The product was dried using a vacuum oil pump, isolating **6** as a waxy solid (210 mg; 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 2H), 8.09 (s, 1H), 7.58 (s, 2H), 4.16 (broad t, 2H), 3.91 (t, *J* = 4.4 Hz, 2H), 3.84 – 3.62 (m, 18H), 3.40 (t, *J* = 5.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 158.5, 130.9, 123.8, 120.3, 70.6-70.4 (multiple peaks), 70.0, 69.5, 67.8, 50.6. LC-MS: a single dominant peak is observed, indicating that the purity of the material is >95%. Mw_{calc} ($C_{20}H_{29}N_3O_{10}$) = 471.46 g/mol, m/z_{obs} = 472.1 $[M+H]^+$.

*Bis-1,3-(UPy-C6-Urea-C12-Ureth-dPEG12)-5-dPEG6-azide-isophthalamide (**7**)*

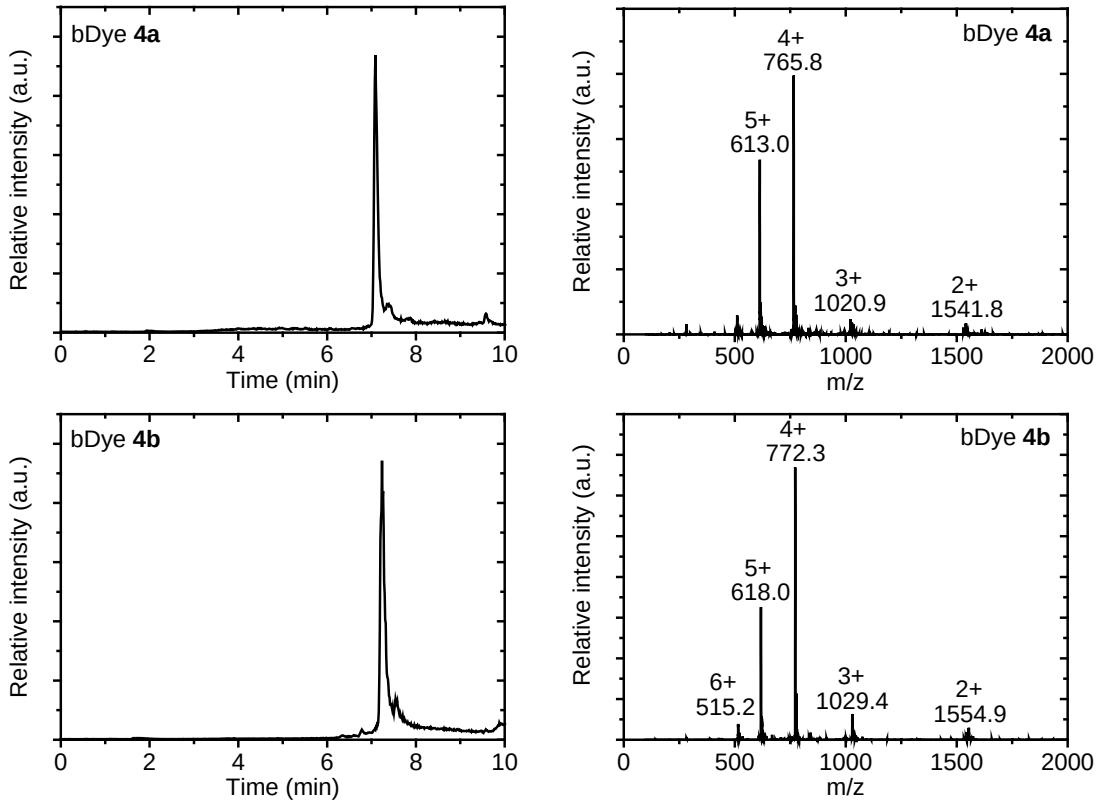
The HCl salt of the UPy-C6-Urea-C12-Ureth-dPEG12-NH_2 building block (50 mg; 45.4 µmol, 2.2 eq.) and **6** (9.7 mg; 20.6 µmol) were dissolved in chloroform (1 mL) and N,N-Diisopropylethylamine (18 µL; 13.3 mg, 0.103 mmol, 5 eq.). Slight heating on a warm water bath was required to dissolve all components. Finally, PyBOP (24 mg; 45.4 µmol, 2.2 eq.) was added. After stirring of the reaction mixture for 2 hours at room temperature, HPLC-MS analysis confirmed that the reaction had been completed. The solvent was removed *in vacuo,* and the remaining solid was subjected to reversed-phase column chromatography. The crude product was dissolved in ACN (1 mL), H_2O (0.5 mL) and a droplet of TFA, and this solution was brought onto a 30 g Biotage SNAP cartridge KP-C18-HS. A gradient of ACN/H_2O mixtures with 0.1% formic acid was used as eluent. Product **7** was isolated as an off-white solid (44 mg; 83%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.16 (bs, 2H), 11.82 (bs, 2H), 10.08 (bs, 2H), 7.96 (s, 1H), 7.65 (bs, 2H), 7.60 (s, 2H), 5.82 (s, 2H), 5.1 (bs, 4H), 5.0 (bs, 2H), 4.20 (m, 6H), 3.86 (t, *J* = 4.7 Hz, 2H), 3.64 (m, 106H), 3.38 (t, *J* = 5.1 Hz, 2H), 3.23 (q, *J* = 6.7 Hz, 4H), 3.14 (m, 16H), 2.24 (s, 6H), 1.65 – 1.54 (m, 4H), 1.46 (m, 8H), 1.40 – 1.32 (m, 8H), 1.25 (m, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 166.6, 159.1, 158.8, 156.4, 154.7, 148.5, 135.9, 117.5, 116.9, 106.5, 70.8-69.6 (multiple peaks), 67.9, 63.7, 50.7, 46.3, 46.2, 41.0, 40.4, 40.0, 39.6, 30.4, 29.9-29.2 (multiple peaks), 26.9, 26.7, 26.5, 26.4, 26.3, 26.2, 19.0. In HPLC-PDA/MS a single dominant peak is observed, indicating that the purity of the material is >95%. HRMS: for molecular formula $C_{120}H_{217}N_{19}O_{40}$ $[M+2H]^{2+}$, $[M+3H]^{3+}$ and $[M+4H]^{4+}$ are calculated at 1283.2843, 855.8588 and 642.1461, and are recorded at 1283.2827 (1.2 ppm), 855.8586 (0.2 ppm) and 642.1458 (0.5 ppm), respectively, with the deviations from the calculated data given in brackets.

*bDye **4a** and bDye **4b***

UPy-N_3-UPy (5.7 or 5.4 µmole, 1.2 eq), Cy3-alkyne or Cy5-alkyne (4.7 or 4.5 µmole, 1.2 eq), copper sulfate (5 mM), $Bim(py)_2$ (4mM) and sodium ascorbate (26 mM) were dissolved in a mixture of DMF and water (1:1) and stirred overnight at room temperature. The reaction mixture was purified on a reversed phase C18 column starting with a gradient of 5-100% acetonitrile in water, and subsequently changed to acetonitrile:THF:water (7:2:1) yielding pure bDye **4a** (25.6% yield, 3.7 mg) and bDye **4b** (52.6% yield, 7.3 mg). LC-MS: bDye **4a**; Mw_{calc} = 3060.85 g/mol, m/z_{obs} = 1541.8 $[M+H+Na]^{2+}$, 1020.9 $[M+3H]^{3+}$, 765.8 $[M+4H]^{4+}$, 613.0 $[M+5H]^{5+}$. bDye **4b**; Mw_{calc} = 3086.89 g/mol, m/z_{obs} = 1554.9 $[M+H+Na]^{2+}$, 1029.4 $[M+3H]^{3+}$, 772.3 $[M+4H]^{4+}$, 618.0 $[M+5H]^{5+}$, 515.2 $[M+6H]^{6+}$

*LCMS spectra of the bivalent UPy dyes **4a** and **4b**. Left: Total ion current (TIC) chromatogram of analytical reversed phase-high performance liquid chromatography (RP-HPLC) with a gradient of 5-100% acetonitrile over a course of 10 minutes, Right: Electrospray ionization-mass spectrometry (ESI-MS) m/z spectrum.*



Methods

Preparation of 2% UPy-Cy3 and 2% UPy-Cy5 stocks. A stock solution of 25 µM UPy-OMe was prepared in MilliQ water, vortexed, stirred for 1.5 hours at 72 °C with occasionally vortexing until a clear solution was obtained. Subsequently, stocks of 50 µM UPy-Cy3 and UPy-Cy5 were prepared in acetonitrile, followed by the injection of 10 µl of the dye stock in 990 µl of the UPy-OMe stock. The mixture was vortexed and annealed for 15 minutes at 45 °C to allow incorporation into the stacks.

Preparation of 2% UPy-Cy3-UPy and 2% UPy-Cy5-UPy stocks. Same as the preparation of 2% UPy-Cy3 and UPy-Cy5 stocks, however, 50 µM stocks of the dyes were diluted in THF:Acetonitrile:water (5:4:1) instead of acetonitrile.

Fluorescence measurements. Emission scans were performed using a 25 µM concentration with an excitation at 540 nm and an emission at 550 nm. Upon mixing the UPy-Cy3 and UPy-Cy5 stocks, emission scans were performed every minute in the first 10 minutes and subsequently every 10 or 15 minutes. The FRET ratio of every emission scan was determined by dividing the maximum fluorescence at around 660 nm by the maximum fluorescence at around 570 nm.

STORM measurements. Stock solutions were diluted from 25 to 12.5 µM in PBS and flown in a chamber between glass microscope coverslips (Menzel-Gläser, No. 1, 24x24 mm) and glass slides which were separated by double-sided tape. The samples were annealed for 2.5 minutes and subsequently washed with PBS (twice) and STORM buffer (twice). STORM buffer contained 50 mM Tris pH 7, an oxygen scavenging system (0.5 mg/mL glucose oxidase, 40 µg/mL catalase), 10% (w/v) glucose and 10 mM 2-aminoethanethiol.

Supporting data

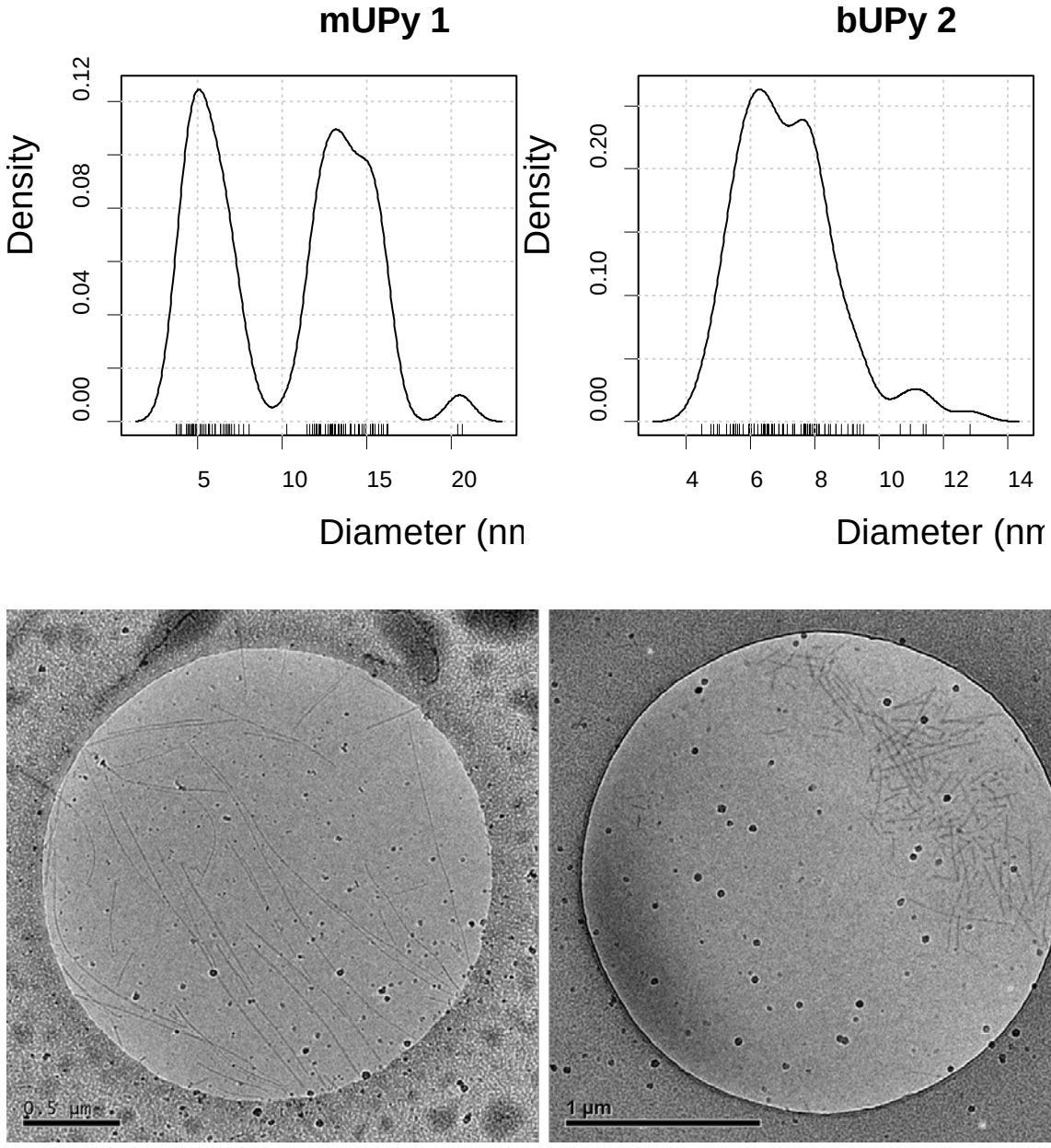


Fig. S1 Additional Cryo-TEM information to Fig. 1. Top: Density plots of the diameter manually measured on 100 random spots in the detailed cryo-TEM images using the measuring tool in ImageJ. Density plots were generated with Rcmdr using a gaussian function and a bandwidth of 1.0. mUPy **1** (left) has a biphasic diameter distribution with presumably single dimers with a diameter of approximately 5 nm, and bundled dimers with a diameter of approximately 14 nm. bUPy **2** displays a mean diameter of 7.15±1.54 nm (standard deviation indicated as error) having a slightly wider diameter as compared to the single dimers of mUPy **1**. Bottom: overview cryo-TEM images of mUPy **1** (left) and bUPy **2** (right) at lower magnification. Long fibrillar structures were observed for mUPy **1** (*c* = 482 µM) and much shorter fibrillar structures for bUPy **2** (*c* = 474 µM). The dark circular objects in the left image are non-vitrified water. Scale bars represent 0.5 µm (left) and 1 µm (right).

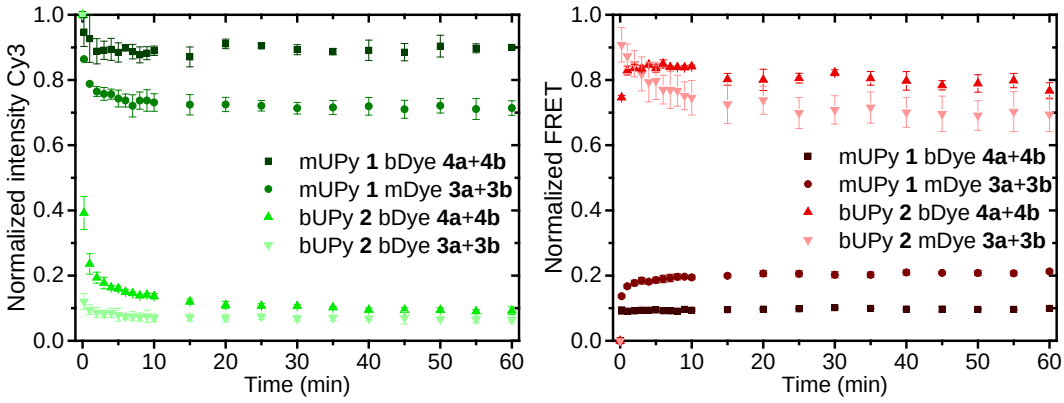


Fig. S2 Normalized plots of emission scans recorded after mixing mDye **3a** and **3b** or **4a** and **4b** incorporated in mUPy **1** or bUPy **2** (c_{Total}=50 µM, excitation at 540 nm, *N*=3, additional data to Fig. 3). Normalized plots were obtained by using the maximum of reference **3a** or **4a** (Cy3, set to 1) and the maximum of reference **3b** or **4b** (FRET, set to 0). The maximal Cy3 intensity was taken at approximately 560 nm, whereas the maximum FRET at approximately 660 nm.

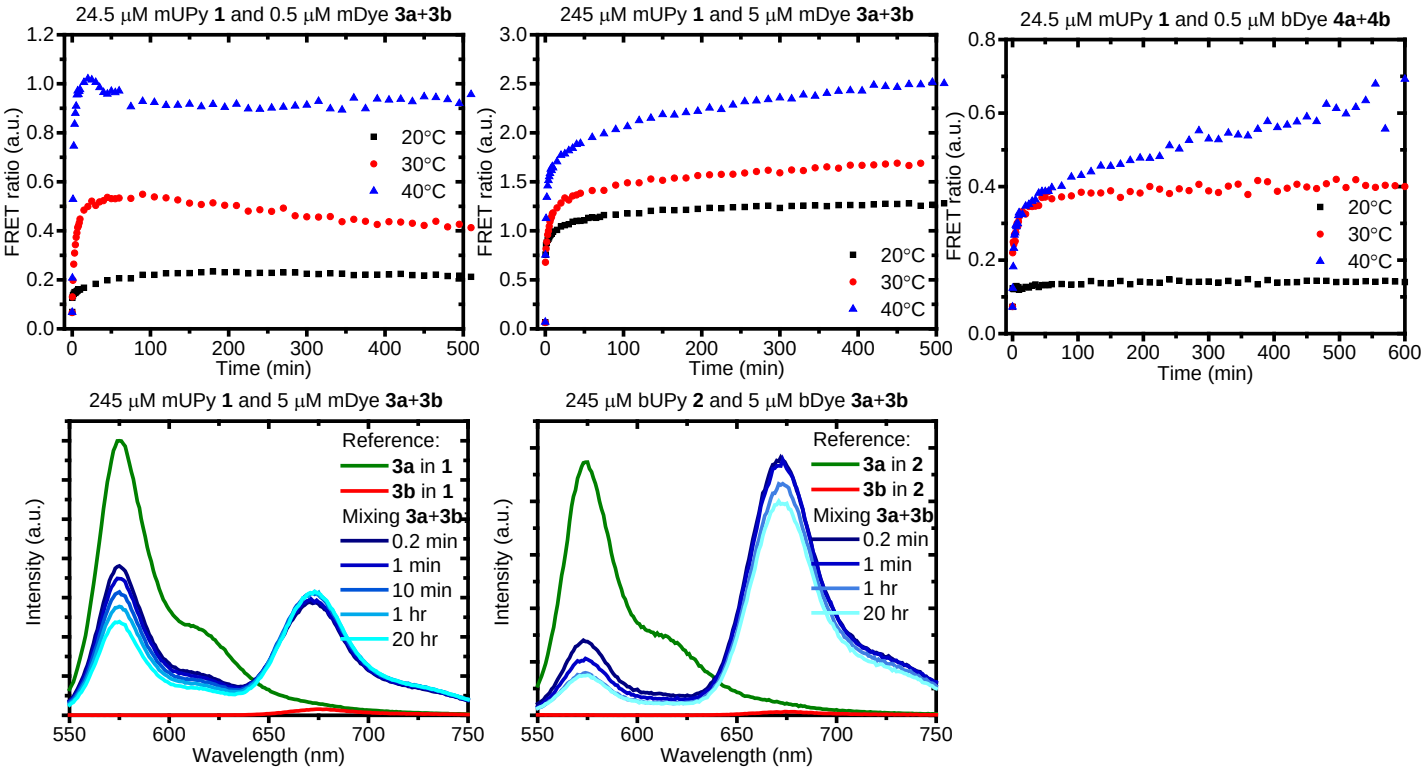


Fig. S3 Fluorescence experiments on elevated temperature and a 10-fold higher concentration upon excitation at 540 nm. Top row: temperature studies of monovalent scaffold **1** with mDyes **3a** and **3b** or bDyes **4a** and **4b** incorporated. Upon increasing temperature, the exchange was accelerated. Bottom row: concentrated samples of mUPy **1** or bUPy **2** with mDyes **3a** and **3b**. At a 10 fold higher concentration, the exchange was slightly accelerated. FRET ratio was obtained by dividing the maximum fluorescence of the acceptor (around 660 nm) by the maximum fluorescence of the donor (around 560 nm).

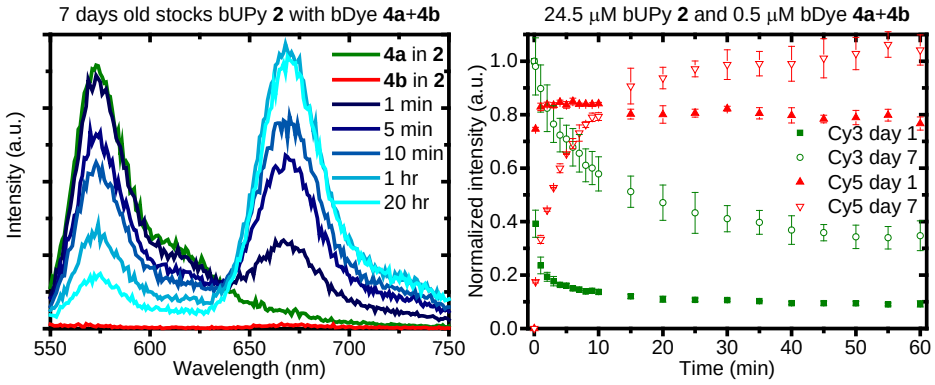


Fig. S4 FRET aging experiment with bivalent scaffold **2** and bivalent dyes **4a** and **4b** (*c* = 24.5 μM bUPy **2** and 0.5 μM bDyes **4** in MilliQ water containing 1% acetonitrile, excitation at 540 nm, *N*=2). Upon aging the stock solutions for 7 days and subsequently mixing, the exchange rate was significantly decreased, probably due to a stronger packing of the monomers within the fibres. FRET ratio was obtained by dividing the maximum fluorescence of the acceptor (around 660 nm) by the maximum fluorescence of the donor (around 560 nm).

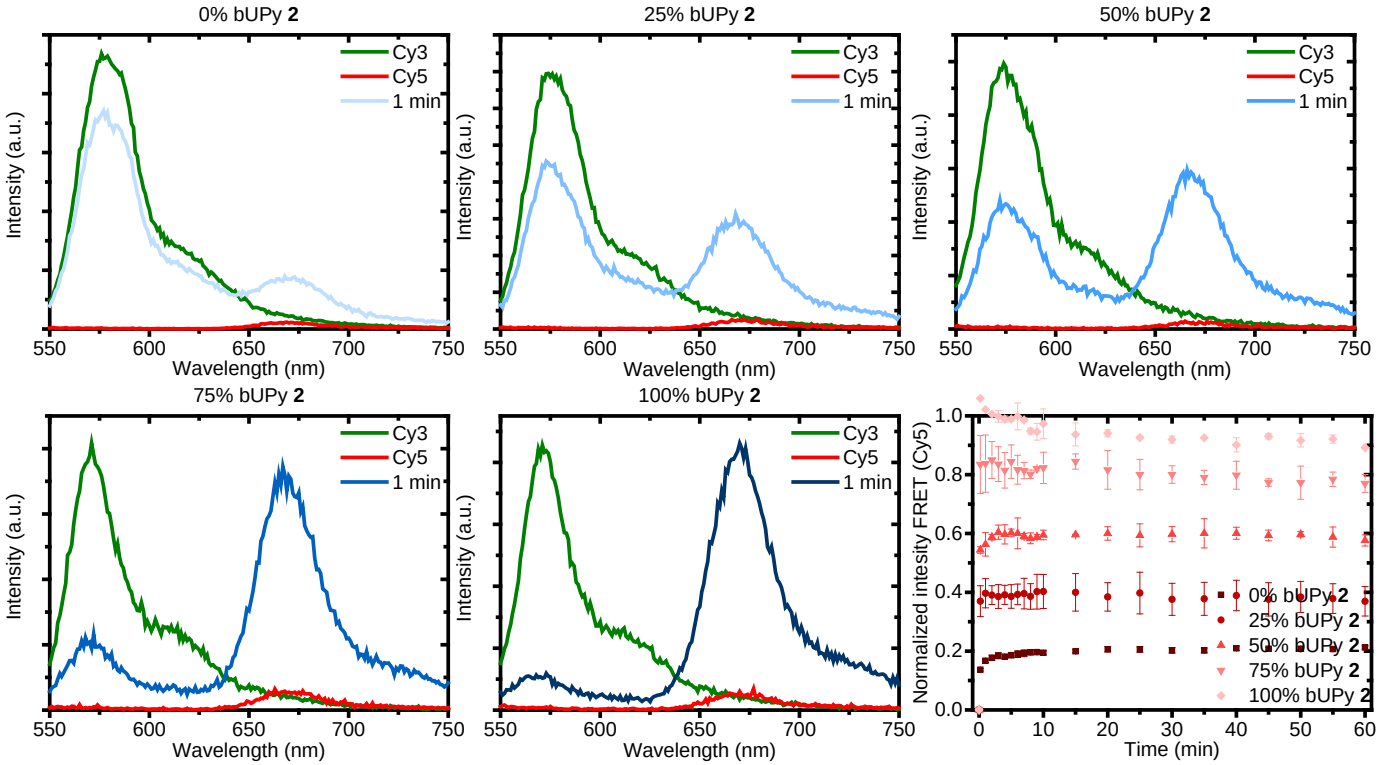


Fig. S5 Co-assembly of bivalent scaffold **2** with respect to monovalent scaffold **1** with 2 mol% of mDyes **3a** and **3b** incorporated upon excitation at 540 nm. From top to bottom, from left to right: 24.5 μM **1** and 0.5 μM **3** (0%); 18.375 μM **1**, 6.125 μM **2** and 0.5 μM **3** (25%); 12.25 μM **1**, 12.25 μM **2** and 0.5 μM **3** (50%); 6.125 μM **1**, 18.375 μM **2** and 0.5 μM **3** (75%); and 24.5 μM **2** and 0.5 μM **3** (100%); Normalized FRET intensity of the different samples (*N*=2). Upon increasing content of bivalent scaffold **2**, the exchange rate was accelerated, indicated by an increased peak at around 660 nm. As a reference, Cy3 and Cy5 (i.e. **3a** and **3b** in designated fibre composition respectively) were measured.

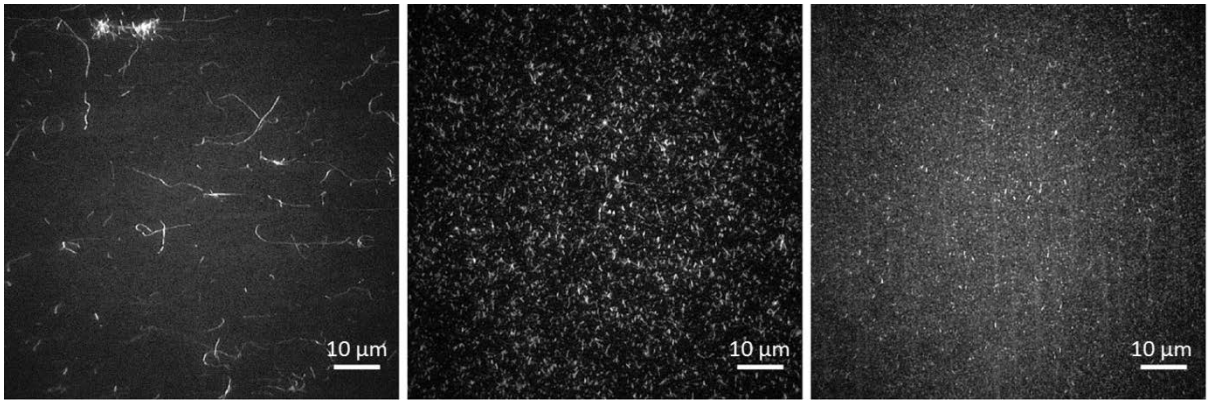


Fig. S6 TIRF images of 12.5 µM solutions of mUPy **1** with 2 mol% mDyes **3a** and **3b** (left), a 1 to 1 mixture of mUPy **1** and bUPy **2** with 2 mol% of bDyes **4a** and **4b** (middle) and bUPy **2** with 2 mol% of mDye **3a** and **3b** (right). Micrometre long fibres were observed for monovalent UPy **1** (left) and smaller, less-defined aggregates for bivalent scaffold **2** (right). After co-assembling mUPy **1** with bUPy **2**, fibre-like structures were observed which were shorter in length than those observed for mUPy **1**, but longer than bUPy **2**. This indicates that both monomers influence the microscopic structures they form and that probably no self-sorting takes place.

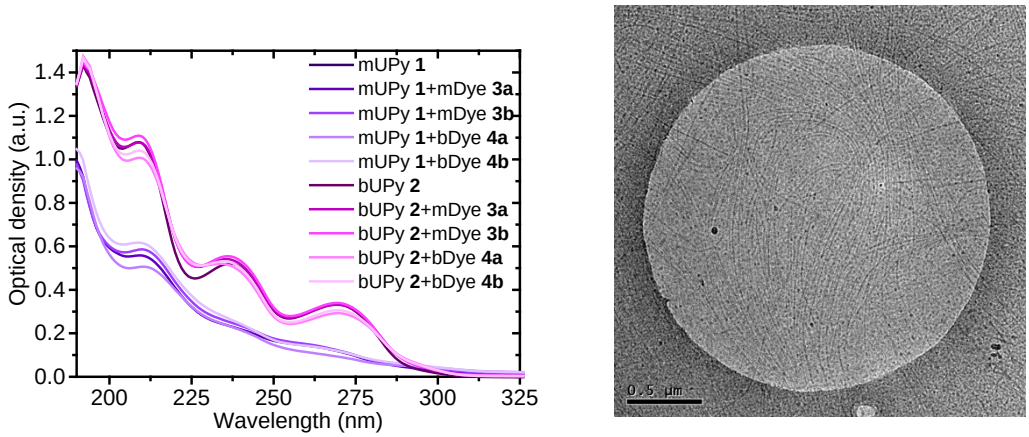


Fig. S7 Investigating the influence of the incorporated dye on the assembly and morphology as studied by UV-VIS and Cryo-TEM. Left: UV-VIS spectra of the multiple samples used with typical absorption bands. Right: Cryo-TEM of 2 mol% mDye **3a** incorporated in monovalent scaffold **1** (*c*=500 µM). The dark circular objects are crystalline ice particles. The low percentage of incorporated dyes (2 mol%) did not influence the stacking behaviour, indicated by similar absorption curves and similar fibrillar structures.

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