Electronic Supplementary Material (ESI) for Chemical Science

Dynamic and bio-orthogonal protein assembly along a supramolecular polymer

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Supporting Information

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General

O-Benzotriazolyl-*N,N,N′,N′*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and *N,N´*-diisopropylethylamine (DIPEA) and trifluoroacetic acid (TFA) were purchased from Biosolve (www.biosolve.nl). Dry *N,N´*-dimethylacetamide (DMA), dry *N,N´*-dimethylformamide (DMF), dry triethylamine (TEA), isopropanol and lithium hydroxide monohydrate were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Oxalyl chloride, 1-chloro-*N,N*,2-trimethyl-1-propenylamine, and magnesium sulfate were purchased from Acros (www.acros.be). Deuterated solvents were bought from Cambridge Isotope Laboratories (www.isotope.com).

All solvents and chemicals were used as received. Water was demineralized prior to use. Chloroform, tetrahydrofuran (THF), and dichloromethane (DCM) (HPLC grade) were degassed with argon and purified by passage through activated alumina solvent column prior to use. Analytical thin layer chromatography (TLC) was carried out using Merck pre-coated silica gel plates (60F-254) using ultraviolet light irradiation at 254 or 365 nm. Manual column chromatography was carried out using Merck aluminium oxide (90 active basic, 0.063-0.2 mm). Preparative recycling GPC was performed using a Shimadzu system equipped with a Shimadzu LC-10ADvp pump, a Jai-Gel 2.5 H and a Jai-Gel 2 H column in series and a Shimadzu SPD-10AVvp UV/Vis detection system performing detection at 275 nm and 325 nm. HPLC grade chloroform was used as the eluent (with a flow of 3.5 ml/min and manual injection was performed with a volume of 2 ml. One cycle through the system took 1 h.) Manual size-exclusion chromatography was performed on BIO RAD BioBeads S-X1 (200-400 mesh) in a long glass column (1.2 m) at atmospheric pressure and a flow rate less than 1 mL/min in DMF.

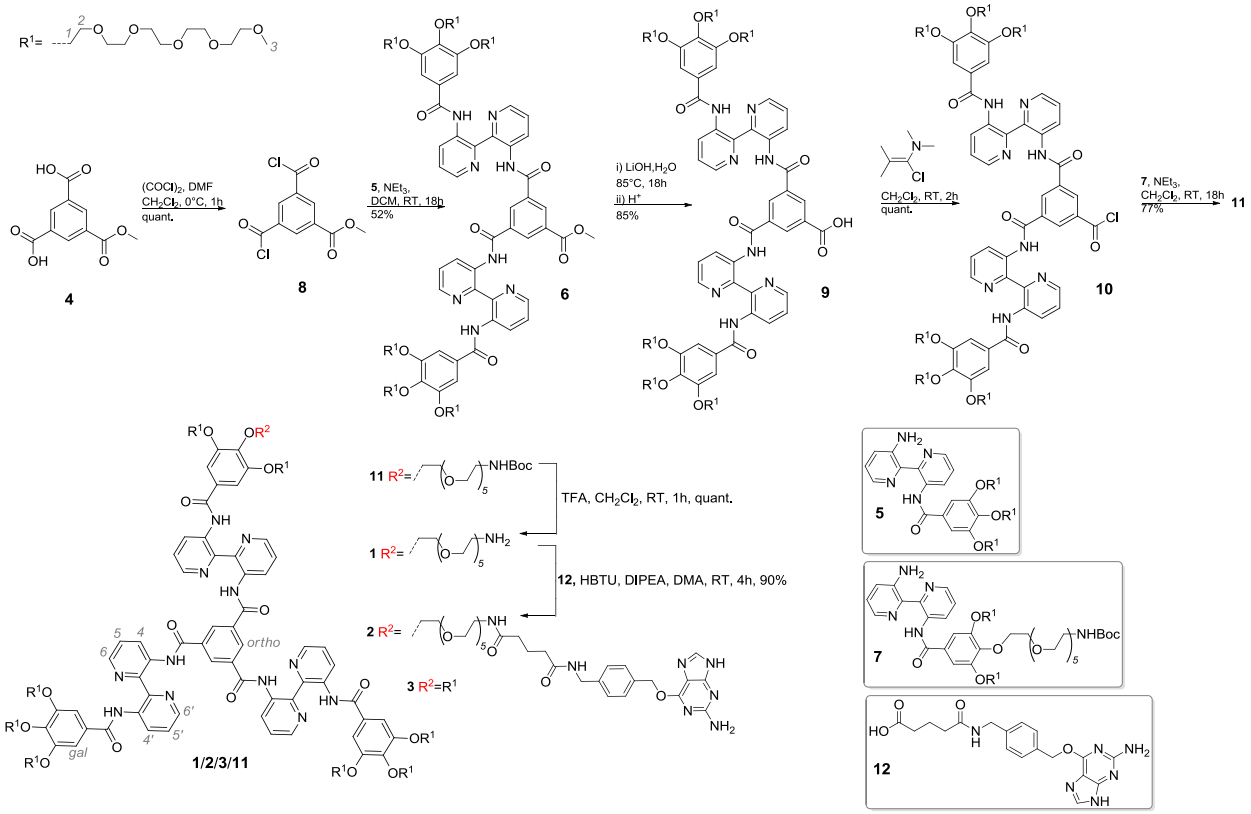
Matrix assisted laser desorption/ionisation time of flight mass spectra (MALDI-TOF-MS) were measured on a PerSeptive Biosystems Voyager-DE PRO spectrometer with a Biospectrometry workstation using 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrix material. M/z values are given in g/mol. Infrared spectra were recorded as a liquid film on a Perkin Elmer Spectrum One 1600 FT-IR spectrometer, equipped with a Perkin Elmer Universal ATR Sampler Accessory. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 MHz (100 MHz for ^{13}C) NMR spectrometer at 298 K. Chemical shifts are given in parts per million (ppm) and the spectra are calibrated to residual solvent signals of $CDCl_3$ (7.26 ppm (^{1}H) and 77.0 ppm (^{13}C)). Splitting patterns are labeled as s, singlet; d, doublet; dd, double doublet; t, triplet; and m, multiplet.

General LC-MS analysis: samples were analyzed using a Shimadzu SCL-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). Analyses were performed using a reversed phase HPLC column (C4 Jupiter, 150 x 2 mm, 5 µm), using an injection volume of 1-4 µL, a flow rate of 0.20 mL/min and typically a gradient (20% to 90% in 10 min, held at 90% for 1 more minute) of acetonitrile in water (both containing 0.1% formic acid) at 298K. Prior to LC-MS the protein samples have been desalted using DyeEx 2.0 Spin Columns (Qiagen). Deconvolution of mass spectra was performed using the ProMassXcali software.

Fluorescence spectra were recorded on a Varian Cary Eclipse photoluminescence spectrometer equipped with a Perkin–Elmer PTP-1 Peltier temperature control system. All fluorescence measurements were performed in phosphate buffer (25 mM, pH 7.5 containing 50 mM NaCl) in quartz cuvettes of 10 mm

light path (Hellma) and 2 mL minimal volume at 20°C. Samples were excited at 410 nm for CFP, 500 nm for YFP or 340 nm for the discotics. The SNAP-tag fusion proteins were expressed and purified as described previously.[1] Protein concentrations were measured with NanoDrop V3.5.2 using A_{515} and a molar extinction coefficient of 84 000 $M^{-1}cm^{-1}$ for YFP-SNAP[2] and A_{435} and a molar extinction coefficient of 32 5000 $M^{-1}cm^{-1}$ for CFP-SNAP[3]. The FRET ratio (YFP/CFP) was calculated by dividing the emission of YFP at 527 nm and the emission of CFP at 475 nm. The FRET ratio (YFP/Disc) was calculated by dividing the emission of YFP at 527 nm and the emission of Disc at 494 nm. The change in FRET ratio is defined as the difference between the FRET ratio at a later measuring point and the FRET ratio at the beginning. Dynamic and static light scattering experiments were performed on an ALVCGS-3 Compact Goniometer, in the angular range of 30 to 150 degrees. The incident beam was produced by a HeNe laser operating at 532 nm. The intensity signal was sent to an ALV5000 digital correlator, using a typical acquisition time of 60 s for each angle.

Synthesis



Scheme S1: Synthesis of Benzylguanine-Disc **2**.

Compound **3**[4], **4**[5], **5**[6], **7**[7] and **12**[8] were synthesized according to literature.

5-Methoxycarbonyl-benzene-1,3-dicarbonyl dichloride (8).

To an ice-cold stirred suspension of 5-methoxycarbonylbenzene-1,3-dicarboxylic acid[5] (**4**, 84.5 mg, 0.377 mmol) in chloroform (2.5 ml) containing one drop of DMF was added dropwise in 15 min a solution of oxalyl chloride (0.0965 mL, 1.131 mmol) in chloroform (2.5 mL). Then, the reaction mixture was allowed to reach room temperature and the progress of the reaction was monitored by ^{1}H-NMR. When no carboxylic acid was left, the mixture was concentrated *in vacuo* and the residue flushed with dry dichloromethane leaving a residue (0.098 g) that was used as such in the next step. ^{1}H-NMR (THF-d8): δ= 9.08 (d, J = 2 Hz, 2 H), 9.05 (t, J = 2 Hz, 1 H), 4.11 (s, CO_2Me, 3H); ^{13}C-NMR ($CDCl_3$) δ: 166.8, 164.2, 137.6, 136.8, 135.1, 132.8, 53.4.

Methyl 3,5-bis{{3'-{3,4,5-tris[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoylamino}-2,2'-bipyridyl-3-aminocarbonyl}}-benzoate (6).

A solution of crude 5-methoxycarbonyl-1,3-benzenedicarbonyl dichloride **8** (50 mg, 0.384 mmol) in dichloromethane (1.25 mL) was added dropwise in 5 min to a stirred solution of monoamine **5**[6] (0.40 g, 0.384 mmol) and triethylamine (0.102 mL, 0.768 mmol) in dichloromethane (5 mL) and the reaction was continued overnight. The mixture was washed with water (3 x 1 mL), the organic layer dried with $MgSO_4$, filtered and concentrated *in vacuo* to leave a residue (0.48 g) that was dissolved in chloroform (3 mL). Purification by column chromatography over alumina (25 g) with chloroform - isopropanol (98:2) gave after evaporation of the appropriate fractions a residue (0.327 g) still containing monoamine. This material was dissolved in water (9 mL) and extracted with wet ethyl acetate (4 x 10 mL) to remove most of the monoamine. The remaining aqueous phase was then extracted with dichloromethane (3x 7.5 mL). The combined dichloromethane phases were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the pure ester (225 mg, 52%). IR (ATR): ν = 3108, 2870, 1730 ($COCH_3$), 1680, 1569, 1099 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ = 15.37 (s, 2H), 14.43 (s, 2H), 9.53 (dd, *J* = 8.5 and 1.7 Hz, 2H), 9.42 (dd, *J* = 8.5 and 1.7 Hz, 2H), 9.22 (t, J = 2 Hz, 1 H), 9.03 (d, J = 2 Hz, 2H), 8.89 (dd, *J* = 4.6 and 1.5 Hz, 2H), 8.46 (dd, *J* = 3.9 and 1.8 Hz, 2H), 7.58 (dd, 2H), 7.56 (dd, 2H), 7.32 (s, 4H), 4.27 (t, 12 H), 4.12 (s, 3H), 3.9-3.5 (m, 108 H), 3.39 (s, 18 H). ^{13}C-NMR ($CDCl_3$): δ = 165.8, 165.8, 163.9, 152.7, 142.4, 142.1, 141.6, 141.0, 140.7, 137.6, 137.3, 136.0, 131.4, 131.3, 131.1, 130.4, 130.0, 129.8, 124.5, 124.3, 108.0, 72.45, 71.9, 70.8, 70.6, 70.5, 70.5, 69.7, 69.4, 59.0, 52.7. MALDI-TOF MS: m/z calcd ($C_{110}H_{164}N_8O_{42}$) 2270.20; found 2293.12 [M^++Na], 2271.14 [M^++H].

3,5-Bis{{3'-{3,4,5-tris[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]benzoyl- amino}-2,2'-bipyridyl-3-aminocarbonyl}}-benzoic acid (9).

To a suspension of methyl ester **6** (212.5 mg, 0.093 mmol) in water (5 ml) was added $LiOH.H_2O$ (19.5 mg, 0.465 mmol). The mixture was heated at 85°C overnight. Monitoring by ^{1}H-NMR showed the disappearance of ester. After cooling to room temperature the aqueous phase was extracted with ethyl acetate (8 x 4 mL) to remove the last traces of monoamine. Then, the aqueous phase was acidified with

aqueous oxalic acid to pH 3-4, after which it was extracted with dichloromethane (3 x 4 mL). Drying of the organic phase with $MgSO_4$ and filtration gave a filtrate that was evaporated to afford the pure acid **9** as a thick oil (170 mg, 80%). IR (ATR): ν = 3079, 2870, 1729 (C=OOH), 1680, 1568, 1097 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ = 15.31 (s, 2H), 14.47 (s, 2H), 9.48 (dd, *J* = 8.5 and 1.7 Hz, 2H), 9.33 (dd, *J* = 8.5 and 1.7 Hz, 2H), 9.06 (t, J = 2 Hz, 1 H), 8.91 (d, J = 2 Hz, 2H), 8.84 (dd, *J* = 4.6 and 1.5 Hz, 2H), 8.42 (dd, *J* = 3.9 and 1.8 Hz, 2H), 7.47 (dd, 2H), 7.44 (dd, 2H), 7.29 (s, 4H), 4.24 (t, 12 H), 3.9-3.5 (m, 108 H), 3.39 (s, 18 H). ^{13}C-NMR ($CDCl_3$): δ = 166.5, 165.6, 163.7, 152.6, 142.2, 141.8, 141.3, 141.1, 140.5, 137.5, 137.4, 135.6, 131.4, 131.4, 131.0, 130.4, 129.7, 129.5, 124.4, 124.3, 108.0, 72.5, 71.9, 71.9, 70.8, 70.6, 70.6, 70.5, 70.5, 70.5, 70.4, 69.7, 69.4, 59.0. MALDI-TOF MS: m/z calcd ($C_{109}H_{162}N_8O_{42}$) 2256.31; found 2278.99 [M^++Na].

3,5-Bis{{3'-{3,4,5-tris[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]benzoyl- amino}-2,2'-bipyridyl-3-aminocarbonyl}}-benzolyl chloride (10).

A solution of 1-chloro-*N,N*,2-trimethyl-1-propenylamine (14.5 mg, 0.11 mmol) in dichloromethane (0.4 mL) was added dropwise in 40 minutes to **9** (0.164 g, 0.073 mmol) dissolved in dry dichloromethane (1.5 mL). The progress of the reaction was monitored by IR. When no carboxylic acid was left, the mixture was concentrated *in vacuo* to remove the excess of 1-chloro-*N,N*,2-trimethyl-1-propenylamine and the residue was used as such in the next step. IR (ATR): ν = 2870, 1759 (C=OCl), 1672, 1568, 1097 cm^{-1}.

Mono-NHBoc-Disc (11).

A solution of crude **10** (0.073 mmol) in dichloromethane (1.2 mL) was added dropwise in 30 min to a stirred solution of monoamine **7**[7] (0.094g, 0.08 mmol) and triethylamine (15 µL, 0.11 mmol) in dichloromethane (0.5 mL) and the reaction was continued overnight. The reaction mixture was washed with 0.1 M aqueous NaOH (2 x 3 mL) and brine (1 x 3 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated *in vacuo.* The excess of **7** was removed using size-exclusion chromatography (Recycling-GPC in chloroform), obtaining pure **11** (191 mg, 77%). IR (ATR): ν = 2873, 1738, 1715, 1672, 1568, 1097 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ= 15.53 (s, 3H), 14.49 (s, 3H), 9.60 (d, *J* = 8.5 Hz, 3H), 9.39 (d, *J* = 8.5 Hz, 3H), 9.28 (s, 3H), 9.05 (d, *J* = 4.5 Hz, 3H), 8.52 (d, *J* = 4.5 Hz, 3H), 7.57 (dd, *J* = 8.5, 4.5 Hz, 6H), 7.35 (s, 6H), 5.03 (s, 1H, N*H*Boc), 4.27 (dd, *J* = 10.8, 5.5 Hz, 18H), 3.90 (t, *J* = 4.8 Hz, 12H), 3.83 (t, *J* = 4.8 Hz, 6H), 3.74 (q, *J* = 4.8 Hz, 18H), 3.70 – 3.59 (m, 110H), 3.53 (dd, *J* = 10.4, 5.4 Hz, 18H), 3.37 (s, 6H), 3.35 (s, 18H), 3.30 (d, *J* = 5.2 Hz, 2H, C*H*$_2$NHBoc), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$): δ=165.96, 164.25, 156.11 (C=OBoc), 152.88, 142.53, 142.41, 141.68, 141.60, 140.91, 137.67, 137.58, 136.28, 130.71, 130.11, 130.04, 129.71, 124.84, 124.49, 112.73, 108.19, 79.25 (*C*(CH_3)$_3$), 72.65, 72.05, 70.98, 70.81, 70.64, 70.36, 69.87, 69.55, 59.15 (OCH_3), 40.55 (CH_2NH), 28.57 (CH_3). MALDI- TOF MS: m/z calcd ($C_{165}H_{251}N_{13}O_{62}$) 3408.81; found 3431.68 [M^++Na], 3309.18 [(M-Boc)$^+$+H].

Mono-NH_2-Disc (1).

To a stirring solution of **11** (33.7 mg, 0.099 mmol) in dichloromethane (2 mL) trifluoroacetic acid (TFA, 0.2 mL) was added dropwise and the reaction was continued for 2 h at room temperature before TFA

was co-evaporated with toluene. After drying, the pure mono-NH_2-Disc **1** was obtained as a sticky yellow compound (34 mg, quantitative). ^{1}H-NMR ($CDCl_3$): δ= 15.53 (s, 2H), 15.49 (s, 1H), 14.49 (s, 3H), 9.60 (d, *J* = 8.5 Hz, 3H), 9.38 (m, 3H), 9.28 (s, 3H), 9.06 (d, *J* = 3.2 Hz, 3H), 8.52 (d, *J* = 3.2 Hz, 2H), 8.49 (d, *J* = 3.2 Hz, 1H), 7.57 (dd, *J* = 8.5, 4.5 Hz, 6H), 7.36 (s, 4H), 7.33 (s, 2H), 4.28 (dd, *J* = 10.0, 5.3 Hz, 18H), 3.97 – 3.88 (m, 12H), 3.88 – 3.80 (m, 6H), 3.79 – 3.71 (m, 18H), 3.70 – 3.58 (m, 110H), 3.59 – 3.48 (m, 18H), 3.37 (s, 6H), 3.36 (s, 18H), 3.23 (s, C*H*$_2$NH$_2$, 2H). ^{13}C NMR ($CDCl_3$): δ= 165.95, 164.23, 152.85, 152.71, 142.42, 142.39, 141.73, 141.68, 141.43, 140.91, 137.65, 137.57, 136.26, 131.20, 130.74, 130.11, 129.70, 124.84, 124.49, 108.12, 107.37, 72.64, 72.03, 71.99, 70.95, 69.99, 69.85, 69.70, 69.51, 69.15, 67.18, 59.14(OCH_3), 59.12(OCH_3), 40.23(CH_2NH_2), 29.82(CH_3). MALDI- TOF MS: m/z calcd ($C_{160}H_{243}N_{13}O_{60}$) 3308.69; found 3309.77[M^++H], 3331.66 [M^++Na].

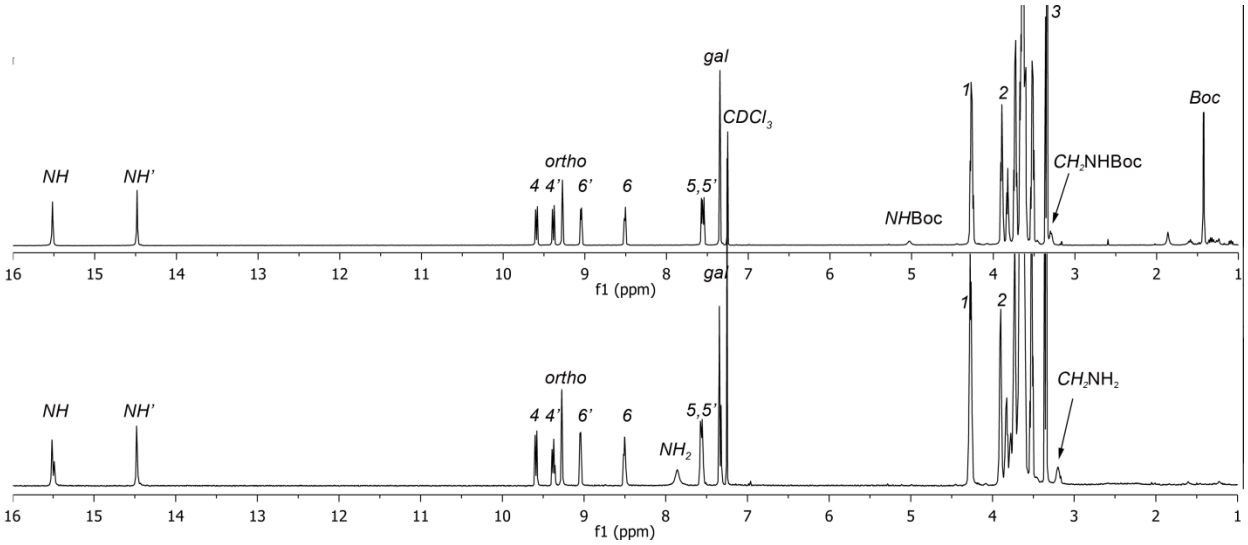


Figure S 1:^{1}H-NMR of Mono-NHBoc-Disc **11** (top) and Mono-NH_2-Disc **1** (bottom) in $CDCl_3$ at 20°C.

Benzylguanine-Disc (2, BG-Disc).

A solution of **12**[8], the benzylguanine-acid conjugate, (5 mg, 13 µmol) together with HBTU (4.7 mg, 13 µmol) and DIPEA (2.2 µL) in dry DMA (250 µL) was incubated for 15 min. 100 µL of this solution (6 µmol) were added to mono-NH_2-Disc **1** (10 mg, 3 µmol) dissolved in dry DMA. The solution was stirred overnight at room temperature. After all the amine discotic had reacted, as followed by MALDI-TOF, the solvent was evaporated and the crude mixture was purified via size exclusion chromatography (SX-1 BioBeads in DMF) to yield BG-Disc **2** (10 mg, 2.7 µmol, 90%).[9] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 15.52 (s, 3H), 14.49 (s, 3H), 9.60 (d, *J*=8.4, 3H), 9.33 (m, 3H), 9.06 (s, 3H), 8.51 (s, 3H), 8.18 (s, 1H, CONH'), 8.07 (s, 1H, guanine), 7.70 – 7.47 (m, 6H), 7.35 (s, 6H), 7.20 – 6.93 (m, 4H, Ar(BG)), 5.51 (s, 2H, BzO), 4.82 (d, 2H, BzNH), 4.35-4.20 (m, 18H), 4.01 – 3.20 (m, 190H), 2.29 (m, 4H, CO*CH*$_2$CH$_2$*CH*$_2$CO), 1.98 (m, 2H, CH$_2$*CH*$_2$CH$_2$CO). MALDI- TOF MS: m/z calcd ($C_{178}H_{261}N_{19}O_{63}$)3675.13; found 3697.36 [M^++Na].

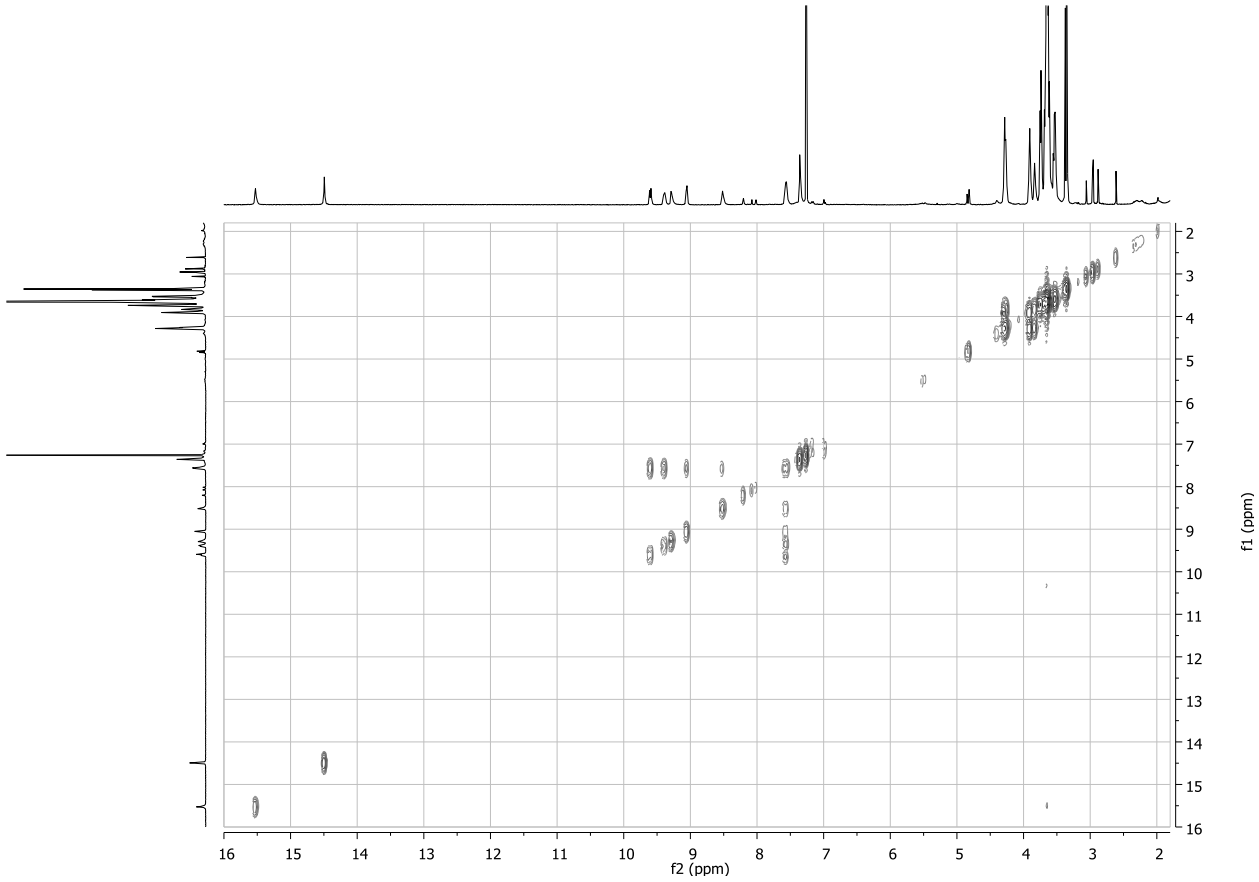


Figure S 2: gCOSY of BG-Disc **2** in $CDCl_3$ at 20°C.

Self-assembly

The self-assembly of C_3-symmetrical disc-shaped molecules leads selectively to the formation of columnar stacks.[4,10,11] The self-assembly is based on strong intramolecular hydrogen bonding between the amide N-H groups and neighboring bipyridine N-atoms. This can be observed experimentally by a downfield shift in the ^{1}H-NMR ($CDCl_3$) signal corresponding to the amide protons, from normally 6-9 ppm to approximately 15 ppm (**Figure S 1**).[10] The intramolecular hydrogen bonding pre-organizes the molecules into a propeller-like conformation, which allows the molecules to form long stacks primarily through π-π stacking.[12,13] In apolar or polar aprotic solvents such as chloroform, DCM, THF, and DMF discotics are typically molecularly dissolved at low concentrations, whereas in polar protic solvents, such as methanol, ethanol and water, the discotics self-assemble (K_{ass} = 1 x 10^8 L/mol) and become highly fluorescent.[4,11] The molecularly dissolved and self-assembled state can be visualized using UV/Vis, fluorescence spectroscopy and dynamic light scattering (DLS) (**Figure S 3**). Upon self-assembly, a red-shift in the UV/Vis spectra and an increase in fluorescence intensity can be observed (**Figure S 3**a & b). The intrinsic fluorescence with a large Stokes shift of the discotics is attributed to the intramolecular double proton transfer within the bipyridine-diamine groups.[6] Upon self-assembly, due to the decreased motion of the molecules within the self-assembling structure, the fluorescent lifetime increases from 0.3 to 4 ns.[14] No correlation function can be obtained for the molecularly dissolved BG-Disc **2** in DCM. In

water, the correlation function of the self-assembled BG-Disc **2** shows that these columnar stacks diffuse through the solution with the same velocity as a sphere with a radius of 200 nm (**Figure S 3**d).

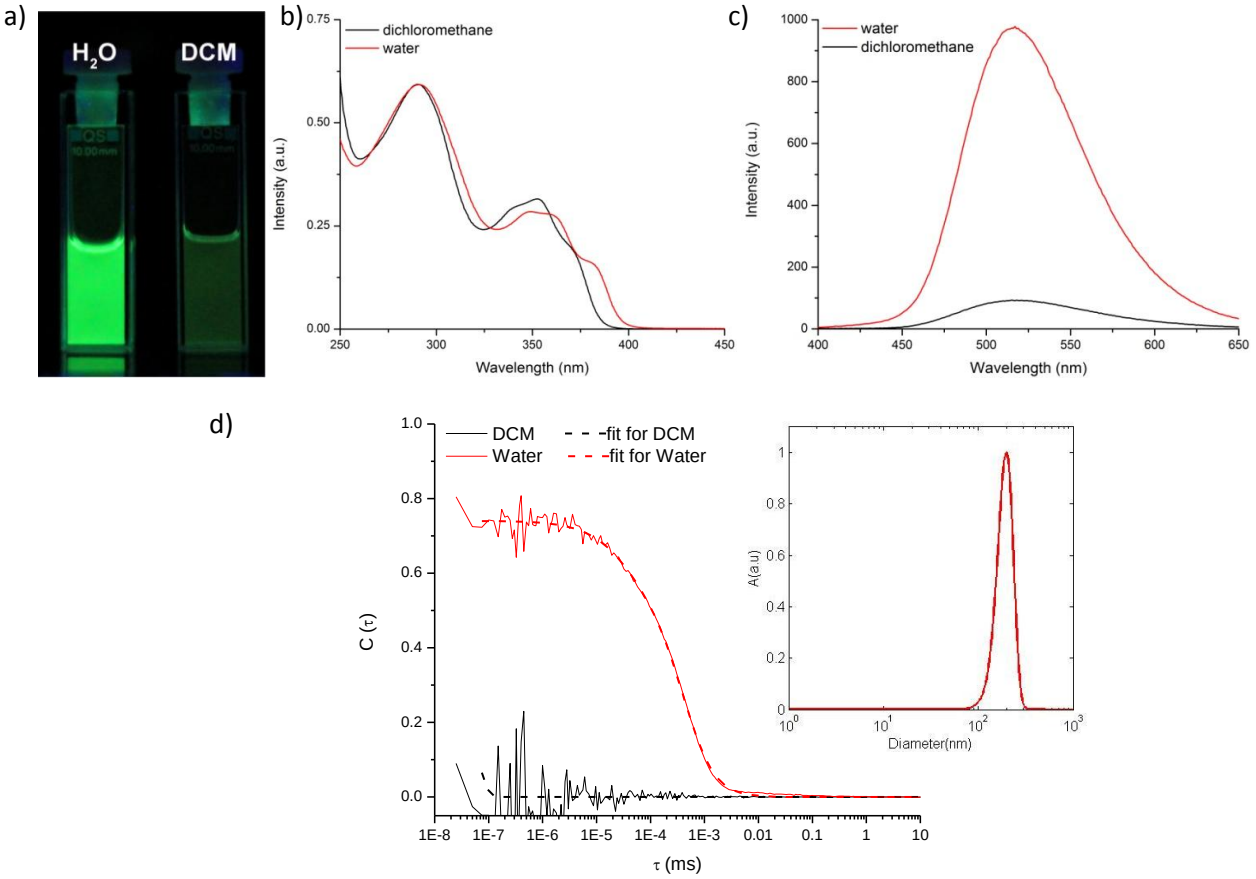


Figure S 3: a) Photographs of these disc solutions in water (left) and in DCM (right) both 100 µM under illumination with UV-light (λex = 360 nm); b) Absorption spectra of BG-Disc **2** (100 µM) in H_2O and DCM at 20 °C. In the absorption spectrum a red-shift is observed in self-assembled state in water; c) Emission spectra (λ_{ex} = 350 nm) of BG-Disc **2** (100 µM) in H_2O and DCM at 20 °C. In the emission spectrum an increase in emission is observed in the self-assembled state in water. d) DLS correlation functions of BG-Disc 2 (10 µM) in H_2O and DCM at 20 °C at 90°. Inset: Cumulant fit of the correlation function of BG-Disc **2** (10 µM) in H_2O. For the data on the Inert-Disc see reference [15].

Functionalization of fluorescent proteins with Discotics

Functionalization of fluorescent proteins with the supramolecular polymer

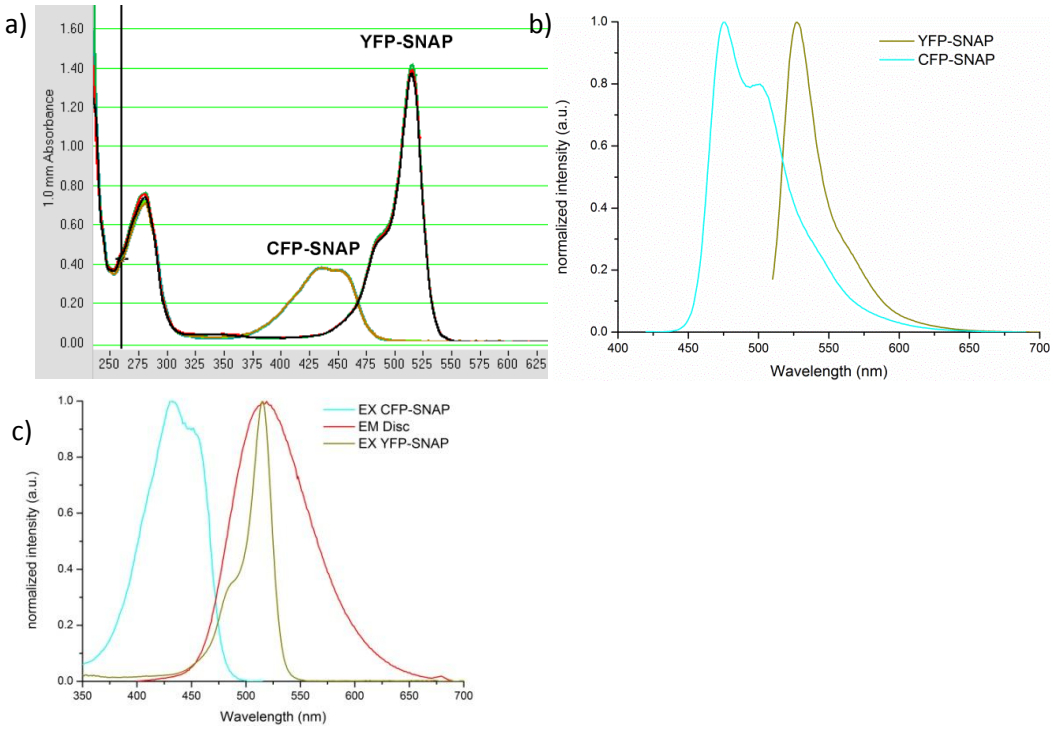


Figure S 4: a) UV spectra of CFP-SNAP and YFP-SNAP (both 160 µM); b) normalized fluorescence spectra of CFP-SNAP (λex = 410 nm) and of YFP-SNAP (λex = 500 nm) both 1 µM[1]; c) Spectral overlap of fluorescent proteins with the discotics; blue = normalized excitation of CFP-SNAP, dark yellow = normalized excitation of YFP-SNAP, red = emission of BG-Disc.

First the coupling efficiencies were systematically optimized by varying the ligation temperature, incubation times and coupling stoichiometry (data not shown). The best coupling efficiencies were achieved using 100 µM BG-Disc **2**/Inert-Disc **3** and a total concentration of 10 µM of SNAP-proteins. The ligation was performed in a sodium phosphate buffer at 37°C in a shaking incubator for up to 3 hours. The ligation was followed by LC-MS, SDS-PAGE and in case of YFP-SNAP by monitoring FRET from the discotics to YFP upon covalent attachment. It has to be noted that the self-assembled discotics fall apart under the ESI-MS conditions and on a denaturing gel.

Table S 1: Expected and found masses of proteins:

	protein	calculated	found
Proteins	CFP-SNAP	49151	49155 (± 2)
	YFP-SNAP	49237	49240 (± 2)
Proteins reacted with **12**	CFP-BG	49385	49387 (± 2)
	YFP-BG	49471	49472 (± 2)
Protein-discotic conjugates	CFP-Disc	52676	52678 (± 2)
	YFP-Disc	52762	52764 (± 4)

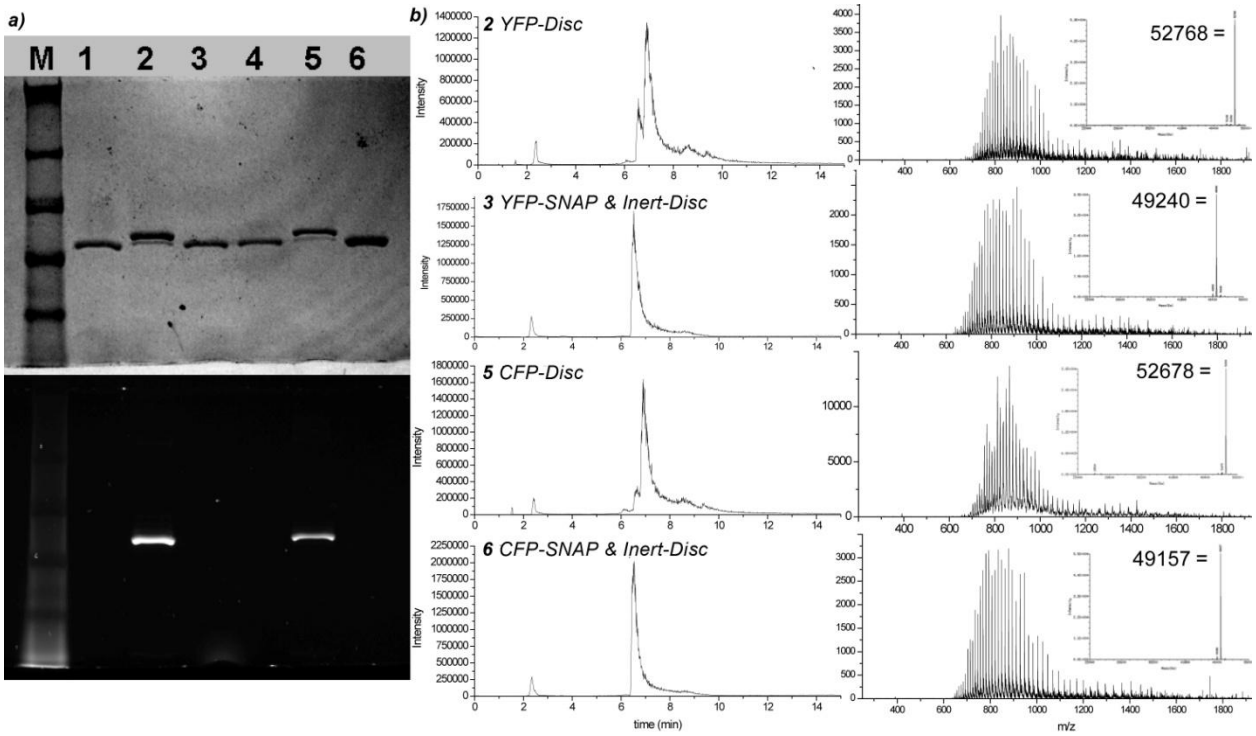


Figure S 5: a) SDS-PAGE gel of CFP- and YFP-SNAP proteins after incubation for 6 hours with buffer, Inert-Disc or BG-Disc. (M= marker, 1= YFP-SNAP, 2=YFP-SNAP + BG-Disc, 3= YFP-SNAP + Inert-Disc, 4= CFP-SNAP, 5= CFP-SNAP + BG-Disc, 6= CFP-SNAP + Inert-Disc). b) LC-MS spectra of these samples (2, 3, 5, and 6) after 6 hours of incubation (TIC, MS and deconvoluted mass). It has to be noted that the self-assembled discotics fall apart under the ESI-MS conditions and on a denaturing gel.

Functionalization of SNAP-proteins with supramolecular polymer vs. small ligand

Since the functionalization is performed in buffer, where the supramolecular polymer is self-assembled, the proteins are reacting with a highly molecularly crowded scaffold. The SDS-PAGE gel and the LC-MS spectra after 3 hours of ligation (**Figure S 5**) show that over 90% of the YFP/CFP-SNAP protein has reacted with the BG-Disc and only small traces of unreacted protein are still present.

To investigate the reactivity of the protein and to compare the functionalization of the BG-covered supramolecular polymer with a small BG-molecule, the ligation reaction was as well performed under the same conditions with **12**. The complete functionalization of CFP-SNAP with the small ligand **12** was achieved during the first 15 minutes of incubation (**Figure S 6**). In contrast, only 50% of the protein was functionalized with the supramolecular polymer after the same time period. After 90 minutes around 90% of the protein was functionalized and longer incubation times did not lead to more functionalization due to the inactivation of the enzyme overtime. In all future experiments the ligation of CFP-SNAP/YFP-SNAP to **2** was stopped after 3 hours through addition of an excess of **12**.

In literature quantitative functionalization of BG-functionalized ligands has often been reported.[1] However, for the functionalization of more complex scaffolds lower yields are common.[16] For self-assembled monolayers (SAMs) consisting of BG-thiols mixed with matrix thiols yields of maximum 78% were observed[17], underlining the highly efficient functionalization of the supramolecular polymer described here.

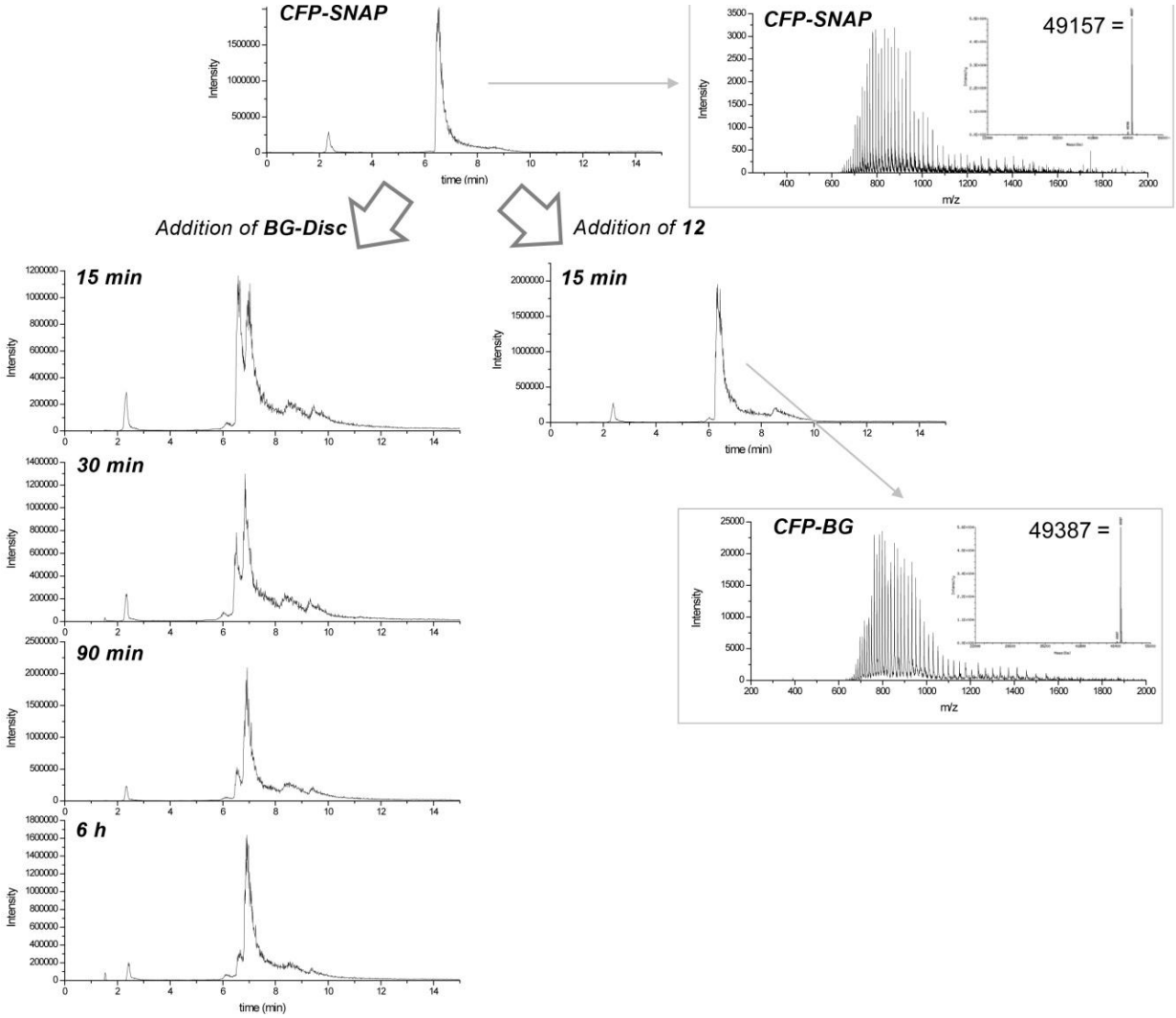
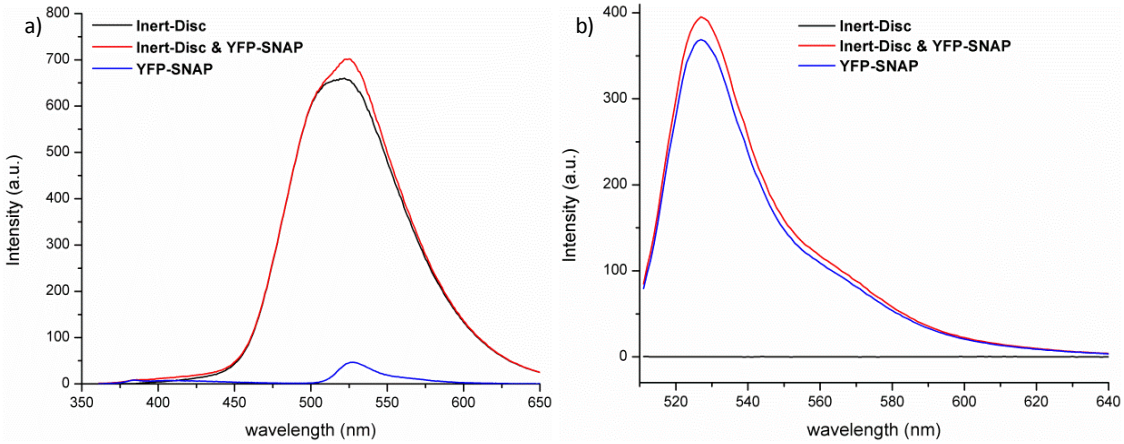


Figure S 6: Comparison between the functionalization of CFP-SNAP (10 µM) with the benzylguanine displaying supramolecular polymer (BG-Disc **2**, 100 µM) and with the small benzylguanine ligand **12** (100 µM) at 37 °C.

FRET from the Disc to the yellow fluorescent protein (YFP)



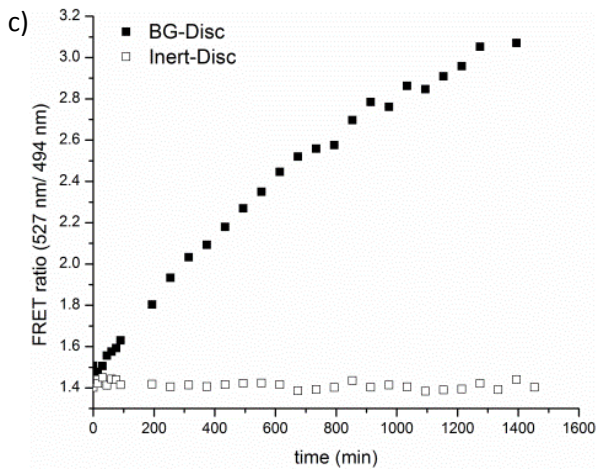


Figure S 7: a) Reference fluorescence spectra of Inert-Disc (20 µM), YFP-SNAP (2 µM) and a mixture of YFP-SNAP (2 µM) with Inert Disc **3** (20 µM) 4 h after mixing excited at 340 nm. At 340 nm, the excitation wavelength of the Disc, YFP is only excited to a small extent (blue line). Results show no background energy transfer in the presence on Inert Disc. b) The same three samples are excited at 500 nm, the excitation wavelength of YFP, showing the equal concentration of the YFP-SNAP protein present and no excitation of the Inert Disc at this wavelength. c) FRET ratios (from Disc to YFP) from the measurement shown in Figure 2 a&b in the main text.

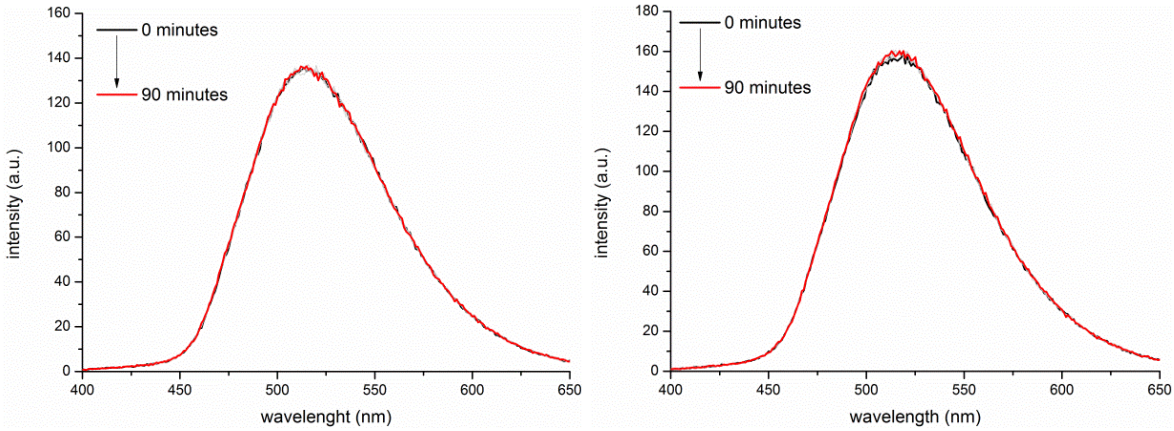


Figure S 8: Reference fluorescence emission spectra of 1 µM CFP-SNAP and 3 µM BG-Disc **2** (left) and of 1 µM CFP-SNAP and 3 µM Inert Disc **3** (right) followed over time at 20°C. As expected no energy transfer is observed from the Disc to CFP, since the spectral overlap is insignificant (see Figure S 4) and CFP has a much lower quantum yield compared to YFP. Excitation wavelength is 340 nm.

The emission of the BG-Disc as well stays stable upon functionalization with CFP-SNAP indicating that the functionalization with fluorescent proteins does not affect self-assembly of the discotics. Due to the excitation of the fluorescent proteins by the laser used in the DLS setup, no DLS measurements could be conducted of the proteins and of the proteins ligated to the discotics.

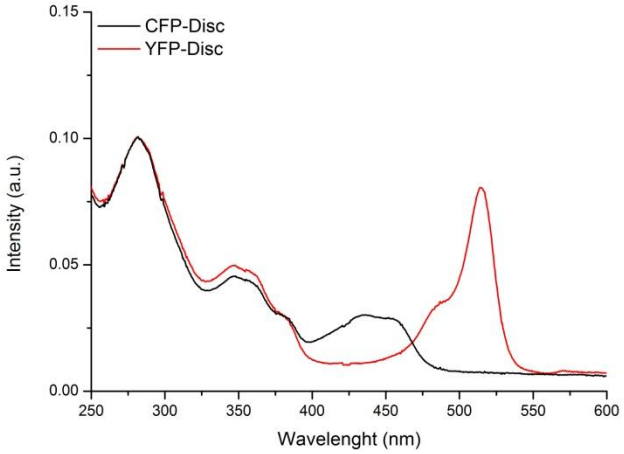


Figure S 9: UV-spectra of BG-Disc **2** (20 µM) ligated to YFP-SNAP (2 µM) or to CFP-SNAP (2 µM).

The red-shift at 395 nm indicates the presence of the self-assembled state after ligation with proteins.

Inducing protein-protein interactions

Ligation of a 1:1 mixture of CFP-SNAP and YFP-SNAP with BG-Disc or Inert-Disc:

Final volume of ligation reaction is 420 µL, containing 100 µM BG-Disc and a 1:1 mixture of 5 µM CFP-SNAP and 5 µM YFP-SNAP. After 3 h of shaking at 37°C the reaction was stopped through addition of a large excess of benzylguanine derivative **12**. 400 µL of this ligation reaction was diluted with 1600 µL of buffer to have a final volume of 2 mL and a final concentration of 20 µM of BG-Disc and 1 µM of each protein. Under the same conditions the 1:1 mixture of 5 µM CFP-SNAP and 5 µM YFP-SNAP was ligated to the Inert-Disc (100 µM). The fluorescence spectra were measured at the excitation wavelength of 410 nm at 20°C (Figure 3, main text).

The LC-MS spectra in Figure S 10 belong to **Figure 3** in the main text, where the fluorescence of these ligation mixtures is measured. Deconvoluting the LC-MS peaks at around 7 minutes confirmed the presence of both proteins in the ligation mixture. The nearly equal intensity of the deconvoluted masses indicated that both proteins ligated with the same efficiency.

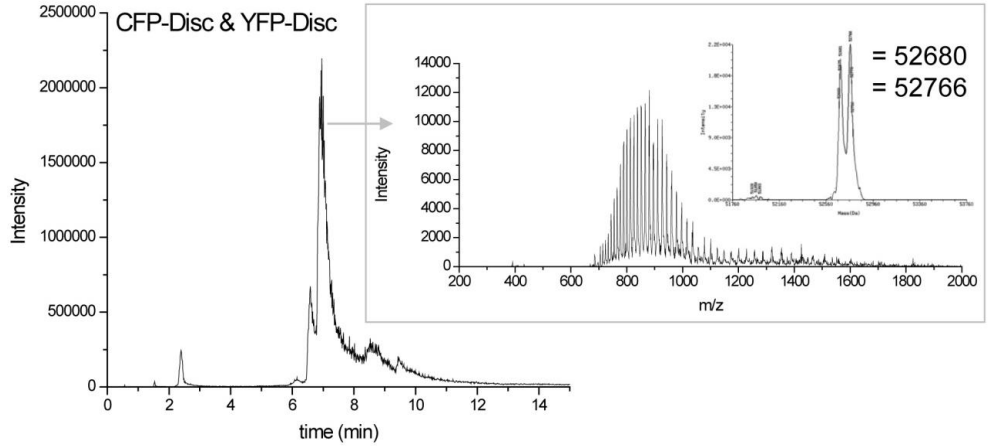


Figure S 10: LC-MS spectra of ligations of a 1:1 mixture of CFP-SNAP (5 µM) and YFP-SNAP (5 µM) with **BG-Disc** (**2**, 100 µM) after 3 h.

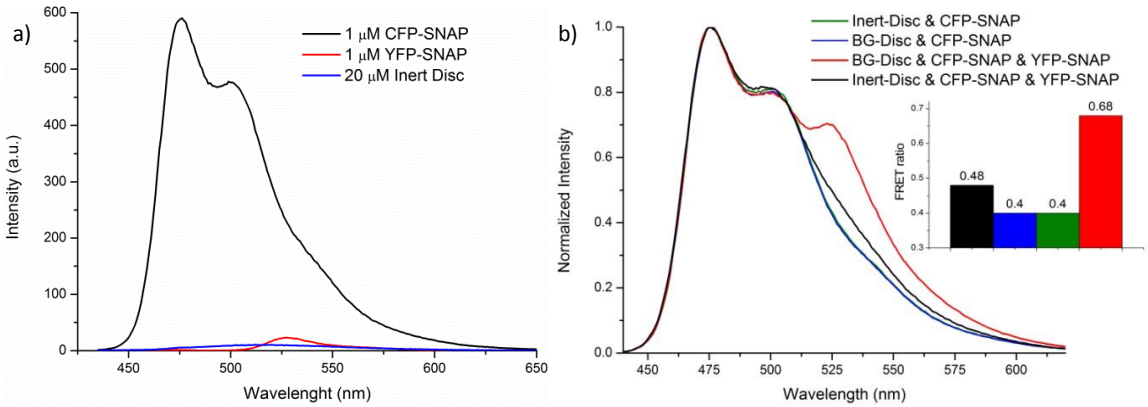


Figure S 11: a) Reference fluorescence emission spectra of 1 μM YFP-SNAP, 1 μM CFP-SNAP and of 20 μM Inert-Disc **3** at 20°C. Excitation wavelength is 410 nm, the excitation wavelength for CFP. At this wavelength only negligible excitation of YFP-SNAP (red trace) and hardly any excitation of the Inert-Disc **3** (blue trace) is observed. b) Normalized reference fluorescence emission spectra (λ_{ex} = 410 nm) of mixtures of BG-Disc and Inert-Disc (both 20 μM) with either CFP-SNAP alone (2 μM) or with a 1:1 mixture of CFP- and YFP-SNAP (each 1 μM). The FRET ratio of CFP-SNAP in presence of either discotic is 0.4. For the mixture of Inert-Disc with CFP-SNAP and YFP-SNAP the FRET ratio increases to 0.48 due to a negligible excitation of YFP at 410 (see Figure S 7). When both proteins are mixed with the BG-Disc, due to energy transfer from CFP to YFP, the FRET ratio increases to 0.68.

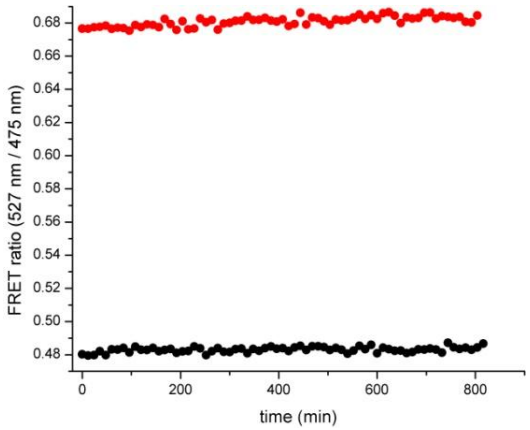
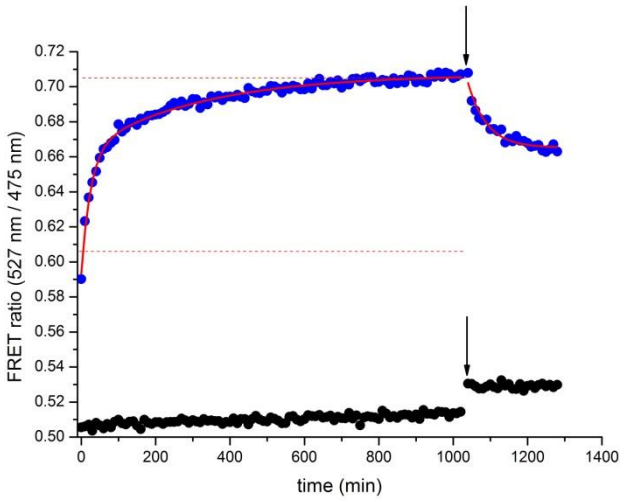


Figure S 12: FRET ratio of the time course of 14 h of the ligation mixtures from Figure 3. The red trace corresponds to the 1:1 mixture of YFP-SNAP (1 μM) and CFP-SNAP (1 μM) reacted for 3 h at 37°C with the BG-discotic **2** (20 μM). The red trace corresponds to the 1:1 mixture of YFP-SNAP (1 μM) and CFP-SNAP (1 μM) incubated for 3 h at 37°C with the inert-discotic **3** (20 μM). The constant FRET ratio of the inert-discotic **3** and CFP-/YFP-SNAP mixture shows that the proteins don't aggregate overtime, whereas the constant FRET ratio of the BG-discotic **2** and CFP-/YFP-SNAP mixture indicates no changes in the structure of the supramolecular polymers.

Dynamic intermixing

Ligation CFP-SNAP or YFP-SNAP with BG-Disc:

Final volume of ligation reaction is 210 μL, containing 100 μM **BG-Disc** and 10 μM CFP-SNAP. After 3 h of shaking at 37° C the reaction was stopped through addition of a large excess of benzylguanine derivative **12**. Under the same conditions YFP-SNAP was ligated to BG-Disc. 200 μL of each ligation reaction were mixed and diluted with 1600 μL of buffer to have a final volume of 2 mL and a final concentration of 20 μM of BG-Disc and 1 μM of each protein. The fluorescence spectra were measured every 10 minutes over the course of 17 h at 10°C at the excitation wavelength of 410 nm.



Model	ExpAssoc		
Equation	y = y0 + A1*(1 - exp(-x/t1)) + A2*(1 - exp(-x/t2))		
Reduced Chi-Sqr	3.38429E-6		
Adj. R-Square	0.98991		
		Value	Standard Error
B	y0	0.59354	0.00168
	A1	0.06969	0.00215
	t1	24.96782	1.56431
	A2	0.04395	0.00132
	t2	312.40976	22.55488

Figure S 13: Non-corrected FRET ratios overtime of a mixture of **3** incubated with YFP-SNAP and of **3** incubated with CFP-SNAP (black) and of a mixture of YFP-SNAP ligated to **2** and of CFP-SNAP ligated to **2** (blue). The arrow indicates the time point at which 20 µM of **3** was added to both mixtures. The increase in energy transfer upon addition of 20 µM Inert-Disc is due to background excitation of the discotics at 410 nm (see **Figure S 11**). In Figure 4 in the main text the FRET ratio is corrected for this background excitation. The red line represents a double exponential growth fit.

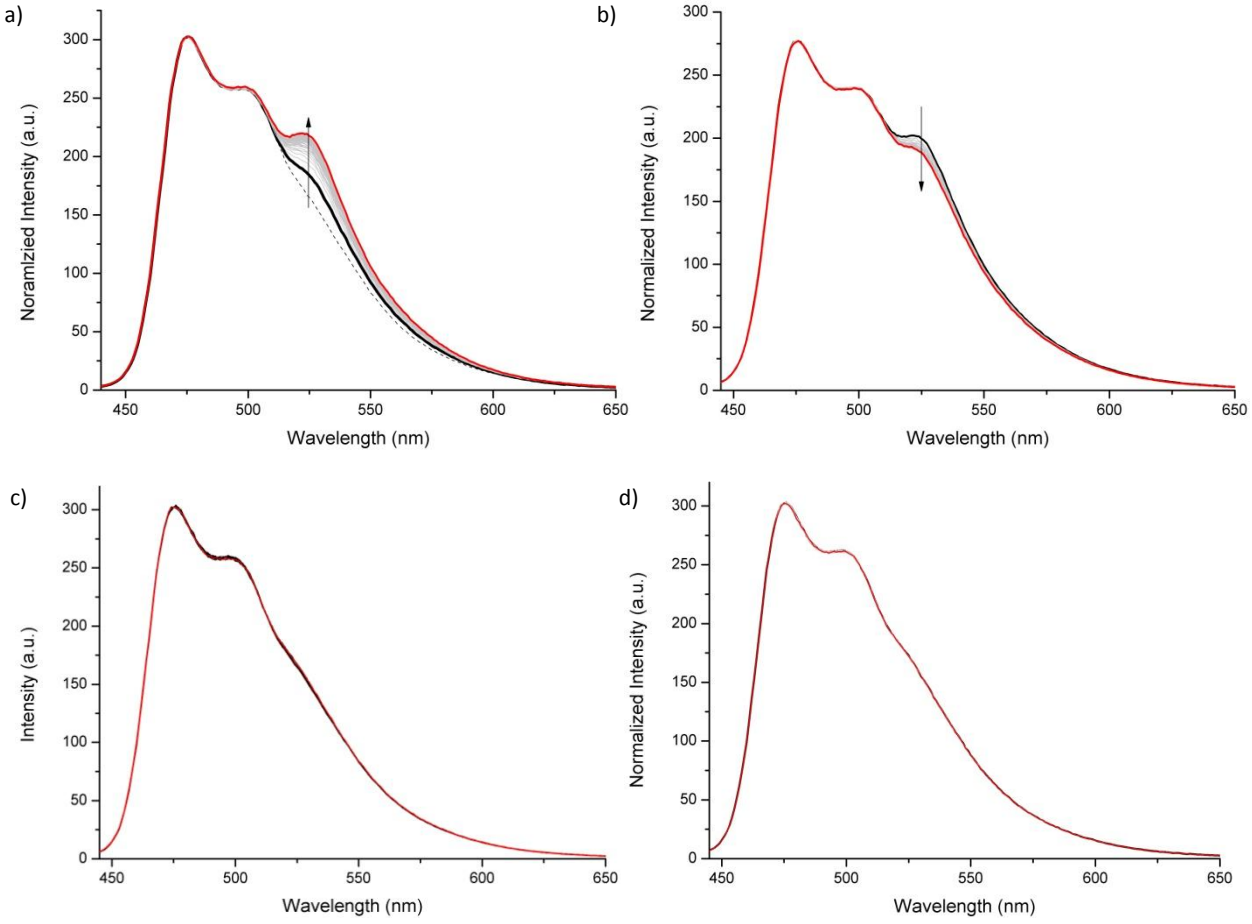


Figure S 14: a) Fluorescence emission spectra (λ_{exc}= 410 nm) over the time course of 17 h of a 1:1 mixture of the two ligations consisting of YFP-SNAP and **2** and of CFP-SNAP and **2** after 3 hours of separate ligation directly after intermixing; t = 0 min (black), t = 1020 min (red). The spectra are normalized to the initial CFP emission at 475 nm. The dashed line shows as the

comparison the emission of the mixture of proteins with the Inert-Disc (see c) where no energy transfer is observed. b) Fluorescence emission spectra (λ_{exc}= 410 nm) over the time course of 4 h of the 1:1 mixture of the two ligation shown in a after addition of Inert-Disc (20 µM); *t* = 0 min (black, 1040 min after start), *t* = 240 min (red, 1280 min after start). The spectra are normalized to the initial CFP emission at 475 nm. c) Fluorescence emission spectra (λ_{exc}= 410 nm) over the time course of 17 h of a 1:1 mixture of YFP-SNAP and **3** and of CFP-SNAP and **3** after 3 hours of separate ligation directly after intermixing; *t* = 0 min (black), *t* = 1020 min (red). The spectra are normalized to the initial CFP emission at 475 nm. d) Fluorescence emission spectra (λ_{exc}= 410 nm) over the time course of 4 h of the 1:1 mixture of the two ligation shown in c after addition of Inert-Disc (20 µM); *t* = 0 min (black, 1040 min after start), *t* = 240 min (red, 1280 min after start). The spectra are normalized to the initial CFP emission at 475 nm. These spectra belong to Figure 4 in the main text.

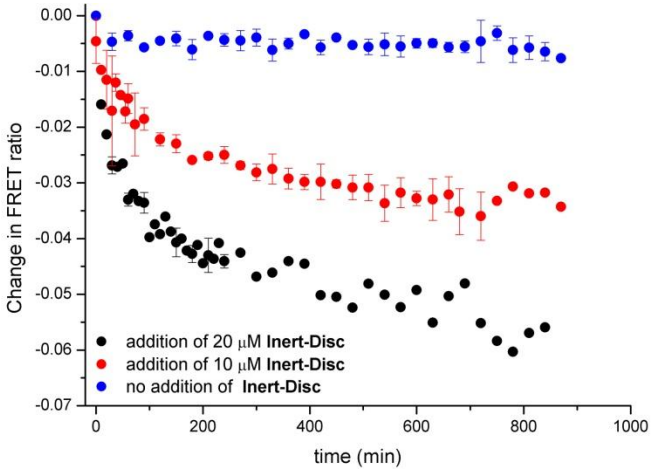


Figure S 15: Change in FRET ratios overtime of an intermixed mixture of YFP-SNAP ligated to **2** and of CFP-SNAP ligated to **2** without (blue) and after addition of 10 µM (red) and 20 µM (black) of **3**.

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