Electronic Supplementary Information

Probing the Self-Assembly and Stability of Peptidic Rod-like Micelles by Aggregation Induced Luminescence

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1. Materials and Instrumentation

Unless stated otherwise, all reagents and chemicals were obtained from commercial sources at the highest purity available and used without further purification. Water was demineralised prior to use. Unless stated otherwise, all reactions were carried out under an inert gas atmosphere and all solvents were dried prior to use. Purification *via* flash chromatography was carried out using silica-gel with an average grain size of 15-40 µm (MERCK). Analysis of the collected fractions was performed *via* TLC on silica coated aluminum sheets (60 F254, MERCK). The solid phase peptide synthesis (SPPS) was carried out on a Peptide Synthesizer CS Bio CS136XT using 2-chloro-tritylchloride resin (1.5 mmol loading) and SPPS-grade reagents.

NMR spectroscopy. NMR spectra were recorded on a BRUKER Avance II 400 and BRUKER Avance III 600 spectrometer. All measurements were carried out in deuterated solvents. The chemical shift (δ) is recorded in parts per million (ppm) and relative to the residual solvent protons.[1] The measured coupling constants were calculated in Hertz (Hz). To analyse the spectra the software MESTRENOVA 9.0.1 was used. The signals were quoted as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

Mass spectrometry. Mass spectra were recorded on the electrospray ionization spectrometers (ESI) Micro Tof (BRUKER) and QTof Ultima 3 (micromass / Waters) using methanol as solvent. Molecules of a high molecular mass were detected using matrix assisted laser desorption ionization-time of flight (MALDI-TOF) spectrometry using an Autoflex Speed (BRUKER).

CD Spectroscopy. CD spectra were recorded on a J-815 (JASCO) using the software Spectra Manager 2.12.00 and processed with Origin Pro 9.1 G. All spectra were recorded at 20°C.

Steady-state photoluminescence spectroscopy. Steady-state photoluminescence spectra were recorded on a Perkin–Elmer LS 50B spectrometer and processed with Origin Pro 9.1 G. All spectra were recorded at 20°C. The experiments using nile red were performed by adding 2 µL of a stock solution in methanol in order to obtain a final concentration of 5 µM.

Time-resolved photoluminescence spectroscopy. Time-resolved photoluminescence measurements were performed with a Fluorolog3 spectrofluorometer equipped with a FluoroHub TCSPC (time-correlated single photon counting) unit (Horiba Jobin Yvon). As excitation source a pulsed NanoLED-370 with an emission wavelength of 370 nm, a repetition rate of 1 MHz and a pulse width of 1.2 ns was used (Horiba Jobin Yvon). The photon arrival times with respect to the excitation pulse were collected in fluorescence decay histograms with a channel width of 119 ps. The overall timing resolution of the setup was quantified by the FWHM (full width at half maximum) IRF (instrumental response function) to 1.5 ns, measured at 370 nm with a scattering solution.

Data analysis. Data analysis was performed with home-written software in Python utilizing a reconvolution fit according to Equation S1. The results are summarized in Table T1, whereat each decay curve could be sufficiently fitted with a sum of two exponential functions. From the individual amplitudes and lifetime components a_n and τ_n, respectively, an intensity weighted average fluorescence lifetime $< \tau >$ was calculated (Equation S2).

$$I_{fl}(t) = \left[\sum_n a_n \exp\left(-\frac{t}{t_n}\right)\right] \otimes \text{irf(t)} + \text{b} \qquad \text{(S1)}$$

$$< \tau >= \sum_n \frac{a_n \tau_n^2}{\sum_m a_m \tau_m} \qquad \text{(S2)}$$

Table T1. Fluorescence lifetime fit parameters

emission wavelength / nm	a_1	τ_1 / ns	a_2	τ_2 / ns	<τ> / ns	χ^2_{red}
450 ± 7.25	0.89	0.78	0.11	5.44	3.00	1.1
460 ± 7.25	0.89	0.81	0.11	5.88	3.18	1.2

HPLC. HPLC was carried out using the following gradient with 0.1% TFA as buffer:

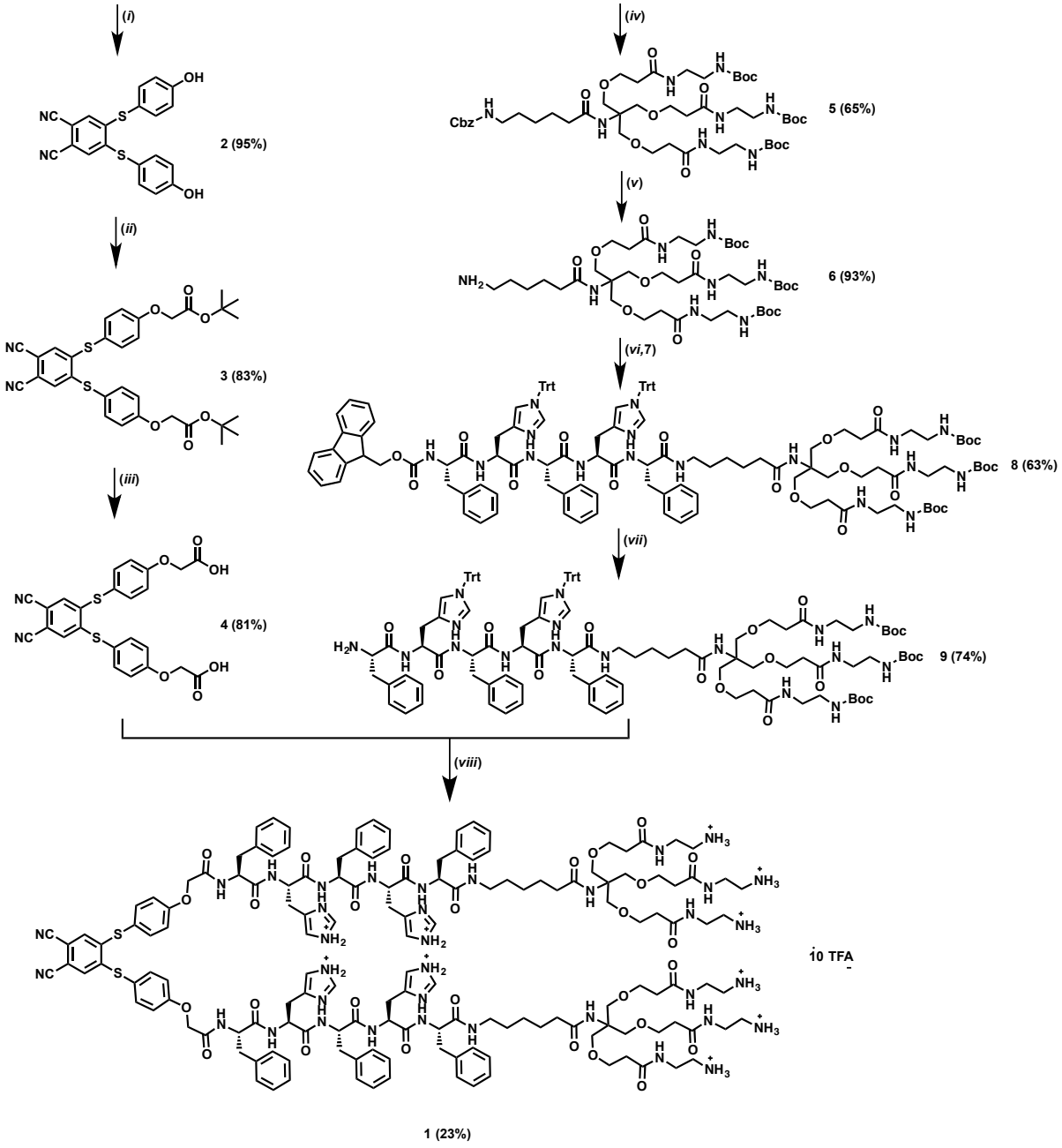
t/min	A (H_2O/ACN = 95/5)	B (ACN)
0	100%	0%
5	100%	0%
50	50%	50%
55	0%	100%
70	0%	100%
75	100%	0%
85	100%	0%

Preparative HPLC was carried out on a semipreparative VariTide RPC Column at 18.9 mL/min flowrate. Analytical HPLC was carried out on a CS-GmbH MultoHigh Phenyl – 5µ column using a 1 mL/min flowrate.

Transmission electron microscopy (TEM). In brief, 5 µL sample droplets were adsorbed for 2 min on freshly glow-discharged copper grids (Electron Microscopy Sciences; CF300-CU) covered by a thin, continuous carbon film. The grids were then negatively stained with 2.0% uranyl acetate (Polysciences) for 1 min before blotting with filter paper (Whatman no. 4). All images were recorded with a FEI Tecnai T12 electron microscope equipped with a LaB_6 cathode and operated at 120 kV. Digital electron micrographs were recorded with a 4k x 4k CMOS camera (TVIPS) under minimal dose conditions.

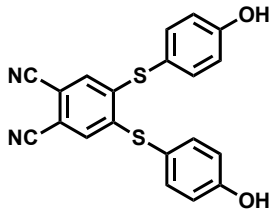
2. Synthesis

Synthetic route of **1**



Scheme 1: (*i*) K_2CO_3 (6 eq), 4-hydroxy-thiophenol (3 eq), 4,5-dichlorophthalonitrile (1 eq), DMF, 24 h, 80°C; (*ii*) **2** (1 eq), K_2CO_3 (19 eq), tert-butyl bromoacetate (6 eq), 48 h, 60°C; (*iii*) **3** (1 eq.), TFA 12.5% in DCM, 1h, r.t.; (*iv*) Cbz-Ahx-Tris[2-(carboxyethoxy)methyl]methylamide (1 eq.), Boc-EDA (5 eq.), PyBOP (4 eq.), DIPEA (2.5 eq.) DCM/DMF (1:2), 10 min, 50°C; (*v*) **5** (1 eq.), Pd/C (10 wt%), H_2, 24 h, r.t.; (*vi*) **7** (1.0 eq.), **6** (1.1 eq), HOAT (1.1 eq), PyBOP, (1.2 eq), DMF, 24 h, r.t.; (*vii*) **8** (1 eq.), piperidine (5% in $CHCl_3$), 2 h, r.t.; (*viii*) 1.) **4** (1 eq), **9** (3 eq), PyBOP (3 eq), DIPEA (3 eq), DMF, 3 h, rt; 2.) TFA/H_2O/TIS (95/2,5/2,5), 1 h, rt.

2 4,5-bis((4-hydroxyphenyl)thio)phthalonitrile



To a stirred solution of potassium carbonate (3.0 g, 15.3 mmol, 6.0 eq.) in dry DMF was added 4-hydroxy-thiophenol (1.0 g, 7.65 mmol, 3.0 eq.) and the remaining suspension was heated under stirring to 80°C for 30 minutes followed by the addition of 4,5-dichlorophtalonitrile (500 mg, 2.55 mmol, 1.0 eq.) and heating was continued for further 24h. The slurry was poured carefully on 1N HCl (150 mL) and the precipitate was filtered. The residue was dissolved in EtOAc (50 mL) and extracted with distilled water until the aqueous layer became neutral. The organic layer was dried over $MgSO_4$ and evaporated to dryness.

Yield: 910 mg (2.43 mmol, 95%).

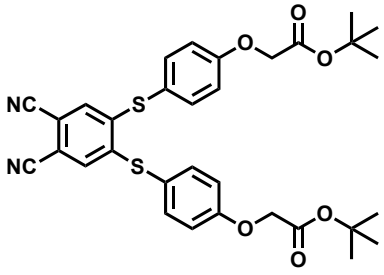
Molecular Formula: $C_{20}H_{12}N_2O_2S_2$ (yellow solid).

^{1}H NMR (400 MHz, DMSO): δ (^{1}H) = 10.25 (s, 2H, OH), 7.45 (d, J = 8.5 Hz, 4H, SCC*H*CH), 6.98 (s, 2H, CNCC*H*) 6.96 (d, J = 8.8 Hz, 4H, SCCHC*H*).

^{13}C NMR (101 MHz, DMSO): δ (^{13}C) = 160.06, 144.18, 137.58, 128.86, 117.91, 115.93, 115.47, 110.87.

ESI-HRMS: Calcd. for $[C_{20}H_{12}N_2O_2S_2Na]^+$: 399.0238, found: 399.0332.

3



To a stirred solution of **2** (100 mg, 0.27 mmol 1.0 eq.) in dry DMF was added finely ground potassium carbonate (1.00 g, 5.1 mmol, 18.9 eq.) and the slurry was heated to 60°C for 1 h, followed by the dropwise addition of bromo-tert-butyl acetic acid (300 mg, 1.54 mmol, 5.7 eq.) and a catalytic amount of postassium iodide (20 mg). The obtained solution was heated for further 48 h after which all solvents were removed in vacuo. The

remaining oil was dissolved in 30 mL of dichlomethane and extracted three times with 20 mL of distilled water. The organic layer was dried over magnesiumsulfate and the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethylacetate as eluent.

Yield: 133 mg (0.22 mmol, 83 %).

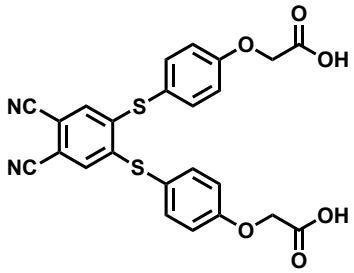
Molecular Formula: $C_{32}H_{32}N_2O_6S_2$ (yellow oil).

^{1}H NMR (400 MHz, $CDCl_3$): δ (^{1}H) = 7.49 (d, J = 8.9 Hz, 4H, SCC*H*CH), 7.03 (d, J = 8.9 Hz, 4H, SCCHC*H*), 6.89 (s, 2H, CNCC*H*), 4.60 (s, 4H, CH_2), 1.50 (s, 18H, CH_3^{tBu}).

^{13}C NMR (101 MHz, $CDCl_3$): δ (^{13}C) = 167.75, 160.49, 144.89, 137.73, 129.50, 119.86, 117.33, 115.91, 111.82, 83.33, 66.19, 28.69.

ESI-HRMS: Calcd. for $[C_{32}H_{32}N_2O_6S_2Na]^+$: 627.1599, found: 627.1609.

4



3 (133 mg, 0.22 mmol, 1.0 eq.) was dissolved in 8 mL of dry dichloromethane followed by the dropwise addition of 1 mL of trifluoroacetic acid. The remaining clear solution was stirred for 1 h followed by the removal off all solvents and freeze-drying.

Yield: 88 mg (0.18 mmol, 81%).

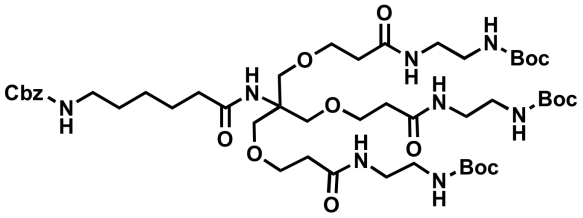
Molecular Formula: $C_{24}H_{16}N_2O_6S_2$ (colorless solid).

^{1}H NMR (300 MHz, DMSO): δ (^{1}H) = 13.13 (bs, 2H, CO_2H), 7.57 (d, J = 8.9 Hz, 4H, SCC*H*CH), 7.12 (d, J = 8.9 Hz, 4H, SCCHC*H*), 7.08 (s, 2H, CNCC*H*), 4.80 (s, 4H, CH_2).

^{13}C NMR (75 MHz, DMSO) δ (^{13}C) = 169.87, 159.68, 143.69, 136.94, 129.52, 118.45, 116.83, 115.70, 111.16.

ESI-HRMS: Calcd. for $[C_{24}H_{16}N_2O_6S_2Na]^+$: 515.0347, found: 515.0372.

5 Cbz-Dendrone (Ddn)



N-Boc-ethylenediamine (1.68 mL, 10.5 mmol, 5.0 eq.) was added to a suspension of Cbz-Ahx-Tris[2-(carboxyethoxy)methyl]methylamide[2] (1.23 g, 2.1 mmol, 1.0 eq.), PyBop (4.37 g, 8.4mmol, 4.0 eq.) and DIPEA (0.85 mL, 5.3 mmol, 2.5 eq.) in 16 mL DCM and 30 mL DMF. The reaction was stirred for 10 minutes at 50°C at 550 mPa. The reaction mixture was concentrated under reduced pressure and the crude product was precipitated out of H_2O. The crude product was purified *via* flash chromatography over SiO_2 (Chloroform/Methanol: 95/5; $R_{f\,(Chloroform/Methanol:\,8/2)}$ = 0.76).

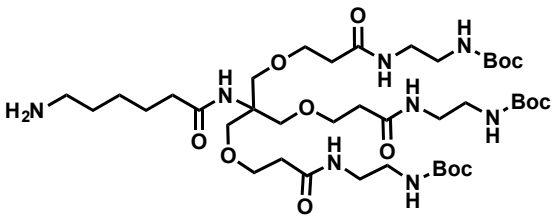
Yield: 1.38 g (1.37 mmol, 65%).

Molecular formula: $C_{48}H_{82}N_8O_{15}$ (colorless sticky solid).

ESI-HRMS (MeOH) (m/z): Calculated for $[C_{48}H_{82}N_8O_{15}Na]^+$: 1033.5797, found: 1033.5814.

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (^{1}H) = 7.85 (t, J = 5.7 Hz, 3H, N*H*CH_2CH_2NH), 7.42 – 7.27 (m, CH^{Ar}, 5H), 7.21 (t, J = 5.6 Hz, Cbz-*NH*, 1H), 6.99 (s, 1H N*H*-C_q,), 6.78 (t, J = 5.7 Hz, 3H, $NHCH_2CH_2N$*H*), 4.99 (s, 2H, CH_2^{Cbz}), 3.61 – 3.49 (m, 12H, CH_2OCH_2), 3.12 – 3.02 (m, 6H, $NHCH_2$), 3.01 – 2.91 (m, 8H, $NHCH_2$), 2.27 (t, J = 6.4 Hz, 6H, CH_2CH_2CO), 2.05 (t, J = 7.4 Hz, 2H, CH_2CO^{Ahx}), 1.55 – 1.16 (m, 33H, CH_2^{Ahx}/CH_3^{Boc}).

6 H-Ddn



5 (359.4 mg, 355.5 mmol) was stirred with 10 wt% Pd/C under an H_2 atmosphere over night. The catalyst was afterwards removed via filtration over Celite®. After removal of the solvent under reduced pressure, the desired product was obtained without further purification.

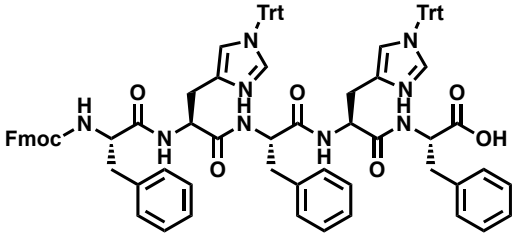
Yield: 293.8 mg (334.9 mmol, 93%).

Molecular formula: $C_{40}H_{76}N_8O_{13}$ (colorless oil).

MALDI-MS (H_2O/ACN) (m/z): $[C_{40}H_{76}N_8O_{13}Na]^+$: 877.5605, found: 877.5609.

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ = 7.89 (t, J = 5.4 Hz, 3H, N*H*CH_2CH_2NH), 7.71 (bs, 3H, NH_3), 7.02 (s, 1H, N*H*-C_q,), 6.80 (t, J = 5.7 Hz, 3H, $NHCH_2CH_2NH$), 3.69 – 3.46 (m, 12H, CH_2OCH_2), 3.12 – 2.89 (m, 12H, $NHCH_2$), 2.73 (t, J = 7.5 Hz, 2H, NH_3CH_2), 2.28 (t, J = 6.5 Hz, 6H, CH_2CH_2CO), 2.07 (t, J = 7.3 Hz, 1H, 2H, CH_2CO^{Ahx}), 1.58 – 1.12 (m, 33H, CH_2^{Ahx}/CH_3^{Boc}).

7 Fmoc-Phe-His(Trt)- Phe-His(Trt)- Phe-OH



The loading of the resin was performed according to a procedure described in literature.[3–5] The appropriate Fmoc-protected histidine (4.5 mmol, 3.0 eq.) was dissolved in 10 mL DCM/DMF (4:1) and added to the 2-chlorotrityl-chloride resin (1.0 g, 1.5 mmol) followed by the addition of 1.2 mL DIPEA. After stirring for 5 min at room temperature additional 3 mL of DIPEA were added. The reaction mixture was stirred for 1 h at room temperature and afterwards treated with 1 mL MeOH. The vessel was drained and the beads were washed consecutively three times each with DCM, DMF, DCM and MeOH. Afterwards the beads were dried under vacuum overnight.

The dried beads were swollen in DCM p.a. for 10 min while shaking the reaction vessel. After sucking off the solution, piperidine (20% in DMF) was added and the vessel was shaken for 20 min. After draining of the vessel the beads were washed four times with DMF and twice with DCM. The resin was treated with a solution of the corresponding protected amino acid (6.0 mmol, 4.0 eq.), HBTU (6.0 mmol, 4.0 eq.) and DIPEA (9.0 mmol, 6.0 eq.) in DMF. After shaking for 1 h the solution was removed and the resin was washed five times with DMF. This procedure was repeated with the corresponding amino acid for every coupling process, starting with the Fmoc deprotection on the resin. Finally the resin is washed with DCM.

The beads were stirred for 45 min in a solution of 2,2,2-trifluoro-ethanol (TFE) and DCM (2:8). Afterwards the solution was drained from the reaction vessel and the beads were washed at least two times with a small amount of DCM. The collected solutions were concentrated under reduced pressure and the product precipitated out of a 0 °C solution

of diethylether and cyclohexane (1:2). The whole procedure was repeated three times and the precipitates were combined and dried under high vacuum.

Yield: 1.86 g (1.29 mmol, 86%).

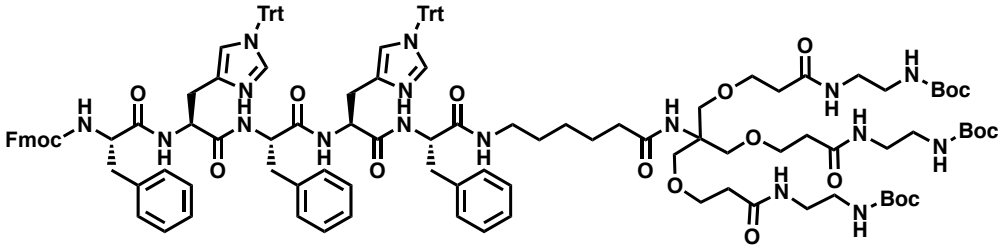
Molecular formula: $C_{92}H_{81}N_9O_8$ (colorless solid).

ESI-MS (MeOH) (m/z): $[C_{92}H_{81}N_9O_8H]^+$: 1440.63, found: 1440.68;

$[C_{92}H_{81}N_9O_8Na]^+$: 1463.61, found: 1463.68.

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (^{1}H) = 8.51 (d, J = 8.1 Hz, 1H, α-N*H*), 8.21 (d, J = 7.8 Hz, 1H, α-N*H*), 8.06 (d, J = 7.6 Hz, 1H, α-N*H*), 8.02 (d, J = 7.6 Hz, 1H, α-N*H*), 7.87 (d, J = 7.5 Hz, 2H, C*H*$^{Ar,\ Fmoc}$), 7.63 – 7.48 (m, 3H, α-N*H*/C*H*^{Ar,Fmoc}), 7.45 – 6.92 (m, 51H, C*H*^{Ar,Trt}/C*H*^{Ar,Phe} /C*H*^{Ar,Fmoc}/C*H*^{Ar,His}), 6.71 (s, 1H, C*H*^{Ar,His}), 6.63 (s, 1H, C*H*^{Ar,His}), 4.54 – 4.36 (m, 4H, α-C*H*), 4.18 (ddd, J = 11.9, 8.8, 3.7 Hz, 1H, α-C*H*), 4.10 – 3.86 (m, 3H, C*H*$_2^{Fmoc}$/C*H*^{Fmoc}), 3.08 – 2.60 (m, 10H, CH$_2^{Phe}$/CH$_2^{Phe}$).

8 Fmoc-Phe-His(Trt)- Phe-His(Trt)- Phe-Ddn



7 (270 mg, 308 µmol, 1.0 eq.) was dissolved in 10 mL DMF and HOAT (46 mg, 339 µmol, 1.1 eq.), **6** (487 mg, 339 µmol, 1.1 eq.) and PyBOP (192 mg, 639 µmol, 1.2 eq.) were added to the stirred solution at room temperature. The reaction mixture was concentrated under reduced pressure after stirring over night and purified using size exclusion chromatography *via* Sephadex LH 20 (MeOH, $R_{f\ (Chloroform/Methanol:\ 9/1)}$ = 0.31).

Yield: 446.6 mg (194.3 µmol, 63%).

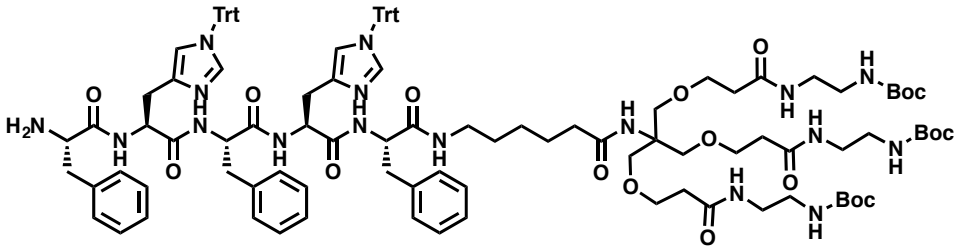
Molecular formula: $C_{132}H_{155}N_{17}O_{20}$ (colorless oil).

MALDI-MS (H_2O/ACN) (m/z): $[C_{117}H_{145}N_{17}O_{18}Na]^+$: 2299.17, found: 2299.16.

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (^{1}H) = 8.50 (d, J = 7.5 Hz, 1H, α-N*H*), 8.21 (d, J = 7.5 Hz, 1H, α-N*H*), 8.09 (d, J = 7.3 Hz, 1H, α-N*H*), 8.07 – 8.01 (m, 1H, N*H*^{Ahx}), 7.97 – 7.80 (m, 6H, α-N*H*/N*H*CH$_2$CH$_2$NH/C*H*^{Ar,Fmoc}), 7.64 – 7.52 (m, 3H, α-N*H*/C*H*^{Ar,Fmoc}), 7.45 – 6.92 (m, 52H, C*H*^{Ar,Fmoc}/C*H*^{Ar,Trt}/C*H*^{Ar,Phe}/C*H*^{Ar,His}/N*H*C$_q$), 6.80 (t, J = 5.7 Hz, 3H, NHCH$_2$CH$_2$N*H*), 6.71 (s, 1H, C*H*^{Ar,His}), 6.59 (s, 1H, C*H*^{Ar,His}), 4.56 – 4.31 (m, 4H, α-C*H*), 4.24 – 4.13 (m, 1H, α-C*H*), 4.11 – 3.86 (m, 3H, C*H*$_2^{Fmoc}$/C*H*^{Fmoc}), 3.62 – 3.44 (m, 12H, C*H*$_2$OC*H*$_2$), 3.15 – 2.59

(m, 24, CH_2^{His}/CH_2^{Phe}/NHCH_2CH_2NH/NHCH_2^{Ahx}), 2.28 (t, J = 6.4 Hz, 6H, CH_2CH_2CO), 2.01 (t, J = 7.4 Hz, 2H, CH_2CO^{Ahx}), 1.44 – 1.00 (m, 33H, CH_2^{Ahx}/CH_3^{Boc}).

9 H-Phe-His(Trt)- Phe-His(Trt)- Phe-Ddn



9 ml chloroform and 1 ml piperidine were added to **8** (400 mg, 174 µmol). It was stirred for 2 hours and another 9.5 ml chloroform and 0.5 ml piperidine were added. It was stirred for further 40 minutes, the solvent was removed under reduced pressure and the remaining solid was dissolved in 10 ml chloroform which was then removed again under reduced pressure. Another 7 ml chloroform were added and precipitated in cold diethylether. The diethylether was decanted and the solid was dissolved in 2 ml DMF and precipitated out of H_2O. After centrifugation the product was freeze-dried.

Yield: 294 mg (129 µmol, 74%).

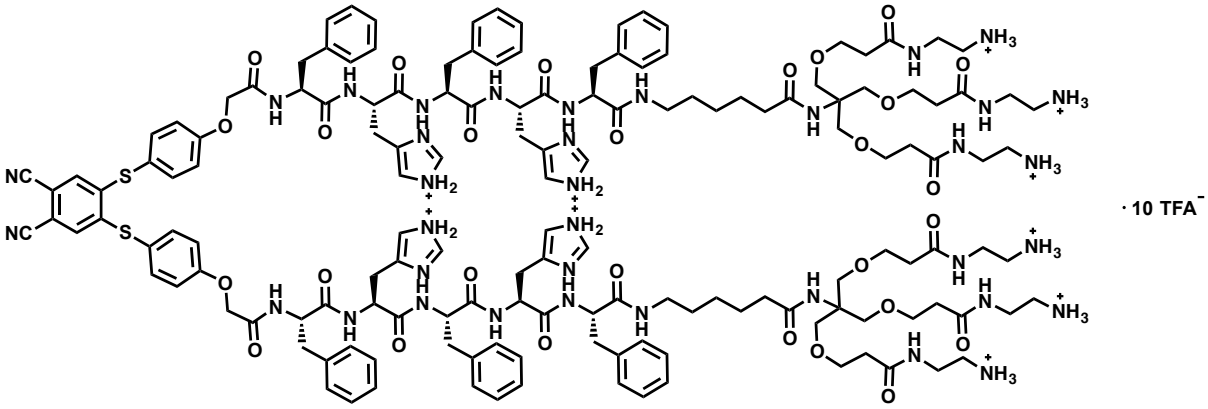
Molecular formula: $C_{117}H_{145}N_{17}O_{18}$ (colorless solid).

MALDI-MS (H_2O/ACN) (m/z): $[C_{117}H_{145}N_{17}O_{18}H]^+$: 2077.10, found: 2077.21;

$[C_{117}H_{145}N_{17}O_{18}Na]^+$: 2099.24, found: 2099.27.

^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ (^{1}H) = 8.78 (d, J = 7.5 Hz, 1H, α-N*H*), 8.23 (d, J = 7.5 Hz, 1H, α-N*H*), 8.07 – 7.97 (m, 2H, α-N*H*/NH^{Ahx}), 7.92 – 7.81 (m, 4H, α-N*H*/N*H*CH_2CH_2NH), 7.43 – 6.92 (m, 48H, C$H^{Ar,Trt}$/C$H^{Ar,Phe}$/C$H^{Ar,His}$/N*H*C_q), 6.79 (t, J = 5.6 Hz, 3H, $NHCH_2CH_2$N*H*), 6.55 (s, 1H, C$H^{Ar,His}$), 6.45 (s, 1H, C$H^{Ar,His}$), 4.53 – 4.28 (m, 4H, α-C*H*), 3.59 – 3.46 (m, 12H, CH_2OCH_2), 3.37 – 3.35 (m, 1H, α-CH^{Phe}), 3.11 – 2.61 (m, 24, CH_2^{His}/CH_2^{Phe}/NHCH_2CH_2NH/NHCH_2^{Ahx}), 2.27 (t, J = 6.5 Hz, 6H, CH_2CH_2CO), 2.01 (t, J = 7.5 Hz, 2H, CH_2CO^{Ahx}), 1.45 – 1.01 (m, 33H, CH_2^{Ahx}/CH_3^{Boc}).

1 BPTP(Phe-His-Phe-His-Phe-Ddn)$_2$



4 (7.0 mg, 13.6 µmol, 1 eq.), **9** (85.0 mg, 40.1 µmol, 3.0 eq.), PyBOP (20.8 mg, 40.1 µmol, 3.0 eq.) and DIPEA (6.5 µL, 40.1 µmol, 3.0 eq.) were dissolved in a small amount of DMF. The reaction mixture was stirred for 3 h at room temperature. After concentration under reduced pressure, the residue was purified via Sephadex LH 20 (MeOH). The first eluating fraction was collected and treated with 5 ml of a mixture of TFA/H_2O/TIS (95/2.5/2.5) and stirred for 1 h. All volatile compounds were removed under reduced pressure and the same amount of deprotecting mixture was added again and stirred for 1 h. The residue was purified via preparative HPLC after concentration under reduced pressure.

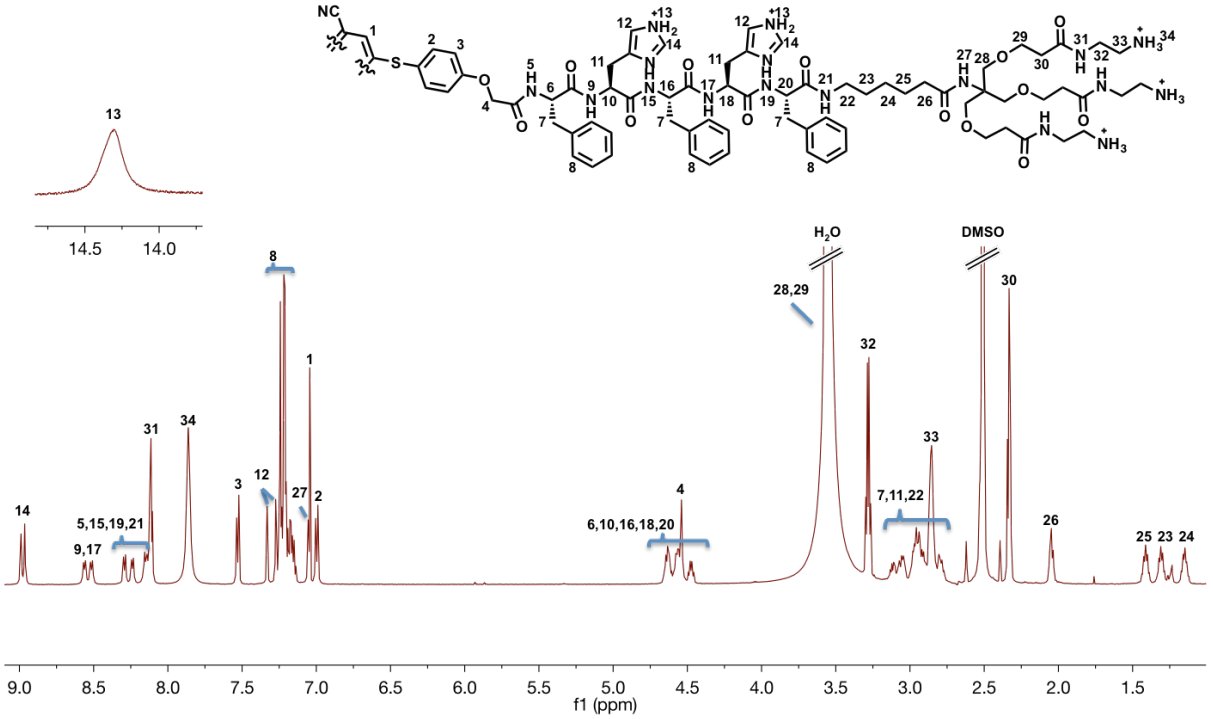
Yield: 12.8 mg (3.318 µmol, 23%).

Molecular formula: $C_{152}H_{198}N_{36}O_{28}S_2$ (colorless solid).

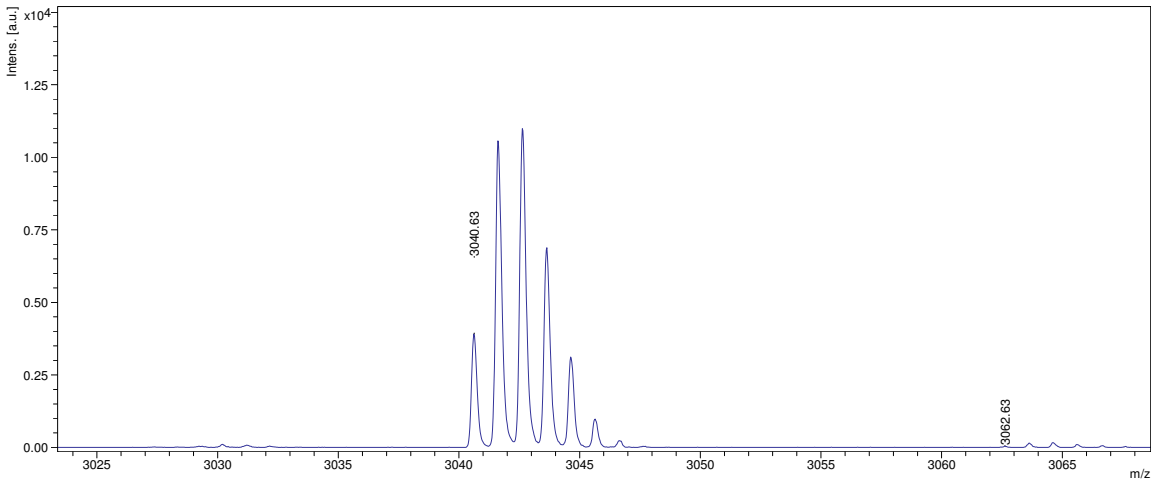
MALDI-MS (H_2O/ACN) (m/z): $[C_{152}H_{198}N_{36}O_{28}S_2Na]^+$: 3040.47, found: 3040.63.

^{1}H NMR (600 MHz, DMSO-d_6, 298 K): δ (^{1}H) = 14.31 (s, 8H, NH_2^{His}), 8.99 (s, 2H, NH_2CHN^{His}), 8.96 (s, 2H, NH_2CHN^{His}), 8.56 (d, J = 8.0 Hz, 2H, α-NH^{His}), 8.51 (d, J = 8.2 Hz, 2H, α-NH^{His}), 8.29 (d, J = 8.0 Hz, 2H, α-NH^{Phe}), 8.24 (d, J = 7.5 Hz, 2H, α-NH^{Phe}), 8.18 – 8.13 (m, 4H, α-NH^{Phe}/NH^{Ahx}), 8.11 (t, J = 5.8 Hz, 6H, NHCH$_2$CH$_2$NH$_3$), 7.86 (s, 18H, NH$_3$), 7.53 (d, *J* = 8.6 Hz, 4H, SCC*H*CH), 7.33 (s, 2H, *CHNH$_2^{His}$*), 7.27 (s, 2H, *CHNH$_2^{His}$*), 7.26 – 7.12 (m, 30H, C$H^{Ar,\ Phe}$), 7.05 (s, 2H, NHC_q), 7.04 (s, 2H, CNCC*H*), 7.00 (d, J = 9.0 Hz, 4H, SCCHC*H*), 4.67 – 4.43 (m, 28H, α-NH^{Phe}/α-NH^{His}/OCH_2CO), 3.59 – 3.55 (m, 24H, CH_2OCH_2), 3.35 – 3.24 (m, 12H, NHCH_2), 3.16 – 2.74 (m, 36H, CH_2^{His}/CH_2^{Phe}/CH_2NH$_3$/NHCH_2^{Ahx}), 2.33 (t, J = 6.6 Hz, 12H, CH$_2$CH_2CO), 2.05 (t, J = 7.5 Hz, 4H, CH_2CO^{Ahx}), 1.47 – 1.35 (m, 4H, CH_2CH$_2$CO^{Ahx}), 1.34 – 1.27 (m, 4H, NHCH$_2$CH_2^{Ahx}), 1.19 – 1.09 (m, 4H, NHCH$_2$CH$_2$CH_2^{Ahx}).

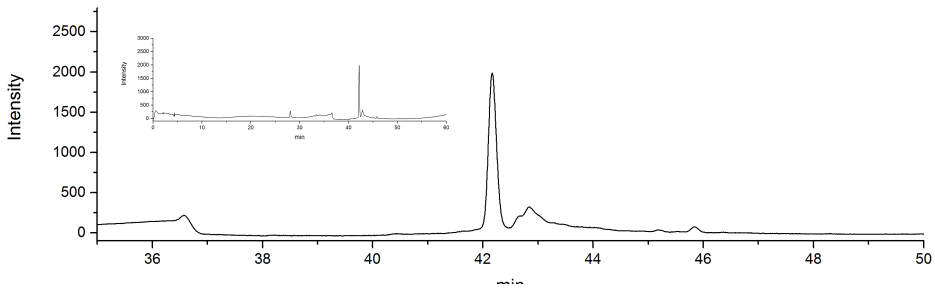
^{1}H-NMR spectrum of **1**



MALDI-MS spectrum of **1**



Analytical HPLC trace of **1**



3. Additional experimental data

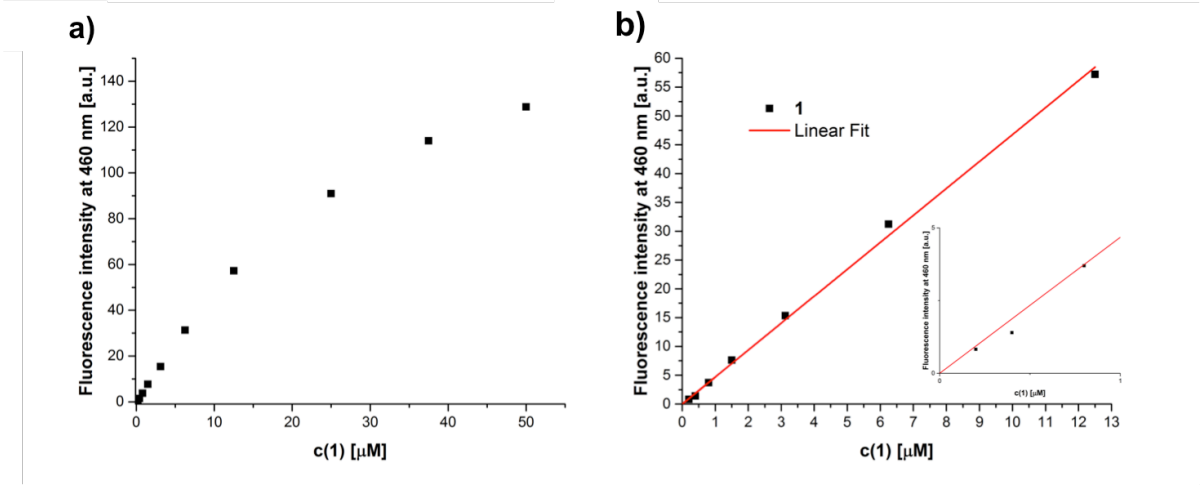


Figure S1: a) Maximum intensity of the fluorescence at λ_{em} = 460 nm (λ_{exc} = 285 nm) at different concentrations of **1** in phosphate buffer (10 mM, pH 7.4). b) Linear fit of the fluorescence intensity at λ_{em} = 460 nm in the concentration regime between 200 nM to 12.5 µM.

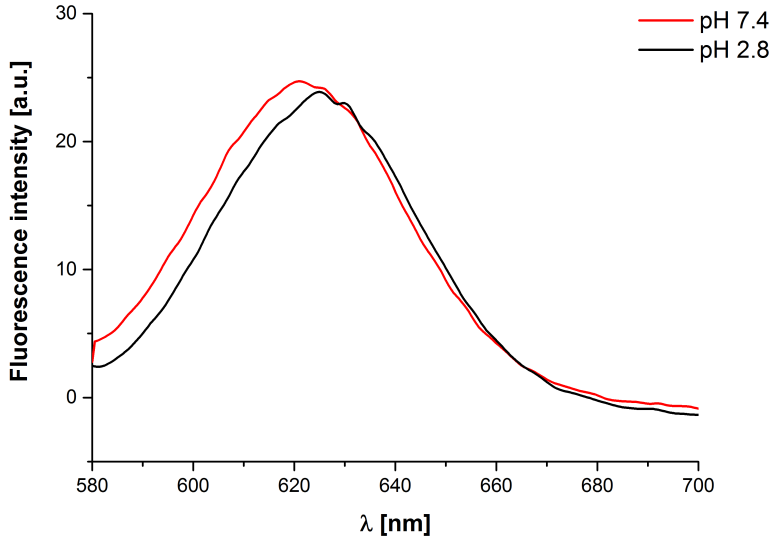


Figure S2: Fluorescence emission of Nile Red (5 µM) and **1** (25 µM) in 10 mM phosphate buffer (λ_{exc} = 550 nm) at different pH values.

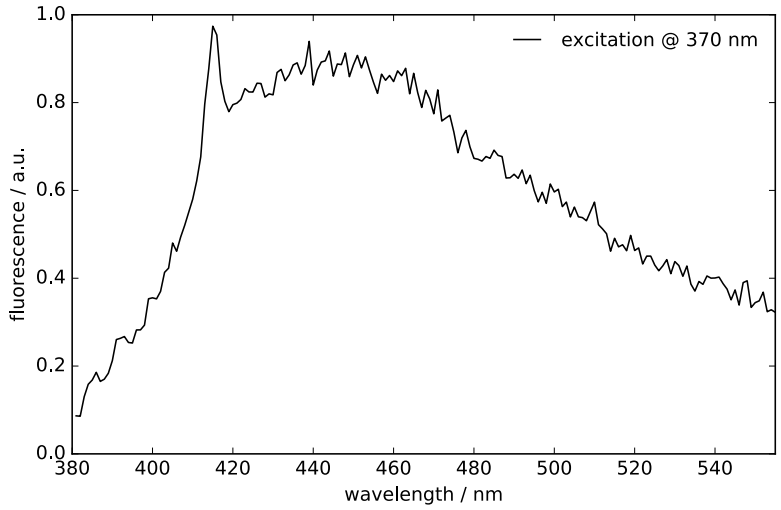


Figure S3: Fluorescence emission of **1** (25 µM) in MeOH (λ_{exc} = 370).

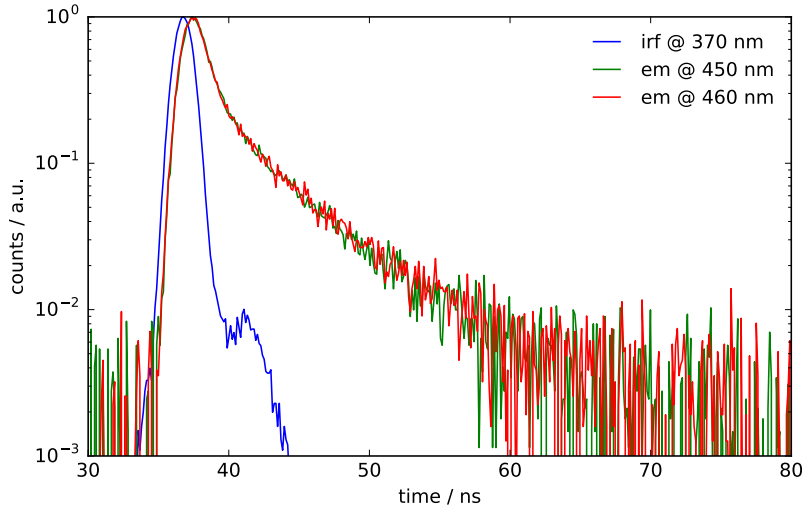


Figure S4: Fluorescence decays of **1** (25 µM) in MeOH at λ_{em} = 450 nm (green curve) and λ_{em} = 460 nm (red curve), with an excitation wavelength λ_{exc} = 370.

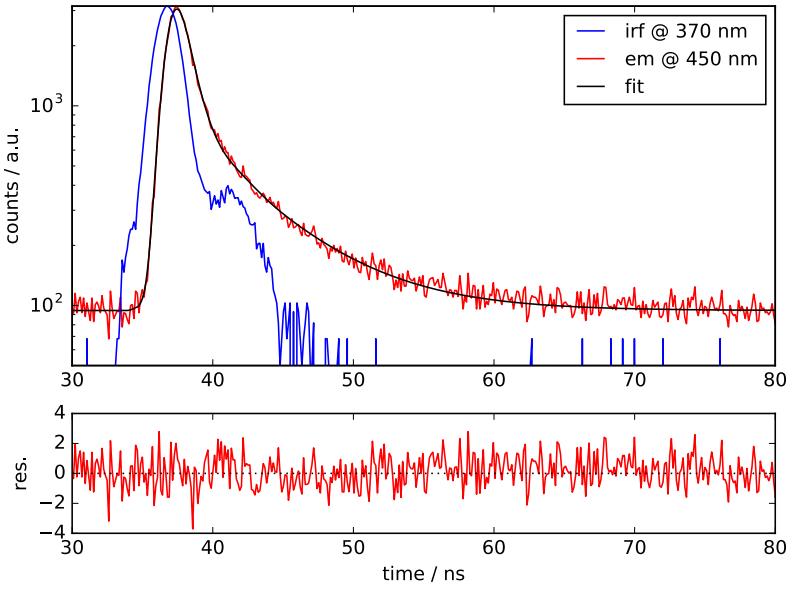


Figure S5: Fluorescence decay of **1** (25 μM) in MeOH at λ_{em} = 450 nm (red curve), with an excitation wavelength λ_{exc} = 370. The decay curve was fitted (black curve) using equation S1, page S3. From the individual amplitudes and lifetime components $\boldsymbol{a_n}$ and $\boldsymbol{\tau_n}$, an intensity weighted average fluorescence lifetime $< \boldsymbol{\tau} >$ was calculated (equation S2 and table T1, page S3).

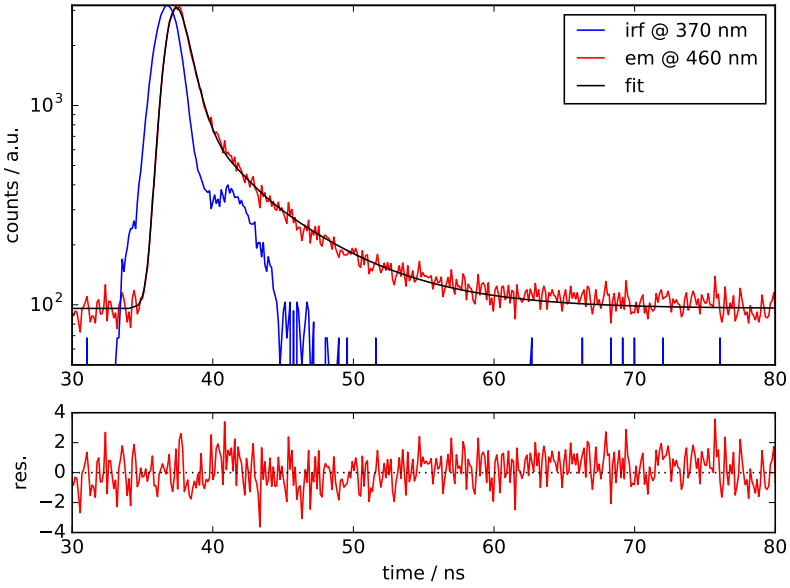


Figure S6: Fluorescence decay of **1** (25 μM) in MeOH at λ_{em} = 460 nm (red curve), with an excitation wavelength λ_{exc} = 370. The decay curve was fitted (black curve) using equation S1, page S3. From the individual amplitudes and lifetime components $\boldsymbol{a_n}$ and $\boldsymbol{\tau_n}$, an intensity weighted average fluorescence lifetime $< \boldsymbol{\tau} >$ was calculated (equation S2 and table T1, page S3).

4. References

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