

Supporting Information  
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# Probing the folding of peptide-polymer conjugates using the $\pi$ -dimerization of viologen end-groups

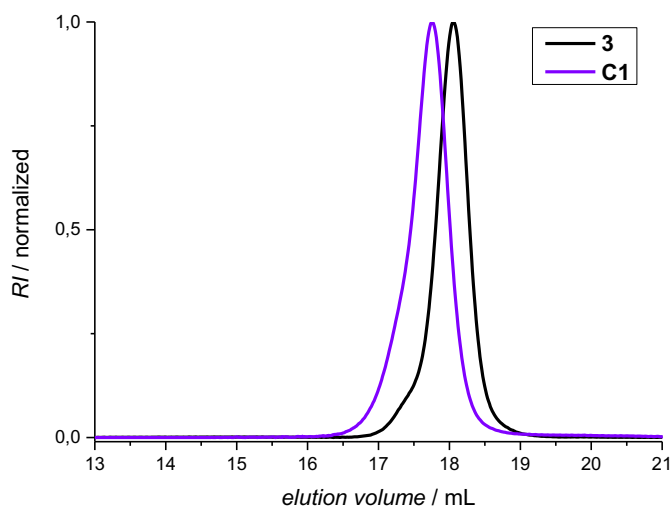
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## 1. Additional Experimental Data

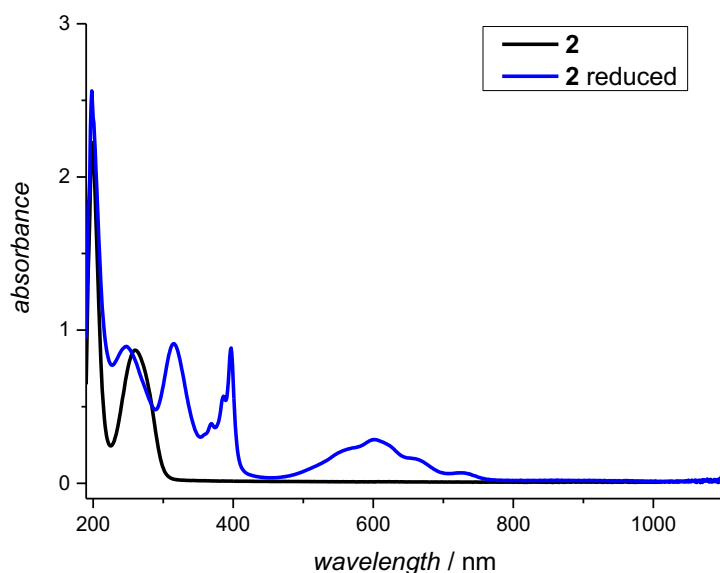


**Figure S1.** GPC elugrams of the PEG (3000) diamine **3** and the conjugate **C1** in HFIP.

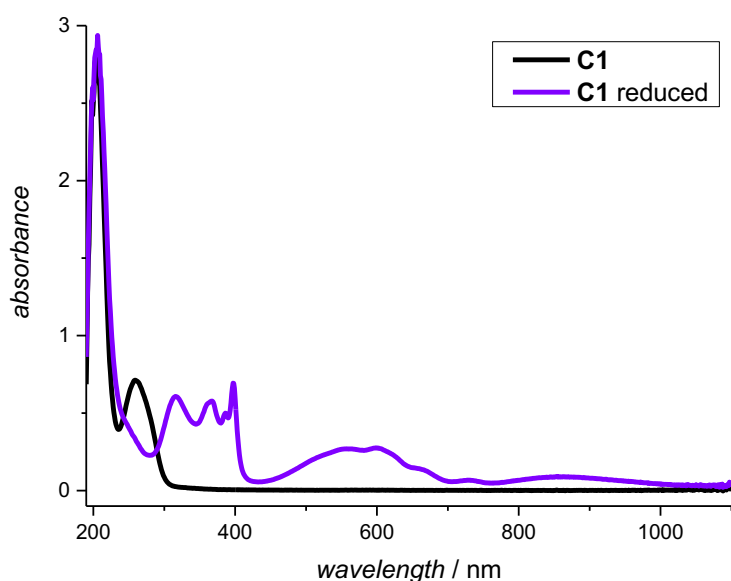
**Table S1:** Analytical data of **3** and **C1**.

	$X_n(\text{NMR})^a$	$M(\text{GPC})^b / \text{g mol}^{-1}$	$\bar{D}^b$
<b>3</b>	68	23000	1.67
<b>C1</b>	68	31000	1.76

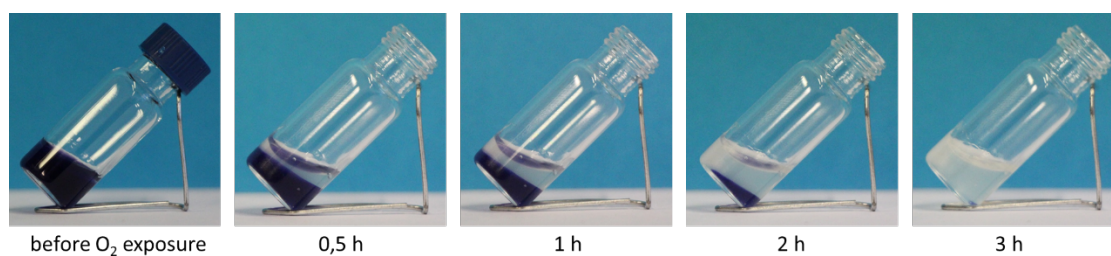
<sup>a</sup> Determined by  $^1\text{H-NMR}$ . <sup>b</sup> Determined by HFIP GPC relative to PMMA standards.



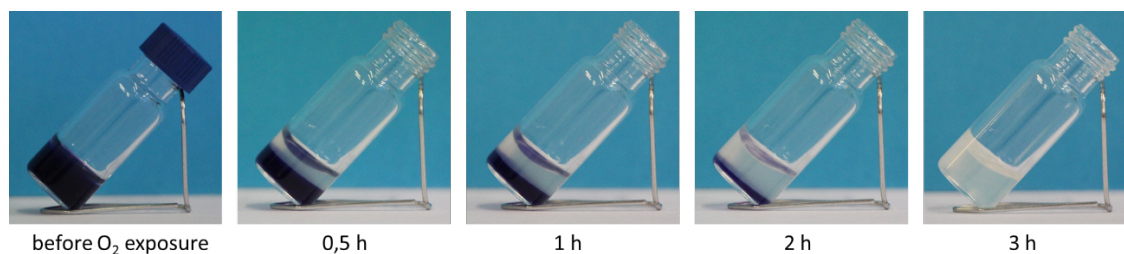
**Figure S2.** UV-Vis absorption spectra of **2** (25  $\mu\text{M}$ ) in 10 mM phosphate buffer at neutral pH in the oxidized state and after one-electron reduction using sodium dithionite (0.2 mM).



**Figure S3.** UV-Vis absorption spectra of **C1** (25  $\mu\text{M}$ ) in 10 mM phosphate buffer at neutral pH in the oxidized state and after one-electron reduction using sodium dithionite (0.2 mM).



**Figure S4.** Hydrogel of **C2** (1.5% w/v) with **C1** (0.2 mM or 0.5% w/v) in 400  $\mu\text{L}$  phosphate buffer (10 mM) and sodium dithionite (0.8 mM) under an argon atmosphere (left picture) and after exposure to air over time.



**Figure S5.** Hydrogel of **C2** (2.5% w/v) with **C1** (0.2 mM or 0.5% w/v) in 400  $\mu\text{L}$  phosphate buffer (10 mM) and sodium dithionite (0.8 mM) under an argon atmosphere (left picture) and after exposure to air over time.

## 2. Instrumentation

### pH-electrode

All pH-values were adjusted using the MI-410 Micro-Combination pH-probe. The electrode was calibrated using Mettler-Toledo certified buffer solutions at pH 4.01 and pH 10. All pH values were adjusted using aqueous NaOH or HCl solutions, the samples were measured after the pH value was stable.

### Mass spectrometry

The mass-spectroscopic analyses were performed by the mass spectroscopic department of the Johannes Gutenberg-University in Mainz. ESI (electrospray ionization) were executed on an Agilent 6545 QTOF-MS spectrometer. All test samples were prepared at a concentration of 0.1 g/L using MeOH as solvent. MALDI samples were measured on an Autoflex maX MALDI-TOF/TOF device. The corresponding matrices used are stated in the synthetic procedures.

### NMR-spectroscopy

NMR spectra were recorded on a BRUKER ARX 300 spectrometer and a BRUKER Avance II 400 spectrometer. All measurements were carried out using DMSO- $d_6$  or  $CDCl_3$  as deuterated solvent. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) relative to the chemical shifts of the residual protons in the deuterated solvent. The spin multiplicities of the signals are assigned as follows: s (br) (singlet (broad)), s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). All measured coupling constants are stated in Hertz (Hz). All NMR spectra were analyzed using the software MestReNova Version: 11.0.4-18998.

### GPC

Gel permeation chromatography (GPC) was performed using HFIP, which contained 3 g L<sup>-1</sup> potassium trifluoroacetate (KTFA) as eluent at 40 °C and a flow rate of 0.8 mL min<sup>-1</sup>. GPC columns were packed with modified silica (PFG columns, particle size: 7  $\mu$ m porosity: 100 Å and 1000 Å, respectively). Poly(methyl methacrylate) standards (PMMA, Polymer Standards Services GmbH) were used for calibration and toluene was used as the internal standard. A refractive index detector (G1362A RID) and an UV/Vis detector (230 nm; Jasco UV-2075 Plus) were used for polymer detection.

### **Circular dichroism (CD) spectroscopy**

All spectra were recorded using a peptide-polymer conjugate concentration of 50  $\mu\text{M}$  in 10 mM phosphate buffer using a quartz cell with a path length of 2 mm or 1 mm. The pH values were adjusted by addition of aqueous HCl and NaOH. CD-spectra were recorded on a J-815 CD spectrometer (JASCO) using the software Spectra Manager 2.08.04. The low peptide-polymer conjugate concentration made sure that the HT signal was lower than 600 V over the whole spectra for  $\lambda > 210$  nm. An average of three scans was reported. All spectra were corrected by the subtraction of the buffer (background). All data was processed using OriginPro 9.1.

### **Transmission electron microscopy (TEM)**

TEM samples were prepared from 50  $\mu\text{M}$  or 100  $\mu\text{M}$  solutions in 20 mM TRIS buffer, the pH was adjusted by the addition of HCl and NaOH. 5 $\mu\text{L}$  sample droplets were absorbed for 1 min on freshly glow-discharged copper grids (CF300-Cu, 300 mesh) coated with a 3-4 nm carbon layer. The grids were then negatively stained using a 2% v/v solution of uranyl acetate for 15 s. TEM samples which were reduced with sodium dithionite were prepared under an argon atmosphere, to prevent the oxidation of the radical cation. TEM images were accomplished on a FEI Tecnai<sup>TM</sup> T12 transmission electron microscope equipped with a BioTWIN lens and a LaB6 cathode operated at 120 kV. Digital electron micrographs were recorded with a 4k x 4k CMOS camera (TVIPS) and a 1k x 1k CCD camera (MegasSYS).

### **UV-Vis Spectrophotometry**

UV/Vis absorption of the samples was analyzed using a Perkin Elmer – Lambda 25 UV-Vis spectrometer. The measurements were executed in 10 mm glass cuvettes. All analyses were performed at room temperature and ambient pressure. A cuvette with 10 mM phosphate buffer was used as reference sample. The instrument settings were: 1 nm slit width, 1 nm wavelength stepping at a speed of 400 nm/min, 200–1100 nm wavelength range.

### 3. Materials and Methods

All reactions involving air- and moisture-sensitive compounds or intermediates were performed under argon atmosphere using standard Schlenk techniques. The glassware was dried in an oven at 120 °C or heat gun dried under high vacuum prior to use. All reagents and solvents were added using disposable syringes and needles through septa. Solids were added using an argon or nitrogen counter flow. Degassing of solvents was achieved by bubbling argon through the solution for at least 10 minutes. The vacuum level used for the removal of organic solvents was about 1 mbar and 0.1 mbar for the removal of water (Christ Alpha 1-2 LD plus freeze dryer).

#### Solvents and reagents

Unless stated otherwise, all solvents and reagents were obtained from commercial sources in the highest purity available and used without further purification. The list of suppliers includes SIGMA-ALDRICH (Sigma-Aldrich Chemie GmbH, Taufkirchen) ACROS ORGANICS (Thermo Scientific GmbH, Nidderau), MERCK (Merck KGaA, Darmstadt), ALFA AESAR (Alfa Aesar GmbH & Co. KG, Karlsruhe), CARBOLUTION Chemicals (Carbolution Chemicals GmbH, Saarbrücken), BACHEM (Bachem, Bubendorf) and IRIS BIOTECH (Iris Biotech GmbH, Marktredwitz). Water was demineralized prior to use. DMF, NMP and Piperidine were purchased in peptide grade quality. Solvents used for air- and moisture-sensitive reactions were purchased anhydrous.

#### Chromatography

Qualitative thin layer chromatography was carried out on silica-coated aluminium sheets (60, F254) with a fluorescence indicator from MERCK. The indication of the analytes was achieved by irradiation of the TLC plates with UV light ( $\lambda = 254$  nm). Alternatively, the plates were dipped into a KMnO<sub>4</sub>, cerium molybdate or ninhydrin solution followed by heating. Size exclusion chromatography was performed using a Sephadex® LH-20 column with methanol as the eluent.

#### Gelation Experiments for Oxygen Diffusion

2 mg of **C1** (final concentration: 0.5 wt% or 0.2 mM) and varying amounts of **C2** were weighed in a vial and set under an argon atmosphere. The mixture was dissolved in 200  $\mu$ L degassed acidic 10 mM phosphate buffer. Afterwards 200  $\mu$ L degassed basic

10 mM phosphate buffer with 1.6 mM sodium dithionite (final concentration: 0.8 mM) was added. The pH value of the final solution was basic, leading to hydrogel formation within minutes. Afterwards the vial was opened and the argon atmosphere was replaced with air. The vial was left open to the atmosphere and the oxidation of the viologen radical cation was followed optically over time.

## 4. Synthesis

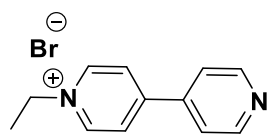
### Synthesis of Peptides via SPPS

The loading of the 2-chlorotrityl chloride resin was performed according to literature procedures.<sup>[1]</sup> The first Fmoc-protected amino acid (2.0 eq. relative to the resin loading capacity) to be coupled to the 2-chlorotrityl chloride resin (1.0 g, loading capacity 1.6 mmol/g) was dissolved in 10 mL DCM. A slight amount of DMF was added due to the low solubility of some amino acids in pure DCM. The solution was added to the vessel containing the resin under an argon atmosphere. DIPEA (2.0 eq. relative to the resin loading capacity) was added and the mixture was shaken for 5 minutes at room temperature. This was followed by the addition of additional DIPEA (3 eq.). The reaction mixture was shaken for one hour at room temperature followed by the addition of MeOH (1 mL/g resin) and shaken for 15 minutes. After draining the vessel, the resin was washed consecutively three times with 10 mL DCM, DMF, DCM and MeOH. The resin was dried *in vacuo* overnight.

The following step-wise chain elongation was performed using a CS136XT peptide synthesizer, which is an automated batch peptide synthesizer. The beads were swollen in DCM while shaking the reaction vessel. After draining the DCM, a piperidine solution (20% in DMF) was added to the vessel, which was shaken for 20 minutes. Afterwards, the vessel was drained and the beads were washed four times with DMF and two times with DCM. After the addition of the Fmoc-protected amino acid (4.0 eq relative to the resin loading capacity), HBTU (4.0 eq) and DIPEA (6.0 eq) in DMF were added to the reaction vessel. After shaking for one hour, the solution was removed and the beads were washed with DMF five times. This procedure was repeated for the following amino acids, starting with the Fmoc deprotection of the resin-bound amino acid. In the final step the resin was washed with DCM.

To cleave the resin-bound peptide the beads were shaken in a mixture of DCM and trifluoroethanol (4/1) for 45 minutes. The solution was drained and the beads washed two times with a small amount of DCM. The collected solutions were concentrated under reduced pressure. The product precipitated out of diethyl ether and was isolated through centrifugation. The procedure was carried out three times.

## 1-ethyl-4,4'-dipyridyl bromide (1)



1

4,4'-dipyridyl (5g, 32.1 mmol, 1 eq.) was dissolved in 40 mL of benzene in a flask equipped with a stir bar. Ethylbromide (24 mL, 320.5 mmol, 10 eq.) was added to the solution and the mixture was stirred under reflux (90 °C) overnight. A green/yellow precipitate could be observed the next day. The solution was cooled down and then stirred with 170 mL of toluene for 2 hours at room temperature. The precipitate was isolated through vacuum filtration and repeatedly washed with toluene. Drying in high vacuum resulted in a pale grey solid.

**Yield:** 4.847g (18.3 mmol, **57%**), pale grey solid.

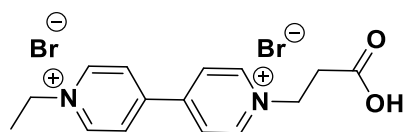
**Molecular formula:** C<sub>15</sub>H<sub>18</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>.

**ESI-HRMS (MeOH) (m/z):** Calculated for [C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup>: 185.1073 found: 185.1073.

**<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>, 298 K):** δ/ppm: 9.28 (d, *J* = 7.0 Hz, 2H, *H*<sub>2</sub>/*H*<sub>6</sub>), 8.89-8.85 (m, 2H, *H*<sub>2</sub>'/*H*<sub>6</sub>'), 8.65 (d, *J* = 6.9 Hz, 2H, *H*<sub>3</sub>/*H*<sub>5</sub>), 8.08-8.03 (m, 2H, *H*<sub>3</sub>'/*H*<sub>5</sub>'), 4.68 (q, *J* = 7.3 Hz, 2H, CH<sub>2</sub>), 1.58 (t, *J* = 7.3 Hz, 3H, CH<sub>3</sub>).

**<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>, 298 K):** δ/ ppm 152.15 (*para*-C), 150.97 (2C, *ortho*-C'), 145.15 (2C, *ortho*-C), 140.87 (*para*-C'), 125.37 (2C, *meta*-C), 121.92 (2C, *meta*-C'), 55.96 (CH<sub>2</sub>CH<sub>3</sub>), 16.34 (CH<sub>3</sub>).

## 1-ethyl-1'-(3-propionic acid)-4,4'-dipyridyliumdibromide (2)



2

**1** (3 g, 11.3 mmol, 1 eq.) and 3-bromopropionic acid (17.3 g, 113 mmol, 10 eq.) were dissolved in 100 mL ACN in a flask equipped with a stir bar. The mixture was stirred under reflux overnight. A green precipitate could be observed the next day. The mixture was cooled down and then stirred with 70 mL toluene for 30 minutes at room temperature. The precipitate was isolated through vacuum filtration and washed with toluene. After recrystallization in ACN the product could be isolated as a light green solid powder.

**Yield:** 3.41 (8.1 mmol, **72%**), light green solid.

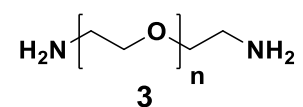
**Molecular formula:** C<sub>12</sub>H<sub>13</sub>BrN<sub>2</sub>.

**ESI-HRMS (MeOH) (m/z):** Calculated for [C<sub>12</sub>H<sub>13</sub>N<sub>2</sub>]<sup>+</sup>: 258.1368 found: 258.1363.

**<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>, 298 K):** δ/ppm: δ 12.79 (s(br), 1H, COOH), 9.46-9.39 (m, 4H, H<sub>2</sub>/H<sub>6</sub>/H<sub>2</sub>'/H<sub>6</sub>'), 8.83-8.78 (m, 4H, H<sub>3</sub>/H<sub>5</sub>/H<sub>3</sub>'/H<sub>5</sub>'), 4.90 (t, *J* = 6.7 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>COOH), 4.74 (q, *J* = 7.3 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.18 (t, *J* = 6.6 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>COOH), 1.60 (t, *J* = 7.3 Hz, 3H, CH<sub>3</sub>).

**<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>, 298 K):** δ/ ppm 171.59 (COOH), 148.95/148.58 (2C, *para-C*/*para-C*'), 146.44 (2C, *ortho-C*'), 145.68 (2C, *ortho-C*), 126.68/126.31 (4C, *meta-C*/*meta-C*'), 56.69 (2C, CH<sub>2</sub>CH<sub>3</sub>/CH<sub>2</sub>CH<sub>2</sub>COOH), 34.40 (CH<sub>2</sub>CH<sub>2</sub>COOH), 16.41 (CH<sub>3</sub>).

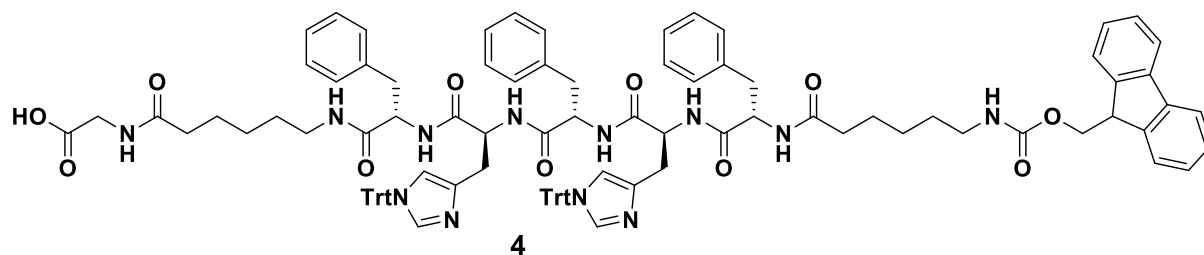
**PEG-diamine  $M_n = 3000$  (**3**)**



The synthesis of **3** was carried out according to a literature procedure.<sup>[1]</sup>

**$^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ , 298 K):**  $\delta/\text{ppm}$ : 3.51 (s(br), 268 H,  $\text{CH}_2\text{CH}_2\text{O}$ ), 3.36 (t,  $J = 5.8$  Hz, 4H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ ), 2.66 (t,  $J = 5.8$  Hz, 4H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ ).

### Fmoc-Ahx-Phe-His(Trt)-Phe-His(Trt)-Phe-Ahx-Gly-OH (**4**)



The synthesis of **4** was carried out according to the procedure “Synthesis of peptides via SPPS”.

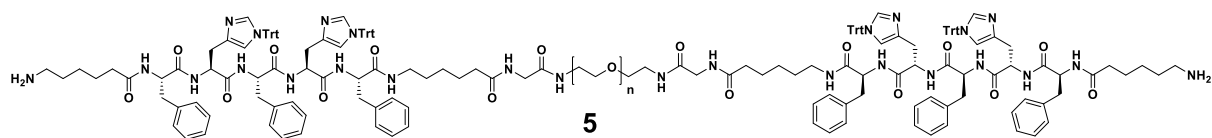
**Yield:** 905 mg (0.31 mmol), colourless solid.

**Molecular formula:** C<sub>106</sub>H<sub>106</sub>N<sub>12</sub>O<sub>11</sub>.

**ESI-HRMS (MeOH) (m/z):** Calculated for [C<sub>106</sub>H<sub>107</sub>N<sub>12</sub>O<sub>11</sub>]<sup>+</sup>: 1723.8177, found: 1723.8164.

**<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, 298 K):** δ/ppm: 12.52 (s, 1H, COOH), 8.49 (d, *J* = 7.4 Hz, 1H, α-NH), 8.16 (d, *J* = 7.5 Hz, 1H, α-NH), 8.10-8.04 (m, 2H, α-NH), 7.97 (t, *J* = 5.7 Hz, 1H, α-NH<sup>Gly</sup>), 7.93-7.86 (m, 4H, α-NH/CH<sup>Fmoc</sup>), 7.67 (d, *J* = 7.5 Hz, 2H, CH<sup>Fmoc</sup>), 7.43-6.99 (m, 52H, α-NH/CH<sup>Fmoc</sup>/Trt/CH<sup>His</sup>/CH<sup>Phe</sup>), 6.68 (s, 1H, CH<sup>His</sup>), 6.57 (s, 1H, CH<sup>His</sup>), 4.48-4.32 (m, 5H, α-CH), 4.31-4.16 (m, 3H, CH<sub>2</sub><sup>Fmoc</sup>/CH<sup>Fmoc</sup>), 3.71 (d, *J* = 5.9 Hz, 2H, CH<sub>2</sub><sup>Gly</sup>), 3.05-2.61 (m, 14H, CH<sub>2</sub><sup>Ahx</sup>/CH<sub>2</sub><sup>Phe</sup>/CH<sub>2</sub><sup>His</sup>), 2.05 (t, *J* = 7.5 Hz, 2H, CH<sub>2</sub><sup>Ahx</sup>), 1.97-1.87 (m, 2H, CH<sub>2</sub><sup>Ahx</sup>), 1.44-1.35 (m, 2H, CH<sub>2</sub><sup>Ahx</sup>), 1.29-1.21 (m, 6H, CH<sub>2</sub><sup>Ahx</sup>), 1.14-1.07 (m, 2H, CH<sub>2</sub><sup>Ahx</sup>), 1.04-0.97 (m, 2H, CH<sub>2</sub><sup>Ahx</sup>).

**H<sub>2</sub>N-Ahx-Phe-His(Trt)-Phe-His(Trt)-Phe-Ahx-Gly-PEG-Gly-Ahx-Phe-His(Trt)-Phe-His(Trt)-Phe-Ahx-NH<sub>2</sub> (5)**

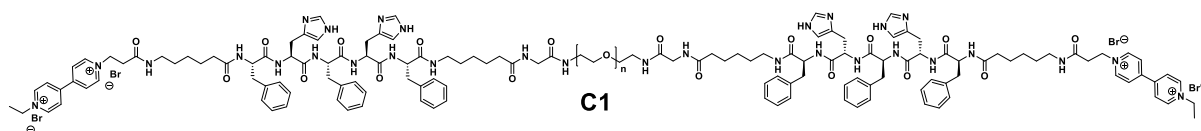


**3** (400 mg, 133  $\mu$ mol, 1 eq.) was dissolved in 8 mL DCM in a flask equipped with a stir bar. **4** (528.7 mg, 306  $\mu$ mol, 2.3 eq.), PyBOP (159.6 mg, 306  $\mu$ mol, 2.3 eq.), HOBt (41.4 mg, 230  $\mu$ mol, 2.3 eq.) and DIPEA (97  $\mu$ L, 533  $\mu$ mol, 4 eq.) were added to the solution. The mixture was stirred overnight at room temperature. Afterwards the mixture was precipitated in diethyl ether. The resulting solid was dissolved in 4 mL of DMF and 1 mL of piperidine was added. The solution was stirred for 45 min at room temperature. After the deprotection all volatiles were removed through reduced pressure. The residue was separated via size exclusion chromatography (Sephadex® LH 20, MeOH). The polymer was dissolved in water and lyophilized resulting in a colourless sticky solid.

**Yield:** 590 mg (97  $\mu$ mol, **73%**), colourless sticky solid.

**<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, 298 K):**  $\delta$ /ppm: 8.49 (d, *J* = 7.5 Hz, 2H,  $\alpha$ -NH), 8.15 (d, *J* = 7.5 Hz, 2H,  $\alpha$ -NH), 8.06 (d, *J* = 7.0 Hz, 2H,  $\alpha$ -NH), 8.01-7.84 (m, 10H,  $\alpha$ -NH), 7.41-6.98 (m, 94H, *Trt*/CH<sup>His</sup>/CH<sup>Phe</sup>), 6.67 (s, 2H, CH<sup>His</sup>), 6.57 (s, 2H, CH<sup>His</sup>), 4.50-4.34 (m, 10H,  $\alpha$ -CH), 3.65 (d, *J* = 5.9 Hz, 4H, CH<sub>2</sub><sup>Gly</sup>), 3.50 (s(br), 264H, PEG-CH<sub>2</sub>), 3.39 (t, *J* = 6.0 Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.20 (q, *J* = 5.8 Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.05-2.61 (m, 28H, CH<sub>2</sub><sup>Ahx</sup>/CH<sub>2</sub><sup>Phe</sup>/CH<sub>2</sub><sup>His</sup>), 2.06 (t, *J* = 7.5 Hz, 4H, CH<sub>2</sub><sup>Ahx</sup>), 1.99-1.90 (m, 4H, CH<sub>2</sub><sup>Ahx</sup>), 1.42-1.35 (m, 8H, CH<sub>2</sub><sup>Ahx</sup>), 1.30-1.20 (m, 8H, CH<sub>2</sub><sup>Ahx</sup>), 1.12-1.00 (m, 8H, CH<sub>2</sub><sup>Ahx</sup>).

## C1

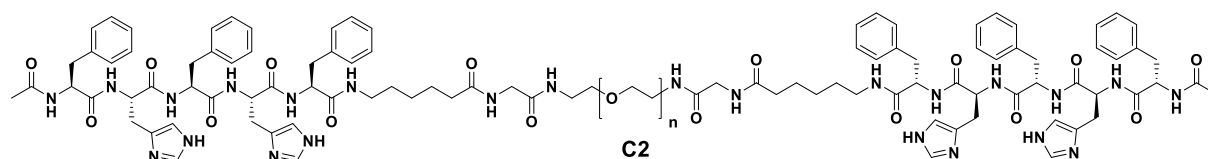


**5** (295 mg, 49  $\mu\text{mol}$ , 1 eq.) was dissolved in 2 mL DMF in a flask equipped with a stir bar. **2** (161.7 mg, 386  $\mu\text{mol}$ , 8 eq.), PyBOP (100.9 mg, 193 mmol, 4 eq.), HOBt (26.1 mg, 193  $\mu\text{mol}$ , 4 eq.) and DIPEA (53  $\mu\text{L}$ , 290  $\mu\text{mol}$ , 6 eq.) were added to the solution. The mixture was stirred for 6 hours at room temperature. Then PyBOP (50.4 mg, 96 mmol, 2 eq.) and HOBt (13 mg, 96 mmol, 2 eq.) were added to the yellow solution, which was stirred for 18 hours. Afterwards a mixture of TFA (2 mL), water (0.1 mL) and triisopropylsilane (0.1 mL) was added. The solution was stirred for 45 min at room temperature. After the deprotection all volatiles were removed through reduced pressure and the solid was codistilled with toluene. The residue was separated via size exclusion chromatography (Sephadex® LH 20, MeOH).

**Yield:** 280 mg (47  $\mu\text{mol}$ , **96%**) slightly yellow sticky solid.

**<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, 298 K):**  $\delta$ /ppm: 9.43-9.38 (m, 8H, *H*<sub>2</sub>/*H*<sub>6</sub><sup>Viologen</sup>/*H*<sub>2</sub>'/*H*<sub>6</sub>'<sup>Viologen</sup>), 8.81-8.77 (m, 8H, *H*<sub>3</sub>/*H*<sub>5</sub><sup>Viologen</sup>/*H*<sub>3</sub>'/*H*<sub>5</sub>'<sup>Viologen</sup>), 8.50-7.97 (m, 20H, *CH*<sup>His</sup>/ $\alpha$ -NH), 7.91 (t, *J* = 5.7 Hz, 2H,  $\alpha$ -NH<sup>PEG</sup>), 7.45-7.11 (m, 34H, *CH*<sup>His</sup>/*CH*<sup>Phe</sup>), 4.90 (t, *J* = 6.4 Hz, 4H, N<sup>+</sup>CH<sub>2</sub>CH<sub>2</sub>), 4.73 (q, *J* = 7.3 Hz, 4H, N<sup>+</sup>CH<sub>2</sub>CH<sub>3</sub>), 4.66-4.41 (m, 10H,  $\alpha$ -CH), 3.66 (d, *J* = 5.9 Hz, 4H, CH<sub>2</sub><sup>Gly</sup>), 3.51 (s(br), 264H, PEG-CH<sub>2</sub>), 3.40 (t, *J* = 5.8 Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.21 (q, *J* = 5.8 Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.11-2.74 (m, 28H, CH<sub>2</sub><sup>Ahx</sup>/CH<sub>2</sub><sup>Phe</sup>/CH<sub>2</sub><sup>His</sup>), 2.10 (t, *J* = 7.5 Hz, 4H, CH<sub>2</sub><sup>Ahx</sup>), 2.00-1.92 (m, 4H, CH<sub>2</sub><sup>Ahx</sup>), 1.60 (t, *J* = 7.3 Hz, 6H, N<sup>+</sup>CH<sub>2</sub>CH<sub>3</sub>), 1.47-1.42 (m, 4H, CH<sub>2</sub><sup>Ahx</sup>), 1.32-1.23 (m, 12H, CH<sub>2</sub><sup>Ahx</sup>), 1.18-1.11 (m, 4H, CH<sub>2</sub><sup>Ahx</sup>), 1.02-0.94 (m, 4H, CH<sub>2</sub><sup>Ahx</sup>).

## C2

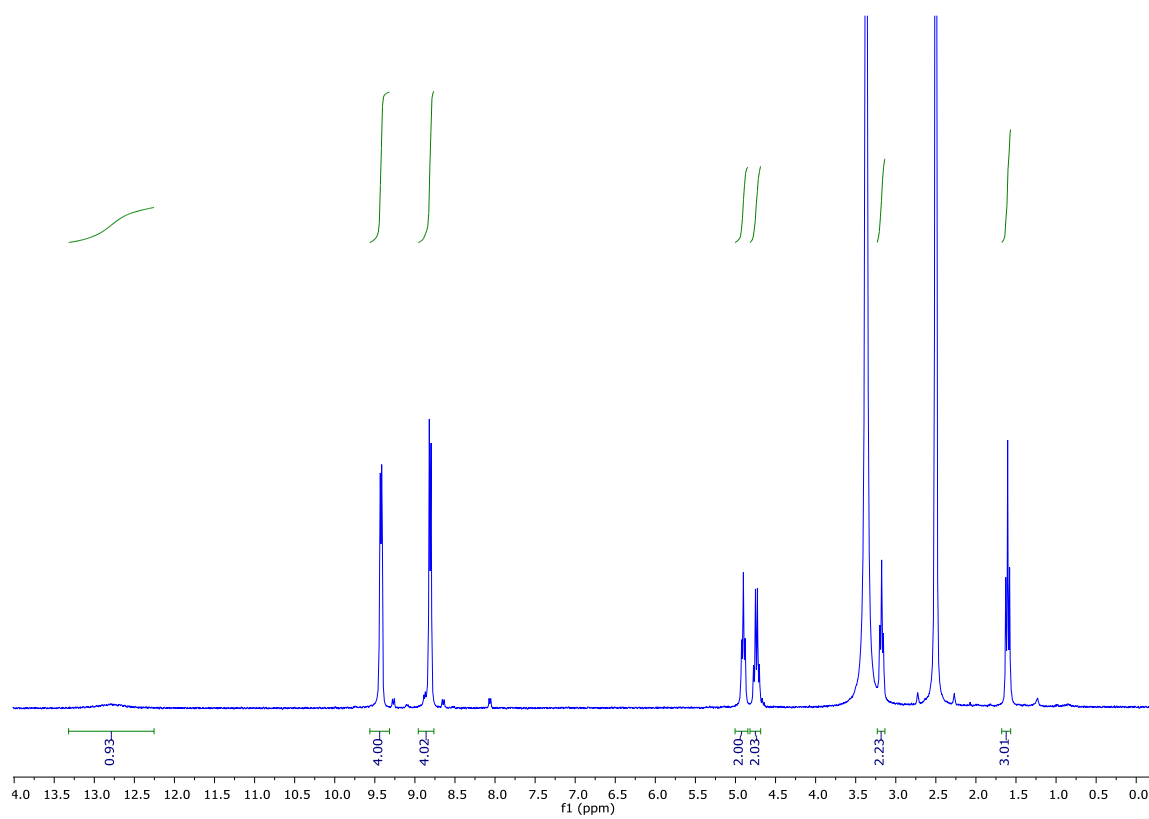


The synthesis of **C2** was carried out according to a literature procedure.<sup>[1]</sup> The utilized PEG-spacer was PEG-3000 ( $X_n = 68$ ).

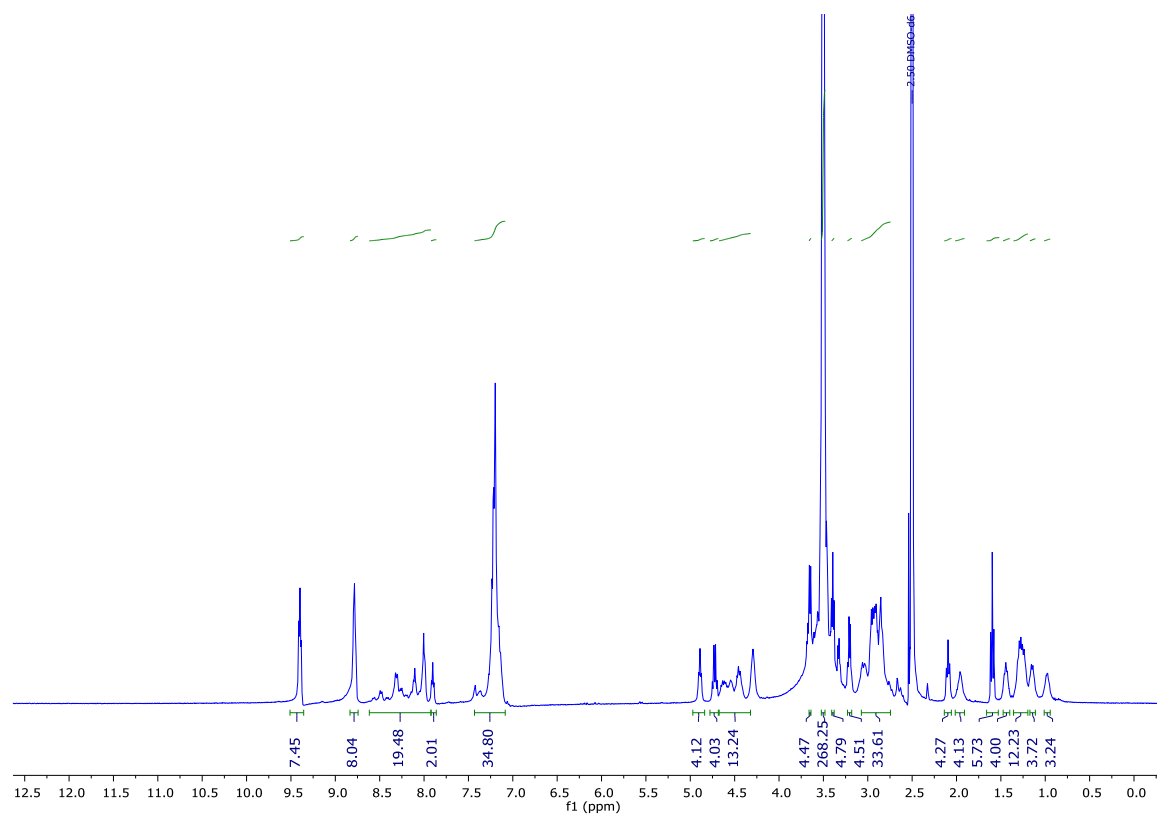
**<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>, 298 K):**  $\delta$ /ppm: 14.14 (s(br), 8H,  $\text{NH}_2^{+\text{His}}$ ), 9.04-8.92 (m, 4H,  $\text{CH}^{\text{His}}$ ), 8.48 (d, 2H,  $\alpha\text{-NH}$ ), 8.35 (d,  $J = 8.2$  Hz, 2H,  $\alpha\text{-NH}$ ), 8.21 (d,  $J = 7.5$  Hz, 2H,  $\alpha\text{-NH}$ ), 8.18-8.08 (m, 4H,  $\alpha\text{-NH}/\text{NH}^{\text{Ahx}}$ ), 8.03 (d,  $J = 7.4$  Hz, 2H,  $\alpha\text{-NH}$ ), 7.99 (t,  $J = 5.6$  Hz, 2H,  $\text{NH}^{\text{Gly}}$ ), 7.90 (t,  $J = 5.5$  Hz, 2H, PEG-NH), 7.48-7.08 (m, 34H,  $\text{CH}^{\text{His}}/\text{CH}^{\text{Phe}}$ ), 4.66-4.34 (m, 10H,  $\alpha\text{-CH}$ ), 3.65 (d,  $J = 6.0$  Hz, 4H,  $\text{CH}_2^{\text{Gly}}$ ), 3.50 (s(br), 264H, PEG-CH<sub>2</sub>), 3.39 (t,  $J = 5.8$  Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.20 (q,  $J = 5.8$  Hz, 4H, PEGCH<sub>2</sub>CH<sub>2</sub>NH), 3.11-2.70 (m, 24H,  $\text{CH}_2^{\text{His}}/\text{CH}_2^{\text{Phe}}/\text{CH}_2^{\text{Ahx}}$ ), 2.10 (t,  $J = 7.5$  Hz, 4H,  $\text{CH}_2^{\text{Ahx}}$ ), 1.74 (s, 6H,  $\text{NHCOCH}_3$ ) 1.50-1.40 (m, 4H,  $\text{CH}_2^{\text{Ahx}}$ ), 1.36-1.26 (m, 4H,  $\text{CH}_2^{\text{Ahx}}$ ), 1.19-1.10 (m, 4H,  $\text{CH}_2^{\text{Ahx}}$ ).

**MALDI-MS (DIT+KTFA, DCM/MeOH = 1/1) (m/z):** Calculated for  $[\text{C}_{234}\text{H}_{391}\text{N}_{24}\text{O}_{83}]^+$ : 4868.8, found: 4867.6; calculated for  $[\text{C}_{234}\text{H}_{390}\text{N}_{24}\text{O}_{83}\text{Na}]^+$ : 4890.8, found: 4890.0.

## 5. Spectra



**Figure S6.**  $^1\text{H}$ -NMR of **2** in  $\text{DMSO-d}_6$ , 400 MHz.



**Figure S7.**  $^1\text{H}$ -NMR of **C1** in  $\text{DMSO-d}_6$ , 400 MHz.

## 6. References

- [1] R. Otter, N. A. Henke, C. Berac, T. Bauer, M. Barz, S. Seiffert, P. Besenius, *Macromol. Rapid Commun.* **2018**, 39, 1800459.