Supporting Information

Thermoresponsive cyclic peptide – poly(2-ethyl-2-oxazoline) conjugate nanotubes

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Instrumentation

NMR spectra were recorded on a Bruker Avance DPX 300 (300 MHz) spectrometer. The TMS peak at 0.00 ppm or residual solvent peak was used as an internal reference. Signals are recorded in terms of chemical shift (in ppm), relative integral, multiplicity, coupling constants (in Hz) and assignment, in that order. The following abbreviations for multiplicity are used: s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet; sx, sextet; br, broad.

Analytical liquid chromatography coupled with mass spectrometry (LC-MS) was run using a Shimadzu 2020 LCMS system equipped with PDA and ESI detectors. A linear gradient of water to acetonitrile (each containing 0.1 % TFA) over 60 min, using C-18 reverse phase columns was used to achieve good separation.

FTIR were performed on a Varian Scimitar 800 FT-IR in DRIFTS (diffuse reflectance infrared fourier transform spectroscopy) mode. DRIFTS was conducted against a KBr background, and ATR was conducted on a ZnSe crystal.

Microwave reactions were performed in a CEM Discover SP microwave reactor. The initial power input was set to 200 W and the 5 ml vessel used was cooled using N2 throughout the reaction to maximize the microwave power input, which averaged 50 W.

A Themo Finnigan LCQ Deca ion-trap mass spectrometer (accurate to within 0.2 – 0.3 Da) was used to verify the masses of the small molecules. For most samples, electrospray ionisiation (ESI) was used as the ionisation source, except for the cyclic peptides where atmospheric-pressure chemical ionization (APCI) was employed. Most samples were run in positive ion mode. Mass calibration was performed using caffeine, MRFA, and Ultramark 1621 (Aldrich) in the *m/z* range of 195 – 1822 Da. All spectra were aquired within the *m/z* range of 200 – 2000 Da with spray voltages ranging from 2 to 5 kV, a capillary voltage of 26 V, and a capillary temperature of 275 °C. Nitrogen was used as a sheath gas (flow: 50 % of maximum). Methanol was taken as the eluent.

Size exclusion chromatography (SEC) was performed on a Polymer Laboratories PL-GPC 50 instrument equipped with a PL-AS RT autosampler and a PL-RI differential refractive index detector. The system was fitted with a PolarGel-M guard column (50 × 7.5 mm) and two PolarGel-M analytical columns (300 × 7.5 mm). Molecular weights were measured relative to a set of narrow molecular weight polystyrene standards (1 – 1600 kDa) with DMF as the eluent at a flowrate of 0.7 ml/min at 50 °C. Samples were prepared by filtration of a solution of 2 – 4 mg/ml polymer through a 0.45 μm filter. Flow rate markers were injected with the polystyrene calibrants and with each sample (0.1 % water in DMF eluent) to calibrate the retention times on each run. 0.1 % (w/w) LiBr was added to the eluent to reduce the effect of hydrogen bonding in the system.

Dynamic light scattering experiments were performed with a 633 nm wavelength laser at 90° with a 400 and 200 μm pinhole between the sample and the detector, in a decalin bath at 25 °C. Correlation functions were fit using Brookhaven software with a CONTIN model. All solvents were filtered through a 20 nm PTFE filter prior to use to eliminate dust. The polymer refractive index (RI) was used to generate a number average distribution. Solvent viscosities and RI values were taken from literature. Where the

exact viscosity and RI of a solvent mixture was not available, the weighted average viscosity was used. Static light scattering experiments were performed over an angle range of 15 – 155°, with a 1 mm pinhole. Toluene was used to calibrate the detector intensity. Temperature dependent experiments were undertaken by monitoring count rate continuously as the temperature was increased from 30 °C by 5°C over 5 minutes, followed by 5 minutes to allow for stabilization, up to 70°C, at which point temperature was increased by 5 °C over 5 minutes followed by 15 minutes to allow for stabilization, up to 80 °C. The aperture spot size was reduced from 400 to 200 μm to improve the accuracy of the measurement. The reversibility of the transition was observed multiple times (>3).

Transmission electron microscopy (TEM) images were taken on a JEOL JEM-1400 microscope at 120 kV. Sample grids were prepared by coating 400 mesh copper grids with a formvar film, and sputter coating with carbon (~10 nm). Images were taken by heating solutions to 80 °C and depositing them onto TEM grids in an oven at 80 °C.

Synthetic procedures:

Cyclo(-L-Trp-D-Leu-L-Lys(N_3)-D-Leu-)$_2$ (1): The linear two arm peptide was prepared on preloaded 2-chlorotrityl chloride resin using standard Fmoc solid phase peptide synthesis protocols (HBTU/*i*Pr_2NEt couplings) and pre-synthesized Fmoc-Lys(N_3)-OH,[1] cleaved from the resin using hexafluoro-2-propanol/CH_2Cl_2 (1:4, 3 x 15 min), and triturated to yield the product as a white solid (1.68 g, 82 % yield relative to UV calculated loading). *m/z* (ESI) 1352.6 ($[M+H]^+$, calc = 1351.8). LCMS revealed a single peak, and so the product was cyclized without further purification.

Under an atmosphere of nitrogen, 4-(4,6-dimethoxy-1,3,5-triazin)-4-methylmorpholinium tetrafluoroborate (DMTMM.BF_4, 118 mg, 0.359 mmol) was added to a solution of linear precursor (404 mg, 0.299 mmol) in anhydrous DMF (6 ml, c = 0.05 M). The resulting mixture was stirred at ambient temperature for 4 d after which time the DMF was removed under reduced pressure. MeOH was then added to provide a suspension which was subsequently cooled in an ice bath and then filtered under vacuum to collect the precipitate which was washed with cold MeOH (10 ml). The filtrate was re-concentrated under reduced pressure, suspended in MeOH and filtered, each time collecting the precipitate. This process was repeated until the filtrate was free of any precipitate. The combined precipitates were dried under high vacuum to provide the desired Boc-protected cyclic peptide (263 mg, 66%) as an off-white solid. *m/z* (APCI) = 1334.0 ($[M+H]^+$, calc = 1333.8). The Boc-protecting groups were removed by treating product with TFA/triisopropyl silane/thioanisole/water (85:5:5:5 v/v/v/v, 3 mL) for 3 h under an atmosphere of nitrogen. The reaction mixture was afterwards concentrated under reduced pressure and then triturated with cold diethylether to give the cyclic peptide **6** as an off-white solid in quantitative yield. ^{1}H-NMR (300 MHz, *d*-TFA) δ ppm: 8.22 (d, *J* = 7.8 Hz, 2H, Trp), 7.76 (d, *J* = 7.2 Hz, 2H, Trp), 7.64 (s, 2H, imidazole), 7.53-7.37 (m, 4H, Trp), 5.32 (t, *J* = 7.4 Hz, 2H, Trp α-CH), 4.95-4.86 (m, 2H, Lys α-CH), 4.81 (m, 6H, Leu α-CH), 3.41 (s, 4H, N_3-CH_2), 1.25-2.00 (m, 28H, Lys & Trp CH_2, Leu CH), 0.95 (br s, 24H, Leu CH_3). *m/z* (APCI) = 1133.9 ($[M+H]^+$, calc = 1133.7). IR (ATR, ZnSe) cm^{-1}: 3277, 2950-2850, 2098, 1687, 1624, 1541.

pEtOx Synthesis: The alkyne-functionalized pEtOx was synthesized following a previously published procedure, modified by replacing the propargyl tosylate initiator with the cheaper propargyl benzenesulfonate.[2]

Conjugation: p(EtOx) DP 20 and 40 (2.1 equivalents / peptide) were conjugated to the di-functional CP (**1**) according to our previous work. The cyclic peptide **1** (15 mg, 0.013 mmol) was suspended in TFE_2 (2.0 g) by sonication for 10 min. To this was added a solution of the polymer (2.1 or 3.0 equiv) and

CuSO4 (4 equiv, 0.053 mmol, 13 mg) in DMF (2.0 g). Sodium ascorbate (0.053 mmol, 10 mg) was added, and the mixture was stirred in a CEM Discover SP microwave reactor at 100 °C for 15 min. The initial power input was set to 200 W and the vessel was cooled using N_2 throughout the reaction to maximize the microwave power input, which averaged 50 W over the course of the reaction. The product purified by passing the concentrated crude mixture over a sephadex LH60 preparative SEC column in methanol. ^{1}H-NMR (300 MHz, *d6*-DMSO + 5 % D2O, (pEtOx40)2-CP) δ ppm: 8.40-7.96 (m, NH, triazole CH), 7.88 (m, NH), 7.61 (d, *J* = 7.75 Hz, 2H, trp H3), 7.28 (d, *J* = 7.88 Hz, 2H, trp H6), 7.10 (s, 2H, trp H2), 7.01 (t, *J* = 7.23 Hz, 2H, trp H4), 6.91 (t, *J* = 7.51 Hz, 2H, trp H5), 4.75-4.07 (m, 16H, 8 α-CH, 4 N-CH_2-C=C, 4 CH_2-OH), 3.35 (br s, 320H, N-CH_2-CH_2-), 2.27 (br s, 160H, CO-CH_2-), 0.97 (br s, 140H, CO-CH_2-CH_3), 0.84-0.64 (m, 16H, leu CH_3).

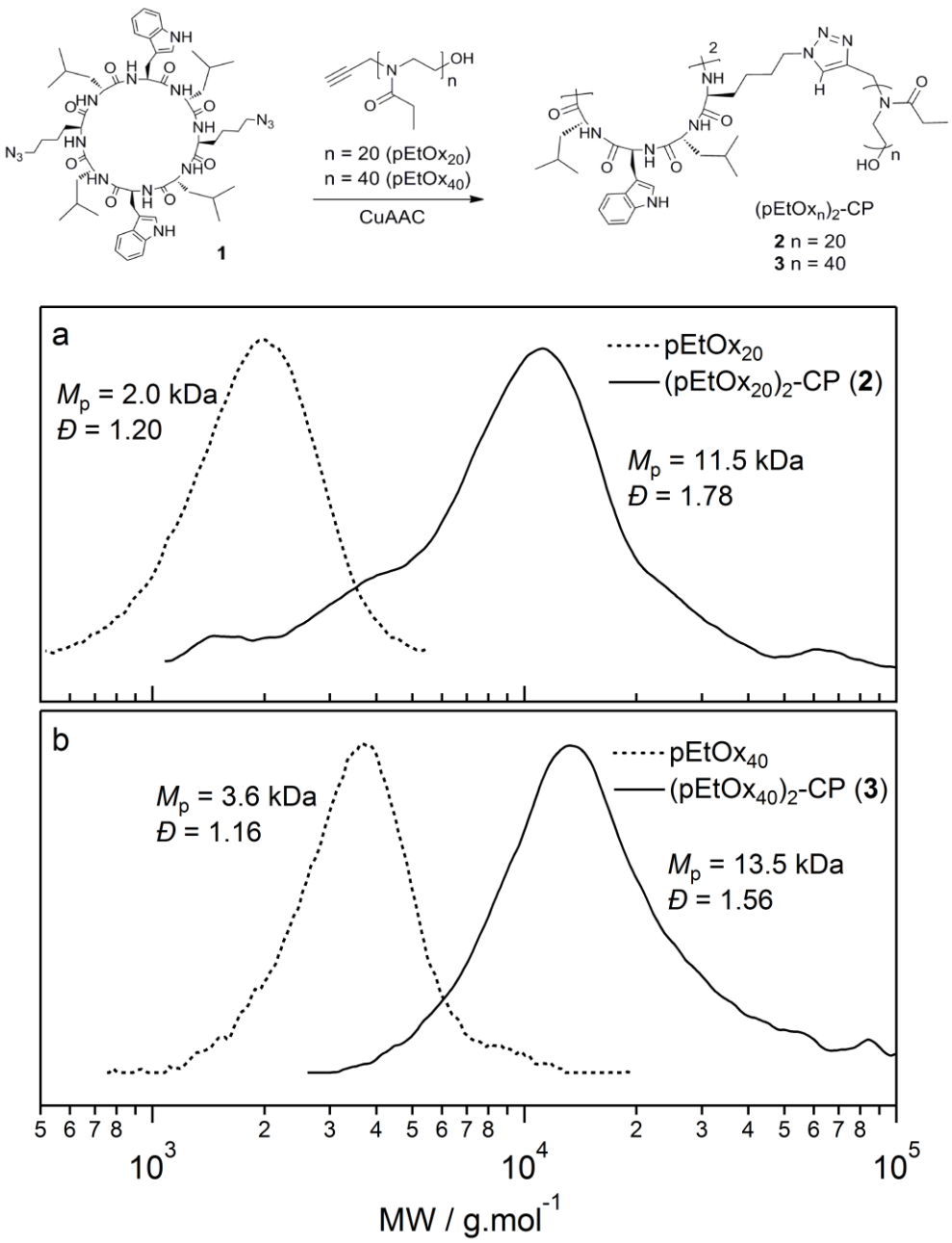

Figure S1: Scheme for conjugate synthesis and SEC traces (in DMF) of pEtOx and $(pEtOx_n)_2$-CP after purification by prep-SEC. a) n = 20 and b) n = 40.

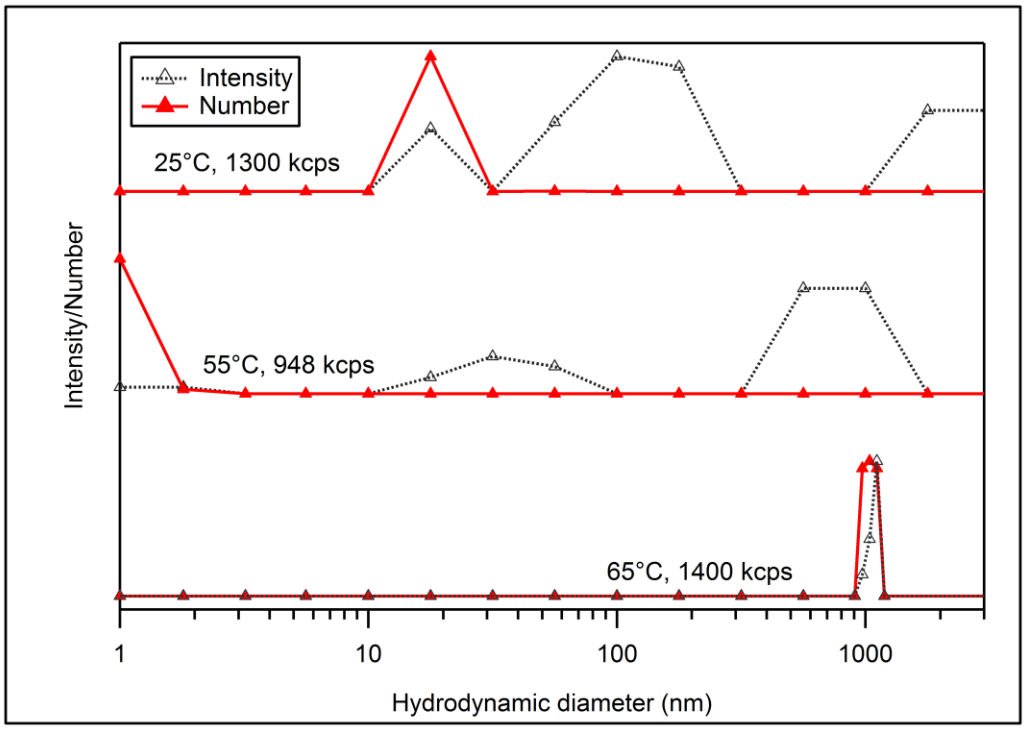

Figure S2: DLS data of a 0.3 mM solution of $(pEtOx_{40})_2$-CP conjugate in water at various temperatures.

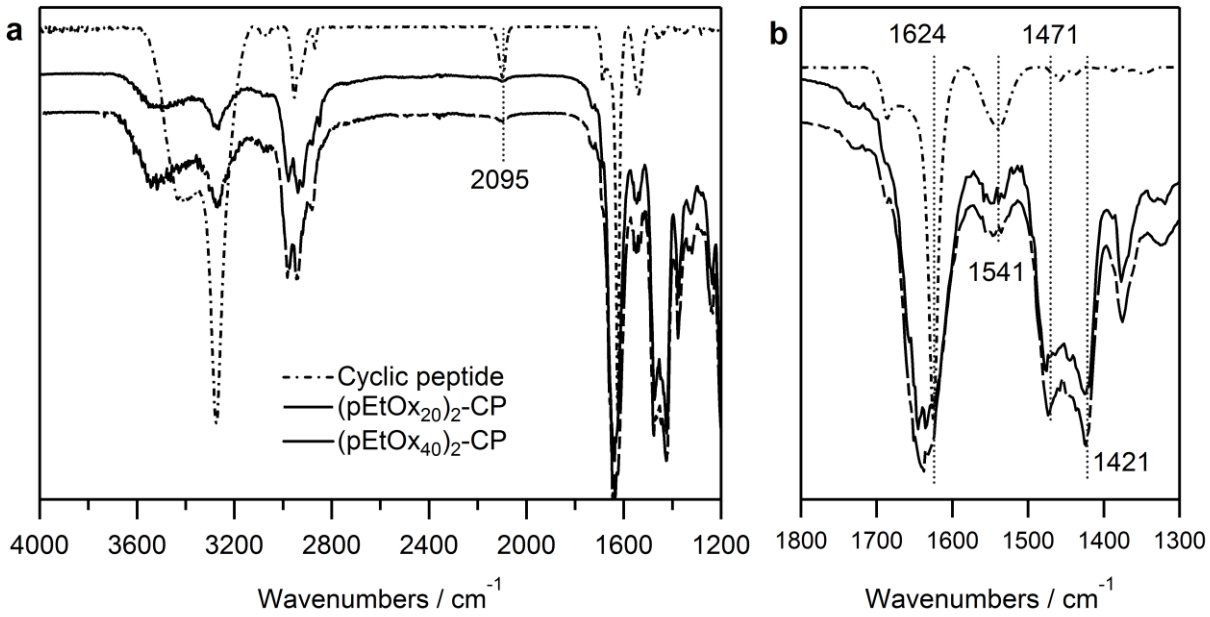

Figure S3: DRIFTS-IR of the cyclic peptide and conjugates in the dry state.

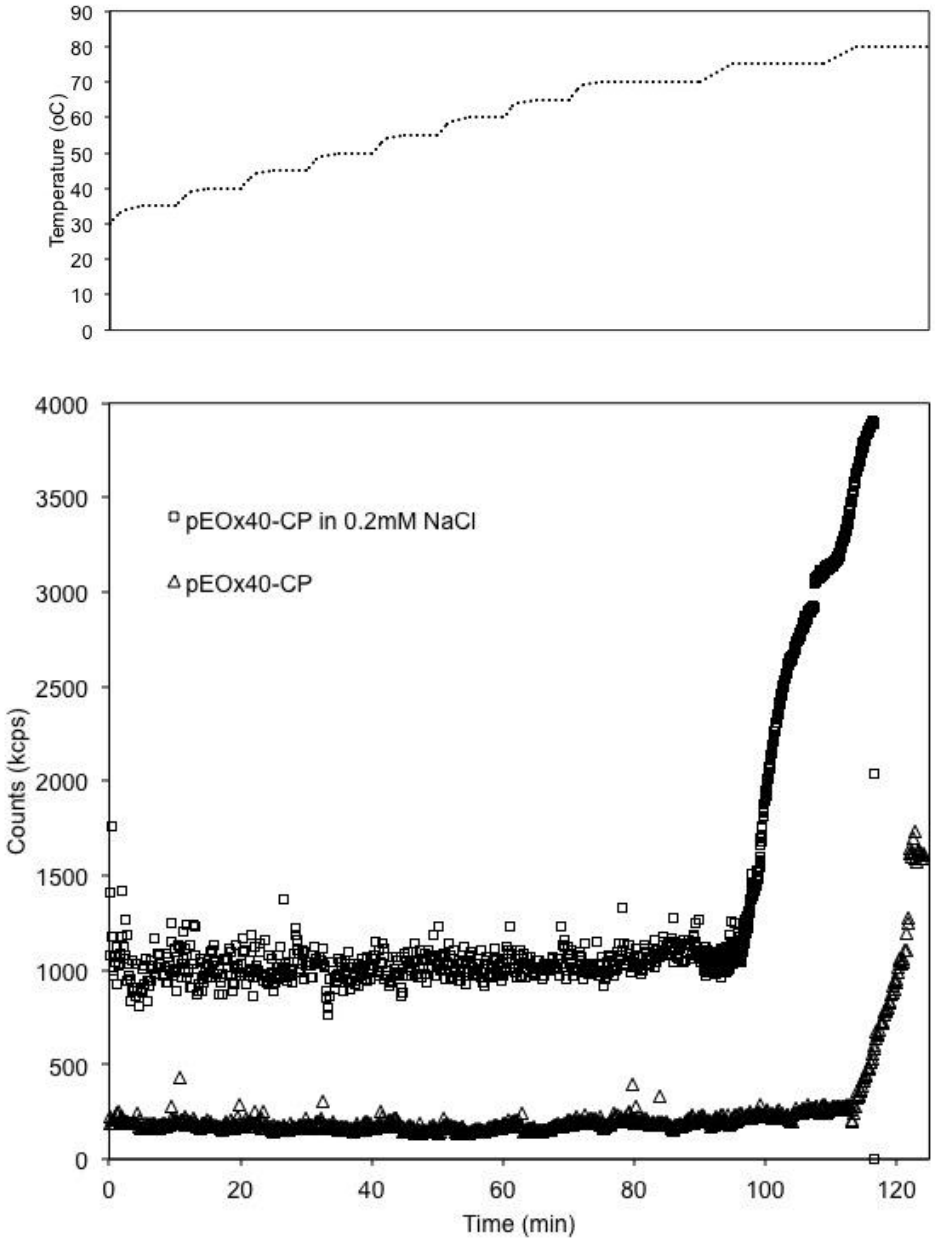

Figure S4: Countrate vs temperature for the $(pEtOx_n)_2$-CP conjugates in water.

References

1. Chapman, R.; Jolliffe, K. A.; Perrier, S. Polym. Chem. 2011, 1956.

2. Fijten, M. W. M.; Haensch, C.; van Lankvelt, B. M.; Hoogenboom, R.; Schubert, U. S. Macromol. Chem. Phys. 2008, 209, 1887.