A modular synthetic platform for the construction of protein-based supramolecular polymers via coiled-coil self-assembly

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SUPPORTING INFORMATION

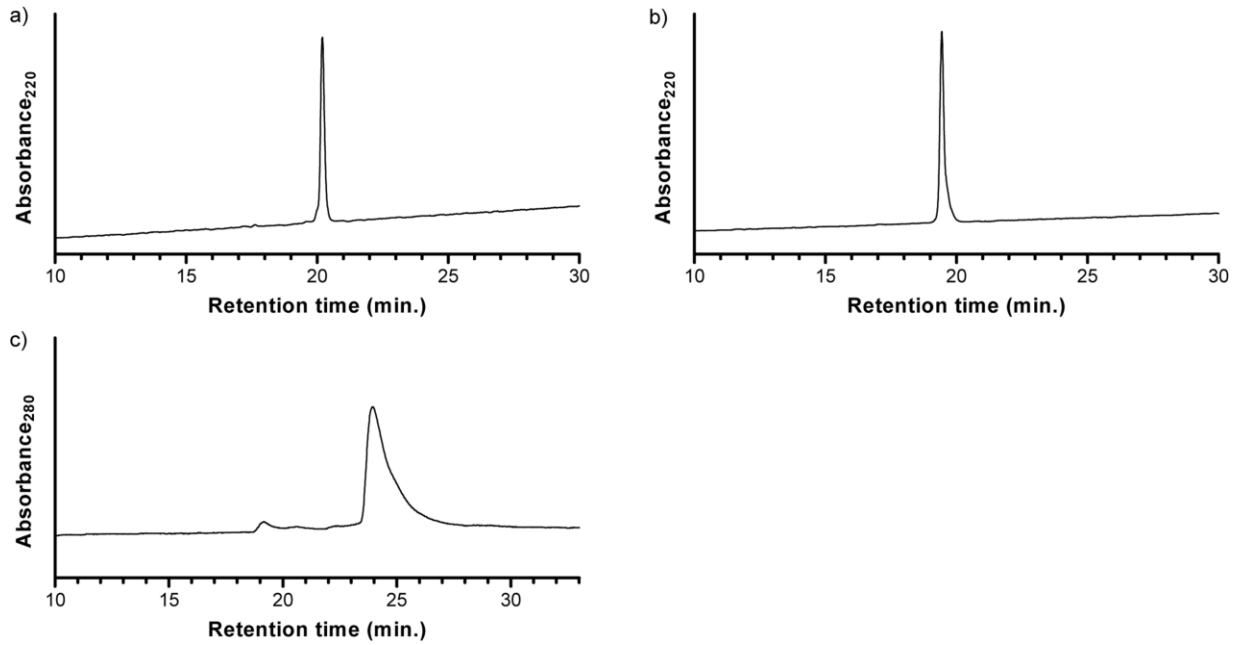

Figure S1. Analytical HPLC chromatograms for purified subunits **8** (a), **9** (b), and **10** (c).

Table S1. MALDI-TOF data for synthetic peptides and subunits.

#	$[M+H]^+$ *m/z* (avg.)	
	Calculated	**Observed**
1	4038.7	4036.9
3	4054.7	4054.7
4	4095.9	4096.7
7	8106.4	8105.4
8	8248.6	8248.3
9	8274.6	8274.7
10	8330.8	8333.0

Experimental Methods:

Synthesis of peptides 1, 3, and 4. Peptides were prepared by standard Fmoc solid-phase synthesis protocols with microwave heating[1] on NovaPEG Rink Amide resin. Cysteine residues in **3** and **4** were coupled at room temperature. Cleavage of each peptide from resin was achieved with 94% trifluoroacetic acid, 2.5% ethanedithiol, 2.5% water, and 1% triisopropylsilane for 2 to 3 hours. Crude peptides were precipitated from the concentrated TFA cleavage solution by the addition of diethyl ether (~40 mL). Following centrifugation, the ether was decanted and the peptide pellet suspended in 0.1% TFA in 20% acetonitrile, 80% water for purification. Peptides were purified by HPLC on a Jupiter or Luna C_{18} preparative column (Phenomenex) using gradients between 0.1% TFA in water and 0.1% TFA in acetonitrile. Identity of the purified peptides was confirmed by mass spectroscopy using a Voyager DE Pro MALDI-TOF instrument.

Synthesis of linker 5a. Protocol adapted from the literature.[2] Ethylene diamine (0.668 mL, 10 mmol) was dissolved in 10 mL chloroform, and the solution cooled on an ice bath. The flask was equipped with two addition funnels, one containing bromoacetyl bromide (2.613 mL, 30 mmol) in 12 mL chloroform and the other containing potassium carbonate (3.3 g, 23.9 mmol) in 12 mL water. The contents of the addition funnels were added simultaneously to the stirred reaction over 15 minutes. After addition was complete, the ice bath was removed, and the reaction allowed to continue at room temperature for 2 h. The aqueous and organic layers were separated and the aqueous phase extracted with ethyl acetate (4 x 100 mL). The combined organic layers were then dried over magnesium sulfate and the solvent removed under vacuum to give 1.66 g (55% yield) of the product as a white solid. ^{1}HNMR (400 MHz, D_2O) δ = 3.84 (4H, s), 3.34 (4H, s). ^{13}CNMR (100 MHz, DMSO) δ = 166.2, 38.5, 29.5. HRMS *m/z* calculated for $C_6H_{11}Br_2N_2O_2$ $[M+H]^+$: 300.9178; found: 300.9182.

Synthesis of linker 5b. Protocol adapted from literature.[3] Ethylene diamine (0.67 mL, 10 mmol) was added to 200 mL of 0.05 M NaOH. Iodoacetyl chloride (1.79 mL, 25 mmol) in 50 mL 1,2-dichloroethane was then added to the mixture and allowed to vigorously stir at room temperature for 10 minutes. A pale yellow precipitate was recovered after vacuum filtration. The precipitate was washed with water and dried under vacuum to give 1.30 g (33% yield) of the product as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6) δ = 8.28 (2H, s), 3.61 (4H, s), 3.09 (4H, s); ^{13}CNMR (125 MHz, DMSO-d_6) δ = 168.3, 39.0, 1.2; HRMS *m/z* calculated for $C_6H_9I_2N_2O_2$ $[M-H]^-$: 394.8753; found: 394.8748.

Synthesis of linker 6. Protocol adapted from the literature.[3, 4] Piperazine (0.8614 g, 10 mmol) was dissolved in 10 mL chloroform and the solution cooled on an ice bath. The flask was equipped with two addition funnels, one containing bromoacetyl bromide (2.613 mL, 30 mmol) in 12 mL chloroform and the other containing potassium carbonate (3.3 g, 23.9 mmol) in 12 mL water. The contents of the addition funnels were added simultaneously to the stirred reaction over 15 minutes. The ice bath was removed, and the reaction allowed to continue at room temperature for an additional 2 h. The aqueous and organic layers were separated and the aqueous phase extracted with ethyl acetate (4 x 100 mL). The combined organic layers were then dried over magnesium sulfate and the solvent removed under vacuum to give 2.54 g (77% yield) of the product as a white solid. ^{1}HNMR (400 MHz, D_2O) δ = 4.05 (4H, s), 3.65 (8H, m); ^{13}CNMR (100 MHz, DMSO-d_6) δ = 165.0, 164.9, 45.7, 45.3, 41.5, 41.2, 28.0, 27.9; HRMS *m/z* calculated for $C_8H_{13}Br_2N_2O_2$ $[M+H]^+$: 326.9344; found: 326.9378.

Synthesis of subunit 7. A 3 mL solution (1.8 mg/mL, ~222 μM) of GCN4-p1 (S_{14}-C) was prepared in 0.1 M phosphate buffer, pH 8 with 15% v/v DMSO. The reaction was allowed to proceed with stirring for 24 h, after which the formation of a precipitate was observed and analysis by HPLC showed significant depletion of the starting material. The solution was diluted with an additional 3 mL of 15% v/v aqueous DMSO to dissolve the precipitate, lyophilized, and redissolved in a 20% aqueous acetonitrile with 0.1% TFA. The crude material was purified by preparative HPLC; the identity and purity of the final product were verified by analytical HPLC and MALDI-MS, respectively.

Synthesis of subunits 8 and 10. A solution of peptide **3** or **4** was prepared in deionized water and the concentration determined by UV absorption. A fresh solution of linker **5a** or **5b** was prepared in DMF (1.5 mM final concentration of linker). A 2.75 mL solution was prepared consisting of 150 μM peptide in 25 mM phosphate buffer, pH 7.0 with 1% acetonitrile. One aliquot of the linker solution (27.5 μL) was added immediately. The reaction was placed in water bath at 65°C and allowed to stir for 2 hours with four additional aliquots of linker being added in 15 minute intervals for the first hour (137.5 μL linker total). To maintain solubility, two aliquots of acetonitrile were added in 30 minute intervals (total of 3% acetonitrile by volume). The reaction was quenched after 2 hours with a 25% acetonitrile/0.01% TFA solution and then purified using preparative HPLC. Fractions containing product were lyophilized and subjected to a second round of purification using semi-preparative HPLC. Identity and purity of the final product were confirmed by analytical HPLC and MALDI-MS, respectively.

Synthesis of subunit 9. A solution of peptide **3** was prepared in deionized water and the concentration determined by UV absorption. A fresh solution of linker **5b** was prepared in DMF (1.5 mM final concentration of linker). A 4 mL solution was prepared consisting of 150 μM peptide in 25 mM phosphate buffer, pH 7.0 with 1% acetonitrile. One aliquot of the linker solution (40 μL) was added immediately. The reaction was placed in water bath at 65°C and allowed to stir for 2 hours with four additional aliquots of linker being added in 15 minute intervals for the first hour (200 μL linker total). The reaction was quenched after 2 hours with a 25% acetonitrile/0.01% TFA solution and then purified using preparative HPLC. Fractions containing product were lyophilized and subjected to a second round of purification using semi-preparative HPLC. Identity and purity of the final product were confirmed by analytical HPLC and MALDI-MS, respectively.

Preparation of stock solutions for biophysical analysis. Peptide and subunit stock solutions were prepared by dissolving lyophilized powder in 1 mL water. A sample for UV analysis was then prepared by combining 25 μL peptide solution with 75 μL 8 M guanidinium HCl pH 7. Absorbance was measured on an Olis HP 8452 UV-VIS spectrometer, and stock concentration calculated using the tyrosine extinction coefficient (ε_{276} = 1450 M^{-1} cm^{-1}).[5]

Circular dichroism spectroscopy. CD measurements were carried out on an Olis DSM17 Circular Dichroism Spectrometer using 1 mm quartz cuvettes. Samples consisted of 100 μM peptide in 10 mM HEPES at pH 7.0. CD scans were carried out from 260 to 200 nm with 5 second averaging times, 1 nm step size, and 2 nm bandwidth at 20 °C. Spectra were corrected for a buffer blank and baseline molar ellipticity at 260 nm. Thermal denaturation experiments were performed using the same sample conditions as above and monitoring at 222 nm between 2°C and 95°C in steps of 5°C after an initial 3°C step. Data were analyzed using OLIS GlobalWorks. Each thermal melt was fit to simple two-state unfolding transition to obtain reported melting temperatures (T_m).

Analytical gel permeation chromatography. Measurements were made on an AKTA Purifier FPLC system equipped with a Superdex 200 column (GE Healthcare). Runs were performed using an isocratic elution buffer consisting of 50 mM HEPES, 150 mM NaCl, pH 7. Samples, prepared immediately before injection from UV-calibrated water stock solutions, consisted of 100 μM peptide or subunit in elution buffer; a 10x buffer stock was added to achieve proper buffer and salt concentrations. Samples for mixing experiments were prepared by combining appropriate volumes of peptide stocks to achieve the desired peptide-to-peptide ratios, followed by the addition of an appropriate volume of 10x buffer. A molecular weight calibration curve for the column was obtained by analyzing a solution of 1 mg/mL each of ferritin, BSA, ovalbumin, and aprotinin.

Molecular dynamics simulations. Molecular dynamics simulations were performed using the GROMACS 4.5 software package[6] and the GROMOS 53a6 force field.[7] The ethylenediamine and piperazine linkers were parameterized using atom types and accompanying restraints for analogous chemical groups already present in the force field. For each linker, a model compound was constructed

consisting of two Ac-Cys-NH_2 fragments cross-linked by the organic bridge in question. Coordinates were energy minimized in vacuum using the steepest descent algorithm. The system was placed in a dodecahedral simulation box, solvated with explicit water (SPC model), and relaxed to constant temperature and pressure with positional restraints on the non-water atoms. The final molecular dynamics simulation was carried out with a time step of 2 fs. Covalent bonds were constrained using the LINCS algorithm. Distance cutoffs were set at 10 Å for both short-range electrostatic and van der Waals interactions. Long-range columbic interactions were treated with the Particle Mesh Ewald method. Temperature was maintained at 298 K using the Nose-Hoover thermostat (time constant of 1 ps), and pressure was maintained at 1 bar using the Parrinello-Rahman barostat (time constant of 2 ps). Each system was equilibrated for 1 ns using the above settings before a running a production simulation of 50 ns. The tertiary amides in compound **12** did not show rotation on the time scale of the simulation, so two parallel runs were performed starting from the two different conformers possible to generate the ensemble shown in Fig. 5. Trajectories were analysed using tools included in GROMACS.

Dynamic Light Scattering. Data were collected on a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Westborough, MA) dynamic light scattering instrument with a 632.8 nm laser at a fixed angle of 90° and a constant temperature of 25°C. Subunit samples (200 µM peptide in 150 mM NaCl, 50 mM HEPES pH 7) were prepared by adding 10X buffer to concentrated stock solutions of subunit in water. The samples were filtered using 0.22 µm filters and allowed to sit for 2 hours before measurements were taken. Measurements were taken in a low volume quartz cuvette with a path length of 1 cm. Three measurements of at least 25 runs were taken for each sample.

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