Supplementary Material (ESI) for Chemical Communications

Supporting Information for
Folding Induced CO_2-Soluble Peptides

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Experimental

Peptide Synthesis and Purification: Peptides were synthesized on an Applied Biosystems Pioneer Peptide synthesizer using Fmoc-protected PEG-PAL amide resin (loading 0.38-0.40 mmol/g). Peptides were synthesized on 0.1 mmole scale. Automated synthesis was used up to the glycosylated amino acid, at which point manual synthesis was practiced to ensure coupling and deprotection of the glycosylated residue and acetylation of the N-terminus. Manual couplings proceeded for 3-10 hours, and deprotections for 20 minutes to 1 hour. Manual couplings and deprotections were monitored by the Kaiser test. The following amino acid protecting groups were used: Lys(Boc). Fmoc-protecting groups were removed using 2% piperidine, 2% DBU in DMF. Amino acids were coupled using HBTU/HOBt activation. The N-terminus was acetylated with 5% acetic anhydride and 6% 2,6-lutidene in DMF. Peptides were simultaneously cleaved and sidechain protecting groups removed with a cocktail of 95% trifluoroacetic acid (TFA), 2.5% triisopropyl silane and 2.5% water for 2-4 hours. The TFA was evaporated with a stream of air, and crude peptides were precipitated with cold ether, extracted into water, and lyophilized.

Crude peptides were purified by reverse phase HPLC on a Waters 600 HLPC with a dual wavelength detector, using a Vydac C_{18} semi-preparatory column (218TP510) with UV detection at 220 and 280 nm. Peptides were purified using a gradient of A (95% H_2O, 5% acetonitrile, 0.1% TFA) and B (95% acetonitrile, 5% H_2O, 0.1% TFA) and typically eluted between 15 – 30% B. After purification, peptides were identified with MALDI mass spectrometry.

Synthesis of Fmoc-Ser(AcGlc)-OH:[1, 2] To a dry 100 mL round-bottom flask, purged with N_2, was added 2.36 g (1.2eq) Fmoc-Ser-OH, 2.34 g (1 eq) pentaacetylglycopyranose, 2.28 mL (3 eq) $BF_3{\cdot}Et_2O$ and 15 mL dry acetonitrile. The mixture was allowed to stir and the reaction was followed by TLC, visualized by UV and a *p*-anisaldehyde stain. When the reaction was complete, the solution was diluted with 30 mL dichloromethane, washed with 1M HCl (aq) and dried over magnesium sulfate. The dichloromethane was then removed by vacuum, leaving a yellowish-white foamy solid. The product was purified by column chromatography (97.5% CH_2Cl_2 / 2% MeOH / 0.5% AcOH up to 90% CH_2Cl_2 / 8% MeOH / 2% AcOH).

CO_2 Solubility Experiments: Approximately 2 to 2.5 mg peptide (2.5 μmol) was introduced to the CO_2 cell along with a stir bar. The cell was closed and the piston adjusted such that the volume of the cell was 3.9 mL. CO_2 was then introduced to a pressure of 4000 psi at 24°C and the peptide was stirred. At this pressure and temperature, the density of CO_2 is 0.959 g/mL, therefore 0.085 mol CO_2 was introduced to the cell. The CO_2 pressure was varied manually with a piston while the peptide was stirred. The cloudpoint was reached

when no particulates were seen in the cell, and mixture became transparent, such that the back of the cell was visualized. Cloudpoint data were observed in five-degree increments from 6 °C to 60 °C. The pressure limit of the cell was 5000 psi, which limited some cloudpoint measurements. Cloudpoint measurements were taken in duplicate.

Circular Dichroism: Peptide concentrations were determined from the Tyr absorbance. CD spectra were acquired on an Aviv 60DS spectropolarimeter. Scans were taken from 180-250 nm at 0-1 °C. Data points were taken every 2 nm, and the scans were repeated in triplicate. Mean residue ellipticities were determined from the CD data according to Equation 1, where A is the raw CD signal in degrees, c is the concentration in dmol/cm^3, l is the path length of the cell in cm, and n is the number of residues in the sequence.

$$[\theta] = A / (c*l*n) \quad (1)$$

1. M. Elofsson, B. Walse and J. Kihlberg, *Tetrahedron Lett*, 1991, **32**, 7613-7616.
2. L. A. Salvador, M. Elofsson and J. Kihlberg, *Tetrahedron*, 1995, **51**, 5643-5656.