Electronic Supplementary Material (ESI) for Biomaterials Science.

Understanding the Metal Mediated Assembly and Hydrogel Formation of a β-Hairpin Peptide

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Supporting Information

1. General information.

Solvents for RP-HPLC were purchased as HPLC grade and used without further purification. All other reagents were used as supplied. Conventional Fmoc protected amino acids and *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) were purchased from GL Biochem (Shangai, China). *N*-methylmorpholine (NMM) and piperidine were purchased from Aldrich (St Louis, USA). Dimethylformamide (DMF, AR grade) and acetonitrile (CH_3CN, HPLC grade) were purchased from Scharlau (Barcelona, Spain). Trifluoroacetic acid (TFA) was purchased from Oakwood Chemical (River Edge, USA). Triisopropylsilane (TIPS) was purchased from Alfa Aesar (Wardhill, MA). The aminomethyl polystyrene resin was purchased from Rapp Polymer GmbH (Tübingen, Germany) and Fmoc-L-Ala-OCH_2-phi-OCH_2CH_2COOH was purchased from PolyPeptide (Strasbourg, France).

Molecular models were prepared with UCSF Chimera v 1.10.2.[1] TEM images were analyzed with ImageJ.[2]

2. Peptide synthesis and purification

Peptide synthesis was performed via the Fmoc/tBu strategy on an aminomethyl-Chemmatrix resin functionalized (0.62 $mmol.g^{-1}$) with a Fmoc-Rink amide linker using a PS3 Synthesiser (Tucson, AZ, USA) at 0.2 mmol scale. The Fmoc group was deprotected with 20% v/v piperidine in DMF 2 x 5 min at room temperature. Amino acid couplings were performed with single couplings 5 eq. of Fmoc-protected amino acid, 4.5 eq. HATU and 10 equivalents of *N*-methylmorpholine in DMF for 20 min at room temperature.

Following completion of the sequence, the peptide was unattached from the resin and the side-chain protecting groups were removed by treating the resin with a mixture of TFA/TIPS/H_2O 95:2.5:2.5 (v/v/v) at room temperature for 2 h.

The crude peptide was precipitated with cold diethyl ether, isolated by centrifugation, and washed with cold diethyl ether (3x), dissolved in 1:1 (v/v) MeCN/H_2O containing 0.1% TFA and lyophilised. The peptide was analysed for purity by LC-MS using ESI in positive mode (Agilent 1120 compact LC system equipped with Agilent 6120 Quadrupole MS and a UV detector at 214 nm (Palo Alto, CA)) using an Eclipse XDB-C8 column (5 µ; 4.6 x 150 mm; Agilent) at 0.3 $mL.min^{-1}$ using a linear gradient from 5 to 85% B in 40 min at 60°C. The solvent system used was A (0.1% formic acid in H_2O) and B (0.1% formic acid in MeCN).

Peptide purification was performed by semi-preparative RP-HPLC (Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector (Milford, MA, USA)) using an X-Terra Prep MS C18 ODB (10 µm, 19 x 300mm), a 0.5 % $B.min^{-1}$ linear gradient from 5 to 85 %B and a 10 mL.min-1 flow rate at room temperature. The solvent system consisted of A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN).

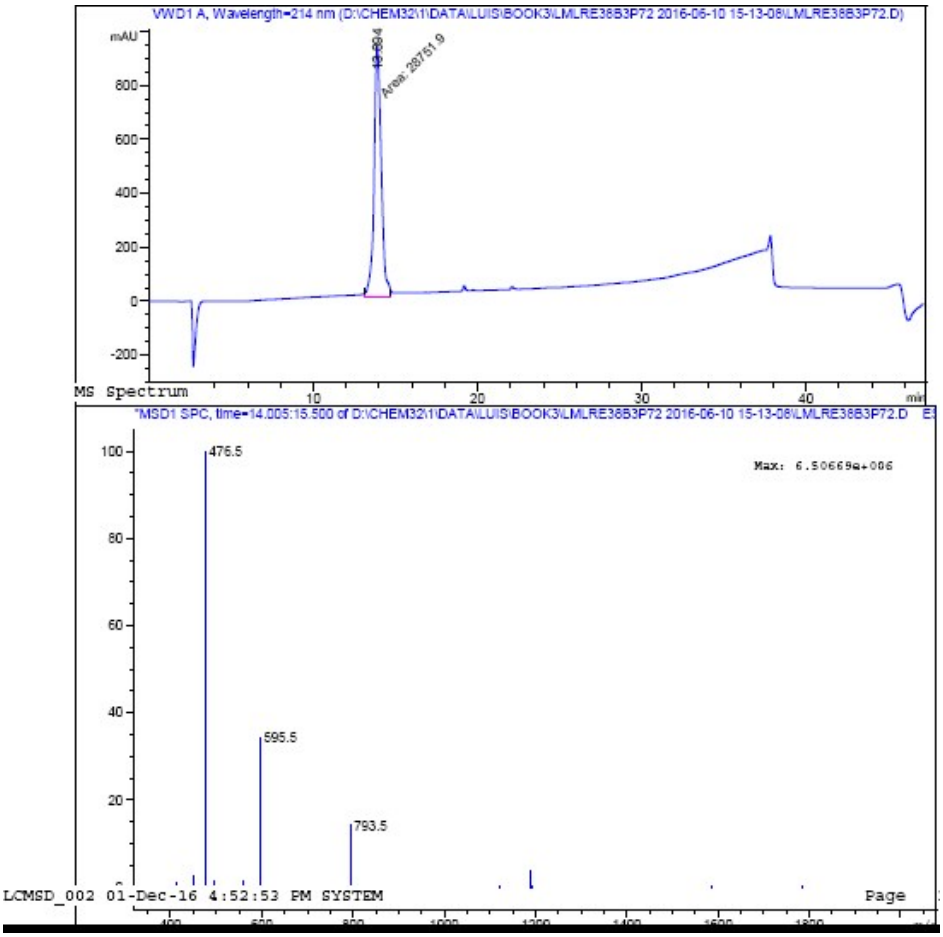

Fig. S1 HPLC and LC-ESI-MS spectrum of **1**. Peak assignment: 476.6 ($[M+3H]^{3+}$, calc. 476.6), 595.4 ($[M+4H]^{4+}$, calc. 595.5) and 793.4 ($[M+5H]^{5+}$, calc.793.7). The HPLC profile was acquired in a Eclipse XDB-C8 column (5 µ; 4.6 x 150 mm; Agilent) at 0.3 $mL.min^{-1}$ using a linear gradient from 5 to 85% B in 40 min at 60°C. The solvent system used was A (0.1% formic acid in H_2O) and B (0.1% formic acid in MeCN). The peptide purity was >99% as judged by integration of the HPLC chromatogram at 214 nm.

3. Hydrogel preparation

Hydrogel formation was visually demonstrated by the tube inversion test.[3] The peptide was first dissolved in water at a 2 wt% concentration followed by 1:1 dilution with 100 mM Tris buffer (pH 7.4). Samples were allowed to stand at room temperature.

Hydrogel formation was also monitored in the presence of inorganic salts NaCl, $CaCl_2$, $MgCl_2$ or $ZnCl_2$. The peptide was first dissolved in water at a 2 wt% concentration and then 100 mM Tris buffer (pH 7.4) containing NaCl (30 mM), $MgCl_2$ (10 mM), $MgCl_2$ (10 mM) or $ZnCl_2$ (10 mM) was added in a 1:1 proportion. Samples were allowed to stand at room temperature and hydrogel formation was examined as described above.

4. Rheology

The rheological measurements were performed on a stress-controlled rheometer (MCR 302, Anton Paar Austria) fitted with a 25 mm diameter plate geometry, with a gap of 0.2 mm. A thin layer of oil was placed in the gap between the plate and sample to prevent evaporation. The samples were prepared as indicated above but this time the MQ grade water and buffer solution were ice chilled before they were placed in the rheometer. Dynamic sweep experiments were carried out following the protocols described in the manuscript.

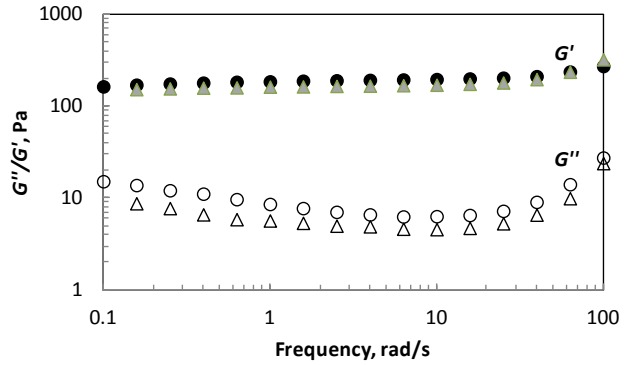

Fig. S2 Frequency sweep (0.2% strain) of 1 wt% **1** in 50 mM Tris buffer (pH 7.4) with 30 mM NaCl (filled and open circles) or 10 mM $ZnCl_2$ (filled and open triangles).

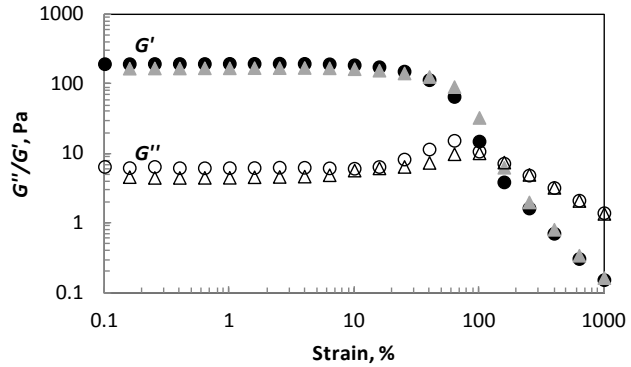

Fig. S3 Strain sweep (6 rad.s^{-1}) of 1 wt% **1** in 50 mM Tris buffer (pH 7.4) with 30 mM NaCl (filled and open circles) or 10 mM $ZnCl_2$ (filled and open triangles).

5. FTIR

ATR-FTIR spectra were recorded in a Perkin Elmer Spectrum 100 FT-IR Spectrometer. Peptide samples were dissolved in 25 mM aqueous HCl and freeze dried (3x) to remove residual TFA. Samples were dissolved in water to a 1 wt% concentration and subsequently diluted (1:1) with 100 mM Tris buffer (pH 7.4) with or without inorganic salts. The final solution was freeze dried and further dissolved in D_2O and freeze dried (3x). Samples were finally dissolved in D_2O to give a 1 wt% peptide concentration. Each spectrum was an average of 36 scans taken at a resolution of 4 cm^{-1}. The spectrum from buffer in D_2O was subtracted as a background.

6. Transmission Electron Microscopy

Sample microstructure was explored using a Tecnai12 electron microscope (FEI) operated at 120kV and equipped with a 2Kx2K GATAN· CCD camera. Carbon-coated copper TEM grids (400 mesh

from Agar Scientific) were glow discharged for 30 s, placed shiny side down on a 10 μL droplet of sample for 10 s, blotted for 10 s before being exposed to 10 μL of double deionized water for 10 s. After blotting for a further 10 s, the grid was finally placed on 10 μL of 2% (w/v) uranyl acetate for 60 s and blotted for 10 s.

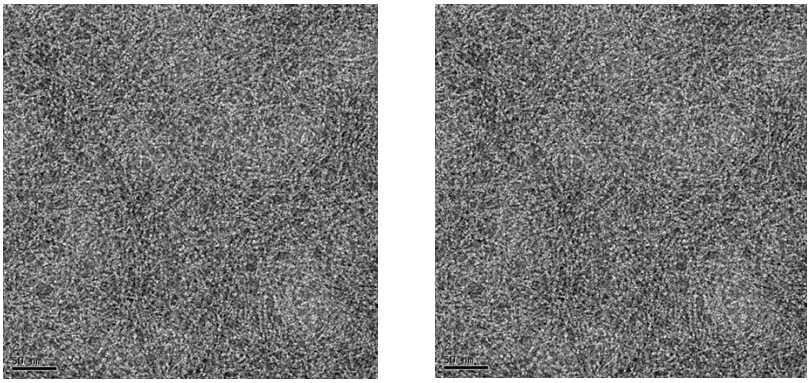

Fig. S4 Transmission electron micrographs prepared from a sample of 1 wt% hydrogel of **1** in 50 mM Tris buffer pH 7.4 with 30 mM NaCl (left) or 10 mM $ZnCl_2$ (right) stained with 2% uranyl acetate. The black scale bar in the left bottom of the images is equivalent to 50 nm.

7. Study of Phe-OMe ester hydrolysis.

Two sets of 200 μL of hydrogels of **1** at 1 wt% in 50 mM Tris buffer (pH 7.4) were prepared in 2 mL LC vials. One set contained 30 mM NaCl and the other 10 mM $ZnCl_2$. An aliquot of 200 μL of 20 mM Phe-OMe dissolved in 50 mM Tris buffer (pH 7.4) containing either 30 mM NaCl or 10 mM $ZnCl_2$ was added on top of each gel, thus giving a final concentration of 10 mM of ester over the entire mixture's volume. A similar protocol was followed for control samples lacking peptide **1**. Vials were sealed and placed in the LC autosampler. Samples (5 μL) from the liquid on top of the gel were drawn at consecutive intervals of time and analyzed by HPLC in an Agilent Technologies 1120 Compact LC system with UV detector at 214 nm using a GraceSmart RP18 (3 μ, 150x4.6 mm) column at a flow of 0.5 $mL.min^{-1}$ using a linear gradient from 5 to 50% B in 25 min at 25°C. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN).

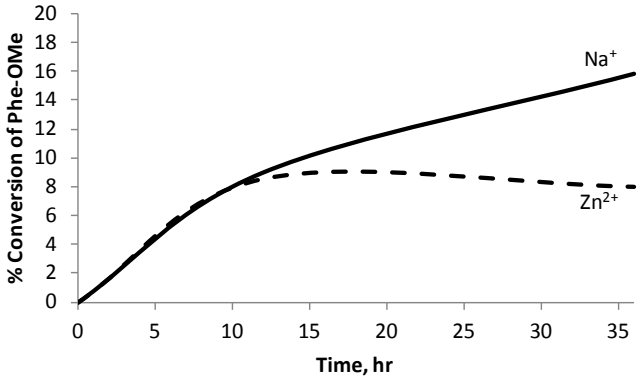

Fig.S5 Kinetics of hydrolysis of Phe-OMe to Phe-OH in hydrogels of **1** prepared at 1 wt% in 50 mM Tris buffer (pH 7.4) in presence of either NaCl or $ZnCl_2$.

An interesting finding of this experiment was that ~50% of the product of hydrolysis (Phe-OH) was specifically retained within the hydrogel while the ester (Phe-OMe) remained distributed uniformly over the entire mixture (Fig. S6). This specific species retention was attributed to the interaction of the negatively charged carboxylate of Phe-OH with the positively charged groups of peptide **1** assumed at the physiological pH.

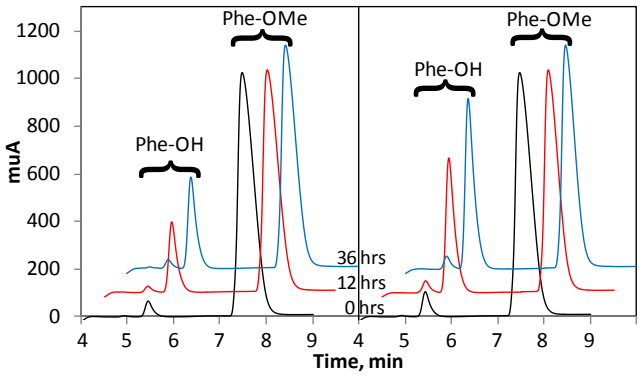

Fig. S6 Representative HPLC profiles of: (left panel) the aqueous layer of a 1:1 mixture initially consisting of a 1 wt% gel of 1 in 50 mM Tris buffer (pH 7.4) and 10 mM $ZnCl_2$ (bottom phase) and an aqueous solution containing 20 mM Phe-OMe in 50 mM Tris buffer (pH 7.4) and 10 mM $ZnCl_2$ (top phase); and (right panel) an aqueous solution initially containing 20 mM Phe-OMe in 50 mM Tris buffer (pH 7.4) and 10 mM $ZnCl_2$. Aliquots of the liquid phases were analyzed by HPLC at 0, 12 and 36 hrs (black, red and blue coloured traces respectively). The HPLC traces at 12 and 36 hrs have been shifted left (by 0.5 and 1 minutes respectively) and up (by 100 and 200 muA respectively) for clarity.

References

1. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605.
2. C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods*, 2012, **9**, 671.
3. R. Jin, C. Hiemstra, Z. Zhong and J. Feijen, *Biomaterials*, 2007, **28**, 2791.