A peptide hydrogel derived from a fragment of human cardiac Troponin C

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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 polystyrene resin functionalised with an Fmoc-L-Ala-OCH_2-phi-OCH_2CH_2COOH acid linker using a PS3 Synthesiser (Tucson, AZ, USA) on 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 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 released from the resin with concomitant removal of the side-chain protecting groups by treatment 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 (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 2%$B.min^{-1}$ 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 same conditions were employed in the analysis of the purified peptide and the mass confirmed by MS using ESI in positive mode.

Purification of crude peptide was performed by semi-preparative RP-HPLC (Dionex Ultimate 3000 (Sunnyvale, CA, USA) equipped with a 4 channel UV detector) at 210, 230, 254, and 280 nm using a X-Terra Prep MS C18, 10 μm, 19x300mm, using a 0.5%$B.min^{-1}$ linear gradient, a flow of 8 $mL.min^{-1}$ at 60 °C. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeCN).

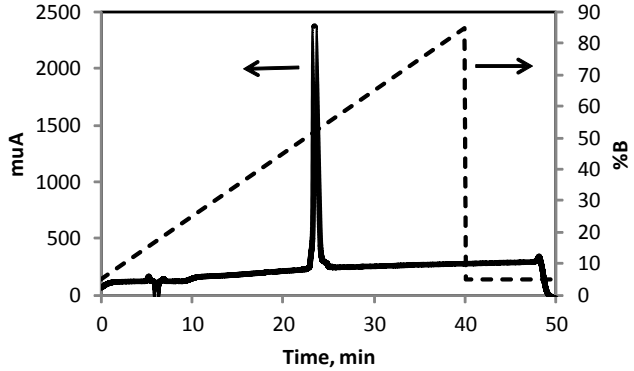

Fig. S1 HPLC profile of **1**.

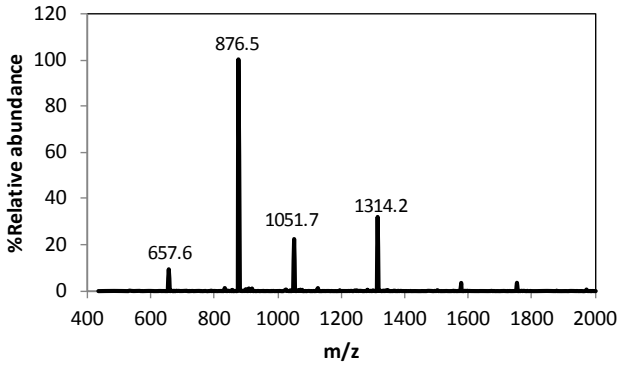

Fig. S2 ESI-MS spectrum of **1**. Peak assignment: $[M + 2H]^{2+}$ obs. 1314.2 (calc. 1314.5); $[M + 3H]^{3+}$obs. 876.5 (calc. 876.6);. $[M + 4H]^{4+}$ obs. 657.6 (calc. 657.7).

3. Hydrogel preparation

3.1 Hydrogel formation in water

Hydrogel formation was monitored visually as a function of pH, temperature and peptide concentration. Water was added to the peptide and the resulting mixture mixed until the sample assumed a homogenous consistency, the pH was then adjusted by adding aliquots of an appropriate NaOH aqueous solution and the volume was adjusted to give the target final concentration. The solutions were allowed to stand at room temperature overnight. Similar experiments were carried out at a fixed peptide concentration of 2.5 wt% and pH and samples were incubated at different temperatures 5, 25, 50, 60, 70 and 90 ºC.

3.2 Hydrogel formation in presence of inorganic salts.

Peptide was dissolved in 50 mM tris buffer pH 7.4. The solution was then split into four vials of equal volumes and aliquots of aqueous solutions of NaCl, $CaCl_2$, $MgCl_2$ and $ZnCl_2$ were separately added to each vial and final volume adjusted to obtain a final concentration of salt of 75, 25, 25 and 25 mM respectively, a 2.5 wt% final peptide concentration and pH of 7.4. The mixtures were allowed to stand at room temperature and hydrogel formation was constantly assessed by inverting the vials. Mixtures were diluted with 50 mM tris buffer at pH 7.4 containing the above mentioned concentration of the corresponding salt, vortexed and allowed to stand at room temperature and hydrogel formation was constantly assessed by inverting the vials. Dilution experiments continued until hydrogel formation was not observed.

Fig. S3 Peptide **1** mixtures at a concentration of 1 wt% in tris buffer pH 7.4 with NaCl (75 mM), $MgCl_2$ (25 mM), $CaCl_2$ (25 mM), and $ZnCl_2$ (25 mM) (left to right) following overnight storage at room temperature.

3.2 Hydrogel formation in phosphate buffer.

Peptide was dissolved in 20 mM phosphate buffer at pH 7.4. The solution was then split into four vials of equal volumes and aliquots of aqueous solutions of NaCl were separately added to each vial to get a final concentration of salt of 0, 10, 50, 150 mM respectively and a 2.5 wt% final peptide concentration. The mixtures were allowed to stand at room temperature and hydrogel formation was constantly assessed by inverting the vials. Mixtures were diluted with 20 mM phosphate buffer at pH 7.4 containing the above mentioned concentrations of NaCl, vortexed and allowed to stand at room temperature and hydrogel formation was constantly assessed by inverting the vials. Dilution experiments continued until hydrogel formation was not observed.

Fig. S4 Peptide **1** mixtures at a concentration of 2.5 wt% in phosphate buffer pH 7.4 with 0, 10, 50 and 150 mM NaCl (left to right) after standing for two hours at room temperature.

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. To prevent evaporation the sample was covered by a thin layer of oil. The sample was allowed to anneal at 37 °C for 1 hr, then at 75 °C for 2 hr and finally the temperature was dropped to 25 °C at a rate of 1 °C·min^{-1}. Dynamic sweep experiments followed as described in the manuscript. The frequency sweep (0.01 to 100 Hz) was performed at a constant temperature of 37 °C and a constant strain of 1%. The temperature sweep was performed by heating the sample from 25 °C to 50 °C then cooling back from 50 °C to 25 °C at a rate of 1 °C·min^{-1}, during which the measurement was performed at a frequency of 1 Hz and a strain of 1%.

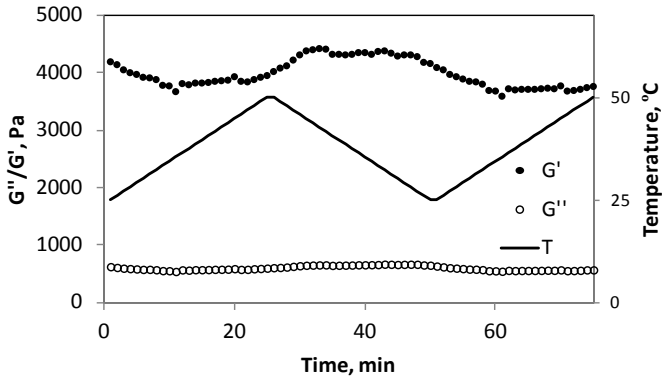

Fig. S5 Dynamic time sweep measurements (6 rad.s^{-1}, 1% strain) for 2.5 wt % **1** in water.

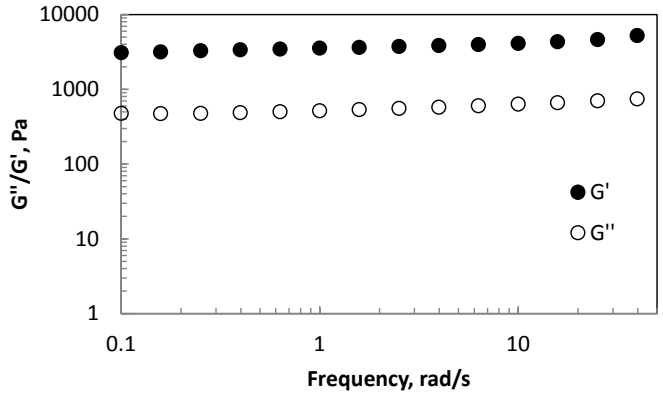

Fig. S6 Frequency sweep (1% strain) of 2.5 wt% **1** in water.

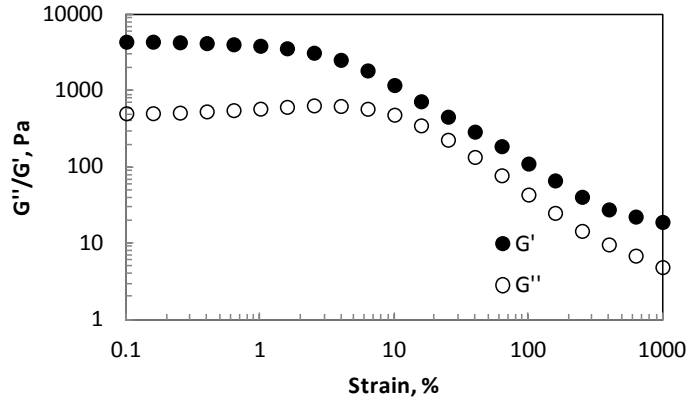

Fig. S7 Strain sweep (6 rad.s^{-1}) of 2.5 wt% **1** in water.

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 further dissolved in D_2O and freeze dried (3x) and samples were dissolved in D_2O at 5 and 1 wt%. Each spectrum was an average of 36 scans taken at a resolution of 4 cm^{-1}. The spectrum from D_2O was subtracted as a background.

Transmission FTIR spectra were also recorded using a Thermo Scientific Nicolet IS5 spectrometer, equipped with a DTGS detector. Samples were sandwiched between two CaF_2 plate windows, with a 0.025 mm thick mylar spacer) in a Specac GS20500 sample cell holder. Spectra were scanned 128 times over the range of 900-4000 cm^{-1}. Samples were dissolved in D_2O, the spectrum from D_2O was subtracted as a background.

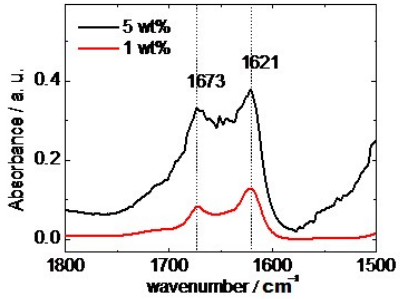

Fig. S8 Transmission FTIR spectra of **1** at 1 and 5 wt% in D_2O.

6. Circular Dichroism.

CD spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). Spectra are presented with absorbance A < 2 at any measured point with a 0.5 nm step, 1 nm bandwidth, and 1 second collection time per step. Solutions were measured using quartz parallel plaques (0.01 mm gap). The spectrum from water was subtracted as background.

7. X-Ray Diffraction (XRD)

XRD was performed on a peptide stalk prepared by drawing a fiber of a 2 wt% peptide solution between the ends of wax-coated capillaries. After drying and separation, a stalk was left on the end of one capillary. The capillary was mounted vertically in the four axis goniometer of a RAXIS IV++ X-ray diffractometer (Rigaku) equipped with a rotating anode generator. The XRD data was collected using a Saturn 992 CCD camera. The sample-to-detector distance was 50 mm.

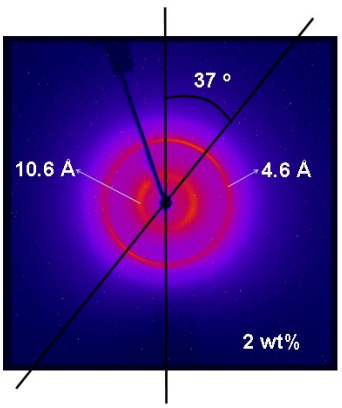

Fig. S9. 2D XRD pattern measured for a stalk dried from a 2 wt% solution of peptide **1**

8. Small angle X-ray scattering (SAXS).

Experiments were performed at the Dutch-Belgian Beamline (DUBBLE) station BM26B of the European Synchrotron Radiation Facility (Grenoble, France).[3] A sample-detector distance D~ 3015 mm and a wavelength λ= 1.033 Å were used to measure the SAXS data. A Dectris-Pilatus 1M detector (pixel resolution= 981 × 1043; pixel size= 172 × 172 μm) was used to record the 2D SAXS scattering patterns. SAXS data was corrected for sample absorption and background scattering. The data was normalized by the intensity of the incident beam in order to correct for primary beam intensity fluctuations. The scattering vector $q = 4\pi \sin\theta/\lambda$ (2θ= scattering angle) was calculated using the standard silver behenate scattering pattern calibration.

The SAXS intensity was fitted to a form factor corresponding to the Porod's approximation for a long infinite cylinder.[4] A Gaussian size distribution was used to describe the polydispersity of the cylinder radius. Briefly, the model depends on the cylinder radius, R, and the polydispersity in the cylinder radius ΔR. A constant background Co was added to the form factor. Sasfit software[5] was used to fit the cylinder form factor to the SAXS curve. The parameters obtained from this fitting were: R= 2.5 nm ΔR = 1 nm and Co= $8x10^{-3}$.

9. 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.

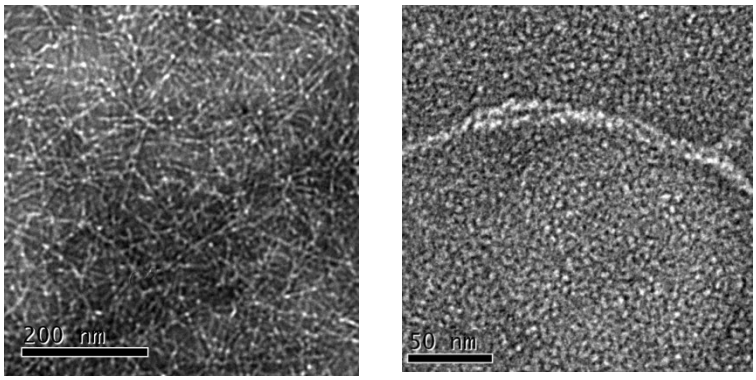

Fig. S10 Electron micrographs of 1 wt% (left), and 0.015 wt% (right) hydrogel sample stained with 2% uranyl acetate.

10. Rationale of the molecular model for peptide 1 fibril assembly.

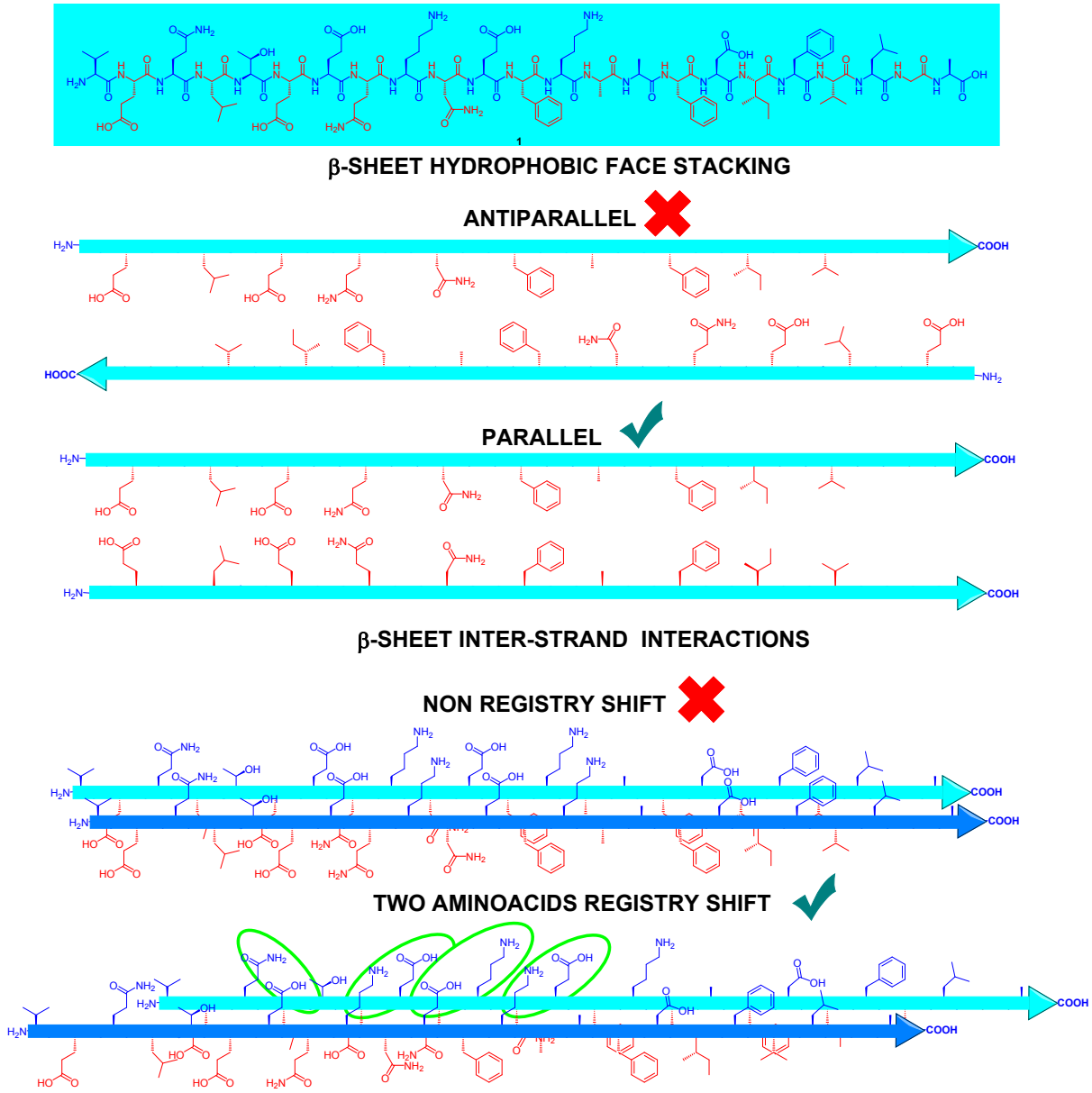

Fig. S11 Possible arrangements for peptide **1** β-sheet assembly into fibrils. Residues in the hydrophilic face of the peptide are coloured dark blue and residues in the hydrophobic face are coloured red. Green coloured ovals enclose favourable cross-strand staggering of complementarily charged residues. All models are viewed along the fibril growth axis that is perpendicular to the β-sheet backbone.

References

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4. G. Porod, *Acta Phys Austriaca*, 1948, **2**, 255.
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