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Supplementary Information for

Fibrils and nanotubes assembled from a modified amyloid-β peptide fragment differ in the packing of the same β-sheet building blocks

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Materials and Methods

Materials. H_2N-AAKLVFF-COOH (95 % pure) was purchased from Peptide Protein Research (Fareham, UK). For analysis by SSNMR the peptide contained selectively ^{13}C labelled amino acids. In one peptide the second alanine from the N-terminus (Ala2) was ^{13}C labelled at the methyl position and Val5 was ^{13}C labelled at the carbonyl position. This peptide is denoted [$^{13}C_2$-AV]AAKLVFF. Another peptide contained a single ^{13}C label at the carbonyl position of Phe7. This peptide is denoted [^{13}C-F]AAKLVFF. Fibrils were prepared by dissolving peptide in water at a concentration of 1 wt %[1] and nanotubes were prepared by dissolving peptide in methanol at a concentration of 1 wt %.[2] Insoluble material was harvested by centrifugation after stirring at room temperature for 5 d and washed several times with water or methanol.

Morphology. Morphologies of the AAKLVFF aggregates were analysed by TEM using negative staining with 4% uranyl acetate. Peptide suspensions (10 µl) were loaded onto carbon coated copper grids and visualised on a Tecnai 10 electron microscope at 100 kV.

Molecular spectroscopy. Peptide samples for FTIR were first left on the bench in 100 mM HCl for 30 minutes to remove residual TFA from peptide synthesis. The peptide sample was then freeze-dried, resuspended in D_2O for 1 hour and then freeze-dried again to remove H_2O. The sample was suspended in 50 µL of D_2O and loaded into a 50 µL FTIR cell, with a 50 µm PTFE spacer. FTIR spectra were recorded using a BioRad FTS-40

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spectrometer with an HgCdTe (MCT) detector. A resolution of 1 cm^{-1} was used throughout and all spectra were averaged over 500 scans. The spectrometer was purged with dry air; any residual water vapour absorption was corrected by subtracting an appropriately scaled spectrum of humid air. Solvent correction was performed by subtracting appropriately scaled spectra of D_2O, measured under the same conditions and at the same temperature as peptide samples.

Solid-state NMR. NMR experiments were performed using a Bruker Avance 400 MHz spectrometer operating at a magnetic field of 9.3 Tesla. Peptide aggregates were sedimented from bulk solution by centrifugation and packed into a 4 mm zirconium rotor. CP-MAS SSNMR experiments were carried out at room temperature. The sample spinning rate was maintained automatically between 4 kHz ±1 Hz. All experiments utilized an initial 4.0-μs ^1H 90° excitation pulse length, 1-ms Hartmann-Hahn contact time at a matched ^1H field of 65 kHz, TPPM proton decoupling at a field of 85 kHz during signal acquisition and a 2-s recycle delay. RR experiments [3, 4] were carried out by adjusting the sample spinning rate (ω_R) to the exact difference between the resonance frequencies ($\Delta\Omega$) of the methyl carbon of Ala2 and the carbonyl carbon of Val5 (n = 1 RR) or to half the frequency difference (n = 2 RR). After cross-polarization, ^{13}C longitudinal difference polarization was created with a nonselective 4-μs $\pi/2$ pulse followed by a train of 18 DANTE pulses, representing an overall π pulse of 30 μs, to invert the ^{13}C spin polarisation for Cα selectively. After a mixing period the ^{13}C magnetization was returned to the transverse plane by a second nonselective $\pi/2$ pulse before digitization of the free-induction decay. A series of experiments using mixing periods of up to 34 ms was performed to measure the time dependence of difference polarization. Curves representing exchange of Zeeman order were obtained from the difference in intensities of the Ala2 methyl carbon and Val5 carbonyl carbon peaks. MQNMR spectra were obtained using a time-reversible double quantum selective pulse sequence [5] with chemical shift refocusing π pulses applied at the ^{13}C frequency during 9.6 ms excitation/preparation periods and an initial condition of longitudinal ^{13}C spin polarization. Spectra for different MQ orders were separated by phase shifting in 32 increments by 360/32 =

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11.25°. The total MQNMR experiment time was approximately 3 days for each sample. The coherence orders excited represent the number of spins that are correlated through a dipolar-coupling network (i.e., with 13C spins separated by ~6.5 Å), and can report on the supramolecular structure of fibrils by identifying clusters or groups of molecules.

Simulation of RR exchange curves. Interatomic distances were determined by comparison of the data with numerically simulated curves.[4] Curves were simulated for dipolar coupling constants d_{CC} corresponding to fixed pairs of ^{13}C-^{13}C distances derived from molecular models of feasible β-strand registrations. The ^{13}C-^{13}C interatomic distance r_{CC} is related to d_{CC} according to the equation:

$$d_{CC} = -(\frac{\mu_0}{4\pi})\frac{\gamma_I\gamma_S\hbar}{r_{CC}^3} \qquad [1]$$

The zero quantum relaxation time (T_2^{ZQ}) also affects the shape of the curve. T_2^{ZQ} is generally not known, but can be estimated from the reciprocal sum of the NMR line widths. A series of curves was calculated by varying T_2^{ZQ} between 1 ms and 5 ms, a conservatively broad range of values, and keeping all other parameters constant. Simulated curves for n = 1 and n = 2 RR were compared against the data for n = 1 and n = 2 RR to minimise the combined χ^2 function:

$$\chi^2 = \hat{N}\left[\sum_{i=1}^{n}\frac{(O_i - C_i)^2}{C_i}\right]_1 + \hat{N}\left[\sum_{i=1}^{n}\frac{(O_i - C_i)^2}{C_i}\right]_2 \qquad [2]$$

Where O_i and C_i are the observed and calculated values at each of *n* mixing times and the subscripts 1 and 2 denote the order of rotational resonance corresponding to the calculations within each square bracket. $\hat{N}$ is an operator normalising each set of values for n = 1 RR and n = 2 RR to a value between zero and 1. This normalisation procedure was followed to ensure that the combined χ^2 value is not biased by either set of data.

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