SUPPORTING INFORMATION

Assessing the effect of aromatic residues placement on α-helical peptide structure and nanofibril formation of 21-mer peptides

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Figure S1. TEM Micrographs of L6, F6, F4, F4W2, and W6 prepared at 1 mM concentration.

*The TEM samples were prepared and imaged as follows: 10 mM peptides solutions were prepared by dissolving lyophilised peptides in MilliQ water and diluting to 2 mM. Then, the 2 mM peptide solutions were mixed with required sodium bicarbonate (and left at room conditions for 2 hours to reach pH 7.4) to final concentration of 1 mM. TEM samples were prepared upon completion of 2-hour titration period by deposition of 4µL of titrated peptide on 200-mesh carbon-coated copper grid and left for 90s. The excess liquid was wicked away and 4µL of aqueous 1% uranyl acetate stain solution was added and briefly mixed on the grid. After 90 s, the excess was removed, and grids were air-dried. The TEM images were collected using Hitachi-H7700 transmission electron microscope.
Figure S2. Free energy landscape for full atomistic models of L6, F6, F4, F4W2, W6, and W2F4 measured up to 1.2 µs (first 200 ns of simulation omitted). Radius of gyration of backbone and RMSD of backbone versus average structure of each peptide over the simulation period used as X and Y axis. Representative models at local minimum energies are represented with their occurrence of simulation time. Purple, green, blue, and red, and orange colours indicate parts of the representative models that are α-helix, random coil, turn, and 3-10-helix, and β-sheet secondary structure. Bar legend for all free energy landscapes are the same shown at top left of the figure with kJ/mol units.
Figure S3. RMSF results for MD simulation of all-atom models up to 1.2 µs (first 200 ns of simulation omitted) of each residue for L6, F6, F4, F4W2, and W6 peptides coloured blue, red, purple, green, and orange, respectively.

Figure S4. MOI(ζ/ξ) of three repeated MD simulations of peptide CG models from different initial peptide distribution in the simulation box.
Figure S5. Snapshot of largest CG cluster for W2F4 at 25 µs with Phe and Trp residues coloured yellow and green, respectively and different peptide chains presented in different colours.

Table S1. Moment of Inertia (MOI) along the principles axis of CG models (at 25 µs) calculated for all designed peptides along with solvent accessible surface area (SASA) of them calculated at initial (after energy minimisation) and end of simulation (at 25 µs). Results are average of three repeated MD simulations of peptide CG models from different initial peptide distribution in the simulation box.

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<th>MOI (z/x)</th>
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<td></td>
<td>X</td>
<td>Y</td>
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Table S2. Secondary structure prediction of L6, F4, F6, F4W2, and W6 peptides at 1 mM, 100 µM, 10 µM, and 1 µM concentrations using BeStSel method. Each class of secondary structure is defined by BeStSel. Total α-helical structure is combination of Helix 1 and Helix 2. Total antiparallel is combination of Anti 1, Anti 2, and Anti 3 while total β-sheet is combination of parallel and anti-parallel.

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Table S3. Fibre diameter calculation for L6, F4, F6, F4W2, and W6 peptides based on AFM images. It should be noted these measurements are from the images in Figure 1 of the manuscript. These are representative images only.

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<th>F4 (pm)</th>
<th>F6 (pm)</th>
<th>F4W2 (pm)</th>
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Average: 1434.1  2612.7  1071.2  5479.1  5467.7

Standard Deviation: 335.2  547  176.3  1040.4  1148.8

References:

Appendix: High performance liquid chromatography (HPLC) and mass spectroscopy (MS) data for each peptide

HPLC data for L6:

Pump A: 0.065% trifluoroacetic in 100% water (v/v)
Pump B: 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow: 1 ml/min
Wavelength: 220 nm

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![Chromatogram](image)

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MS data for L6:

Positive
Intensity

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m/z
HPLC data for F6:

Column: 4.6mmx250mm, Agela
Solvent A: 0.1% trifluoroacetic in 100% acetonitrile
Solvent B: 0.1% trifluoroacetic in 100% water
Gradient:

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<td>25min</td>
<td>71%</td>
<td>29%</td>
</tr>
<tr>
<td>25.1min</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>30min</td>
<td>STOP</td>
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Flow Rate: 1.0ml/min
Wavelength: 214nm
Volume: 10ul

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MS data for F6:
HPLC data for **W6**: 

- **Column**: 4.6mmx250mm, Sinochrom ODS-Bp 5um
- **Solvent A**: 0.1% trifluoroacetic in 100% acetonitrile
- **Solvent B**: 0.1% trifluoroacetic in 100% water
- **Gradient**
  - 0.01min: 10% A, 90% B  
  - 25min: 67% A, 33% B  
  - 25.1min: 100% A, 0% B  
  - 30min: STOP
- **Flow Rate**: 1.0ml/min
- **Wavelength**: 214nm
- **Volume**: 10ul

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S - 11
MS data for **W6:**
HPLC data for **F4W2**:

- **Column**: 4.6mmx250mm, Agela
- **Solvent A**: 0.1% trifluoroacetic in 100% acetonitrile
- **Solvent B**: 0.1% trifluoroacetic in 100% water
- **Gradient**
  - 0.01min: 43% A, 57% B
  - 25min: 68% A, 32% B
  - 25.1min: 100% A, 0% B
  - 30min: STOP
- **Flow Rate**: 1.0ml/min
- **Wavelength**: 214nm
- **Volume**: 10μl

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MS data for **F4W2**:
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<tr>
<td>Gradient</td>
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MS data for F4:

- $\text{[M+3H]}^3^+$ at 888.20
- $\text{[M+4H]}^4^+$ at 651.35
- $\text{[M+5H]}^5^+$ at 521.25