**Supporting Information**

# Control of Strand Registry by Attachment of PEG Chains to Amyloid Peptides Influences Nanostructure

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## Experimental

### Experimental Methods

*Materials*

Fmoc-Phe-Wang resin (100-200 mesh, 0.66 mmol $g^{-1}$ substitution), Fmoc-PEG-OH(Mol. Wt: 1544.8), Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Leu-OH and Fmoc-Lys(Boc)-OH were purchased from Novabiochem (UK). Trifluoroacetic acid (TFA), piperidine and triisopropylsilane (TIPS) were purchased from Sigma-Aldrich. Fmoc -PEG-COOH (Mol. Wt: 575.65) and Fmoc-PEG-COOH (Mol. Wt: 839.96) were purchased from Quanta Biodesign Limited. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), diisopropylethylamine (DIPEA) and N-methylpyrrolidone (NMP) were obtained from AGTA Bioproducts (UK). Water (HPLC grade), acetonitrile (HPLC grade), Methanol (MeOH) and dichloromethane (DCM) and diethyl ether were purchased from Fisher Scientific (UK).

*General Synthesis Methods*

The (Fmoc)-PEG-FFKLVFF conjugates shown in Scheme 1 were synthesized by solid phase methods using standard FastMoc chemistry [Fmoc (9-fluorenylmethyloxycarbonyl) protecting group and activation by HBTU/HOBt]. A 0.25 mmol scale synthesis was conducted with a fully automated peptide synthesizer (433A Applied Biosystems), which allowed for direct conductivity monitoring of Fmoc deprotection. Onto the pre-loaded Fmoc-Phe-Wang resin the complete sequence of the peptide was assembled from the C-terminus towards the N-terminus. The retulted resin was dispersed in NMP in a flask, followed by addition of Fmoc-PEG-COOH (2-3 equiv), HBTU/HOBt (2-3 equiv) and DIEA (4-6 equiv). The mixture was stirred at room temperature for 48h, filtered, washed with NMP, MeOH and DCM, respectively. The obtained resin was divided into two parts for the following reactions: part one was directly applied to cleavage reaction with an acidic cleavage

cocktail (95% TFA, 2.5% water, and 2.5% TIPS) concomitant with deprotection of lysine's ε-amino-Boc groups to give Fmoc-PEG-FFKLVFF conjugates; for part two after the Fmoc protecting group was removed by a solution of 20% piperidine/NMP, it was submitted to cleavage reaction to generate PEG-FFKLVFF conjugates. In the cleavage reaction, the mixture was stirred at room temperature for approximately 4 h and filtered. The obtained solution was concentrated and precipitated in cold diethyl ether and the crude product was separated by centrifugation and decanting the supernatant. Semi-preparative reverse phase HPLC (Perkin Elmer 200) was used for purification of the product and the following HPLC condition was employed: the eluents were 0.1% TFA in HPLC grade water and 0.1% TFA acetonitrile; during HPLC process a gradient solvent was used, where the acetonitrile portion increased linearly from 0% to 90% over 20 min and then decreased linearly to 0% over 10 min. A sample injection (injected with Perkin Elmer Series 200 Autosampler) was run on a C18 preparative column (Vp 250/10 Nucleosil, Macherey-Nagel, 7μm, 250mm× 10 mm) for 30 min with flow rates 4 ml/min at 35°C (Perkin Elmer Series 200 Peltier Column Oven), where a UV/vis detector (Perkin Elmer Series 200 UV/VIS Detector) monitored the sample elution at 238 or 254 nm. The fractions of the (Fmoc)-PEG-FFKLVFF conjugates were collected, followed by lyophilization to give a white solid, which was characterized by ESIMS and $^1$H NMR.

*Synthesis of Peptide* **n5-Fmoc**

RP-HPLC retention time $t_R$ = 19.90 min; MS(ESI)m/z for $C_{83}H_{109}N_9O_{17}$ $[M+H]^+$calcd 1504.80, found $[M+H]^+$, 1504.80. $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.80 (dd, $J_1$ = 24.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.93 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.31 -2.01 (m, 10H), 2.43 (m, 2H), 2.82-3.21 (m, 10H), 3.57 (m, 26H), 4.17 (m, 3H), 4.37 (m, 4H), 4.64 (m, 3H), 7.24 (m, 22H), 7.41 (t, J = 7.2 Hz, 2H), 7.66 (d, J = 7.2 Hz, 2H), 7.83 (d, J = 7.6 Hz, 2H),7.89 - 8.20, [amide-H, -$NH_2$].

*Synthesis of Peptide* **n5**

RP-HPLC retention time $t_R$ = 14.53 min; MS(ESI)m/z for $C_{68}H_{99}N_9O_{15}$ $[M+H]^+$calcd 1282.72, found $[M+H]^+$, 1282.73; $[M+2H]^{2+}$ calcd 641.86, found 641.87. $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.82 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.94 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.40 - 1.76 (m, 8H),1.85 (m, 1H), 1.96 (m, 1H), 2.43 (m,

2H), 2.66-3.08 (m, 12H), 3.65 (m, 24H), 4.13 (t, J = 7.2 Hz, 1H), 4.40 (m, 2H), 4.56 (q, J = 5.2 Hz, 1H), 4.64 (m, 3H), 7.24 (m, 20H), 7.90 - 8.20, [amide-H, -$NH_2$].

*Synthesis of Peptide* **n11-Fmoc**

[001] RP-HPLC retention time $t_R$ = 20.10 min; MS(ESI)m/z for $C_{95}H_{133}N_9O_{23}$ $[M+H]^+$calcd 1768.96, found $[M+H]^+$, 1768.96. $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.78 (dd, $J_1$ = 24.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.93 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.44 (m, 2H), 1.54 (m, 1H), 1.67 (m, 4H), 1.79 (m, 1H),1.88 (m, 1H), 1.97 (m, 1H), 2.46 (m, 2H), 2.84-3.22 (m, 10H), 3.61 (m, 50H), 4.09 (t, J = 8.0 Hz, 1H), 4.22 (t, J = 6.8 Hz, 1H), 4.38 (m, 5H), 4.50 (m, 1H), 4.64 (m, 2H), 7.23 (m, 18H), 7.33 (t, J = 7.2 Hz, 4H), 7.41 (t, J = 7.2 Hz, 2H), 7.67 (d, J = 7.6 Hz, 2H), 7.82 (d, J = 7.6 Hz, 2H),7.92, 8.07, 8.20, [amide-H, -$NH_2$].

*Synthesis of Peptide* **n11**

RP-HPLC retention time $t_R$ = 14.63 min; MS(ESI)m/z for $C_{80}H_{123}N_9O_{21}$ $[M+2H]^{2+}$calcd 773.9496, found $[M+2H]^{2+}$, 773.9489; $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.81 (dd, $J_1$ = 24.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.93 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.40 - 1.80 (m, 8H),1.86 (m, 1H), 1.97 (m, 1H), 2.45 (t, J = 6.0Hz, 2H), 2.80-3.18 (m, 12H), 3.66 (m, 48H), 4.15 (t, J = 7.6 Hz, 1H), 4.46 (m, 3H), 4.68 (m, 3H), 7.23 (m, 20H), 7.90 - 8.20, [amide-H, -$NH_2$].

*Synthesis of Peptide* **n27-Fmoc**

RP-HPLC retention time $t_R$ = 20.50 min;MS(ESI)m/z for $C_{127}H_{197}N_9O_{39}$ $[M+H]^+$calcd 2474.96, found $[M+H]^+$, 2474.37. $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.78 (dd, $J_1$ = 24.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.92 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.40 - 2.01 (m, 10H), 2.47 (m, 2H), 2.83-3.21 (m, 10H), 3.63 (m, 114H), 4.08 (t, J = 7.6 Hz, 1H), 4.22 (m, 1H), 4.38 (m, 5H), 4.64 (m, 3H), 7.24 (m, 18H), 7.33 (t, J = 7.6 Hz, 4H), 7.41 (t, J = 7.6 Hz, 2H), 7.67 (d, J = 6.8Hz, 2H), 7.81 (d, J = 7.6 Hz, 2H),7.92, 8.07, 8.20, [amide-H, -$NH_2$].

*Synthesis of Peptide* **n27**

RP-HPLC retention time $t_R$ = 14.78 min; MS(ESI)m/z for $C_{112}H_{187}N_9O_{37}$ $[M+H]^{2+}$ calcd 2253.73, found 2253.31. $^1$H NMR (400 MHz Methanol-$d_4$, ppm): 0.79 (dd, $J_1$ = 26.0 Hz, $J_2$ = 6.8 Hz, 6H), 0.94 (dd, $J_1$ = 20.0 Hz, $J_2$ = 6.4 Hz, 6H), 1.40 - 2.01 (m,

10H), 2.48 (m, 2H), 2.84 - 3.22 (m, 12H), 3.65 (m, 112H), 4.09 (t, J = 7.6 Hz, 1H), 4.39 (m, 3H), 4.55 (m, 1H), 4.64 (m, 2H), 7.26 (m, 20H), 7.90 - 8.20, [amide-H, -$NH_2$].

**Preparation of Solutions**. The three PEG-peptides were soluble in water and so self-assembly was studied in aqueous solution. The three Fmoc-PEG-peptides were insoluble in water, but were soluble in acetontitrile/water mixtures (the solvent for the HPLC purification) and so this mixed solvent system was used for the self-assembly studies. The measured (Mettler Toledo FiveEasy pH meter) pH value was 4.5 for all peptides at 1 wt% (in water for **n5**, **n11** and **n27** or 1:1 acetonitrile/water for **n11-Fmoc** and 2:1 acetonitrile/water for **n5-Fmoc** and **n27-Fmoc**).

**FTIR.** Spectra were recorded using a Nexus-FTIR spectrometer equipped with a DTGS detector and a multiple reflection attenuated total reflectance (ATR) system. Solutions of the three PEG-peptides in $D_2O$ (1 wt.%) and of the three Fmoc-PEG-peptides in $D_2O/CD_3CN$ (1 wt%) were sandwiched in ring spacers between two $CaF_2$ plate windows (spacer 0.0125 mm). All spectra were scanned 128 times over the range of 4000-950 $cm^{-1}$.

**Circular Dichroism.** CD spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). Solutions of the PEG-peptides (1 wt%) were loaded in parallel plaque cells (Hellma quartz Suprasil®), with a 0.1 mm pathlength. The CD data were measured using 1 sec acquisition time per point and 0.5 nm step. The post-acquisition smoothing tool from Chirascan software was used to remove random noise elements from the averaged spectra. A residual plot was generated for each curve in order to verify whether or not the spectrum has been distorted during the smoothing process. The CD signal from the water was subtracted from the CD data of the peptide solutions.

**Cryogenic-Transmission Electron Microscopy (Cryo-TEM).** Experiments were performed at Unilever Research, Colworth, Bedford, UK. Sample preparation was carried out using a CryoPlunge 3 unit (Gatan Instruments) employing a double blot technique. 3 µl of sample was pipetted onto a plasma etched (15 s) 400 mesh holey carbon grid (Agar Scientific) held in the plunge chamber at approx 90% humidity. The samples were blotted, from both sides for 0.5, 0.8 or 1.0 s dependant on sample viscosity. The samples were then plunged into liquid ethane at a temperature of -170 ˚C. The grids were blotted to remove excess ethane then transferred, under liquid nitrogen to the cryo TEM specimen holder (Gatan 626 cryo holder) at -170 ˚C. Samples were examined using a Jeol 2100 TEM operated at 200 kV and imaged using a Gatan Ultrascan 4000 camera and images captured using DigitalMicrograph software (Gatan).

**X-ray Diffraction (XRD).** Measurements were performed on stalks prepared by drying filaments from solutions containing 5-7% of the conjugate, in water in the case of the PEG-peptides and acetonitrile/water (2:1) mixtures in the case of the Fmoc-PEG-peptides. Solutions of the peptide were suspended between the ends of wax-coated capillaries and dried. The stalks were mounted (vertically) onto 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.

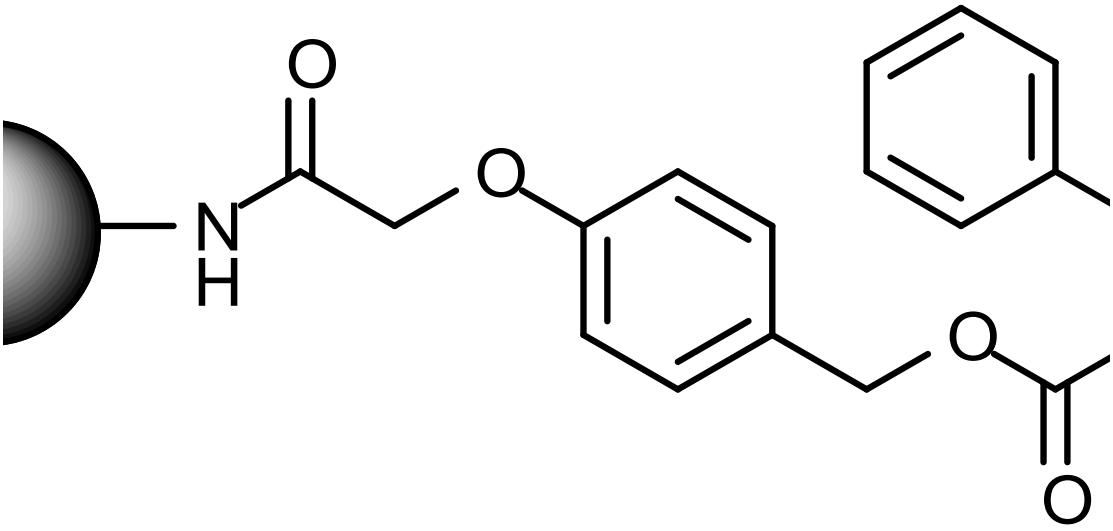
**Small-Angle X-ray Scattering (SAXS)**. Experiments were performed on beamline ID02 at the ESRF, Grenoble, France. The sample was mounted into a glass capillary flow-through cell within a metal chamber heated by a water bath. Data were collected using a FReLoN Kodak CCD detector and since no orientation was observed were reduced to one-dimensional form using the software SAXSUtilities. The data presented are averages of ten frames, taken at different sample positions. The sample-

to-detector distance was 1.2 m and the X-ray wavelength was $\lambda$ = 0.995 Å. The $q = 4\pi\sin\theta/\lambda$ scale (here $2\theta$ is the scattering angle) was calibrated using silver behenate. The same configuration was used to obtain *in situ* WAXS data, recorded using a linear AVIEX PCCD-4284 CCD detector.

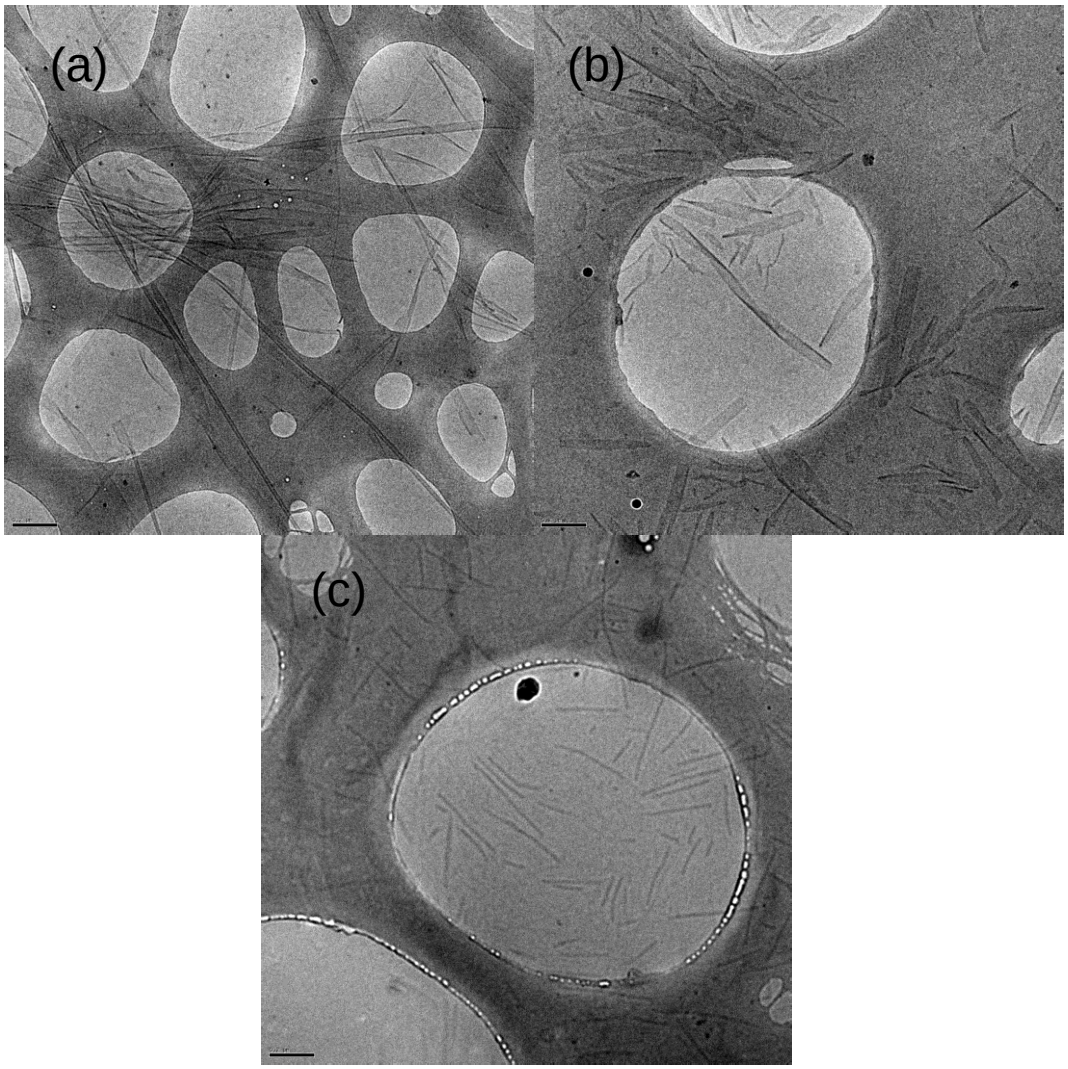
**SI Table 1**. XRD peak positions (in Å).* Shoulder peaks are denoted sho.

| Sample<br>Assignment | **n27** | **n11** | **n5** |
|---|---|---|---|
| Long spacing | 34-36 (first order) | 27.2 (sho.) | 26.9 |
| Second order | 18 | 14.2 | 13.9 |
| Third order | 12.5 | | |
| β-sheet spacing | 10.2 | 10.8 (sho.)/9.65 | 10.9 |
| Unassigned | 6.22 | | |
| β-strand spacing | 4.86 | 4.87 | 4.86 |
| Unassigned | 4.35 | 4.38 | |
| $C_\alpha$-$C_\alpha$ spacing | | 3.93 | 3.93 |

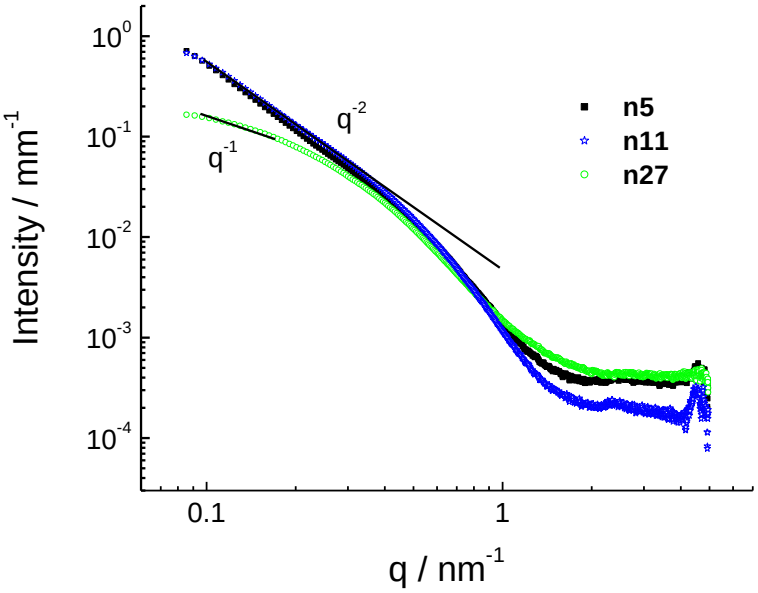
* N.B. As the long spacing peaks are observed close to the beamstop, the uncertainty in d spacing is larger, estimated at ±1 Å. For the second and third order peaks the uncertainty is approximately ±0.2 Å and ±0.01 Å for other peaks



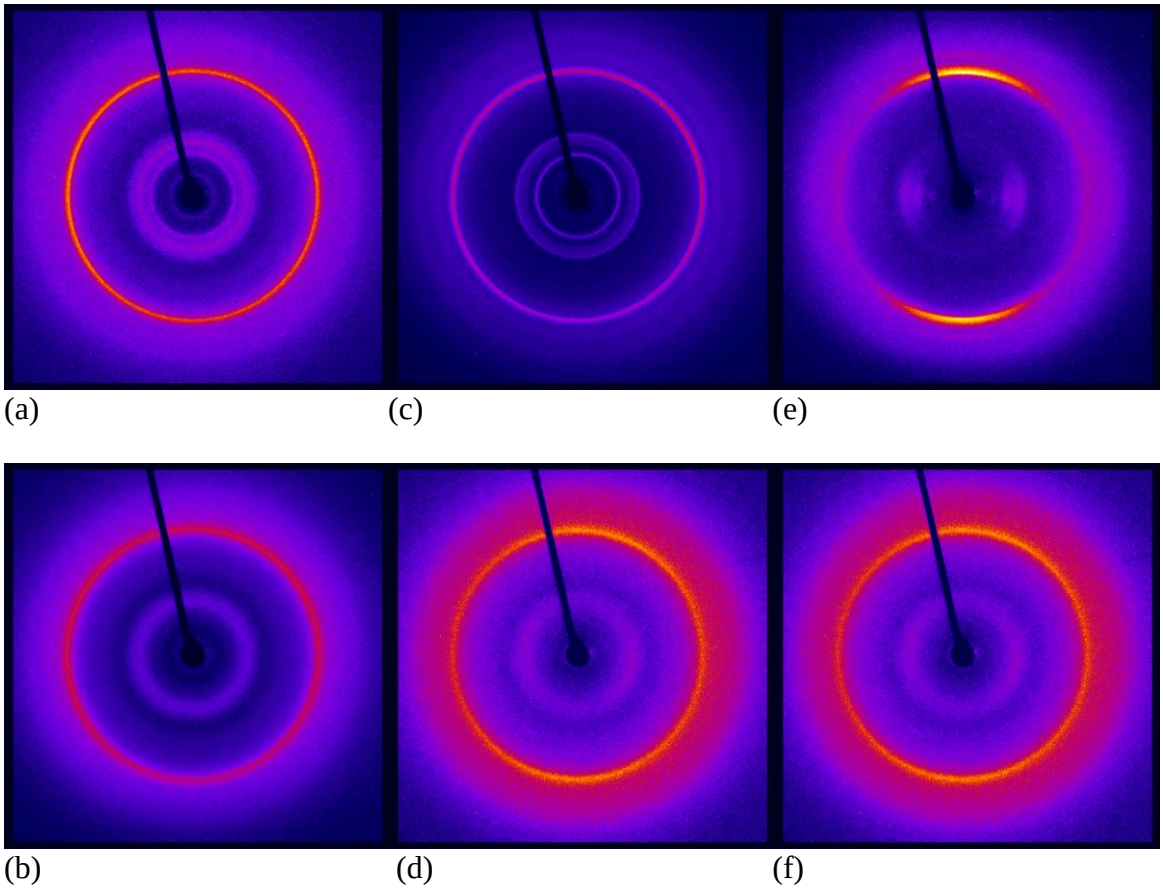

**Scheme 1** Synthesis of (Fmoc)-PEG-FFKLVFF conjugates




**SI Fig.1** Additional cryo-TEM images showing nanostructures in 1 wt% solutions of (a) **n5**, (b), **n11**, (c) **n27**. The scale bar indicates 200 nm.




**SI Fig.2**. SAXS data for 1 wt% solutions of PEG-FFKLVFF conjugates. At low *q* the scattering profiles for **n5** and **n11** show the scaling behaviour $q^{-2}$ expected for sheet-like objects whereas for **n27**, a $q^{-1}$ scaling is observed, consistent with cylindrical fibrils.




**SI Fig.3**. Fibre XRD patterns, (a) **n5**, (b) **n5-Fmoc**, (c) **n11**, (d) **n11-Fmoc**, (e) **n27**, (f) **n27-Fmoc**.