

Supplementary information

Decoupling the effects of hydrophilic and hydrophobic peptide moieties at the neuron-nanofibre interface

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Synthesis and characterisation of lysine containing tetrapeptides

Initial amino acid loading

2-chlorotriptyl chloride resin (100-200 mesh; 1% DVB; 1.1 mmol/g) (500 mg, 0.55 mmol) was weighed into a 10 mL polypropylene syringe equipped with a porous polypropylene frit (Torviq SF-1000), which was used as the reaction vessel. The resin was washed with dichloromethane (3 × 5 mL) before being allowed to swell in dichloromethane (5 mL) for at least 0.5 h prior to the loading of the first amino acid.

A solution of Fmoc-AA-OH (3 equiv.) was dissolved in a mixture of dry dichloromethane (2 mL), *N,N*-dimethylformamide (2 mL) and *N,N*-diisopropylethylamine (DIPEA) (8 equiv., 0.8 mL) and taken up into the syringe with resin and stirred overnight using an orbital shaker. The resin was then washed with dichloromethane (3 × 4 mL) and *N,N*-dimethylformamide (DMF) (3 × 4 mL).

N-terminal Fmoc deprotection

A solution of 20% (v/v) piperidine in DMF (2 × 4 mL) was added to the resin once for 1 min, then a fresh aliquot was taken up again and stirred for 10 mins. The solution was subsequently expelled and the resin washed with DMF (5 × 4 mL). The resulting resin-bound amine was used immediately in the next peptide coupling step.

Amino acid coupling

The next amino acid (3 equiv., masses as below) was dissolved in a 0.45 M DMF solution of 1-hydroxybenzotriazole hydrate (HOBt·H₂O)/*N,N,N',N'*-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (3 equiv.) and DIPEA (6 equiv., 0.6 mL) and this coupling solution added to the resin and stirred for 45 mins using an orbital shaker. The solution was expelled and the resin washed with DMF (5 × 4 mL).

Amino acid	Mass used (mg)
Fmoc-Phe-OH	640
Fmoc-Lys(Boc)-OH	773

After another *N*-terminal Fmoc deprotection, iterative couplings were performed in order to build up the required peptide sequence.

Cleavage of the peptide

After the final coupling step, the resin was washed with DMF (3 × 4 mL) and dichloromethane (3 × 4 mL). A solution of 1:9 dichloromethane: trifluoroacetic acid with three drops of water was then added to the resin, and the resin stirred for 2 hours using an orbital shaker. The cleavage solution was then expelled, the resin washed with dichloromethane (2 × 4 mL) and the solvents evaporated

under a stream of nitrogen. The resulting residue was lyophilised and purified by semi-preparative HPLC using gradient of acetonitrile/water with 0.1% formic acid to give a white fluffy solid.

Characterisation data for **Fmoc-FFKK**: IR: 3279 (m), 3063 (w), 3033 (w), 2939 (w), 2866 (w), 1633 (s), 1533 (s), 1451 (m), 1398 (m), 1320 (w), 1287 (w), 1259 (m), 1201 (s), 1182 (m), 1133 (s), 1083 (w), 1033 (m), 836 (w), 799 (w), 755 (m), 739 (s), 722 (m), 697 (s); ¹H NMR (400 MHz, DMSO-d₆) δ 8.26-8.18 (m, 1H), 7.89-7.83 (m, 2H), 7.78 (d, *J* = 6.3 Hz, 0.5H), 7.60 (t, 0.5H), 7.43-7.32 (m, 2H), 7.26-7.13 (m, 6H), 4.59 (br. s, 1H), 4.28-4.06 (m, 2H), 3.95 (q, *J* = 6.1 Hz, 1H), 3.38 (q, *J* = 5.1 Hz, 1H), 3.09-3.01 (m, 1H), 2.81-2.89 (m, 2H), 2.72, (br. s, 3H), 1.67-1.52 (m, 4H), 1.34 (br. s, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 174.71, 170.59, 170.37, 165.31, 142.56, 139.41, 137.43, 129.33, 129.19, 128.93, 128.15, 127.96, 127.62, 127.30, 127.07, 126.18, 125.23, 121.39, 120.04, 55.93, 53.80, 53.27, 52.20, 46.52, 31.0, 26.82, 26.37, 22.38, 21.51; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4122, found 791.4127.

Characterisation data for **Fmoc-FKFK**: IR: 3282 (m), 3062 (w), 3033 (w), 2939 (m), 2868 (w), 1638 (s), 1574 (m), 1532 (s), 1450 (m), 1398 (m), 1347 (w), 1287 (w), 1257 (m), 1202 (s), 1180 (m), 1134 (s), 1083 (w), 1034 (m), 837 (w), 800 (w), 739 (s), 698 (s); ¹H NMR (400 MHz, DMSO-d₆) δ 8.71 (t, *J* = 6.3 Hz, 1H), 8.38 (s, 1H), 8.05 (dd, *J* = 8.8 Hz, 1H), 7.89-7.83 (m, 3H), 7.63-7.53 (m, 1H), 7.43-7.38 (m, 3H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.30-7.17 (m, 11H), 4.37-4.20 (m, 3H), 4.17-4.08 (m, 2H), 3.82 (br. s, 2H), 3.09 (br. s, 1H), 3.06 (br. s, 1H), 2.87-2.82 (m, 2H), 2.79-2.66 (m, 5H), 1.68 (br. s, 1H), 1.53 (br. s, 3H), 1.29 (br. s, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 174.07, 173.01, 170.88, 169.65, 142.57, 139.42, 138.32, 138.02, 129.38, 128.94, 128.14, 128.10, 127.30, 126.16, 121.40, 120.04, 55.47, 55.11, 51.88, 32.58, 32.20, 27.42, 27.06, 22.01; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4122, found 791.4120.

Characterisation data for **Fmoc-FKKF**: IR: 3285 (m), 3068 (w), 3033 (w), 2945 (m), 2873 (w), 1639 (s), 1529 (s), 1450 (m), 1397 (m), 1332 (w), 1291 (w), 1258 (w), 1201 (s), 1185 (m), 1132 (s), 1083 (m), 1033 (m), 837 (w), 800 (w), 757 (w), 740 (s), 722 (s), 700 (s); ¹H NMR (400 MHz, DMSO-d₆) δ 8.23 (d, *J* = 8.3 Hz, 0.5H), 8.00 (d, *J* = 8.3 Hz, 0.5H), 7.90-7.83 (m, 2H), 7.70 (t, *J* = 6.5 Hz, 1H), 7.45-7.40 (m, 2H), 7.36-7.30 (m, 1H), 7.27-7.23 (m, 2H), 7.21-7.09 (m, 4H), 4.49-4.44 (m, 1H), 4.29-4.20 (m, 1H), 3.95-3.92 (m, 1H), 3.12-3.07 (m, 1H), 2.90-2.82 (m, 1H), 2.76-2.67 (m, 3H), 1.75-1.68 (m, 1H), 1.60-1.48 (m, 3H), 1.36-1.29 (m, 2H), 1.24-1.20 (m, 1H). ¹³C NMR (101 MHz, DMSO-d₆) δ 171.40, 170.82, 157.84, 155.89, 143.90, 143.73, 140.72, 137.75, 129.24, 129.08, 128.94, 128.07, 127.94, 127.66, 127.30, 127.08, 126.26, 125.30, 121.39, 120.13, 120.04, 65.68, 54.20, 53.22, 46.65, 37.68, 31.29, 26.80, 26.53, 22.16; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4122, found 791.4126.

Characterisation data for **Fmoc-KFFK**: IR: 3278 (m), 3060 (w), 3031 (w), 2944 (m), 2873 (w), 1635 (s), 1531 (s), 1451 (m), 1399 (m), 1337 (w), 1291 (w), 1254 (w), 1201 (s), 1182 (m), 1130 (s), 1080 (m), 1044 (m), 837 (w), 799 (w), 758 (w), 740 (m), 722 (s), 698 (m); ¹H NMR (400 MHz, DMSO-d₆) δ 8.23 (d, *J* = 8.5 Hz, 0.5H), 8.00 (d, *J* = 8.5 Hz, 0.5H), 7.91-7.83 (m, 2H), 7.70 (t, *J* = 6.3 Hz, 1H), 7.45-7.40 (m, 2H), 7.36-7.30 (m, 1H), 7.27-7.08 (m, 5H), 4.48-4.45 (m, 1H), 4.31-

4.18 (m, 1H), 3.98-3.93 (m, 1H), 3.11-3.08 (m, 1H), 2.94-2.82 (m, 1H), 2.77-2.67 (m, 3H), 1.77-1.68 (m, 1H), 1.62-1.47 (m, 3H), 1.37-1.32 (m, 2H), 1.26-1.15 (m, 1H). ¹³C NMR (101 MHz, DMSO-d₆) δ 170.92, 143.89, 140.72, 139.41, 137.56, 129.24, 129.06, 128.93, 128.07, 127.65, 127.29, 127.07, 126.25, 125.59, 121.39, 120.03, 54.34, 54.18, 53.28, 46.64, 31.30, 31.04, 26.79, 26.52, 22.16; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4122, found 791.4118.

Characterisation data for **Fmoc-KKFF**: IR: 3279 (m), 3066 (w), 3034 (w), 2942 (m), 2868 (w), 1634 (s), 1537 (s), 1452 (m), 1397 (m), 1338 (w), 1293 (w), 1266 (w), 1201 (s), 1189 (m), 1130 (s), 1083 (m), 1046 (m), 838 (w), 799 (w), 756 (w), 738 (m), 722 (s), 696 (m); ¹H NMR (400 MHz, DMSO-d₆) δ 8.05 (d, *J* = 7.9 Hz, 0.5H), 7.94 (d, *J* = 7.9 Hz, 0.5H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.70 (t, *J* = 6.7 Hz, 1H), 7.46-7.40 (m, 2H), 7.36-7.31 (m, 1H), 7.25-7.11 (m, 5H), 4.51-4.45 (m, 1H), 4.32-4.19 (m, 1H), 3.94-3.88 (m, 1H), 3.08 (dd, *J* = 15.6, 5.1 Hz, 0.5H), 2.99 (dd, *J* = 14.2, 3.8 Hz, 0.5H), 2.93-2.88 (m, 1H), 2.80-2.69 (m, 2H), 1.62-1.40 (m, 4H), 1.30-1.18 (m, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 173.20, 171.59, 170.83, 170.61, 157.95, 155.88, 143.88, 140.74, 138.20, 137.57, 129.20, 128.94, 128.01, 127.95, 127.67, 127.31, 127.11, 126.13, 125.27, 121.40, 120.15, 65.62, 54.60, 54.44, 53.65, 52.30, 46.67, 36.87, 31.47, 31.29, 26.62, 26.53, 22.29, 21.67; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4122, found 791.4124.

Characterisation data for **Fmoc-KKFF**: IR: 3286 (m), 3063 (m), 3034 (w), 2944 (m), 1663 (s), 1635 (s), 1524 (s), 1450 (m), 1412 (w), 1395 (m), 1334 (w), 1254 (m), 1201 (s), 1182 (s), 1130 (s), 1082 (w), 1032 (w), 837 (w), 800 (w), 757 (w), 739 (m), 722 (m), 697 (m); ¹H NMR (400 MHz, DMSO-d₆) δ 8.35 (d, *J* = 8.3 Hz, 1H), 7.90-7.87 (m, 4H), 7.72-7.69 (m, 3H), 7.42 (t, *J* = 7.8 Hz, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.25-7.15 (m, 10H), 4.37-4.13 (m, 6H), 3.96-3.91 (m, 1H), 3.09-2.89 (m, 4H), 2.76 – 2.64 (m, 5H), 1.58-1.17 (m, 12H). ¹³C NMR (101 MHz, DMSO-d₆) δ 172.99, 170.92, 155.95, 143.79, 140.73, 138.25, 137.97, 129.53, 128.91, 128.07, 127.67, 127.07, 125.99, 125.28, 120.15, 65.54, 54.90, 54.16, 46.67, 37.47, 37.33, 32.77, 31.31, 27.12, 26.58, 22.45, 22.30; HR-MS (ESI): calcd for C₄₅H₅₄N₆O₇ + H⁺: 791.4120, found 791.4196.

Viscosity measurements

Viscosity measurements were performed on an Anton Paar MCR 302 rheometer using a 25 mm stainless steel parallel plate geometry configuration and analysed using RheoPlus v3.61 software. Typical viscosity measurements involved casting 550 μL of a peptide solution at 0.5% (w/v) onto one of the stainless steel plates, lowering the other plate to the measurement position, and monitoring viscosity as a function of shear rate (1-100 s⁻¹). A Peltier temperature control hood and solvent trap was used to reduce evaporation and maintain a temperature of 25 °C. The rheology plots displayed are an average of at least three repeats for each point and error bars denote two standard deviations from the log-averaged mean.

Scanning electron microscopy measurements

SEM imaging was undertaken on a NanoSEM 450 fitted with a through lens detector at an operating voltage of 5 kV, with a working distance of between 4.9-5.7 mm used. Peptide samples

were lyophilised after semi-preparative HPLC purification and lightly dusted onto double sided carbon tape, with any excess peptide removed. The sample was then coated with a 30 nm layer of platinum using a Leica EM ACE600, where the thickness was monitored using a film thickness monitor.

Atomic force microscopy measurements

Peptide solutions were prepared at 0.5% (w/v) in water and cast onto a freshly cleaved mica substrate, followed by spreading of the drop over the mica using a glass slide, with the excess liquid wicked away using capillary action. These samples were left to dry in air overnight. Imaging was undertaken on a Bruker Multimode 8 atomic force microscope in Scanasyt mode in air, whereby the imaging parameters are constantly optimised through the force curves that are collected, preventing damage of soft samples. Bruker Scanasyt-Air probes were used, with a spring constant of 0.4 - 0.8 N/m and a tip radius of 2 nm.

Small angle neutron scattering measurements

Peptide solutions were prepared at 1% (w/v) in D₂O and transferred to a demountable titanium cell of 2 mm path length. Measurements were performed at detector distances of 2 and 14 m. Isotropic scattering patterns were radially averaged and combined for a q range of 0.006 – 0.35 Å⁻¹, where $q = 4\pi\sin(\theta)/\lambda$, and λ is neutron wavelength (5 Å) with a wavelength spread, $\Delta\lambda / \lambda$ 12% and 2θ is the scattering angle. Data was corrected for the background, empty cell scattering and the sensitivity of the individual detector pixels. The data was reduced using IgorPro software¹ employing NIST macros specific to QUOKKA² to an absolute intensity scale.

Data was modelled using SasView,³ with a flexible cylinder model chosen for each scattering pattern, based upon AFM characterisation data. The scattering length density (SLD) of the peptide was calculated to be 1.7×10^{-6} , with this value and the SLD of the solvent (D₂O) fixed. Other parameters were allowed to vary freely and following a few optimisation cycles, the background was fixed. After this, multiple different starting points were used for the Kuhn length, radius and length of the cylinder, to ensure that a global, physically realistic minimum was found.

Primary neuronal cultures

Primary neurons were obtained from pregnant C57BL6 mice of embryonic day 16.5. Briefly, the abdominal cavity of time-mated females was opened to remove the uterus. Embryos were placed on ice, decapitated and brains removed. After meninges were carefully removed, cortices and hippocampi were dissected and incubated with trypsin (Sigma) at 37 °C for 15–20 min, followed by trituration with fire-polished glass Pasteur pipettes (Livingstone) to obtain single cell solutions. Cells were counted using a hemocytometer and plated onto glass coverslips in Dulbecco's Modified Eagle Medium (Life technologies) medium containing 10% heat-inactivated fetal bovine serum (Hyclone). Medium was changed to Neurobasal containing B27 supplement and Glutamax

(all Life technologies) for continued culturing. All procedures were approved by the Animal Ethics Committee of the University of New South Wales, Australia.

Cell viability measurements

Cytotoxicity measurements were performed using an Alamar Blue colorimetric assay on primary neuronal cells. Each experiment was repeated three times. To a 24-well plate, 150 μL of peptide sol was added in triplicate and incubated overnight. Surrounding wells were supplemented with water to ensure hydration. After removal of excess solution, cells were seeded atop the peptide coated wells and incubated for 24 hours, before 10 μL Alamar Blue was added to each well, followed by further incubation for a further 4h. Control wells included cell-free peptide coating, a poly-D-lysine substrate and a negative control of 20% (v/v) DMSO. The absorbance at 570 nm and 596 nm was recorded using a BioRad Benchmark plate reader.

Circular dichroism measurements

CD measurements were performed using a ChirascanPlus CD spectrometer, with data collected between wavelengths of 180 – 500 nm with a bandwidth of 1 nm, sample ratio of 0.1 s/point and step of 1 nm. In a typical experiment, 0.5% (w/v) peptide sols were prepared and diluted 1:20 in milliQ water. Temperature was kept constant at 20 °C and all experiments were repeated three times and averaged into a single plot.

Zeta potential measurements

The zeta potential of nanofibre solutions was measured using a Malvern Instruments Zetasizer NanoZS, equipped with a He-Ne laser beam with a wavelength of 633 nm and scattering angle of 173°. Measurements were performed in folded capillary cells (Malvern Instruments, DTS1070) using peptide sols prepared at 0.5% (w/v). Each measurement was performed in triplicate and averaged into a single plot.

Contact angle measurements

Contact angle measurements were performed on a Kruss Drop Shape Analyzer DSA30 whereby a small (< 100 μL) droplet was dispensed automatically from a 1 mL syringe onto a glass coverslip coated with a peptide nanofibre solution as described for the cell viability measurements. Contact angles were determined by through a manual method whereby the edges and top of the droplet was user-defined, followed by contact angle calculation through Kruss ADVANCE software.

Fibre labelling experiments

A 0.5% (w/v) solution of peptide nanofibres was diluted 2 \times to 0.25% (w/v) (or 3.16 mM). To this, a solution of FITC in carbonate buffer (2.2 equivalents, 7 mM) was added and the solutions stirred overnight in the dark. A 100 μL aliquot was removed from each solution and diluted into 900 μL methanol. The extent of labelling was confirmed through analytical HPLC using a Phenomenex

XBridge C₁₈ 5 μm (4.8 × 150 mm) column, with an acetonitrile/water gradient of 5-95% over 30 mins.

Solid state and gel state NMR measurements

The ¹³C solid state NMR experiments were performed on a Bruker Biospin Avance III 300 MHz spectrometer with a wide bore 7 T superconducting magnet operating at frequencies of 75 MHz and 300 MHz for the ¹³C and ¹H nuclei respectively. Approximately 80 mg of each sample was packed into 4 mm outer diameter zirconia rotors with Kel-F® caps and spun to 6.5 kHz at the magic angle for measurement. The ¹³C NMR spectra were acquired using a ¹H-¹³C Hartman-Hahn cross-polarisation with a 1 ms contact pulse ramped from 70-100% for polarisation transfer. The ¹³C and ¹H 90 ° pulse lengths were 4 μs and 3.5 μs respectively. Total Suppression of Spinning Sidebands (TOSS) scheme was used to ensure that the no overlap of the spinning sideband signals with the isotropic peaks are observed, and Spinal-64 ¹H decoupling scheme with a 75 kHz decoupling field strength were used during acquisition. Recycle delays of 2 s were used and up to 1 k transients were signal averaged for sufficient signal-to-noise.

The ¹H gel state NMR experiments were performed on a Bruker Biospin Avance III 700 MHz spectrometer with a standard bore 16.4 T superconducting magnet operating at frequencies of 700 MHz for the ¹H nuclei. Approximately 30 μL of each gel sample prepared in D₂O was packed into Kel-F® insert for a 4 mm outer diameter zirconia rotor and spun to 5 kHz at the magic angle in a HrMAS probe. The ¹H NMR spectra were acquired using a pre-saturation pulse to suppress the residual H₂O signal.

Tethered bilayer lipid membrane experiments

Tethered bilayer lipid membranes (tBLMs) were fabricated using pre-prepared tethered benzyl-disulfide (tetra-ethyleneglycol) n = 2 C₂₀-phytanyl tethers (DLP) and benzyl-disulfide-tetra-ethyleneglycol-OH spacers (TEGOH) in the ratio of 1:9, as described previously (SDx Tethered Membranes Pty. Ltd., Australia).⁴ To these tethering chemistries, 8 μL of a 3 mM solution of a mobile lipid-phase mixture of 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) lipids (Sigma-Aldrich) dissolved in ethanol, was added. After a 2 min incubation, a rapid solvent exchange, to form the tBLM, was undertaken with a buffer consisting of 100 mM NaCl and 10 mM tris at pH 7.2. Membrane conduction measures were determined using swept frequency AC electrical impedance spectroscopy using a TethaQuick electrical impedance spectrometer (SDx Tethered Membranes Pty. Ltd., Australia). To determine membrane conduction, phase and impedance magnitude data was collected using a 50 mV peak-peak AC excitation (2000 Hz – 0.1 Hz). The data were then fitted to an equivalent circuit consisting of a Constant Phase Element (CPE) in series with a Resistor/Capacitor (RC) element representing the lipid membrane, and an extra resistor in series representing the NaCl/Tris buffer. In this circuit, the CPE represents the imperfect capacitance created by the chemically coated gold tethering electrode.⁵ Data were fitted with the assistance of a proprietary adaptation of a Levenberg–Marquardt fitting routine. Peptides were

initially dissolved in water at a concentration of 1% (w/v) (approximately 10 mM) and diluted to the appropriate concentration either using the NaCl/tris buffer described above (main text, Figure 6a-f) or DMEM containing 10% FBS (Figure S12)

Peptide disassembly by analytical HPLC

Peptide coverslips were prepared and immersed in DMEM as described previously. At the conclusion of seven days, the supernatant was removed and stored at 4 °C. Calibration curves were constructed using serially diluted peptide solutions in methanol from 0.5% (w/v) to 0.005% (w/v), corresponding to a concentration range of approximately 60 μ M to 6 mM. Supernatant media was diluted 1:1 in methanol and analysed using a XBridge C₁₈ 5 μ m (4.8 \times 150 mm) column, with an acetonitrile/water gradient of 5-95% over 25 mins.

SDS-PAGE of adsorbed serum proteins on peptide nanofibres

Peptide coverslips were prepared as described previously, before being immersed in complete 293T media (DMEM, 10% FBS, penicillin/streptomycin) for 24h. After 24h, unbound proteins were washed away using PBS (3 washes) and 50 μ L sample buffer added to the coverslips. A cell scraper was used to suspend any peptide or deposited protein into the sample buffer, which was boiled for 3 minutes at 95 °C before 10 μ L was loaded onto a 2% agarose SDS-PAGE gel. The gel was run at 130 V for 45 minutes before being subjected to a modified silver staining procedure. The stained gel was visualised under visible light using a BioRad ChemiDoc MP.

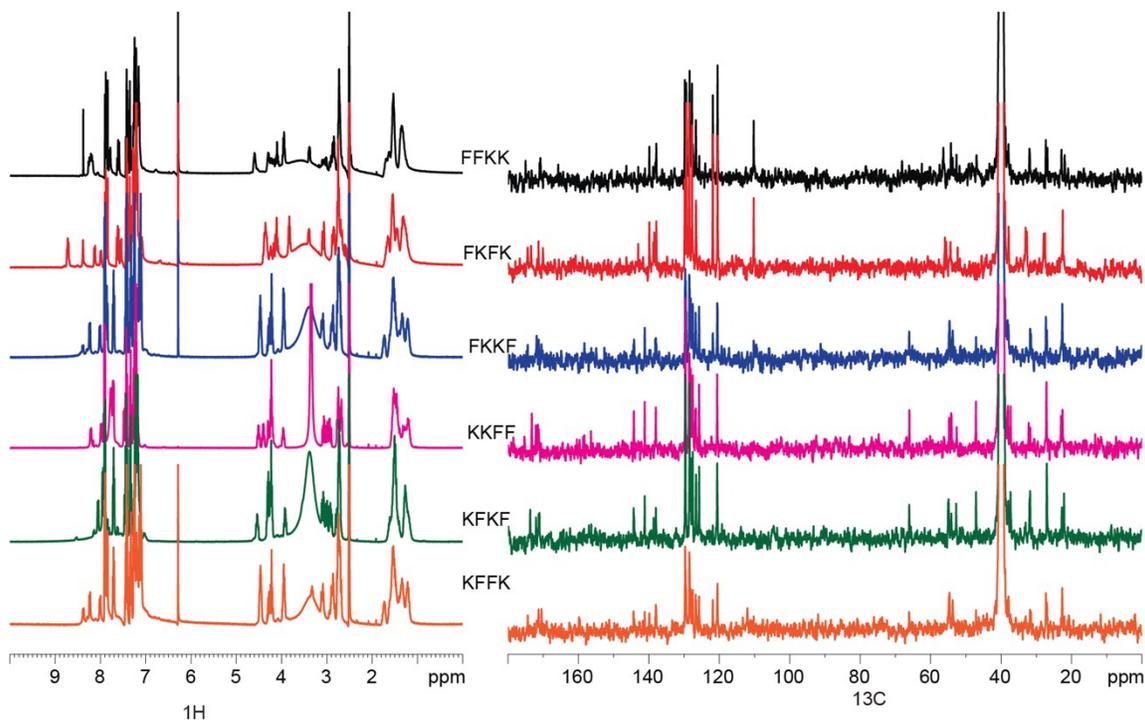


Figure S1 – ¹H NMR and ¹³C NMR of lysine containing tetrapeptides.

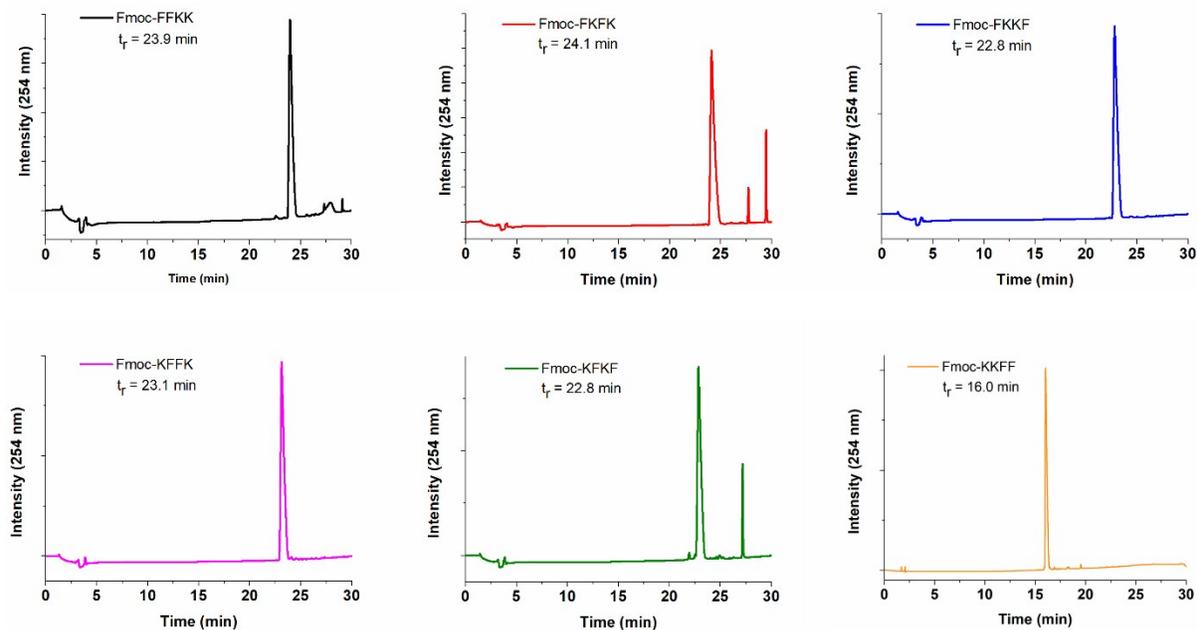


Figure S2 – Analytical HPLC trace of lysine tetrapeptides. Based on peak area, the purity of all peptides is >93%. Fmoc-KKFF displays a different t_r due to the use of a different solvent gradient.

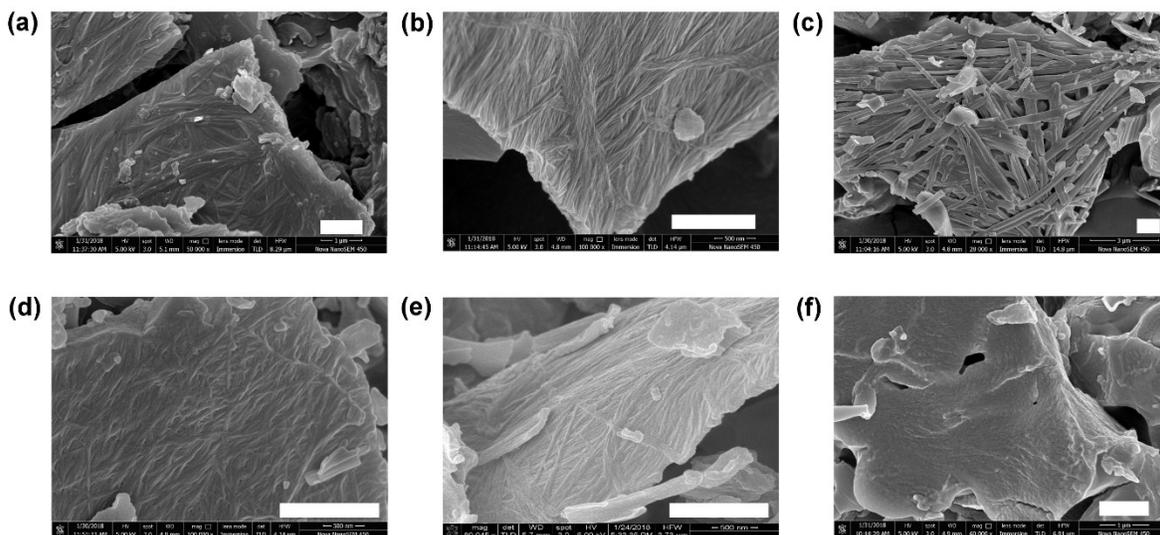


Figure S3 – SEM images showing the fibrous nature of tetrapeptides (a) Fmoc-FFKK, (b) Fmoc-FKFK, (c) Fmoc-FKKF, (d) Fmoc-KFFK, (e) Fmoc-KFKF and (f) Fmoc-KKFF upon lyophilisation after semi-preparative HPLC purification. This confirms that solid state NMR studies have been performed on the peptide nanofibre state. Scale bar represents 1 μm .

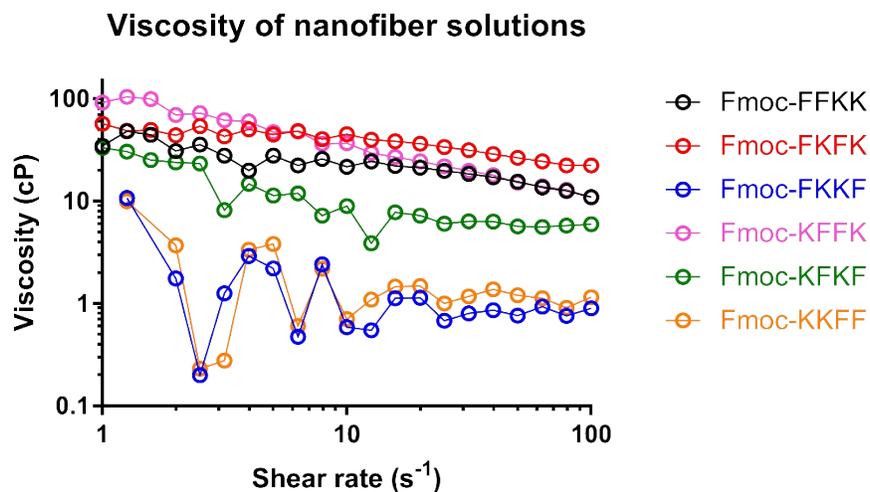


Figure S4 – Viscosity measurements of lysine containing tetrapeptides, recorded at 0.5% (w/v).

Table S1 – Fibre sizes (in nm) as measured from AFM images of tetrapeptides. Each diameter is the average of at least 20 individual fibre measurements.

Fmoc-FFKK	Fmoc-FKFK	Fmoc-FKKF	Fmoc-KFFK	Fmoc-KFKF	Fmoc-KKFF
5.8 ± 1.6	4.9 ± 1.3	6.1 ± 0.9	6.2 ± 1.7	7.6 ± 2.5	4.3 ± 0.9

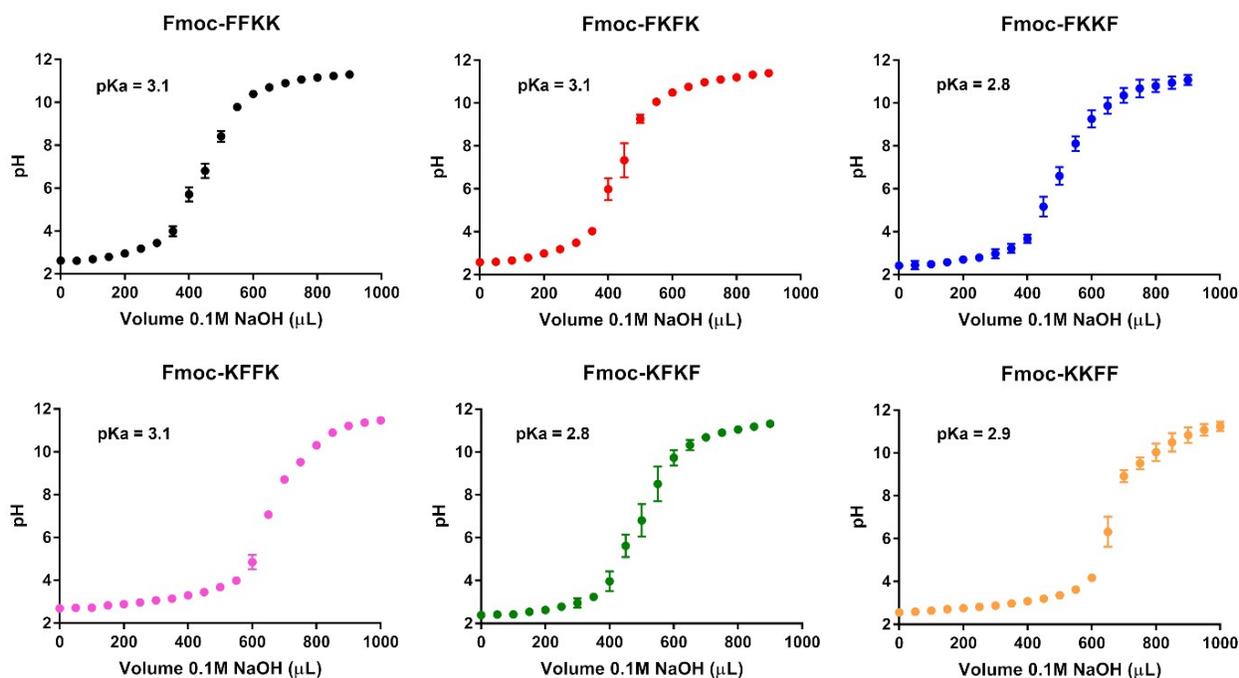


Figure S5 – pK_a determination for each of the lysine tetrapeptides. Peptides were dissolved in water at 1 mg/mL (0.1% (w/v)) and 2 equivalents 0.1 M HCl, before addition of 0.1 M NaOH. Calculated pK_a values are very similar, ranging from 2.8 (Fmoc-KFKF) to 3.1 (Fmoc-FFKK).

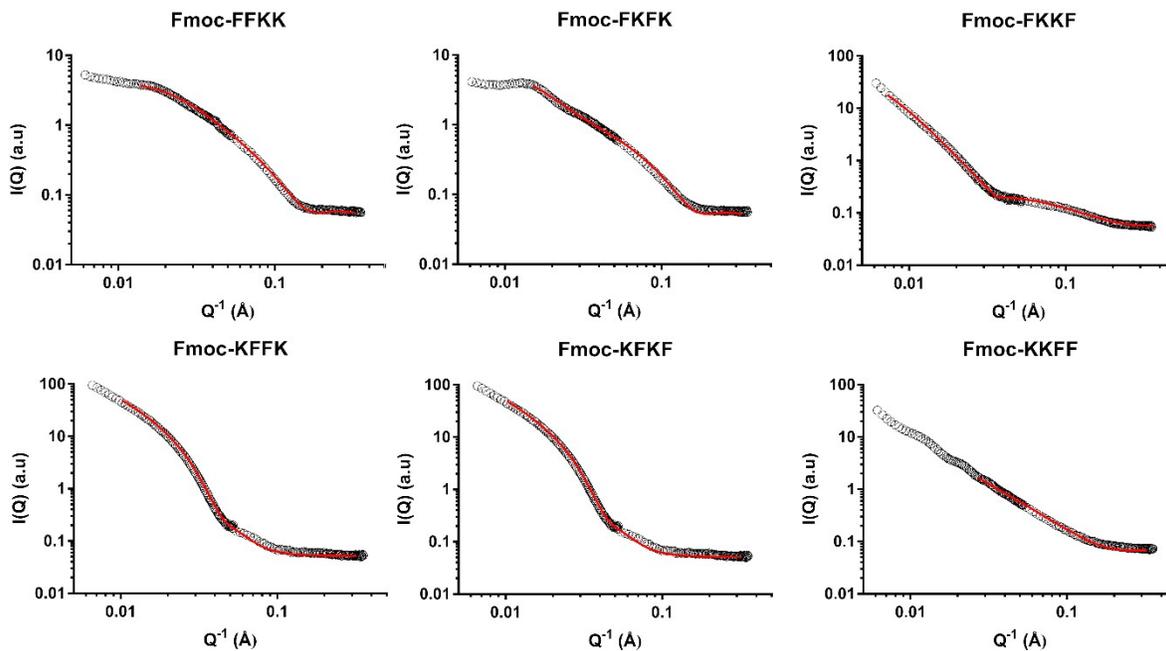


Figure S6 – SANS data for the six tetrapeptides in D_2O at 1% (w/v) fitted to a flexible cylinder model, with the exception of Fmoc-FKKF, which was fit to a combination of a fractal model at low q ($0.007 - 0.03 \text{ \AA}^{-1}$) and a flexible cylinder model at high q ($0.03 - 0.3 \text{ \AA}^{-1}$). Good agreement between the model and collected data is observed in all cases.

Table S2 – Outputs from SANS model fitting for each tetrapeptide above (Figure S6).

	Fmoc- FFKK	Fmoc- FKFK	Fmoc- FKKF (low q)	Fmoc- FKKF (high q)	Fmoc- KFFK*	Fmoc- KFKF	Fmoc- KKFF
Fibre length (Å)	435 ± 12	668 ± 10		54 ± 4	1152 ± 200	4861 ± 200	5900 ± 300
Kuhn length (Å)	56 ± 3	167 ± 2		59 ± 6	288 ± 5	13 ± 3	18 ± 4
Radius (Å)	21.3 ± 0.1	21.2 ± 0.1		11.8 ± 0.1	85 ± 0.1	16.2 ± 0.1	15.1 ± 0.2
χ^2	2.77	10.94		3.45	6.9	5.53	16.49
Fractal dimension			2.79 ± 0.02				
Correlation length (Å)			154 ± 5				
χ^2			3.49				

* denotes that a polydispersity function (PDI = 0.2) was applied to the radius of the fibre, as consistent with AFM images shown in Figure 1.

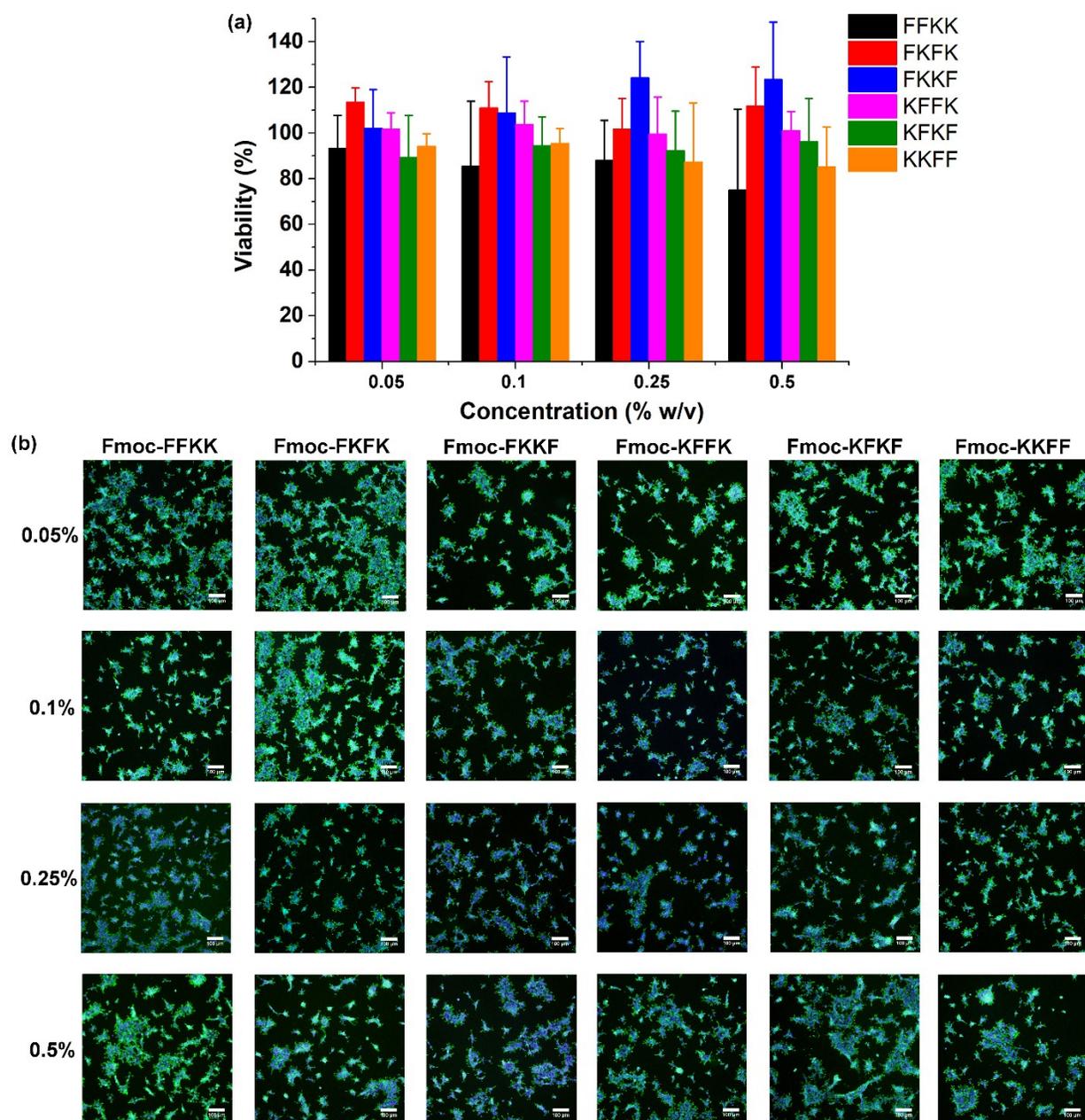


Figure S7. Evaluation of nanofibre surfaces for culturing robust, immortalised HEK293T cells. Glass coverslips were coated with a peptide nanofibre solution at specified concentrations and HEK cells seeded atop these substrates at 50,000 cells/well. Cells were fixed after 24h and stained for F-actin (phalloidin, green) alongside DAPI. (a) Cell viability was quantified through an Alamar Blue assay (24h) with poly-D-lysine coated glass used as a positive viability control. (b) Representative images for each peptide at various concentrations. Scale bar represents 100 μm.

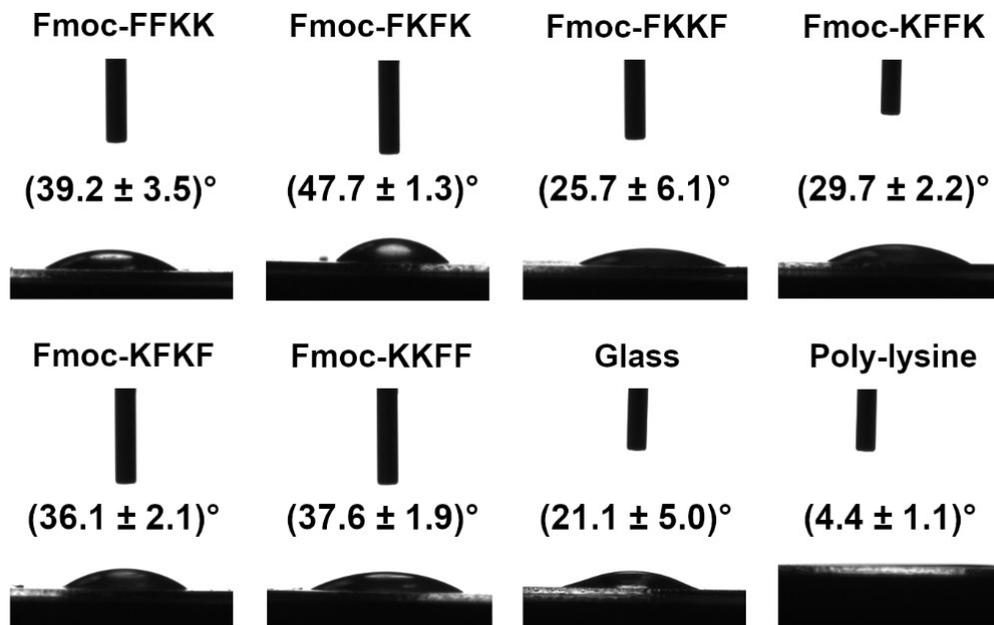


Figure S8 – Representative contact angle images for a tetrapeptide coated glass coverslips, with controls of water and poly-D-lysine also presented. It is clear that Fmoc-FKFK is the most hydrophobic of the coverslip coatings, consistent with nanofibre labelling results.

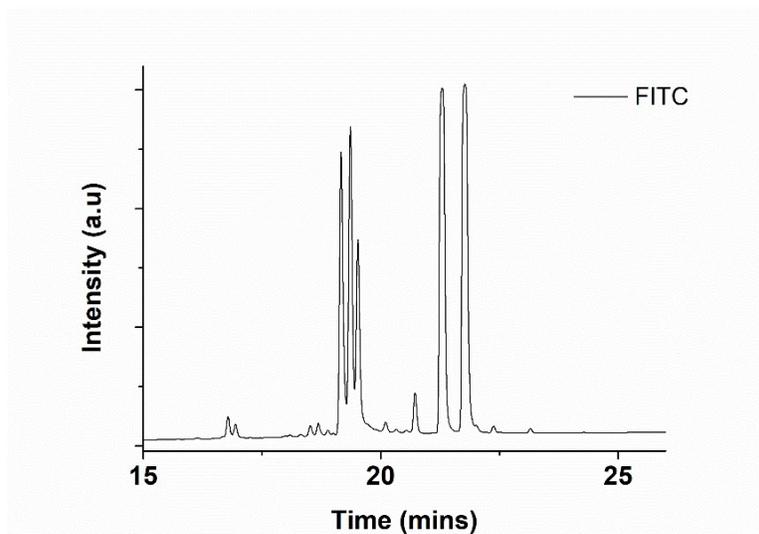


Figure S9 – Analytical HPLC trace for fluorescein isothiocyanate (FITC), with a mixture of isomers clearly evident.

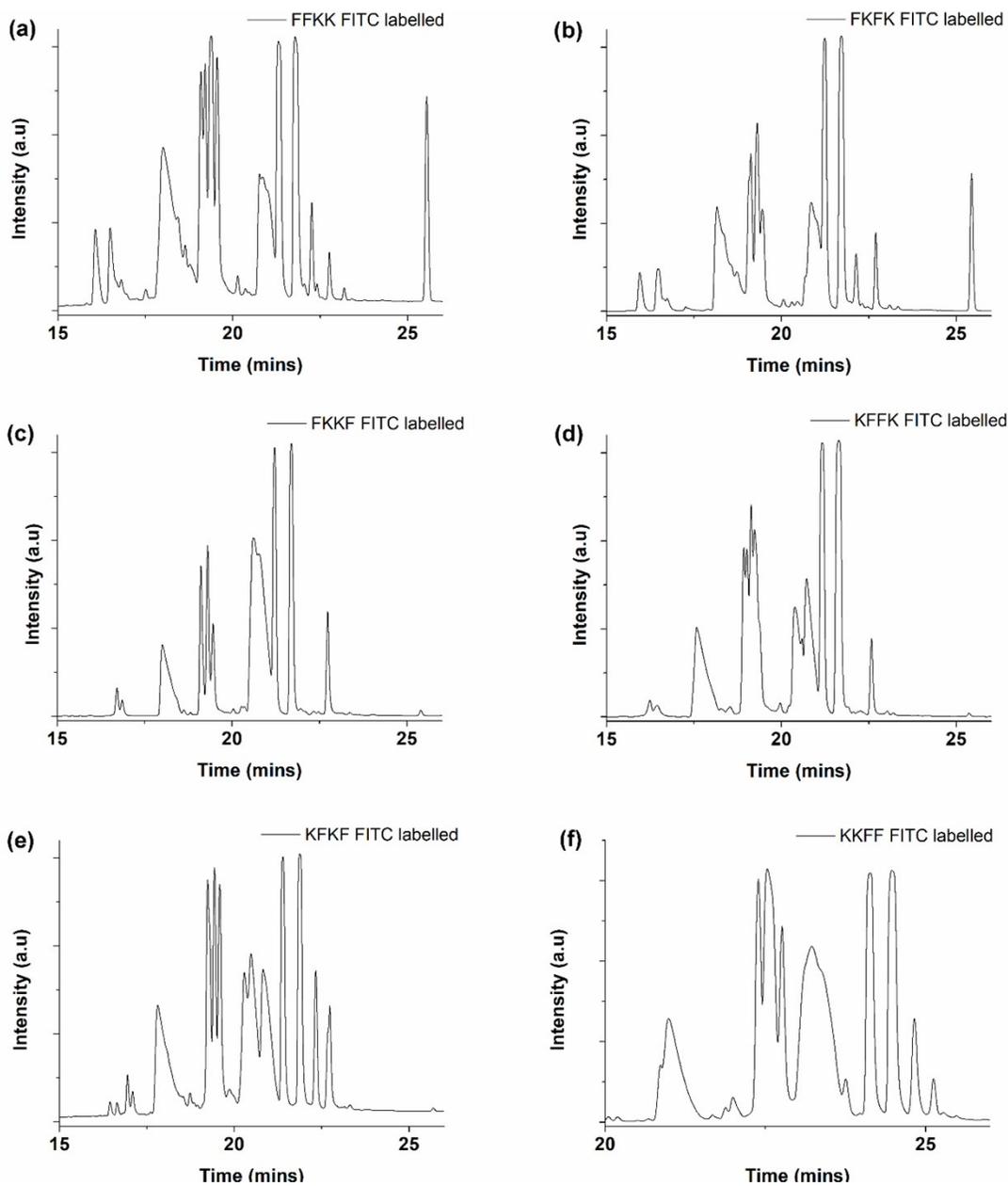


Figure S10 – Analytical HPLC for FITC-labelled, lysine containing tetrapeptides. The degree of labelling was obtained through comparing peak areas for the peak at ~18 mins (21 mins for Fmoc-KKFF) representing unlabelled peptide, and ~21 mins (23 mins for Fmoc-KKFF) which denotes the FITC labelled peptide.

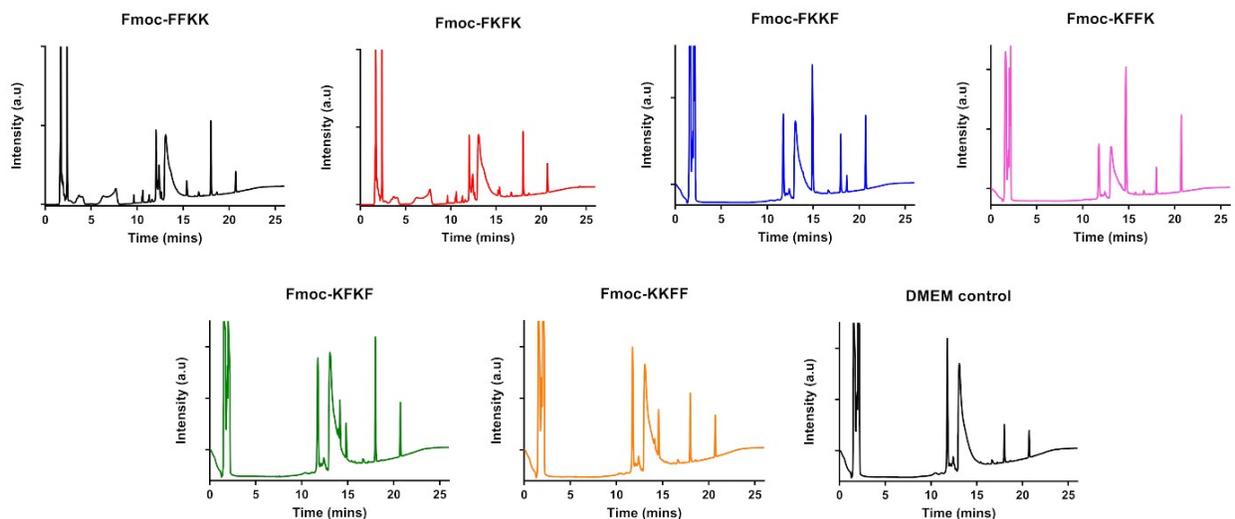


Figure S11 – Analytical HPLC monitoring degradation and leaching of peptide coatings into culture media. The peak at approximately $t_r = 15$ minutes is indicative of the tetrapeptide and while Fmoc-FKKF and Fmoc-KFFK exhibit higher levels of these peptides in the supernatant media, their concentration is still $< 10 \mu\text{M}$ as determined through comparison to peptide standards.

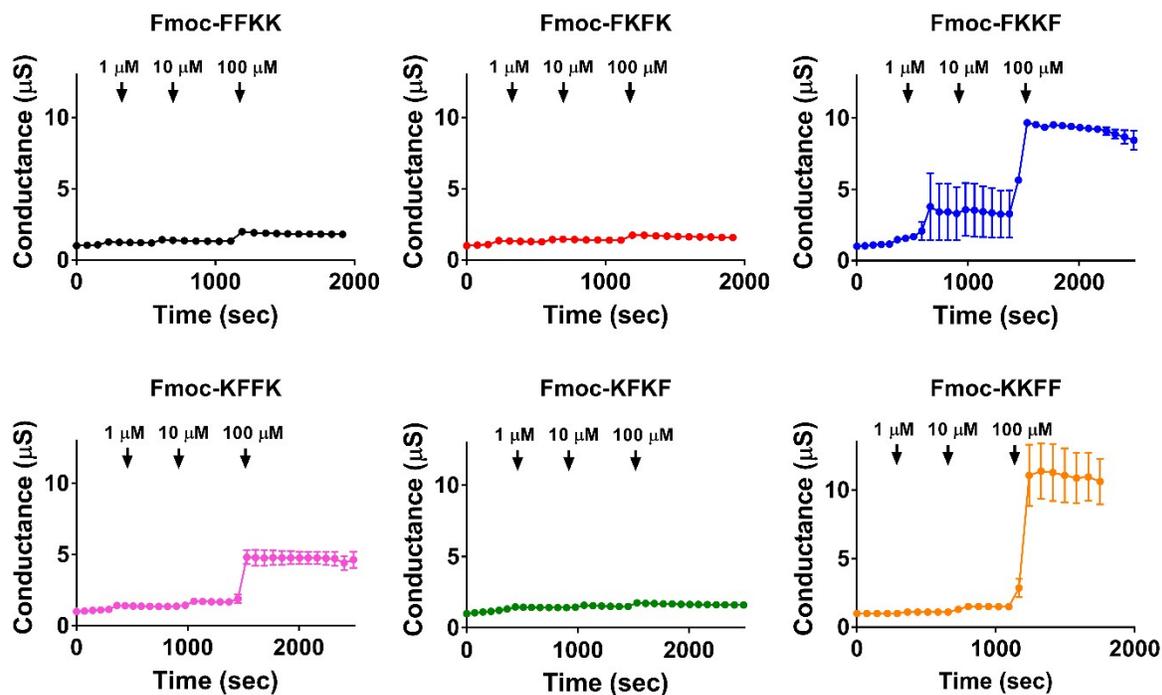


Figure S12. Electrical impedance spectroscopy performed on sparsely tethered bilayer lipid membranes (tBLMs) consisting of 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) lipids, with changes in conductance monitored upon treatment of the membranes with tetrapeptide nanofibre solutions diluted into DMEM containing 10% FBS.

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