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Supplementary information

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1. Peptide synthesis

All solvents and reagents used for the peptide synthesis were identical to those described in Medini *et al*.[1] The peptides were synthesized as previously described.[1] Briefly, a rink amide linker (0.2 mmol) was coupled to aminomethyl resin (loading 1 mmol/g) using DIC (diisopropylcarbodiimide) (0.4 mmol in CH_2Cl_2 (3 mL) for 2 h). The syntheses of each peptide were carried out on a 0.1 mmol scale using an automated microwave synthesizer, Biotage Initiator® [Alstra Software, Uppsala, Sweden]. The Fmoc group was deprotected using a standard protocol.[2] Fmoc protected amino acids were coupled using HATU (O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) in DMF (dimethylformamide) as the activating agent and $i$$Pr_2EtN$ (*N*,*N*′-diisopropylethylamine) in NMP (*N*-methylpyrrolidine) as the base. Coupling times were 5 min at 25 W at a maximum temperature of 70 °C throughout the synthesis. The final N^{α}–Fmoc group was removed for each peptide (**1**, **2** and **3**). Finally, they were reacted for 30 min at room temperature with 20% acetic anhydride in DMF using $i$$Pr_2EtN$ (10 equivalents) as a base to afford a *N*-terminus acetylated peptide. Following peptide chain assembly, the crude peptides were cleaved from the resin with simultaneous removal of side-chain protecting groups using a standard protocol.[2] The crude peptides were purified by reversed-phase high performance liquid chromatography (RP-HPLC)). Samples were purified on a Gemini C-18 column (10 × 250 mm, 5 µm, 110 Å; Phenomenex, CA, USA) at no higher concentration than 2 mg/ml to avoid self-assembly.

2. Sample preparation

Mixture **A** was prepared by combining peptide (**1**) littleSven and peptide **2** (Phe) at a 1:1 ratio, both in powder form. MilliQ water was added to achieve a final concentration of 1% *w/v*, followed by sonication for ~ 5 min at room temperature. Mixture **B** (peptide (**2**) Phe and (**3**) Tyr) and **C** (peptide (**1**) littleSven and (**3**) Tyr) were prepared using the same protocol. Mixtures **A**, **B** and **C** were in solution at day 1 and, with the exception of mixture **C,** showed an increase in viscosity without achieving self-supporting gelation on day 4.
Daily aliquots were taken from each mixture for TEM and MALDI-imaging analysis.
As a control, each peptide was analysed individually by dissolving the desired peptide in MilliQ water to achieve a final concentration of 0.5% w/v.

3. Transmission electron microscopy

All aliquots for each mixture were treated as follows. An aliquot was applied onto a copper mesh grid overlaid with continuous carbon and the experiment was carried out as described in Medini *et al*.[1] Briefly, a 40 μl aliquot of the sample at 1% *w/v*, was adsorbed onto a glow-discharged-cleaned grid for 1 min. The grid was then deposed onto a water drop (40 μl) for a wash step for 15 sec. Excess solution was removed with filter paper (Whatman #1), and the sample was negatively stained with uranyl acetate for 30 sec. Excess liquid was blotted with filter paper and the grid was allowed to air-dry. Images were acquired using an FEI Tecnai 12 electron microscope operated at 120 kV. Control images are shown in Figure *S1*. The fiber length was measured using ImageJ (NIH, http://rsb.info.nih.gov/ij).

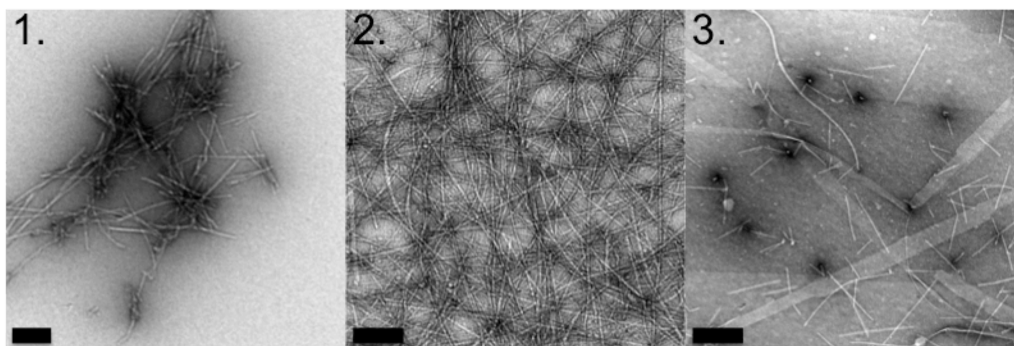

Figure S1. TEM images of peptides (**1**), (**2**) and (**3**) at 5 mg/ml (0.5% w/v). The scale bar represents 200 nm.

4. MALDI-imaging

4.1 Matrix application

A matrix solution of 5 mg/ml α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich) in 66% CH_3CN: MilliQ water with 0.1% (v/v) trifluoroacetic acid was prepared just prior to matrix application. Once the droplets had dried completely on the glass slides, the matrix was introduced using a robotic sprayer (TM Sprayer, HTX Technologies). Then, 6 passes of the sprayer (0.08 mL/min flow rate, 3.0 mm spacing, 1200 mm/min pass speed) was applied to each slide over the entire surface. A calibration spot (saturated solution of red phosphorus in ACN) was applied to each slide. The slides were then placed inside a desiccator overnight to ensure complete drying of the matrix.

4.2 MALDI-imaging experiment

Once removed from the desiccator, the slides were placed into the metal slide holder (Bruker Daltonics) and scanned using a flatbed optical scanner (Epson, Perfection V370 Photo). The slides were then placed directly into the MALDI source of a SolarixXR FT-ICR so that the slide positions in the holder were not changed. The instrument was calibrated using red phosphorus prior to each MALDI-MSI run with a maximum acceptable mass error of 0.1 ppm over the entire mass range. A sample spot was selected for optimization of the MALDI laser conditions. FlexImaging software (Bruker Daltonics) was used to align the MALDI target with the optical image previously obtained. For the imaging experiment, a new sample spot was selected and the software was set to raster the laser over the sample at a 50 μm spatial resolution (2500 μm^2 pixel size) with 100 laser shots per pixel. Each pixel results in a mass spectrum of that area. Once the

experiment was complete, the software combined all the spectra over the entire droplet with the optical image to produce a 2D map of the peptide signals. The spectra were analyzed by mass or by area depending on the desired information.

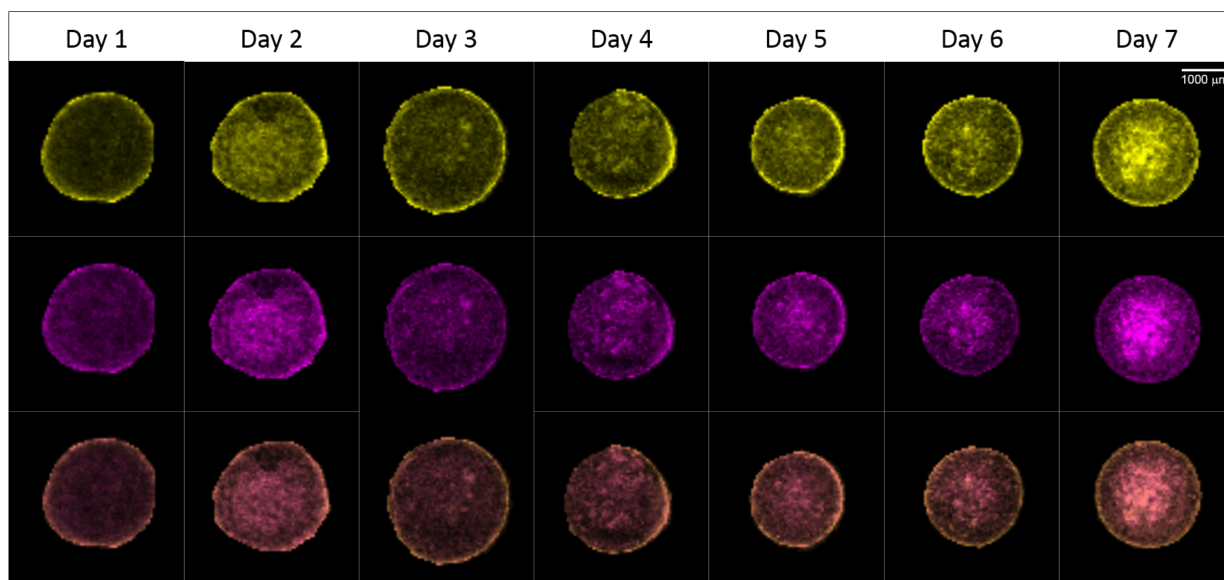

Figure S2. Time series of mixture **B** (peptide (**2**) in magenta and peptide (**3**) in yellow) from day 1-7. MALDI-imaging (first row (**2**) only, second row (**3**) only, third row (**2**) and (**3**)).

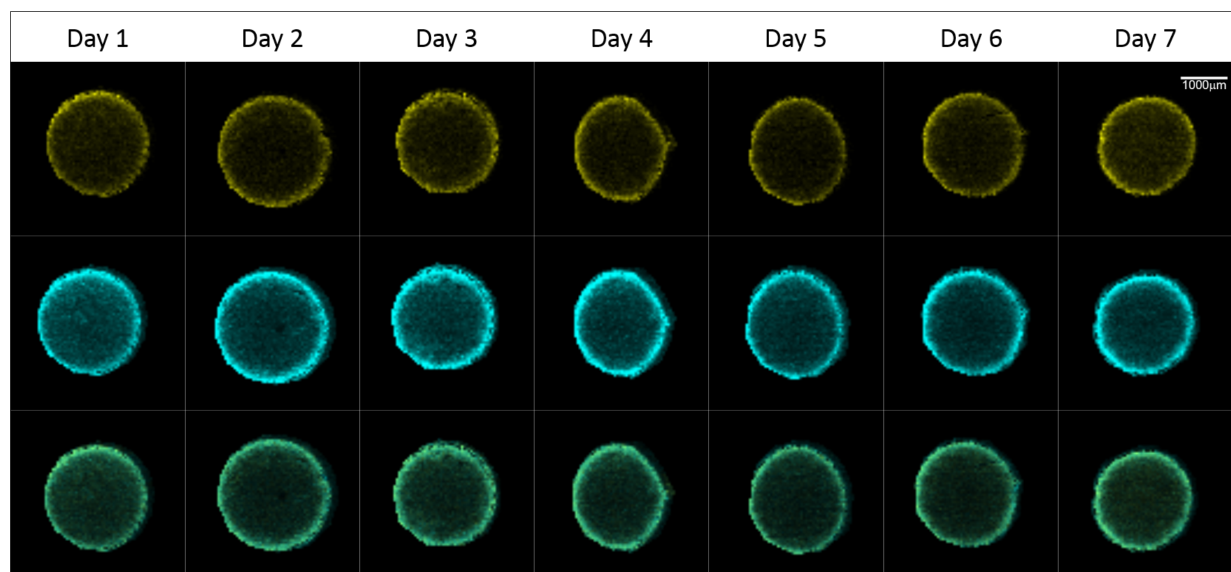

Figure S3. Time series of mixture **C** (peptide (**1**) in cyan and peptide (**3**) in yellow) from day 1-7. MALDI-imaging (first row (**1**) only, second row (**3**) only, third row (**1**) and (**3**)).

References.

(1) Medini, K.; Mansel, B. W.; Williams, M. A.; Brimble, M. A.; Williams, D. E.; Gerrard, J. A. *Acta Biomaterialia* **2016**.

(2) Kamalov, M.; Yang, S.; Harris, P. W.; Cooper, G. J.; Brimble, M. A. *Synlett* **2014**, *25*, 1835.