Supporting Information for

Rational design of a hexapeptide hydrogelator for controlled-release drug delivery

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General

PBS solutions were obtained by the addition of Aldrich pellets to 200 ml of mQ water (except for IR analyses for which NMR grade D_2O was used). The solution was freshly prepared before use.

Peptide synthesis

All Fmoc-amino acids were purchased from Novabiochem®, activator (N,N,N′,N′-Tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate, TBTU) was purchased from Chem-Impex International® and Fmoc-Lys(Boc)-Wang resin was purchased from Fluorochem LtD®. All other reagents and solvents were purchased from Sigma Aldrich and used without further purification. MBG-1 was synthesized on Fmoc-Lys(Boc)-Wang resin

using standard solid-phase Fmoc-chemistry.[30] The crude peptide was cleaved by a cocktail containing 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS), and 2.5% water volume fraction. The side chains were also deprotected during cleavage. TFA was removed by rotary evaporation under reduced pressure. The crude peptides were precipitated and washed three times by cold diethyl ether. Subsequently, the crude peptides were dissolved in water and lyophilized before purification.

Characterization of peptide MBG-1

H-Phe-Glu-Phe-Gln-Phe-Lys-OH (4, MBG-1). Preparative HPLC yielded the desired compound (white powder, 26%) HPLC (standard gradient): t_{ret}, 10.9 min. HRMS (ESP^+) found m/z 845.4202 $[M + H]^+$, $C_{43}H_{57}N_8O_{10}$ requires 845.4192.

Salt exchange

The TFA salt of MBG1 (2mg) was dissolved in a 1N HCl solution (1mL) and lyophilized. This procedure was repeated twice. Next, the obtained powder was dissolved in mQ water (1mL) and lyophilized to eliminate any residual traces of HCl. The peptide's integrity was verified by HPLC analysis.

High-resolution Mass Spectrometry (HRMS)

HRMS data was recorded with a Micromass QTOF-micro system. Mass spectra were recorded with a LCMS-MS triple-quadrupole system. Analytical HPLC was performed on an Agilent 1100 Series system with a Supelco Disovery BIO Wide Pore RP column (25 cm × 4.6 mm, 5 μm). Flow rates of 0.3 ml/min were used and detection was done at 215 and 254 nm. The solvent system consisted of 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B).

Rheological Analysis

For experimental procedure, see manuscript.

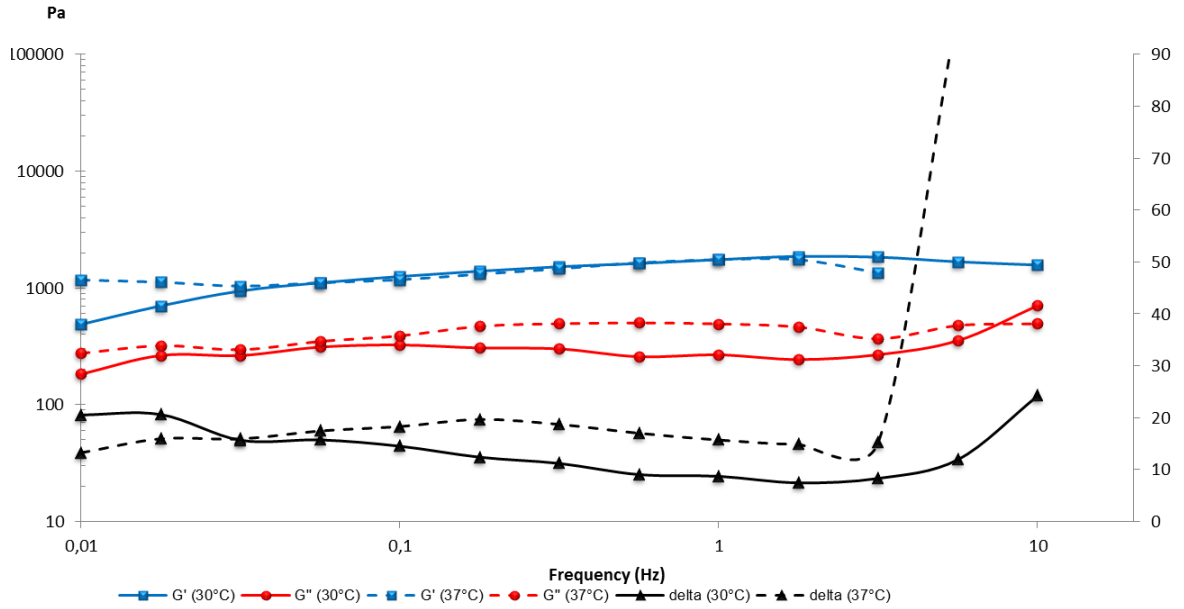

Figure S1. Frequency sweep rheological analysis of reference peptide 2 elastic (G'), viscous (G'') moduli and delta values at both 30°C and 37°C.

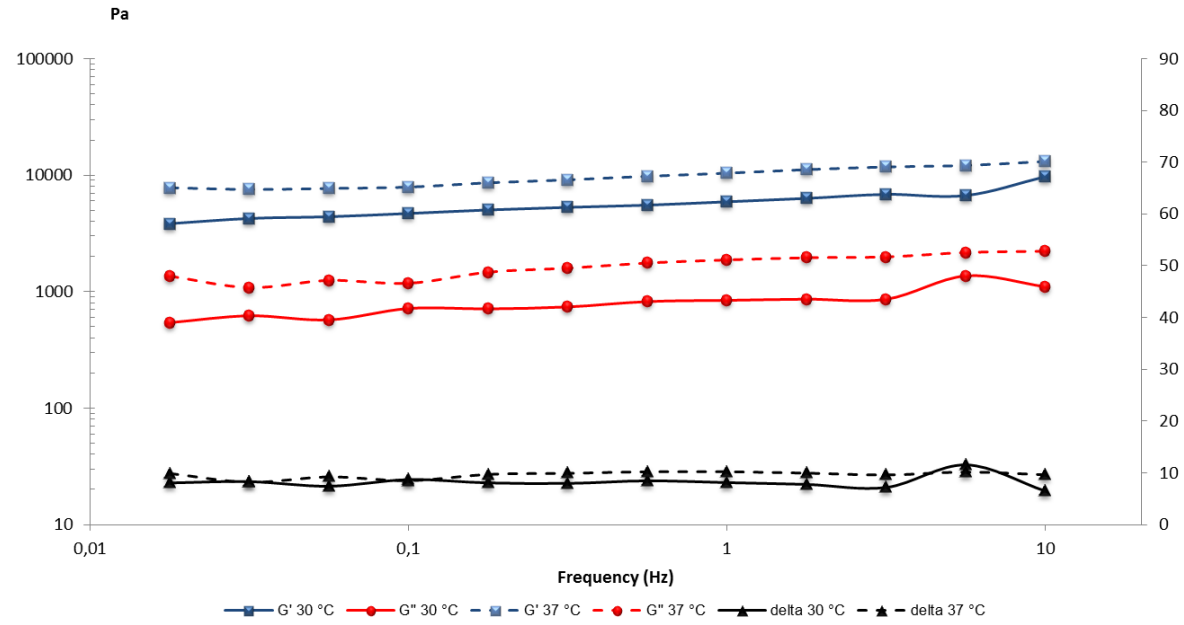

Figure S2. Frequency sweep rheological analysis of peptide MBG-1 elastic (G'), viscous (G'') moduli and delta values at both 30°C and 37°C.

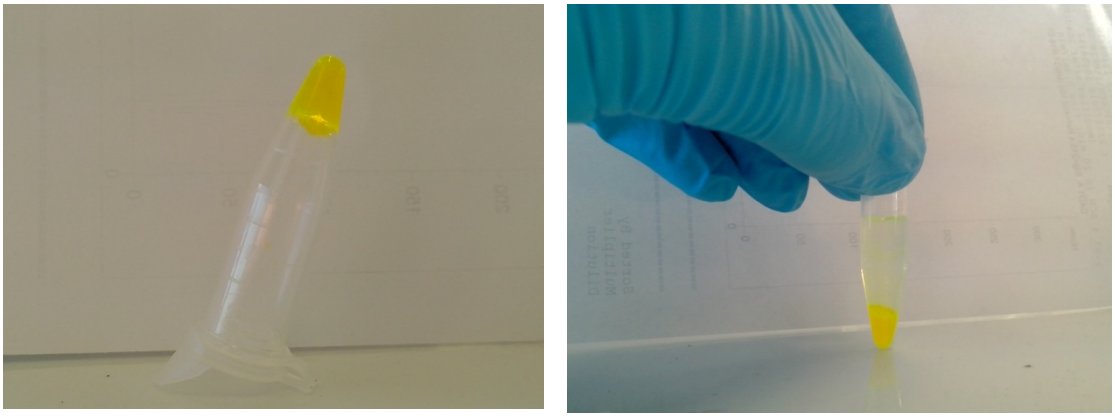

Figure S3. Pictures of the self-assembled hydrogel of MBG-1 encapsulated with 0.1% w/v fluorescein sodium.

Circular Dichroism

The secondary structure of the peptides was analysed using a 0.1 cm quartz cell on a Jasco J815 Spectropolarimeter, with 1 s integrations, 1 accumulation and a step size of 1 nm with a band width of 1 nm over a range of wavelengths from 200 to 270 nm. Peptide samples were freshly prepared directly in the CD cell, and spectra were recorded after 2h. Measurements were repeated at least 3 times and their average was plotted.

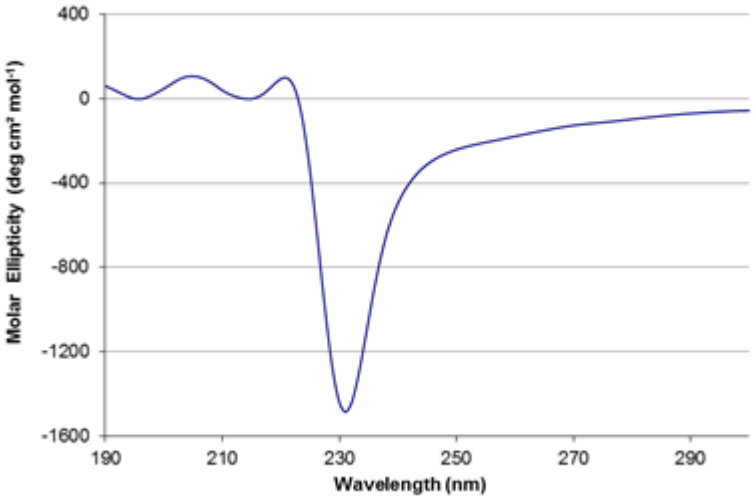

Figure S4. CD spectrum of MBG-1 at 10 mg/ml in a mixture of 1:1 PBS/water.

In CD spectra classical β-sheets are usually associated with a maximum around 200 nm and a minimum between 214 and 217 nm.[I] As depicted in **Figure S4**, a maximum around 205 nm and a minimum at 231 nm were observed in the CD spectrum of MBG-1. Lee et al.[II] recently reported a hydrogel with the same very specific maximum at 205 in combination with a minimum at 230 nm. Distorted β-sheets[III] or π- π interactions[IV] were held responsible for this specific CD spectrum indicating that the same might be true for the hydrogels of MBG-1.

Fluorescence microscopy

Fluorescein coated dextran (MW: 500 KDa) was dissolved in PBS water (2 mg/ml to obtain a final loading of 0.1% w/v). The resulting solution was then added to a solution of MBG-1 dissolved in mQ water. Upon addition of the flurorescein-dextran/PBS solution the gel formed instantly. The images were recorded on a Leica DM2500P equipped with 40 X (NA 0.75) objective and a Leica 360FX CCD camera.

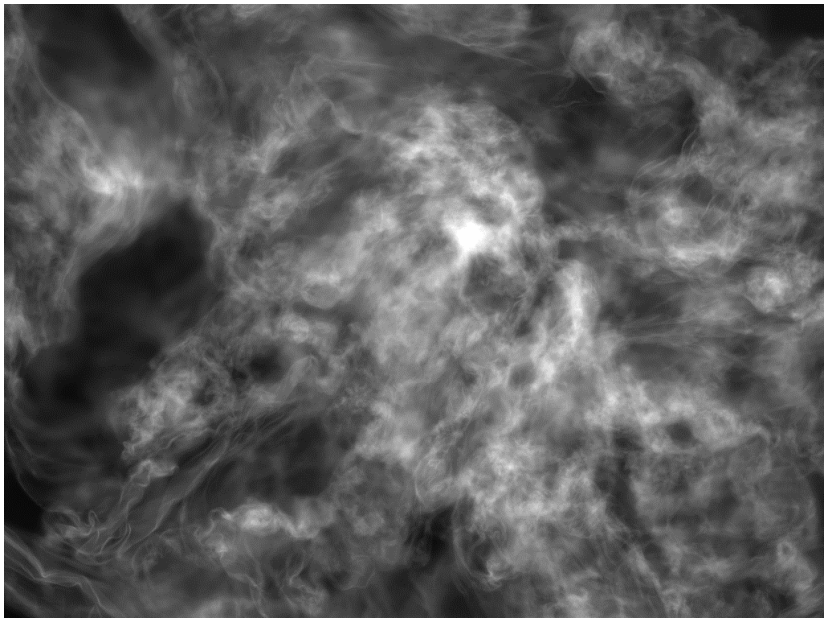

Figure S5. Microscopic view of the heterogeneous structure of MBG-1/fluorescein-dextran (500 KDa)

References

[I] S. M. Kelly, N. C. Rice*, Curr. Protein Pept. Sci.* **2000**, *1*, 349.

[II] N. R. Lee, C. J. Bowerman, B. L. Nilsson, *Biomacromolecules* **2013**, *14*, 3267.

[III] M. C. Manning, M. Illangasekare, R. W. Woody, *Biophys. Chem.* **1988**, *31*, 77.

[IV] C. J. Bowerman, D. M. Ryan, D. A. Nissan, B. A. Nilsson, *Mol. Biosyst.* **2009**, *5*, 1058.