# Supplementary Information

# Synthesis and characterization of mono S-lipidated peptide hydrogels: a platform for the preparation of reactive oxygen species responsive materials

Aakanksha Rani,[a,b,c] Luis M. De Leon-Rodriguez,*[a] Iman Kavianinia,*[a,b,c,d] Duncan J. McGillivray,[a,b,c] David E. Williams[a,b,c] and Margaret A. Brimble*[a,b,c,d]

[a] School of Chemical Sciences, The University of Auckland, 23 Symonds St., Auckland 1010, New Zealand

[b] School of Biological Sciences, The University of Auckland, 3A Symonds St., Auckland 1010, New Zealand

[c] MacDiarmid Institute for Advanced Materials and Nanotechnology, New Zealand

[d] Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, 3A Symonds St., Auckland 1010, New Zealand

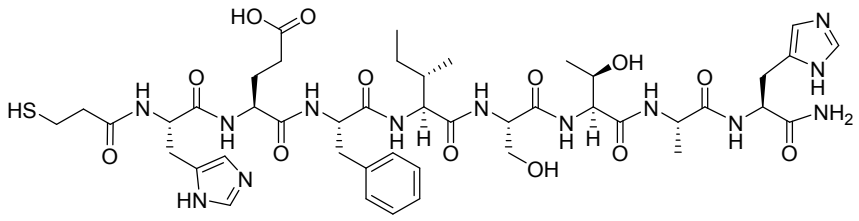
**Corresponding Authors**
Email: m.brimble@auckland.ac.nz , l.deleon@auckland.ac.nz, i.kavianinia@auckland.ac.nz

# Table of Contents

# Table of Figures

# 1. Peptide Synthesis

## 1.1 Synthesis of 3



**3**

Peptide **3** was synthesised using Fmoc SPPS according to **general method A** and **B**, then purified according to **general method C**. Purified peptide **3** was obtained as a white solid after lyophilization (70.1% yield); $R_t$ = 9.1 min; *m/z* (ESI-MS) 1028.4 ([M+H]$^+$ requires 1028.4), **Fig.S1**.

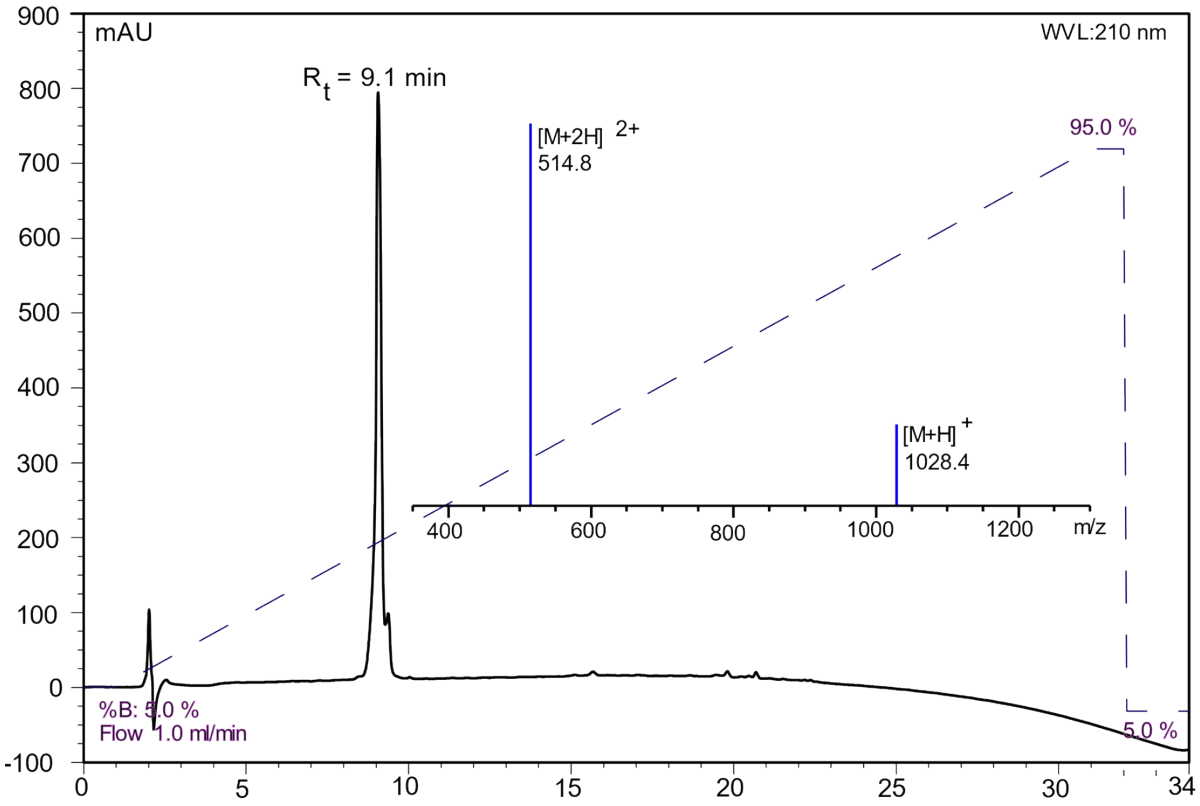
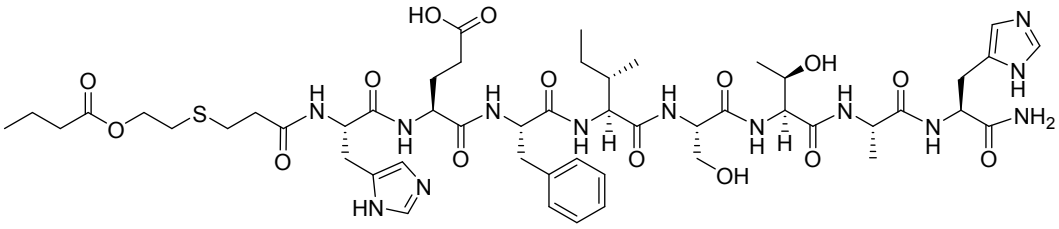



**Figure S1**. RP-HPLC and ESI-MS traces of **3** (*ca.* 96% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.2 Synthesis of 5a



**5a**

Peptide **3** was synthesised using Fmoc SPPS following **general method A** and **B**. The crude product was purified following **general method C**. Purified **3** (10 mg, 10 µmol) was dissolved in 1 mL NMP solution, containing vinyl butyrate (23 µL, 0.2 mmol, 20 eq.), TIPS, TFA and *t*-nonyl mercaptan as per **general method D** for 15 min. The crude product of **5a** was purified following **general method C**. Purified lipidated peptide **5a** was obtained as a white solid after lyophilization (55.0 % yield); $R_t$ = 12.1 min; *m/z* (ESI-MS) 1143.0 ($[M+H]^+$ requires 1142.5), **Fig. S2**.

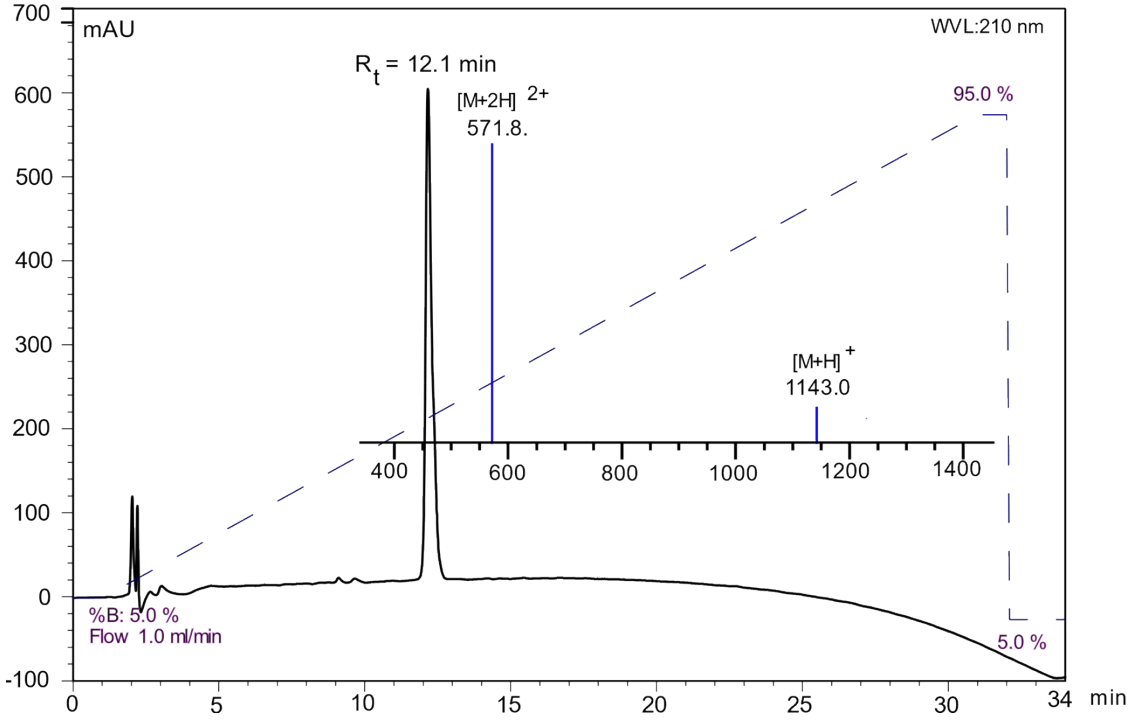
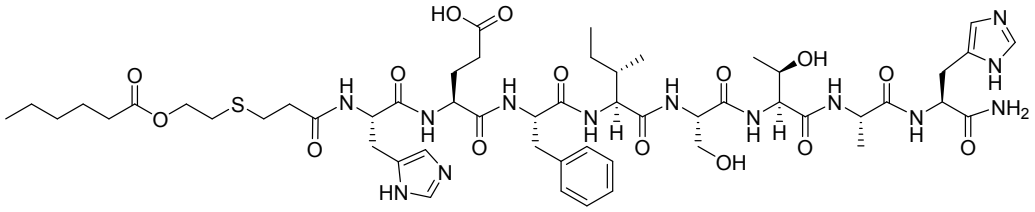



**Figure S2**. RP-HPLC and ESI-MS traces of **5a** (*ca.* 98% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.3 Synthesis of 5b



**5b**

Peptide **3** was synthesised using Fmoc SPPS following **general method A** and **B**. The crude product was purified following **general method C**. Purified **3** (10 mg, 10 µmol) was dissolved in 1 mL NMP solution, containing vinyl hexanoate (32 µL, 0.2 mmol, 20 eq.), TIPS, TFA and *t*-nonyl mercaptan as per **general method D** for 15 min. The crude product of **5b** was purified following **general method C**. Lyophilisation yielded purified **5b** as a white solid 51.3% yield); $R_t$ = 14.1 min; *m/z* (ESI-MS) 1170.5 ($[M+H]^+$ requires 1170.6), **Fig. S3**.

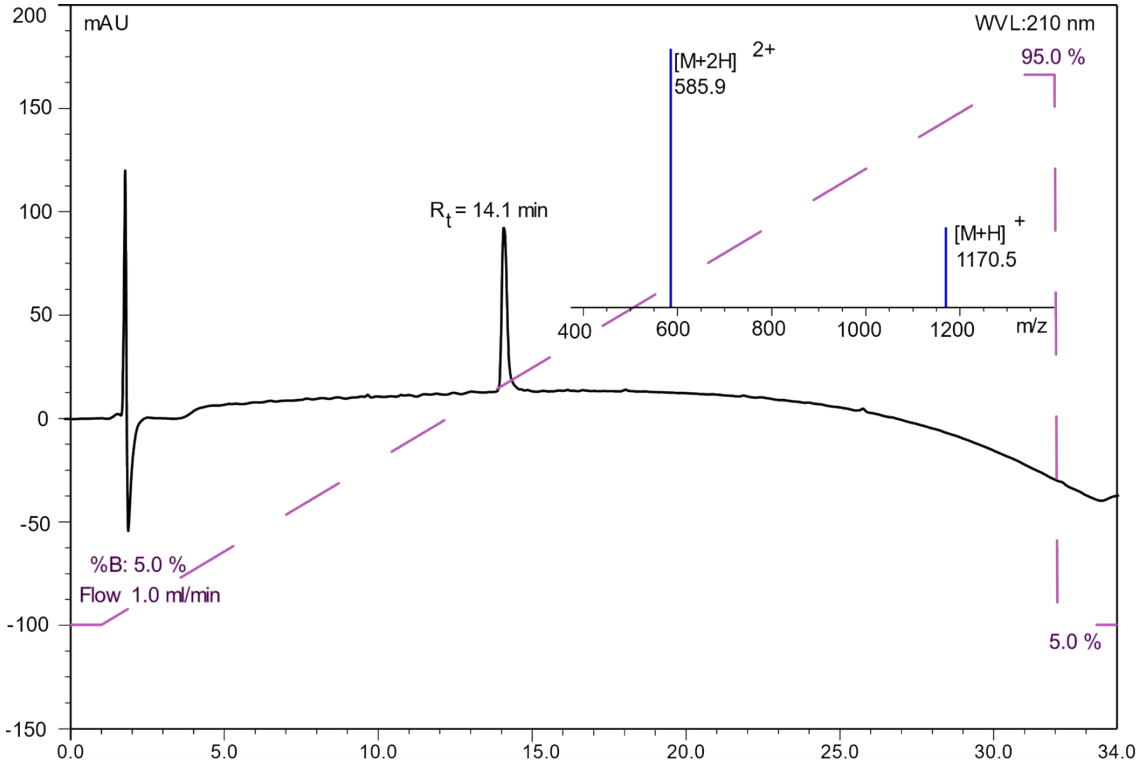
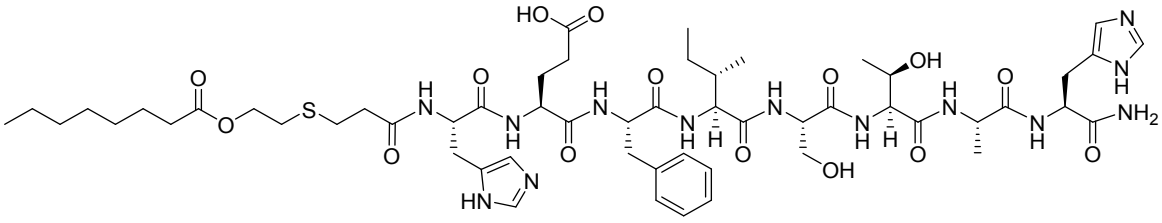



**Figure S3**. RP-HPLC and ESI-MS traces of **5b** (*ca.* 99% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.4 Synthesis of 5c



**5c**

Peptide **3** was synthesised using Fmoc SPPS following **general method A** and **B**. The crude product was purified following **general method C**. Purified **3** (10 mg, 10 μmol) was dissolved in 1 mL NMP solution, containing vinyl octanoate (38 μL, 0.2 mmol, 20 eq.), TIPS, TFA and *t*-nonyl mercaptan as per **general method D** for 15 min. The crude product of **5c** was purified following **general method C**. The purified lipidated peptide **5c** was obtained as a white solid after lyophilization (50.2 % yield); $R_t$ = 15.9 min; *m/z* (ESI-MS) 1198.5 ([M+H]$^+$ requires 1198.6), **Fig. S4**.

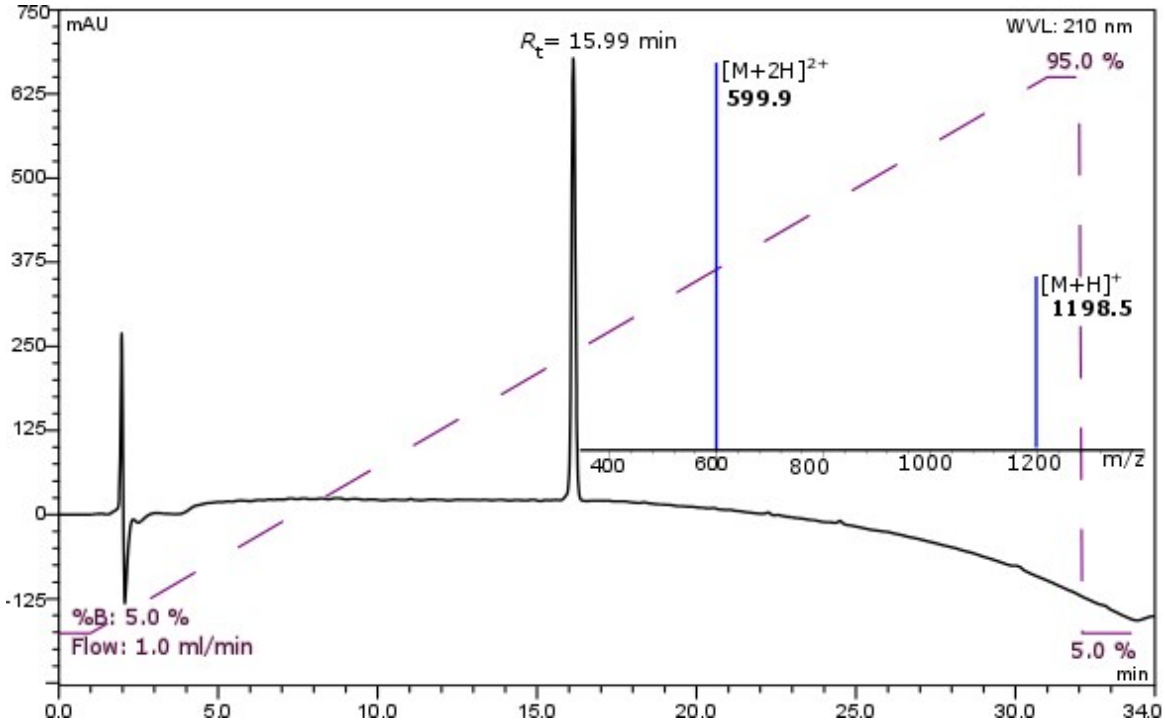
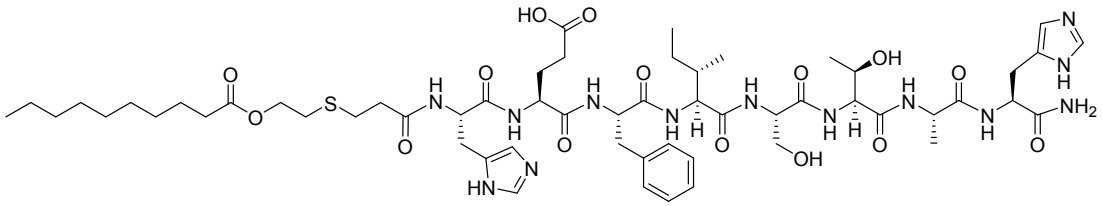



**Figure S4**. RP-HPLC and ESI-MS traces of **5c** (*ca.* 99% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 μm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.5 Synthesis of 5d



**5d**

Peptide **3** was synthesised using Fmoc SPPS following **general method A** and **B**. The crude product was purified following **general method C**. Purified **3** (10 mg, 10 µmol) was dissolved in 1 mL NMP solution, containing vinyl decanoate (45 µL, 0.2 mmol, 20 eq.), TIPS, TFA and *t*-nonyl mercaptan as per **general method D** for 15 min. The crude product of **5d** was purified following **general method C**. Purified lipidated peptide **5d** was obtained as a white solid after lyophilization (52.8 % yield); $R_t$ = 17.5 min; *m/z* (ESI-MS) 1227.1 ([M+H]$^+$ requires 1226.6), **Fig. S5**.

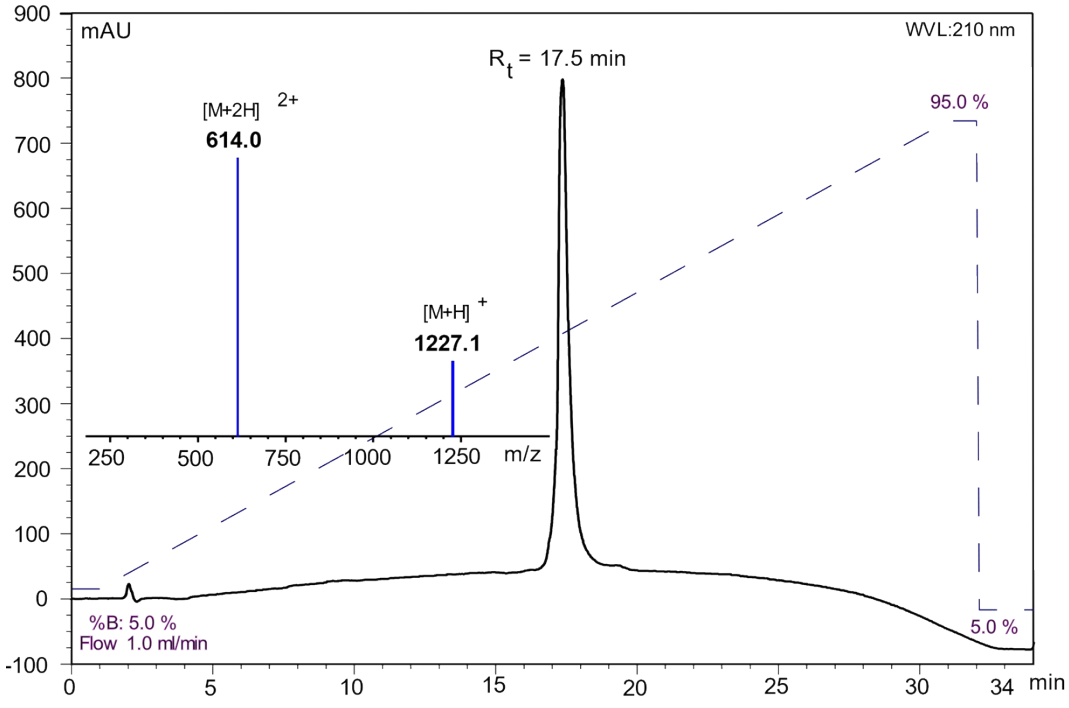
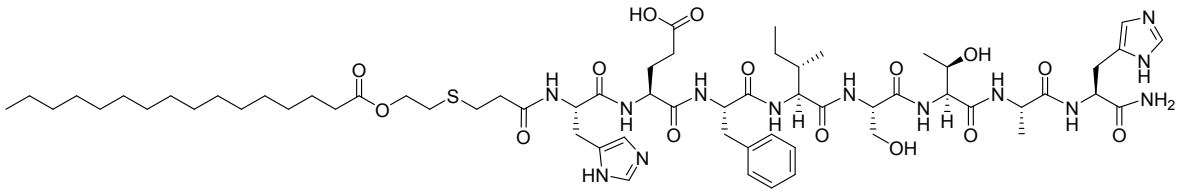



**Figure S5**. RP-HPLC and ESI-MS traces of **5d** (*ca.* 99% purity as judged by peak area of RP-HPLC at 210 nm); Agilent ZORBAX 300SB-C3, (3 mm × 150 mm; 3.5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.6 Synthesis of 5e



**5e**

Peptidyl **3** was synthesised using Fmoc SPPS following **general method A** and **B**. The crude product was purified following **general method C**. Purified **3** (10 mg, 10 μmol) was dissolved in 1 mL NMP solution, containing vinyl palmitate (65 μL, 0.2 mmol, 20 eq.), TIPS, TFA and *t*-nonyl mercaptan as per **general method D** for 15 min. The crude product of **5e** was purified following **general method C**. Purified lipidated peptide **5e** was obtained as a white solid after lyophilization (51.8 % yield); $R_t$ = 23.4 min; *m/z* (ESI-MS) 1310.8 ([M+H]$^+$ requires 1310.7), **Fig. S6**.

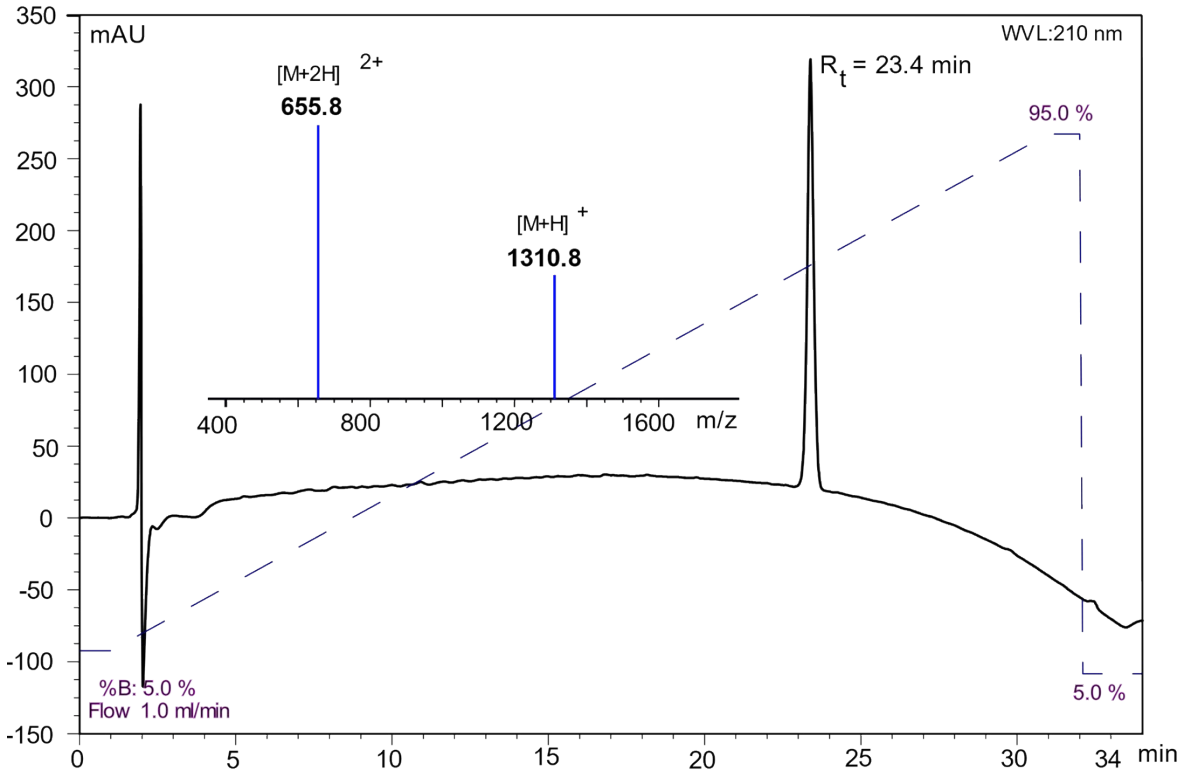
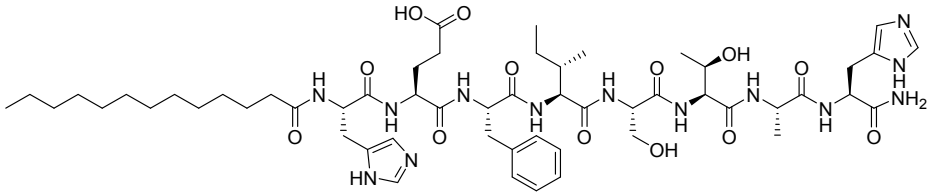



**Figure S6**. RP-HPLC and ESI-MS traces of **5e** (*ca.* 99% purity as judged by peak area of RP-HPLC at 210 nm); Agilent ZORBAX 300SB-C3, (3 mm × 150 mm; 3.5 μm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.7 Synthesis of 5f



**5f**

Peptidyl resin **2** was synthesised using Fmoc SPPS following **general method A** and **E**. The crude product of **5f** was purified following **general method C**. Purified lipidated peptide **5f** was obtained as a white solid after lyophilization (50.0 % yield); $R_t$ = 17.9 min; *m/z* (ESI-MS) 1136.7 ([M+H]$^+$ requires 1136.7), **Fig. S7**.

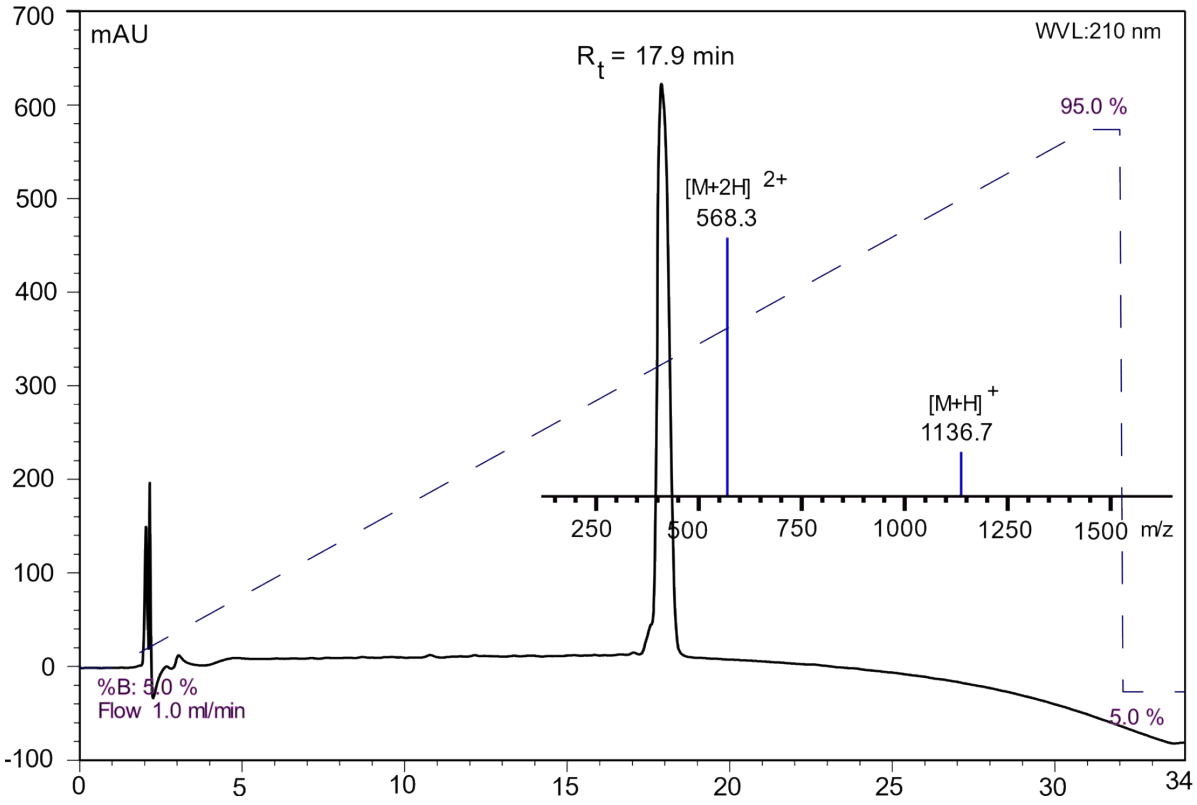
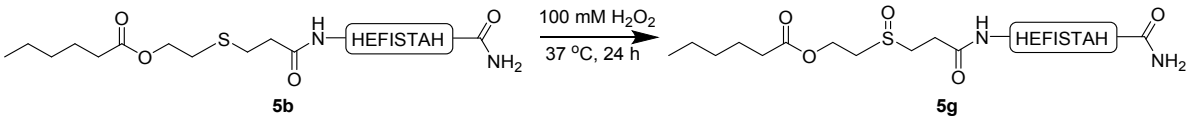



**Figure S7**. RP-HPLC and ESI-MS traces of **5f** (*ca.* 98% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 1.8 Oxidation of peptide 5b in the presence of $H_2O_2$




**5b** **5g**

Purified peptide **5b** was dissolved in the 100 mM $H_2O_2$ at a 2 wt% concentration at 37 °C. Reaction progress was monitored by RP-HPLC analysis (**Fig. S8**). Upon reaction completion, the desired peptide was purified using **General method C**. Purified **5g** was obtained as a white solid after lyophilization in a 70 % yield; $R_t$ = 12.5 min; *m/z* (ESI-MS) 1187.0 ([M+H]$^+$ requires 1185.6), **Fig. S9**.

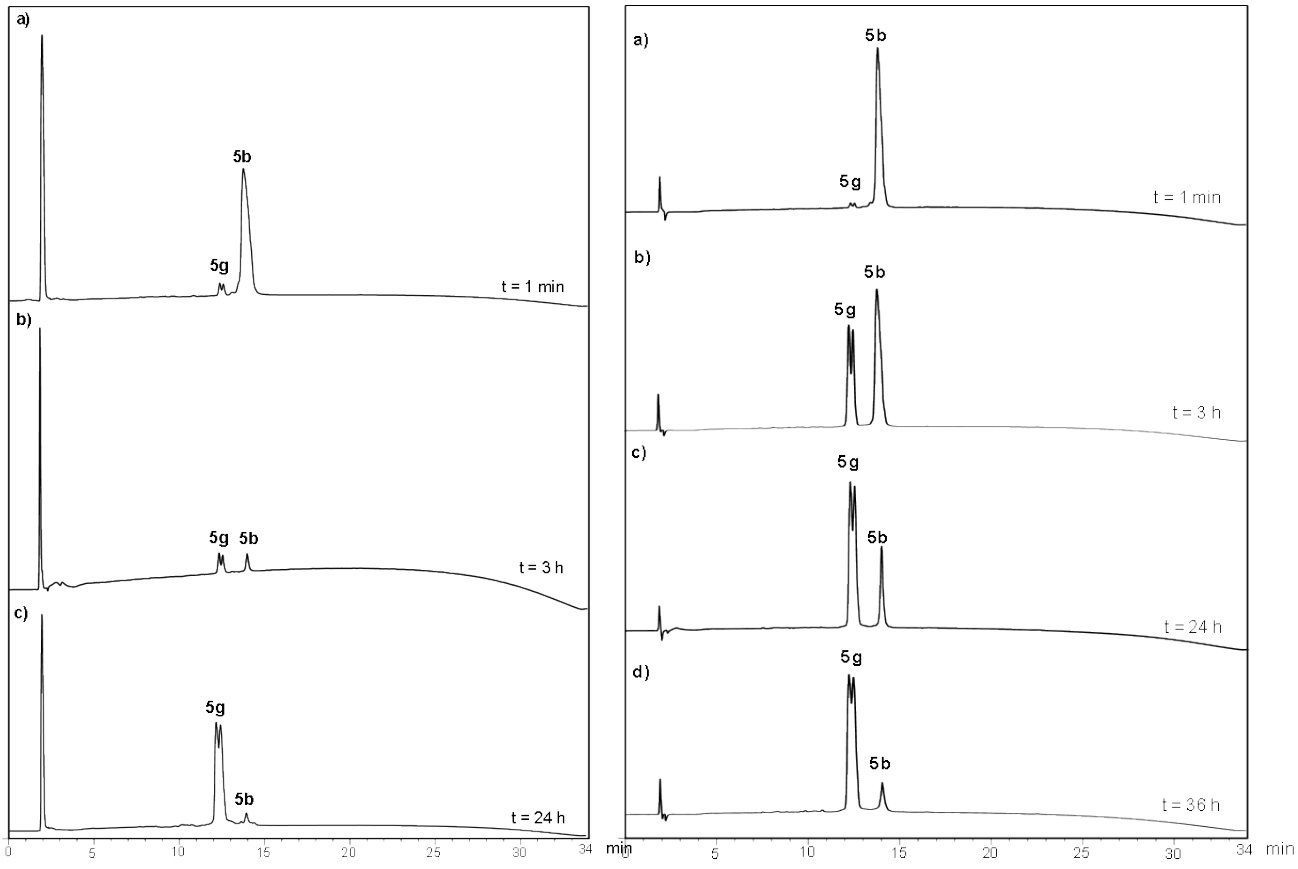
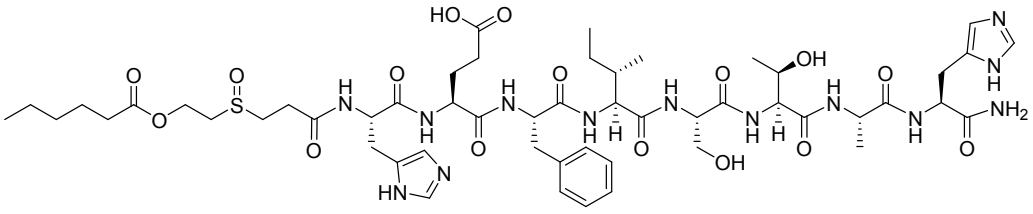



**Figure S8**. RP-HPLC of an aliquot of 2wt% hydrogel **5b** containing 100 mM (left) and 20 mM (right) $H_2O_2$ at pH 7.0 after a) 1 min, b) 3 h and c) 24 h and 36 h (RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 µm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.



**5g**

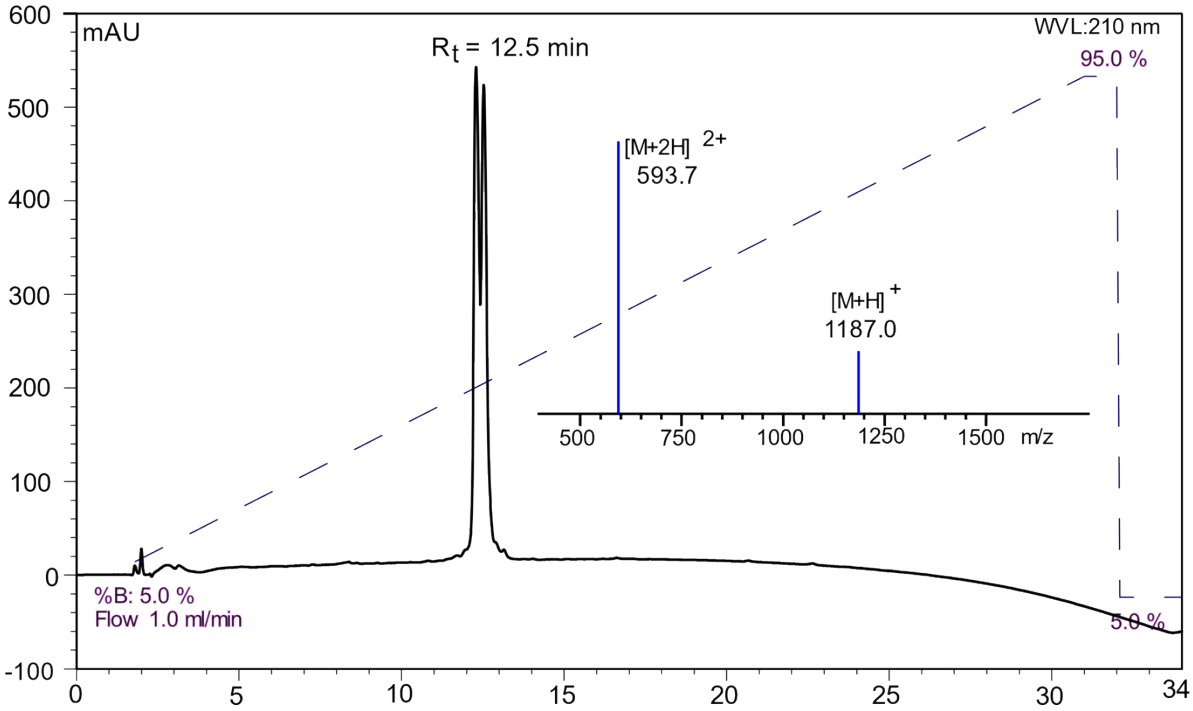



**Figure S9**. RP-HPLC and ESI-MS traces of **5g** (*ca.* 98% purity as judged by peak area of RP-HPLC at 210 nm); XTerra® MS C18, (4.6 mm × 150 mm; 5 μm), linear gradient of 5% B to 95% B over 30 min, *ca.* 3% B per minute at r.t. 1.0 mL/min.

## 2. Other Figures

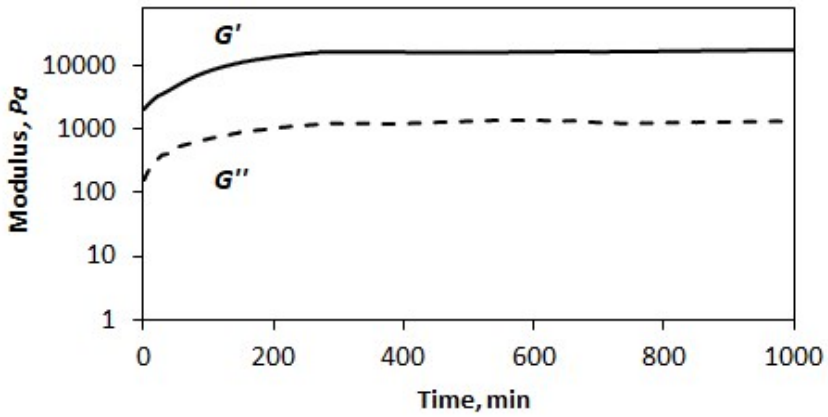



**Figure S10**. Storage ($G'$) and viscous ($G''$) moduli of a 2 wt% hydrogel of **5b** in water pH 7 at 37 °C as a function of time ($\omega$ = 6 rad.s$^{-1}$ and $\gamma$ =0.2% strain).

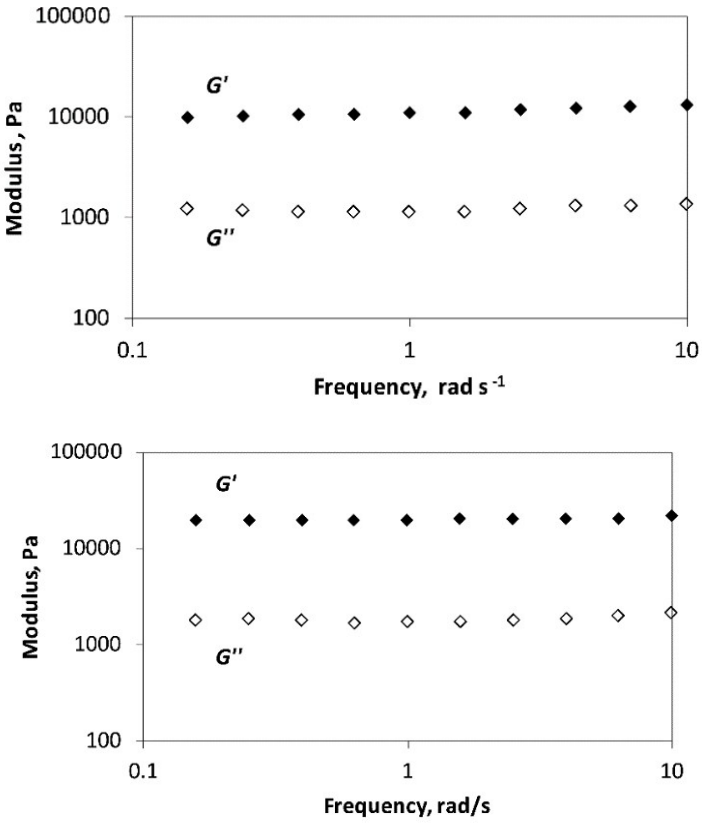



**Figure S11**. Storage ($G'$) and loss moduli ($G''$) of a 2 wt% hydrogel of peptide **5b** in $H_2O$ at pH 7 as a function of frequency ($\gamma$ =0.2% strain). After first dynamic time sweep measurement (**Fig. 1**, region I) (left) and after temperature ramp sweep experiments (**Fig. 1**, region IV) (right).

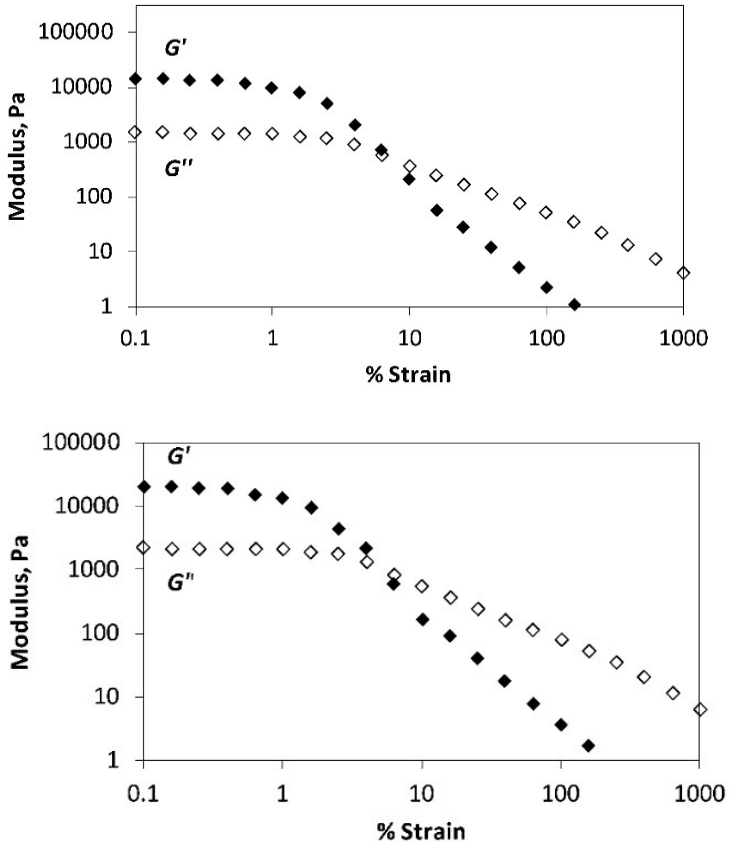



**Figure S12**. Storage ($G'$) and loss moduli ($G''$) of a 2 wt% hydrogel of peptide **5b** in $H_2O$ at pH 7 as a function of strain (frequency = 6 rad.s$^{-1}$). After first dynamic time sweep measurement (**Fig. 1**, region I) (left) after temperature ramp sweep experiments (**Fig. 1**, region IV) (right).

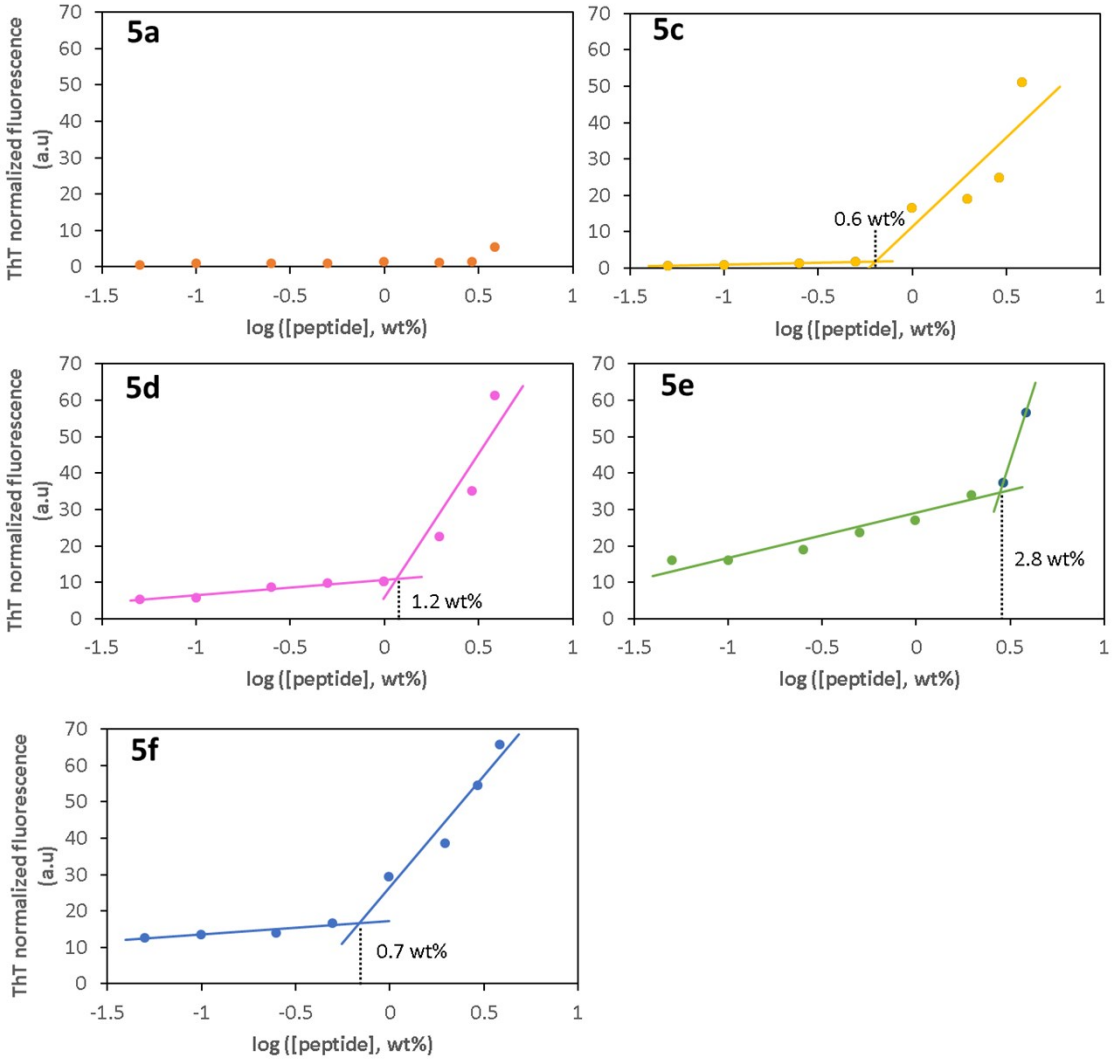



**Figure S13**. Thioflavin (ThT) fluorescence assay plots of **5a**, **5c**, **5d**, **5e**, and **5f**. The fluorescence intensity was normalized relative to the one of the ThT fluorescence assay of **5b** at the highest peptide concentration.

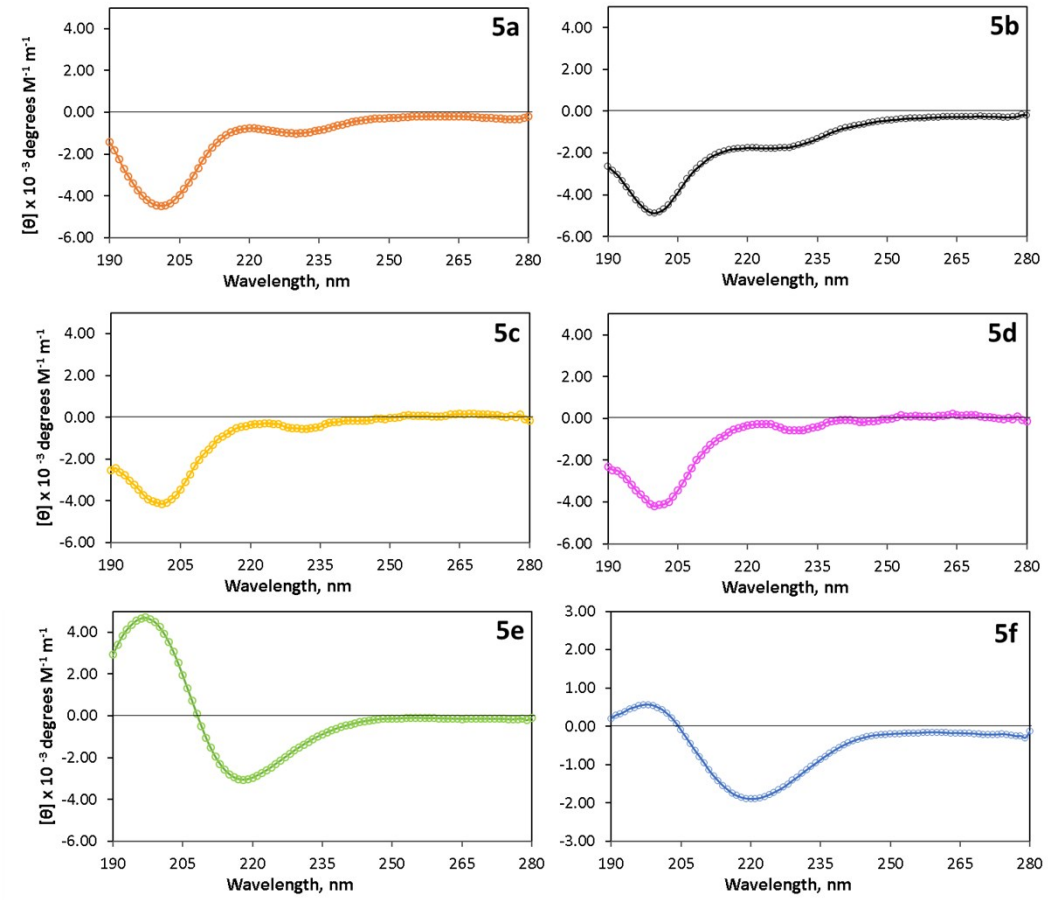



**Figure S14**. Circular dichroism spectrum of a 0.05 wt% solution of peptides **5a-5f** in $H_2O$ at pH 7. The solution of **5b**was prepared by dilution of a 2 wt% hydrogel sample. The solutions of peptides **5a** and **5c-5f** were prepared from a 5 wt% solution aged for 12 h and then diluted to 0.05 wt%. All the spectra were acquired right after sample preparation.

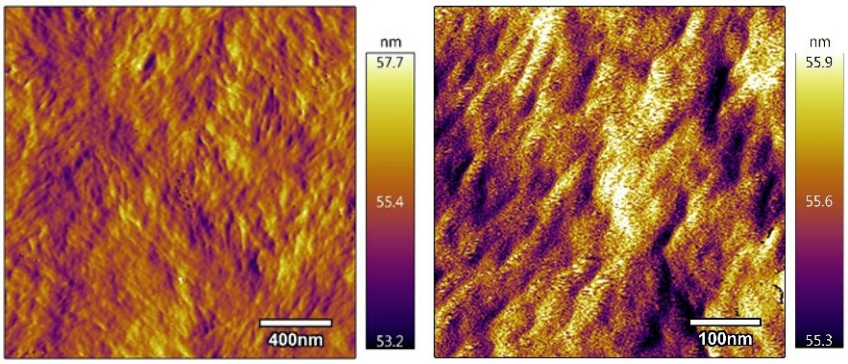



**Figure S15**. AFM phase image of a 2 wt% sample of peptide **5b** showing a fibrillar nanostructure.

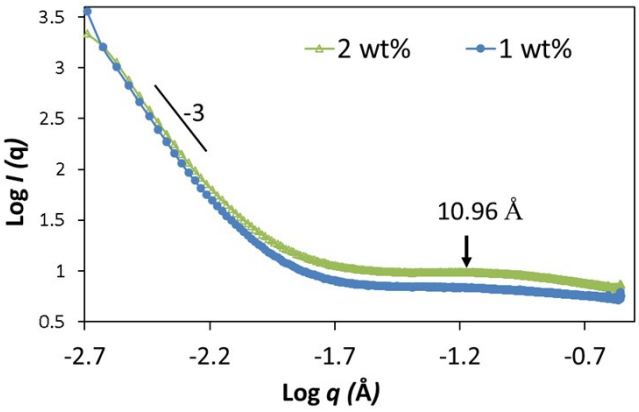



**Figure S16**. SAXS profiles of samples of **5b** prepared at 2 wt % (red open squares) and 1 wt % (green open triangle).

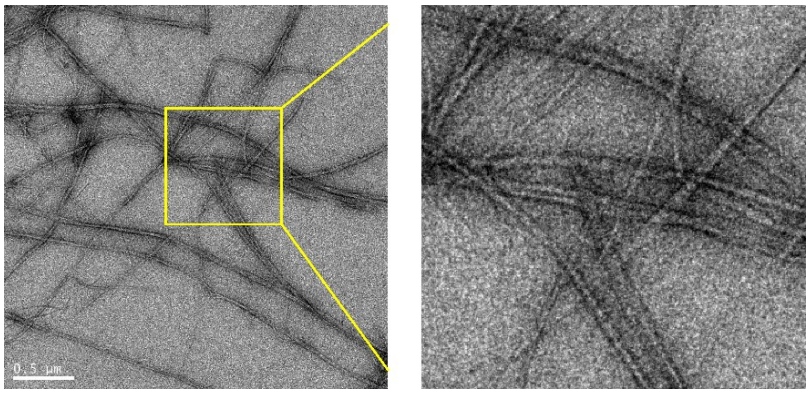



**Figure S17**. TEM image of **5b** sample at pH 7 prepared from 2 wt% hydrogel for 12 h and then diluted to 1 wt%.

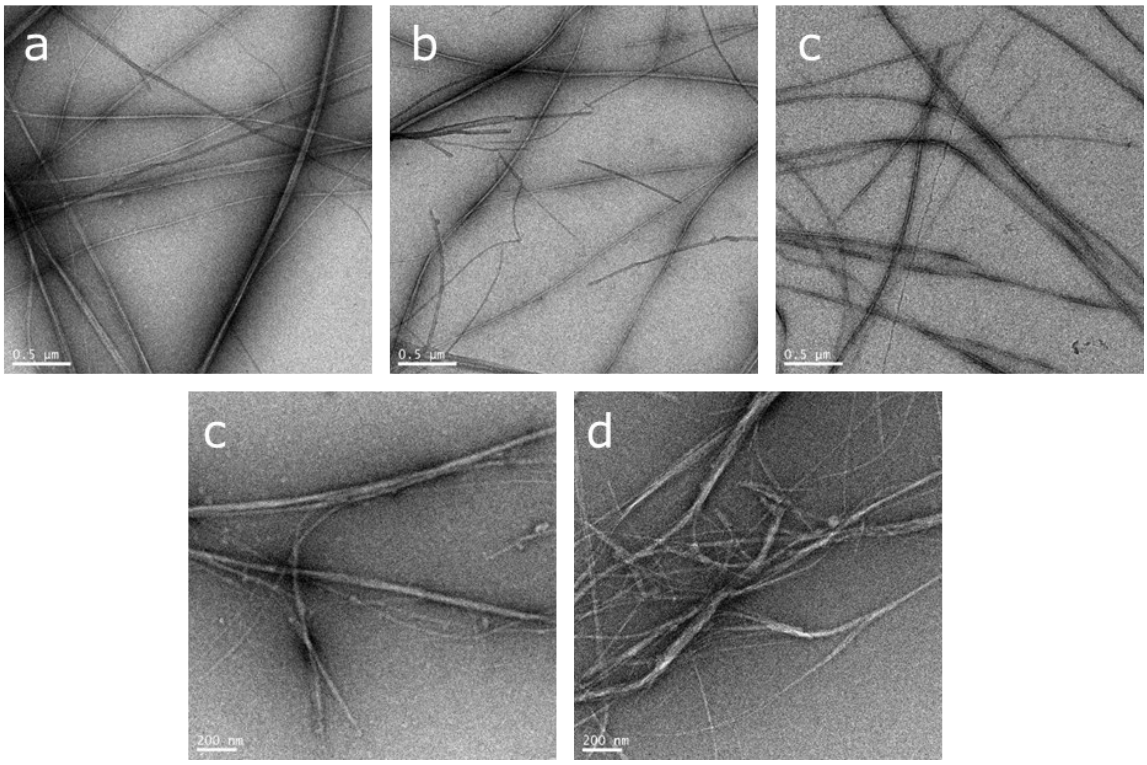



**Figure S18**. TEM image of **5b** at 1 wt% concentration aqueous solution at a) pH 2, b) pH 4, c) pH 6, pH 8 d) pH 10. The pH was adjusted by the addition of 1 M aqueous HCl or NaOH.

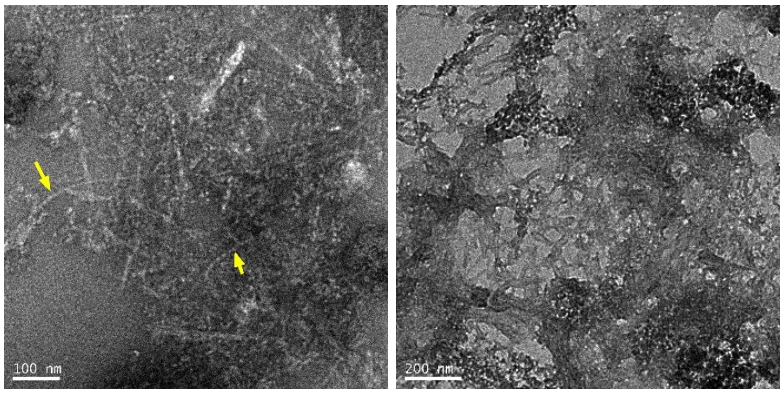



**Figure S19**. TEM image of samples of **5b** in water at 0.1 wt% (left) and 0.05 wt% (right). The yellow arrows point to examples of some fibres from which the width was measured.

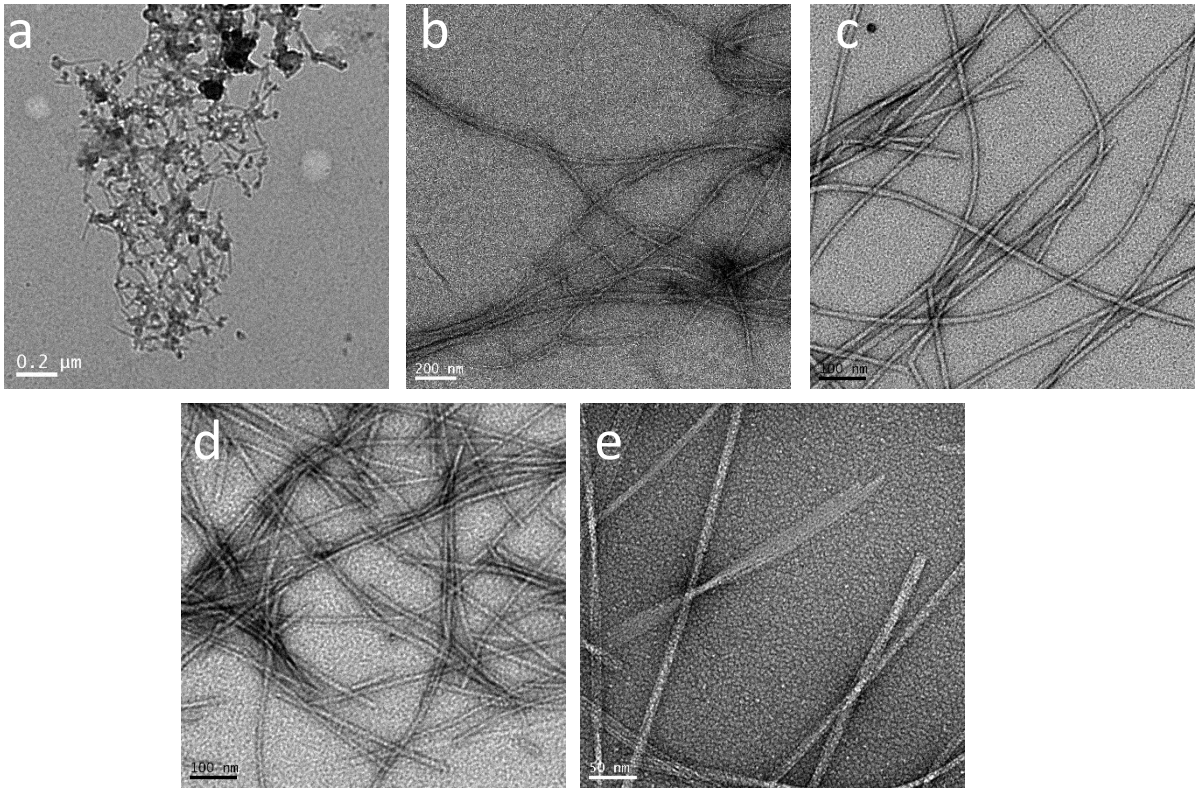



**Figure S20**. TEM images of **5a**, **5c**, **5d**, **5e** and **5f** samples prepared from a 5 wt% solution for 12 h and then diluted to 1 wt%.

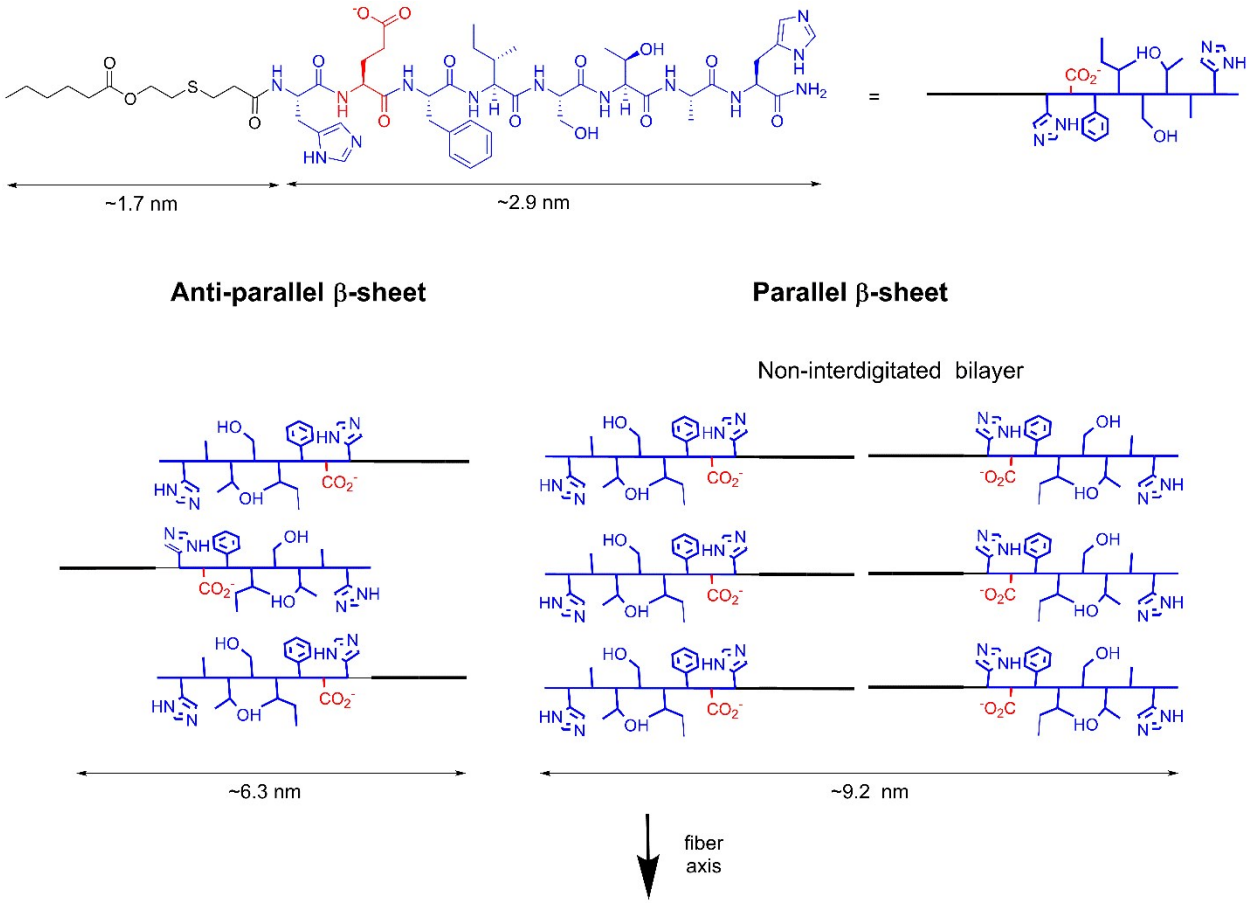



**Figure S21**. Anti-parallel and parallel β-sheet assembled structures of peptide **5b**.

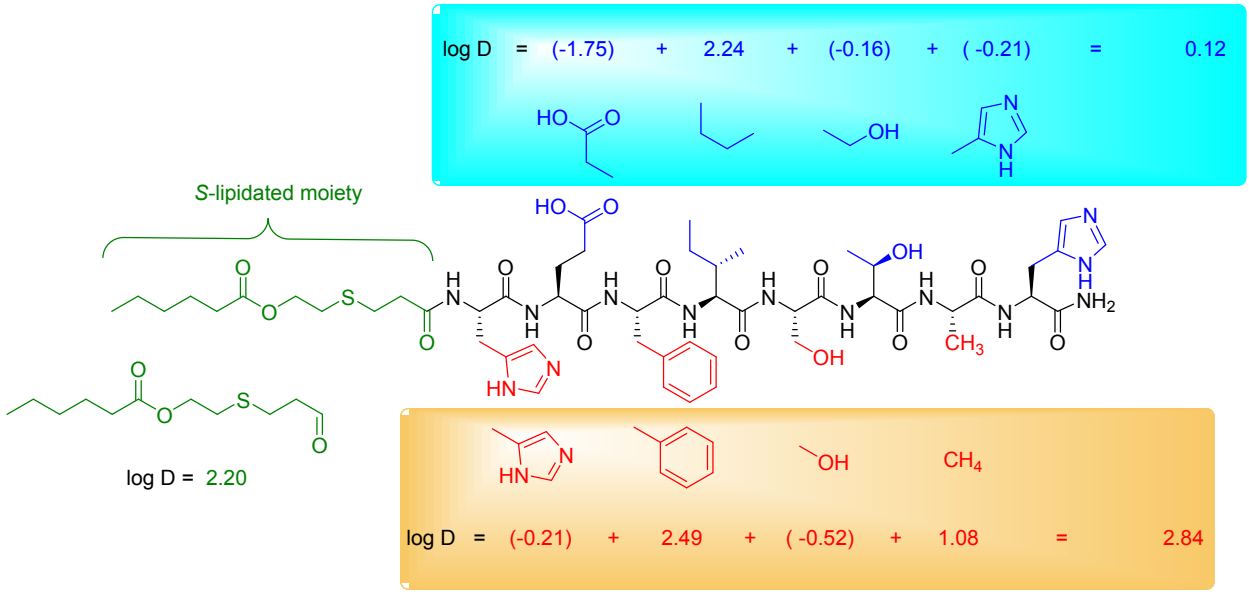



**Figure S22**. Predicted log D values for the side chain groups and *S*-lipidated fragment of **5b**.

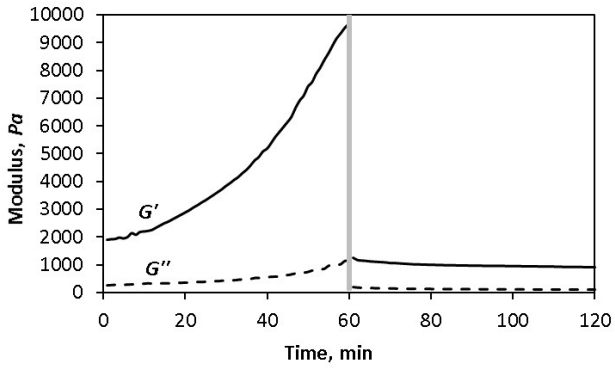



**Figure S23**. Storage ($G'$) and loss ($G''$) modulus of a 3 wt% hydrogel of **5b** in water and after addition of $H_2O_2$ 100 mM (indicated by the solid grey line) to give a 2 wt% final concentration as a function of time ($\omega$ = 6 rad.s$^{-1}$ and $\gamma$ = 0.2% strain).

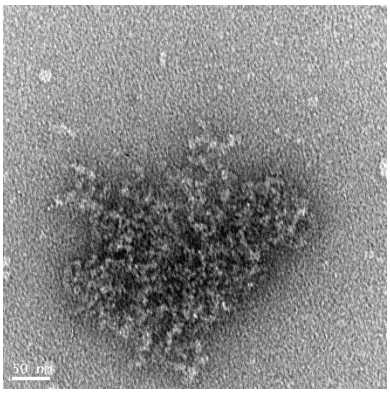



**Figure S24**. TEM image of a sample of the reaction mixture of **5b** with $H_2O_2$ after oxidation completion. The concentration of the peptide in the sample deposited in the grid was 0.4 wt%

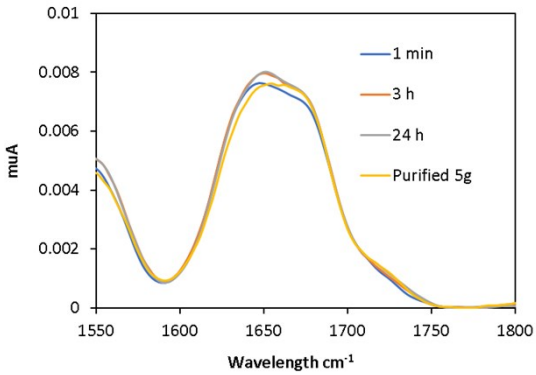



**Figure S25**. ATR-FTIR of a 2 wt% hydrogel sample of **5b**, 1 min after (blue), 3 h (orange), 24 h (grey) after addition of $H_2O_2$; and of a 2 wt% solution of purified peptide **5g** (yellow).

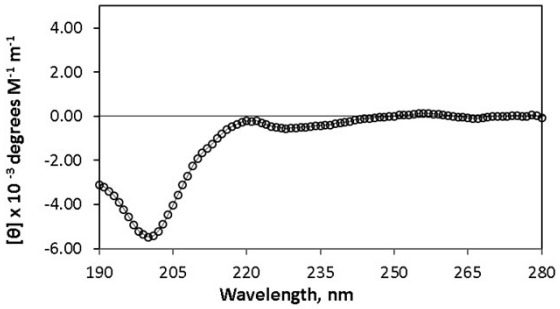



**Figure S26**. Circular dichroism spectrum of a solution of peptide **5g** at a 0.05 wt% concentration.

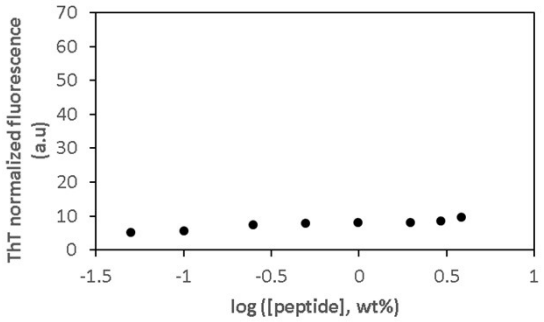



**Figure S27**. Thioflavin (ThT) fluorescence assay plot of **5g**. The fluorescence intensity was normalized relative to the one of the ThT fluorescence assay of **5b** at the highest peptide concentration.

# 3. References

1 C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods*, 2012, **9**, 671-675.