

Supporting Information

Biocatalytic and thermoreversible hydrogel from a histidine-containing tripeptide

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1. Materials and Methods

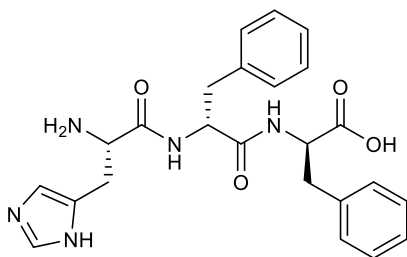
2-chlorotrytil resin, O-Benzotriazole-N,N,N,N'-tetramethyl-uronium-hexafluorophosphate (HBTU), and Fmoc protected *N*-trityl-L-histidine and D-phenylalanine were purchased from GL Biochem (Shanghai) Ltd. All solvents were purchased of analytical grade from Merck. Piperidine, trifluoroacetic acid (TFA), *N,N*-diisopropyl ethyl amine (DIPEA), triisopropyl silane (TIPS) were from Acros. Sodium dihydrogen phosphate and disodium hydrogen phosphate were from BDH AnalaR. High purity Milli-Q-water (MQ water) with a resistivity greater than 18 M Ω cm was obtained from an in-line Millipore RiOs/Origin system. ^1H -NMR spectra were recorded at 400 MHz and ^{13}C -NMR spectra were recorded at 100 MHz on a Varian Innova Instrument with chemical shift reported as ppm (in DMSO or MeCN with tetramethylsilane as internal standard). ESI-MS spectra were recorded on an Agilent 6120 single quadrupole LC-MS system.

2. Peptide synthesis

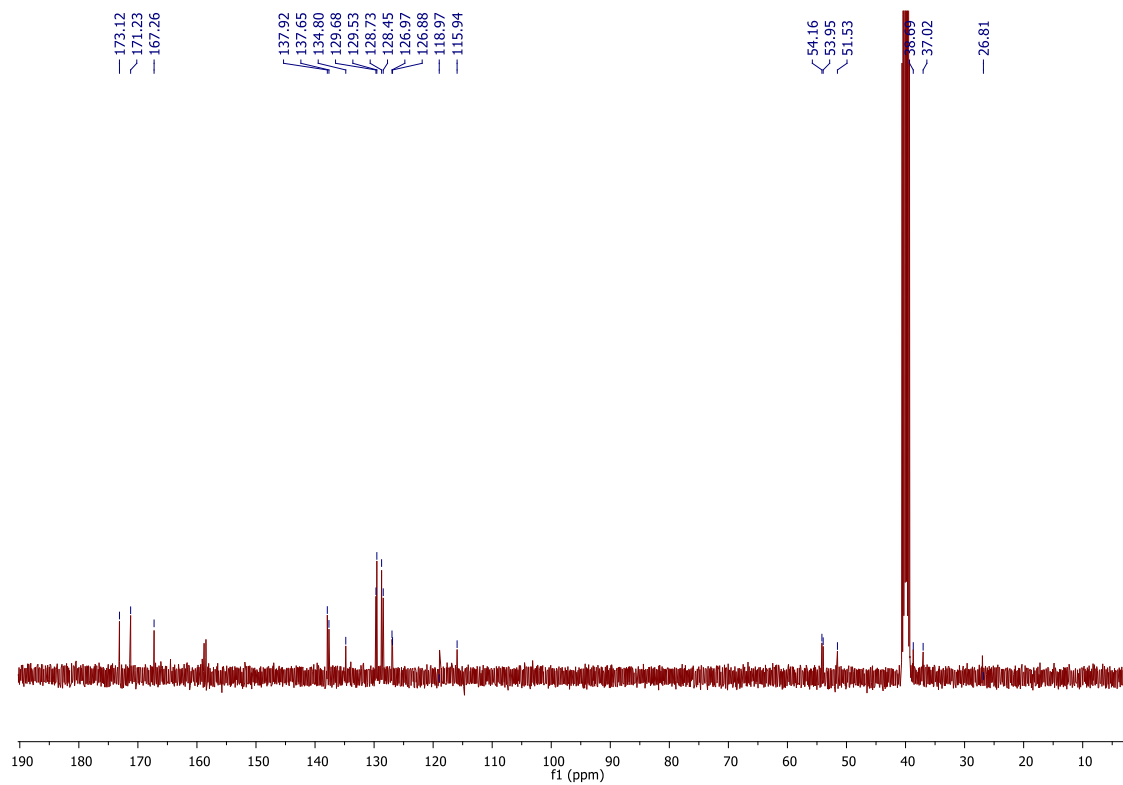
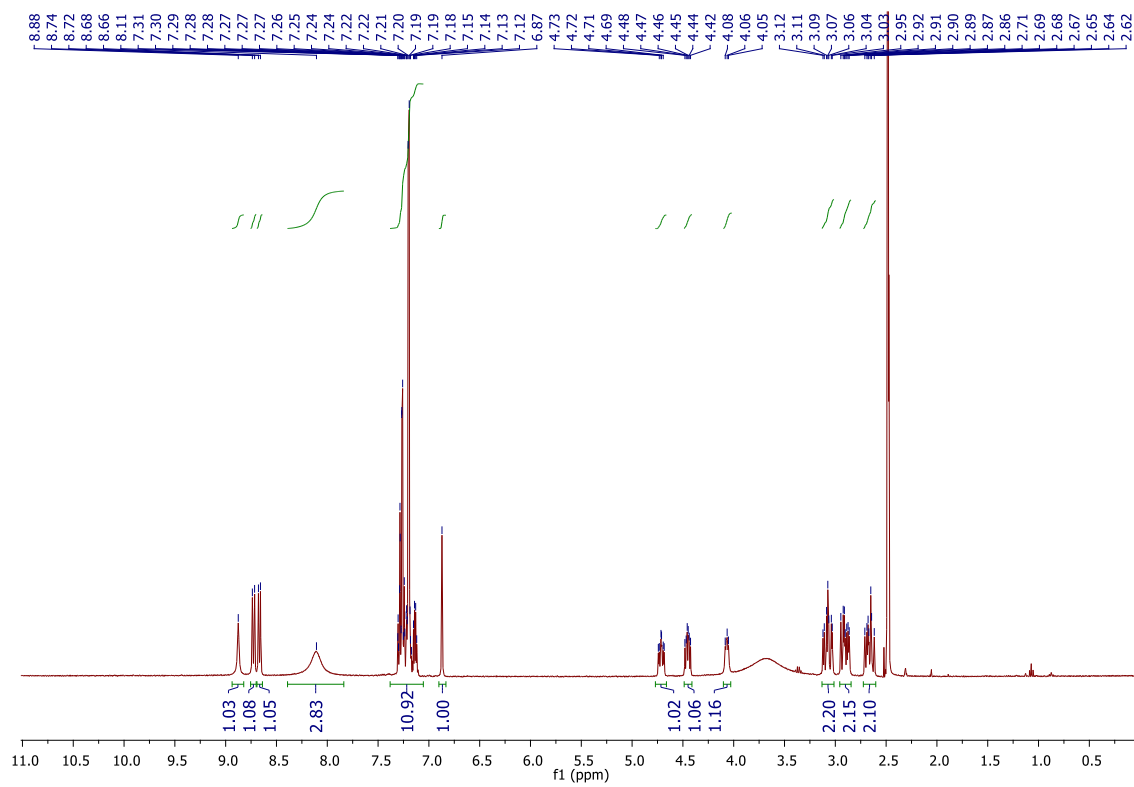
The peptide was synthesised using standard Fmoc solid phase peptide synthesis with HBTU activation. Briefly, Fmoc-amino acid deprotection was performed in a sintered funnel, with continuous stirring, in 20% piperidine in *N,N*-dimethylformamide (DMF) for 20 minutes (2 x 10 minutes) until both bromophenol blue and acetaldehyde/chloranil tests were positive. HBTU activation was performed with 2.5 equiv. of Fmoc-amino acid, 2.0 equiv. of HBTU and 2.0 equiv. of HOAt in DMF (4 mL for every equiv. of resin), with DIPEA (2 mL of a 1 M solution in DMF for every equiv. of resin). Coupling was performed at room temperature for 1.5 h in a sintered funnel with continuous stirring, and completeness was monitored by both bromophenol blue and acetaldehyde/chloranil tests after thorough washes with DMF and DCM. Final cleavage was obtained using a mixture of TFA/DCM/TIPS/water (47.5:47.5:2.5:2.5). The crude peptide was too hydrophobic to be precipitated in cold ether, thus the majority of TFA was evaporated under argon flow, and the remaining oil was dissolved in a mixture of acetonitrile/water

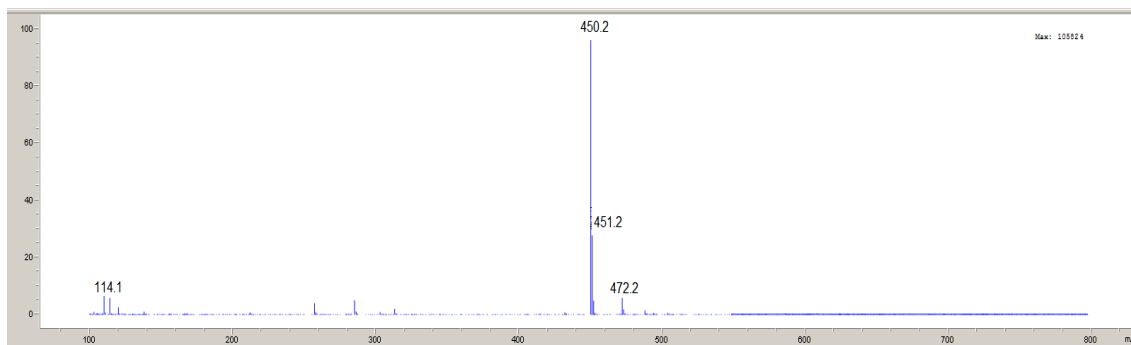
and then purified by reverse-phase HPLC (Agilent Technologies). The HPLC Agilent 1260 Infinity system was equipped with a preparative gradient pump (1311B), semipreparative C-18 column (Kinetex, 5 microns, 100 Å, 250 x 10 mm, Phenomenex), autosampler (G1329B), Photodiode Array detector (G1315C). The gradient used consisted of acetonitrile (MeCN) / water with 0.1% TFA with the following program: t = 0–2 min. 25% MeCN; t = 12 min. 50% MeCN; t = 15 min. 95% MeCN; t = 16 min. 95% MeCN (t_R = 6.8 min). The compound was then freeze-dried to yield the corresponding peptide as a white fluffy powder. Peptide identity was verified by ESI-MS, ^1H -NMR and ^{13}C -NMR.

3. L-His-D-Phe-D-Phe spectroscopic data

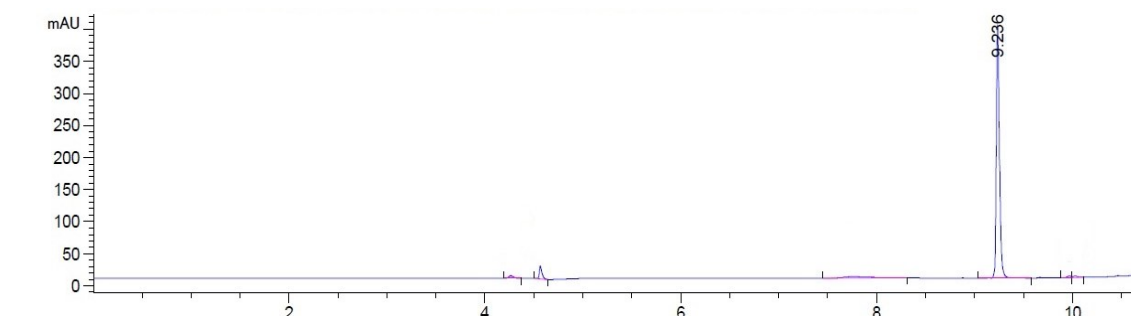


^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.89 (s, 1H, NH), 8.73 (d, J = 8.8 Hz, 1H, NH), 8.67 (d, J = 7.9 Hz, 1H, NH), 8.12 (s, 3H, NH_3^+), 7.34 – 7.06 (m, 11H, Ar), 6.87 (s, 1H, CH), 4.71 (m, 1H, αCH), 4.44 (m, 1H, αCH), 4.07 (dd, J = 7.9, 4.9 Hz, 1H, αCH), 3.15 – 3.01 (m, 2H, βCH_2), 2.98 – 2.83 (m, 2H, βCH_2), 2.75 – 2.59 (m, 2H, βCH_2). **^{13}C NMR** (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 173.1, 171.2, 167.3 (3 x CO); 137.9, 137.7, 134.8, 129.7, 129.5, 128.7, 128.5, 127.0, 126.9 (Ar); 54.2, 54.0, 51.5 (3 x αC); 38.7, 37.0, 26.8 (3 x βC). **MS (ESI):** m/z 450.2 ($\text{M}+\text{H}$) $^+$ 472.2 ($\text{M}+\text{Na}$) $^+$ $\text{C}_{24}\text{H}_{27}\text{N}_5\text{O}_4$ requires 450.2

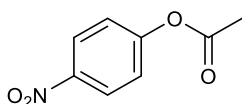




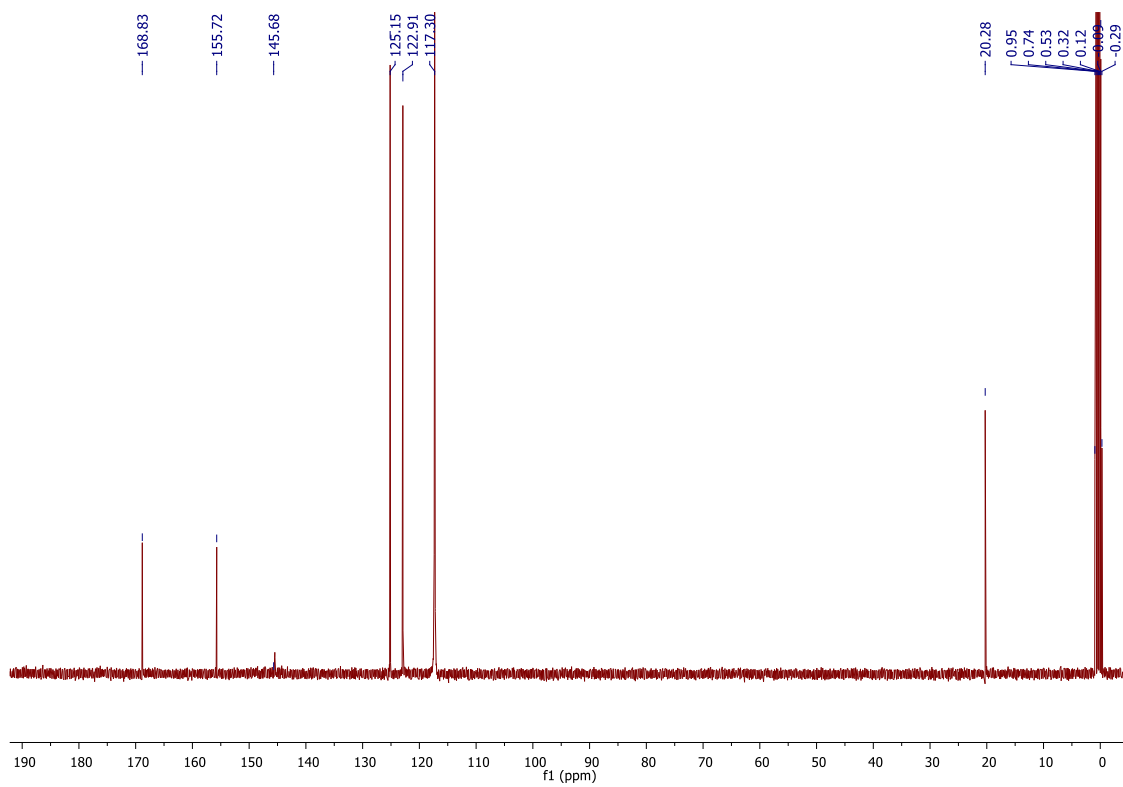
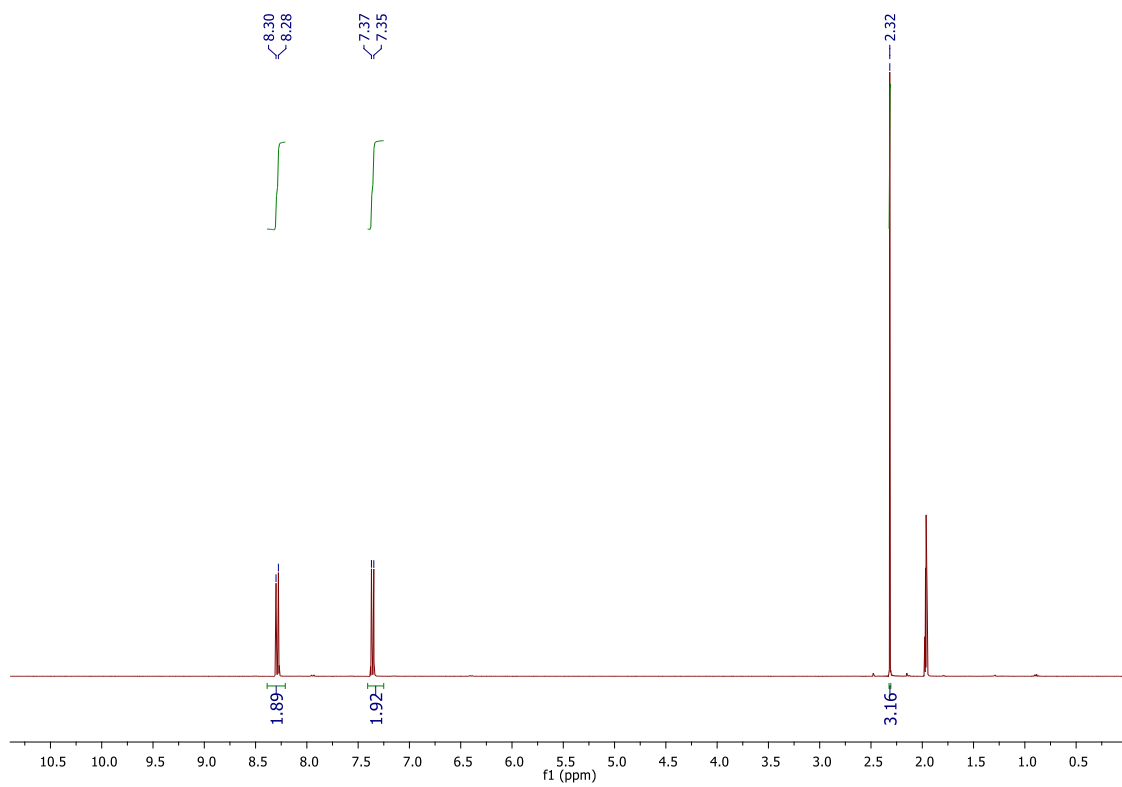
Analytical LC purity >97% (solvent gradient: 5 % to 95% MeCN 0.1 % TFA in 11 min.)



4. p-nitrophenyl acetate synthesis and spectroscopic data



Procedure. Synthesis of 4-nitrophenyl-acetate was done according to standard literature methods. 0.20 mmol mmol of 4-nitrophenol, 0.24 mmol acetic anhydride and 1 % DMAP catalyst were dissolved in 5 mL CH_2Cl_2 . The reaction mixture was stirred at room temperature for 2 h and then extracted with HCl 0.1 M and dried over anhydrous sodium sulfate. Evaporation of dichloromethane yielded the ester as an oily material which was precipitated as a white solid in ethanol. **^1H NMR** (400 MHz, $\text{MeCN-}d_3$) δ (ppm): 8.29 (d, $J = 9.2$ Hz, 2H, Ar), 7.36 (d, $J = 9.2$ Hz, 2H, Ar), 2.32 (s, 3H, CH_3). **^{13}C NMR** (100 MHz, $\text{MeCN-}d_3$) δ (ppm): 168.8 (CO), 155.7, 145.7, 125.2, 122.9 (Ar), 20.82 (Me).

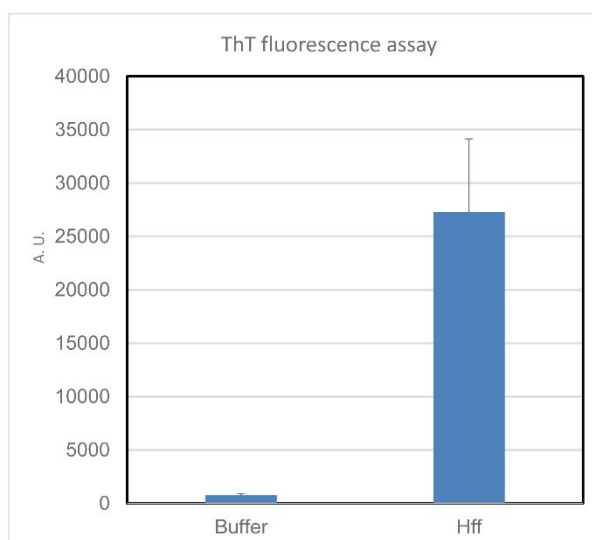


5. Peptide gelation

The peptide L-His-D-Phe-D-Phe-COOH was dissolved in sodium phosphate buffer (0.1 M pH 11.8) to a final concentration of 50 mM, getting a final pH of 7.2 ± 0.1 . All buffer solutions were filtered (0.2 μm) prior to use.

6. ThT test for amyloid-like structure detection

Gel precursor solution (0.12 ml) was prepared as described above and immediately put on wells of Greiner 96 U Bottom Transparent Polystyrene. After 4 hours, 30 μl of a solution of Thioflavin T (22.2 μM in 20 mM glycine-NaOH pH 7.5, filtered with a 0.2 μm filter) were added in the wells. After 15 minutes, the fluorescence emission was analysed using a Tecan Infinite M1000 pro, selecting an excitation wavelength of 446 nm and an emission wavelength of 490 nm, with a bandwidth of 20 nm. Each condition was repeated twice in triplicate. Average and standard deviations were calculated and plotted with Excel.



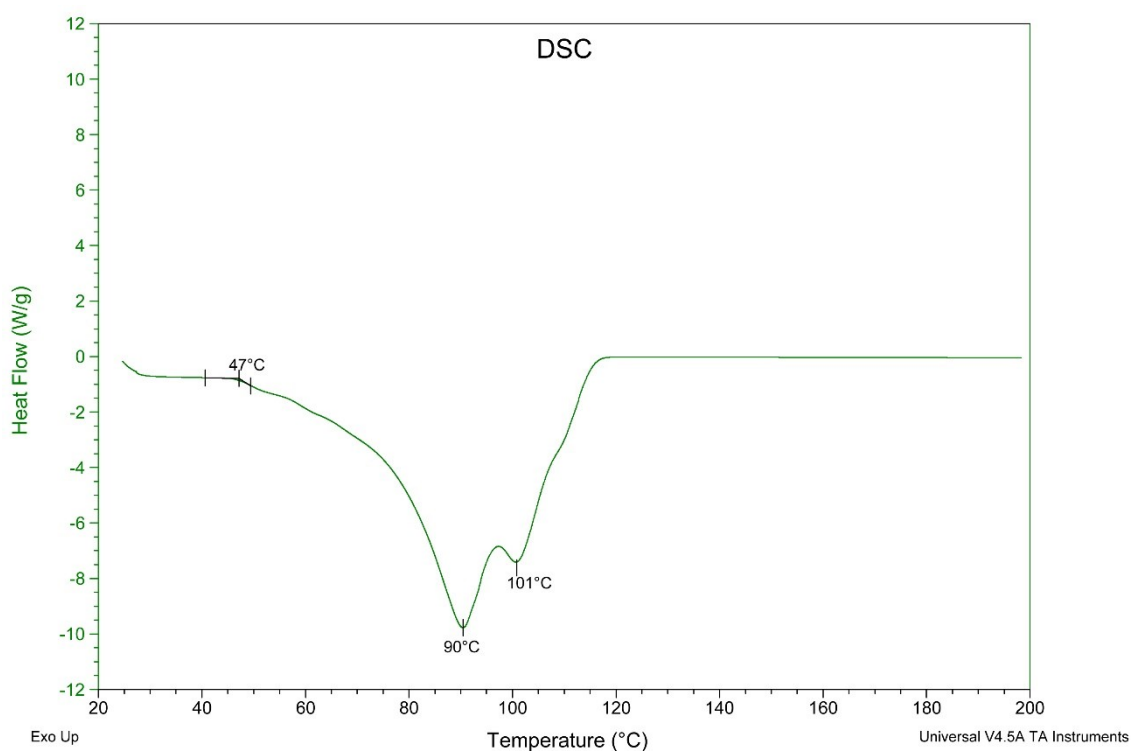
7. FT-IR spectroscopy

FT-IR spectra were registered using KBr-pellet method. The gel was prepared and after 24 h it was dried under vacuum. Then, it was mixed with KBr to make the pellet. The

spectrum was acquired from 400 to 4000 cm^{-1} , at 1 cm^{-1} resolution and 128 accumulations on a Perkin Elmer System 2000.

8. Differential Scanning Calorimetry (DSC)

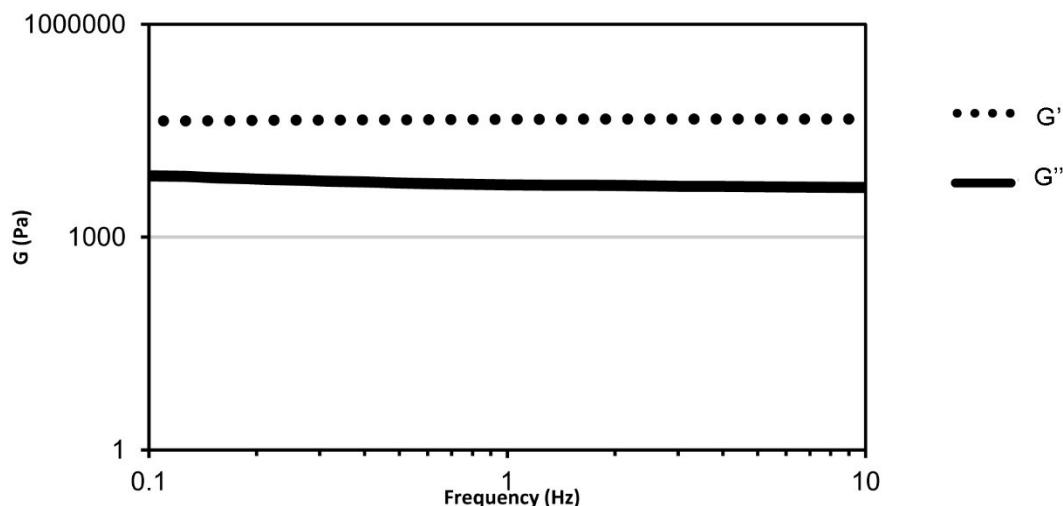
DSC data were collected on a Q100 calorimeter (TA Instruments). The hydrogel samples were prepared directly in the DSC pans (20 μl). Pans were closed with their lids, and measurements started after 20 min at room temperature. DSC scans started with an isotherm at 25 $^{\circ}\text{C}$ for 10 min, followed by a 5 $^{\circ}\text{C min}^{-1}$ ramp up to 200 $^{\circ}\text{C}$ (*i.e.*, below peptide decomposition temperature). Measurements were repeated twice.



9. Rheometry

Dynamic time sweep rheological analysis was conducted on a Malvern Kinexus Ultra Plus Rheometer with a 20 mm stainless steel parallel plate geometry. The temperature was maintained at 25 $^{\circ}\text{C}$ using a Peltier temperature controller. Samples were prepared *in situ* and immediately analysed with a gap of 1.00 mm. Time sweeps were recorded for

1 hour, using a frequency of 1.00 Hz and a controlled stress of 5.00 Pa. After 1h, frequency sweeps were recorded using a controlled stress of 5.00 Pa and then stress sweeps were recorded using a frequency of 1 Hz.



10. Circular Dichroism

A 0.1 mm quartz cell was used on a Jasco J815 Spectropolarimeter, with 1s integrations, 1 accumulation and a step size of 1nm with a bandwidth of 1nm over a range of wavelengths from 220 to 280 nm at 25 °C (Peltier). Samples were freshly prepared directly in the CD cell and the spectra immediately recorded.

11. TEM

TEM micrographs were acquired on Jeol, JEM 2100, Japan, at 100 kV. TEM grids (copper-grid-supported lacey carbon film) were first exposed to the UV-ozone cleaner (UV-Ozone Procleaner Plus) for 45 mins to make the grid surface more hydrophilic. Then, 24-hours-aged gels were precisely deposited on a TEM grid, dried for 15 mins at RT, and contrasted by aqueous tungsten phosphate solution (pH 7.4).

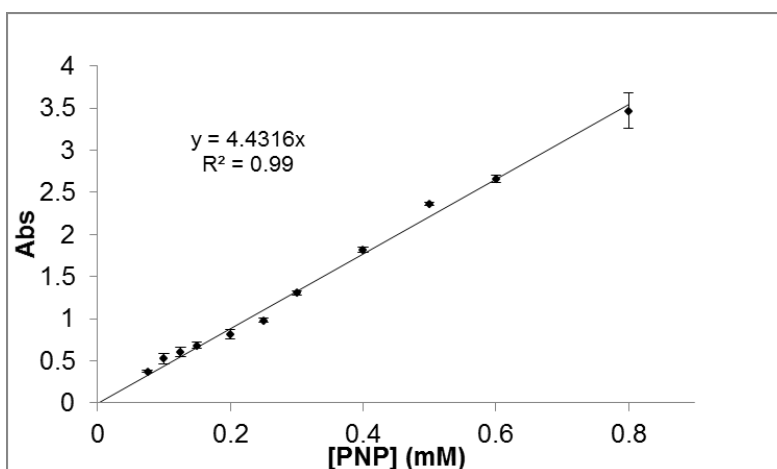
12. AFM

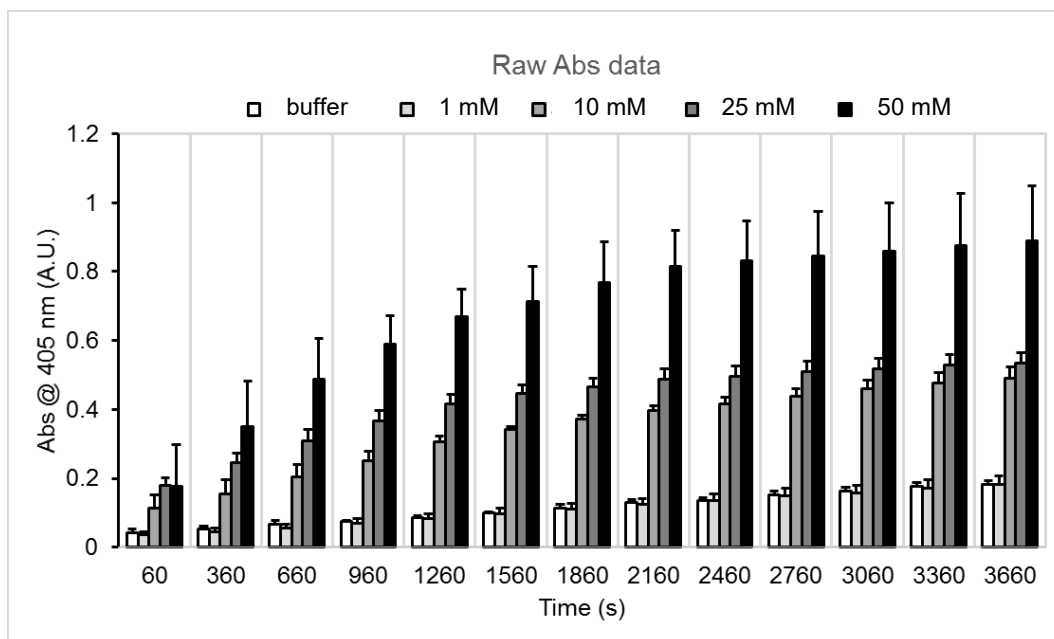
Gels were prepared as described above and after 24 hours they were spread on a mica surface. Atomic Force Microscopy (AFM) measurements were performed using a Nanoscope V microscope (Digital Instruments Metrology Group, model MMAFMLN) in tapping mode in air at room temperature, using standard $\mu\text{masch}^{\circledR}$ SPM probe (HQ:NSC15/AIBS) with tip height 12-18 μm , cone angle $<40^{\circ}$ (Resonant frequency 325kHz, force constant of $\sim 40 \text{ N m}^{-1}$). Image analysis was performed with Gwiddion software.

13. Esterase activity assay

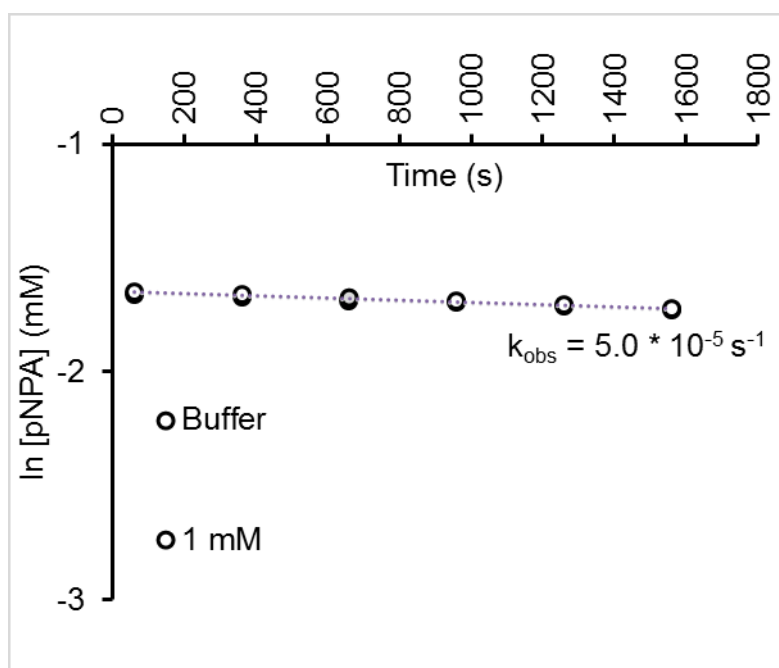
Gel precursor solutions were prepared as described above and 98 μl of phosphate buffer 0.1M pH 11.8 were immediately put on wells of Greiner 96 U Bottom Transparent Polystyrene. Final pH was 7.2 ± 0.1 . Controls were run in phosphate buffer 0.1M at the same pH, with a final peptide concentration of 1 mM, 10 mM, 25 mM, or buffer alone. After 4 hours, 2 μl of a solution of pNPA (10 mM in MeOH) were added to the wells. Absorbance at 405 nm was monitored for 1 hour on a Tecan Infinite M1000 pro. Each condition was repeated twice in triplicate. Average and standard deviations were calculated and plotted with Excel.

Calibration curve using pNP standard solutions

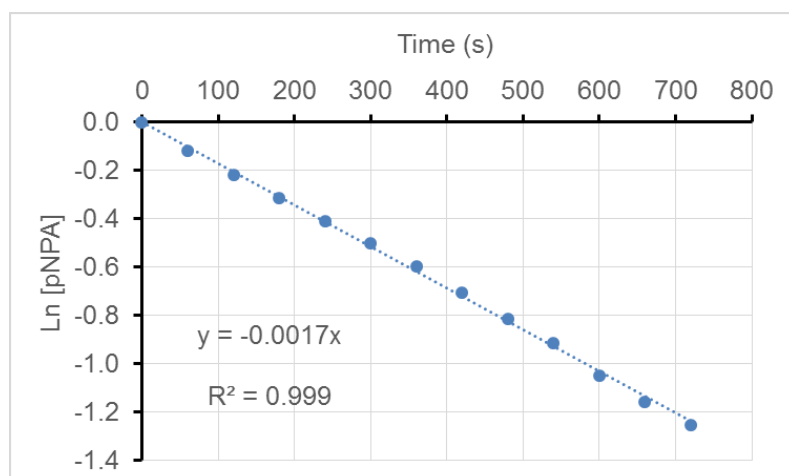
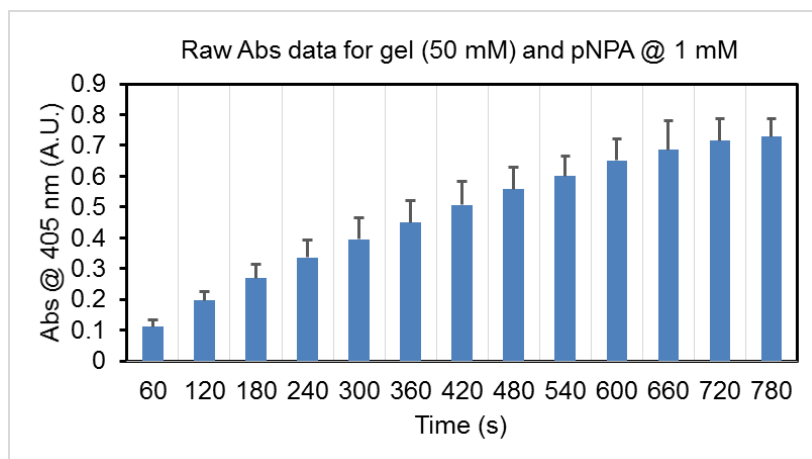




K_{obs} for buffer and peptide (1mM) controls

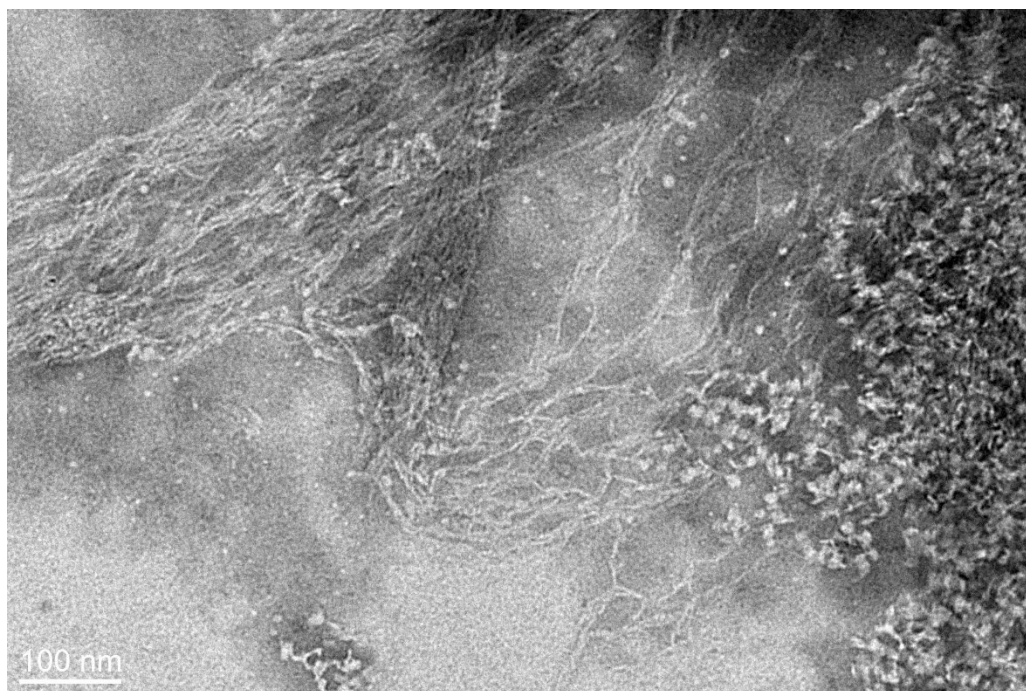
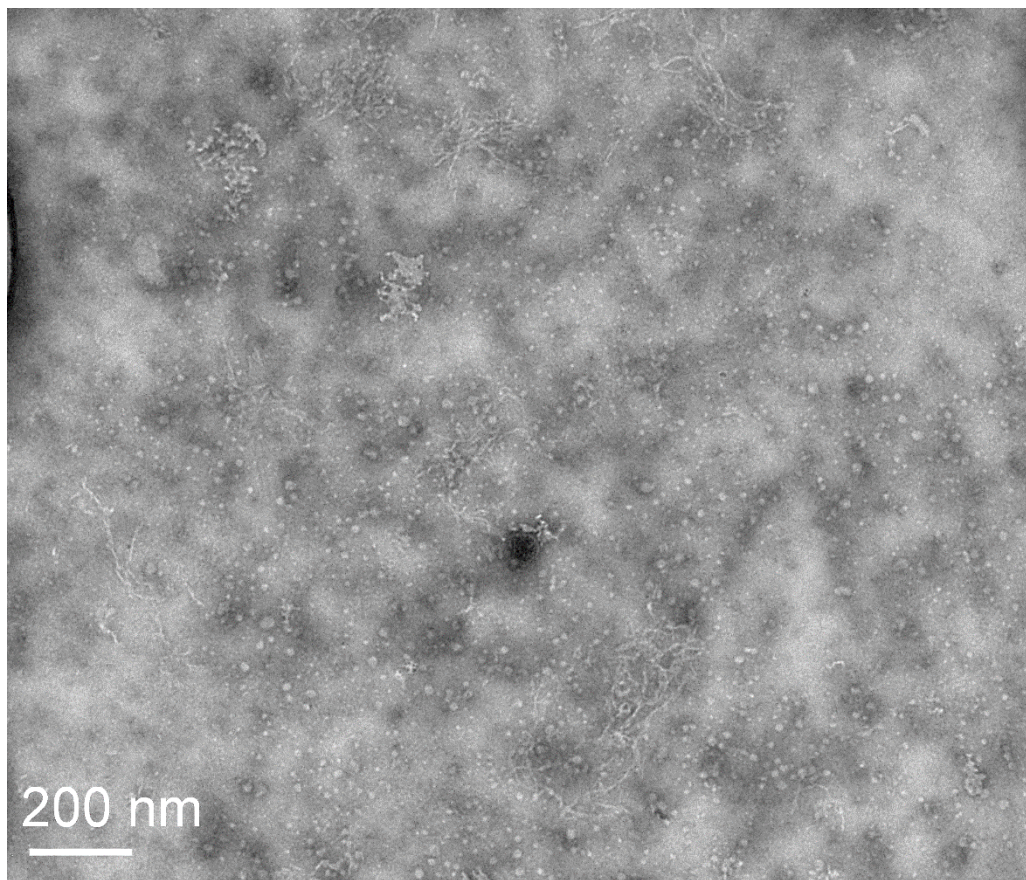


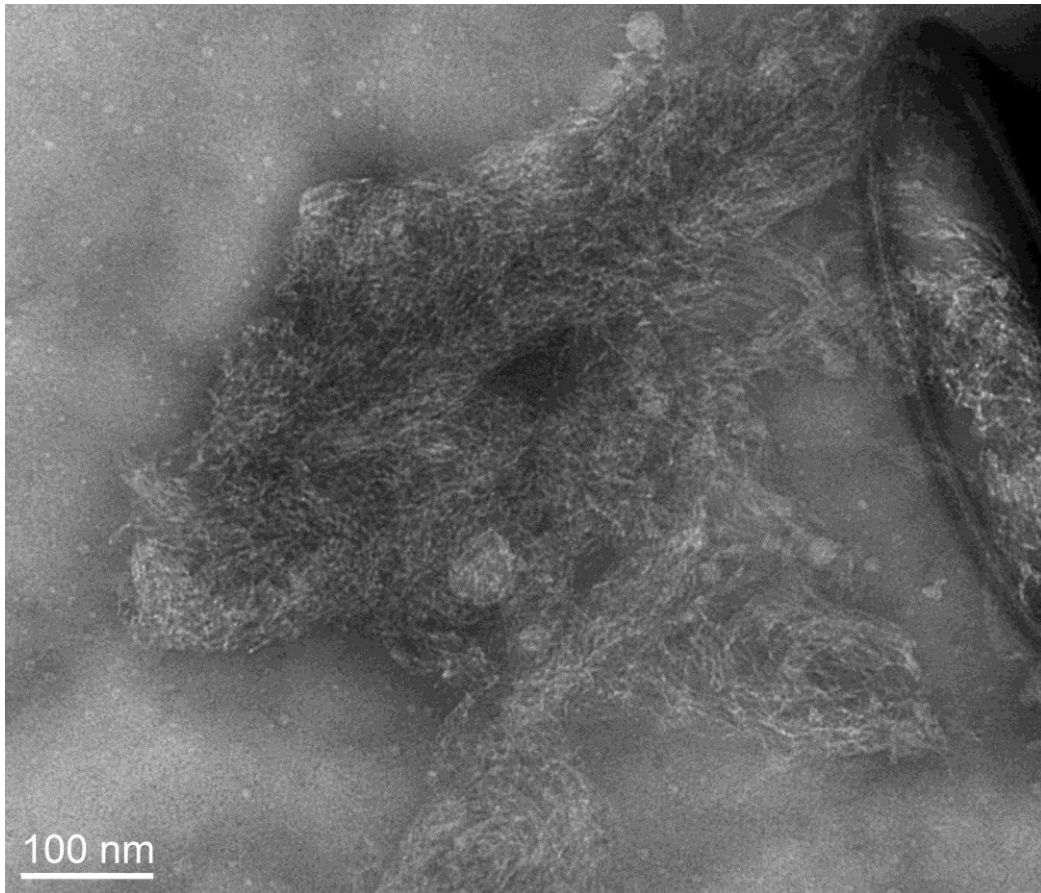
The gel (50 mM peptide) was also tested for catalytic activity as described above, but with higher amount of substrate (1 mM pNPA). $K_{\text{obs}} = 1.7 \times 10^{-3} \text{ s}^{-1}$. Data is shown below.



14. TEM images of peptide below mgc

Peptide at 25 mM





Peptide at 10 mM:

