Electronic Supplementary Information

Kinetically Trapped Self-Assembly in Synthetic Nucleopeptide and Nucleotides

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Instrumentation:

NMR Experiments: All NMR studies were carried out on a Bruker Ascend 500 MHz NMR instrument at 25 ^{0}C. Variable temperature ^{1}H NMR were carried out from 25 °C to 85 °C. Compounds concentrations were in the range 1–10 mmol in DMSO-d_6 or 10 % D_2O (90% H_2O).

Mass Spectrometry: Mass spectrometry was recorded on a LC MS QToF HRMS System XEVO G3 mass spectrometer by positive mode electrospray ionization.

Field Emission Scanning Electron Microscopy (FE-SEM): FE-SEM images were recorded in a FE-SEM MIRA instrument. A small portion of all samples (gel or solution) were placed on microscope cover glasses. These samples were dried first in air and then in vacuum and coated with gold and recorded at 5 keV. The fiber widths were calculated by using ImageJ software.

Transmission Electron Microscopy (TEM). The morphologies of all gels have been studied by using TEM at room temperature (25 °C). 36 mM hydrogel was prepared in phosphate buffer pH 7.2, 100 mM. 10μL of these gels were further diluted to 1mL of the same buffer and 5 μL of these dilute solutions were placed on a TEM grid (300 mesh size Cu grid) coated with a carbon film. The grid was allowed to dry by slow evaporation in air, and then allowed to dry separately in a vacuum overnight. Images were taken using UHR-FEG-TEM JEM-2100F at 200 kV. The fiber widths were calculated by using ImageJ software.

ANS Fluorescence study: Fluorescence spectroscopic studies of fresh and enzyme treated hydrogels in a sealed cuvette were carried out in a Perkin-Elmer FL 6500 Fluorescence Spectrometer instrument. All the experiments were carried out with excitation slit 5 nm and emission slit width 10nm. The excitation wavelength was fixed at 375 nm. The concentration of ANS dye in each sample was 5 μM.

HPLC Study: High-performance liquid chromatography was performed in an Agilent 1260 Infinity LC System. The analytes were chromatographically resolved on a reverse-phase (Eclipse Plus C18, 4.6 x 250 mm, 5 μm) analytical LC column. For the detection of AMP/ATP, the mobile phase was composed of solvent A (water, 1% TFA) and solvent B (Methanol). The gradient elution program was 10 % solvent B over 10 min at a steady flow rate of 0.5 ml min^{-1}. A standard solution of 40 mM AMP provided a sharp peak at 6.8 min and a standard solution of 40 mM ATP gave a sharp peak at 4.9 min.

Materials:

Thymine-1-acetic acid, Fmoc-L-Leucine, Fmoc-L-Valine, Fmoc-L-Phenylalanine, Adenosine triphosphate (ATP), Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), Wang resin, 1-hydroxybenzotriazole (HOBt), Diisopropylcarbodiimide (DIC) Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), N,N-Diisopropylethylamine (DIPEA), Trifluoroacetic acid (TFA), Piperidine, Triisopropylsilane (TIS) and 4-Dimethylaminopyridine were purchased from Sigma Aldrich. Buffer solutions were made using Na_2HPO_4 and NaH_2PO_4 using a Metler Toledo FP20-Bio Kit w/ LE410 pH sensor.

Methods:

Solid-Phase Synthesis of Nucleo-tripeptides.

All nucleopeptides were synthesized (0.3 mmol scale) by conventional Fmoc-solid phase peptide synthesis and coupled on the solid phase using diisopropylcarbodiimide (DIC)/HBTU/HOBt amide coupling chemistry. Wang resin swelled in DCM in a fritted, capped syringe for 30 min and then rinsed with dimethylformamide (DMF). The resin was mixed with a coupling solution (3 equiv of Fmoc-protected amino acids or 3 equiv of nucleobase derivatives, 3 equiv of HOBt, and 3 equiv of HBTU) for 60 min. The coupling reaction was followed by washing with DMF (5 mL, 3 times) and DCM (5 mL, 3 times). 5 mL of 20% piperidine in DMF was used for deprotecting the Fmoc group (20 min × 2). After washing with 5 mL of DMF (3 times) and 5 mL of DCM (3 times) Fmoc deprotection and coupling were repeated until achieving the desired nucleopeptide. Cleavage from the resin and side group deprotection were simultaneously completed by a 2.5−3 h reaction with 5 mL of 95:2.5:2.5 trifluoroacetic acid/water/triisopropylsilane (TFA/H_2O/TIS). The mixture was then collected and the TFA was removed under vacuum. After concentrating in vacuo, the resulting viscous solution was precipitated with cold diethyl ether. Scavenged protecting groups were removed by washing with cold diethyl ether (×3) and centrifugation, and then the product was dried under a gentle stream of N_2. The peptide was characterized by ^{1}H NMR and mass spectrometry.

Thymine-Leu-Val-Phe-COOH (**1**): ^{1}H NMR (500 MHz, DMSO-D6): δ 11.25 (s,1H, NH- Thymine), 8.32-8.30 (d,1H, NH, *J*=8), 8.18-8.17 (d,1H, NH, *J*=8), 7.76-7.74 (d, 1H, NH, *J*=9), 7.39 (s,1H, CH-Thymine), 7.27-7.17 (m, 5H, Phe), 4.46-4.13 (m,5H, CH2, α-Hs), 3.06-2.87 (m,2H, β-H), 1.94-1.90 (m, 1H, β-H), 1.74 (s, 3H, CH3), 1.61-1.34 (m, 3H, β-H and γ-H), 0.87-0.78 (m, 12H, CH3).

HRMS (m/z) 542.2409 $(M-H)^+$

Preparation of hydrogel: Nucleopeptide **1** was dissolved in 300 µL of 100 mM phosphate buffer by heating at >70°C to obtain a clear solution. ATP was added to this solution which results in a turbid solution. After a heating and cooling cycle followed by sonication, the solution turned clear and self-assembled into a hydrogel within 10-15 minutes. Gel formation was tested by inverted vial method. The amount of nucleotides kept 1.1 equivalent to the nucleopeptide **1** for all the samples.

Determination of Minimum gelation concentration: A series of glass vials prepared each containing different concentrations of the compound **1** dissolved in 300µL of 100 mM phosphate buffer. After the mixture cooled, the vials were inverted to test hydrogel formation. The lowest concentration at which the gel does not flow when the tube is inverted is considered the Minimum Gelation Concentration.

Determination of gel melting point (T_{gel}): Gel melting temperature was determined by heating gels in a thermostat-controlled water bath at a heating rate of 2 °C/ 5 minute until the gel was melted. The calculated error range was found to be ±1 °C.

pK_a determination: The pK_a of the nucleopeptide was determined by pH-metric method. Initial pH of the nucleopeptide **1** (1 mM) was adjusted to 2.45 and the pH was increased by 1N NaOH solution. The amount of base added was plotted as a function of pH, which allows determination of the pK_a.

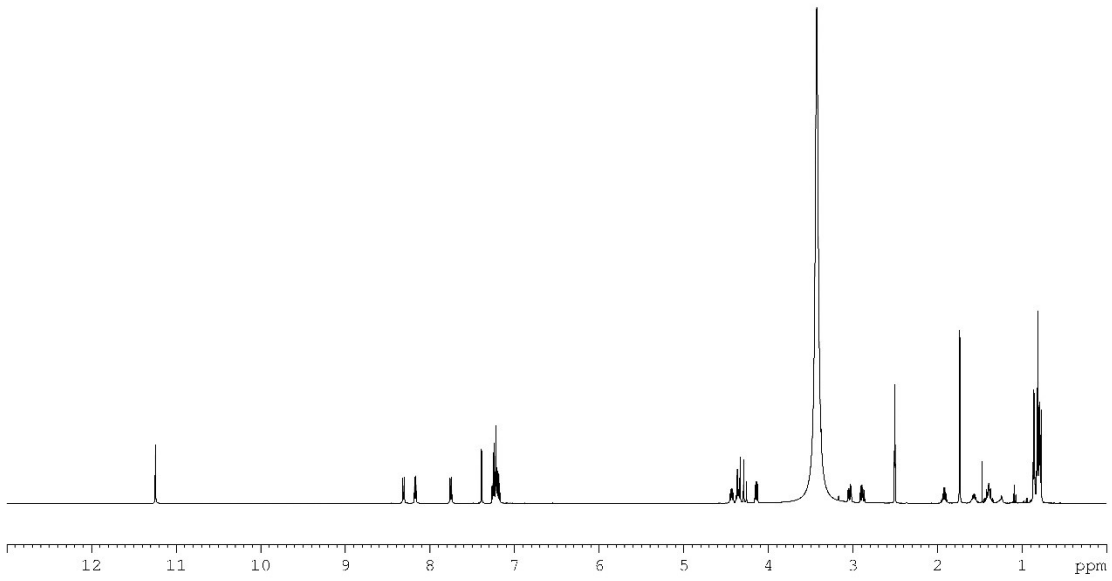

Fig. S1: ^{1}H NMR in DMSO-D6 of nucleopeptide **1**.

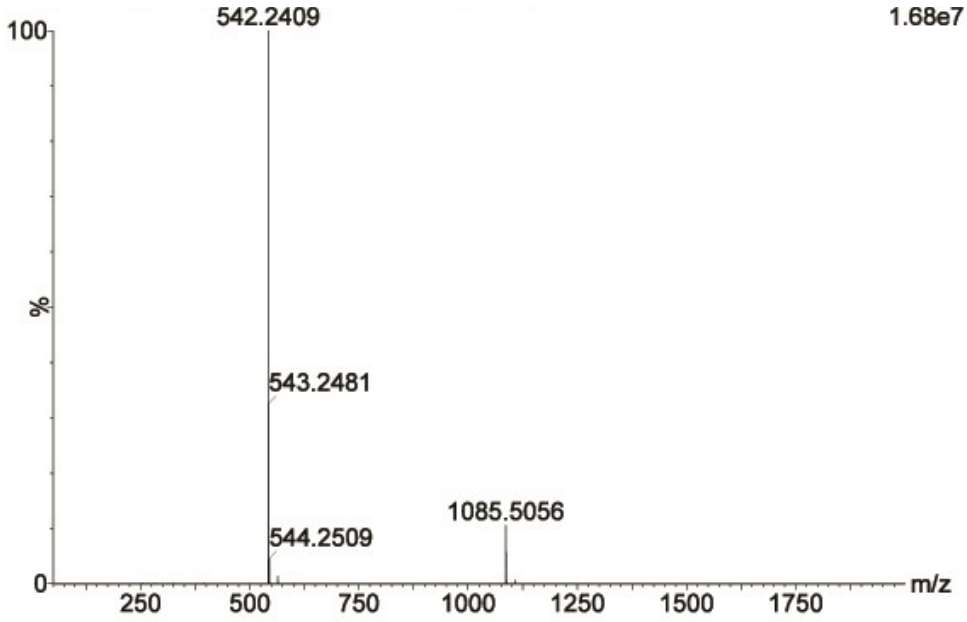

Fig. S2: HRMS of nucleopeptide **1**.

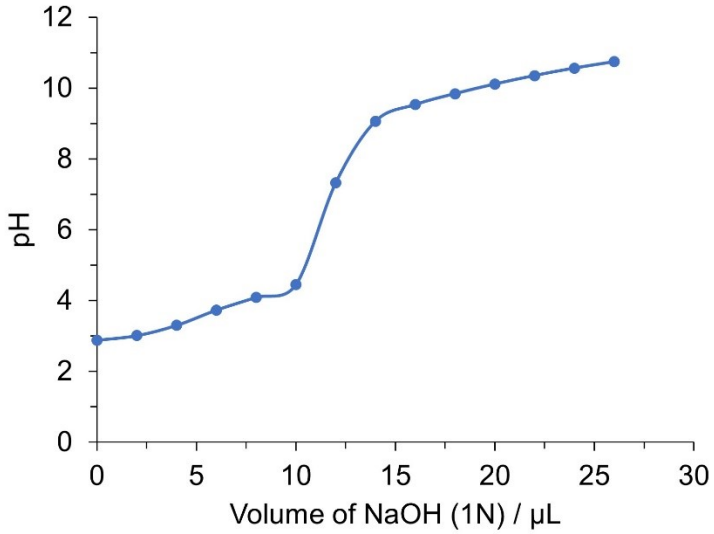

Fig. S3: The pK_a plot of the nucleopeptide **1**. The pK_a value was determined to 6.75.

Fig. S4: Solution of 36 mM nucleopeptide **1** in 100 mM phosphate buffer at pH 7.2

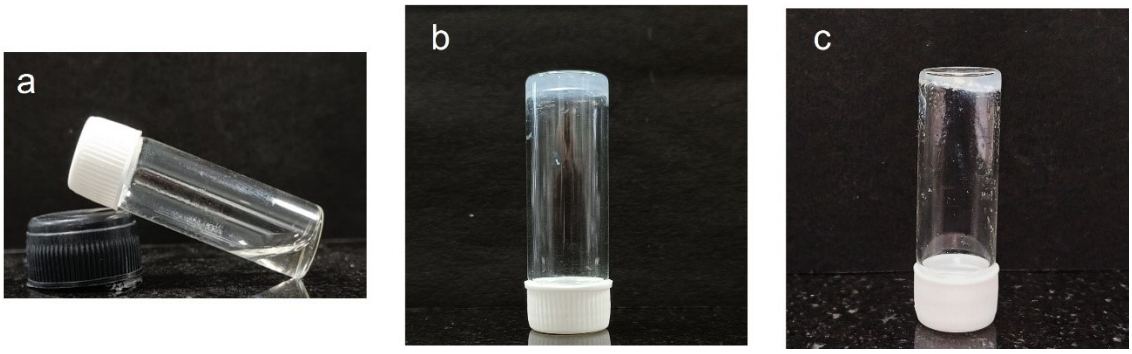

Fig. S5: Images of 36 mM **1** a) 5 min after addition of ATP (40 mM), b) after 15 min, formed hydrogel and c) 7 hrs, after the addition of 3 U/mL of apyrase. All experiments were performed in 100 mM phosphate buffer at pH 7.2.

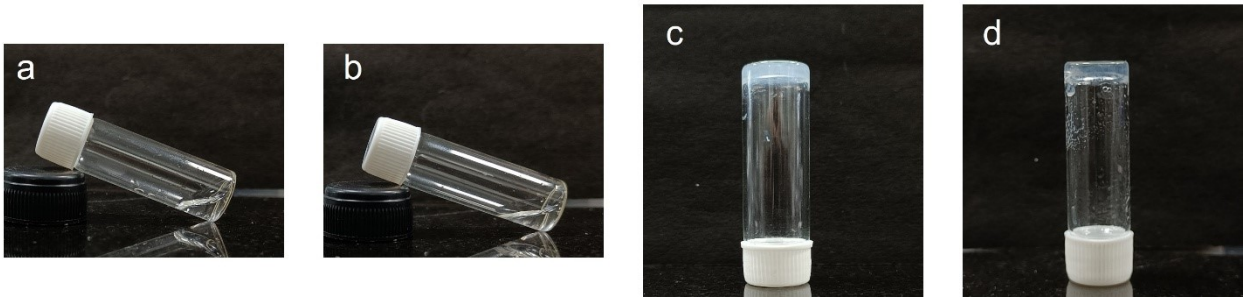

Fig. S6: Concentration dependent images of **1**-ATP (**1**: ATP ; 1: 1.1 equivalent) at a) 20 mM, b) 30 mM, c) 36 mM and d) 40 mM in 100 mM phosphate buffer at pH 7.2. The hydrogel does not form lower than 36 mM of nucleopeptide **1**, hence, the MGC was considered to 36 mM.

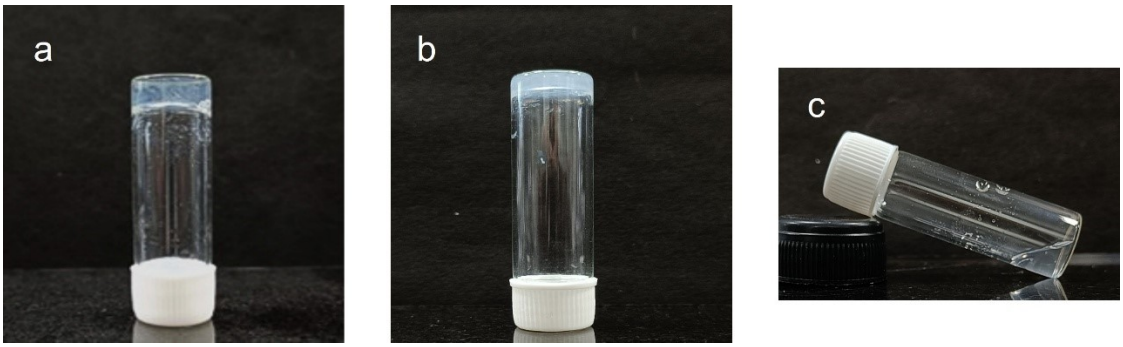

Fig. S7: Images of 36 mM of **1**-ATP hydrogel in 100 mM phosphate buffer at pH a) 6.5, b) 7.2 and c) 8.0. The solubility was less at pH 6.5 and the hydrogel does not form at higher pH 8.0. The concentration of nucleopeptide **1** and ATP were 36 mM and 40 mM respectively.

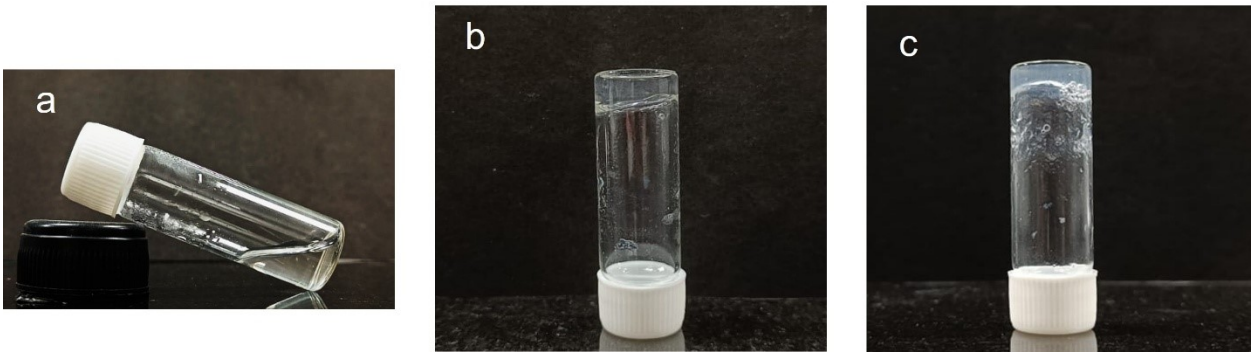

Fig. S8: Images of 36 mM of **1** and 40 mM of ADP a) after 15 min, b) after 48 hrs, and c) 4 hrs, after the addition of 3U /mL of apyrase to 48 hrs aged **1**-ADP hydrogel in 100 mM phosphate buffer at pH 7.2.

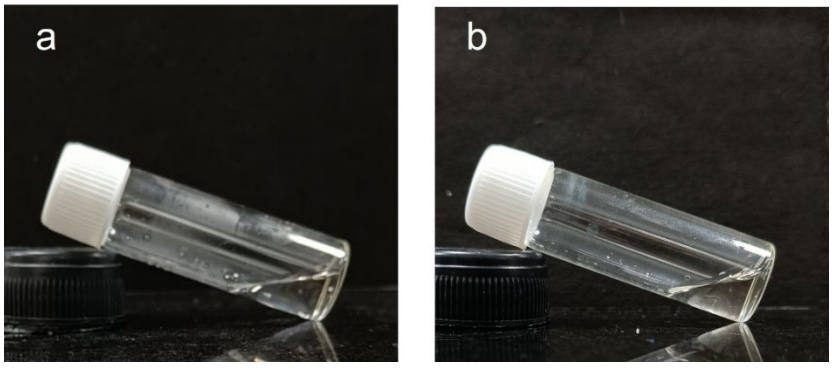

Fig. S9: Images of 36 mM of **1** and 40 mM of AMP after a) 15 min, and b) 7 days in 100 mM phosphate buffer at pH 7.2.

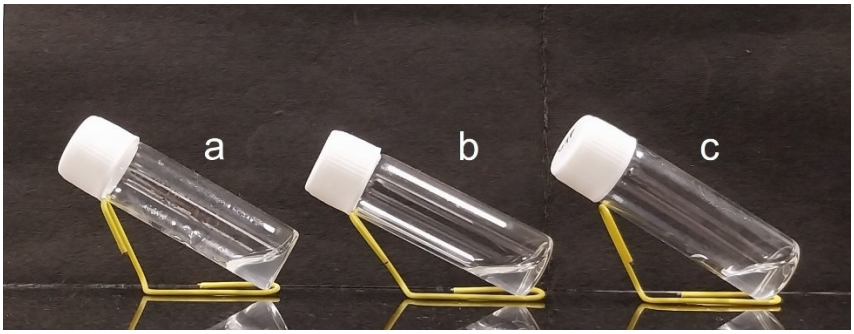

Fig. S10: Images of a) **1**-CTP solution, b) **1**-GTP solution, c) **1**-UTP solution in 100 mM phosphate buffer at pH 7.2. 36 mM of **1** and 40 mM of nucleotides were present for all the samples.

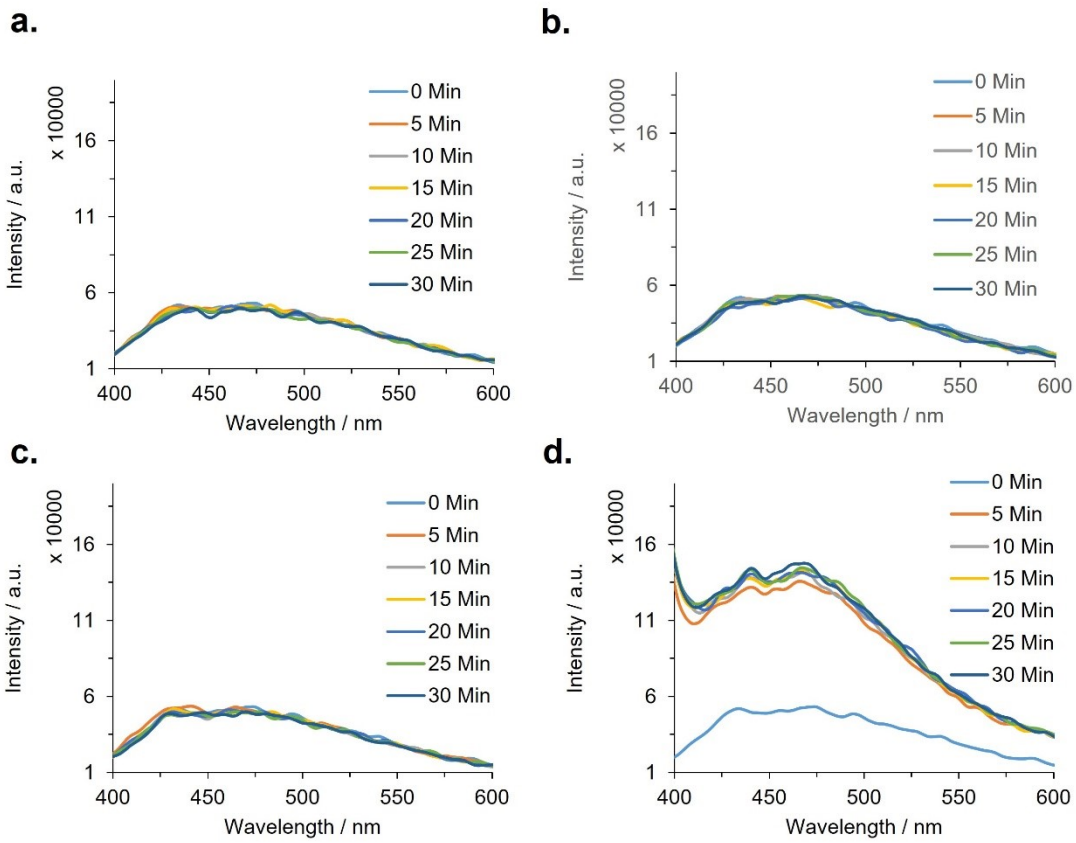

Fig. S11: Emission spectra obtained from **a**) **1**, **b**) **1**-AMP, **c**) **1**-ADP and **d**) **1**-ATP. The emission from **1**-ATP solution showed a drastic increase in emission immediately after addition of ATP to the solution of **1**. The compound itself and mixtures of AMP and ADP showed no change in emission with time. The emission was studied by mixing ANS dye as a probe for hydrophobicity. All studies carried out in phosphate buffer (100 mM) at pH 7.2.

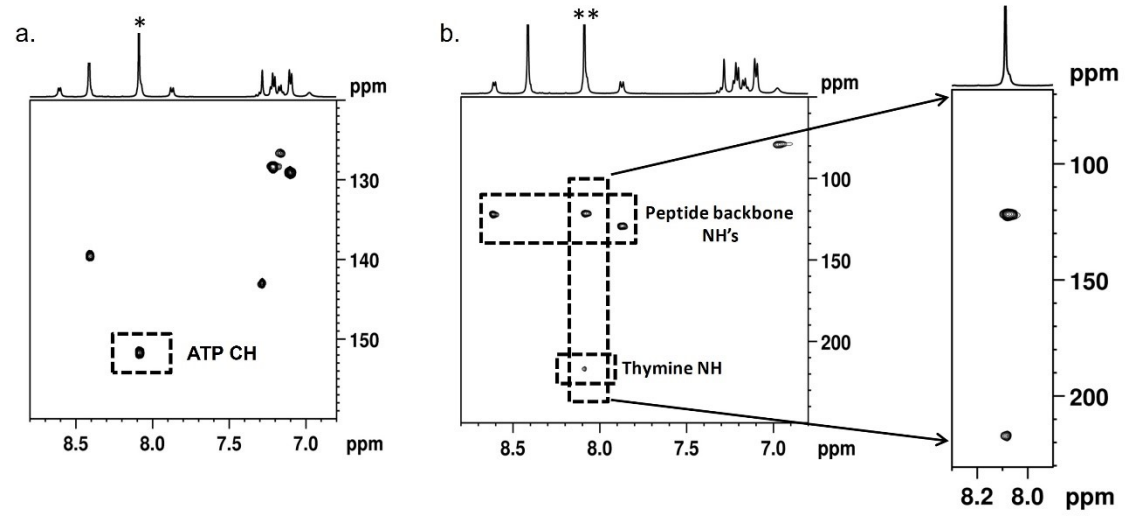

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Fig. S12: a) ^{13}C-HSQC at 5 ºC of **1**-ATP hydrogel showing superimposed spectrum of CH overlapped with -NH Thymine and one -NH of peptide. b) ^{15}N-HSQC at 5 ºC of **1**-ATP hydrogel showing superimposed spectrum of -NH Thymine overlapped with ATP CH and one NH of peptide. Experiments were performed in 10 % D_2O, 90 % H_2O using 36 mM of **1** and 40 mM ATP in 100 mM phosphate buffer at pH 7.2.

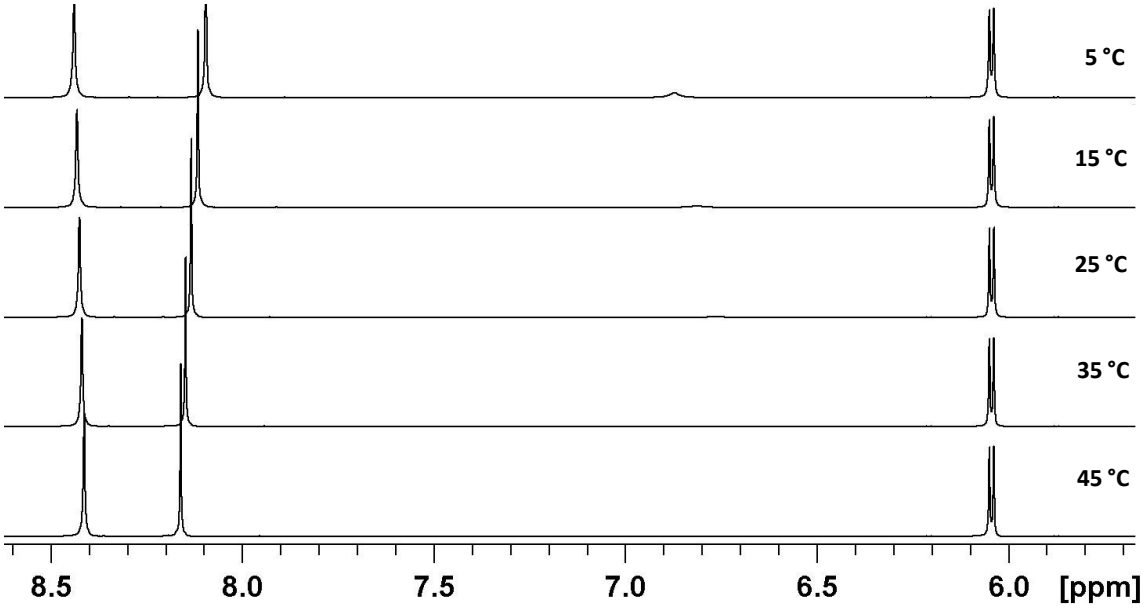

Fig. S13: Variable temperature **(**VT) ^{1}H NMR (10 % D_2O, 90 % H_2O) of 40 mM ATP solution in 100 mM phosphate buffer at pH 7.2.

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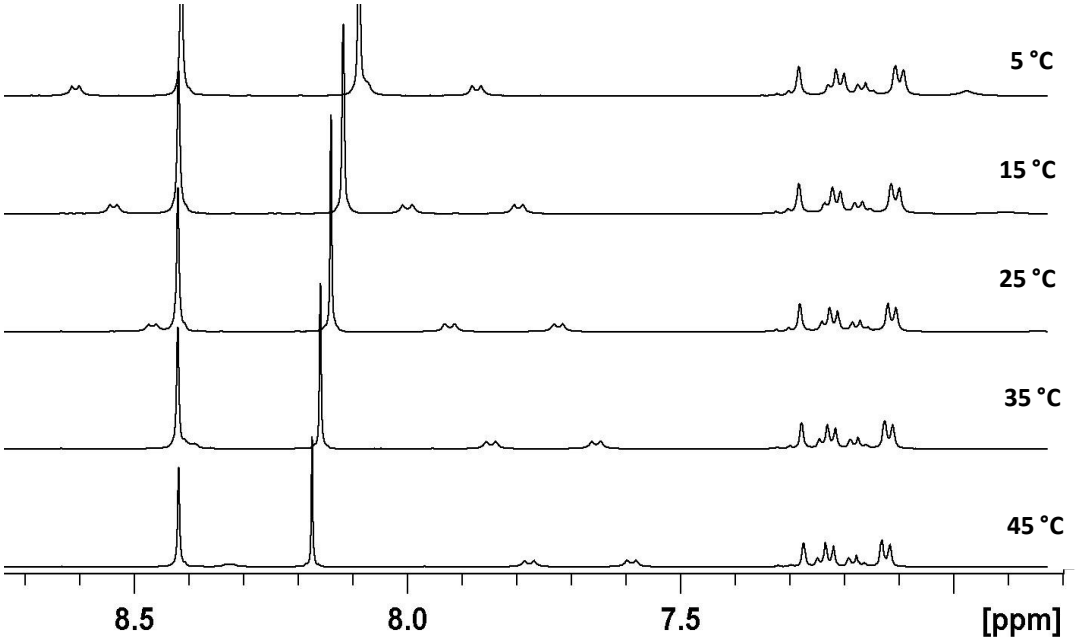

Fig. S14: Variable temperature (VT) ^{1}H NMR (10 % D_2O, 90 % H_2O) of 36 mM of **1** and 40 mM ATP hydrogel in 100 mM phosphate buffer at pH 7.2.

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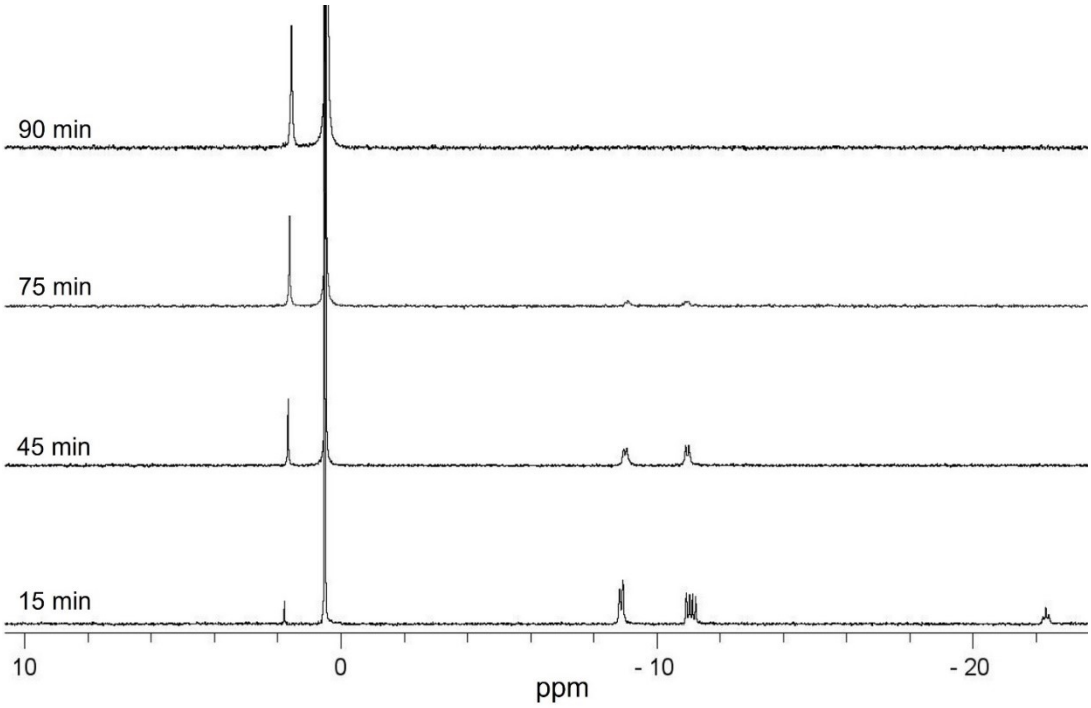

Fig. S15: ^{31}P NMR (10 % D_2O, 90 % H_2O) of ATP hydrolysis by apyrase in 100 mM phosphate buffer at pH 7.2. The experiment showed 40 mM of ATP hydrolyzed within 90 min. using 3U/mL apyrase.

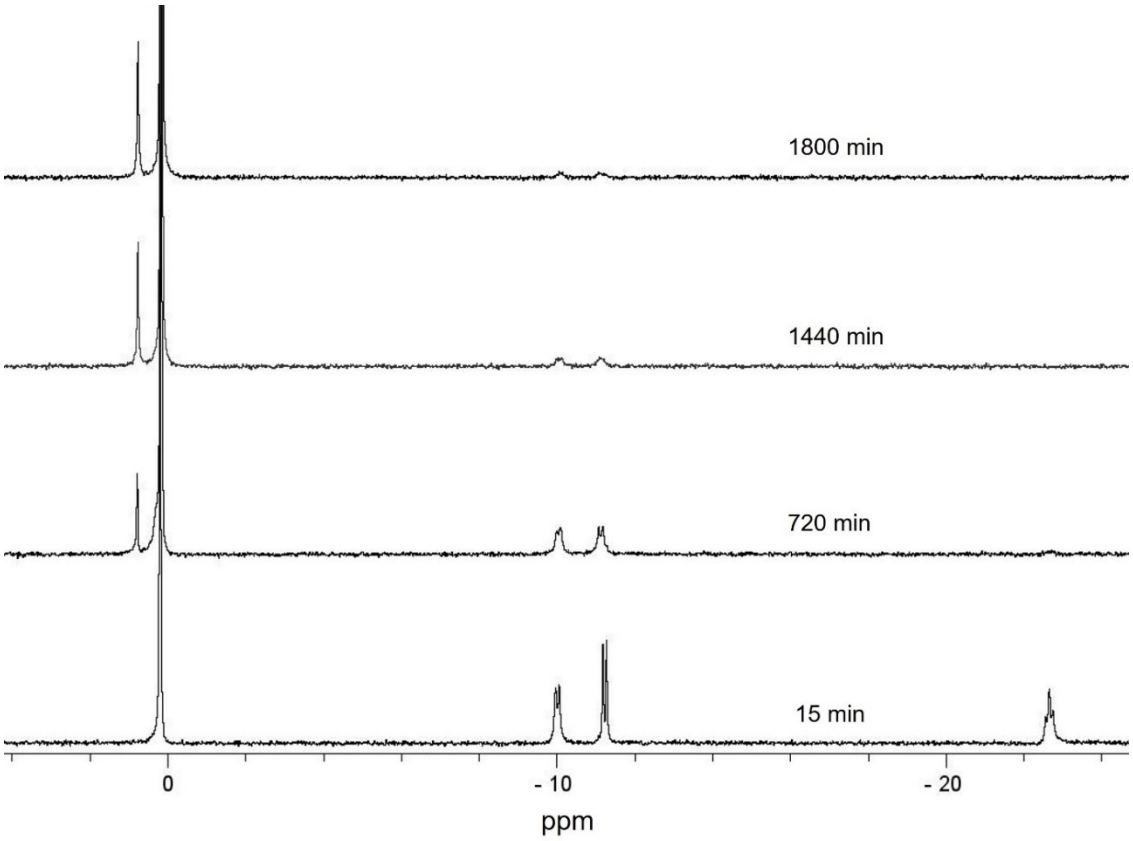

Fig. S16: ^{31}P NMR (10 % D_2O, 90 % H_2O) of 40 mM ATP hydrolysis in a **1**-ATP hydrogel by 1.5 U/mL apyrase in 100 mM phosphate buffer at pH 7.2. The experiment was monitored for 30 hrs.

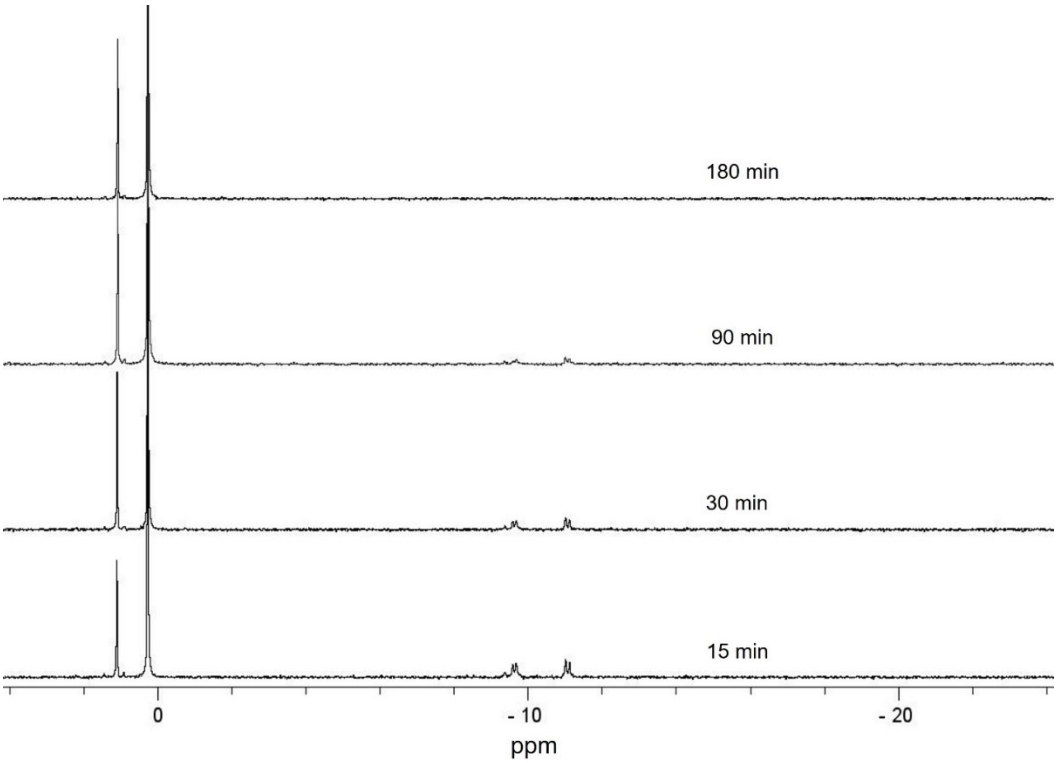

Fig. S17: ^{31}P NMR (10 % D_2O, 90 % H_2O) of ADP hydrolysis in a **1**-ADP hydrogel by 3 U/mL apyrase in 100 mM phosphate buffer at pH 7.2. The experiment showed 40 mM of ADP hydrolyzed within 180 min.

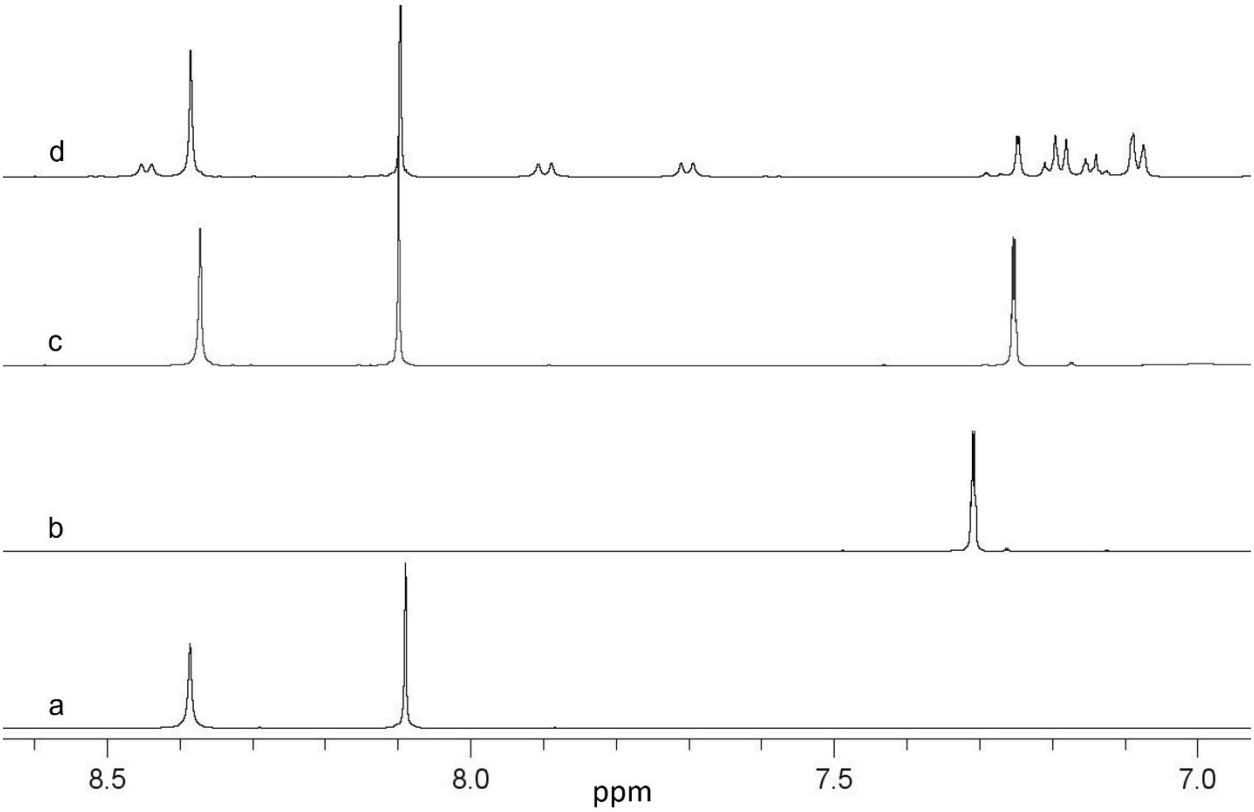

Fig. S18: ^{1}H NMR (10 % D_2O, 90 % H_2O) of a) ATP, b) Thymine-acetic acid, c) ATP-Thymine-acetic acid and d) **1**-ATP hydrogel at 25 ˚C. The Thymine-acetic shows similar peak patterns as **1**-ATP hydrogel, however, does not form hydrogel in same condition, indicates the peptide backbone helps in self-assembly. All experiments recorded in phosphate buffer (100 mM) at pH 7.2.

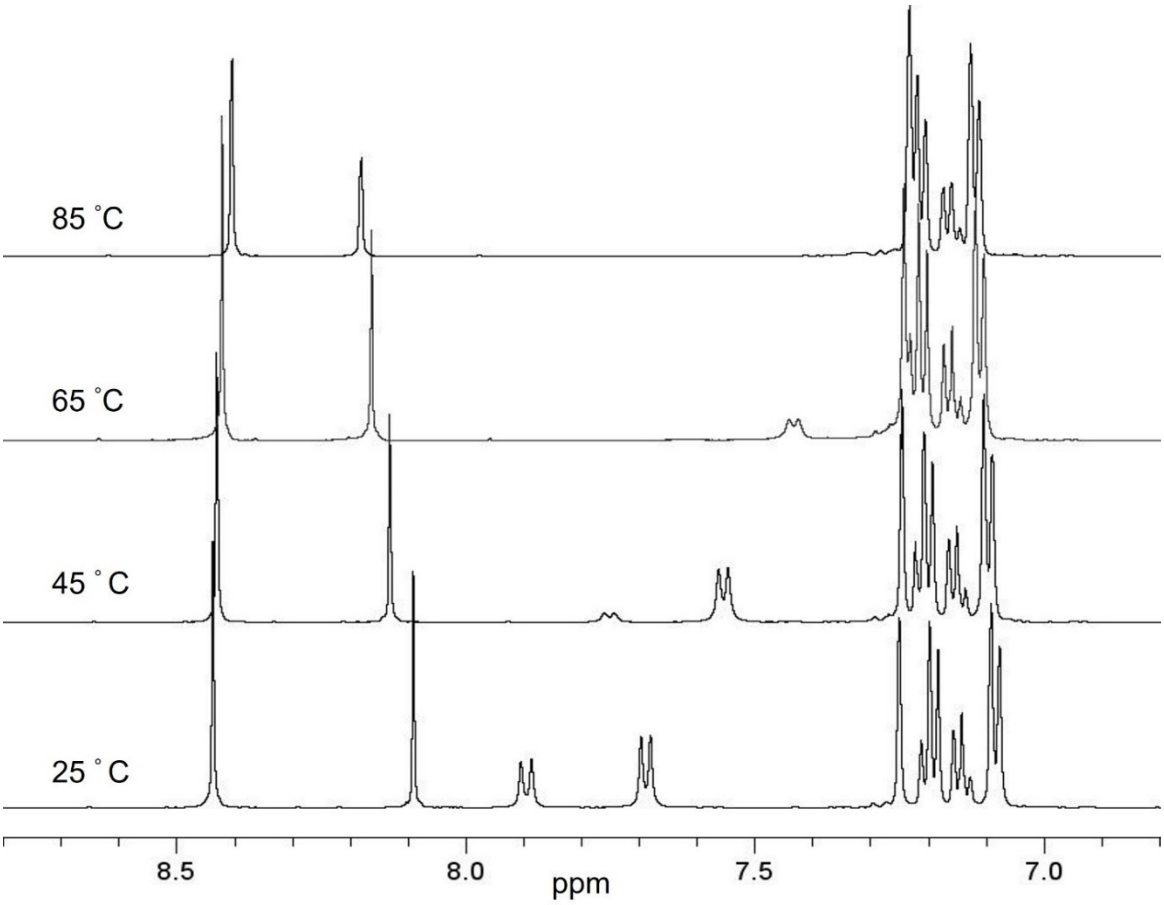

Fig. S19: Variable temperature **(**VT) ^{1}H NMR (10 % D_2O, 90 % H_2O) of 36 mM **1** and 40 mM AMP solution in 100 mM phosphate buffer at pH 7.2.

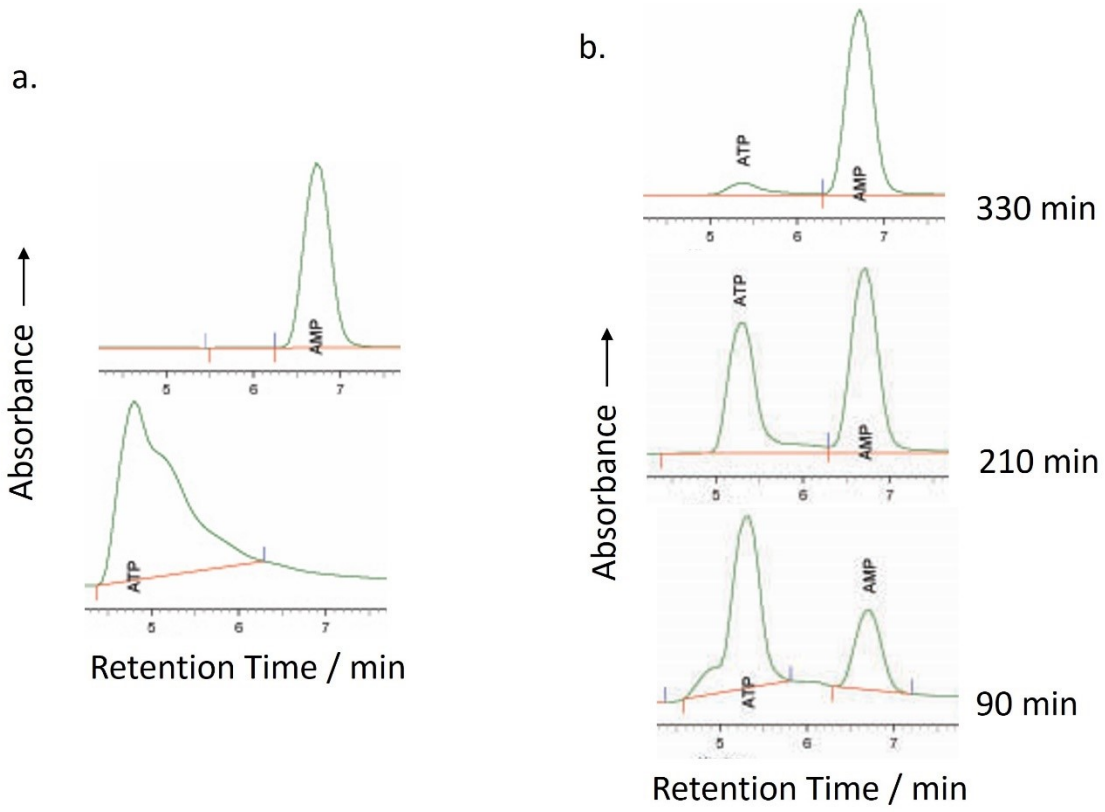

Fig. S20: HPLC chromatogram shows a) standard peaks at retention time 4.9 min and 6.8 min corresponds to ATP and AMP respectively. b) The hydrolysis of ATP by apyrase shows almost 90 % decrease in concentration of ATP after 330 min. Solutions were made in phosphate buffer at pH 7.2 (100 mM). The mobile phase was composed of solvent A (water, 1% TFA) and solvent B (Methanol). The gradient elution program was 10 % solvent B over 10 min at a steady flow rate of 0.5 ml min^{-1}.

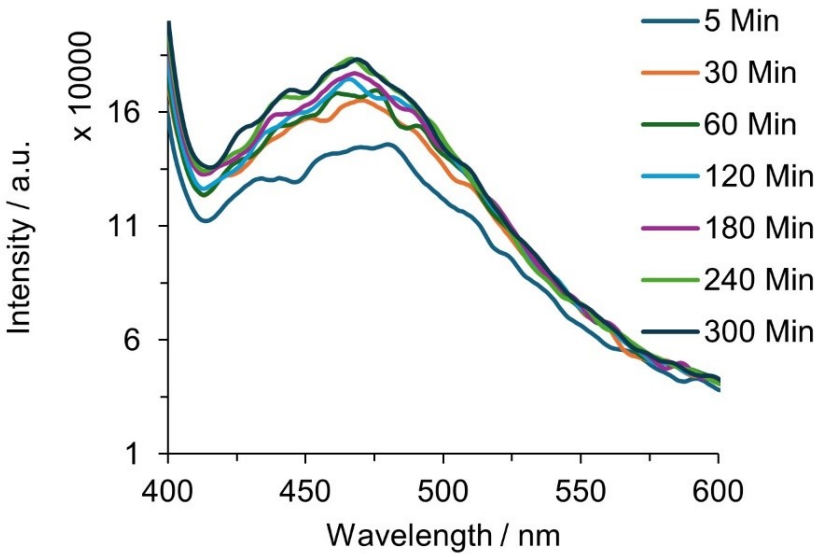

Fig. S21: Emission spectra obtained after addition of apyrase enzyme to the **1**-ATP hydrogel. The spectra show a continuous increase in emissions at 470 nm after addition of apyrase to the hydrogel. The emission was studied by mixing ANS dye as a probe for hydrophobicity. The study carried out in 100 mM phosphate buffer at pH 7.2.

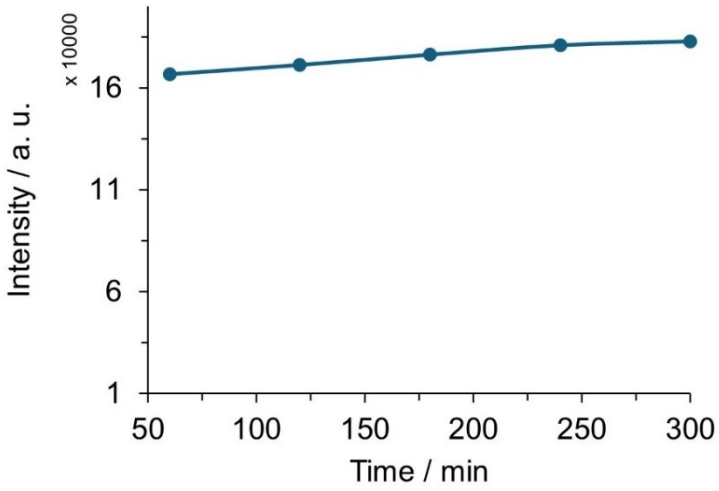

Fig. S22: Emission spectra of the **1**-ATP hydrogel (60 min aged) after addition of apyrase enzyme shows further increase in emission (3U/mL apyrase added to the 30 min aged hydrogel of **1**-ATP)

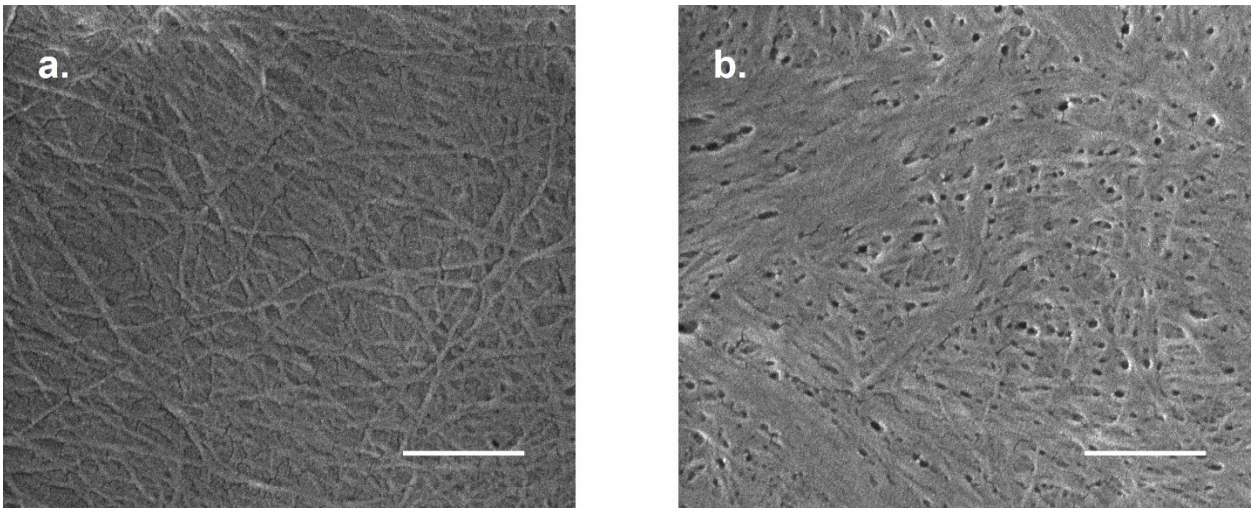

Fig. S23: FE-SEM image of a) **1** and ADP hydrogel shows three dimensional fibrous self-assembly with an average diameter 25 (±4) nm. b) **1**-ADP hydrogel after hydrolysis by apyrase shows a thicker fibrous nano architecture similar what has been observed for 1-ATP hydrogel after hydrolysis. The average diameter after hydrolysis of **1**-ADP hydrogel was 39 (± 8) nm. The scale bar is 500 nm.

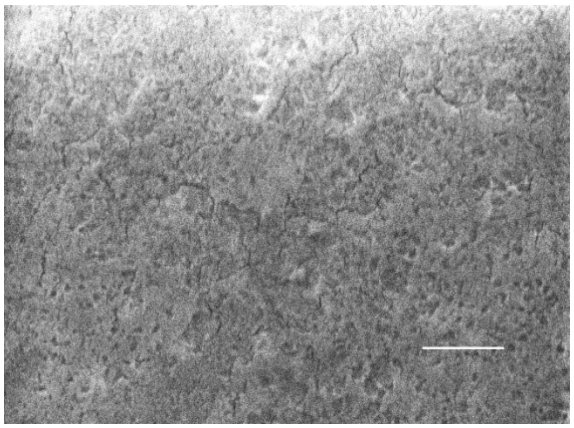

Fig. S24: FE-SEM image of **1** and **AMP** solution (non-hydrogel) shows no characteristic self-assembly. Concentration maintained 36 mM in phosphate buffer (100 mM) at pH 7.2. The scale bar is 500 nm.