Supporting Information

Macrocycle-ATP mediated contraction and expansion of hydrogel actuator

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Table of Contents

Material and Instrumentation

All solvents and reagents were obtained from commercial sources and used as received without further purification unless otherwise specified. ^{1}H NMR, and ^{13}C NMR (400 MHz) spectra were recorded on an Agilent MR400 spectrometer.

Syntheses

Texas-sized box (**TXsb**):

TXsb was synthesized according to previously reported literature procedure.[1]

Polymer Network	*x* (mol%)	*y* (mol%)	*z* (mol%)
Gel0	0.5	99.5	0
Gel1	0.5	49.25	49.25
Gel2	0.5	33.17	66.33
Gel4	0.5	19.9	79.6

Scheme S1: Free radical polymerization of the hydrogels **Gel0**, **Gel1**, **Gel2**, and **Gel4**, with varying ratios of VBA and DMAEMA.

Preparation of **Gel2**:

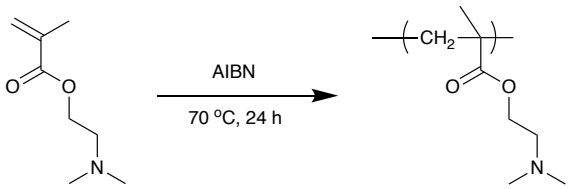
4-Vinyl benzoic acid (6.7 mmol), 2-(dimethylamino)ethyl methacrylate (DMAEMA) (13.3 mmol), and 0.1 mmol bisacrylamide (BAM) were dissolved in 2 mL dimethyl sulfoxide (DMSO). After which 0.1 mmol azobisisobutyronitrile (AIBN) was completely dissolved in the solution. N_2 was bubbled through the solution for 30 minutes, and the solution was

separated into 350 µL aliquots in 2 mL vials and left at 70 °C in an oven for 24 hours. Following this, the glass vials were broken to remove the gels and placed in a deionized (DI) water bath for 3 days to remove unreacted reagents, changing the water every 24 hours. Then the gels were left to air dry for 24 hours and in an oven at 50 °C overnight to dry completely. This provided disk shaped samples of **Gel2** of approximately 1 cm diameter.

Preparation of **Gel0**, **Gel1**, and **Gel4**:

The same procedure as above was followed as for **Gel2**, only adjusting the measurements for the differing y and z mole percentages as shown in the table above.

Polymer **pDMAEMA**:



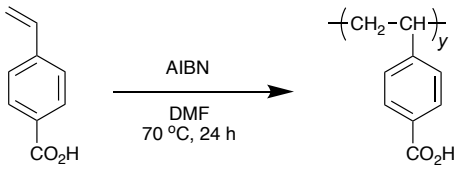
DMAEMA **pDMAEMA**

Preparation:

AIBN (0.0126 mmol) was dissolved in DMAEMA (2.508 mmol) in a scintillation vial and bubbled with N_2 for 30 minutes. The vial was then left in the oven at 70 °C for 24 hours. This provided the polymer **pDMAEMA** as a translucent solid, which was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.05 (bs, 2H), 2.56 (bs, 2H), 2.26-2.35 (bm, 6H), 1.65-2.08 (bm, 2H), 0.75-1.15 (bm, 3H). GPC: M_n = 65 kDa M_w = 124 kDa PDI = 1.90

Polymer **pVBA**:



VBA **pVBA**

Preparation:

VBA (1.254 mmol) and AIBN (0.0126 mmol) were added to a vial and dissolved in 0.2 mL dry DMF. The solution was then bubbled with N_2 for 30 minutes and left in the oven at 70 °C for 24 hours. The resulting viscous solution was completely dissolved in 50 mL methanol and transferred to a round bottom flask, followed by evaporation of volatiles and leaving under high-vacuum overnight. This provided the polymer **pVBA** as a white solid. ^{1}H NMR (400 MHz, MeOD) δ 7.45-7.95 (bm, 2H), 6.31-7.02 (bs, 2H), 1.05-2.39 (bm, 3H). GPC: M_n = 65 kDa M_w = 127 kDa PDI = 1.95

Polymer Characterization

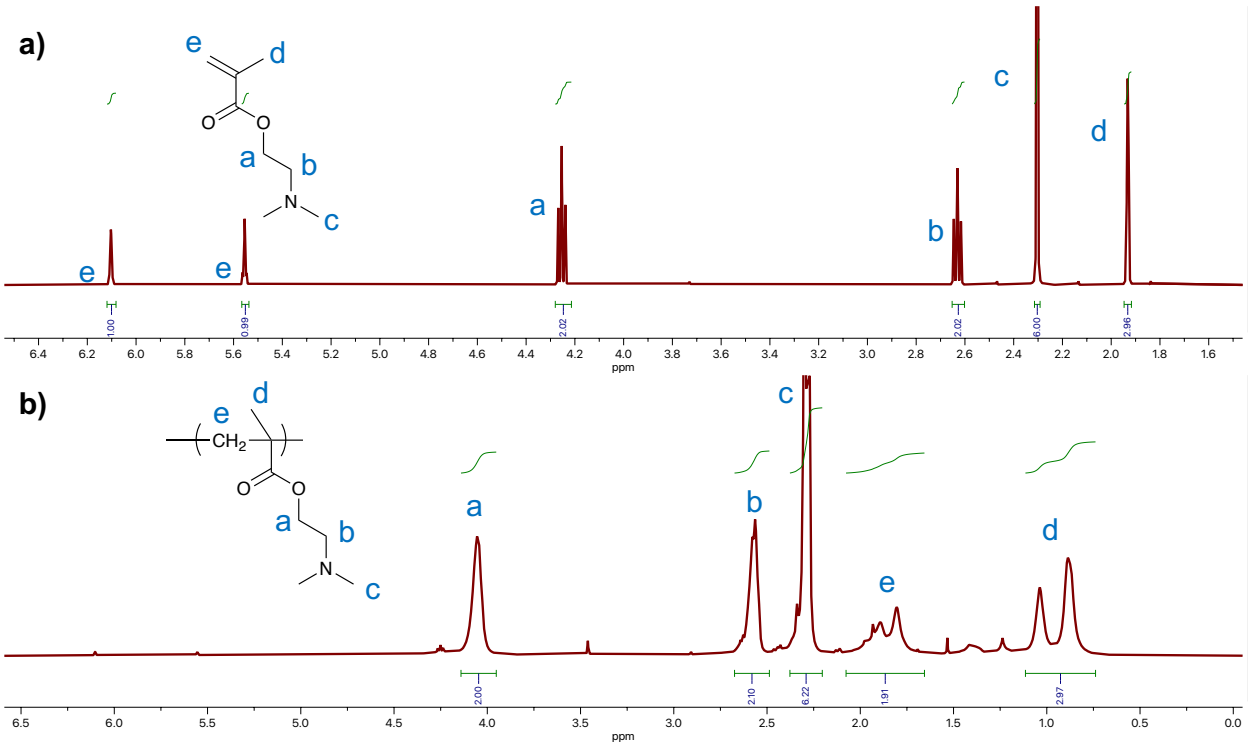


Figure S1. ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K) of: a) monomer 2-(dimethylamino)ethyl methacrylate (DMAEMA) and b) **pDMAEMA**.

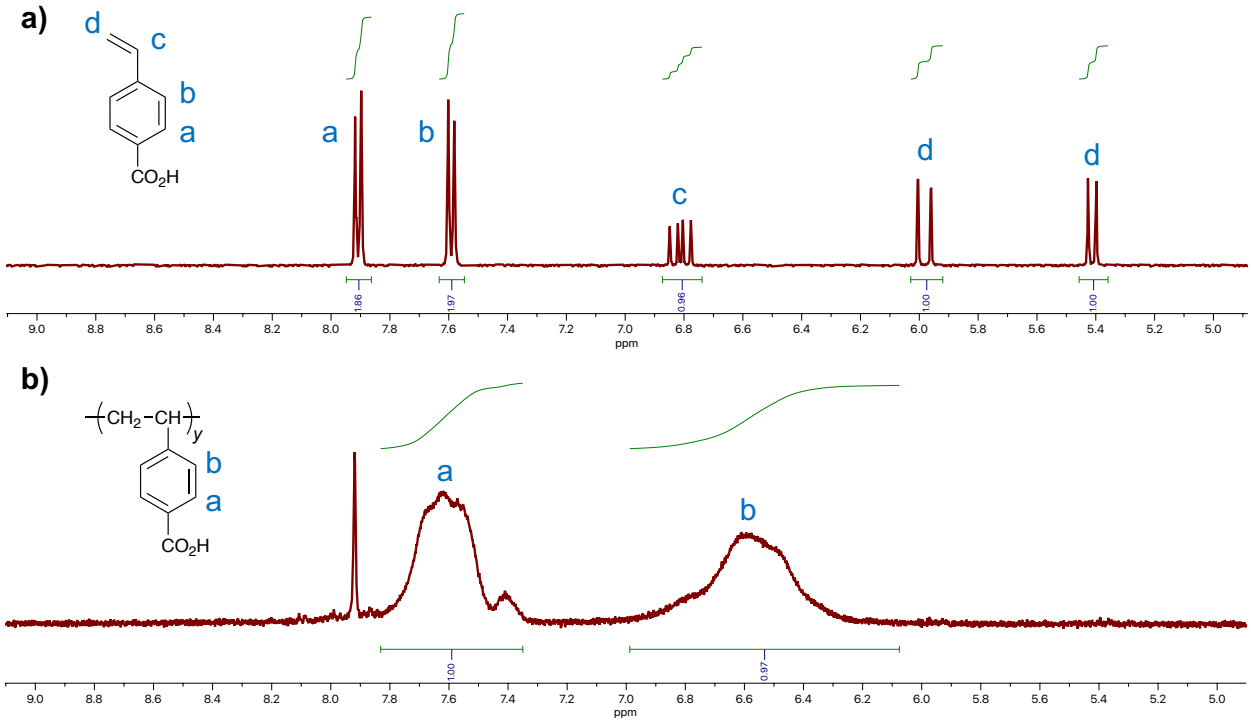


Figure S2. ^{1}H NMR spectra (400 MHz, DMSO-d_6, 298 K) of: a) monomer 4-vinylbenzoic acid (VBA) and b) **pVBA**.

Host-guest interaction studies

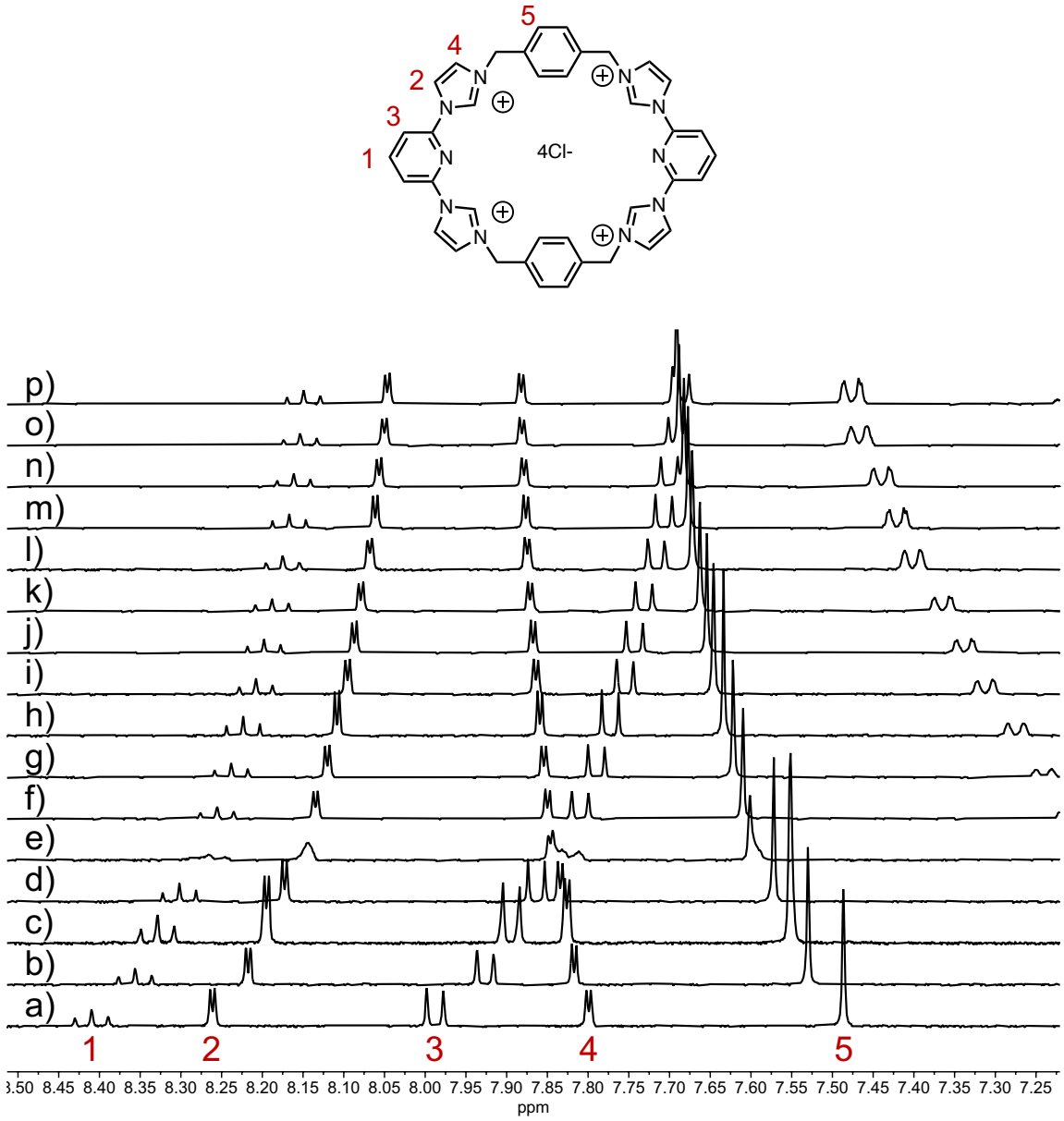


Figure S3. Partial ^{1}H NMR spectra (400 MHz, D_2O, 298 K): of **TXsb** recorded at a concentration of 0.50 mM in the presence of differing concentrations of tetramethylammonium (TMA) benzoate (a) 0.0 mM; (b) 0.1 mM; (c) 0.2 mM; (d) 0.3 mM; (e) 0.4 mM; (f) 0.5 mM; (g) 0.6 mM; (h) 0.7 mM; (i) 0.8 mM; (j) 0.9 mM; (k) 1.0 mM; (l) 1.1 mM; (m) 1.2 mM; (n) 1.3 mM; (o) 1.4 mM; (p) 1.5 mM.

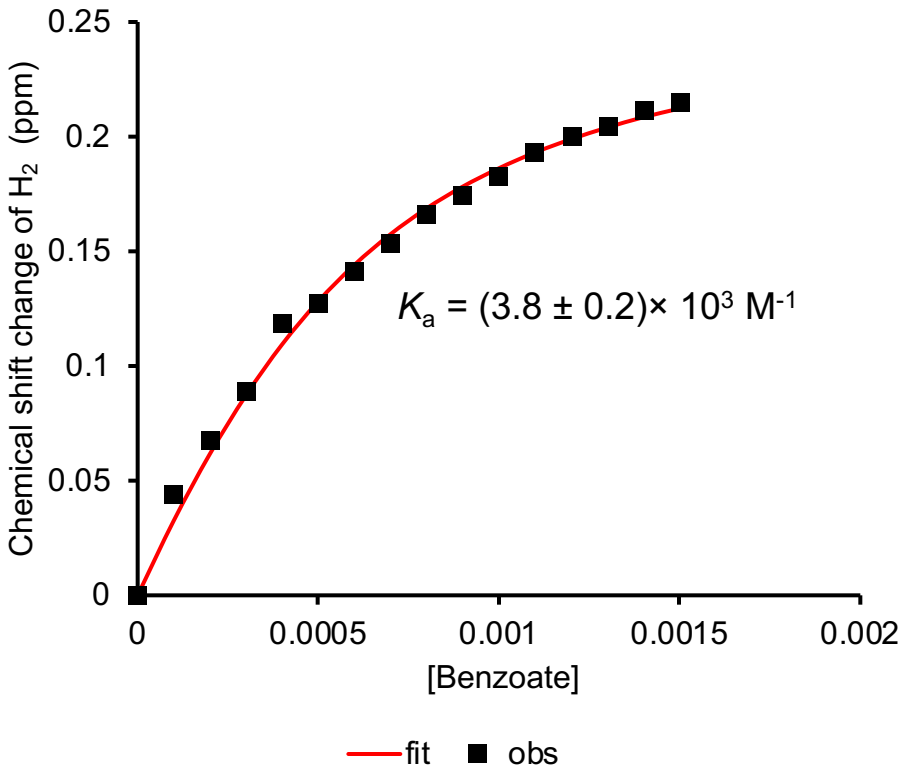


Figure S4. Changes in the chemical shift corresponding to H_2 on **TXsb** as a function of added tetramethylammonium benzoate. Non-linear curve-fitting was obtained with the online fitting tool Bindfit[2] on supramolecular.org in accord with the 1:1 Nelder-Mead method.

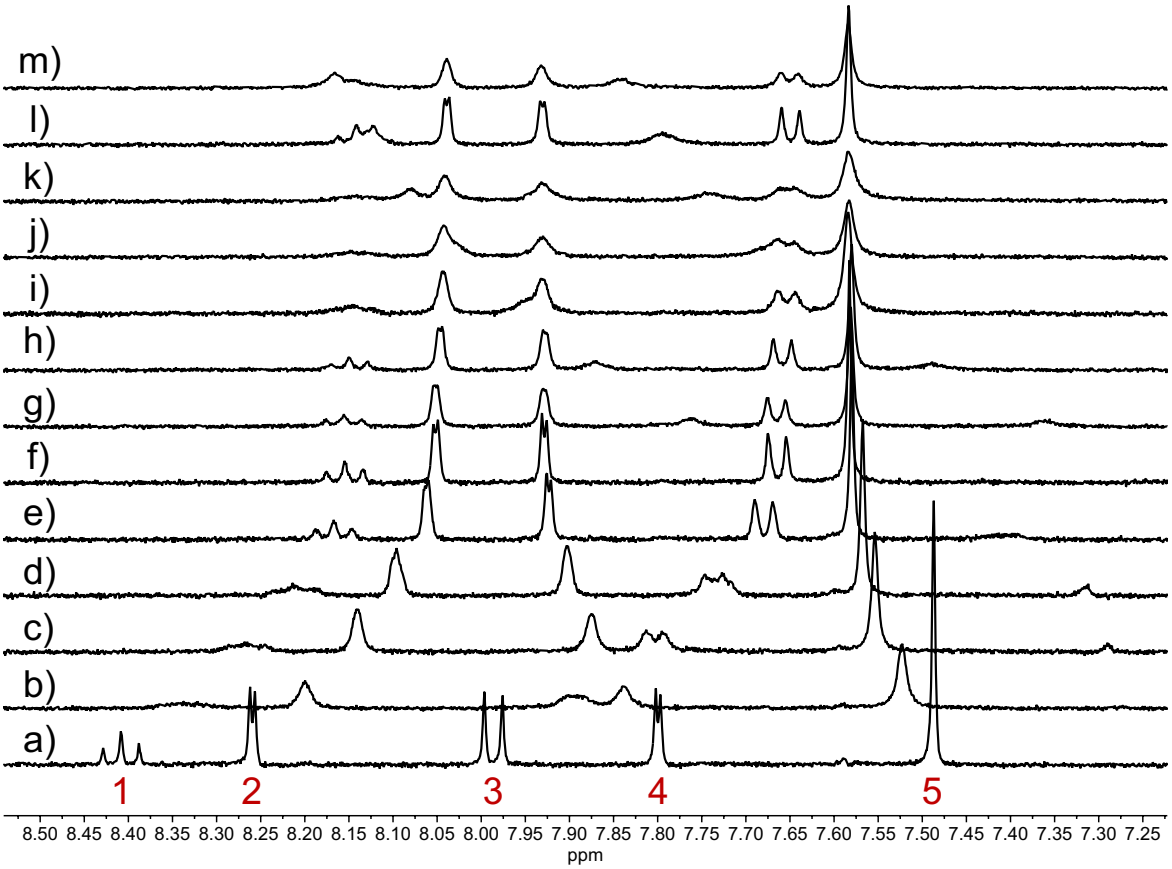


Figure S5. Partial ^{1}H NMR spectra (400 MHz, D_2O, 298 K): of **TXsb** recorded at a concentration of 0.50 mM in the presence of differing concentrations of ATP disodium salt (a) 0.0 mM; (b) 0.12 mM; (c) 0.24 mM; (d) 0.37 mM; (e) 0.48 mM; (f) 0.6 mM; (g) 0.73 mM; (h) 0.85 mM; (i) 0.99 mM; (j) 1.1 mM; (k) 1.25 mM; (l) 1.4 mM; (m) 1.5 mM

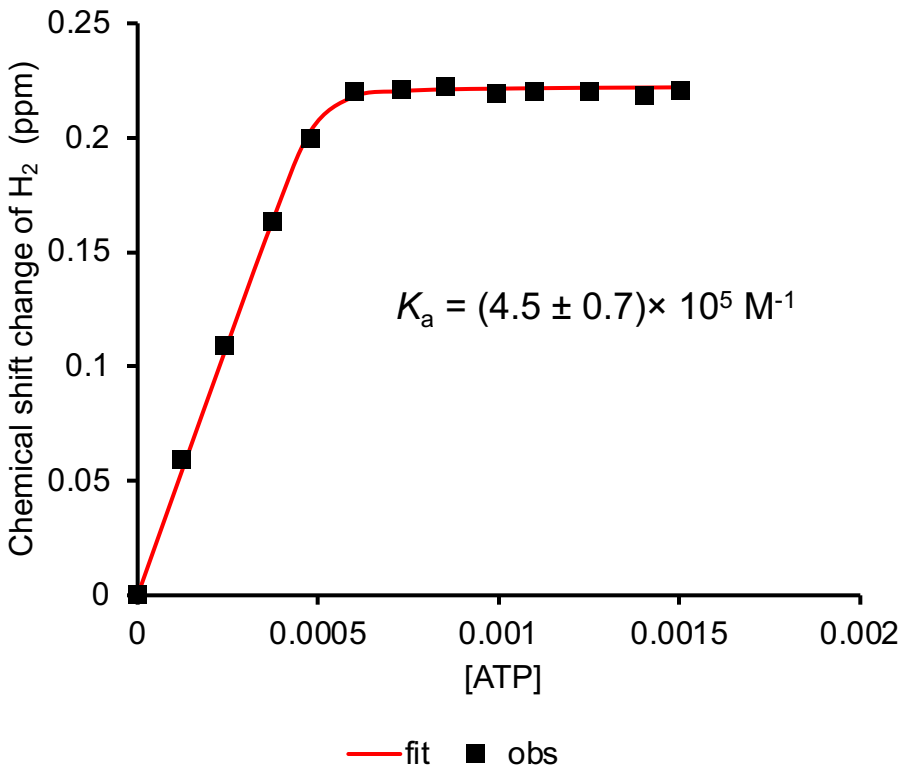


Figure S6. Changes in the chemical shift corresponding to H_2 on **TXsb** as a function of added ATP. Non-linear curve-fitting was carried out using the online fitting tool Bindfit on supramolecular.org in accord with the 1:1 Nelder-Mead method.

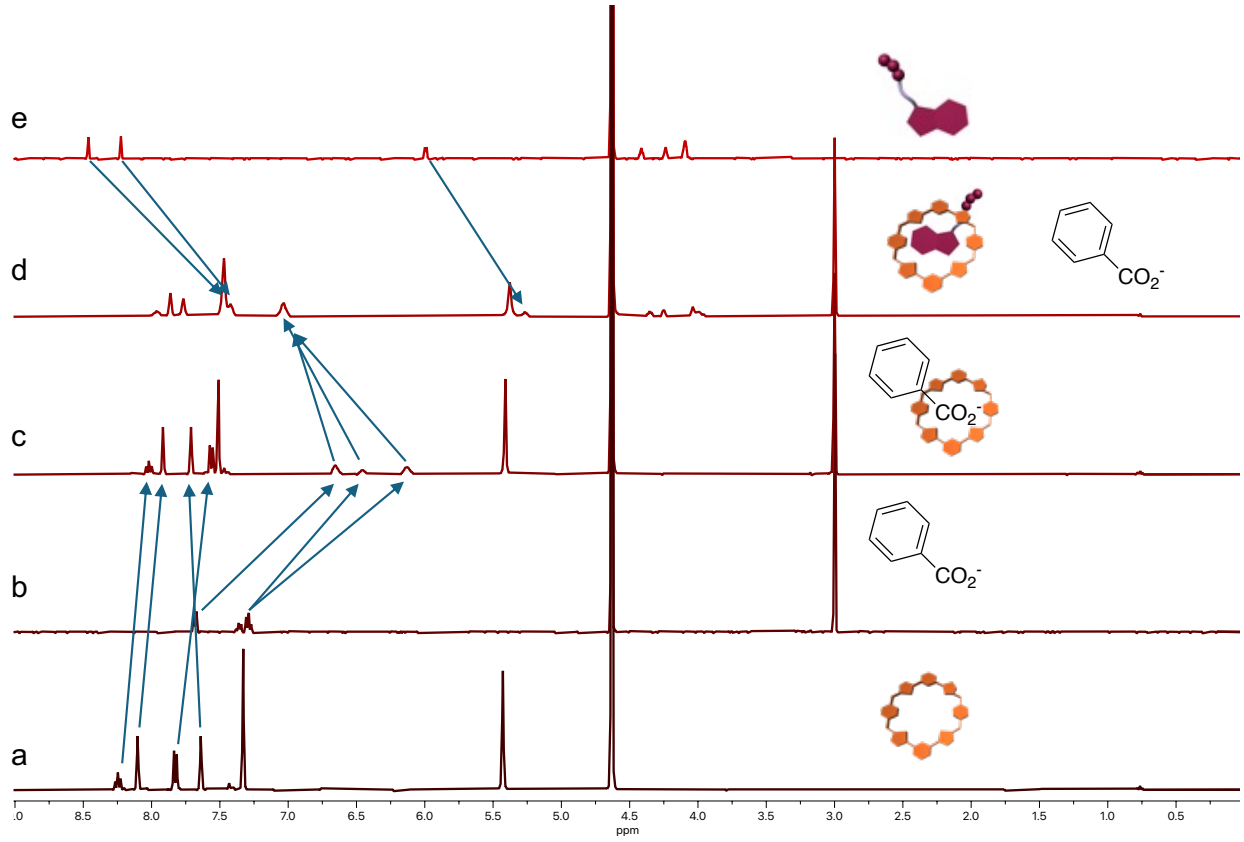


Figure S7. ^{1}H NMR spectra (500 MHz, D_2O, 298 K) of (a) 3.00 mM **TXsb**; (b), 3.00 mM benzoate; (c) **TXsb** and benzoate (3.00 mM each); (d) **TXsb**, benzoate, and ATP; (e) 3.00 mM ATP.

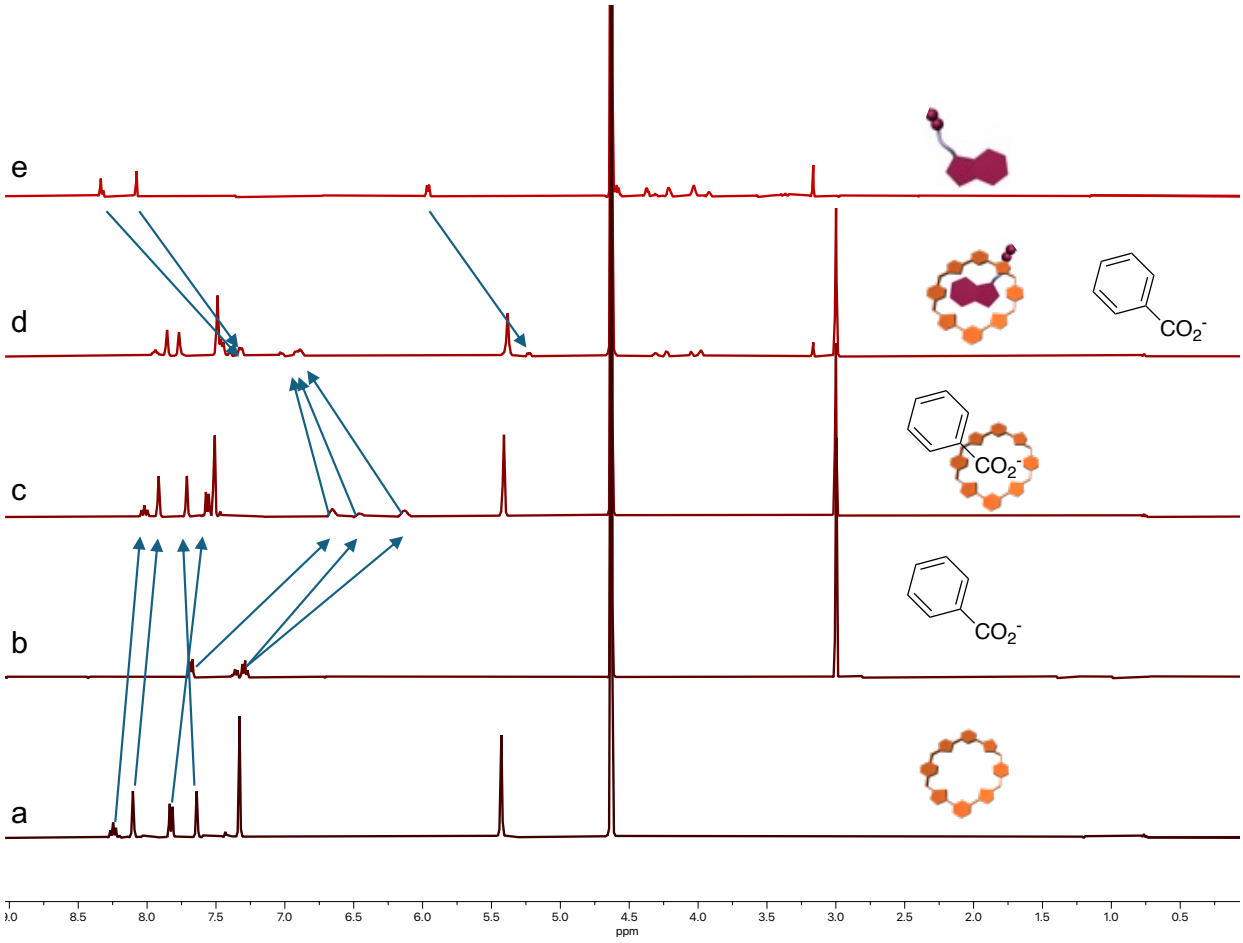


Figure S8. ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of (a) 3.00 mM **TXsb**; (b), 3.00 mM TMA benzoate; (c) **TXsb** and benzoate (3.00 mM each); (d) **TXsb**, benzoate, and ADP; (e) 3.00 mM ADP.

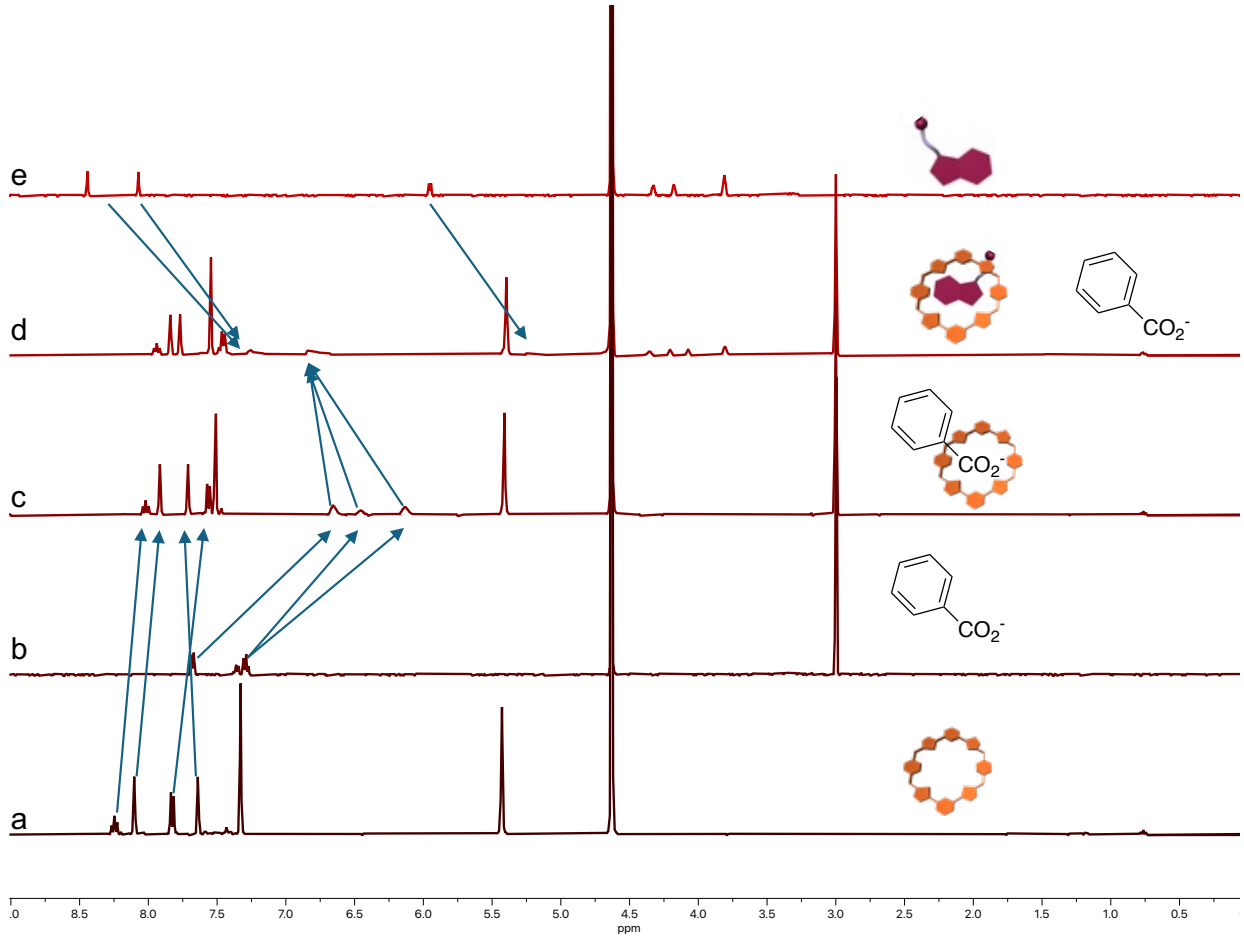


Figure S9. ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of (a) 3.00 mM **TXsb**; (b), 3.00 mM TMA benzoate; (c) **TXsb** and TMA benzoate (3.00 mM each); (d) **TXsb**, TMA benzoate, and AMP; (e) 3.00 mM AMP.

Swelling Studies

Gels were rehydrated in 20 mL DI water and weight measurements were taken every 24 hours until the gels reached equilibrium (2 days in water). This was taken as the baseline state of the gels. These hydrated gels were then placed in 20 mL solutions of 200 μM **TXsb** in water. The gels reached equilibrium in 4 days. Following this, the gels were transferred to 20 mL solutions of 200 μM ATP, ADP, AMP, or P_i. These studies required 2 days to reach equilibrium. For the ATPase enzyme studies, Apyrase from potatoes was used from Sigma-Aldrich containing ~3.0 units/mg protein. 1 unit of Apyrase was added to solutions of **Gel2** in ATP. Equilibrium was reached at 2 days. Swelling ratios were determined by the following equation.

Equation 1:

$$Swelling\ ratio = \frac{W_s - W_d}{W_d}$$

Where W_s = starting weight, and W_d = dry weight

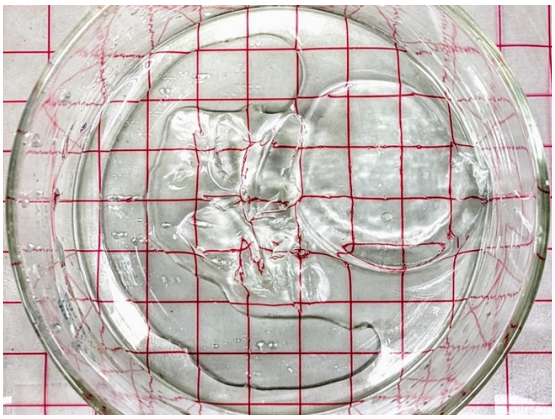


Figure S10. Swelling of **Gel2** observed after the addition of an ATPase enzyme (Apyrase) leading to gel breakdown. 1 cm x 1 cm grid for scale.

SEM Imaging

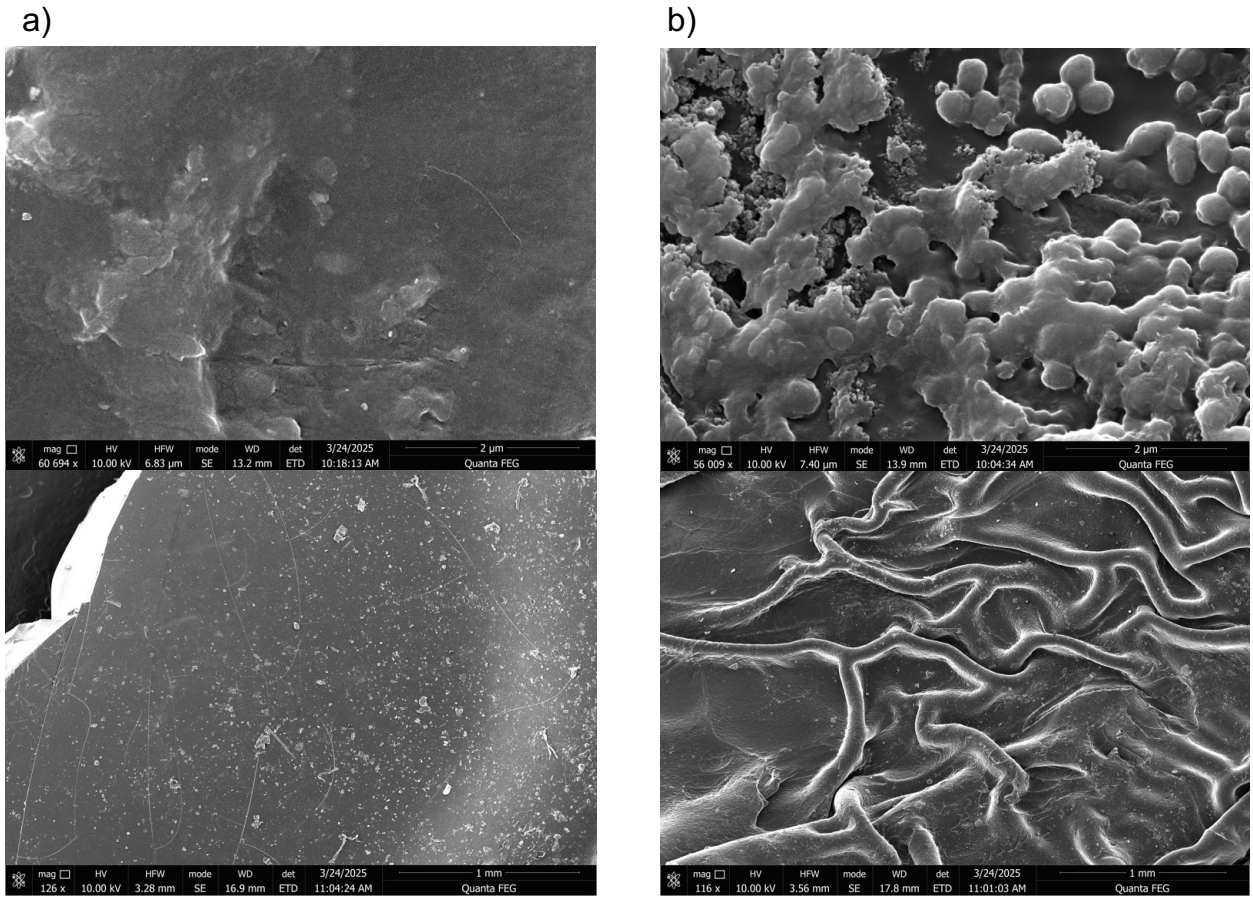


Figure S11. SEM micrographs of **Gel2** in the presence of (a) ATP and (b) **TXsb**

Rheological Testing

Storage modulus (G') and loss modulus (G'') of **Gel2** in various swelling states were characterized using a TA Instruments Discovery Hybrid Rheometer (DHR 20). An 8 mm parallel steel plate geometry was employed with a stainless-steel Peltier plate base. A strain sweep was performed at a frequency of 10 rad/s under an axial force of 0.5 N, with strain ranging from 0.1% to 10%, to identify the linear viscoelastic region. Subsequently, a frequency sweep was conducted from 1 rad/s to 100 rad/s within the linear viscoelastic region at a strain of 0.1% or 1%.

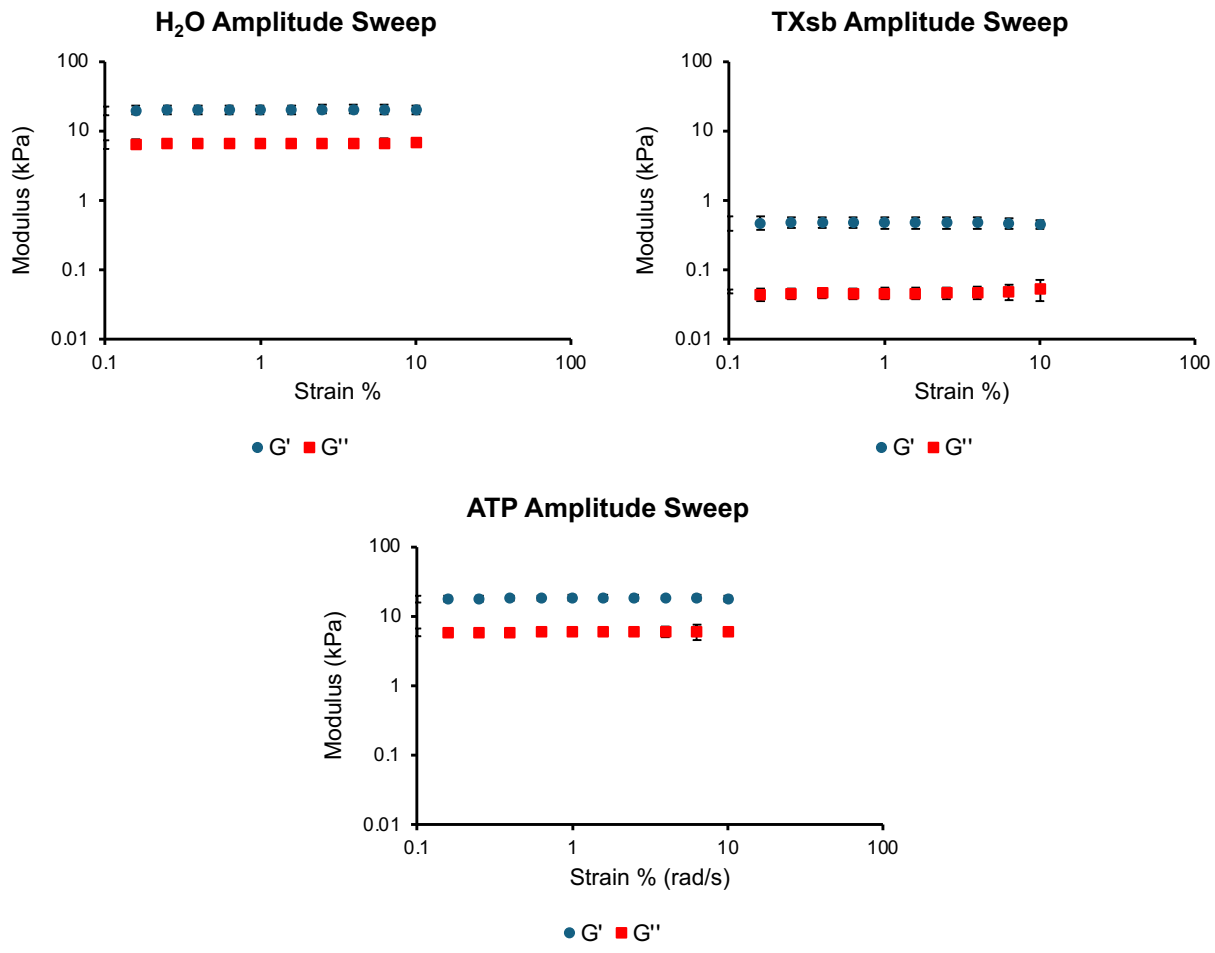


Figure S12. Amplitude sweep rheograms of **GelI2** in H_2O and in the presence of **TXsb** and ATP.

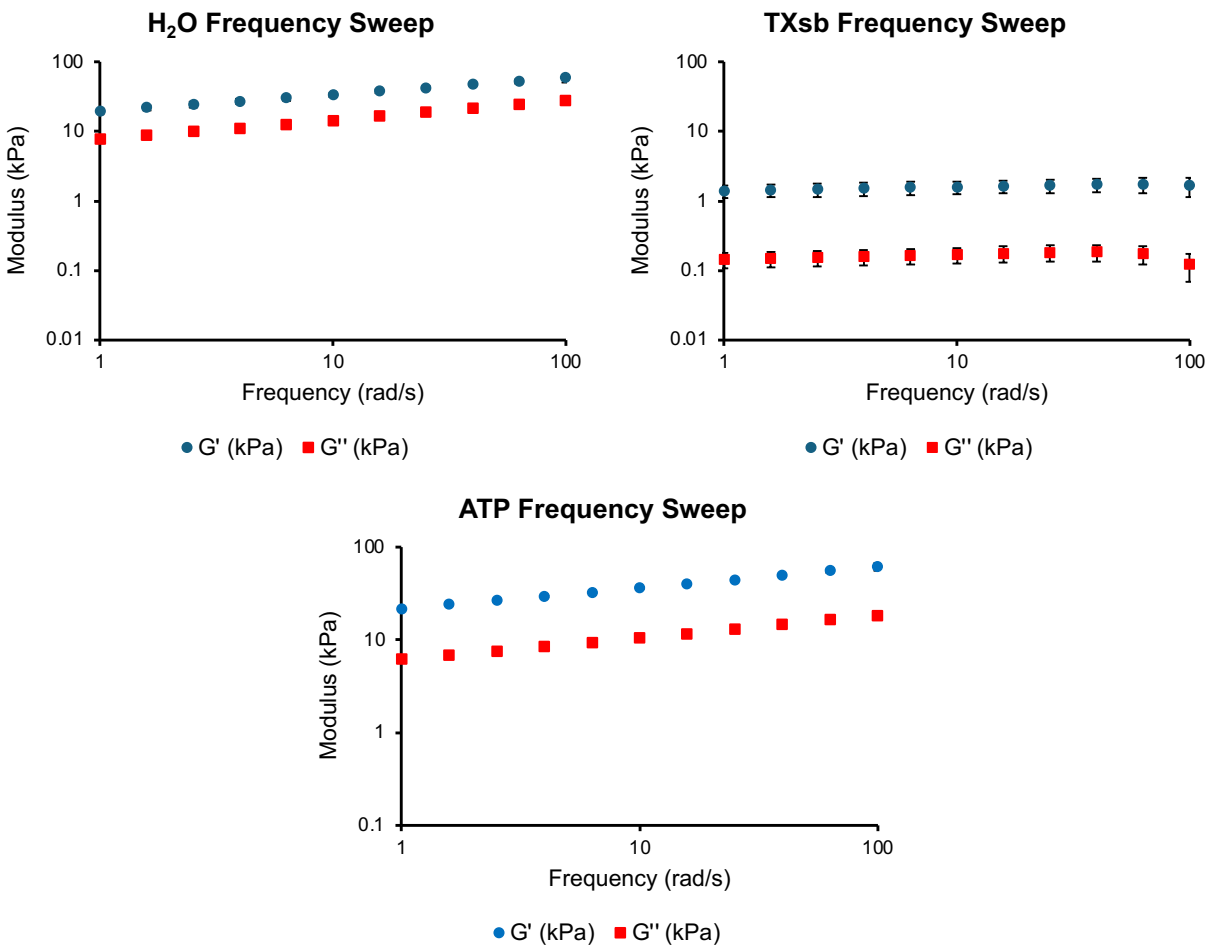


Figure S13. Frequency sweep rheograms of **Gel2** in H_2O and in the presence of **TXsb**, and ATP.

Supporting References

1. H.-Y. Gong, B. M. Rambo, E. Karnas, V. M. Lynch and J. L. Sessler, *Nat. Chem.*, 2010, **2**, 406
2. P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305.