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Macrocycle-ATP mediated contraction and expansion of a hydrogel actuator†

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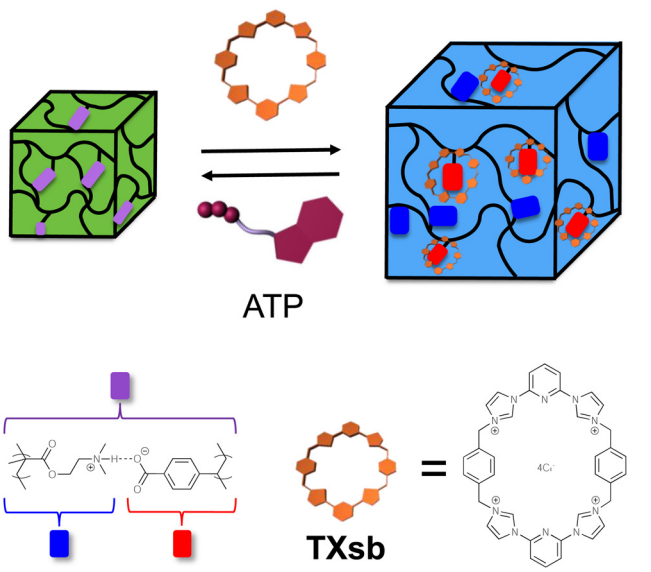
Hydrogel actuators that respond to specific environmental stimuli are attracting increasing interest. Reported here is a hydrogel actuator system that is selectively responsive to adenosine triphosphate (ATP) over its hydrolysis products.

Hydrogels are a class of hydrophilic three-dimensional polymer networks that have been studied extensively in recent years for applications in bioscience and material science.[1–3] Their hydrophilic nature and porosity allow hydrogels to absorb and retain large amounts of water. This gives rise to shape and volume changes as water absorption causes swelling of the hydrogels, while water release leads to shrinking. This property has led to the development of hydrogel actuators, a class of hydrogels that convert the chemical energy of intermolecular and intramolecular interactions into motion *via* swelling and/or deswelling. In favourable cases, the resulting physical changes can elicit work-like functions. Appropriately designed hydrogel actuators can thus be viewed as rudimentary mimics of contracting and extending muscles. To date, a number of chemical stimuli have been used as the triggers to promote actuator behaviour. These include electricity, light, magnetic field, pH, redox, solvent change, and temperature, and supramolecular host–guest interactions.[4–10] Collectively, these have allowed a wide variety of applications, such as drug delivery, self-healing, and adhesive materials to be pursued.[11–14] These advances notwithstanding, there are few examples where biologically relevant species are used as the "fuels" to drive muscle-like elongation and shrinking behaviour.

Here we show that the tetra-cationic macrocycle "Texas-sized box" (**TXsb**) developed in the Sessler group in 2010[15] can act as a key component in a hydrogel actuator that responds to adenosine triphosphate (ATP). The system is based on an ionically cross-linked hydrogel that contains benzoic acid and tertiary amine groups tethered to the polymer backbone. Controlled expansion and contraction are then mediated by binding competition between benzoate anions and ATP to the **TXsb** contained within the gel (Scheme 1). Binding of **TXsb** to the benzoate units in the gel in water releases the ionic crosslinks and increases the number of free quaternary amine moieties. This increases the hydrophilicity of the gel, causing the gel to swell as its capacity to hold water increases. Competitive binding of ATP to **TXsb** reverses this expansion since the **TXsb** binds the triphosphorylated nucleotide more favourably than the benzoate groups in the hydrogel. This allows the ionic crosslinks on the hydrogel backbone to reform, thus returning the gel to its contracted state. Applied in this way, ATP induces contraction analogous to what is seen in a muscle. To our knowledge this is the first example of an ATP-macrocycle mediated actuator.



Scheme 1 Illustration showing macrocycle-ATP responsive swelling of the hydrogels of this study in aqueous media.

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The key design hypothesis underlying our approach to creating an ATP responsive actuator was that the triphosphate moiety, being highly charged, would out compete the benzoate functionality present in a hydrogel for the cationic **TXsb**, and the presence or absence of this competitive binding would control the degree of hydrogel crosslinking and resulting degree of swelling. With this vision in mind, the crosslinked hydrogel shown in Scheme S1 (ESI†) was designed. It was prepared *via* the azobisisobutyronitrile (AIBN) initiated radical polymerization of 2-(dimethylamine)ethyl methacrylate (DMAEMA), 4-vinyl benzoic acid (VBA) and *N*,*N*′-methylenebisacrylamide (BAm) using varying ratios of DMAEMA : VBA. The mole percent of covalent crosslinker BAm was limited to 0.5%, while the hydrogen bonding between the benzoic acid and the basic tertiary amine units of DMAEMA act as additional non-covalent crosslinks. To characterize the polymerization and confirm non-covalent crosslinking, linear polymers of VBA and DMAEMA (**pVBA** and **pDMAEMA**) lacking the covalent crosslinking of the polymer chains were prepared as model systems. They were synthesized in a similar fashion to the gels *via* radical polymerization initiated by AIBN.

Fig. S1 and S2 (ESI†) present the ^{1}H NMR spectra of both monomeric and polymeric forms of VBA and DMAEMA. The broadening of the peaks in the spectra of the polymer along with the disappearance of the vinyl protons of the monomers are taken as evidence that high molecular weight macromolecules were formed. The number average molecular weight (M_n) for both linear polymers **pDMAEMA** and **pVBA** was found to be 65 kDa, as determined by gel permeation chromatography (GPC) (Fig. S4 and S5, ESI†). Combining DMSO solutions of **pDMAEMA** and **pVBA** resulted in instant gelation as shown in Fig. 1.

The ratio of DMAEMA and VBA in the gel composition was found to define the degree of swelling in water, as well as the swelling response in the presence of **TXsb** (Fig. 2). Gels were placed in water for two days to reach equilibrium, and weighed to calculate the swelling ratio according to the following equation:

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

where W_s is the swollen gel weight and W_d is the dry gel weight. The gels were then placed in 200 μM solutions of **TXsb** macrocycle and allowed to stand for 4 days to reach equilibrium. Each swelling study was done with batches of 10 gels. **Gel0**, composed of purely VBA, underwent little to no swelling, a finding ascribed to its hydrophobic nature. **Gel1** contains equal parts DMAEMA and VBA. It was found that the swelling response was largely restricted in the

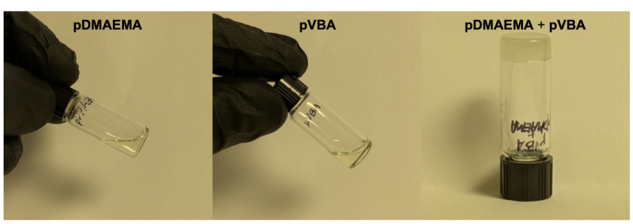
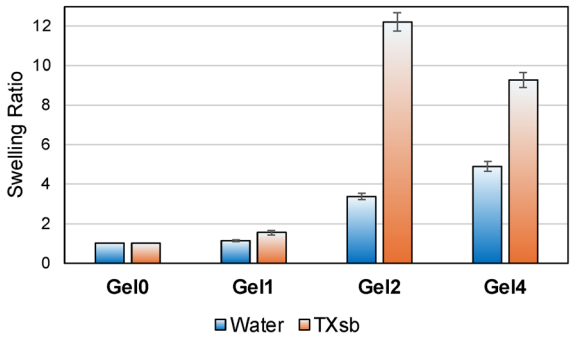


Fig. 1 Photos showing from left to right DMAEMA, VBA, and the gel formed when **pDMAEMA** and **pVBA** are dissolved in DMSO and combined.



Polymer Network	VBA (mol%)	DMAEMA (mol%)	DMAEMA:VBA
Gel0	99.5	0	0
Gel1	49.25	49.25	1
Gel2	33.17	66.33	2
Gel4	19.9	79.6	4

Fig. 2 Hydrogel swelling ratios in water and in the presence of **TXsb** macrocycle and gel composition ratios.

case of this gel. This could reflect the fact that the bulk of the DMAEMA units are involved in the non-covalent crosslinking of the polymeric framework, resulting in a material largely resilient to swelling. To address this issue, **Gel2** and **Gel4** were synthesized to maintain an excess of amine units on the polymer backbone. **Gel2**, with a 2 : 1 ratio of DMAEMA : VBA, was found to have the greatest swelling ratio increase from water upon treatment with **TXsb**, making it the preferred candidate for developing an actuator system. **Gel4** underwent the largest degree of initial swelling in water, presumably reflecting the substantial number of free hydrophilic amine moieties. On the other hand, **Gel4** only underwent a two-fold increase in size in the presence of **TXsb**. This is consistent with the lower ratio of benzoate groups and a reduction in the level of host–guest interactions.

Tetramethylammonium (TMA) benzoate was chosen as a model substrate to investigate the macrocycle/anion host–guest interactions in water using proton NMR spectroscopy. In the spectra (Fig. S3, ESI†), the chemical shifts of the protons on both **TXsb** and TMA benzoate can be observed, confirming the host–guest complexation of **TXsb** and the benzoate anion, with a binding constant (K_a) value of $(3.8 \pm 0.2) \times 10^3$ M^{-1} (Fig. S4, ESI†). The host–guest binding properties of **TXsb** and a wide array of aryl-carboxylate hosts, including benzoate, have been reported by our group.[16] Similarly, the host–guest binding interactions of **TXsb** and ATP were confirmed by an NMR spectroscopic titration experiment (Fig. S5 and S6, ESI†). Dramatic shifts in the **TXsb** protons are taken as evidence of **TXsb**-ATP host–guest interaction. The corresponding binding constant (K_a) was determined to be $(4.5 \pm 0.7) \times 10^5$ M^{-1}, a value two orders of magnitude higher than recorded for benzoate. To test further the competition between ATP and benzoate for the **TXsb**, a proton NMR spectroscopy experiment was carried out. To a mixture of equal amounts **TXsb** and benzoate, 1 equivalent of ATP was added. As can be seen in Fig. 3, treating **TXsb** with benzoate induced a notable upfield shift in the benzoate proton signals. However, after the addition of ATP, the same proton

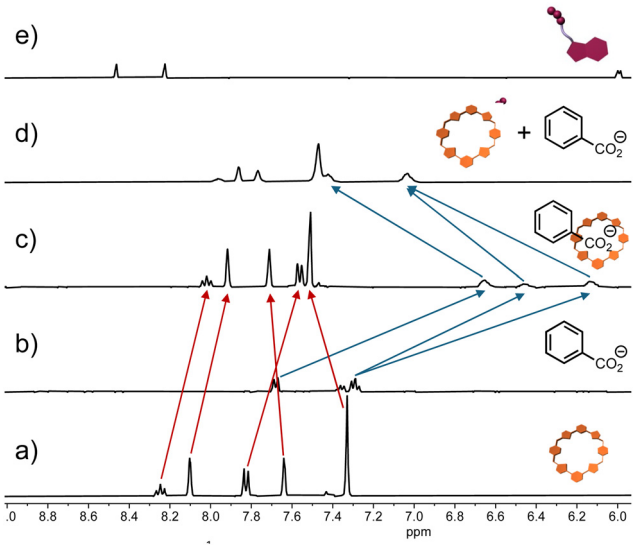


Fig. 3 Top: Partial ^{1}H NMR spectra (400 MHz, D_2O, 298 K): (a) 3.00 mM **TXsb**; (b) 3.00 mM benzoate; (c) **TXsb** and benzoate (3.00 mM each); (d) **TXsb**, benzoate, and ATP (3.00 mM each); (e) ATP.

signals shift back downfield to the values seen for the uncomplexed form. Based on these results, it was expected that hydrogel expansion induced by **TXsb**–benzoate complexation could be reversed by ATP and that this competition would lead to shrinking of the gel.

In metabolic processes, such as muscle contraction, ATP is consumed by being hydrolysed and converted to adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and inorganic phosphate (P_i). AMP and ADP contain 1 and 2 anionic phosphate groups, respectively. Although they possess fewer anionic charges than ATP, their ability to outcompete benzoate in interacting with **TXsb** was tested. In analogy to what was seen for ATP, ADP and AMP were found to displace effectively benzoate (Fig. S7–S9, ESI†). However, ADP and AMP caused less of a downfield shift in the benzoate proton signals than ATP. Upon addition of equal amounts of ATP, ADP and AMP, the benzoate protons shift 0.87, 0.73 and 0.62 ppm, respectively. This leads us to suggest that ATP would be more effective than ADP or AMP in promoting the contraction of the gels.

After maximum swelling was achieved with **TXsb**, samples of **Gel2** were placed in solutions containing 200 μM aqueous ATP, ADP, and AMP, and P_i, respectively. Of these, only ATP caused a significant deswelling response, as reflected in a shrinking of the gels back to baseline swelling levels in water (Fig. 4a). In contrast, treatment with ADP caused a minimal decrease in size by 7%, while AMP and P_i caused a very slight increase of 4%, which was within the standard error. We thus conclude that ATP provides a selective gel shrinking actuator response. Fig. 4b presents images of **Gel2** showing the swelling observed in water, followed by treatment with **TXsb** and ATP, respectively. Of note is that at the end of this sequence the gel treated with ATP had returned to the same size as it was in water alone. The **TXsb** expansion and ATP-mediated contraction effects proved repeatable as determined by placing first in fresh

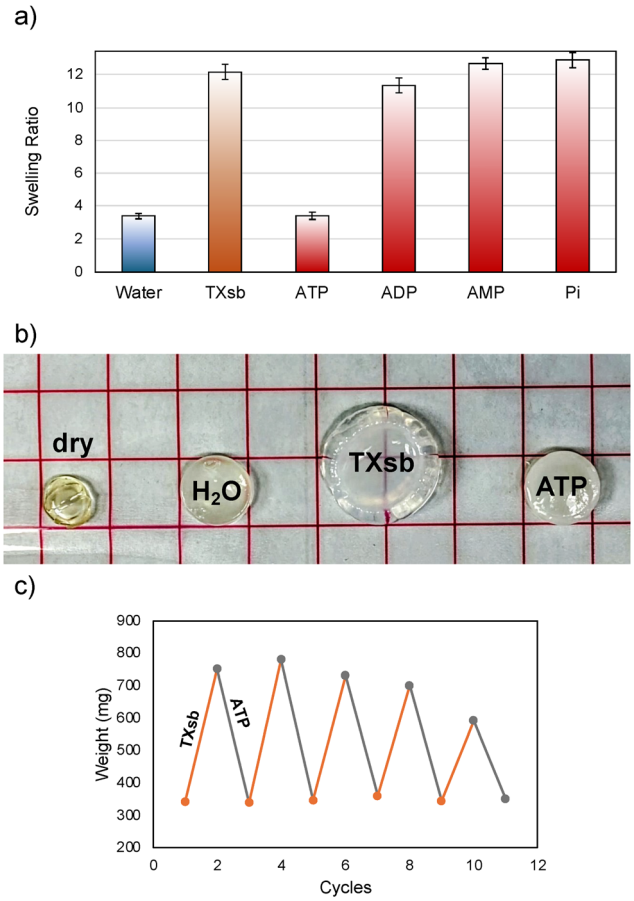


Fig. 4 (a) **Gel2** swelling ratios at equilibrium in water, followed by swelling response upon treatment with **TXsb**, and subsequent shrinking by 1 molar equivalent of ATP, ADP, AMP, and P_i relative to **TXsb**. (b) **Gel2** swelling in various environments (from left to right): dry, water, **TXsb**, and ATP. A 1 cm × 1 cm grid is provided for scale. (c) **Gel2** subject to repeated cycles of expansion and contraction by **TXsb** and ATP.

solutions of 200 μM **TXsb** and then those containing 200 μM ATP (Fig. 4c). Five complete cycles of expansion and contraction were demonstrated before a noticeable dip in performance was observed. The microscopic changes in gel morphology were investigated by scanning electron microscopy (SEM). The shrunken **Gel2** in ATP exhibits a uniform and crystalline morphology, while the expanded gel in the presence of **TXsb** shows globular deformations on the gel surface after swelling, along with wrinkling of the surface topography. The resulting stress is proposed to lead to performance degradation (Fig. S11 ESI†).

As noted above, little to no contraction is observed in the presence of ADP, AMP, or one equivalent of P_i. We thus reasoned that the enzyme catalysed hydrolysis of ATP would cause reswelling of the gel. With this goal in mind, an ATPase enzyme, commonly called an apyrase, which is known to hydrolyse ATP and ADP forming the byproducts ADP, AMP, and P_i[17] was employed to catalyse the hydrolysis of ATP in the presence of **Gel2**. This caused the gels to expand dramatically to the point of breaking apart. As shown in Fig. 5a, upon addition of ATPase, the ATP-shrunken gels expanded over 8-fold. As 200 μM of ATP is used for gel contraction, this can be rationalised by the potential influx

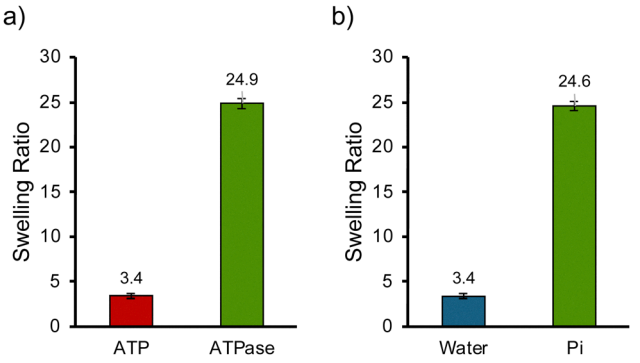


Fig. 5 Swelling ratios of **Gel2** upon (a) addition of ATPase enzyme to ATP-shrunken hydrogels and (b) addition of 400 μM P_i to hydrogels at equilibrium in water.

of 400 μM of P_i upon ATP hydrolysis. The resulting relatively high anion concentration out-competes the benzoate anions in the gel leading to breakdown of the gels (Fig. S10, ESI†). The effect of this excess in P_i was tested by adding 400 μM ammonium phosphate to samples of **Gel2** at equilibrium in water (Fig. 5b). This addition also resulted in an over 8-fold swelling response and breakdown of the gels. Fragmentation of the gels precluded detailed studies. However, an effort was made to collect the pieces and weigh them. While material losses are likely, these qualitative studies supported the conclusion that an expansion of at least 8-fold could be produced through exposure of the ATP-bearing material to apyrase or treatment with an excess of P_i. Additionally, rheological testing of **Gel2** was conducted for the different swelling states in H_2O, **TXsb**, and ATP (Fig. S12 and S13 ESI†). As expected, the contracted gels in H_2O and ATP exhibit very similar storage (G') and loss moduli (G''). In contrast, the expanded gels in **TXsb** display over 10-fold lower G' values. This reduction is ascribed to a loss in the number ionic crosslinks in the hydrogel networks, resulting in a softer and less elastic material. This large decrease in stiffness caused by the expansion of the gel in **TXsb** helps explain why the even greater expansion induced by ATPase leads to gel breakdown since the gel begins to lose elastic behaviour, deforming under the stress of its own expansion.

In summary, we have developed a stimulus-responsive hydrogel whose extent of contraction and swelling could be controlled *via* successive treatment with the "Texas-sized box", **TXsb**, and ATP, respectively. The swelling is attributed to bonding interactions between **TXsb** and benzoate anions in the gel, which reduce the extent of crosslinking to ammonium groups in the gel, thus promoting swelling. ATP out-competes the benzoate moieties as a substrate for the carboxylates and its addition leads to contraction of the hydrogel. The present work thus underscores how effective polymer design, including the nature of the backbone, coupled with tuning of molecular interactions *via* addition of ionic substrates, can be used to create hydrogel actuator systems, including ones such as presented here, that show "muscle-like" contraction features when exposed to the biological "energy currency", ATP.

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Data availability

All data are included either in the main text or the ESI.†

Conflicts of interest

There are no conflicts to declare.

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