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Chemical-mediated translocation in protocell-based micro-actuators

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Artificial cell-like communities participate in diverse modes of chemical interaction but exhibit minimal interfacing with their local environment. Here we develop an interactive micro-system based on the immobilization of a population of enzyme-active semipermeable proteinosomes within a helical hydrogel filament to implement signal-induced movement. We attach large single polynucleotide/peptide microcapsules at one or both ends of the helical protocell filament to produce free-standing soft micro-actuators that sense and process chemical signals to perform mechanical work. Different modes of translocation are achieved by synergistic or antagonistic enzyme reactions located within the helical connector or inside the attached microcapsule loads. Mounting the micro-actuators on a ratchet-like surface produces a directional push-pull movement. Our methodology opens up a route to protocell-based chemical systems capable of utilising mechanical work and provides a step towards the engineering of soft microscale objects with increased levels of operational autonomy.

The design and construction of communities of artificial cell-like entities (protocells) capable of synergistic and antagonistic modes of interaction is a key consideration in synthetic protobiology. Membrane-bounded protocells in the form of supramolecular lipid and polymer vesicles¹⁻³, semi-permeable protein-polymer microcapsules (proteinosomes)⁴, organoclay/DNA microcapsules⁵, inorganic colloidosomes⁶, and surfactant-stabilized water-in-oil microemulsions^{7,8} have been prepared and used as biomimetic models of confined enzyme activity^{9,10}, membrane gating⁶, growth and division^{11,12}, cytoskeleton assembly^{13,14}, *in vitro* gene expression^{15,16}, autonomous movement^{5,17}, and artificial phagocytosis¹⁸ and parasitism¹⁹. In addition, membrane-free coacervate micro-droplets have been exploited as molecularly crowded synthetic protocells²⁰⁻²⁴ capable of *in vitro* gene expression²⁵, enhanced

protein and RNA catalysis²⁶⁻²⁸, non-equilibrium electric field-induced energization²⁹, chemical sensing³⁰, artificial predation³¹ and morphogen-mediated differentiation³². Diverse modes of chemical and genetic signalling have been demonstrated within aqueous dispersed communities of artificial protocells comprising lipid vesicles³³, binary populations of vesicles and proteinosomes³⁴, colloidosome suspensions³⁵, 2D arrays of coacervate micro-droplets³⁶, or proteinosomes containing DNA logic gates³⁷. Alternatively, protocell populations have been assembled into spheroids of chemically ligated proteinosomes³⁸, networks of hemi-fused lipid-stabilized water-in-oil emulsion droplets³⁹⁻⁴¹ and hydrogels with embedded emulsion^{42,43} or coacervate⁴⁴ droplets to produce models of synthetic prototissues.

While studies on protocell-protocell interactions are burgeoning, minimal attention has been paid to protocell-environment interactive systems in which endogenous activities associated with populations of cell-like entities regulate functional changes in the surrounding milieu. To address this concept and provide a proof of principle, we immobilize a population of enzyme-active semipermeable proteinosomes within a mechanically energized environment as a path towards the fabrication of protocell-based micro-actuators capable of chemically induced movement. Specifically, we use microfluidics to prepare helical filaments of calcium alginate containing urease-loaded proteinosomes and implement the coupling of endogenous enzyme activity to the coordinated release of the elastic potential energy stored in the surrounding hydrogel environment. As a consequence, the helical filaments increase in pitch via an internally driven process of chemomechanical transduction associated with urease-mediated removal of Ca^{2+} ions from the crosslinked hydrogel matrix. We show that contraction of the extended soft spring-like microstructures occurs in the presence of a protocell-based glucose oxidase (GOx)-mediated Ca^{2+} influx, indicating that the degree of translocation can be controlled by the judicious employment of spatially distributed antagonistic enzyme reactions. Given these observations, we construct micro-actuator prototypes based on exploiting the endogenous release of elastic energy in the helical protocell filaments as a “drive-shaft” capable of performing work on polynucleotide/peptide microcapsule loads attached at one or both ends of the coiled hydrogel. The microcapsules consist of a semi-permeable DNA/protamine membrane and have been previously used as models of enzyme-powered protocell buoyancy⁵. We encapsulate enzymes within the loading elements to implement functional outputs in surface-attached filaments comprising a single microcapsule and increase the modes of endogenous transmission in free-standing micro-

actuators by using bilaterally attached microcapsules that operate synergistically or antagonistically with respect to pitch elongation along the helical connector. Finally, by mounting the micro-actuators on a substrate comprising a ratchet-like surface morphology, we implement directional movement of the proteinosome-containing helical filaments via a push-pull mechanism of translocation. Overall, our results provide a step towards the fabrication of protocell-based chemomechanical micro-systems capable of utilising mechanical work stored in their local environment and offers opportunities for the engineering of soft stimuli-responsive microscale objects with increased levels of operational autonomy.

Results

Microfluidic preparation of protocell-embedded helical hydrogel filaments. Helical filaments consisting of dispersed populations of water-filled ultra-small (mean size, 2.4 μm) proteinosomes (Supplementary Fig. 1 and Movie 1) immobilized within a coiled calcium alginate hydrogel were prepared by glass capillary-based microfluidics (Fig. 1a and Supplementary Fig. 2). The ultra-small proteinosomes were prepared by sonication-mediated emulsification of a buffered solution of cationized bovine serum albumin/poly(N-isopropylacrylamide) (BSA-NH₂/PNIPAAm) nanoconjugates in 2-ethyl-1-hexanol, followed by membrane crosslinking and transfer to aqueous solution (Supplementary Fig. 3). The protocells were dispersed in aqueous sodium alginate (2-4 wt%, low molecular weight) and injected into an outer stream of aqueous calcium chloride (2 wt%). Contact between the two channels at the orifice resulted in rapid Ca²⁺-mediated crosslinking of the alginate inner stream such that the proteinosome population became immobilized within the incipient hydrogel fibre during transit along the capillary. Significantly, the alginate/proteinosome solution was injected at a higher flow velocity compared with the calcium chloride outer phase to spontaneously transform the linear stream of the viscous inner phase into a helical trajectory in accordance with the liquid rope-coil effect^{45,46}. For example, centimetre-long helical hydrogel filaments with regular thickness (50-80 μm) and uniform pitch (210 μm) were routinely prepared at inner and outer flow rates of 0.55 and 8 mL/h using respective orifice diameters of 50-80 and 550 μm (Fig. 1b and Movie 2). Fluorescence microscopy images of the coiled filaments prepared in the presence of fluorescein isothiocyanate (FITC)-membrane-

labelled proteinosomes showed a homogeneous distribution of captured microcapsules throughout the hydrogel matrix (Fig. 1c, Supplementary Fig. 4 and Movie 3). Typically, the number of embedded proteinosomes occupying a single pitch of the helical micro-architecture was approximately 1.3×10^3 as estimated from fluorescence-activated cell sorting (Supplementary methods). The protocell loading could be readily increased or decreased without affecting the integrity of the hydrogel microstructure (Supplementary Fig. 5). High magnification confocal fluorescence microscopy images of the hydrogel filaments (Fig. 1d) and corresponding line profiles across individual embedded proteinosomes (Supplementary Fig. 6) confirmed that the protocells remained intact after immobilization. Moreover, confocal laser scanning microscopy images of individual hydrogel-entrapped protocells containing encapsulated macromolecules such as FITC-tagged dextran ($M_w = 150$ kD) or small dye molecules (FITC) showed retention (Supplementary Fig. 7) or leakage (Supplementary Fig. 8) of the guest molecules, respectively, indicating that the semi-permeable properties of the proteinosome membrane remained operational within the immobilized population. In general, the helical filaments were stable in water for more than two months (Supplementary Fig. 9) and could withstand temperatures up to 70 °C for over 12 hours without significant structural destruction or loss of the embedded proteinosomes (Supplementary Fig. 10).

We assessed the potential of the microfluidic procedure as a general platform for the fabrication of proteinosome-embedded hydrogel filaments with variable morphology, composition and mechanical activation. Firstly, we systematically adjusted the ratio of the flow velocity of the sodium alginate/proteinosome and calcium chloride inner and outer phases, respectively. At a constant outer flow rate of 8 mL/h, changing the flow rate of the inner stream from 0.4 to 1.2 mL/h resulted in helical filaments with decreasing pitch lengths ranging from 490 to 85 μm (Figs. 1e-h). Coiling of the inner stream was due to the viscous force between the inner and outer phase that derived from the difference in flow velocities. As a consequence, increasing the mismatch resulted in enhanced coiling of the filaments and possibly introduced higher levels of frustrated compression into the twisted hydrogels during microfluidic processing. This mechanism was consistent with the transformation of increasing amounts of kinetic energy into stored elastic potential energy in the helical filaments. Interestingly, more extreme changes in the ratio of flow velocity produced filaments with linear, wave-like or zig-zag morphologies (Supplementary Fig. 11). Secondly, we used the

microfluidic procedure to co-locate different types of protocells within the coiled hydrogel filaments as a step towards the anisotropic organization of communities of artificial cells. For example, populations of proteinosomes with different membrane fluorescence tags, ultra-small colloidosomes (mean size, 5 μm , Supplementary Fig. 12) or mixtures of proteinosomes and colloidosomes could be readily dispersed in aqueous sodium alginate and used as the inner phase to construct helical hydrogels with co-located communities of intact protocells (Supplementary Fig. 13).

Protocell-mediated energy release and helical extension. The above results indicate that *in situ* crosslinking of the helical hydrogel filaments during transit through the microfluidic capillary resulted in fixation of the coiled morphology and the concomitant storage of mechanical energy. To verify the storage of elastic potential energy within the helical protocell filaments we determined their spring-like elastic behaviour by fixing small glass weights to the free end of attached coiled filaments (Fig. 2a). Reversible elongation/relaxation of the helical filaments was observed for changes in pitch of up to 30% (Fig. 2b), consistent with elastic behaviour under these conditions. This was in agreement with plots of displacement against force that showed a linear relationship in accordance with Hooke's Law with a spring constant of approximately 16 mN m^{-1} for proteinosome-embedded helical hydrogel filaments prepared with an initial pitch of $220 \mu\text{m}$ (Fig. 2c).

As a step towards the chemically induced macroscale extension of the helical filaments, we implemented the coupling of enzyme activity within the immobilized proteinosomes to the controlled release of the stored mechanical energy of the coiled hydrogel. In so doing, we sought to use collective microscale processes to transform the elastic potential energy into kinetic energy via an endogenous process of chemomechanical transduction. Specifically, we encapsulated urease within the proteinosomes prior to formation of the helical filaments and then exposed millimetre-long sections of the coiled hydrogels to aqueous urea to initiate the *in situ* production of carbonate ions and concomitant decomplexation of the calcium alginate crosslinks (Fig. 3a). Optical microscopy images revealed that the helical pitch (initial value, $164 \mu\text{m}$) increased within minutes of adding the enzyme substrate and continued progressively for approximately 1 h to a length of $520 \mu\text{m}$ (Figs. 3b, Supplementary Fig. 14 and Movie 4). In contrast, addition of urea to a dispersion of straight filaments of the urease/proteinosome-containing crosslinked hydrogel gave minimal elongation

(Supplementary Fig. 15), indicating that movement of the coiled microstructure was principally associated with the release of stored mechanical energy rather than osmotically induced swelling. This mechanism was consistent with plots of the time-dependent changes in normalized pitch, helical diameter and filament thickness, which showed a three-fold increase over 1 h in pitch but negligible lateral swelling (Fig. 3c). We also monitored the time-dependent changes in pH and Ca^{2+} ion concentration in the aqueous solution external to the helical filaments during protocell-mediated urease-induced elongation (Fig. 3d). Although the pH increased from 7 to 9 within 20 min after addition of urea, control experiments indicated that the rise in pH was not directly responsible for helical elongation (Supplementary Fig. 14). In contrast, while the concentration of Ca^{2+} typically remained low (ca. 2.5×10^{-3} mM), and below the solubility constant for bulk CaCO_3 precipitation (ca. 7×10^{-2} mM) (Fig. 3d), a white precipitate of CaCO_3 was observed when a large volume (0.5 g) of the proteinosome-embedded helical hydrogel filaments was added to a bulk solution of urea (5 mL, 60 mM) (Supplementary Figs. 16,17), consistent with an energy release mechanism based on disruption of the crosslinks due to carbonate-mediated chelation of the localized calcium ions.

The enzyme-mediated conversion of elastic energy to kinetic energy in the helical filaments was investigated by systematically varying the urea and urease concentrations as well as the crosslinking density and the filament diameter of the hydrogel network. At constant encapsulated enzyme concentrations (15 mg/mL) and hydrogel crosslinking density (2 wt% alginate), increasing the concentration of urea increased the speed of the helical elongation (Fig. 3e). Low substrate concentrations (10 or 30 mM urea) gave low and constant speeds of elongation over 1 h (2.3 and 5.1 $\mu\text{m}/\text{min}$, respectively), while urea concentrations of 60 or 90 mM produced rapid increases in the pitch (11.1 and 17.5 $\mu\text{m}/\text{min}$, respectively) over an initial period of 10 min, followed by slower rates of elongation as the residual elastic energy in the filament became depleted (Fig. 3e). In all cases, the extent of elongation achieved after 12 h was similar and approximately $1150 \pm 90 \mu\text{m}$ (Supplementary Fig. 18). Similar increases in the rate of helical elongation were observed when the proteinosome-encapsulated urease concentration was changed between 5 to 25 mg/mL (Fig. 3f), indicating a direct correlation between the rate of chemomechanical transduction and enzyme activity. In general, the helical fibres became softer as they extended with a concomitant rapid decrease in the spring constant until the stored elastic energy was depleted (Fig. 3g). Changing the calcium alginate concentration from 2 to 3 wt% at urease and urea

concentrations of 15 mg/mL and 60 mM, respectively, had a marked inhibitory effect on the release rate of the stored elastic potential energy (Fig. 3h), presumably due to the greater resilience of the hydrogel network at higher levels of crosslinking. In contrast to the crosslinking density, we noted that changes in the filament diameter did not have a significant influence on the response behaviour (Supplementary Fig. 19). Taken together, the above results indicate that it is possible to couple the endogenous enzymatic activity of an immobilized population of micrometre-sized protocells to the transformation of mechanical energy stored within the surrounding helically configured soft environment to generate unidirectional macroscale movement.

Protocell-induced contraction of extended helical filaments. Given that urease-active proteinosomes could be immobilized in energized physical environments akin to a compressed spring and collectively exploited for the chemomechanical transduction of the stored elastic potential energy, we investigated whether contraction of the extended filaments could be implemented by introduction of a protocell-mediated Ca^{2+} flux. Our aim was to connect the protocell activity within the coiled filaments to an extraneous population of glucose oxidase (GOx)-containing proteinosomes to produce a network of synthetic microscale agents that could operate antagonistically with respect to the extension and contraction of the helical hydrogel filaments (Fig. 4a). For this, we immersed a helical filament containing a population of urease-encapsulated proteinosomes in aqueous urea to induce elongation, followed by transfer of the extended helix into an aqueous suspension of GOx-containing proteinosomes (Fig. 4b). We then added glucose and a suspension of CaCO_3 particles to initiate the formation of gluconic acid within the non-immobilized proteinosomes and thereby increase the Ca^{2+} concentration in contact with the extended helical filaments via acid-mediated CaCO_3 dissolution (Figs. 4c,d). Time-dependent fluorescence microscopy images of individual filaments indicated that slow retraction of the helical extension occurred in the presence of the GOx-containing proteinosomes when activated by glucose (Fig. 4e), indicating that both energy release (pitch elongation) and pitch contraction could be induced by antagonistic enzyme activities associated with the different protocell organizations. The rate of pitch elongation within the first hour (mean value, 82 $\mu\text{m}/\text{h}$) was considerably faster than the speed of contraction (mean value, 7 $\mu\text{m}/\text{h}$); as a consequence, it often took 12 h or so for the helical pitch to be restored to the original value (Fig. 4f). We attributed these

differences to the exergonic and endergonic nature of the extension and contraction processes, respectively. No significant filament contraction was observed in control experiments undertaken in the absence of the GOx-encapsulated proteinosomes or CaCO_3 particles (Fig. 4g). Measurement of the pitch contraction associated with pre-extended filaments produced under different urease/urea reaction times indicated that reversible expansion/contraction was obtained provided that the helical hydrogels were not elongated more than *ca.* 170-180 μm , including pre-extensions close to the original pitch (*ca.* 86 μm). The pitch was not contracted to values much less than 86 μm due to close contact of the helical turns in the native filaments. Progressive increases in the levels of incomplete contraction were observed for helical pitch extensions beyond 180-200 μm (Fig. 4h,i and Supplementary Fig. 20), indicating that irreversible disintegration of the crosslinking network occurred at high levels of decalcification.

Having established that reversible elongation and contraction of the helical hydrogel/protocell filaments could be achieved in the presence of an extraneous population of GOx-containing proteinosomes and CaCO_3 particles, we sought to endogenously couple the release and re-storage of elastic energy by co-encapsulation of urease and GOx within the hydrogel-immobilized proteinosomes (Fig. 5a). Integrating the antagonistic enzyme activities within individual protocells in the presence of extraneous CaCO_3 particles enabled both urea/urease-mediated extension and glucose/GOx-induced contraction to be sequentially actuated within individual hydrogel filaments (Fig. 5b). Time-dependent changes in the helical pitch indicated that the rates of urease-mediated extension were relatively rapid (typically 1 h) and independent of the number of hydrogel filaments added to the reaction system (Fig. 5c). In contrast, the recovery times (rate of contraction) were much slower and strongly dependent on the total GOx concentration (number of added helical filaments) included in the experimental protocol (Fig. 5c and Supplementary Fig. 21). We attributed the slow recovery to the delay in attaining a bulk rather than local H^+ concentration sufficient to dissolve the extraneous CaCO_3 particles.

Based on the above observations, we explored the possibility of implementing a dynamic steady state in helical filament extension by counterbalancing the enzyme activities of the embedded protocell population with those of a surrounding population of extraneous GOx-containing proteinosomes by establishing appropriate simultaneous rates of Ca^{2+} ion removal and production. Under optimum conditions, the pitch elongation was offset from a

typical value of 350 μm (no extraneous proteinosomes) to a minimal elongation of *ca.* 25 μm (Supplementary Fig. 22), indicating that antagonistic enzyme activities within the protocell network could regulate movement of the helical hydrogel filaments.

Enzyme-mediated translocation in protocell-based actuators. Having established that the protocell/hydrogel helical filaments are capable of endogenously controlled chemomechanical transduction using chemical signals from the external environment, we sought to exploit the enzyme-mediated pitch extension and contraction as a step towards the construction of a chemically driven protocell-based micro-actuator. To demonstrate the utilization of the released elastic energy in the form of work, we fixed one end of a helical filament containing immobilized urease-encapsulated proteinosomes to a plastic surface under water and showed that the urea-induced extension force was sufficient to translocate a single microcapsule attached electrostatically to the free end of the hydrogel spring. Typically, a single positively charged chitosan-functionalized silica colloidosome (mean size, 200 μm) (Supplementary Fig. 23 and SI Methods) could be displaced along a horizontal plastic surface under water over distances greater than 1 mm without disassembly of the coupled microstructure (Fig. 6a and Movie 5)., The attached colloidosome moved at a mean velocity of *ca.* 10 $\mu\text{m min}^{-1}$ (Fig. 6b), which was equivalent to a kinetic energy of 10^{-22} W and a theoretical output power for each immobilized proteinosome within the helical hydrogel filament of 10^{-26} W (Supplementary Fig. 24 and SI Methods). Increasing the size of the attached colloidosome above a critical mass inhibited translocation across the plastic substrate due to the increase in frictional force that counterbalanced the work produced by the extending helical filament (Supplementary Fig. 25).

We used the above procedure to couple the mechanical work undertaken by the proteinosome-embedded helical hydrogel to a simple functional output that was dependent on translocation of the attached microcapsule into a contact-dependent chemically active environment. For this, we reduced the weight of the filament-attached particle by replacing the single colloidosome with a lower density DNA/protamine microcapsule (mean size, 400 μm , Supplementary Fig. 26) to facilitate translocation, and chemically activated the attached capsule with encapsulated horseradish peroxidase (HRP) (SI Methods). A thin slab of an agarose hydrogel loaded with the HRP co-substrate *o*-phenylenediamine (*o*-PD) was placed approximately 800 μm from the attached DNA/protamine microcapsule such that urea-

induced extension of the helical filament resulted in chemomechanical translocation of the microcapsule onto the agarose surface (Fig. 6c). As a consequence of direct contact, green fluorescence was observed specifically within the microcapsule when aqueous hydrogen peroxide (H_2O_2 , HRP co-substrate) was present in the external environment (Figs. 6d,e). We attributed the onset and progressive increase in fluorescence intensity over 20 min (Fig. 6f) to the contact-dependent localized uptake of *o*-PD into the semi-permeable DNA/protamine microcapsule followed by HRP-mediated peroxidation in the presence of H_2O_2 and formation of the fluorescent product 2,3-diaminophenazine (2,3-DAP) initially within the filament-attached microcapsule. In contrast, 2,3-DAP fluorescence was not observed over the same time period when no helical extension and translocation of the microcapsule occurred due to the absence of urea (Fig. 6f), indicating minimal background levels of *o*-PD in the surrounding environment.

Endogenous transmission in free-standing protocell-based micro-actuators. Given that individual surface-anchored protocell/hydrogel helical filaments could perform work on a single microcapsule, we developed free-standing micro-actuators with two oppositely attached loading elements as a step towards integrated enzyme-powered microstructures capable of chemomechanical movement. The prototypes were based on a symmetrical or asymmetrical bilateral arrangement of a pair of DNA/protamine semipermeable protocells (mean size, 300 μm) connected through a single Ca alginate helical filament (mean length, 1.0 mm) that acted as an enzyme-powered drive shaft to perform work on the oppositely attached loading elements (Fig. 7a). Moreover, by housing urease within the hydrogel-immobilized proteinosomes or alternatively inside the attached microcapsules, we operated the micro-actuators in two different modes of endogenous transmission based on processing the urea input signal directly within the helical drive shaft or indirectly within the interior of the DNA/protamine protocells.

We first tested whether urease activity within the helical connector was sufficient to translocate a symmetrically arranged pair of attached microcapsules. To achieve this, we added urea to the external solution and monitored changes in the spacing between the two opposing microcapsules associated with removal of Ca^{2+} ions from the crosslinked hydrogel due to the *in situ* proteinosome-mediated production of carbonate. Fluorescence microscopy images showed a progressive increase in the spacing between the attached microcapsules,

indicating that the output power from the free-standing proteinosome/hydrogel filament was sufficient to move the microcapsules in opposite directions (Fig. 7b). Typically, the loading elements moved at a constant velocity of 6 $\mu\text{m}/\text{min}$ over a period of 1 to 2 h after addition of urea to give a 1.5 times increase in the interparticle spacing (Fig. 7c). As a consequence, the positioning of the two attached DNA/protamine microcapsules was spatiotemporally dependent on the extent of transmission arising from proteinosome-mediated urease activity within the interconnecting hydrogel filament.

As an alternative mode of transmission, we modified the above system such that the production of carbonate ions occurred specifically within both of the attached loads but not in the helical drive unit (Fig. 7d). As such, we sought to construct a free-standing protocell-based micro-actuator in which the loading elements regulated their own movement rather than being moved by enzyme activity in the helical connector. Addition of urea to the micro-actuator resulted in pitch extension and concomitant increase in the spacing between the loading elements (Fig. 7e), consistent with removal of Ca^{2+} ions from the crosslinked hydrogel matrix and release of the stored elastic potential energy. Movement of the attached single protocells occurred symmetrically in opposite directions such that the spacing between them doubled typically over a period of 20 min (Movie 6). The apparent output power was higher than that observed when only a single urease-containing microcapsule was attached to a free-standing helical filament (x1.5 increase spacing over 20 min; Supplementary Figs. 27 and 28 and Movie 7), suggesting that both microcapsules operated in unison to extend the helical connector and activate their own movement.

To programme a cycle of consecutive pitch extensions and contractions in the free-standing helical micro-activators, we positioned the microstructures in a Z-shaped flow chamber and exposed them to alternate flows of aqueous urea and CaCl_2 (Supplementary Fig. 29). Time-dependent optical images showed symmetrical pitch extensions and contractions of the micro-actuator due to the influx of urea or CaCl_2 , respectively (Fig. 7f and Movie 8). We sought to bias the changes in pitch by using a ratchet-like surface to direct the chemically driven movement of helical filament micro-actuators comprising a bilateral arrangement of attached urease-containing microcapsules. For this, we mounted single micro-actuators onto the jagged surface of a butterfly wing (Supplementary Fig. 30) such that the helical axis was parallel to the direction of overlap of the chitin scales (Fig. 7g), and placed the samples in a Z-shaped flow chamber. The micro-actuator was placed on the butterfly wing without physical

attachment. Sequential addition of aqueous urea and CaCl_2 gave rise to a two-stage mode of translocation that was constrained to one direction along the stepped surface (Fig. 7h). Measurement of the positional displacements incurred by the two attached microcapsules demonstrated a ratchet-like “push-pull” mechanism that involved out of phase pinning and sliding of the interconnected microcapsules (Fig. 7i).

Based on the above methodology, we constructed an integrated protocell-operated micro-actuator comprising an asymmetric arrangement of two enzyme-active DNA/protamine microcapsules that operated antagonistically with regard to the removal or addition of Ca^{2+} ions from or to the helical drive unit. Release of the stored elastic energy (pitch extension) was accomplished as above by attachment of a urease-containing microcapsule, while simultaneous contraction of the pitch was achieved endogenously by positioning a microcapsule containing both GOx and CaCO_3 particles at the opposite end of the connecting hydrogel spring (Fig. 8a). Addition of urea alone gave rise to asymmetric extension of the pitch and a 1.5 times increase in spacing between the coupled microcapsules over 20 min (Fig. 8b). Time-dependent optical and fluorescence microscopy images showed that the increase in pitch was preferentially located adjacent to the urease-containing microcapsule, while the helical structure remained essentially unchanged close to the inactive GOx-containing loading element (Figs. 8c,d), confirming the presence of a carbonate diffusion gradient along the coiled connecting unit. In contrast, simultaneously activating the GOx-containing load by addition of glucose (2 mM) along with urea (5 mM) produced anisotropic extension in the pitch of the helical drive shaft after 20 min (Fig. 8e). Further increases in the glucose : urea ratio inhibited the pitch extension such that transmission of the carbonate trigger to the micro-actuator was dynamically muted as the removal and addition of Ca^{2+} reached a steady state (Figs. 8f,g). The anisotropic translocation behaviour was also influenced by the length of the helical drive unit (Supplementary Fig. 31). Measurements of the pitch extension over a constant time period for the different arrangements of the DNA/protamine protocells showed a progressive decrease in the output power as the urease activity became less effective (Fig. 8h). Control experiments confirmed that the antagonistic mode of operation was determined by the mass balance associated with the flux of Ca^{2+} ions rather than dampening of the increase in local pH by GOx activity (Supplementary Fig. 32).

Discussion

In this paper, we describe a protocell/environment interactive system based on the

immobilization and distribution of large numbers of urease-active semi-permeable proteinosomes within mechanically energized helical hydrogel filaments. The protocell/hydrogel helical filaments are readily produced by microfluidic processing to produce centimetre-long coiled filaments with controllable width and pitch. The methodology is straightforward and readily adaptable provided that any practical constraints associated with jamming of the protocells in the input orifice during microfluidic injection are circumvented. A key outcome of our methodology is the storage of elastic potential energy within the environment of the protocell population by *in situ* Ca^{2+} -mediated crosslinking of the sodium alginate/proteinosome input stream. The latter is activated into a coiled trajectory by the viscous force between the inner and outer phases such that the crosslinked hydrogel filaments are loosely analogous to a compressed spring; as a consequence, controlled release of the elastic energy results in pitch elongation (kinetic energy). This energy transformation and concomitant movement at the macroscale can be driven endogenously by urease/urea activity within the proteinosomes, which results in the localized production of carbonate and subsequent removal of Ca^{2+} ions from the crosslinked hydrogel matrix. Conversely, the elongated helical filaments can be contracted endogenously in the presence of glucose and CaCO_3 by co-encapsulation of GOx and urease in the immobilized proteinosomes. As a consequence, programmable changes in protocell reactivity can be chemically coupled to mechanical work in the local environment in a reversible fashion. Alternatively, contraction of the extended helical filaments can be achieved exogenously via a population of extraneous GOx-containing proteinosomes capable of releasing aqueous Ca^{2+} ions from CaCO_3 particles by GOx/glucose-mediated acidification.

An important advantage of using the immobilized protocell-based micro-reactors is that high and controllable local urease concentrations are distributed as “hot-spots” throughout the hydrogel without deleterious enzyme-matrix interactions during the fabrication and operation of the helical microstructures. Also, integrating enzymes into the hydrogel via protocell encapsulation rather than direct incorporation provides benefits in terms of accessing more complex chemical pathways, enabling the spatial separation of antagonistic enzymes (via immobilization of mixed proteinosome populations for example) and minimising diffusive loss of the enzyme to the external environment when the hydrogel matrix is stimulated by auxiliary chemicals.⁵⁵ Moreover, by confining the urease activity and controlling the population number density of the protocells within the hydrogel, the secretion of

carbonate is modulated so that release of the elastic energy is locally controlled to produce pitch extensions over extended periods of time without degradation of the filament morphology. We also note that the enclosure of enzymes within the proteinosomes enables fluxes and gradients of enzyme substrates and products to be generated across the semi-permeable protein-polymer membrane; in principle, this could be used as a selective trigger to release the elastic potential energy by gating the diffusion of molecules across the proteinosome membrane, which is known to exhibit thermoresponsive behaviour^{4,38}.

We use the chemomechanical transformations associated with the protocell/hydrogel helical filaments to design and construct microscale objects that function as prototypes of proteinosome-based micro-actuators. By coupling the endogenously powered helical drive shaft to bilaterally attached enzymatically active DNA/protamine protocells, we implement on-board processing of incoming signals to regulate the chemomechanical response. Moreover, depending on the arrangement of the loading elements, symmetric or asymmetric pitch extensions, as well as synergistic or antagonistic modes of endogenous transmission can be achieved in the protocell-based micro-actuators. In each case, encapsulation of the enzymes and their competitive or complementary interplay are key requirements for localizing the chemomechanical responses and determining the extent of endogenously driven movement. As a consequence, placing an external bias on the system by mounting the micro-actuators on a ratchet-like surface leads to directional movement via a push-pull mechanism of translocation. Currently, only limited “walk cycles” have been achieved due to the slow response times for the “push” stage, and further studies will attempt to optimise this step via modifications in enzyme and substrate concentrations and parameters used to operate the flow chamber.

Many current approaches to the fabrication of micro-structured polymer/hydrogel actuators rely directly on local or global stimuli such as pH, temperature, light or magnetism to trigger repeatable mechanical transformations⁴⁷⁻⁵². In comparison, polymer/hydrogel actuators driven by enzyme-powered chemical stimulation are limited. In this respect, self-propelling nano- and micro-motors have been constructed by the implementation of enzymatic conversions in polymer capsules and vesicles.^{5,53-54} In other studies, entrapped urease was used to programme the one-way disassembly of alginate fibres containing embedded oil droplets as well as trigger local pH changes leading to the coordinated disassembly of neighbouring hydrogel beads.⁵⁵ In contrast, the chemomechanical

transformations describe herein occur without disintegration of the hydrogel matrix or disruption of the homogeneous protocell distribution (Supplementary Fig. 33), enabling the changes in shape to be rendered reversible by judicious control of the coupling between endogenous protocell reactivities and the degree of crosslinking in the surrounding external environment. However, our prototype systems are constrained by slow contraction rates (typically 12 hours to achieve full contraction) and limited reversibility (two complete cycles). This is due to their dependence on high rates of Ca^{2+} mass transfer for fast and effective pitch contraction and the onset of urease degradation at high Ca^{2+} ion concentrations. One possible strategy to circumvent these constraints could involve small-volume containment of the polymer/hydrogel actuators so that changes in the bulk Ca^{2+} ion concentrations are more responsive to the enzyme reactions localized within the protocells. Alternatively, a redox-active metal-ion could be used as the crosslinking agent to increase the “on/off” sensitivity of the pitch extension and contraction mechanisms.

In conclusion, our results demonstrate a pathway to the design of interactive protocell network/environment systems, provide a step towards the use of immobilized microscale reactors for reversible chemomechanical movement and offer opportunities for the engineering and endogenous control of stimuli-responsive soft microscale materials.

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Author contributions

N. G., M. L. and S. M. conceived the experiments; N. G. undertook the experiments; L. T. assisted with constructing the bilateral micro-actuators; A. J. P. and B. V. V. S. P. Kumar prepared DNA/protamine microcapsules; N. G., M. L., L. T. and S. M. undertook the data analysis; L. T. contributed to the preparation of the manuscript; N. G., M. L. and S. M. wrote the manuscript. All authors commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available from the website or from the authors.

Fig. 1 Microfluidic preparation of protocell-embedded helical hydrogel filaments. **a**, Scheme showing microfluidic device used to fabricate helical calcium alginate hydrogel fibres containing populations of ultra-small proteinosomes. **b**, Bright-field and corresponding fluorescence microscopy images of a helical hydrogel/protocell filament. Green fluorescence originates from the embedded population of FITC-membrane-labelled proteinosomes. The sample was prepared at inner (sodium alginate/proteinosomes) and outer (CaCl_2) flow rates of 0.55 and 8 mL h^{-1} , respectively; scale bar, $500 \text{ }\mu\text{m}$. **c**, High magnification of **(b)** showing regular helical pitch of $210 \text{ }\mu\text{m}$ and homogeneous distribution of the proteinosome population; scale bar, $200 \text{ }\mu\text{m}$. **d**, Confocal laser scanning microscopy image of localized section of a helical filament showing the presence of a dispersed population of intact FITC-labelled proteinosomes immobilized within the hydrogel matrix; scale bar, $20 \text{ }\mu\text{m}$. **e-g**, Bright field and corresponding fluorescence microscopy images showing protocell/hydrogel filaments with different pitches. Samples were prepared at a constant outer phase (CaCl_2) flow rate of 8 mL h^{-1} and inner phase (sodium alginate/proteinosomes) flow rate of 0.4 mL h^{-1} (**e**), 0.7 mL h^{-1} (**f**) and 1.0 mL h^{-1} (**g**); scale bar, $400 \text{ }\mu\text{m}$. **h**, Plot of helical pitch length against inner phase flow rate for protocell/hydrogel filaments prepared at a constant outer phase flow rate of 8 mL h^{-1} . Error bars represent the standard deviation ($n=3$).

Fig. 2 Protocell-mediated elastic behaviour. **a**, Optical photograph showing micro-measuring system used to determine the spring constant of a coiled protocell/hydrogel filament. A single proteinosome-embedded helical hydrogel filament (**H**) is attached to the end of a pair of tweezers (**T**) and a small glass weight (**G**) is used to provide the extension; scale bar, 1 mm. **b**, Plot of the helical pitch of a protocell/hydrogel filament against cycles of extension and relaxation (open arrows) showing elastic behaviour of the hydrogel spring. **c**, Plot of normalized displacement (ΔX) against normalized external force (F) for a helical filament showing linear dependence with a spring constant (k) of 16 mN/m.

Fig. 3 Protocell-mediated energy release and helical hydrogel filament elongation. **a**, Scheme showing enzyme-mediated extension of a helical protocell filament. Release of elastic potential energy is triggered by addition of urea to the coiled calcium alginate filament due to urease activity within the immobilized proteinosomes (left graphic). A multi-step endogenous mechanism is responsible for chemomechanical transduction (right graphic); (1) diffusion of urea into the hydrogel matrix and penetration of the substrate through the proteinosome semi-permeable membrane (dashed blue circle) leads to the internalized enzyme-mediated transformation of urea to ammonia and carbon dioxide; (2) release of the hydrolysed products (ammonium hydroxide and carbonate) through the protocell membrane into the surrounding hydrogel results in decomplexation of Ca^{2+} ions from the alginate crosslinks and transformation of the elastic potential energy into kinetic energy. **b**, Time series of optical microscopy images showing elongation of a helical protocell/hydrogel filament after addition of urea (60 mM; time t_0). The helical pitch doubles within 60 min. Scale bar, 500 μm . **c**, Plots of time-dependent changes in the normalized pitch, helical diameter and filament thickness determined from images in (**b**). **d**, Time-dependent plots of pH (blue line) and Ca^{2+} concentration (red line) in the external solution containing helical filaments of immobilized urease-encapsulated proteinosomes. **e**, Time-dependent increases in pitch for helical filaments of urease-encapsulated proteinosomes after exposure to different urea concentrations (10, 30, 60, 90 mM). **f** plots as in (**e**),

but for helical filaments prepared at different encapsulated urease concentrations (5, 15, 25 mg/mL) and constant urea concentration. **g**, Plot of measured spring constants against changes in pitch for protocell/hydrogel helical filaments after different levels of enzyme-mediated extension. Regions of the plot that exhibit elastic (blue) or non-elastic (red) behaviour are shown. **h**, Plots as in **(e)** but for helical filaments prepared at alginate concentrations of 2, 3 or 4 wt% and constant values of urea and urease. Error bars represent the standard deviation ($n \geq 3$).

Fig.4 Protocell-induced contraction of extended helical hydrogel filaments. **a**, Scheme showing enzyme-driven Ca^{2+} -mediated energy release (pitch extension) and pitch contraction in a protocell network comprising a helical Ca^{2+} -crosslinked alginate filament containing urease-encapsulated proteinosomes (orange circle) and exogenous GOx-containing proteinosomes (blue circle). CaCO_3 particles (grey circle) are added to the external environment as a source of Ca^{2+} ions. Addition of urea or glucose (Glc) respectively removes or adds Ca^{2+} ions to the crosslinking sites in the hydrogel. In the latter case, GOx activity in the extraneous proteinosomes produces gluconic acid (GlcA; via D-glucono- δ -lactone) and hydrogen peroxide, which releases Ca^{2+} ions into the medium via CaCO_3 dissolution. **b**, Fluorescence microscopy image showing a single urease-containing helical protocell filament (green fluorescence) surrounded by a dispersed population of GOx-containing proteinosomes (red fluorescence). Green and red fluorescence originate from FITC- or RITC-labelled proteinosomes, respectively. Scale bar, 200 μm . **c,d**, Time-dependent plots of corresponding changes in pH (**c**) and Ca^{2+} concentration (**d**) after addition of glucose to a suspension of elongated helical protocell/hydrogel filaments in the presence of a dispersed population of GOx-containing proteinosomes (GOxP) and CaCO_3 particles (red plots). A buffered decrease in pH is observed while the Ca^{2+} concentration in the supernatant increases over 6 h. Control experiments undertaken without CaCO_3 show a rapid non-buffered decrease in pH (green line in (**c**)), while in the absence of GOxP no significant changes in pH or Ca^{2+} concentration are observed (blue lines). **e**, Time-series of fluorescence microscopy images of urease/urea-driven pitch elongation (left column) and GOx/glucose-mediated pitch contraction (right

column) for a single protocell/hydrogel filament. See (a) for mechanistic details; scale bar, 400 μm . f, Time-dependent changes in helical pitch (line plot) and deformation velocity (bars) associated with the enzyme-mediated extension and contraction of the helical filament shown in (e). Changes in pitch elongation (red bars and black line) and pitch contraction (blue bars and black line) are shown. Half-life (τ) of the extension and contraction are 30 min and 4 h, respectively. g, Differences in pitch contraction (normalized length) for hydrogel-immobilized helical filaments after urease-mediated extension. The elongated filaments were immersed in aqueous glucose solutions (3 mL, 100 mM) containing (i) GOxP (100 μL) and CaCO_3 (4 mg); (ii) CaCO_3 particles (4 mg); or (iii) GOxP (100 μL). h, Time-dependent contractions in pitch for pre-extended protocell/hydrogel filaments immersed in a mixture of GOx-containing proteinosomes, CaCO_3 particles and glucose. Blue dash line indicates the initial pitch before elongation (ca. 86 μm). Helical filaments extended beyond a pitch of 200 μm showed incomplete reversibility. i, Pitch contraction measured in distance (red line) and percentage change (blue line) for protocell/hydrogel filaments pre-extended to different pitch values (x axis) and then immersed in a mixture of GOx-containing proteinosomes, CaCO_3 particles and glucose. The initial pitch before elongation was ca. 86 μm .

Fig.5 Protocell-mediated reversible contraction of helical hydrogel filaments. **a**, Scheme showing reversible helical filament extension and contraction driven by proteinosome-mediated endogenous urease and GOx reactivity. Reaction network is as shown in Fig. 4a but with GOx and urease co-encapsulated within the hydrogel-immobilized proteinosomes. **b**, Time-series of fluorescence microscopy images of a single RITC-labelled protocell/hydrogel filament showing urease/urea-driven pitch elongation (0-1 h) followed by GOx/glucose-mediated pitch contraction (1-13 h). Experimental set-up as in (a). Scale bar, 400 μ m. **c**, Time-dependent changes of normalized helical pitch associated with enzyme-mediated extension and contraction; experimental set-up as in (a). Urea (60 mM) was added to trigger the extension ($t = 0$ h); 1, 25, 50 or 100 pieces of elongated filaments were immersed in aqueous glucose solutions (0.5 mL, 100 mM) containing CaCO₃ particles (0.5 mg) to induce contraction ($t = 1$ h). Although the rapid relative rate of extension is approximately the same, the recovery time (rate of contraction) is strongly influenced by the GOx concentration (number of added helical filaments). The black dashed line delineates the turn-over between extension and contraction. Error bars represent the standard deviation ($n=3$). Experiments were repeated three times and analysed using multiple separate coils. All individual results were incorporated into data shown.

Fig. 6. Enzyme-mediated translocation in protocell-based micro-actuators. **a**, Time series of bright field (left) and fluorescence microscopy images (right) showing directional horizontal movement under water of a silica colloidosome linked to one end of a surface-attached protocell/hydrogel helical filament undergoing urease-powered extension. Green fluorescence in the helical filament arises from hydrogel-immobilized FITC-labelled urease-containing proteinosomes. Red fluorescence in the colloidosome arises from encapsulated RITC-labelled horseradish peroxidase (HRP). The colloidosome is displaced by a distance of 1.1 mm in 2 h. Scale bar, 400 μm . **b**, Time-dependent plot of the colloidosome displacement shown in (a). The colloidosome is translocated at an approximately constant velocity (mean, 10 $\mu\text{m min}^{-1}$) over a period of 120 min. Error bars represent the range of variation ($n=2$). **c**, Scheme illustrating use of a urea-responsive helical Ca alginate filament of urease-containing proteinosomes (green) with attached single HRP-containing microcapsule (grey) as a simple protocell-based micro-actuator. Addition of urea in the presence of H_2O_2 results in translocation of the filament-attached microcapsule onto a neighbouring α -PD-loaded agarose hydrogel (blue) and subsequent contact-dependent peroxidation to give a green fluorescent output (2,3-DAP) in the microcapsule (green). **d,e**, Observations for set-up shown in (c); bright field (top) and fluorescence (bottom) microscopy images of a protocell/helical filament with attached DNA/protamine HRP-containing microcapsule before (**d**) and 20 min after (**e**) chemomechanical induced translocation. Green fluorescence in the helical filament arises from hydrogel-immobilized FITC-labelled urease-containing proteinosomes. Appearance of green fluorescence in the microcapsule on contact with agarose hydrogel arises from 2,3-DAP production. Dashed circle in (**d**) delineates the position of the non-fluorescent microcapsule before addition of urea. Scale bars, 400 μm . **f**, Time-dependent plots showing increase in fluorescence intensity (displayed as a grey value) associated with a filament-attached HRP-containing microcapsule in contact with a α -PD-loaded agarose hydrogel and in the presence (red) or absence (blue) of urea.

Fig. 7. Endogenous transmission in protocell-based micro-actuators. **a**, Fluorescence microscopy images showing integrated soft micro-actuators with a bilateral arrangement of DNA/protamine microcapsule loading elements and protocell/hydrogel helical drive shaft.. Red and green fluorescence in the microcapsules arise from encapsulated RITC- or FITC-labelled bovine serum albumin, respectively; green fluorescence in the helical filament originates from immobilized FITC-membrane-labelled proteinosomes. Scale bar, 400 μm . **b**, Graphic (top) and fluorescence microscopy images showing micro-actuator with helical filament connector containing urease (URE)-loaded proteinosomes (green spring) and symmetrical arrangement of attached (enzyme-free) DNA/protamine microcapsules (red spheres). The time series (0 to 120 min) shows the helical pitch extension and concomitant bilateral displacement of the two attached microcapsules after addition of urea. Fluorescence labels as in (a). Scale bar, 400 μm . **c**, Time-dependent plot showing progressive increase in spacing between the attached microcapsules shown in (b). Error bars represent the standard deviation ($n=3$). **d**, Schematic showing synergistic mode of endogenous transmission in a free-standing protocell-based micro-actuator. Design as shown in (b) (graphic) except that urease is housed only in the two attached microcapsules. **e**, Fluorescence microscopy images before (top) and 20 min after (bottom) addition of urea showing pitch extension and associated bilateral displacement of two urease-containing microcapsules in a micro-actuator (UU, as shown in d). Fluorescence labels as in (a). Scale bar, 400 μm . **f**, Bright field images showing two consecutive cycles of elongation and contraction for micro-actuator UU before (top) and after exposure to alternative inputs of aqueous urea (+ u) and CaCl₂ (+ Ca); scale bar, 400 μm . **g**, Scheme showing directional bias in the movement of a protocell-based micro-actuator UU when positioned on a substrate with ratchet-like surface texture and subjected to sequential additions of aqueous urease and CaCl₂. **h**, Time series of optical microscopy images showing directional movement of a micro-actuator UU along the ratchet-like tiling pattern of a butterfly wing. Layers of periodically overlapping chitin scales (vertical stripes) constrain the movement to one direction along the steps (white arrow). Images are recorded before (top), and 4 and 8 min after addition of urea (+ u), and then 4 min after addition of CaCl₂ (+ Ca) and indicate a

two-stage movement of the interconnected microcapsules (blue-green objects). The helical hydrogel filament connector is not visible in the side light source used for imaging. White dashed lines indicate the initial location of the protocell-based actuator. Scale bar, 200 μm . **i**, Time-dependent plots showing ratchet-like “push-pull” movement of the two connected microcapsules shown in **(h)**. The microcapsule located at the leading edge of the steps (right capsule) is displaced by *ca.* 240 μm in one direction (right displacement) over 8 minutes after addition of urea due to pitch extension of the interconnecting helical hydrogel filament. In contrast, the opposing microcapsule (left capsule) is pinned against the step riser. Addition of CaCl_2 (after 4 min) induces pitch contraction, releasing movement of the left microcapsule towards its counterpart, which in turn becomes pinned on the jagged surface.

Fig. 8. Antagonistic modes of transmission in protocell-based micro-actuators **a**, Scheme showing micro-actuator with proteinosome-containing helical hydrogel filament connector (green spring) and two attached DNA/protamine protocells containing encapsulated urease (URE, **U**, red sphere) or GOx and CaCO_3 particles (**G**, green sphere); experiments are coded as U_xG_y for urea and glucose substrate concentrations, x and y , respectively). The enzymes operate antagonistically to indirectly remove or add Ca^{2+} ions from or to the drive shaft. **b**, Fluorescence microscopy images before (top) and 30 min after (bottom) addition of urea in the absence of glucose to the micro-actuator displayed in (**a**) showing asymmetric pitch extension and translocation. Micro-actuator label, U_5G_0 (no glucose) Red or green fluorescence in the urease- and GOx-containing microcapsules arise from co-encapsulation of RITC-BSA or FITC-BSA, respectively. Green fluorescence in the helical filament originates from FITC-labelled proteinosomes (no urease). Scale bar, 400 μm . **c**, Time series of optical microscopy images showing asymmetric extension of micro-actuator U_5G_0 . Scale bar, 400 μm . **d**, Time-dependent asymmetrical changes in helical pitch associated with different positions along the drive shaft (P_{1-5}) identified in (**c**). **e,f**, Fluorescence microscopy images before (top) and 20 min after (bottom) addition of urea and glucose to a micro-actuator configured as displayed in (**a**). Substrate concentrations: 5 mM urea and 2 mM glucose (U_5G_2) showing anisotropic pitch extension (**e**), or 2 mM urea and 10 mM glucose (U_2G_{10}) showing minimal change in pitch (**f**). The microcapsules operate in opposition with regard to helical extension (urease) or contraction (GOx) to give different modes of translocation depending on the respective enzyme activities. White dashed lines indicate the initial mid-point of the pitch superimposed on the images before and after helical translocation. Scale bars, 400 μm . **g**, Time-dependent plots showing the normalized spacing between two microcapsules for different protocell-based micro-actuators prepared with one free end and one attached urease-containing microcapsule (**U**); two attached urease-containing microcapsules (**UU**). Labels U_5G_0 , U_5G_2 and U_2G_{10} as in (**b**), (**e**) and

(f), respectively. **h**, Plot showing percentage of helical extension for various protocell-based micro-actuators. Data taken after 20 min. Labels as described in (g). Error bars represent the standard deviation (n=3). Experiments were repeated three times and analysed using multiple separate coils. All individual results were incorporate into data shown in (d), (g) and (h).

Methods

Materials

The following chemicals and materials were used without further purification. Round glass capillary (inner diameter 0.58 mm, outer diameter 1.00 mm), square glass capillary (inner diameter 1.05 mm), epoxy resin, calcium chloride (CaCl_2), sodium alginate, 50% hydrophobic silica nanoparticles, urea, urease (40 U/mg), CaCO_3 particles (5-6 μm in diameter), glucose oxidase (GOx, 139 U/mg), glucose, H_2O_2 (30% v/v), o-phenylenediamine, agarose, albumin from bovine serum, NHS-PEG₁₆-NHS, fluorescein isothiocyanate (FITC), rhodamine B isothiocyanate (RITC), deoxyribonucleic acid sodium salt (DNA) from salmon testes (MW $\approx$ 1300 kDa), protamine sulfate salt from salmon (Grade X).

Optical and confocal microscopy

Optical microscopy experiments were carried out using a Leica DMI 3000B optical microscope. Fluorescence imaging was performed using a Leica DFC 310FX, and dye molecules were excited by using specific filters. Confocal microscopy imaging was performed on a Leica SP5-II laser scanning microscope attached to a Leica DMI 6000 inverted epifluorescence microscope and equipped with a X10 or X20 objective.

Preparation of microfluidic chips

Capillary microfluidic chips were used to prepare the helical protocell filaments. Round glass capillary tubes with inner and outer diameters of 550 μm and 1000 μm , respectively, were tapered to 50-80 μm by using a Sutter Instrument (P-97). Untapered glass capillaries were used as collection tubes. A tapered glass capillary and collection tube were coaxially inserted into a square capillary (inner diameter of the square capillary, 1050 μm). "5 Minutes" epoxy resin was used to seal the tube system. Inner phase and outer phase solutions were injected into the microfluidic chip by using mechanical pumps.

Preparation and characterization of ultra-small urease-containing proteinosomes

Ultra-small proteinosomes (mean size, 2.4 μm) were prepared by sonication according to previous work.⁵⁶ In brief, proteinosomes were prepared by mixing 30 μL of an aqueous solution (pH=8.5 $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer containing 15 mg/mL BSA-NH₂/PNIPAAm, 5, 15 or 25 mg/mL urease) and 2 mg NHS-PEG₁₆-NHS (protein-polymer membrane crosslinker) with 2-ethyl-1-hexanol (0.5 mL) and emulsifying the mixture by sonication for 3-5 min. After 48 hours, the sedimented crosslinked proteinosomes were transferred to water by continuous washing three times with 70% ethanol/water followed by pure water. The proteinosomes were stored in 100 μL aqueous solution.

Dynamic light scattering (DLS) was used to determine the hydrodynamic mean size of the proteinosomes. The proteinosome concentration was determined by fluorescence-activated cell sorting (FACS). Typically, the proteinosome dispersion (100 μL per batch) was diluted 100, 200 and 400 times before counting to match the boundary conditions of the FACS instrument. The concentration of the proteinosome in the original proteinosome solution was estimated to be 1.5×10^6 per μL .

Preparation of ultra-small urease and GOx-containing proteinosomes

Ultra-small urease and GOx-containing proteinosomes were prepared by mixing 30 μL of an aqueous solution (pH=8.5 $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer) containing 15 mg/mL RITC-BSA-NH₂/PNIPAAm, 5 mg/mL

urease and 50 mg/mL GOx) and 2 mg NHS-PEG₁₆-NHS (protein-polymer membrane crosslinker) with 2-ethyl-1-hexanol (0.5 mL) and emulsifying the mixture by sonication for 3-5 min. After 48 hours, the sedimented crosslinked proteinosomes were transferred to water by continuous washing three times with 70% ethanol/water followed by pure water. The proteinosomes were stored in 100 μ L aqueous solution.

Preparation of ultra-small colloidosomes

35 mg of partially hydrophobic silica nanoparticles were dispersed into 2 mL of toluene and used as the oil phase. An aqueous phase containing enzymes (urease, GOx, 25 μ L, 20 mg/mL) and FITC- or RITC-labelled enzymes (5 μ L, 5 mg/mL) was mixed with the oil phase and sonicated for 4 minutes to produce a water-in-oil Pickering emulsion. 10 μ L tetramethoxysilane (TMOS, silica membrane crosslinker) was then added and the emulsion rotated for 24 hours followed by continuous washing with 70% ethanol/water, 50% ethanol/water (x2) and pure water to transfer the colloidosomes into an aqueous phase.

Preparation of protocell-embedded helical hydrogel filaments

An aqueous suspension of ultra-small urease-containing proteinosomes (2 batches, 200 μ L) was dispersed in aqueous sodium alginate (final concentration, 2-4 wt%) and the mixture used as the inner phase in the microfluidic device. Aqueous calcium chloride (CaCl₂; 2 wt %, 180 mM) was used as outer phase. The flow rates of the inner and outer phases were varied between 0.4-1.2 mL/h and 6-12 mL/h, respectively. The microfluidic chip was arranged vertically to avoid jamming of the helical hydrogel filament, which was collected in water. Typically, 3 wt% sodium alginate/proteinosome solution, 2 wt % CaCl₂, and inner and outer phase flow velocities of 0.5 mL/h and 8 mL/h, respectively, were used.

Determination of number density of entrapped proteinosomes

The number of proteinosomes used to prepare the helical protocell/hydrogel filaments was estimated to be typically 3×10^5 per μ L (addition of 200 μ L (1.5×10^6 per μ L) proteinosomes to sodium alginate (final volume, 1 mL). The volume for a single pitch (**V**) was given by:

$$V = \pi r^2 (D^2 \pi^2 + P^2)^{0.5}$$

$$(V = 3.14 \times [30 \times 30 \mu\text{m}] \times ([500 \times 500 \mu\text{m}] \times [3.14 \times 3.14] + [200 \times 200 \mu\text{m}])^{0.5} = 4.473 \times 10^{-3} \mu\text{L}).$$

where **r** represents the radius of the filament; **D** the diameter of the helix; **P** the pitch length of the helix. Thus, the number of proteinosomes contained within a single pitch (**N**) of the helical filament was estimated to be 1.3×10^3 ($= 3 \times 10^5 \times 4.473 \times 10^{-3}$).

Measurement of the spring constant of protocell/hydrogel helical filaments

We used a home-made system to measure the spring constant of the protocell/hydrogel helical filaments. We attached one end of the helical filament to a fixed tweezer, and then the other end was fixed to a small piece of a glass slide using glue. The weight of the glass slide and the glue was measured using a Thermogravimetric Analyser. To offset the influence of buoyancy in the aqueous solution, the external force **F** was estimated to be 50% of the gravitational force, given that the densities of the glass slide and aqueous solution were approximately 2 g/mL and 1 g/mL, respectively.

We used a microfocal camera to record the helical filaments before and after applying different external forces (different weights of the glass attachment). The displacement of the helical filament (ΔX) was measured from optical images. The spring constant was calculated by using the formula:

$$k = F / \Delta X$$

To investigate the variation in the spring constant with changes in the pitch elongation generated by urease activity in the embedded proteinosomes, we immersed a helical filament (prepared using 3 wt% alginate) into aqueous urea (60 mM) to increase the pitch to a steady state value and then measured the spring constant of the extended coiled filament using the above procedures.

Protocell-mediated energy release in helical hydrogel filaments

Miliimetre-long helical hydrogel filaments containing ultra-small urease-containing proteinosomes produced using a 3 wt% sodium alginate/proteinosome solution and 2 wt % CaCl_2 crosslinking solution were manually sectioned using a scalpel. Each piece of the helical filament contained between 4-10 pitches. Before protocell-mediated energy release, the helical filaments were washed with deionized water (x2). One piece of the helical filament was then added to aqueous urea (10-90 mM, typically 60 mM; 2mL) and the mixture poured into a plastic petri dish and homogenised by gentle pipetting. A transparent lid was placed over the petri dish to avoid water evaporation; no external stirring was carried out. Multi-time optical and fluorescence microscopy images were recorded to analyse the extension process. Extension of the helical pitch was manually measured by using ImageJ software. Controlled experiments were carried out by replacing the urea solution with aqueous ammonia (pH=9, total volume; 2 mL).

Preparation of GOx-containing proteinosomes

Proteinosomes were prepared by mixing 60 μL of an aqueous solution (pH=8.5 $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer) containing 4 mg/mL BSA- NH_2 /PNIPAAm, 25 mg/mL GOx and 2 mg NHS-PEG₁₆-NHS (protein-polymer membrane crosslinker) with 2-ethyl-1-hexanol (1 mL) and emulsifying the mixture by shaking by hand for 1 min. After 48 hours, the sedimented crosslinked proteinosomes were transferred to water by continuous dialysis three times with 70% ethanol/water followed by pure water. The proteinosomes were stored in 50 μL aqueous solution.

Enzyme-mediated contraction in extended protocell/hydrogel helical filaments

Small pieces of the urease-containing helical hydrogel filaments (initial pitch, 80-90 μm) were elongated by addition of urea (60 mM) for different time periods to produce coiled filaments with different pitch extensions. The pre-extended filaments were immersed into an aqueous mixture of GOx-containing proteinosomes (100 μL proteinosome and 0.9 mL water), CaCO_3 particles (4 mg) and glucose (3 mL, 100 mM) to induce contraction. The system was gently shaken for 12 h in a mechanical shaker to homogenize the changes in pH and Ca^{2+} concentration, which in some experiments were monitored in real time. Changes in the helical extension were imaged by fluorescence microscopy. Control experiments was carried out by immersing the elongated helical protocell colonies into solutions containing (i) CaCO_3 (4 mg) and glucose (3 mL, 100 mM) or (ii) GOx-encapsulated proteinosomes (100 μL) and glucose (3 mL, 100 mM). Experiments were repeated three times and analysed using 3 pieces of separate coils.

Steady-state filament extension/contraction in protocell networks

Small pieces of the urease-containing helical hydrogel filaments (initial pitch, 90-100 μm) produced using a 3 wt% sodium alginate/proteinosome solution and 2 wt% CaCl_2 crosslinking solution were added to an aqueous mixture containing urea (60 mM), GOx-containing proteinosomes (100 μL proteinosomes in 0.9 mL water), CaCO_3 particles (4 mg) and glucose (3 mL, 100 mM) and homogenised by gentle pipetting. A transparent lid was placed over the petri dish to avoid water evaporation; no external stirring was carried out. Multi-time optical and fluorescence microscopy images were recorded to analyse the extension process under the antagonistic urease and GOx enzyme reactions. Experiments were repeated three times and analysed using 3 pieces of separate coils. Extension of the helical pitch was measured manually by using ImageJ software.

Endogenous reversible extension and contraction of protocell/hydrogel helical filaments

Small pieces of helical hydrogel filaments (4-10 pitches in length; number of pieces, 1, 25, 50 or 100) containing a population of immobilized urease/GOx-encapsulated proteinosomes were added to a single well of a 64-well plate and elongated by addition of urea (60 mM) for 1 hour. The extended filaments were then immersed into an aqueous mixture of CaCO_3 particles (0.5 mg) and glucose (0.5 mL, 100 mM) to induce GOx-mediated contraction. The system was gently shaken for 12 h in a mechanical shaker to homogenize any changes in pH and Ca^{2+} concentration. Time-dependent changes in the helical extension and contraction were imaged by fluorescence microscopy. As the GOx reactivity in a single piece of the helical hydrogel filament was not sufficient for effective contraction of the extended helical filaments, we increased the retraction efficiency by increasing the number of helical filaments used in each experiment under constant CaCO_3 (0.5 mg) and glucose (0.5 mL, 100 mM) concentrations. Experiments were repeated three times and analysed using 3 pieces of separate coils.

Determination of supernatant Ca^{2+} concentrations

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the concentration of Ca^{2+} released into the aqueous phase after protocell-mediated *in situ* decomplexation of the crosslinked calcium alginate hydrogel helical filaments. Samples were centrifuged for 10 minutes at 10000 rpm to remove the hydrogel filaments, and the concentration of Ca^{2+} in the supernatant determined by ICP-OES.

Preparation of positively charged chitosan-functionalized colloidosomes

Water (100 μL , pH = 7, PBS buffer) was added to a dodecane (2 mL) suspension of partially hydrophobic silica nanoparticles (7.5 mg/mL) and emulsified by shaking by hand for 30 seconds to produce a Pickering water-in-oil emulsion. Tetramethoxysilane (TMOS, 5 μL) and (3-glycidyloxypropyl)trimethoxysilane (5 μL) were added to the emulsion and shook for 24 hours to produce ultra-large water-in-oil colloidosomes with a semipermeable silica nanoparticle membrane. The colloidosomes were then transferred to a continuous aqueous phase using a washing cycle involving 70 % ethanol/water, 50 % ethanol/water, 50 % ethanol/water and pure water. The water-in-water colloidosomes were functionalized with chitosan by reaction of the epoxide groups on the silica membrane with amine groups of the cationized polysaccharide. Typically, an aqueous solution of chitosan (1% wt, 2mL) was added to an aqueous dispersion of the colloidosomes (1 mL) and the mixture stirred for 12 hours to produce positively charged colloidosomes. The chitosan-functionalized colloidosomes were kept in water before use. In most experiments, FITC- or RITC-labelled horseradish

peroxidase (HRP) (10 μ L, 5 mg/mL) was included in the encapsulated aqueous phase to visualize the colloidosomes by fluorescence microscopy,

Preparation of semipermeable DNA/protamine microcapsules

Droplets of an aqueous solution of DNA (4 mg/mL) were injected into an aqueous solution of protamine (1 mL, 0.5 mg/mL) using a procedure described elsewhere.⁵⁷ Enzymes (HRP, GOx, urease; typical concentrations, 2 or 10 mg/mL) were encapsulated within the DNA/protamine microcapsules by dissolving the proteins in the DNA solution prior to injection. In most experiments, FITC- or RITC-labelled BSA (10 μ L, 1 mg/mL) were added to 1 mL of DNA aqueous solution to visualize the DNA/protamine microcapsules by fluorescence microscopy.

Enzyme-mediated translocation in protocell-based actuators

Single chitosan-functionalized colloidosomes were attached manually to one end of the helical filaments (3 wt% calcium alginate) comprising urease-containing proteinosomes. The free end of the filaments was then manually attached to the surface of a plastic petri dish using underwater glue. Translocation of the single colloidosomes was initiated by addition of urea (5 mL, 60 mM).

Filament-attached DNA/protamine semipermeable microcapsules containing encapsulated HRP (2 mg/mL) were translocated using the above procedure onto the surface of an agarose hydrogel (5 mL, 100 mg) containing entrapped *o*-phenylenediamine (*o*-PD, 0.1 mg) in the presence of urea (60 mM) and H₂O₂ (1 mM). Before use, the *o*-PD-loaded agarose gel was wiped with a tissue to remove any surface *o*-PD. Control experiments using the same concentrations of urea, H₂O₂ and *o*-PD-loaded agarose were carried out by fixing single DNA/protamine microcapsules directly onto the plastic petri dish in the absence of the helical filaments.

Two single DNA/protamine microcapsules containing RITC-BSA were attached manually to the ends of single helical filaments (3 wt% calcium alginate) comprising urease-containing proteinosomes. Translocation of the DNA/protamine microcapsules was initiated by addition of urea (5 mL, 60 mM).

Endogenous transmission in free-standing protocell-based micro-actuators

Two single DNA/protamine microcapsules containing urease (10 mg/mL) were attached manually to the ends of single helical filaments (3 wt% calcium alginate) comprising non-enzymatically active ultra-small proteinosomes (no urease). Urea (5 mL, 5 mM) was added to initiate extension of the connecting hydrogel spring. As a comparison, a single DNA/protamine microcapsule containing urease (10 mg/mL) and RITC-BSA was attached to only one end of the helical filaments (3 wt% calcium alginate) comprising non-enzymatically active ultra-small proteinosome (no urease); the other end of the filament remained free. Urea (5 mM, 5 mL) was added to initiate extension of the connecting hydrogel spring.

Alternatively, the above filaments were attached at opposite ends to single DNA/protamine microcapsules containing encapsulated urease (10 mg/mL) and RITC-BSA or containing GOx (10 mg/mL), CaCO₃ (1 mg/mL) and FITC-BSA to produce an antagonistic pair of signalling microscale agents. Transmission and translocation of the asymmetrical micro-actuator was initiated by addition of urea and glucose at different concentrations: (i) 5 mM urea, 0 mM glucose; (ii) 5 mM urea, 2 mM glucose; and (iii) 2 mM urea, 10 mM glucose. Multi-time optical and fluorescence microscopy images were recorded to analyse the extension process. All the experiments were repeated three times and analysed using 3 pieces of separate coils. Extension of the helical pitch was manually measured by using ImageJ software.

Kinetic energy and output power calculations

Kinetic energy associated with the movement of a single filament-attached colloidosome was calculated as $\frac{1}{2}mv^2$ where v was the determined mean velocity of displacement ($10 \mu\text{m min}^{-1}$), and m the mass of a single colloidosome determined from the measured density ($\rho \approx 1.2 \text{ g/mL}$) of a sample of *ca.* 200 colloidosomes using $m = [4/3\pi R^3]\rho$ where R was the mean radius of the colloidosome ($100 \mu\text{m}$). The value of m was estimated as $5 \times 10^{-6} \text{ g}$, to give a kinetic energy of 10^{-22} W . The number of proteinosomes (n) estimated with a helical filament of 7-pitch length was estimated as 9.1×10^3 . The output power (P) was then given by:

$$P = \frac{1}{2}mv^2/n \approx 10^{-26} \text{ W}$$

Measurement of colloidosome density

We used a home-made system to measure the density of a colloidosome. A square glass capillary with precise length (L) and width (w) was sealed from one end. An aqueous dispersion containing *ca.* 200 colloidosomes was injected into the square glass capillary. The supernatant (water) was carefully withdrawn. The height (h) of the colloidosome dispersion in the square glass capillary was determined as well as the total mass (M). The colloidosome density (ρ) was given by:

$$\rho = \frac{M - 33\%Lwh\rho_1}{67\%Lwh}$$

where ρ_1 is the density of water (1 g/mL). We performed three measurements; the average colloidosome density was $\approx 1.2 \text{ g/mL}$.

pH monitoring of microcapsules containing urease or GOx/CaCO₃ mixtures

The pH increase associated with the urease-activity of DNA/protamine microcapsules when attached to one or both ends of a helical protocell filaments were determined by immersing one or two urease (10 mg/mL)-containing microcapsules into urea aqueous solution (5 mM , 1 mL). pH changes associated with the GOx-activity of DNA/protamine microcapsules were determined by immersing a GOx (10 mg/mL) and CaCO₃ (1 mg/mL)-containing microcapsule into glucose aqueous solution (10 mM , 0.5 mL). Control experiments were carried out by replacing the GOx/CaCO₃-containing microcapsule by microcapsules containing only GOx (10 mg/mL).

Preparation of Z-shaped PDMS flow chamber

10 g of PDMS monomer and 1 g of PDMS-crosslinker were mixed and poured into a 5.5 cm -diameter petri dish. Crosslinking of the PDMS elastomer was carried out at 70°C in an oven for 6 hours . A Z-shaped channel *ca.* 1 cm in width was manually cut in the crosslinked PDMS elastomer using a scalpel. Two inlet tubes for injecting aqueous solutions of urea or CaCl₂ were connected at one end of the channel, and a single outlet tube was connected at the opposite end of the channel. Liquid injection was actuated by mechanical pumps. Liquid withdrawal was actuated by a peristaltic pump.

Consecutive elongation and contraction of free-standing protocell-based micro-actuator

Protocell-based micro-actuators were prepared by manually attaching two single DNA/protamine microcapsules containing urease (10 mg/mL) to the ends of single helical hydrogel filaments ($3 \text{ wt\%}$ calcium alginate) comprising non-enzymatically active ultra-small proteinosomes (no urease). A single micro-actuator was immersed in 2 mL water present within a Z-shaped PDMS flow chamber. Aqueous

solutions of urea (5 mM, 2 mL/h) and CaCl₂ (1 wt%, 2 mL/h) were injected sequentially into the flow chamber for fixed periods of time to induce consecutive elongation and contraction of the micro-actuator.

Directional movement of protocell-based micro-actuators

Single protocell-based micro-actuators comprising two urease-containing DNA/protamine capsules joined by a helical hydrogel filament containing immobilized proteinosomes was mounted on a small fragment of a butterfly wing (*Danaus chrysippus*, Plain tiger) and placed inside a Z-shaped flow chamber containing 2 mL water. The orientation of the micro-actuator was carefully adjusted such that the filament axis was perpendicular to the direction of corrugation associated with the jagged ratchet-like texture of the wing scales. Aqueous urea (5 mM, 2 mL/h) was flowed into the chamber for 4 min to induce pitch elongation. The micro-actuator was then left for 4 min to allow further pitch elongation, after which a stream of aqueous CaCl₂ solution (1 wt%, 2 mL/h) was flowed through the device for 4 min to induce contraction of the micro-actuator. The injection cycle was then repeated.

References (for Methods section only)

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Data availability

All data supporting the results and conclusions are available within this paper and the Supplementary Information. Source data are provided with this paper. Copies of the raw data can be obtained via the link: https://figshare.com/articles/figure/NCHEM-20071608A_zip/14331317.