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Higher-Order Behaviours in Bio-Inspired Materials

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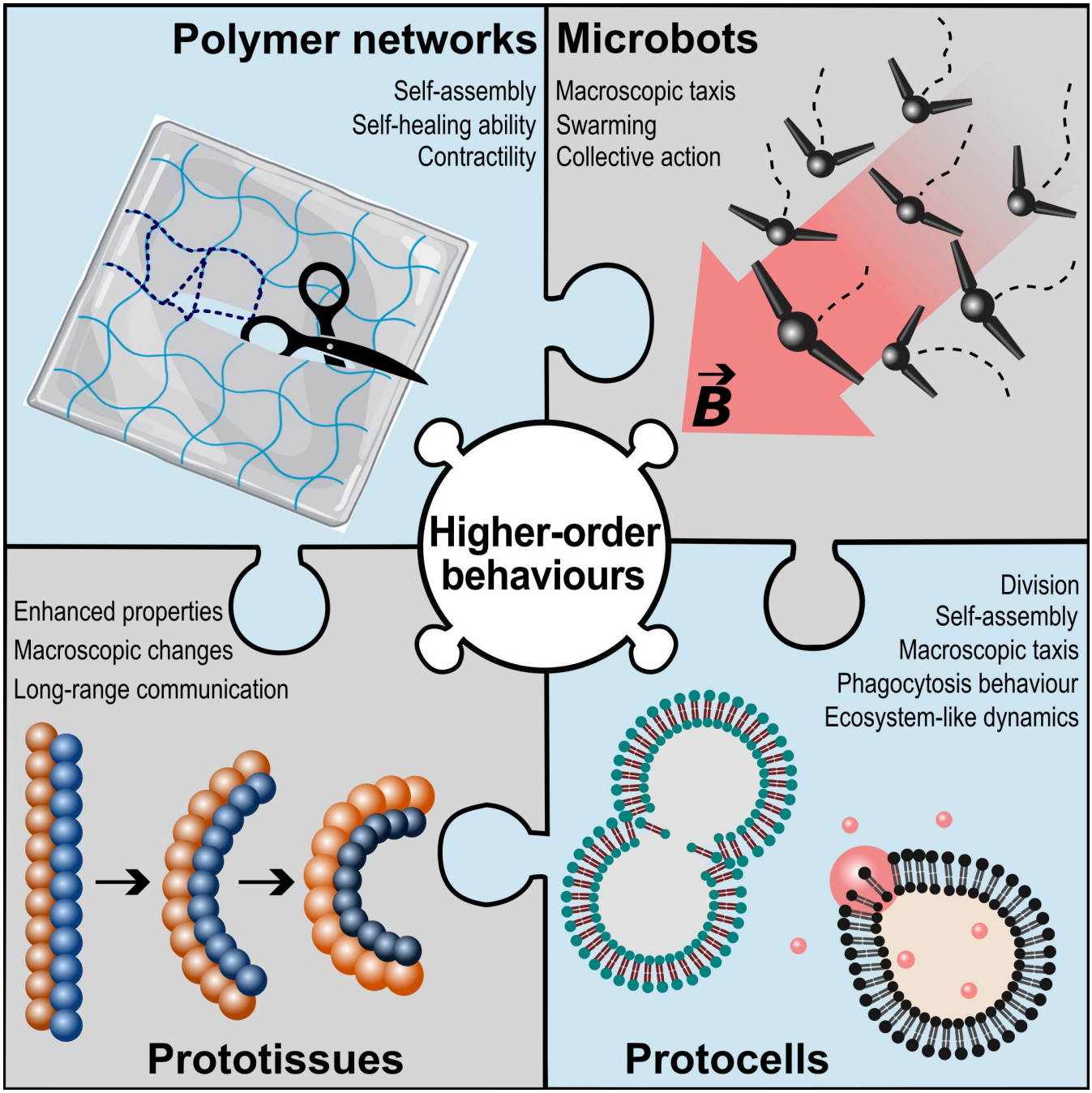
Pierangelo Gobbo has been nominated for the special collection Systems Chemistry Talents by Board Member Pasquale Stano



Bio-inspired approaches in materials science and systems chemistry are yielding a variety of stimuli-responsive and dynamic materials that are gradually changing our everyday life. However, the ability to chemically program these materials to exhibit macroscopic higher-order behaviours such as self-assembly, contractility, swarming, taxis, chemical communication, or predator-prey dynamics remains an ongoing challenge. While still in its infancy, the successful fabrication of bio-inspired materials displaying higher-order behaviours not only will help bridging the gap between living and non-living matter, but it will also contribute to the development of advanced materials for potential applications ranging from tissue engineering and biotechnology, to soft robotics and regenerative medicine. Our Mini-Review will systematically discuss the higher-order behaviours developed thus far in bio-inspired systems, namely (i) polymer networks (ii) microbots, (iii) protocells, and (iv) prototissues. For each system it will provide key examples and highlight how the emergent behaviour could be chemically programmed.

1. Introduction

Emergence is ubiquitous to Nature, and it can be found across all levels of organisation, from fractal patterns in snowflakes to human consciousness. While there is no consensus on the exact definition of emergence, the scientific community across all fields defines emergence as "the whole is bigger than the sum of its parts".[1] It is generally accepted that emergence is hierarchical and organised in levels, meaning that the "parts" or "units", which are on a lower level, are able to interact with one another and generate a novel property of the "whole", "collective" or "ensemble", which is on a higher level.[1] Similarly, the term *higher-order behaviour* is used in a variety of ways, ranging from the human ability to process thoughts[2] to rudimental life-like behaviour achieved in synthetic cells.[3] While in the physical and life sciences these concepts are progressively evolving, in this Mini-Review we define *higher-order behaviours* as those properties or phenomena resulting from *emergence*.

Cell migration,[4] quorum sensing in bacteria,[5] wolf packs,[6] and human crowds[7] are all examples of higher-order behaviours in biological systems. While higher-order behaviours are widely found in natural systems, they still need to be fully understood.[4,8] In particular, pinpointing exactly the origin of these behaviours is not trivial, especially given that there is not a unanimous definition of emergence. Nonetheless, some general principles may be highlighted as potential contributors. For example, it is widely accepted that these behaviours arise from local-level communication within the individuals that comprise the ensemble, resulting in cooperative interactions.[1] These cooperative interactions rely on information exchange between the single units, their neighbours, and the external environment, even if this communication is not intended or explicit.[8a,9] Organisation and hierarchy have also been postulated to be at the basis of emergence,[1a,d] particularly in living systems.[9b] Consequently, bio-inspired materials that are organised or hierarchical may also be expected to display higher-order behaviours. Understanding higher-order behaviours will not only contribute to the elucidation of the natural world, but it will also lead to innovative technologies in many different fields ranging from robotics, artificial intelligence, biotechnology, and materials science.

Bio-inspired materials are artificial materials that aim to display similar structures, properties, or functions to those of living systems without necessarily producing an identical replica.[10] Compartmentalisation is often regarded as one of the key pillars of life.[11] As such, bio-inspired materials are generally designed with compartmentalised structures to mimic the hierarchical organisation of living systems.[12] Bio-inspired materials also seek to replicate the adaptability and out-of-equilibrium properties of living systems. "Adaptive" materials can respond dynamically to their environment and learn from it, taking a step beyond simple stimuli responsive materials.[13] Out-of-equilibrium materials can be defined as materials at a state that is away from thermodynamic equilibrium. An out-of-equilibrium state could be either a local energy minimum (kinetically trapped state), or a dissipative state. A kinetically trapped state does not require energy to be maintained, while on the contrary a dissipative state requires an external flow of energy (sometimes also referred to as "fuel") to maintain the steady state conditions. Keeping a steady state can lead to processes such as structural reconfigurations, self-assembly, or production of chemical signals.[14] As such, these materials require careful chemical programming, to achieve the desired adaptability and responsiveness to external stimuli. Similarly to their natural counterparts, in bio-inspired materials, the individual constituent units interact with each other based on simple rules and fundamental chemical and physical principles. Through these small-scale interactions between the constituent parts, the system as a whole could exhibit a higher-order behaviour such as, the ability to self-assemble and integrate components into complex architectures,[8b] self-healing, macroscopic movement, swarming, phagocytosis-like behaviour, ecosystem-like dynamics (*i.e.*, predator-prey or retaliation dynamics), and communication based on diffusible chemical signals. In this Mini-Review we consider and discuss the most relevant examples of materials endowed with higher-order

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behaviours that have been developed so far, with the aim of providing the reader with a broad overview of the research field. For convenience, we have classified these materials into four categories: (i) polymer networks, (ii) microbots, (iii) protocells, and (iv) prototissues. From a general perspective, this work aims to highlight how the synergistic combination of fundamental concepts of synthetic chemistry and materials chemistry with key elements of systems chemistry can lead to the next generation of bio-inspired, out-of-equilibrium materials. These materials could find applications in diverse areas of science and technology, from tissue engineering and biotechnology, to soft robotics and medicine.[10]

2. Polymer Networks

One of the key higher-order behaviours seen in polymer-based materials is their ability to self-assemble into integrated higher-order structures that display enhanced properties compared to the individual polymer starting materials. Due to their ease of synthesis and functionalisation, polymers can be engineered to emulate the composition or properties of soft living tissues. This has resulted in a wide variety of applications in the biotechnological, medical and clinical sectors. One of the most important and highly functionalisable polymer networks is the hydrogel. Hydrogels present a high water content that gives them mechanical properties that resemble those of biological tissues, making them suitable tools for mimicking complex living tissue features.[15] A key component of living tissues is the extracellular matrix (ECM). The ECM is comprised of a peculiar fibrous architecture,[16] which guarantees the cell's optimal conditions for cellular processes such as spreading, migration, proliferation and differentiation. Xie and co-workers obtained an ECM mimic by reinforcing a hyaluronic acid (HA)-based hydrogel with self-assembled peptide fibres *via* the inclusion of dynamic imine bonds. The self-assembled fibres endowed the hydrogel network with higher stability and enhanced network dynamics.[16] This captured the inherent biophysical and biochemical features

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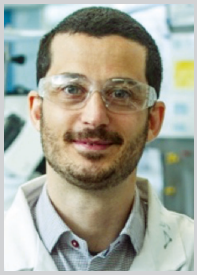
Beatrice Rosetti graduated in Chemistry from the University of Trieste (Italy) in 2020. She continued her studies at the University of Trieste under the supervision of Prof. Silvia Marchesan. In 2022, she obtained her Masters's in Chemistry, studying insulin fibrillation inhibition using various peptides. She is currently a PhD student in the group of Prof. Pierangelo Gobbo, working on developing new photo-sensitive protocellular materials.

Nicoletta Braidotti is a Materials Engineer. She obtained her PhD in Nanotechnology, co-funded by the University of Trieste, Department of Physics, and CNR-IOM, Trieste, in March 2024. Her research focuses on cell mechano- transduction, by using both 2D and 3D cell cultures jointly with independent fabrication of polymeric biocompatible substrates. She used several imaging and mechanical probing techniques characterised by novel multimodal combinations. Significant attention was conferred to the field of cardiac diseases and heart mechanobiology. She is currently a postdoctoral researcher in the group of Prof. Pierangelo Gobbo, working on mechanical characterization of prototissues by using a micro-/nano-indenter.



Pierangelo Gobbo received his PhD in 2016 at the University of Western Ontario (Canada). In 2016 he joined the research group of Prof. Stephen Mann, FRS at the University of Bristol (UK) as an NSERC of Canada Postdoctoral Fellow first, and then as an EU Marie Curie Postdoctoral Fellow. In 2019 he established his independent research group at the University of Bristol and started his work on biomimetic tissue-like materials. In 2021 he moved his research group to the University of Trieste (Italy), where he is currently working as an Associate Professor of Organic Chemistry.

of native ECM, allowing optimal cell spreading, mechanotransduction, cell-cell interactions, and enhanced cellular energy metabolism (Figure 1a).[16] The inclusion of primary amines into the self-assembled fibre structure allowed for easy conjugation of bioactive molecules such as cinnamaldehyde, a component used for treating osteoporosis. Integration of cinnamaldehyde within the hydrogel resulted in increased activity, showing that the integrated self-assembled hydrogel is not only an ECM-mimic but also an optimal vehicle able to promote successful bone regeneration in animal models.[16]

In addition to their ECM-mimicking features, bio-inspired hydrogels have been used for applications in tissue regeneration because of their biocompatibility, hydrophilicity, low toxicity, and injectability.[17] For example, articular cartilage regeneration is challenging due to its inability to spontaneously self-repair.[17a,18] Notably, Ciu *et al.* reported a nanocomposite comprised of a chitosan-based hydrogel integrated with articular cartilage stem cells and mesoporous silica nanoparticles loaded with anhydroicaritin, a small bioactive molecule. This cell-hydrogel nanocomposite could be injected into cartilage and successfully promote cell proliferation, differentiation, and ECM production, thereby promoting cartilage regeneration.[17a] Specifically, the ability to promote self-healing in cartilage was attributed to the synergistic nature of the nanocomposite architecture (the 3D hydrogel network and mesopore channels of silica nanoparticles), which allowed the sustained release of anhydroicaritin. Furthermore, integrated hydrogels with hierarchical architecture and mechanical properties closely similar to the *in vivo* ECM have been highlighted as suitable scaffolds for patient-derived tumour organoid growth.[19] These results highlight the importance of self-assembled hydrogel networks with integrated nano-structures and showcase the potential applications of these bioinspired materials in regenerative medicine, such as preclinical *in vitro* models for testing cancer treatments[19–20] or photothermal therapy for antibacterial treatments.[21]

Another key higher-order behaviour exhibited by hydrogels and used for applications in tissue regeneration is the ability to self-heal. Self-healing can be considered a higher-order behaviour because it arises from the ability of the already formed whole system to re-assemble after breaking, maintaining, therefore, its identity as a single material, rather than reverting back to its constituent parts. A key example of a self-healing hydrogel has been reported by Ma and co-workers, who assembled a double-network hydrogel based on the host-guest complexation and covalent bonding of HA and poly(γ-glutamic acid).[17b] In addition to the reversible host-guest complexation, the hydrogel was designed to contain dynamic hydrazone bonds between the polymer chains. These factors, as well as hydrogen bonding and physical entanglement of the two polymers, allowed it to heal itself. This allowed the double-network hydrogel to closely emulate the physiological dynamical viscoelasticity of native ECM, promote cellular delivery and stem cell fate regulation, and preserve cell survival by protecting cells from shear stresses. Significantly, this self-healing hydrogel efficiently promoted cartilage regeneration in mice.[17b] Another example of a self-healing hydrogel for applications in regenerative medicine applications was reported by Wang *et al.*[22] The authors engineered a dynamic hydrogen-bonded polycitrate-polyethylene glycol-polyethyleneimine-based hydrogel loaded with mesenchymal stem cells-derived extracellular vesicles *via* electrostatic bonds. The presence of

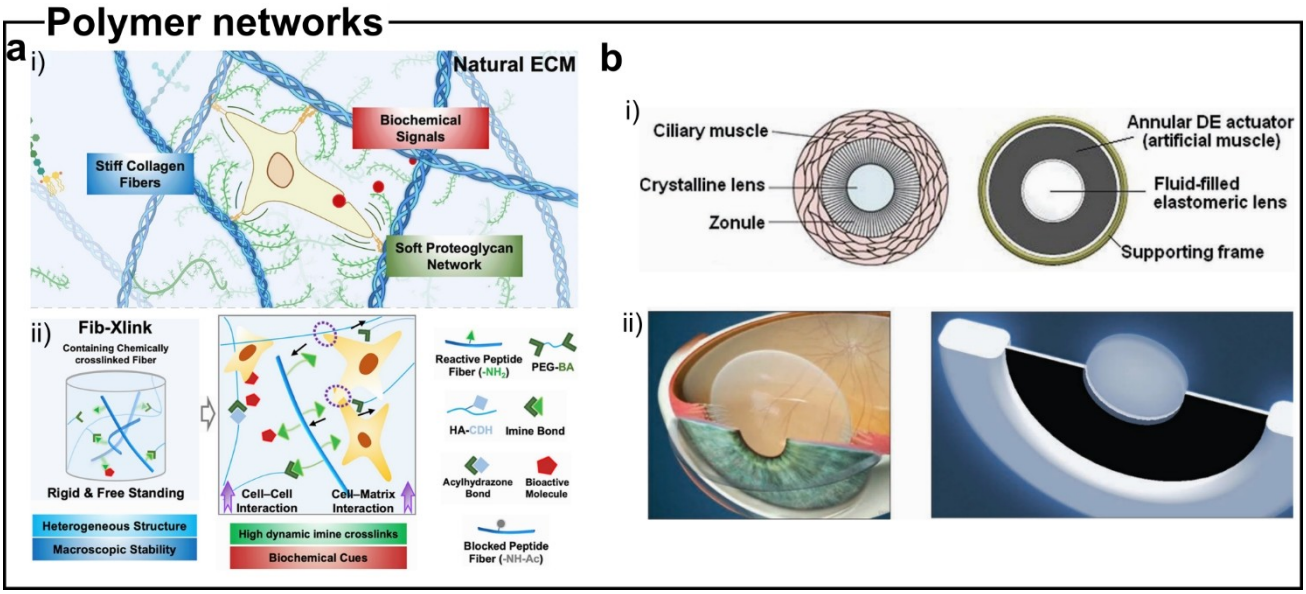


Figure 1. Higher-order behaviour in polymer networks. a, i) Schematic representation of a natural extracellular matrix (ECM); ii) Scheme describing the functioning principles of a synthetic bio-inspired hydrogel with integrated bioactive molecules capable of acting together to emulate the key functions of a natural ECM and promote enhanced responses in living cells. Adapted with permission from reference [16]. Copyright (2023) American Chemical Society; b, i) Schematic representation of the key components of a human eye and comparison to a bio-inspired polymer-based device, capable of collective muscle-like contractions; ii) Schematic cross-section of a human eye; iii) Schematic cross-section of the bio-inspired lens shown in i). Adapted from reference [25]. Copyright (2021) from Wiley.

non-covalent bonds was ascribed to as the cause for its self-healing and adhesive properties; combined, these properties, as well as the ability to slowly release the extracellular vesicles, allowed for the successful treatment of spinal cord injuries. Overall, these examples show the advantages of using hydrogels that display one or more higher-order behaviours, such as integrated self-assembled structures, synergistic behaviour, and the ability to self-heal, particularly in the regenerative medicine field.

While hydrogels can be a good example of bio-inspired materials that can be chemically programmed to display interesting higher-order behaviours, other bio-inspired self-assembled polymeric materials have been developed. For example, Li and co-workers induced the self-assembly of polyacrylonitrile, a piezoelectric polymer, into fibres to obtain nanogenerators capable of self-organizing into a three-dimensional (3D) environment for cellular growth. These materials showed sensibility to forces produced by cells (0.1–10 nN), which could turn into a potential of up to millivolts, electrically stimulating nearby living cells.[23] This approach based on mechano-electrical transduction is promising in the case of heart injury, where there is a consistent loss of the contractile and excitable cells, cardiomyocytes, which are replaced by non-contractile fibrotic scar tissue. Therefore, the restoration of heart electrical conduction resulted in a good chance for promoting myocardial repair.[24] This could be helped by employing such nanogenerators,[23] for example, by electrically inducing stem cell differentiation into cardiomyocytes for direct injection and heart failure treatment or similar approaches.

Another interesting higher-order behaviour that has been engineered using polymer networks is contractility. In Nature, muscular fibres exhibit a higher-order behaviour as they work together synergistically to achieve large-scale contractions. Carpi *et al.* used polymer-based elastomer membranes to mimic the muscle contractions that occur in the human eye to produce electrically tuneable lenses. Additionally, their system mimicked the natural architecture of the human eye, combining a synthetic muscle-like annular actuator and a dielectric elastomer (Figure 1b).[25] Remarkably, these lenses could achieve a relative change in focal length comparable to a human eye, showcasing how interconnecting two systems can allow them to join forces to yield emergent properties.

3. Microbots

Microbots or *nanorobots*, depending on their size, are micro or nanoparticles that can use the chemical energy of the surroundings to transform it into kinetic energy and thereby move autonomously, showing a smart interplay with their environment in order to fulfil specific tasks.[26] The autonomous movement of microbots has the potential to reshape the world of precision medicine. In this section, we focus on the two key higher-order behaviours that are most seen in microbots: autonomous taxis and swarming behaviour. The strategies for driving such groups of nanorobots (often referred to as *swarms*) include using magnetic, optical, or chemical gradients, which are exploited to promote long-range collective and coordinated actions (Figure 2). For example, Tang *et al.*[28] showed that sequential magnetic and acoustic actuation enabled the delivery of immunomagnetic beads, aiming at an accurate cancer therapy *in vivo*. The authors took advantage of taxis as a higher-order behaviour to drive the microbots across the inherent physical barriers associated with the tumour and its hostile environment, leading to local immunosuppression. To do so, the authors modified magnetic beads with a chimeric antigen receptor T (CAR T) using click chemistry. Significantly, the microbot could avoid obstacles and move against a flow of fluids *via* magnetic guidance, while acoustic propulsion enabled tissue penetration (Figure 2a). Furthermore, owing to its asymmetric shape, the microbot displayed an enhanced response to acoustic actuation. It also demonstrated a simulated response approximately 5 times higher to the same radiation force compared to CAR T cells. In tumour tissue models, the microbot achieved between 2- and 10-times greater depth penetration than magnetic-driven CAR T cells or acoustic-driven CAR T cells, respectively, showing distinctive properties over the individual constituents. Remarkably, these microbots could not only move to previously hard-to-access areas but also showed enhanced anticancer activity compared to the individual parts owing to higher target accuracy and greater tissue penetrability. The potentiality of magnetic microbots is indeed associated with their access to distant and intricate small places, such as microfluidic channels and ultra-small vessels within the organism, which makes them an instrumental new technology to perform therapeutic roles with minimum invasion to living systems.

Using a different approach than magnetic actuation, the in-situ generation of gas, Chen *et al.*[29] highlighted the potential impact of autonomously moving microbots in medicine. The authors obtained a 230 nm-sized asymmetric nanowire made of N-doped TiO_2 on one end and gold at the other. Visible light was used to excite the semiconductor. After charge separation, electrons were transferred to gold to cause hydrogen evolution *via* a water-splitting reaction. The gas generated at the gold tip, H_2, propelled these nanobots, generating movement away from the gas formation and irradiation points, showing a material that could propel itself in vitreous tissues using only visible light and without the need for connected wires. Remarkably, the nanorobot was able to move autonomously approximately 15 μm at a speed of around 1.7 $\mu m\,s^{-1}$ inside the vitreous tissue. This approach overcomes the challenge of the passive diffusion of drugs through the vitreous tissue often faced in ophthalmology, opening the door for minimally invasive treatments in high-precision ophthalmology.

In addition to medical applications, microbots with programmable taxis have found applications in the separation and purification of chemicals. An interesting example of enzymatically-driven chemotaxis was presented in the work by Mathesh *et al.*, where they took a step forward towards miniaturisation. Most microbots have 3D architectures.[28,30] In contrast, the authors designed a self-propelling 2D nanomotor by exploiting the hydrophobic interactions between graphene oxide and catalase (Figure 2b).[31] By placing this nanomotor in a concen-

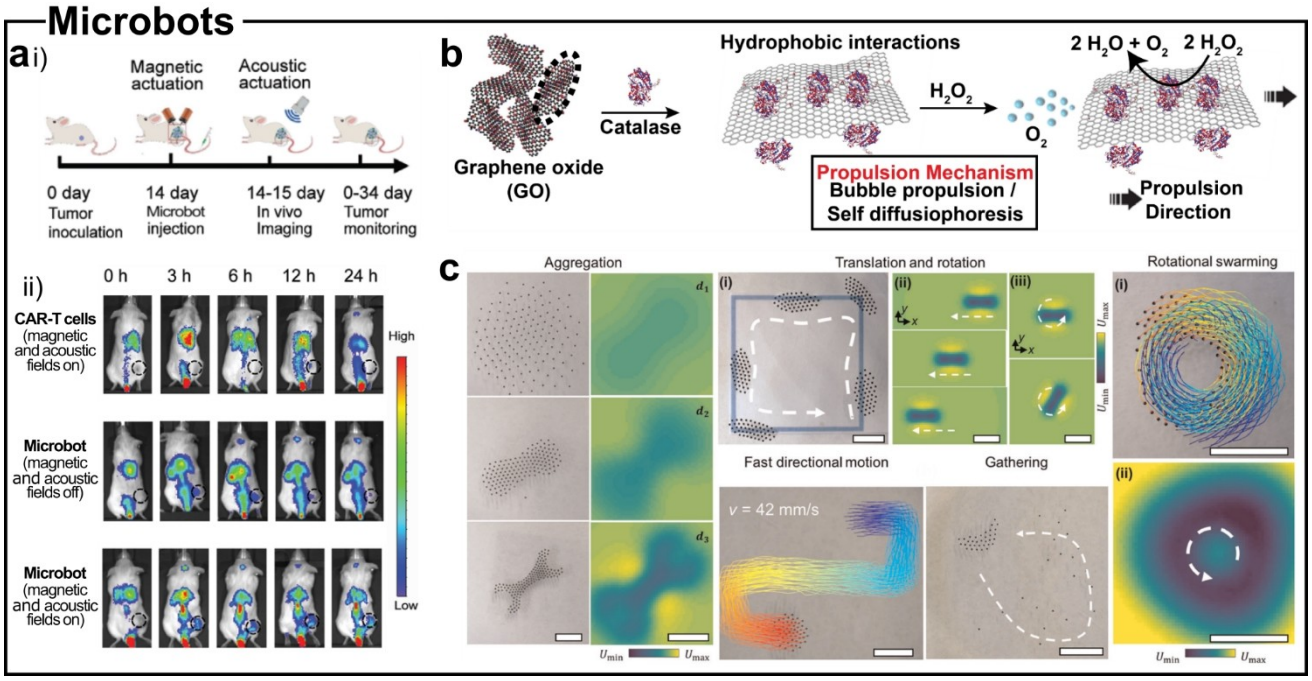


Figure 2. Higher-order behaviour in microbots. a, i) Schematic representation of the delivery procedure of the microbots to a living mouse for the treatment of tumours. ii) Methodology employed to monitor the position of cells and microbots with the actuation off or on. The tumour area is indicated with a dotted circle. Microbots with the actuation on can reach the target area significantly more efficiently. Adapted with permission from reference [28]. Copyright (2023) from Wiley; b, Schematic representation of the self-propulsion mechanism of a graphene oxide/catalase 2D microbot. Reproduced with permission from reference [31]. Copyright (2021) from Wiley; c, Magnetically controlled swarming behaviour of microbots showing collective movement. Reprinted with permission from reference [33]. Copyright (2020) SAGE Publications.

tration gradient of H_2O_2, chemotaxis and self-propulsion were achieved using an ultra-low concentration of fuel (0.003 % of H_2O_2), using the oxygen that is produced by the enzymatic reaction. Using a nanoparticle-tracking analysis, the authors showed that their nanomotor travelled around 4 μm could move with a maximum speed of *ca.* 8 $\mu m\,s^{-1}$. In addition to moving horizontally, this nanomotor exhibited buoyancy. It was, therefore, capable of negative geotaxis, a unique higher-order behaviour of this particular nanobot. Applications in remediation were proven *via* the removal of methylene blue (85 % efficiency), which was recollected by the graphene oxide sheets through multivalent interactions (π-π stacking, hydrophobic and electrostatic interactions).

Zhang and co-workers showed a similar example of programmable taxis in microbots, self-propelling microdroplets, by making a ternary combination of butylparaben, ethanol and water.[32] Under specific conditions, these microbots displayed two higher-order behaviours, as they could spontaneously self-assemble *via* liquid-liquid phase separation and self-propel, achieving maximal velocities of *ca.* 160 $\mu m\,s^{-1}$. Such movement was a consequence of the severe concentration gradient that formed upon phase segregation of the ternary mixture. The dynamics of the system were very complex since the self-propulsion of the microdroplets was shown to cause a replenishing flow between the walls of the container towards the place where phase separation occurred. This drove the mixture out-of-equilibrium and led to a complex, repeating set of micro-transporting events. This is a notable result because the transport of liquid in confined spaces finds several applications in the separation and purification of chemicals by membranes and other related technological processes.

Beyond simple taxis, Dong *et al.*[33] have shown the possibility of designing a general approach to obtain bidimensional static and dynamic formations of swarms of magnetic microbots. The microbots were made of microparticles of a neodymium–iron–boron (NdFeB) alloy and their casting onto resins to yield 20–50 wt % of NdFeB microparticles. The microrobots had radii between 50 μm to 175 μm and were up to 260 in total quantity. The authors set multiple static formations of the swarm *via* self-repelling interactions of the microrobots, arranged using diverse patterned magnetic potential energy maps. Likewise, dynamic behaviours were achieved *via* temporal and spatial programming of external magnetic fields. This allowed for the reversible control of the microbots, thereby replicating many swarming patterns typically observed in Nature, such as directional motion, rotational swarming and aggregation. These microrobotic swarms were, therefore, capable of higher-order collective tasks such as grasping, caging, and pushing, which demonstrated the cooperativity among multiple individual microbots, hence displaying a type of behaviour that cannot be performed by the individual microrobots (Figure 2c). While there is still room to improve such swarms because the actuation range is *ca.* 10 mm, this is a remarkable example of a higher-order behaviour as not one single nanobot can be attributed to having the ability to form patterns individually. Instead, the pattern formation emerges

when they are all placed together, showcasing system or community-level higher-order behaviour.

4. Protocells

Protocells are complex artificial life-like systems that mimic at least one key feature of living cells (*e.g.*, communication, growth and division, information processing, etc.).[34] In the field of bottom-up synthetic biology, these micro-compartmentalised systems were initially used to study the origin of life.[35] More recently, the concept of protocells has expanded beyond the origin of life to include out-of-equilibrium systems with potential applications in materials science, soft matter chemistry, and medicine.[36] As synthetic life-like systems, protocells exhibit the broadest range of bio-inspired higher-order behaviours, ranging from macroscopic movement to mimicking the integrated structures of living systems to ecosystem-like dynamics (*e.g.*, phagocytosis and predator-prey dynamics). Protocells can also exhibit the ability of reconfiguring their structures and self-transforming. In this Mini-Review, we consider these two higher-order behaviours as umbrella terms, due to their broadness. In fact, in the literature examples of self-transformation and structural reconfiguration range from simple membrane reconfiguration to more complex behaviours such as the growth of a cytoskeleton or the ability to grow and divide.

Polyoxometalate coacervate vesicles are a noteworthy example of simple membrane reconfiguration. Williams and co-workers demonstrated that inherently membraneless coacervate microdroplets, which are metastable, would spontaneously reconfigure upon the addition of phosphotungstic acid. The resulting protocells displayed a three-tiered structure comprised of an internal aqueous lumen, a coacervate sub-shell and a polyoxometalate membrane. Significantly, these reconfigured protocells retained their coacervate-like properties but displayed significantly increased stability.[37] Further examples of structural reconfiguration are highlighted later in the text.

A good example showcasing the higher-order behaviour of a protocell community to achieve a collective macroscopic change, was shown by Gobbo *et al.* The authors showed that a community of protocells trapped within a large capsule was capable of producing enough oxygen to lift it.[38] To do so, they membranised poly(diallydimethylammonium) chloride (PDDA) and adenosine 5′-triphosphate (ATP) coacervates using a synthetic enzyme capable of converting H_2O_2 into water and O_2 gas. Encapsulation of multiple of these protocells within a semi-permeable aminoclay/DNA membrane and exposure to H_2O_2 resulted in large amounts of *in-situ* O_2 production, which caused the macrocapsule to become buoyant (Figure 3a). A follow-up and more complex strategy for controlling the movement of protocells was later reported by Peschke *et al.*[39] The authors assembled semi-permeable protamine/DNA protocells and used the enzymes glucose oxidase (GOx) and catalase to power the system. Remarkably, they demonstrated several modes of oscillatory movement by regulating the protocell buoyancy, which was done using chemical gradients. To do so, the protocells were placed at the bottom of a compartment in contact with H_2O_2. The H_2O_2 was decomposed by catalase to yield O_2, thereby promoting the buoyancy of the protocells. Upon reaching the top of the compartment, the protocells came into contact with glucose, the substrate for GOx, which consumed the evolved endogenous oxygen, causing a geotactic movement. Such oscillatory movement could be sustained for 3 hours, and the protocells moved around 10 mm, displaying a variety of regular and damped oscillatory patterns. This core example opens the possibility of designing and consistently exploiting protocell systems for sequential delivery tasks on the microscale. Certainly, the chemotaxis associated with the movement of macroscopic bioinspired devices caused by chemical gradients in their surroundings is a fundamental leap forward for the construction of motile microdevices.

An important step towards the ambitious goal of building a cell is the mimicking of the cell's complex structures and functions. For example, the cell's skeletal framework plays a structural key role. In protocells, higher-order behaviours such as molecular self-assembly and structural reconfiguration can be exploited to achieve the integration of several components, yielding cytoskeleton-like structures that reinforce the overall protocell and endow it with emergent properties. Using DNA nanotechnology, Arulkumaran *et al.*[40] developed giant unilamellar vesicles (GUVs) with cyto- and exo-skeletons comprised of oligonucleotides, which could anneal into nanotubes or fibres. As seen with living cells, this synthetic cytoskeleton greatly stabilised the protocells, reinforcing their mechanical, functional and osmotic stability. This effect was attributed to the high contact and surface area between the nanotubes and the membrane of the protocells, which stemmed from the ability of the tubes to form hydrogels at very low concentrations (Figure 3b). In another work, Gao and co-workers[41] reported the construction of a membrane to encircle coacervate microdroplets obtained with the inclusion of a monolayer of Janus Au-polyethylene glycol (PEG) nanoparticles. This system was able to selectively capture nearby protocells that became inclusion guests. The authors employed an unlocking strategy of the membrane mediated by ligand dissociation of the Au-PEG nanoparticles using either light or chemical cleavage. The cleavage of the membrane exposed the coacervate interior to the outer environment, conducting to contact-dependent sequestration of external colloidal particles, which display attractive interactions with the coacervate. This work showed that the combination of a triggerable membrane permits the design of protocell networks mimicking integrated complex cytoplasmatic behaviours, including artificial networking, sorting, and symbiosis. This higher-order behaviour would permit a synergy driven by the coordinated behaviour of an entire population of protocells, envisioning collective resilience to environmental perturbation, multiple tasking, and functional collaboration, aiming eventually at applications in molecule delivery and microreactor technology.

Protocells have also been engineered to mimic in a rudimental manner the higher-order behaviour of living cells to divide and reproduce. For example, Dreher and co-workers[42] reported protocells with the ability to divide into two or more

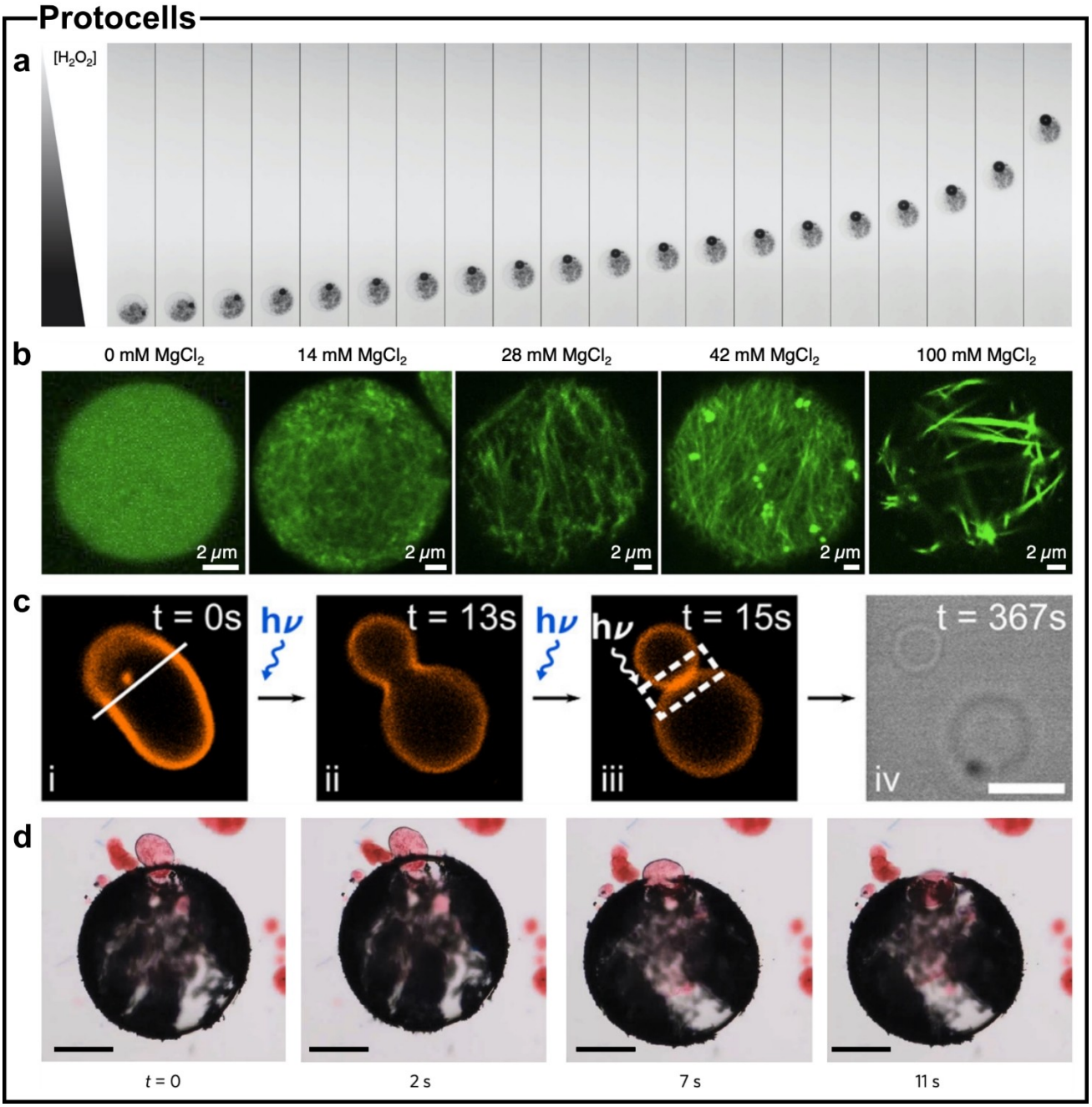


Figure 3. Higher-order behaviour in protocells. a, Composite images showing the collective generation of oxygen and subsequent chemotaxis of a macrocapsule containing catalytic protocells. Reproduced with permission from reference [38]. Copyright (2020) Springer Nature; b, Fluorescence microscopy images of a protocell cytoskeleton comprised of self-assembled DNA nanotubes and fibres formed at different concentrations of $MgCl_2$. Reproduced with permission from reference [40]. Copyright (2023) Springer Nature; c, Fluorescence and brightfield microscopy images showing the division of a protocell in the presence of the photosensitiser Chlorin e6 and under light irradiation. Reproduced with permission from reference [42]. Copyright (2021) ACS Publications; d, Phagocytosis-like behaviour shown by a magnetic Pickering emulsion droplet. Reproduced with permission from reference [43]. Copyright (2017) Springer Nature.

daughter protocells. Using a photosensitiser, Chlorin e6 (Ce6), the authors could selectively induce the peroxidation of unsaturated lipids within the GUVs' membrane. First, the protocells were osmotically deflated, which resulted in a spontaneous increase in the curvature that triggered a morphology change from a spheroidal to a dumbbell-like shape. In a second step, Ce6 was added to the aqueous media and the protocell was irradiated with light. Irradiation of Ce6 generated reactive oxygen species, which induced the formation of hydroperoxy groups next to the double bonds on the outer layer of the membrane, forcing the membrane to curve. Thus, within seconds of illumination, a tightening of the neck occurred, leading to fission and finally, the release of two newly formed spherical daughter GUVs (Figure 3c).

In a different approach towards achieving higher-order behaviours, Rodríguez-Arco and collaborators developed a system that used water-in-oil magnetic Pickering emulsion (MPE) droplets and colloidosomes to achieve phagocytosis-like behaviour (Figure 3d).[43] At high concentrations of oleic acid (2 mg mL^{-1}), a controlled opening could be generated on the shell of MPE droplets. Using a magnet, these magnetic droplets could be directed towards a population of much smaller colloidosomes, which were immediately and spontaneously engulfed by MPE droplets. This generated turbulence associated with a Marangoni convective flow, destabilised the iron-oxide membrane and gave the MPE droplets the ability to propel themselves. The authors were able to improve the system by using enzyme-loaded colloidosomes and encapsulating the complementary enzyme substrates within the MPE droplets to trigger structural and functional changes. By loading the colloidosomes with either catalase, lipase, or alkaline phosphatase they could promote buoyancy, membrane reconstruction, or hydrogelation, respectively, within the MPE droplets upon the engulfment of the colloidosomes.[3a]

More recently, Xu and co-workers[44] were able to combine the higher-order behaviours of protocell self-assembly, division, and phagocytosis, to achieve protocells that could mimic the cell cytosol, capture, and digest a pathogen. Using the Pickering emulsion technique, they were able to encapsulate bovine serum albumin (BSA)/poly(N-isopropylacrylamide) (PNIPAM) bioconjugates, dextran and artificial organelles within a BSA/PNIPAM-based protocell membrane called proteinosome. The intrinsically porous membrane of the proteinosome allowed the pathogens, Ab(1–40) peptides, to be phagocytised by the protocell. Once inside the protocell, the artificial organelles could efficiently capture and digest the pathogens owing to their protein-digestive properties. The dextran increased the viscosity of the internal lumen, providing a cytosol-like environment that prevented aggregation and protected the protein/polymer membrane of the proteinosomes from its organelles. Overall, this work exemplifies how careful chemical programming of small-scale interactions at a local level can, when combined, result in the emergence of not one but several complex and higher-order behaviours.

Single-protocell populations can achieve higher-order behaviours that range from chemotaxis to uptake and digestion of pathogens. However, their potential can be further explored by bringing together multiple communities of distinct protocells to study and emulate complex ecosystem dynamics that are seen in Nature. For example, Qiao *et al.* developed artificial communities of interacting protocells that exhibited a rudimentary form of predatory behaviour. This was achieved by combining a binary population of positively charged protease K-sequestered PDDA/ATP coacervate microdroplets and negatively charged BSA/PNIPAM proteinosomes. The coacervate protocells were able to kill the proteinosome population and take up their payload. This process was driven by electrostatic forces and consisted of an initial electrostatic attachment, a subsequent protease-induced proteinosome disassembly, followed by a payload transfer and release of the modified coacervate population.[45] In a later paper, Qiao and co-workers[46] showed diverse predation strategies that could be coupled temporally and spatially to yield an "eye-for-an-eye" behaviour in a system that is capable of antagonistic interactions mediated by enzymes. This consortium was formed by (*a*) GOx-containing proteinosomes, which were sensitive to the action of proteases; (*b*) pH-sensitive polypeptide-mononucleotide coacervates containing proteinase K, and (*c*) coacervate droplets made of polymer-polysaccharides that were pH-resistant and bound to (*a*). The initial input to this system was glucose, which caused the secretion of H^+ from (*a*), causing the disassembly of (*b*) and subsequent release of protease K. Protease K was then taken up by (*c*) which brought the delayed contact-dependent killing of the proteinosomes and, therefore, the termination of glucose oxidase activity (Figure 4b). This is a nice example of mesoscale coordination *via* programmable response-retaliation dynamics. Such a response is only possible by combining antagonistic interactions between diverse members of an interacting consortium and is thereby enabled by the spatial positioning of individual protocells and the dynamics and efficiency of the internalised reactions.

Interesting perspectives of this work include the determination of population dynamics within these protocell consortia, *via* the precise control of the amount of predatory proteinosomes. These examples showcase the higher-order ecosystem-like dynamics that can arise when carefully tuning the properties of individual protocells to recognise and respond to stimuli produced by a neighbouring population. Finally, the ability to communicate is one of the key higher-order behaviours that form the basis of life.[47] Communication within protocells has been studied extensively and reviewed.[36b,48] Thus, here we will focus on the emergence of properties that arise from bringing into proximity and spatially localising protocell populations capable of communicating with each other. For example, in Taylor *et al.*,[49] the authors use microfluidics to generate Janus-like alginate capsules with two spatially segregated enzyme-loaded colloidosome populations. Significantly, chemical signalling within the spatially localised communities gives rise to increased enzyme kinetics compared to a homogeneous distribution of the same colloidosome populations (Figure 4c). Buddingh and co-workers[50] reported similar findings on signal propagation in protocell communities. Their work highlights the importance of the small-scale interactions between the individual units which, when acting collectively result in higher-order behaviours. The authors assembled a two-population sender-receiver protocell populations loaded with enzymes. The sender protocells were capable of detecting ATP or adenosine 5′-diphosphate (ADP) and converting it into a signalling molecule, adenosine 5′-monophosphate (AMP). Receiver protocells could produce a response – the conversion of NAD^+ to NADH. Interestingly, in the presence of the sender protocells, the receiver protocells produced an enhanced response compared to when no sender protocells were nearby. Remarkably, this response could be amplified to up to 200 times the diameter of the sender protocells, which was exploited to propagate a signalling front across a 5 mm channel (Figure 4c).

Together, these examples highlight the importance of the hierarchical organisation of protocells within a 3D space,

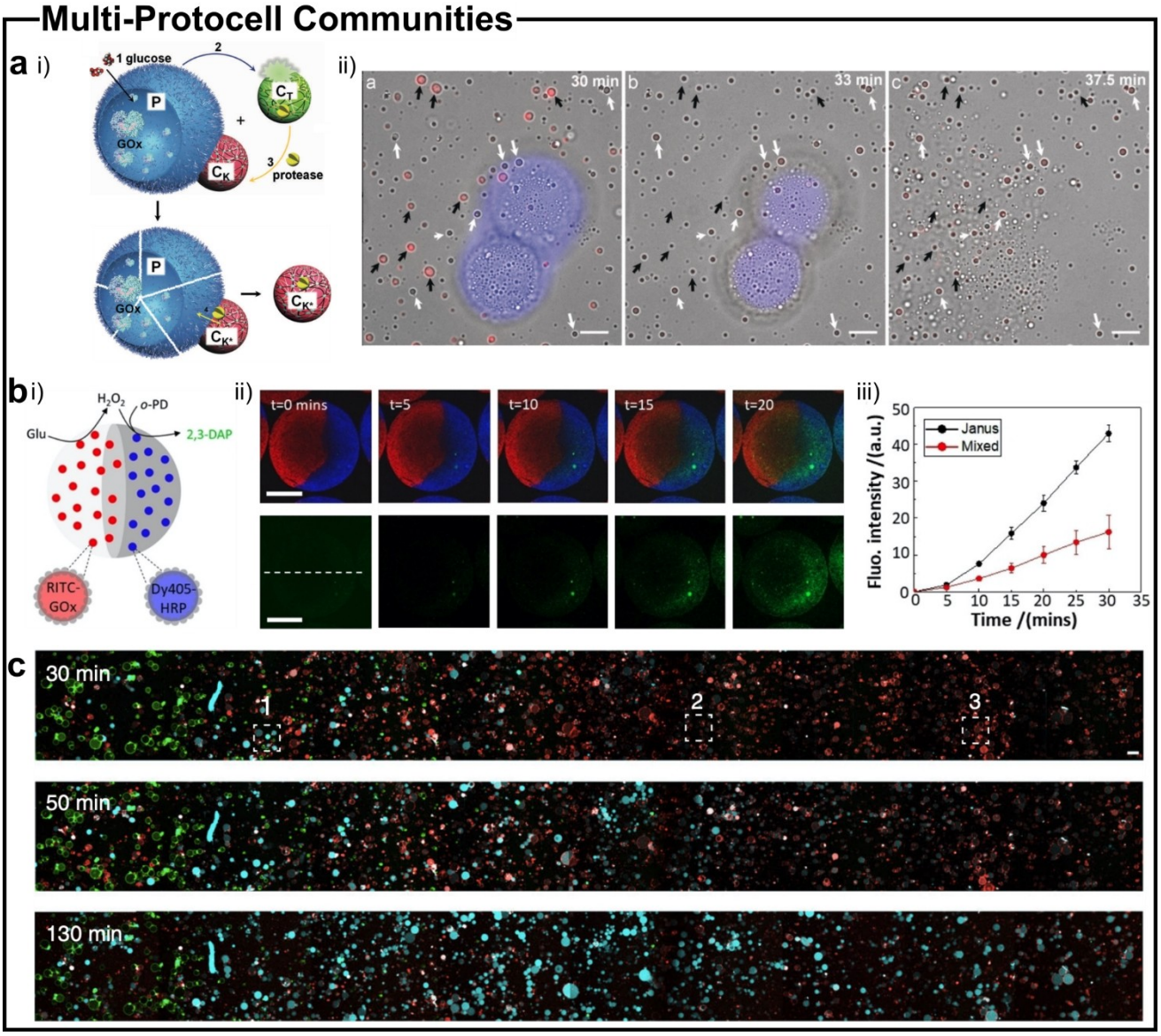


Figure 4. Higher-order behaviour in multi-protocell communities. a, Ecosystem-like dynamics in three protocell populations. i) Scheme showing the "an eye-for-an-eye" dynamics; GOx-containing proteinosomes can increase the pH of the environment, causing the pH-sensitive, protease-loaded coacervates to disassemble. A second pH-resistant coacervate population that is bound to the proteinosome can take up the protease and cause the death of the proteinosome. ii) Overlay of brightfield and fluorescence microscopy image showing the system highlighted in i). The GOx-proteinosomes are shown in blue. Black and white arrows indicate the pH-sensitive and protease-loaded coacervates (red fluorescence), and pH-resistant coacervates, respectively. Adapted with permission from reference [46]. Copyright (2019) Wiley; b, i) Enzyme-mediated communication between two spatially localised protocell communities (red and blue fluorescence). The response appears as green fluorescence. ii) Plot showing the time-dependent fluorescence increase of multi-population capsules in either a mixed (red plot) or a Janus (black plot) configuration, showing how spatial localisation of the protocells within a 3D structure can result in an enhanced response. Adapted with permission from reference [49]. Copyright (2023) Wiley; c, Fluorescence imaging monitoring of the directed propagation of a signalling chemical (cyan fluorescence) from a population of sender protocells (green fluorescence) and receiver protocells (red fluorescence) over time. Adapted with permission from reference [50]. Copyright (2020) Springer Nature.

showcasing how bringing together protocells can result in emergent behaviours.

5. Prototissues

In Nature, emergence, and consequently higher-order behaviours arise from the 3D organisation of individual units.[1,9b] Spatially interlinked cells that can communicate and collaboratively work as a unit towards achieving a function are known as tissues. Similarly to natural tissues, *prototissues*, or *protocellular materials*, are artificial networks of spatially interlinked protocells that can communicate and display higher-order behaviours.[51] The most notable examples of higher-order behaviour in prototissues include long-range communication and signal propagation, the emergence of novel properties, such as macroscopic deformation, and the enhancement of

properties compared to individual or non-crosslinked protocells (Figure 5).

Several examples of communication inside tissue-like materials composed by different type of protocells have been reported. This is shown by combining the specialisation of individual protocell types with the emergent spatiotemporal biochemical response of the complex structure. In detail, several authors exploit an enzymatic cascade, of two or more enzymes, to demonstrate the possibility of developing a complex structure capable of communication. Some examples of enzymatic cascade between two highly used enzymes, such as GOx and HRP, within synthetic tissues have been reported in the last few years.[52] Liu *et al.* produced a tubular prototissue-like vessel capable of modulating a bioactive output. They studied a way to spatially segregate chemically communicating populations of enzyme-loaded polymer/DNA coacervate with phospholipidic membrane, within hydrogels that organise concentrically and specifically. Significantly, they demonstrated that the functionality of the prototissue depends on the spatial organisation of the modules, because transposing or removing any modules resulted in different outputs.[53]

The Bayley group pioneered the first strategy to assemble tissue-like structures by using a 3D printing technique.[54] This technique involved printing picoliter aqueous droplets either in a bulk oil solution or in oil droplets in an oil-in-water emulsion. The oil phases contained lipids which assembled around the water droplets to create single-lipid monolayer compartments. When in contact with each other, neighbouring compartments would rearrange into semi-permeable lipid bilayers. The individual droplets could be loaded with varying concentrations of salts, causing them to swell due to osmosis. Significantly, this resulted in macroscopic deformation, yielding complex 3D-folded structures (Figure 5a).[54a] Additionally, long-range intra-tissue communication was achieved by connecting the protocells with the pore-forming protein α-hemolysin (α-HL), and creating an ionically conductive path across the otherwise insulating tissue. Booth *et al.* improved on this work by making this communication light-activated[54b] and light-controlled.[54c] To do so, they designed and mixed with the oil phase a light-activated DNA system - a transcription/translation promoter, which would only express a protein upon being irradiated with ultraviolet light - and a cell-free protein expression system.[54b]

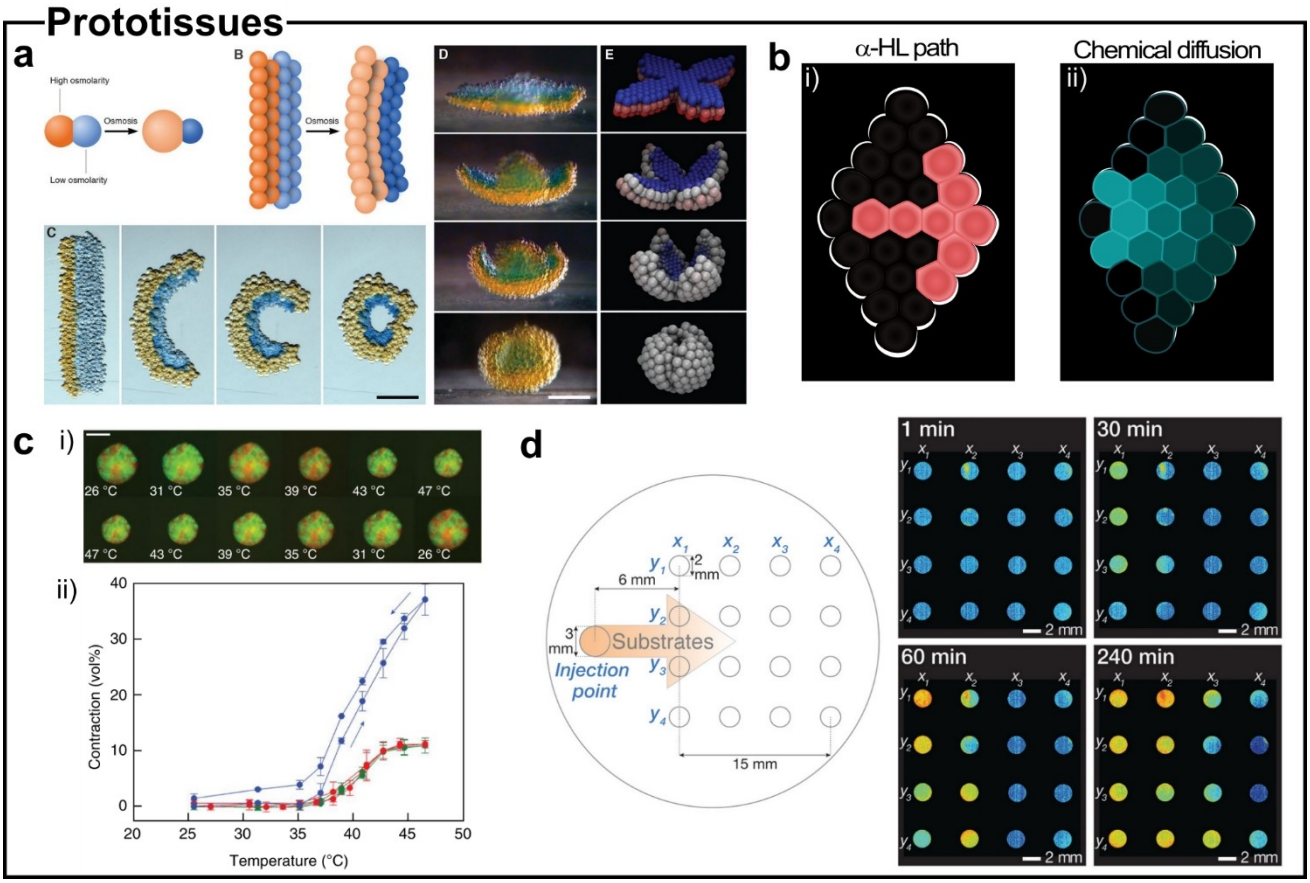


Figure 5. Examples of high-order behaviour in prototissues. a, Semi-permeable droplets arranging in 3D-priting tissue-like structure showing a collective macroscopic deformation due to the osmotic swelling of individual protocells. Reproduced from reference [54a]. Reprinted with permission from AAAS.; b, Controlled long-range communication in prototissues. i) Path created with the pore-forming protein α-hemolysin (α-HL), connecting neighbouring protocells. ii) long-range diffusion of a fluorescent chemical through the tissue following the pattern shown in i). Scheme adapted from reference [55]; c, i) Reversible thermal contraction of a prototissue. ii) Comparison between the reversible thermal contraction of prototissues (blue) with individual proteinosomes (green and red), showcasing the increased contraction in the prototissues. Reproduced from reference [56]. Copyright (2018), with permission from Springer Nature; d, Spatially organised protocellular materials in a 4x4 2D array, showcasing their collective ability to detect chemical gradient fronts. Adapted from reference [57]. Copyright (2021), with permission from Springer Nature.

Using α-HL, they were able to connect the compartments and show the light-controlled expression of a protein.[54b] Incorporating a reversible photoswitch allowed for patterning of the tissue, resulting in a gradient of protein expression, something that could not be achieved by 3D printing only.[54c] In a similar system, Dupin and co-workers[55] used α-HL to propagate a chemical signal through a prototissue and achieve long-range communication across the material (Figure 5b).

Gobbo *et al.* used proteinosomes to produce thermo-contractile tissues. To do so, they used proteinosomes prepared with a thermoresponsive polymer, poly(*N*-isopropylacrylamide-co-methacrylic acid) and functionalised with azide or strained alkyne (bicyclononyne) groups. Using the Pickering emulsion technique, the proteinosomes could be crosslinked through bio-orthogonal chemistry yielding thermoresponsive synthetic prototissues spheroids. Notably, the prototissues displayed enhanced contractions compared to caged but non-bound proteinosomes, contracting to 37% and 11% of their initial volume, respectively (Figure 5c). This contractability was then exploited to turn on and off enzyme-based communication between the caged protocell populations with a temperature ramp, demonstrating a *ca.* 53% decrease in the initial reaction rate of the enzyme cascade at 45 °C *vs* 25 °C due to the thermally-induced contraction of the prototissue.[56] These results showcased the importance of spatially interlinking individual units to produce a coordinated response that resulted in enhanced and emergent properties. Galanti *et al.*[57] improved on this work to develop a method to assemble centimetre-sized free-standing protocellular materials reproducibly. When placed and immobilised into a 2D array, these prototissues could collectively respond to concentration fronts of chemical gradients (Figure 5d). This system used a bio-inspired thermoresponsive polymer to assemble thermoresponsive protocells, which could be interlinked to form thermoresponsive prototissues which displayed enhanced and emergent properties. These prototissues could be spatially organised into 2D arrays to display collective behaviour. Overall, this system beautifully illustrates that, as seen in living systems, when synthetic materials are brought together, interconnected and spatially localised, they can join forces and exhibit enhanced capabilities greater than the sum of their constituent parts.

6. Summary and Outlook

The understanding and replication of the concept of "emergence", ubiquitous to living systems, remains one of the major challenges of modern science. In particular, the transition from simple bio-inspired materials to bio-inspired out-of-equilibrium material systems endowed with higher-order behaviours still requires exploration and innovation.

This Mini-Review has provided an overview of the state-of-the-art systems that have successfully replicated some higher-order behaviours, focusing on polymer networks, microbots, protocells, and prototissues. By reporting key examples within each category, we showcased the strategies that gave rise to rudimental higher-order behaviours such as self-assembly, contractility, swarming, taxis, chemical communication, or predator-prey dynamics, and highlighted that the careful chemical programming of the material systems played a pivotal role.

Achieving emergence, or higher-order behaviours in synthetic materials, involves overcoming several challenges. The majority of the higher-order behaviours reported in the bio-inspired materials developed so far are based on observations or serendipity. Therefore, a detailed understanding of the fundamental physical and chemical processes underlying the principles and mechanisms that result in higher-order behaviours is still needed. The successful design and assembly of bio-inspired materials that can display higher-order behaviours as seen in living systems will require the improvement and development of new chemical systems and experimental methodologies. These will need to be developed with the aim to achieve higher reproducibility and scalability. Ensuring consistent reproducibility, performance, and scalability is essential for the use of these bio-inspired materials in real-world applications. Moreover, if we consider applications in tissue engineering and regenerative medicine, there will also be the need to consider the compatibility and interaction of the material with living systems. While many bio-inspired materials are not inherently toxic to living organisms, their working conditions may be incompatible with biomedical applications. Going beyond simple biocompatibility to achieving a seamless integration between bio-inspired and living systems is a crucial challenge yet to be addressed. Historically, hydrogels have pioneered the integration between living and bio-inspired systems. As mentioned in section 2, these materials have already been successfully utilised to replicate the complex environments of living tissues, such as the ECM. In addition to theragnostic applications, successful hydrogel-based therapies included the treatment of kidney diseases,[29,58] wound dressing,[59] wound healing[60] and heart tissue repair.[61] Indeed, hydrogel-based technologies set the basis for the development of integrated, low-cost, and sustainable technologies that other bio-inspired materials may follow.

From a general perspective, the bio-inspired materials and the higher-order behaviours discussed contribute to narrowing the gap between non-living and living matter setting a new paradigm for materials science. As these advanced materials stand at the crossroads of materials science and systems chemistry, they represent a milestone in a pathway that leads to unprecedented technological advancements with potential applications in areas ranging from tissue engineering to regenerative medicine and soft robotics.

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Conflict of Interests

The authors declare no conflict of interest.

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[1] a) T. Veloz, *Front. Bioeng. Biotechnol.* **2021**, *9*; b) S. Baron, *Philos. Stud.* **2020**, *177*, 2207–2226; c) S. Kalantari, E. Nazemi, B. Masoumi, *J. Organ. Comput. Electron. Commer.* **2020**, *30*, 224–265; d) G. G. L. Guardia, P. J. Miranda, *Axiomathes* **2022**, *32*, 1059–1103; e) J. de Haan, *Ecol. Complex.* **2006**, *3*, 293–301.
[2] F. Toates, *Conscious. Cogn.* **2006**, *15*, 75–118.
[3] a) L. Rodríguez-Arco, B. V. V. S. P. Kumar, M. Li, A. J. Patil, S. Mann, *Angew. Chem. Int. Ed. Engl.* **2019**, *58*, 6333–6337; b) N. Gao, S. Mann, *Acc. Chem. Res.* **2023**, *56*, 297–307.
[4] P. Friedl, D. Gilmour, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 445–457.
[5] R. Popat, D. M. Cornforth, L. McNally, S. P. Brown, *J. R. Soc.* **2015**, *12*, 20140882.
[6] J. Bräuer, K. Stenglein, F. Amici, *J. Comp. Psychol.* **2020**, *134*, 211–221.
[7] J. Drury, *Curr. Opin. Psychol.* **2020**, *35*, 12–16.
[8] a) B. Ladoux, R.-M. Mège, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 743–757; b) E. A. Kane, T. E. Higham, *Integr. Comp. Biol.* **2015**, *55*, 146–165.
[9] a) F. Ji, Y. Wu, M. Pumera, L. Zhang, *Adv. Mater.* **2023**, *35*, 2203959; b) Q. Yang, P. Liberali, *Curr. Opin. Cell Biol.* **2021**, *72*, 81–90.
[10] S. M. Allameh, A. Lenihan, in *Biomimicry for Materials, Design and Habitats* (Eds.: M. Eggermont, V. Shyam, A. F. Hepp), Elsevier **2022**, pp. 25–56.
[11] a) P. L. Luisi, *Origins Life Evol. Biospheres* **1998**, *28*, 613–622; b) D. E. Koshland, *Science* **2002**, *295*, 2215–2216.
[12] a) N. A. Yewdall, A. F. Mason, J. C. M. van Hest, *Interface Focus* **2018**, *8*, 20180023; b) F. Kiakojouri, V. De Biagi, L. Abbracciavento, *Biomimetics* **2023**, *8*, 95.
[13] A. Walther, *Adv. Mater.* **2020**, *32*, 1905111.
[14] N. Giuseppone, A. Walther, in *Out-of-Equilibrium (Supra)molecular Systems and Materials* **2021**, pp. 1–19.
[15] B. L. Dargaville, D. W. Hutmacher, *Nat. Commun.* **2022**, *13*, 4222.
[16] X. Xie, Z. Li, X. Yang, B. Yang, Z. Zong, X. Wang, L. Duan, S. Lin, G. Li, L. Bian, *J. Am. Chem. Soc.* **2023**, *145*, 15218–15229.
[17] a) P. Cui, P. Pan, L. Qin, X. Wang, X. Chen, Y. Deng, X. Zhang, *Bioact Mater* **2022**, *19*, 487–498; b) X. Ma, R. Yang, P. Wang, S. Liu, H. Xu, Z. Ye, B. Chi, *Mater. Today Chem.* **2022**, *23*, 100648; c) A. B. W. Brochu, S. L. Craig, W. M. Reichert, *J. Biomed. Mater. Res. Part A* **2011**, *96 A*, 492–506; d) D. L. Taylor, M. in het Panhuis, *Adv. Mater.* **2016**, *28*, 9060–9093.
[18] M. L. Vainieri, A. Lolli, N. Kops, D. D'Atri, D. Eglin, A. Yayon, M. Alini, S. Grad, K. Sivasubramaniyan, G. J. V. M. van Osch, *Acta Biomater.* **2020**, *101*, 293–303.
[19] E. Prince, J. Cruickshank, W. Ba-Alawi, K. Hodgson, J. Haight, C. Tobin, A. Wakeman, A. Avoulov, V. Topolskaia, M. J. Elliott, A. P. McGuigan, H. K. Berman, B. Haibe-Kains, D. W. Cescon, E. Kumacheva, *Nat. Commun.* **2022**, *13*, 1466.
[20] T. Ahmed, *In vitro models* **2023**, *2*, 1–23.
[21] Y. Guo, B. Xie, M. Jiang, L. Yuan, X. Jiang, S. Li, R. Cai, J. Chen, X. Jiang, Y. He, G. Tao, *J. Nanobiotechnol.* **2023**, *21*, 266.
[22] C. Wang, M. Wang, K. Xia, J. Wang, F. Cheng, K. Shi, L. Ying, C. Yu, H. Xu, S. Xiao, C. Liang, F. Li, B. Lei, Q. Chen, *Bioact Mater* **2021**, *6*, 2523–2534.
[23] T. Li, C. Shi, F. Jin, F. Yang, L. Gu, T. Wang, W. Dong, Z.-Q. Feng, *Sci. Adv.* **2021**, *7*, eabh2350.
[24] L. M. Monteiro, F. Vasques-Nóvoa, L. Ferreira, P. Pinto-do-Ó, D. S. Nascimento, *NPJ Regen.* **2017**, *2*, 9.
[25] F. Carpi, G. Frediani, S. Turco, D. De Rossi, *Adv. Funct. Mater.* **2011**, *21*, 4152–4158.
[26] D. Jin, L. Zhang, *Nat. Mach. Intell.* **2020**, *2*, 663–664.
[27] S. Fu, B. Chen, D. Li, J. Han, S. Xu, S. Wang, C. Huang, M. Qiu, S. Cheng, X. Wu, L. Zhang, S. Du, T. Xu, *Adv. Intell. Syst.* **2023**, *5*, 2300267.
[28] X. Tang, Y. Yang, M. Zheng, T. Yin, G. Huang, Z. Lai, B. Zhang, Z. Chen, T. Xu, T. Ma, H. Pan, L. Cai, *Adv. Mater.* **2023**, *35*, 2211509.
[29] B. Chen, M. Ding, H. Tan, S. Wang, L. Liu, F. Wang, H. Tian, J. Gao, Y. Ye, D. Fu, J. Jiang, J. Ou, D. A. Wilson, Y. Tu, F. Peng, *Appl. Mater. Today* **2022**, *27*, 101455.
[30] J. G. Lee, R. R. Raj, N. B. Day, C. W. I. V. Shields, *ACS Nano* **2023**, *17*, 14196–14204.
[31] M. Mathesh, E. Bhattarai, W. Yang, *Angew. Chem. Int. Ed.* **2022**, *134*, e202113801.
[32] X. Zhang, J. B. You, G. F. Arends, J. Qian, Y. Chen, D. Lohse, J. M. Shaw, *Soft Matter* **2021**, *17*, 5362–5374.
[33] X. Dong, M. Sitti, *Int. J. Rob. Res.* **2020**, *39*, 617–638.
[34] C. Xu, S. Hu, X. Chen, *Mater. Today* **2016**, *19*, 516–532.
[35] a) I. Gözen, E. S. Köksal, I. Põldsalu, L. Xue, K. Spustova, E. Pedrueza-Villalmanzo, R. Ryskulov, F. Meng, A. Jesorka, *Small* **2022**, *18*, 2106624; b) J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* **2001**, *409*, 387–390.
[36] a) W. Jiang, Z. Wu, Z. Gao, M. Wan, M. Zhou, C. Mao, J. Shen, *ACS Nano* **2022**, *16*, 15705–15733; b) A. Rebasa-Vallverdu, S. Valente, A. Galanti, M. Sbacchi, F. Vicentini, L. Morbiato, B. Rosetti, P. Gobbo, *EurJOC* **2023**, *26*, e202300529.
[37] D. S. Williams, A. J. Patil, S. Mann, *Small* **2014**, *10*, 1830–1840.
[38] P. Gobbo, L. Tian, B. V. V. S. Pavan Kumar, S. Turvey, M. Cattelan, A. J. Patil, M. Carraro, M. Bonchio, S. Mann, *Nat. Commun.* **2020**, *11*.
[39] P. Peschke, B. V. V. S. P. Kumar, T. Walther, A. J. Patil, S. Mann, *Chem* **2023**, *10*, 600–614.
[40] N. Arulkumaran, M. Singer, S. Howorka, J. R. Burns, *Nat. Commun.* **2023**, *14*, 1314.
[41] N. Gao, C. Xu, Z. Yin, M. Li, S. Mann, *J. Am. Chem. Soc.* **2022**, *144*, 3855–3862.
[42] Y. Dreher, K. Jahnke, M. Schröter, K. Göpfrich, *Nano Lett.* **2021**, *21*, 5952–5957.
[43] L. Rodríguez-Arco, M. Li, S. Mann, *Nat. Mater.* **2017**, *16*, 857–863.
[44] X. Xu, S. Moreno, M. Gentzel, K. Zhang, D. Wang, B. Voit, D. Appelhans, *Small Methods* **2023**, *n/a*, 2300257.
[45] Y. Qiao, M. Li, R. Booth, S. Mann, *Nat. Chem.* **2017**, *9*, 110–119.
[46] Y. Qiao, M. Li, D. Qiu, S. Mann, *Angew. Chem. Int. Ed.* **2019**, *58*, 17758–17763.
[47] R. Merindol, A. Walther, *Chem. Soc. Rev.* **2017**, *46*, 5588–5619.
[48] a) P. J. Grimes, A. Galanti, P. Gobbo, *Front. Mol. Biosci.* **2021**, *8*; b) V. Mukwaya, S. Mann, H. Dou, *Commun. Chem.* **2021**, *4*; c) L. Aufinger, F. C. Simmel, *Chem. Eur. J.* **2019**, *25*, 12659–12670.
[49] H. Taylor, N. Gao, S. Mann, *Angew. Chem. Int. Ed.* **2023**, *62*.
[50] B. C. Buddingh', J. Elzinga, J. C. M. Van Hest, *Nat. Commun.* **2020**, *11*.
[51] P. Gobbo, *Biochem. Soc. Trans.* **2020**, *48*, 2579–2589.
[52] X. Zhang, C. Li, F. Liu, W. Mu, Y. Ren, B. Yang, X. Han, *Nat. Commun.* **2022**, *13*, 2148.
[53] S. Liu, Y. Zhang, X. He, M. Li, J. Huang, X. Yang, K. Wang, S. Mann, J. Liu, *Nat. Commun.* **2022**, *13*, 5254.
[54] a) G. Villar, A. D. Graham, H. Bayley, *Science* **2013**, *340*, 48–52; b) M. J. Booth, V. R. Schild, A. D. Graham, S. N. Olof, H. Bayley, *Sci. Adv.* **2016**, *2*, e1600056; c) M. J. Booth, V. Restrepo Schild, S. J. Box, H. Bayley, *Sci. Rep.* **2017**, *7*, 9315.
[55] A. Dupin, F. C. Simmel, *Nat. Chem.* **2019**, *11*, 32–39.
[56] P. Gobbo, A. J. Patil, M. Li, R. Harniman, W. H. Briscoe, S. Mann, *Nat. Mater.* **2018**, *17*, 1145–1153.
[57] A. Galanti, R. O. Moreno-Tortolero, R. Azad, S. Cross, S. Davis, P. Gobbo, *Adv. Mater.* **2021**, *33*, 2100340.
[58] G. Feng, J. Zhang, Y. Li, Y. Nie, D. Zhu, R. Wang, J. Liu, J. Gao, N. Liu, N. He, W. Du, H. Tao, Y. Che, Y. Xu, D. Kong, Q. Zhao, Z. Li, *J. Am. Soc. Nephrol.* **2016**, *27*, 2357–2369.
[59] a) B. George, N. Bhatia, A. Kumar, G. A, T. R, S. S. K, K. Vadakkadath Meethal, S. T. M, S. T. V, *Scientific Reports* **2022**, *12*, 13735; b) H. Ren, Z. Zhang, X. Cheng, Z. Zou, X. Chen, C. He, *Science Advances* **2023**, *9*, eadh4327.
[60] Y. Guo, B. Xie, M. Jiang, L. Yuan, X. Jiang, S. Li, R. Cai, J. Chen, X. Jiang, Y. He, G. Tao, *Journal of Nanobiotechnology* **2023**, *21*, 266.
[61] L. Zhang, T. Li, Y. Yu, K. Shi, Z. Bei, Y. Qian, Z. Qian, *Bioact Mater* **2023**, *20*, 339–354.

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