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Perspective

Motility of microscopic swimmers as protocells

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SUMMARY

Encoding life-like behavior in prebiotically plausible compartments, for example growth and reproduction, has been a major focus of protocell research. We argue that, in addition to these essential features of dynamic protocellular systems, motility could have also contributed to a significant degree in the emergence, survival, and complexification of protocells because it would endow these compartments with autonomous behavior and with the possibility to adapt to the environment. Therefore, we have looked into plausible mechanisms by which motility can be imparted to synthetic compartments in fluids, with a special focus on the prebiotic relevance of these compartments. The discussion is organized based on salient features of motile behavior, such as random walk, gravitaxis, chemotaxis, predator-and-pray, stop-and-go, and helical trajectories. In the conclusion, we suggest a motility-based scenario for the formation of stable vesicles from early lipids. The sophisticated movement of these compartments in aqueous environements could also find applications in healthcare, e.g., to deliver cargo.

THE BIGGER PICTURE

Challenges and opportunities:

- Purposeful movement is a hallmark of life, but its molecular origins remain mostly unknown.Research on the motile behaviour of protocells contributes to plausible scenarios on the molecular origins of life.
- Achieving controlled and directional mass transport in complex and possibly even crowded environments will facilitate the development of complex materials, including neuromorphic materials.
- Research on the autonomous movement of protocells as nano- and micro-motors paves the way toward innovative healthcare approaches.

INTRODUCTION

From the earliest times, movement has been seen as a hallmark of life. Leonardo Da Vinci alluded to it when writing "il moto è causa d'ogni vita" in the Codex Trivulanius (1487).[1] Later it was the motion of the first microbes, or "animalcules," that led van Leeuwenhoek to believe that they were living organisms rather than inanimate matter.[2] Indeed, all living systems move to survive, although for each living system, the fundamental physico-chemical mechanisms that are involved are entirely different, and if there was the possibility of a common ancestor for motility, then it has not been found.[3]

The human mind intuitively recognizes the presence of life; however, it is difficult to define what life is with chemical concepts; instead, scientists have set some minimal criteria, such as compartmentalization, metabolism, and reproduction. We argue that autonomous movement, which includes swimming, locomotion, and shape transformation, might be an additional defining feature. Autonomous movement would have provided such a substantial evolutionary advantage to living systems, from the earliest stages, that it became the norm in living systems.

In modern life forms, the result of millions of years of evolution, movement is supported by biomolecular machines, e.g., famously myosin and the bacterium flagellar motor. These biomolecular machines are complex protein systems that have likely emerged at a late stage in chemical evolution. In contrast, we are interested in the fundamental physical and chemical mechanisms that would have supported directional and possibly purposeful movement at the earliest stages. Before the emergence of life, prebiotic Earth was covered by an ocean in which small molecules

would have been too diluted for chemical reaction networks to develop. Therefore, there is a general agreement that life emerged in primeval microscopic compartments, called protocells,[4–7] where chemicals would concentrate and possibly also be preserved from disruption by the environment.

Examples of how motility would have supported protocell survival and their evolutionary predominance include the following:

(1) In comparison to diffusion and Brownian motion, either a random walk or a directed type of motility can significantly increase an organism's or protocell's chances of finding a new patch of nutrients in a heterogeneous environment.[8]
(2) In growth and self-replication, motility is crucial in order to gather and assemble building blocks into a new protocell.[9]
(3) In an ecological context, the advantage brought by motility can match that brought by growth, allowing a fast-growing and a fast-moving population to co-exist.[10]

The above examples show that, besides being a hallmark of life, motility dialogues greatly with other cell functions. This synergy is rarely explored in current models, with perhaps the only example being the interplay between chemotaxis and physical autocatalysis that was evidenced by our group.[11] In turn, growing protocell models have been studied specifically in an inert setting,[12] for motion introduces a complexity that makes it experimentally challenging to measure other associated properties. Growth, on the other hand, complicates models of motility because it undermines the constant particle number constraint. However, it is clear that expanding colonies are affected by both growth rates and migration rates, with migration creating flows in its own medium, that can hamper or facilitate growth.[13]

Three mechanisms for the motile behavior of microscopic objects have been proposed so far (Figure 1A).[14] They are as follows: (1) movement by the production of gas bubbles (propulsion or diffusiophoresis), (2) micellar propulsion mediated by Marangoni flows, and (3) flagellar movement. Early work on the autonomous propulsion of colloidal particles in water has featured the use of enzymes, or other catalytically active compounds, to decompose hydrogen peroxide into water and dioxygen. Large bubbles of dioxygen can move droplets by propulsion, whereas smaller bubbles tend to be associated with diffusiophoresis. These seminal works have set the stage for research on protocell motility; however, they were limited to the use of hydrogen peroxide as a reagent that induces movement. Certainly, there was some hydrogen peroxide on early Earth,[15] but likely in small and sparse quantities; therefore, it is not likely that this mechanism would have predominated on the late Hadean Earth. Moreover, bubble production by the decomposition of hydrogen peroxide is not general to a broad range of systems and environments, and it requires specific catalysis and is limited to only one specific type of reagent. We thus set to unravel some of the fundamental chemical and physical mechanisms by which protocells could have moved with a purpose in the primeval ocean. By definition, motile behavior arises from asymmetry (be it in shape or gradients); in contrast, protocell models are typically symmetric because spherical geometries are associated with the most stable interfaces. This discussion will keep a special focus on those sources of asymmetry that enable motile behavior.

Beyond the catalytic decomposition of hydrogen peroxide, one plausible mechanism for prebiotic motile behavior is based on the fact that interfacially active molecules would have been present in primeval waters. We refer to such interfacially active molecules

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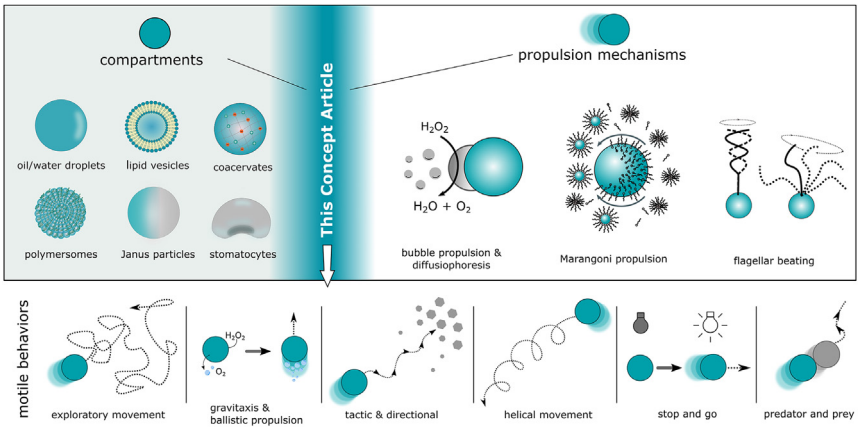

Figure 1. Scheme of possible motile behaviors that can be imparted to compartments by exploiting different propulsion mechanisms

The mechanisms discussed are schematized at the top right section of the figure. Bubble propulsion and diffusiophoresis are achievable through the incorporation of catalase enzymes in the compartment: the hydrogen peroxide is decomposed to produce O_2. The produced gas can cause the diffusiophoresis of the compartment (small bubbles or molecules) or else, when produced in higher amounts (large bubbles), the propulsion of the compartment. Marangoni propulsion is based on the solubilization process of a droplet immersed in a lipid-rich solution (above the critical micellar concentration). During this process, micelles uptake lipid molecules from the droplets interface and generate an interfacial tension gradient. As a result, to re-equilibrate itself, the droplet moves toward regions with higher concentration of empty micelles, where the interfacial tension is lower. The interfacial tension gradient can also be generated by a reaction that modifies the lipid structure. Flagellar beating relies on the presence of cilia or flagellar-like structures (such as microtubules), which, through a whip-like and circular movement, allow the propulsion of compartments.

as surfactants; surfactants of biological origin typically fit the definition of lipids, a term that we also use in a prebiotic context. In this scenario, the protocells would be oil droplets, and they would move because a system with many small droplets stabilized by a surfactant is thermodynamically lower in energy than a system with few large droplets stabilized with a small portion of surfactant and a lot of solvent-filled micelles. In other words, surfactant molecules are more stable at the oil-water interface than just in solution as micelles; therefore, they dissolve the oil to form swollen micelles.[16–18] Furthermore, micellar solubilization leads to an asymmetric distribution of surfactant along the droplet interface. This, in turn, creates a convective internal flow of water and lipids toward parts of the interface lacking surfactant molecules (and higher in interfacial tension). The flow at the interface pulls adjacent liquid along with it and generates Marangoni propulsion of the droplet in the opposite direction, namely where the concentration of empty micelles is higher (and interfacial tension is lower). This mechanism is intrinsically chemotactic.[19] The movement proceeds as long as the droplets do not completely shrink and dissolve (Figure 2A). These motile droplets display life-like behavior, including growth and splitting.[7] Their speed typically ranges from 0.2 to 1 body length per second.[20]

Other strategies are based on mimicking cilia and flagella. Forward propulsion through ciliary or flagellar beating is a non-reciprocal movement that is challenging to engineer synthetically; typically, recreating protrusions is not enough to produce a net displacement. For example, these protrusions should be able to undergo non-reciprocal shape transformations in a synchronized fashion.

Hereafter, we review and discuss the literature according to the type of motile behavior that emerges in these synthetic systems, be it typical protocells (droplets,

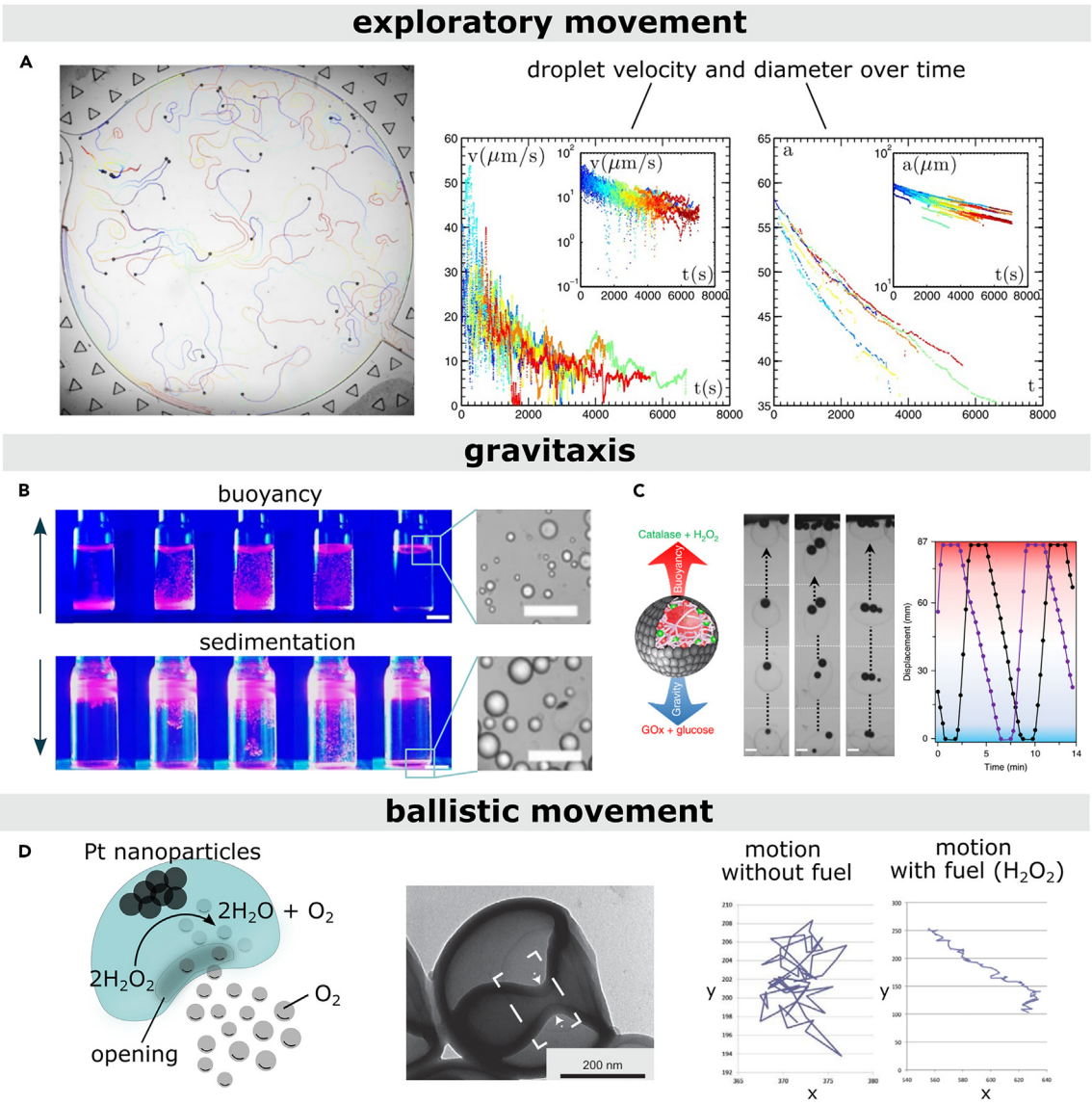

Figure 2. Protocells with exploratory, gravitactic, and ballistic movement

(A) Water droplets displaying self-propulsion by micellar solubilization. Their speed decreases over time due to the reduced number of empty micelles available for water solubilization in the surroundings of the droplets, as the propulsion mechanism progresses. Due to water solubilization, the size of the droplets also decreases over time.

(B) Changes in the density of oil droplets, given by a lipase-mediated reaction, cause their vertical movement toward the top or bottom of the vial.

(C) The activity of a catalase enzyme incorporated in microcapsules produces oxygen bubbles, which cause the buoyancy of the microcapsules. This buoyancy is offset by the introduction of glucose and glucose oxidase. Overall, the capsules display cycles of upward and downward vertical movement.

(D) Jet-like propulsion of a stomatocyte. Its opening is controlled by the dialysis of the solvent, causing the release of oxygen bubbles that are produced internally by decomposition of hydrogen peroxide.

(A) is reprinted with permission from Izri et al.,[21] APS. (B) is reprinted with permission Wang et al.,[22] John Wiley and Sons. (C) is reprinted from Kumar et al.[23] with permission from Springer Nature. (D) is adapted with permission from Wilson et al.,[24] Springer.

vesicles, and coacervates) or artificial compartments (polymersomes, Janus particles, and stomatocytes) (Figure 1). We selected works reporting a motility regime that is plausible for protocells swimming in the primeval ocean: low Reynolds number, microscopic compartments, and aqueous medium. The compartments are of a wide size, ranging from 10 to 100 nm (liposomes), 0.01 to 1 μm (polymersomes and stomatocytes), 1 to 10 μm (Janus particles), and 5 to 50 μm (coacervate and oil

droplets). Liposomes (lipid vesicles in general) closely resemble modern cells and are one of the main protocell models since Morowitz and Deamer proposed them as self-assembled and replicating compartments.[25] Coacervate droplets were one of the first chemical models of protocells, proposed by Oparin and Haldane, and have recently regained relevance as similar structures were found in modern cells.[26–29] Oil droplets are typically disregarded as protocells, but our group has put forward their potential relevance as precursors of lipid vesicles, where water-free condensation reactions could take place.[20]

We also note that because they resemble cells, protocells could contribute to the development of artificial cells. These motile protocells can also be considered as nano- or micro-motors that can swim in fluids with a purpose, e.g., and deliver cargo. Therefore, some aspects of this research could be translated into the clinic for targeted drug delivery and diagnostics.[30–33]

EXPLORATORY MOVEMENT

Exploratory movement is observed in the bacterial world: the *E. coli* bacterium performs what is known as a biased random walk, which alternates between straight paths and tumbles; whenever it senses that it is swimming up a gradient, the frequency of tumbles decreases substantially.[34] When the environment is not known or cannot be sensed, constant, random movement can be enough to provide good coverage without requiring energy costly structures.[35,36] In the absence of sufficiently strong gradients, in spite of its chemotactic character, micellar propulsion results in random walks (Figure 2A).[16] Although dependent on multiple factors, robust motility has been described for active droplets of diverse compositions. A variety of compounds can be used, such as tetradecyltrimethylammonium bromide (TTAB)[37] and Triton-X[38] as amphiphiles, and octanol[11,16] and bromooctane[38] as oils.

Lifespan is a limitation of active droplets driven by micellar solubilization. To address this issue, Hanczyc and coworkers have coupled a bond-forming chemical reaction to the system[39]: the motion of oil droplets originates from the alkaline hydrolysis at the interface of a surfactant precursor within the oil droplet, which generates four asymmetric processes around the droplet. In this case, the interfacial tension gradient is coupled with a local pH gradient. The oil droplet moves away from the acidified solution, just around the droplets, toward fresh solution. Since it is chemotactic to a local pH gradient, this behavior is overall not directional, but it shows potential in coupling protocell function to chemical reactivity.

Remarkably, the motility of water droplets has been observed in one chemical system so far; this was first reported by Dauchot and coworkers.[21] The system involves monoolein (1-oleoyl-rac-glycerol) as a surfactant. This surfactant is prebiotically plausible because monoglycerides have been hypothesized to have had a role in the stabilization of membranes in the pH and ionic strength conditions characterizing the early oceans.[40] The oil is squalane and can also be replaced by tetradecane. The system tolerates additives such as sodium chloride and colloids in the water, indicating that it may be compatible with biologically and prebiotically relevant chemistries.

As the interfacial tension between continuous and discrete phases decreases, motion becomes less robust and more challenging. There is one example of interfacial tension-driven propulsion of water-in-water droplets. This system takes advantage of demixing phases to create an interfacial gradient and thus operates in the

absence of any surfactant.[41] Ban and coworkers found that these droplets, when put out of phase equilibrium conditions, can move as fast as 40 μm/s for up to 5 min, which is substantially shorter than when the water droplets move in oil. The authors refer to the Korteweg effect, which is the Marangoni effect equivalent for thick, undefined interfaces. Other authors have found that enzymatic reactions that disrupt the phase equilibrium in water-in-water droplets can also drive motion. Saleh and coworkers prepared droplets of DNA nanostars in an aqueous solution via liquid-liquid phase separation.[42] When a DNA-cleaving enzyme is added, the droplets slowly dissolve, but they do so by first shrinking and forming inner vacuoles. As the vacuole grows and reaches the surface, its bursting leads to the motion of the droplet in the opposite direction. This mechanism is also similar to bubble production, which we discuss in the next section.

GRAVITAXIS AND BALLISTIC PROPULSION

Life makes use of vertical movement in water, with or against gravity—positive or negative gravitaxis. Gravitaxis is particularly important to organisms that depend on light because it allows them to prevent sedimentation and regulate their exposure to sunlight, and it is reasonable to assume that protocells would have also evolved vertical motility, as sunlight would have been even more crucial as an energy source. Here, we show that protocells can exhibit such behavior, provided that their buoyancy properties are sensitive to their surroundings.

Mann and coworkers made use of a change in density to get protocells to swim upward.[22] Droplets of tributyrin were coated with a layer of a lipase enzyme, which catalyzes the hydrolysis of tributyrin above 17°C. The reaction lowers the droplet's density, which then becomes buoyant in the aqueous phase. When a reservoir of tributyrin is placed on top of the suspension, the vertical movement is controlled with temperature (Figure 2B). The oil droplets have also been incorporated inside proteinosomes as active organelles that can control overall motion and pH. The incorporation of organelles that impart new functions to a protocell resonates with the systems chemistry approach to the origin of life problems and with a heterogeneous scenario of multiple protocellular structures on early Earth.

Vertical movement can also be induced by gas bubbles. In previous work from Mann and coworkers, double-stranded DNA was encapsulated in organoclay sheets to form a giant protocell (Figure 2C).[23] This microcapsule (diameter 200–500 μm) is semi-permeable and can thus encapsulate catalase, an enzyme that retains its activity after encapsulation. Once hydrogen peroxide is added, oxygen bubbles are produced inside the capsule. The bubbles fuse in one big bubble blocked in the organoclay membrane, and the capsule moves vertically at ca. 10–40 mm/s. The upward movement can be offset by the further addition of glucose oxidase to the microcapsules and glucose to the solution. The buoyancy is opposed by the consumption of oxygen, causing a downward movement. Although these capsules can be quite fragile, the principle of buoyancy can be applied to other systems—replacing organoclay with protamine, a work done in the same group (unpublished data), increases stability from a few hours to multiple days.[43]

The production of gas bubbles can be exploited to promote ballistic propulsion not only in the vertical direction; if the gas bubbles can somehow detach from the protocell, the resulting recoil force can produce microscopic motion. The system developed by van Hest and coworkers on stomatocytes provides a widely used strategy where motion is supported by the production of oxygen bubbles.[24] Stomatocytes

are bowl-shaped aggregates that can encapsulate platinum nanoparticles. The opening of the stomatocytes can be controlled by gradually removing the solvent via dialysis, allowing oxygen bubbles formed by the decomposition of hydrogen peroxide to flow out of the cavity. The resulting movement is vertical jet-like propulsion with an average speed of 23 μm/s (Figure 2D). As the propulsion is highly affected by the shape and structure of the protocell, it is possible to get slow or fast motors with linear, circular, or run-and-tumble trajectories.[44] Gravitational forces clearly have a role in the gravitation settling of moving protocells; these effects have been recently studied, analyzed, and compared with the performances of micellar propulsion.[45]

A similar effect can be obtained with protocells bearing gas-producing enzymes on the interface. The previous group replaced the stomatocytes with coacervate droplets and the platinum catalysis with catalase enzyme to produce motile coacervate droplets.[46] The coacervate droplets were framed with a membrane containing catalase, distributed unevenly, and incorporated into giant unilamellar vesicles. In this case, the droplets move because of (self-) diffusiophoresis; oxygen is generated by catalase unevenly around the droplet, causing the solvent to flow and, as a consequence, propelling the droplets. The mean-square displacement was found to depend on both the hydrogen peroxide concentration the and size of the coacervate droplet. The result is an exploratory movement in random directions of the coacervates inside the vesicles.

CHEMOTAXIS AND OTHER FORMS OF DIRECTIONAL BEHAVIOR

Chemotaxis is the directional movement toward or away from a chemical signal.[34] It enables microorganisms to overcome diffusion to both seek resources and avoid poisons—e.g., sperm cells swimming toward an egg or the hunt of white blood cells for pathogens.[47,48] Marine bacteria *Vibrio* are highly chemotactic, even more so than *E. coli*. It probably evolved this way so that, despite the turbulence of its environment, it would still be able to sense and reach nutrient patches before they disappear.[49] Chemotaxis is also a strategy to impart microorganisms and droplets with the ability to solve mazes containing gradients of a chemical signal.[19,50] In this section, we discuss how directional motion can be imparted to microscopic swimmers by a multitude of strategies: using Marangoni propulsion, which is intrinsically chemotactic, and an external or self-generated gradient; incorporating enzymes at the interface of protocells to bias their movement in a gradient; or finally, with protocells capable of ciliary beating.

Directional movement along a concentration gradient can be found in the work of Ces and coworkers, which is concerned with aqueous two-phase systems.[51] Dextran-rich droplets that were dispersed in a continuous phase rich in polyethylene glycol (PEG) and stabilized with liposomes were shown to move toward an external source of water. The water generates a concentration gradient of PEG, which in turn creates an interfacial tension gradient. The directional movement is further supported by the asymmetry in the liposome coverage induced by the Marangoni flows (Figure 3B). Our group has further exploited the chemotactic nature of Marangoni propulsion to demonstrate that a mutualistic relation can develop between motility and chemical reactivity.[11] Hexanethiol droplets submerged in a solution of a water-soluble lipid precursor are initially static. As a Michael reaction takes place between 1-hexanethiol in the droplets and 2-methacrylyloxyethyl phosphorylcholine, a lipid is formed, which assembles micelles that sustain the movement. In return, the movement of the droplets brings the reagents together actively by chemotactic

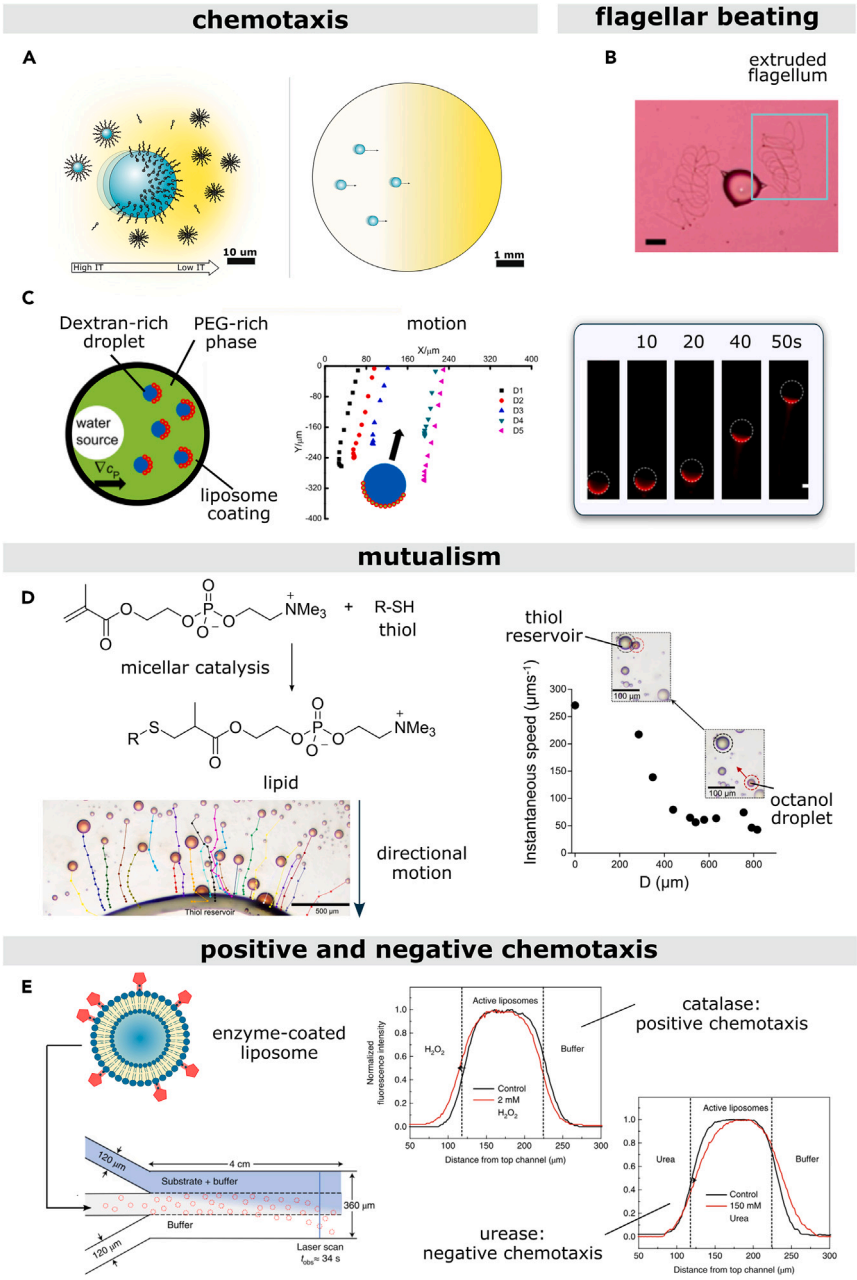

Figure 3. Compartments capable of tactic and directional motion

(A) Schematic depiction of chemotaxis in micellar propulsion. These droplets are chemotactic toward self-generated local gradients of empty micelles, resulting in long-distance random trajectories (left). Directional trajectories are possible when an external gradient of micellar concentration is imposed on a large scale in the chamber where the experiment is performed (right).

(B) The cooling of tetradecane droplets causes the extrusion of flagella that propel the protocells.

(C) Micellar propellers of an aqueous two-phase system of PEG/dextran display chemotaxis toward a water source due to the asymmetric interfacial distribution of liposomes and the better affinity of PEG for water.

(D) The directional motion of micellar propellers toward a lipid precursor also affects the reaction progress, establishing a mutualistic relation between protocellular motion and chemical reactivity.

(E) Liposomes display positive and negative chemotaxis depending on the binding affinity between the enzyme coating and the substrate in a concentration gradient.

Figure 3. *Continued*
(B) is reprinted with permission from Cholakova et al.,[52] Springer Nature. (C) and (D) are reprinted under CC BY 4.0 agreement from Zhang et al.[51] and Babu et al.,[11] respectively. (E) is adapted with permission from Somasundar et al.,[53] Springer Nature.

propulsion, and overall, with the effect of mixing, a mutualistic relationship is established between lipid production and chemotactic motion (Figure 3D).

Marangoni flows are not restricted to lipid-stabilized water/oil emulsions, as discussed in the exploratory movement section. Interfacial tension gradients can be generated in the liquid-liquid phase-separated system studied by Laurino and Dufresne[54]: bovine serum albumin (BSA) protein and PEG separate in a BSA-rich phase (droplets) and a PEG-rich continuous phase. Since this system is aqueous, it is possible to incorporate enzymatic activity in the droplets. Urease partitions inside the droplets, and when urea is added to the mixture, it generates ammonia. Consequently, a pH gradient is generated in the surroundings of the droplets, affecting the interfacial tension between the two liquid phases and resulting in a convective internal flow. The droplets are thus able to move directionally toward regions of high pH, which, in this case, when very high, affects drastically the interfacial dynamics of the systems and drives them to dissolution.[55,56]

Directionality has also been engineered in mechanisms other than micellar motility. Sen and coworkers described a way to direct both positive and negative chemotaxis of enzyme-coated liposomes.[53] In this example, the activity of the enzyme affects the movement because binding lowers the chemical potential of the system, creating a driving force for movement toward the substrate. However, when the interaction between liposomes and products is unfavorable, the liposomes can instead shift away from the buffer with substrate and product. The authors demonstrate this chemotactic behavior by generating a substrate gradient in a microfluidic device. Catalase-coated liposomes, which decompose hydrogen peroxide to produce water and dioxygen, show positive chemotaxis toward hydrogen peroxide. In contrast, urease-coated liposomes show negative chemotaxis, shifting away from high concentrations of urea. This negative chemotaxis is likely caused by the product of the reaction (ammonium carbonate) that interacts unfavorably with the phospholipids of the vesicle. ATPase-covered liposomes show both effects depending on substrate concentration: for low ATP, positive chemotaxis prevails; for high concentrations of ATP, the repulsions between reaction products and the phospholipids give rise to negative chemotaxis (Figure 3E).

The previous examples refer to protocells suspended in the swimming medium, typically water. If protocells interact with a patterned substrate, then motion can also be directed by the geometric pattern. In research reported by Schwille and coworkers,[57] cell-sized liposomes adhered to an asymmetric pattern, and consequently they under went deformation. In turn, this deformation promotes further coating, generating a mechanochemical feedback loop that drives the motion of the liposomes across the pattern. This mechanism of motion shows analogies with the actin/myosin cell migration machinery and relies essentially on the presence of a geometrical pattern.[58]

Finally, directional and persistent movement emerges from the beating of flagella or cilia, a strategy adopted by the majority of modern microorganisms. It is difficult to imagine how protocells that lacked specialized proteins would have developed structures complex enough for non-reciprocal movement. As a reference for the requirements to produce such a system, Aizenberg and coworkers[59] have mimicked

flagellar beating by inducing non-reciprocal shape transformations in filaments composed of liquid crystal elastomers and azobenzene linkers. The filaments are attached on a glass surface and move toward a light source, but they have not yet been adapted in a protocellular context due to the difficulty of obtaining the same structural properties with a prebiotically plausible composition, although some DNA coacervates exhibit liquid crystallinity.

A more plausible flagellum mimic has been shown in compartments as simple as tetradecane droplets, although they are also not typical protocells. Denkov and coworkers showed that droplets of tetradecane in an aqueous solution of surfactant (Brij 58) undergo a liquid-to-plastic phase transition.[52] The alkane droplets spontaneously extrude elastic filaments. Thus, these tail-like structures push the droplets and make them swim. The number of filaments and the movement of the droplets can be tuned by controlling the cooling rate, selected based on specific oil-surfactant combinations (Figure 3B). It would be interesting to explore whether this protrusion mechanism is susceptible to a chemical gradient and can therefore lead to directional and chemotactic motion.

HELICAL MOTILITY

Helical trajectories are largely prevalent for unicellular organisms swimming in water.[9,60] It is likely that this prevalence of helical trajectories is due to the fact that helical propulsion proves more resistant to external perturbations, as it contains both a linear momentum and an angular momentum[61]

Based on this observation, recent research has focused on the design of motile chiral protocells, namely liquid crystal droplets. In fact, some protocellular models, such as peptide/DNA coacervates, do exhibit liquid crystallinity. In micellar propulsion, chiral droplet organization induces helical self-propulsion.[62] The solubilization of the droplets generates Marangoni flow, and the droplets swim with a helical trajectory, with handedness always opposite to that of the liquid crystal. The helical trajectories emerge from the spiral organization of the liquid crystal confined in a droplet when $N = 2d/p$ is ca. 20 (where N is the chirality confinement ratio, d is the diameter, and p is the pitch). In the disclination lines forming the spiral feature, the liquid crystal is disorganized; therefore, the dissolution happens easier, and the Marangoni flow is faster, which gives a spin to the droplet.[63]

Persistence in directionality is key for cells to swim safely from one point to another. However, this persistence in directionality has to be coupled with re-orientation strategies so that the cells can explore the space for nutrients, chemicals, and sources of light. Swimming microorganisms have thus developed unique re-orientation mechanisms; the vast majority of aquatic microorganisms and cells like zooplankton, ciliates, and bacteria move along helical pathways.[64] Chiral motile droplets also display deterministic reorientations in response to external cues. Under illumination, the rotation of the molecular motors inverts the cholesteric helix in the droplets, which undergoes a precession along its helical trajectory. The result is that also the handedness of the trajectory of the droplets is inverted. Deterministic re-orientation under the combined effects of inversion of helical trajectories and precession rotation is called helical klinotaxis and has been found in sperm cells and green algae.[48,60] Such active, chirality-driven re-orientation mechanisms are indeed required to explore the environment (Figure 4B).

Besides chirality, helical motility can be achieved by controlling the physical parameters describing Marangoni propulsion. Maass and her coworkers showed that by

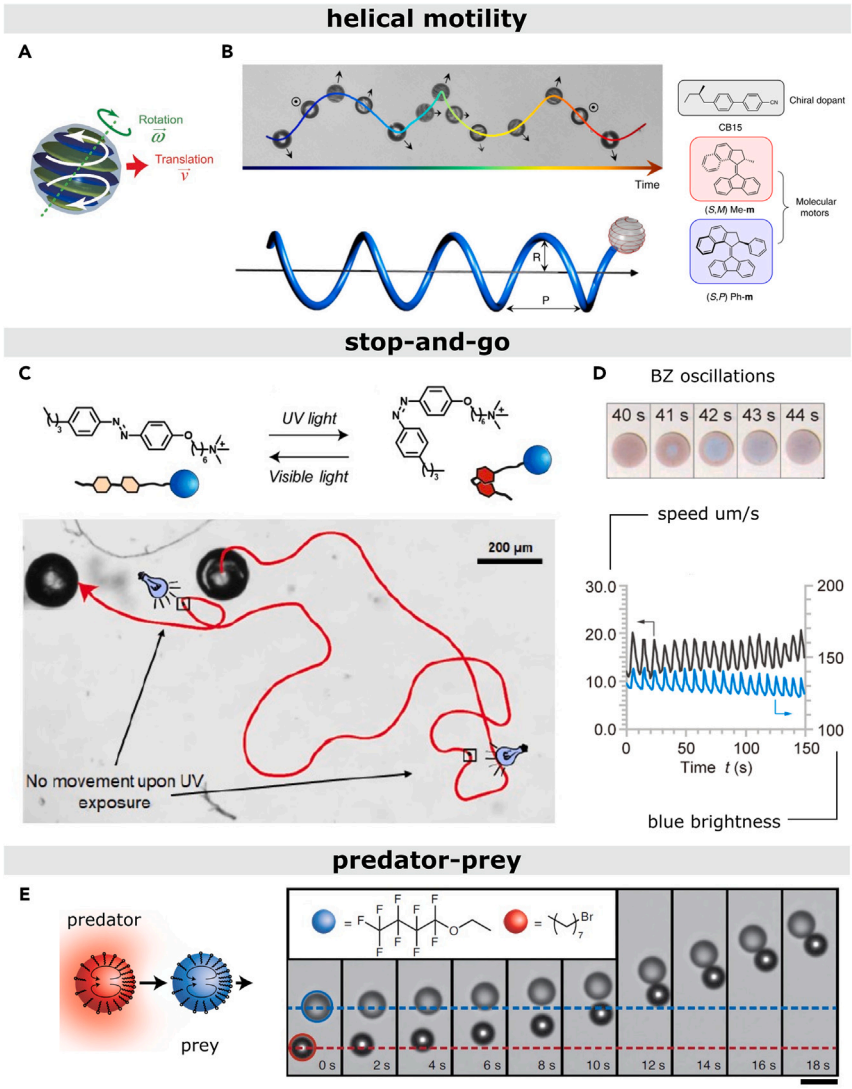

Figure 4. Complex motile behavior displayed by artificial compartments

(A) The anisotropy of liquid crystal droplets is capable of inducing helical motion, for example, in Marangoni propellers.

(B) Droplets of chiral liquid crystals invert their direction of propagation as the handedness of the droplets photo-inverts. The directionality of this reorientation is endogenous and determined by the confinement of the liquid crystal helix in the droplet.

(C) Light can be used to initiate and stop Marangoni propulsion. When an azobenzene moiety is incorporated in the surfactant, depending on its *cis-trans* isomerization, the surfactant can propel or stop the movement of oil droplets.

(D) The BZ oscillatory reaction integrated in water-in-oil droplets affects the interfacial properties of the system. Therefore, the water-based protocells also display an oscillatory speed pattern.

(E) Droplets of two halogenated oils with different solubilization rates move as a result of Marangoni propulsion and chase each other, as in predator-prey natural behavior.

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varying the Peclet number *Pe*, namely the ratio between advective and diffusive transport in fluids, swimming droplets display different types of trajectories.[66] The Peclet number can be modified by changing the concentration of the surfactant or the size of the droplet. For intermediate *Pe* values (6.8–8.7), the droplets follow helical trajectories, whereas for *Pe* above 10.9, they follow random trajectories.

STOP AND GO

In swimming unicellular organisms, running is suitable for finding new nutrient patches, whereas halting allows the bacteria to exploit a patch to the maximum before moving on. This behavior was reproduced by Nakata et al., by coupling the Belousov-Zhabotinsky (BZ) reaction to the monoolein-stabilized water-in-oil droplets. As the reaction oscillates, the speed of the droplets oscillates.[65,67]

Adapting trajectories to achieve optimal exposure to sunlight is sometimes the greatest strategy for survival. This behavior can be displayed by nematic droplets in a concentrated solution of photoswitchable azobenzene surfactant.[37] This surfactant undergoes *trans*-to-*cis* isomerization upon UV irradiation, accompanied by an increase in critical micellar concentration and the cessation of the droplet movement, caused by the fact that the *cis*-isomer surfactant is not effective for liquid crystalline droplets. The stop-and-go cycles were repeated six times by alternating UV and visible light illumination. Overall, the mechanism relies on the fact that the movement of the droplets is gated by photoswitchable micelles (Figure 4C).

In addition to light, temperature can also be used to control stop-and-go cycles of motile droplets by affecting their micellar solubilization.[68] In the work by Maass and coworkers, droplets immersed in surfactant water displayed Marangoni propulsion, provided that the surfactant concentration, e.g., TTAB, was substantially larger than critical micellar concentration. When coupled with a cosurfactant (PF127), above a critical temperature, micelles composed of both surfactant and cosurfactant were formed, and TTAB was subtracted from the micellar solubilization process, driving the droplet's propulsion and arresting their swimming. This process is reversible; therefore, by cooling down the system, pure TTAB micelles formed again, and the movement of the droplets was restored.

PREDATOR AND PREY BEHAVIOR

Motility is not only a means for individual compartments to remain competitive but also a way for protocells to interact. Predator/prey interactions are ubiquitous in the living world, from microscopic to ecological systems, and play a key role in evolution.[69,70] For protocells, predator/prey behavior can be described as the connected movement of two different compartments: one is "chasing" (predator) the other compartment, which is instead "escaping" (prey). The combination of both results in a complex movement that is determined by the chemical composition of each of the two populations.

Zarzar and coworkers recreated this scenario[38] by selecting two populations of oil droplets and a surfactant that dissolves only one of these two oils preferentially (bromo-octane). The movement of the bromo-octane droplets creates a trail of oil-filled micelles, which repels the nearby droplets. Subsequently, the fluorinated oil droplets uptake solubilized bromo-octane from the filled micelles, causing a decrease in the interfacial tension in the region between the two different oil droplets. The net result of these combined processes is that the bromo-octane droplets move toward the prey, which swim far from the predators toward regions with

lower concentrations of filled micelles. Overall, this predator-prey behavior can be interpreted in terms of asymmetric rates of oil exchange between droplets (Figure 4E).[71]

CHALLENGES AND OPPORTUNITIES

Motility can be imparted to a range of prebiotically relevant compartments—some with a sharp interface (liposomes and droplets) and some with a thicker interface, like aqueous two-phase system droplets. Importantly, motility is not a single behavior, and we demonstrated here how it can manifest in active compartments in a variety of ways, meaning that in a prebiotic context, it can grant the protocell the ability to forage its surroundings, efficiently locate nutrients, and even interact with and chase other protocells.

In translating the knowledge from artificial compartments to prebiotically plausible protocells, the mechanisms described here are still difficult to generalize; e.g., micellar propulsion, minimalistic as it is, depends on an excess of primeval lipids, whose prebiotic origin remains to be elucidated. Some examples of diffusiophoresis and bubble propulsion rely on an enzyme coating of the compartments, which would delay the emergence of motility for some millions of years. Therefore, we envision that those efforts to develop droplets that are able to create and sustain asymmetrical surfactant coverage (e.g., using chemical reactions that produce lipids or droplet material) will be essential to achieve autonomous protocells. Motile water droplets in oil could have been forerunners of vesicular protocells in the early ocean; a scenario that becomes plausible when considering the converging set of evidence that an oil slick could have covered the early ocean.[20,72] This oil slick could have been a source and a reservoir for lipids, would have introduced an interface in the prebiotic environment that could potentially promote the formation of vesicles,[73] and would furthermore trap particles, which would take on circular trajectories under confinement.[20,64]

Besides the compositional challenge, we note that the motile behavior closest to modern life forms arises from the most complex supramolecular organizations. The anisotropy of liquid crystal droplets and the extrusions of cooled tetradecane droplets lead to persistence in motion. Because of this, we argue that it is worth exploring protocells with more complex supramolecular morphologies, which is one of the research fields associated with supramolecular systems chemistry. So far, the focus has been very much on symmetrical liposomes and droplets; however, there is a wealth of different morphologies to explore, such as vesicle colonies that form on surfaces, multiphase aqueous droplets, and phase-separated lipid vesicles. We hope that these examples will inspire protocell research to include prebiotically plausible molecules and chemical reactions in motile systems. We envision that making use of all the various forms of motility available to protocells will expand their repertoire of functional behavior. The endowment of protocells with autonomy, the capacity to adapt to their environment and even alter it eventually, and the possibility to interact, communicate, and compete with other protocells may be among the fundamental contributions that complex motile behavior can offer to research on the molecular origins of life.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to the collation of the literature, analysis, and writing of the text.

DECLARATION OF INTERESTS

N.K. is a member of the advisory board of *Chem*.

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