

Autonomous Droplet Architectures

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Abstract The quintessential living element of all organisms is the cell—a fluid-filled compartment enclosed, but not isolated, by a layer of amphiphilic molecules that self-assemble at its boundary. Cells of different composition can aggregate and communicate through the exchange of molecules across their boundaries. The astounding success of this architecture is readily apparent throughout the biological world. Inspired by the versatility of nature’s architecture, we investigate aggregates of membrane-enclosed droplets as a design concept for robotics. This will require droplets capable of sensing, information processing, and actuation. It will also require the integration of functionally specialized droplets into an interconnected functional unit. Based on results from the literature and from our own laboratory, we argue the viability of this approach. Sensing and information processing in droplets have been the subject of several recent studies, on which we draw. Integrating droplets into coherently acting units and the aspect of controlled actuation for locomotion have received less attention. This article describes experiments that address both of these challenges. Using lipid-coated droplets of Belousov-Zhabotinsky reaction medium in oil, we show here that such droplets can be integrated and that chemically driven mechanical motion can be achieved.

Keywords

Fluid robots, molecular robotics, proto-organism, lipid membranes, self-assembly, Belousov-Zhabotinsky reaction

1 Introduction

Organisms consist of one or more membrane-enclosed fluid drops. The contents of these cells as well as the composition of their membranes vary widely across species and across cell types within multicellular organisms. Common to all of them is an intricate organization and sophisticated molecular machinery that is so far only partially understood. Faced with this complexity, the question of what the simplest cell might look like arises. It has been contemplated in the context of the origin of life and more recently in the context of synthetic biology. The latter aims at finding a recipe for the fluid and the membrane that would endow a drop prepared according to the recipe with the phenomena ascribed to living cells, namely, self-maintenance, reproduction, and evolvability [25].

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While synthetic biology aims at building living organisms, the field of robotics has a long tradition of building crude analogues of organisms. Here the goal is lifelike behavior, which is far less ambitious than the aim of reproducing the characteristics of life; though some of the latter might be desirable, they are in general not considered necessary in a robot. Given the universal success of membrane-enclosed fluid drops as an architectural principle throughout the biological world, one can ask whether this concept could be usefully employed for the more modest aims pursued in robotics. Accordingly, the focus of the present article is on the extent to which current laboratory techniques allow for the implementation of simple robots with drops of fluid and with aggregates of such drops.

For a drop to exist as a defined entity, a degree of immiscibility is required between at least two phases (gas or liquid), so that the cohesion of molecules can give rise to surface tension. This in turn brings about the shape of a droplet. For example, we could have a drop of water in an air phase or, to hinder evaporation, in an oil phase. The phase boundary constitutes an interface that is selective with regard to the molecules that will pass across. In the water drop submerged in oil, ionic substances would preferentially stay within the droplet, while hydrophobic substances would leave the droplet and dissolve in the oil phase. Some molecules possess spatially oriented hydrophobic and ionic parts. These so-called *aliphatic* substances will enter the oil phase with one part and enter the water drop with the other part. Consequently, aliphatic molecules self-assemble at the phase boundary and can coat the entire drop, resulting in a membrane.

A membrane around the drop has three important consequences. Firstly, it will alter the interface between the drop and its environment. Secondly, it can prevent adjacent drops from merging and thus enable the construction of stable aggregates. And thirdly, the membrane can itself act as a phase boundary that allows, for example, the compartmentalization of ionic drops in an ionic environment. In double emulsions or cells, more than two phases are present, and typically there is an intermediate partially immiscible phase. In double emulsions there can be an aqueous-in-oil-in-aqueous configuration. In cells there is the internal phase; the liquid lipid bilayer, which acts as a boundary; and an external outer phase, which can be liquid or gaseous. Numerous topologies can be assembled according to this concept, and hundreds of aliphatic substances (in particular, lipids and surfactants) are readily available. These membrane-forming components are typically used in combinations to fine-tune the properties of the interface. Even without considering the contents of the drop and the possibility of inserting more specialized molecules (such as pores or selectively binding molecules) into the membrane, the combinatorics of the aliphatic molecules spans a rich space of potential constructs. For simplicity we will refer to any small quantity of fluid enclosed by a membrane as a *droplet* (see Figure 1).

Droplets and small networks of droplets can be created easily by hand with pipettes, Petri dishes, and simple mixtures of oils and surfactants. Where large quantities of droplets are required, microfluidics can be employed to generate several thousand droplets within minutes [2]. Hierarchical droplet architectures where several droplets are encapsulated within a single larger droplet can also be fabricated manually [39] and by microfluidic technology [30]. The relative ease of their production has led

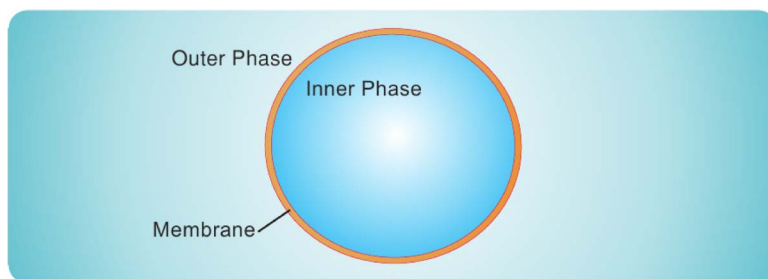


Figure 1. A droplet is a liquid-filled volume separated from, and interfaced with, its environment by one or more layers of aliphatic molecules that form a membrane around the droplet. Often the outer phase is organic and the inner phase is ionic, with a single layer of aliphatic molecules separating the phases. For biological cells both phases are ionic and separated by a double layer of lipids.

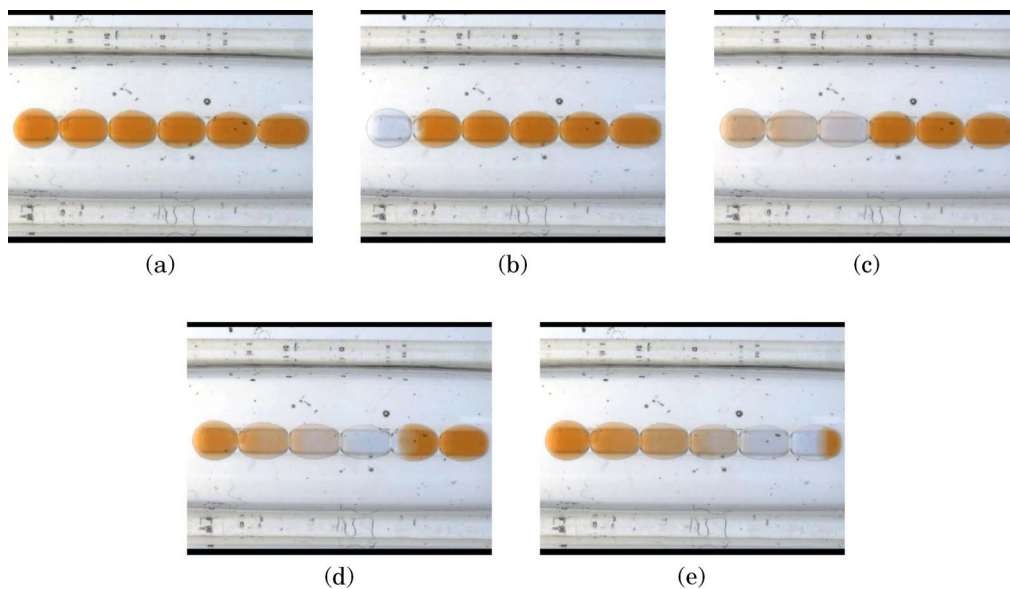


Figure 2. Neuron-type transduction in a droplet chain. Droplets filled with Belousov-Zhabotinsky (BZ) reaction medium are immersed in oil. The BZ droplets are arranged in a 1-mm-wide trench and prevented from merging by synthetic lipids, which have been added to the oil phase and have self-assembled at the droplet surface. The sequence of images shows the propagation of an excitation from left to right through the droplet chain.

to droplets being utilized in many research activities, such as protein engineering, novel particle synthesis, and drug delivery systems [37]. In the last context, Štěpánek convincingly put forward the idea that droplets equipped with sensory and actuation capabilities could be viewed as autonomous chemical robots. His group considers remote-controlled and fully autonomous chemical robots and their interactions [11].

More advanced—even lifelike—behaviors are possible with similar droplet systems. For example, Sumino and colleagues demonstrated that an oil droplet could interrogate a glass surface that had been selectively treated with acid. The droplet was able to detect and avoid acid-treated areas [33]. Hanczyc showed that a randomly moving droplet could respond to the introduction of a pH gradient, adjusting its movement towards an area of high pH [13]. Browne and coworkers were able to demonstrate the self-division of oil droplets within an acidic aqueous solution [6]. The same group has since showed that a droplet can be used to solve a maze by following a pH gradient set up between the entrance and exit of the maze [24]. The information processing of the autonomous droplets described above is performed at the interface by the rearrangement of surfactant molecules. Nevertheless, these higher-level droplet systems indicate an ability to take environmental signals, apply some information processing to the signals, and then perform some action based on the result of the information processing. Consequently we refer to these systems as *autonomous droplets*, following the terminology of [14].

The environment of such droplets can also be formed by other droplets, leading to multi-droplet architectures. An example of a multi-droplet structure in which droplets communicate by means of chemical signals is shown in Figure 2. Lipid-coated droplets of the excitable Belousov-Zhabotinsky (BZ) reaction medium under oil form lipid bilayer membranes at the interfaces between droplets. In the droplets at rest, the catalyst is dark red (reduced state). An excitation, visible by the light color of the catalyst (oxidized state), travels across the interface from droplet to droplet, mediated by chemical transmitters. After a droplet has been excited, a refractory period prevents immediate further excitation and gives rise to directional travel of the excitation.

If we analogize individual autonomous droplets with single-cell organisms and aggregates of multiple droplets with tissues, the question arises whether there is a droplet analogue to multicellular

organisms. This would require an integrated form of the droplet aggregate that is interconnected not only functionally but also physically. Several different techniques are available to achieve this. Anchoring of single-stranded DNA to the lipid layer has been used to assemble droplets with droplet-to-droplet specificity [12]. Here we focus on an even simpler technique to integrate heterogeneous droplets into a single concerted system—droplets assembled as a multisome containing within it a number of smaller functional droplets [39]—and consider its application to the BZ medium.

The BZ reaction is capable of forming complex patterns. Order can be imposed on the patterns, temporal or spatial, to bring about functionality from the BZ medium, for example, elementary chemical logic gates [31] and logic circuits [1]. At the micro scale, the application of spatial order has led to the discovery of lifelike behaviors reminiscent of quorum sensing [36]. Typically spatial order is imposed on BZ at the micro scale through compartmentalization as droplets [3].

The compartmentalization of BZ mixtures as lipid-coated droplets surrounded by an oil phase allows for the exploration of unconventional computing architectures such as artificial wet neuronal networks. In the context of constructing functional networks of lipid-coated BZ droplets, we have directed our efforts toward optimizing droplet stability and inter-droplet communication. However, the chemical compositions of the reaction medium in a droplet, the lipid layer enclosing the droplet, and the oil phase surrounding the droplet can also be optimized with other objectives in mind. BZ droplets exhibit a rich repertoire of behaviors that might be selected for such optimization [9]. Figure 3 shows samples of the phenomena we have observed in our laboratory. In this report, we indicate the viability of the application of BZ autonomous droplets through observations from the literature and specific results from our own laboratory.

2 Design of a BZ Autonomous Droplet

The fundamental features of an autonomous droplet are the ability to sense environmental signals, carry out some information processing on those signals, and subsequently perform an action. In our autonomous droplet design, we combine several BZ droplets to form a single overall larger autonomous droplet (Figure 4). Our current design sees the BZ droplets formed in an organic phase as reverse micelles, with lipids providing compartmentalization. Other approaches to compartmentalizing the BZ droplets could be achieved through using surfactants, polysaccharides (chitosan-alginate polymers), or inorganic membranes [3, 10, 7]. At present, however, given the potential of engineered protein pores to modulate the flow of information between droplets [15, 27], we have adopted a lipid-based approach.

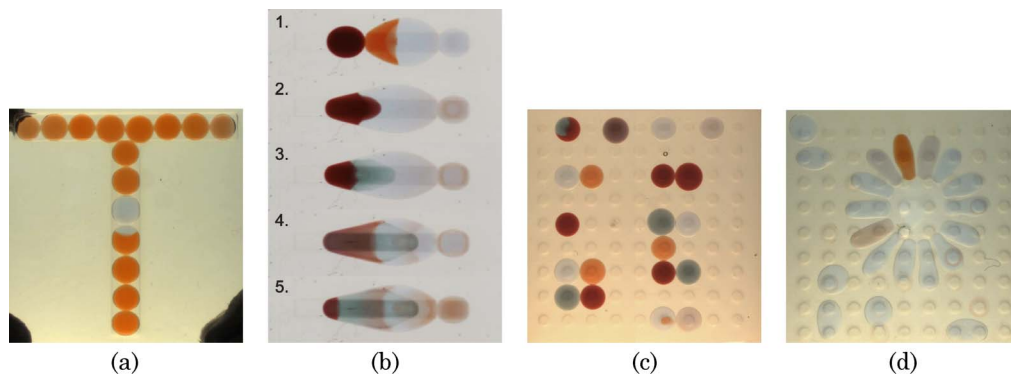


Figure 3. BZ droplets as a flexible model system. (a) A signal passes between droplets in a simple signal-processing network. (b) Complex oscillatory behavior is observed upon fusion of droplets with different BZ mixtures. (c) Two types of droplets, arranged on a microfabricated regular grid; the droplets communicate their chemical state to neighbors through exchange of chemical signals. (d) Spontaneous pattern formation induced by modifying the oil phase. The droplets in the images vary in diameter from 2 to 2.5 mm.

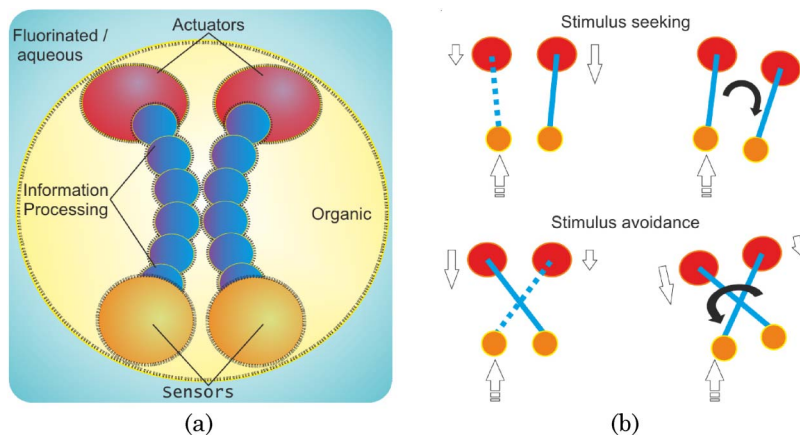


Figure 4. (a) The concept of an autonomous droplet containing an internal network of functional units. (b) Using a Braitenberg vehicle connection scheme (see text), behaviors such as stimulus seeking and avoidance could be programmed into the droplet.

As a starting point, our droplet will be constructed with a simple information-processing scheme based on Braitenberg's vehicles [5], which requires only a pair of light sensors connected to a pair of actuators. Following Figure 4b, if each light sensor is connected to an adjacent actuator and the effect of increased illumination is to inhibit actuation, then in this configuration the droplet will be capable of light-seeking behavior. Similarly, if each light sensor is connected to the opposite actuator, increased illumination will cause the droplet to turn away from the light. To implement such a crossing-over of the signal path, the 3D structure of the droplets on a surface is not sufficient. In addition, control over the signal flow, for example through membrane proteins [4, 17], is required. More complex behavior arises when there are mixed connections between sensors and actuators.

A BZ medium prepared with a ruthenium catalyst becomes photosensitive and leads to inhibition of the reaction when irradiated. At the macro scale this has been exploited to produce a photochemical memory device [22], while at the micro scale it has allowed for programmed synchronization of an array of oscillating BZ droplets [8]. In a similar manner, droplets containing ruthenium catalyst may provide the light-sensing capability of our autonomous droplet.

Networks of BZ droplets contained within an immiscible medium offer much potential as an information-processing scheme. Already studies have been conducted on the effects of altering parameters such as pH, droplet size, and application of temperature gradients on oscillations as well as overall pattern formation [38, 32]. Particularly interesting is the coupling between BZ droplets. During the course of the BZ reaction a number of inhibitory and excitatory intermediates are produced with different hydrophobic/hydrophilic properties, which could be modulated to create artificial neuronal networks. There are oil-soluble intermediates (inhibitory bromine ions and excitatory bromine dioxide radicals) as well as water-soluble intermediates (excitatory bromous acid and inhibitory bromide ions). The coupling between droplets can therefore be optimized for information processing through careful spatial arrangement of the droplets.

Most reported chemically derived motions from the BZ reaction have utilized polymer gels. In such systems the catalyst is incorporated within a polymer gel structure [28, 40, 41]. The gel-catalyst construct is then immersed within catalyst-free BZ media that proceed to react, causing changes in the oxidation state of the catalyst. The changes in oxidation state lead to abrupt alterations of the gel volume, giving rise to cyclic swelling and deswelling. This behavior has been harnessed to produce peristaltic-action object transporters, artificial cilia, and self-walking gels [29, 35, 26]. Although not used in our autonomous droplet design (Figure 5), it is certainly conceivable that BZ gels could be incorporated as part of the design in the future.

Reports of motile BZ droplets are far more rare. Spontaneous motion driven by chemical oscillation has been observed for a 1-mm-diameter BZ droplet, either floating or immersed in oleic acid

[18–20]. Similarly, by using a light-sensitive catalyst, light-directed motion was shown possible with a BZ droplet floating in oleic acid [21]. The motion of these BZ droplets was far less prominent than that exhibited by previous examples of motile droplet systems described in the introduction. Typically the displacement achieved by these droplets in a singular movement was less than 200 μm , with the droplet remaining in close proximity to its origin throughout the course of the reaction. The limited motion displayed by these systems makes their selection for use in creating autonomous droplets, for now, unrealistic. However, as we shall see next, larger displacements can be achieved in BZ droplets.

3 Belousov-Zhabotinsky Droplet Actuators

The shape of a drop of liquid resting on a solid surface is determined by the surface tension between droplet, surface, and surrounding phase. The surface tension arises from the balance of cohesive forces between the molecules of the liquid on the one side and the adhesive forces between liquid and solid on the other. The surface tension between a solid and an aqueous liquid can be determined from measurements of the angle between the solid-aqueous interface and, in the present context, the aqueous-oil interface. The so-called *contact angle*, when measured to exceed 90° (as in the case of more spherical droplets), indicates greater cohesion of molecules within the droplet and decreased wetting of the surface. We assume that the changes in droplet shape are driven predominantly by changes in surface tension between the BZ droplet and the surface upon which it rests. To test our assumption, profiles of a BZ droplet in contact with a surface can be measured optically during the course of a reaction and the contact angles determined.

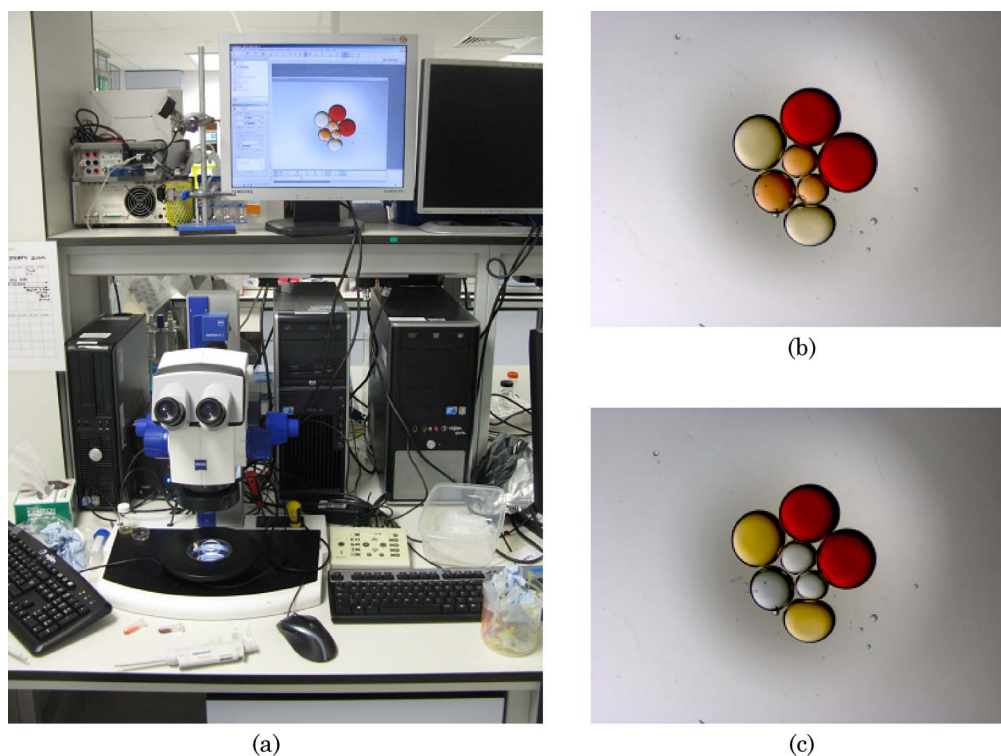


Figure 5. Laboratory setup for the autonomous droplet concept. (a) An early prototype autonomous droplet is created by hand under a standard light microscope. (b) Seven BZ droplets are encapsulated within an organic oil droplet containing natural lipids. (c) The organic oil droplet is submerged within an immiscible fluorinated oil. In this early prototype some of the droplets exhibited limited oscillation.

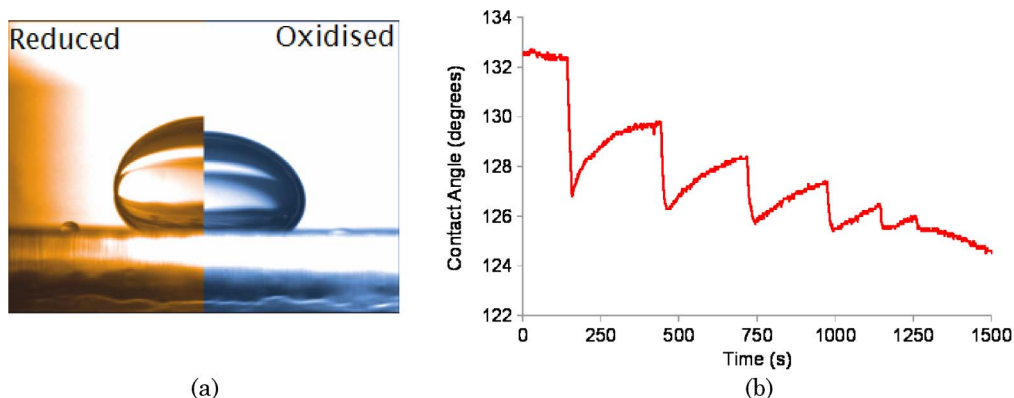


Figure 6. An oscillating BZ droplet on a surface. (a) A false color image created by combining images of the BZ droplet in reduced and oxidized states. (b) Contact angle measurements recorded from the oscillating BZ droplet. A 4- μ l BZ droplet was pipetted on to a flat UV glue sheet (NOA81 + 1% APTES) that had been immersed in a lipid-oil solution (40 mg/ml lecithin in decane). Contact angle measurements were recorded every second using a DSA 30 drop shape analysis system (Kruss, Hamburg) connected to a computer for automated data capture.

Shape changes induced by the BZ reaction have been investigated previously with the malonic acid version of the reaction. Here we make use of cyclohexanedione (CHD) [23] as substrate instead of malonic acid. We have measured oscillation-coupled shape changes of CHD BZ droplets in contact with a surface (Figure 6). Moreover, as shown in Figure 7, we have found that directionally specific shape changes of CHD BZ droplets can be induced when using a patterned substrate. Note that by combining the CHD medium with a bathoferroin catalyst [34] even larger displacements can be obtained from slugs of similar volume on the same substrate (Figure 7e–h).

Utilizing 3D-printing technology and fabrication methods described in [16], we are creating devices to explore the combined effects of geometry, surface treatments, and oil formulations to support mobile BZ droplets. Our preliminary results illustrate that BZ droplets provide an experimental model system that captures some of the functional flexibility prerequisite for evolvability. The results also point towards the feasibility of chemically powered autonomous droplets.

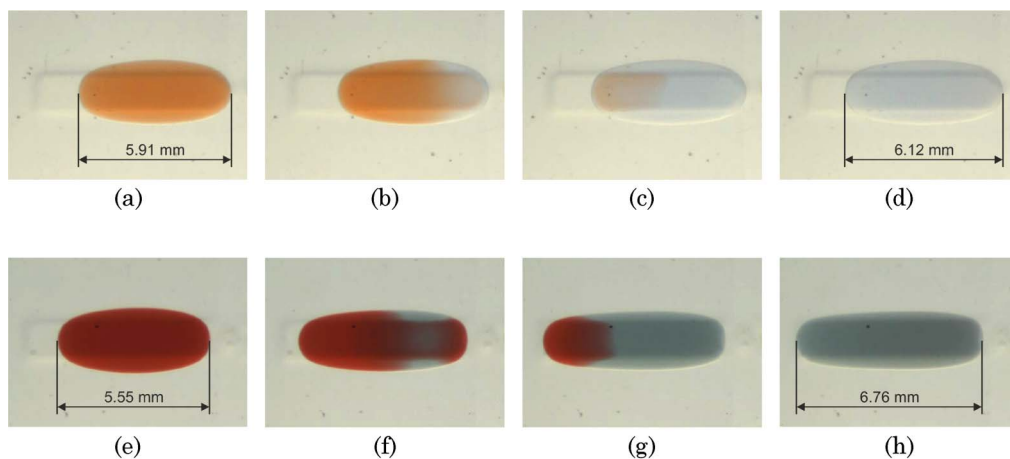


Figure 7. Oscillation-dependent shape changes in Belousov-Zhabotinsky slugs contained within an oil-filled trench device. Both BZ slugs experience shape changes following the direction of the trench as they oscillate from the reduced to the oxidized state. (a)–(d): The BZ slug containing a medium developed for information transmission shows some shape change. (e)–(h): A much more pronounced actuation is possible if the medium is optimized with actuation as an objective. The flexibility of the BZ droplets to adapt to different objectives hints at the possibility of using evolutionary methods for functional specialization of droplets.

4 Conclusions

By the middle of the 19th century it became apparent that the smallest unit showing the properties of life is the cell and that all organisms are composed of cells. Arguably the aspect of the cell that can be reconstituted from synthetic components most easily is the lipid bilayer of its membrane; given suitable conditions, it self-assembles. Even simpler to form are water droplets in oil coated with a single layer of lipids or surfactants. Techniques to compartmentalize mixtures of water-soluble substances by means of self-assembled membranes are now well developed, with manual (pipetting) and automated (microfluidics) methods available for a variety of topologies and phase combinations. The self-assembly of the interfaces in these architectures not only enables convenient mass production at the nanometer scale, but also offers self-repair as long as a surplus of membrane constituents is available. A droplet becomes functional on filling it with an active chemical medium, and the interface (i.e., the membrane composition) of the droplet can be adapted to its function.

We have shown here how the concept of a single autonomous droplet, which may be analogized to a single cell organism, could be extended to aggregates of functionally diverse droplets. The specialized droplets can be considered as modules from which droplet architectures can be composed as long as the compatibility of the interfaces is maintained, as in the example of the Belousov-Zhabotinsky droplet system considered here. Starting from a droplet design that was engineered for signal transduction but exhibited some shape change on excitation, we have investigated how this chemically driven actuation can be enhanced to arrive at droplets that convert chemical energy into mechanical motion.

A fully functional Braitenberg droplet architecture is still a few steps away. Nevertheless, the progress so far indicates that droplets are versatile building blocks even if the synthetic droplets used are far simpler than what is found in nature. Droplets are easy to produce, to manipulate, and to observe at the macro scale. But, as nature amply demonstrates, they can be scaled down to micrometer size and have a virtually unlimited upgrade path in functionality. We therefore view autonomous droplets as amenable to evolutionary procedures and as a promising new direction in robotics.

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