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Biomimicry of Cellular Motility and Communication Based on Synthetic Soft-Architectures

Lei Wang, Shidong Song, Jan van Hest, Loai K. E. A. Abdelmohsen,* Xin Huang,* and Samuel Sánchez*

Cells, sophisticated membrane-bound units that contain the fundamental molecules of life, provide a precious library for inspiration and motivation for both society and academia. Scientists from various disciplines have made great endeavors toward the understanding of the cellular evolution by engineering artificial counterparts (protocells) that mimic or initiate structural or functional cellular aspects. In this regard, several works have discussed possible building blocks, designs, functions, or dynamics that can be applied to achieve this goal. Although great progress has been made, fundamental—yet complex—behaviors such as cellular communication, responsiveness to environmental cues, and motility remain a challenge, yet to be resolved. Herein, recent efforts toward utilizing soft systems for cellular mimicry are summarized—following the main outline of cellular evolution, from basic compartmentalization, and biological reactions for energy production, to motility and communicative behaviors between artificial cell communities or between artificial and natural cell communities. Finally, the current challenges and future perspectives in the field are discussed, hoping to inspire more future research and to help the further advancement of this field.

1. Introduction

In nature, cells are sophisticated membrane-bound units that contain the fundamental molecules of life, possessing various functions to keep organisms alive.^[1] Research in the area of synthetic cells (or protocell models) has recently increased its activities to shed light on prebiotic forms of early life, especially toward understanding the structure, function and their evolution process.^[2] Even though understanding the process of cell evolution is a challenging and conceptive goal, yet the reliable design and engineering of synthetic cells is the first step toward that direction. Therefore, some representative synthetic cell models have been created based on liposomes,^[3] polymersomes,^[4] proteinosomes,^[5] capsosomes,^[6] dendrimersomes,^[7] or colloidosomes,^[8] among others. Besides structure, cells possess multifunctionality in order to exist or even thrive on Earth, such as compartmentalization, growth

and division. However, this is not enough; as cells should also be able to adjust themselves to various harsh environments by signal sensing, cellular information processing, subsequently adaptive changes and communicating with other cells, which would be the basic evolution requirements for survival. Toward fully understanding of these cellular characteristics, many efforts have been devoted in this field with fast developments. Although several reviews have discussed the fundamentals, designs, functions or dynamics, respectively,^[4,10–14] there is not any that discusses the tactic or communicative behaviors of protocell models. Therefore, it is timely to summarize the current progress in this field, and to present the existing challenges in cellular mimicry toward the next step for smart artificial biosystems.


Herein, we have summarized the representative works using soft systems for cellular mimicry in the past few years, following the main outline of cellular evolution, from basic compartmentalization, and biological reactions for energy production, to motility behavior and communicative behaviors between artificial cell communities or between artificial and natural cell communities. Finally, we discuss the current challenges and future perspectives in the field, hoping to inspire more future research.

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2. Biomimetic Soft Systems

Inspired by nature, researchers aim to create complex, multi-functional systems that are able to show life-like behaviors. Indeed, research toward protocellular systems is gaining increased attention. One significant feature in natural systems is compartmentalization, which is an essential element of all living systems—it allows complex reactions to occur with high efficiency while providing stabilizing and spatially ordered micro and/or nano environments for diverse (complex) enzymatic networks. Membrane-bound biological systems (e.g., mitochondria, endosomes, or the nucleus) are examples of this compartmentalization strategy. The membranes confine, for example, functional enzymes and genetic information, while facilitating and controlling molecular transport between different organelles. Engineering synthetic replicas of the biological systems has attracted the attention of many research groups across the globe. The demonstration of life-like behaviors based on artificial compartments while mimicking intricate structural features of biological systems is still a challenging task. In general, biological compartments are soft, allow selective exchange of small molecules, and are able to undertake various reactions without unwanted cross-talks. Bearing this feature in mind, in this section we will highlight the recent advances in developing soft synthetic compartments, ranging from liposomes, proteinosomes, coacervates, and polymersomes to capsosomes, as is shown in **Figure 1**. Moreover, the advantages and disadvantages of synthetic soft compartments as cell mimics will be critically assessed. Since detailed fabrication strategies have already been described in some excellent reviews, then will not be discussed here.^[3,4,9]

2.1. Giant Liposomes

Liposomes are the first reported synthetic replicas of biological compartments. They possess aqueous lumens delineated by lipid bilayers. Similar to their biological counterpart, liposomes are capable of encapsulating enzymatic cargoes, nucleic acids and small molecules and thus, they represent an excellent class of synthetic biomimics. The practical and conceptual elegance of liposomes is apparent in their self-assembled structure, which, upon modification, can become permeable and in contrast to more complex architectures they are fabricated from biocompatible phospholipids. In general, liposomal membranes are not permeable; however, innovative solutions such as the insertion of channel proteins (such as OmpF, α -hemolysin, or gramicidin) or DNA nanopores have been utilized so that the permeability issue can be overcome. Indeed, transmembrane porins render liposomes more versatility for artificial cells research. For example, Noireaux and Libchaber utilized giant liposomes as a platform for cell-free protein synthesis, they successfully reconstituted α -hemolysin into the lipid membrane without disruption, which allowed external feeding of medium (amino acids, nucleotides) to enter liposomes and efficiently prolonged *in vitro* transcription and translation mediated protein expression.^[11]

Liposomes are either unilamellar (with a single bilayer) or multi lamellar (with multiple bilayers). Due to their single lipid bilayer, unilamellar liposomes are more similar to the structure



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Xin Huang received his Ph.D. from Jilin University, China, in 2009. Then he joined Centre for Advanced Macromolecular Design (UNSW, Australia) as a post-doctoral researcher. In 2010, he continued his research supported by Alexander von Humboldt Foundation under the guidance of Prof. Brigitte Voit at Leibniz

Institute of Polymer Research Dresden, Germany. In 2012, he was granted a Marie Curie Research Fellowship in Prof. Stephen Mann's group at University of Bristol, UK. From 2014, he was appointed as full professor at Harbin Institute of Technology, China. His main research interests lie in the construction and application of proteinosomes, toward the biomimicry of protocells, artificial organelles, or nanoreactors.



Samuel Sánchez (Ph.D. Chemistry) is currently an ICREA Professor, Group Leader, and Deputy Director at the Institute for Bioengineering of Catalonia. Previously he was postdoc at NIMS, Japan (2009–2010); group leader at IFW Dresden (2010–2013) and at Max Planck Institute for Intelligent Systems, Stuttgart (2013–

2017), Germany. His group activities span from catalytic nano and micro motors to 3D Bioprinted actuators aiming at engineering life-like systems across different length scales.

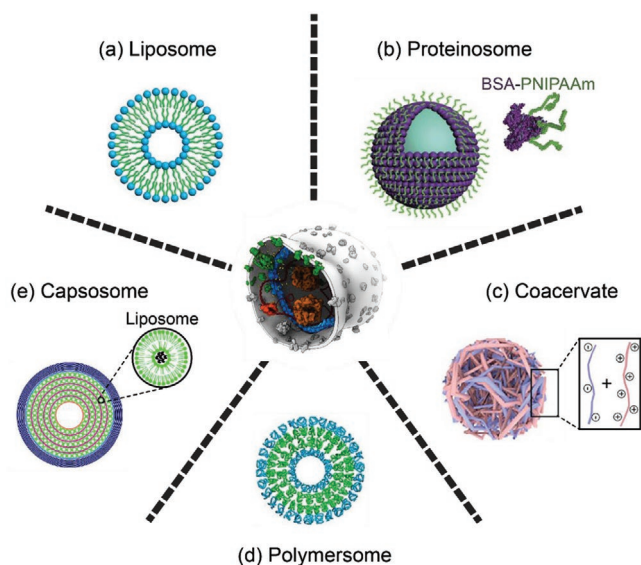


Figure 1. Systematic overview of biomimetic scaffolds. Center image shows an artistic impression of a synthetic cell (Credit: Graham Johnson/BaSyC consortium). a–e) Potential constructs as cell mimetics—which are soft, semipermeable and compartmentalized systems. a,d) Reproduced with permissions.^[10c] Copyright 2014, National Academy of Sciences. b) Reproduced with permissions.^[10a] Copyright 2013, Springer Nature. c) Reproduced with permissions.^[10d] Copyright 2019, American Chemical Society. e) Reproduced with permissions.^[10b] Copyright 2010, American Chemical Society.

of eukaryotic cells.^[12] The size of liposomes ranges from few nanometers to several hundreds of micrometers. Generally, few nanometer-sized (<100 nm), several hundred nanometer-sized (100–1000 nm) and micrometer-sized liposomes (>1 μm) are referred to as small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and giant unilamellar vesicles (GUVs), respectively. With eukaryotic cells ranging from 10–100 μm , GUVs serve the best as cell models, therefore we will focus more on GUVs (Figure 2a).

Liposomes are kinetically stable vesicles; their structure is trapped in a metastable state and thus, the formation pathway, as well as the conditions of internal and external phases (such as difference in osmotic pressure), have a great impact on their stability.^[9f] An example of liposome fabrication methods is thin film hydration, and such fabrication method involves controlled hydration of dry lipid films deposited from an organic solution onto a solid surface (Figure 2b). Another well-known method of liposome formation is electroformation, where liposomes are obtained from aqueous solution with generally low amount of salt.^[9f] The electroformation method employs the deposition of a thin lipid film on an electrode (Figure 2c), followed by aqueous hydration in presence of electric field (with direct or alternating current) that speeds up the hydration process.^[9d,14] This hydration process can be controlled by adjusting the electric field parameters (e.g., voltage and frequency),^[15] which directly impacts the kinetics and the period of the liposome formation. Additionally, liposomes generated from this method are more uniform, with a percentage of unilamellar vesicles higher than that from the thin film hydration method.^[9d] Although hydration and electroformation methods

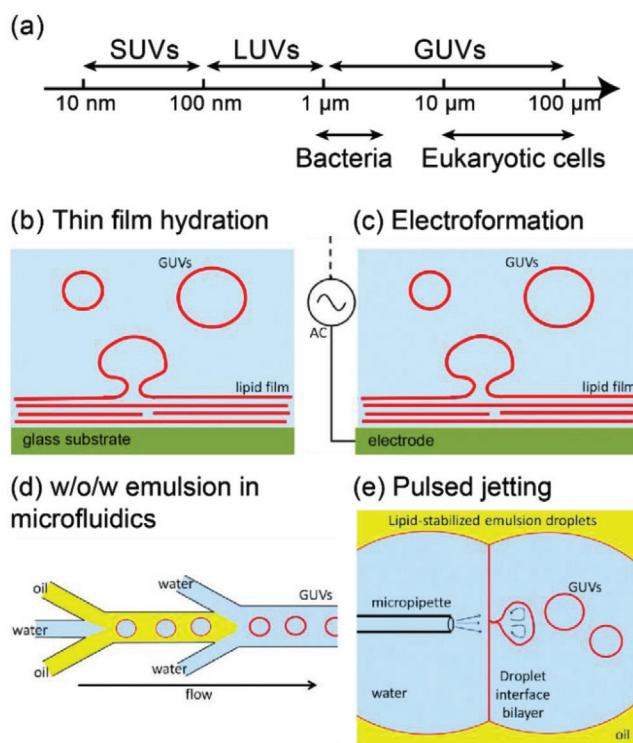


Figure 2. a) Comparison of sizes of liposomes and bacteria and eukaryotic cells. b–e) Schematic illustrations of several GUV formation methods. Adapted with permission.^[13] Copyright 2015, Royal Society of Chemistry.

are fairly common for the formation of giant liposomes, they result in relatively low encapsulation efficiency of cargoes, limiting their utility in the fields of (bio)catalysis and drug delivery carriers, where high encapsulation efficiency is highly desired. Interestingly, the electroformation method was modified by Pott et al. to obtain GUVs from deposits of aqueous solutions of SUVs and LUVs under high ionic strength condition (up to $250 \times 10^{-3} \text{ M NaCl}$),^[16] making such methodology also suitable under more physiologically relevant conditions. Indeed, these findings have advanced the potential of such methodology in the field of mimicking life-like systems and designing biological mimics. Still, one concern related to this method is that the applied electric field could influence the structure of proteins with charges changing during encapsulation and could lead to unnatural behaviors or loss of activity.^[17]

Moreover, various microfluidic techniques have been employed to facilitate liposome formation while reducing reaction volumes and maintaining relatively high throughput. Microfluidics was incorporated to the commonly used GUV formation methods, such as stable w/o emulsions and w/o/w emulsions (Figure 2d).^[18] Utilizing microfluidics, highly stable monodisperse liposomes (that last for at least 26 days) encapsulating cells and proteins were generated.^[18] Another method for liposome formation is the pulsed jetting methodology. This methodology involves the formation of a planar bilayer membrane right at the outlet of a micron-sized nozzle, followed by aqueous phase jetting from the same nozzle into the bilayer and forcing the membrane bent to form a vesicle (Figure 2e).^[19] Liposome size can be tuned by changing the jetting volume and

speed. An advantage of this method is the excellent encapsulation ability, which was demonstrated by using various biorelevant molecules, such as DNA^[19] and pore-forming proteins,^[20] though it is not clear if the functionality and structure of the biological encapsulants were maintained after applying the jetting process with high shear pressure. Additionally, large-sized encapsulants such as latex beads (diameter of 500 nm) have been directly jetted into liposomes by this method that bypasses the molecular weight dependent uptake selectivity.^[20]

2.2. Proteinosomes

It is well known that proteins are nature's highly versatile building blocks and possess broad functions, thus offering another platform for the preparation of artificial cells. Therefore, proteins are ideal building blocks for the "bottom-up" construction of biomimetic compartments, namely: proteinosomes. Proteinosomes are formed from either natural amphiphilic protein (e.g., lipase) or synthetic protein amphiphiles (e.g., natural hydrophilic protein such as bovine serum albumin (BSA) conjugated to synthetic hydrophobic polymer). The concept of proteinosomes was first introduced by Mann group in 2013.^[10a] They prepared amphiphilic protein-polymer conjugates, which self-assembled on an oil droplet-based template to produce microscale compartments (Figure 3a). Similarly, proteinosomes can also be obtained by layer-by-layer (LbL) deposition of proteins on a solid spherical template followed by crosslinking and core removal (Figure 3b).^[21]

The structure of proteinosomes, prepared by Huang and co-workers, was maintained by means of a temperature-responsive

semipermeable membrane that consisted of a closely packed monolayer of conjugated protein-polymer building blocks. The permeability of the membrane was tuned by first assembling the proteinosomes from different proteins, which was followed by subsequent removal of one or more protein species from the membrane. This method is called "self-sacrificing"—utilizing this strategy, Liu et al. prepared a hybrid semipermeable microcapsule comprising three building blocks, i.e., polymer conjugated dextran, lysozyme, and BSA.^[22] Lysozyme was cut off by tris(2-carboxyethyl)phosphine (TCEP), which cleaves disulfide units and BSA was hydrolyzed (degraded) using protease enzyme. By the combination of chemical cleaving of the disulfide units, and the following enzymatic degradation process, both the lysozyme and BSA were removed from the membrane, turning it permeable toward (macro)molecules with molecular weight cutoff of the membrane ranging from 10 to 71 kDa.

The composition of proteinosomes' membrane is not only limited to proteins and polymers, but it can be extended to inorganic nanoparticles.^[23] To this end, Wu et al. reported the formation of hybrid proteinosomes by utilizing proteins/inorganic metal clusters/poly(*N*-isopropyl acrylamide) (PNIPAAm) as building blocks. This hybrid membrane is robust, ideal for supporting interfacial catalytic activity and excellent platform to immobilize enzymes on the interface of oil/water.^[23,24] Due to the diversity and the broad characteristics of inorganic nanoparticles, unlimited features such as photoluminescence, self-rehydration, enhanced antibacterial effect, and interfacial catalysis were installed. These capabilities have further broadened the applications of proteinosomes in the fields of bottom-up synthetic biology, bioinspired microstorage/microreactor, as well as drug/gene delivery.

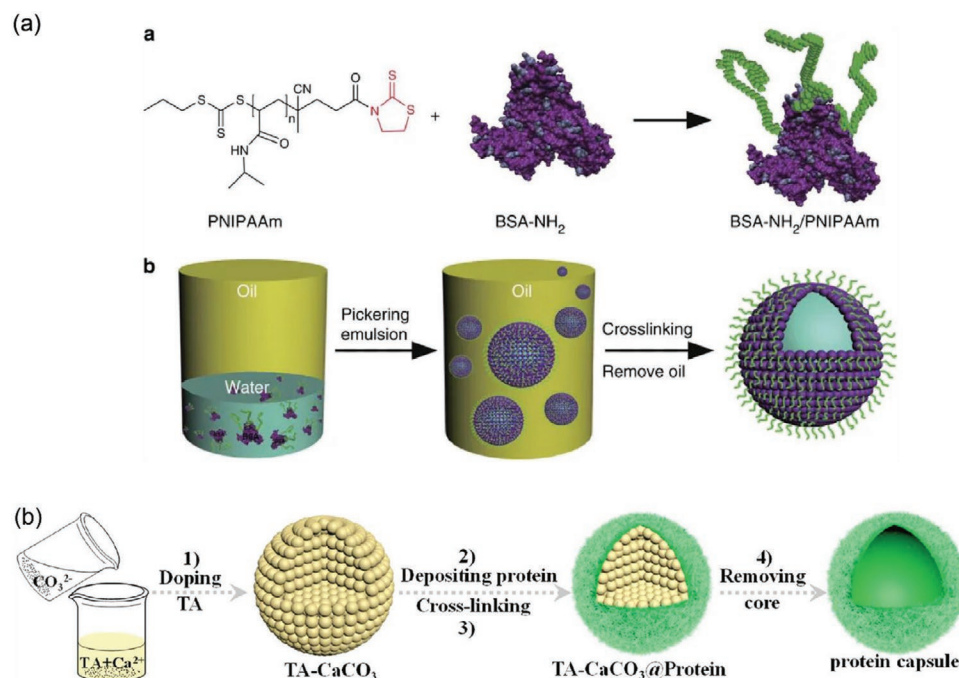


Figure 3. Preparation procedure of proteinosomes. a) Functionalization of protein-polymer nanoconjugates (BSA-NH₂/PNIPAAm) in (a). Use of nanoconjugates for the spontaneous assembly of proteinosome microcompartments in oil, and their transfer into a bulk water phase. Reproduced with permission.^[10a] Copyright 2013, Springer Nature. b) Protein deposition on a spherical inorganic CaCO₃ template followed by crosslinking and core removal. Reproduced with permission.^[21] Copyright 2019, American Chemical Society.

2.3. Coacervates

Coacervate microdroplets are membrane-free compartments formed through the interaction between oppositely charged polyionic (macro)molecules. A membrane-less coacervate was reported by Oparin as a prebiotic protocell model and a basic metabolic unit resembling those formed on the early Earth before the formation of membranes.^[25] Membrane generation is a complex process and the exchange of compounds between a membrane-delimited compartment and external environment requires complicated enzymes and proteins that did not exist in prebiotic time.^[26] Complex coacervates assembled from oppositely charged synthetic polyelectrolytes or bio-macromolecules (polysaccharides, polypeptides and nucleotides) have been widely studied and investigated as mimics of crowded intracellular environment, providing a useful platform for the modeling cellular behavior *in vitro*. Coacervates formed from nucleotide small molecules and polycationic species have been investigated as a novel protocell model. The properties and behavior of the nucleotide-based coacervates are unique as the viscoelastic, crowded phase can readily sequester a host of molecular and macromolecular components dependent upon their charge and hydrophobicity. The efficacy of the crowded internal environment of coacervates has been utilized to activate a complex multienzyme system (minimal polyketide synthase) with up to 20-fold rate increase—due to enrichment of enzymes and stabilizing of enzyme complex arising from interacting with crowded environment^[27]

The energetics of coacervation have gained a great deal of interest over the last decade.^[28] It could be intuitive to consider coacervation between oppositely charged polyelectrolytes is electrostatic-driven process, and thus enthalpy driven. Interestingly, Priftis et al. has proved the entropy-driven nature of coacervation.^[29] They performed isothermal titration calorimetry (ITC) measurements on multiple pairs of polypeptides and polyelectrolytes. ITC is a technique which provides information over the thermodynamic parameters of interactions in solution. From ITC measurements information on binding energy, Gibbs free energy, entropy, and enthalpy changes can be obtained. The ITC results showed almost no heat release during coacervation and enthalpic contribution is almost absent.^[29,30] These observations have led to the conclusion that the major driving force during coacervation is favorable entropy gain, which is a result of the release of trapped counterions during polyelectrolyte association.

Membrane-free coacervates assembled from peptide/nucleotide and polymer/nucleotide are able to endure dynamic environmental changes (e.g., pH and temperature) and selectively sequester and concentrate molecules.^[31] Significantly, polymer/nucleotide coacervate which was assembled from (poly(diallyldimethylammonium) chloride (PDDA) and adenosine triphosphate (ATP)) displayed the sequestration ability toward more diverse range of objects, including nanoparticles and proteins.^[31a] Recently, Kumar et al. reported the encapsulation of an organelle chloroplast in a polymer/polysaccharide coacervate (Figure 4a). Such a complex was able to undertake light-induced electron transfer, which is one step in photosynthesis, representing an excellent example of a synthetic cell with a functional organelle.^[32]

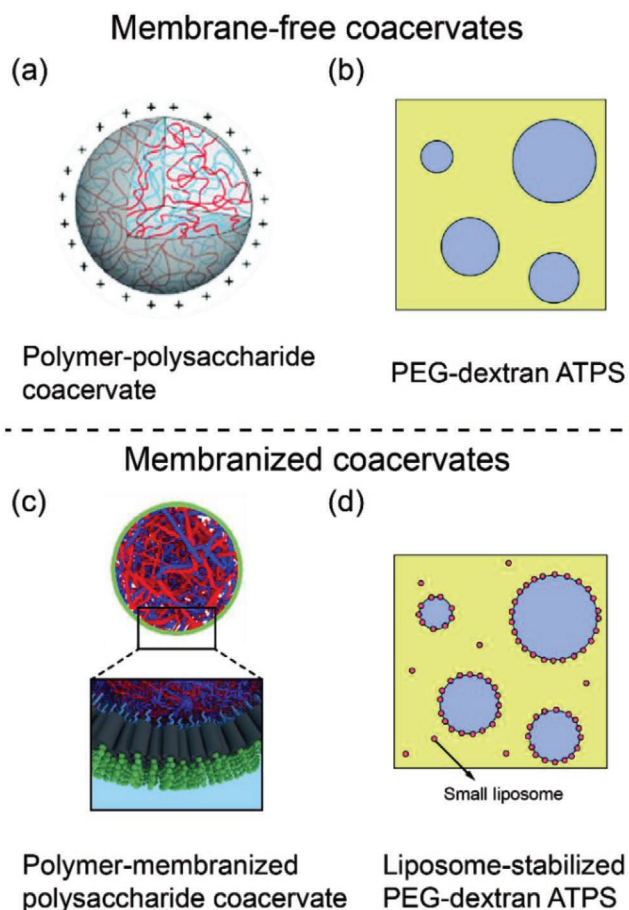


Figure 4. Schematic illustration of membrane-free and membranized coacervates. a) Polymer/polysaccharide coacervate microdroplets comprised of positively charged poly(diallyldimethylammonium chloride) (PDPA) and negatively charged carboxymethyl-dextran (CMDX). b) Aqueous two-phase system (ATPS) with dextran-rich phase dispersed in continuous PEG-rich phase. c) Hierarchical structure of a triblock polymer-membranized coacervate with oppositely charged amylose biopolymers in the coacervate core. d) Small liposome-stabilized ATPS coacervates. a) Reproduced with permission.^[32] Copyright 2018, Royal Society of Chemistry. b,d) Reproduced with permission.^[35] Copyright 2012, American Chemical Society. c) Reproduced with permission.^[34] Copyright 2017, American Chemical Society.

The coacervates' membrane-less nature renders them a rudimentary form of protocells, which lacks a strong resemblance to eukaryotic cells.^[2,33] Such coacervate systems are sensitive to ionic strength and easily dissociated at sufficiently high salt concentration. To overcome this complication, van Hest and co-workers reported a semipermeable membrane-stabilized coacervates,^[34] which assembled from oppositely charged amylose derivatives, were stabilized by the addition of a terpolymer, comprised of a hydrophilic poly(ethylene glycol) segment, a hydrophobic poly(caprolactone-gradient-trimethylene carbonate) segment and a negatively charged poly(glutamic acid) segment (Figure 4c). Abdelmohsen and co-workers utilized such protocell system to mimic cellular compartmentalization, emulating the cellular logic of eukaryotic organisms.^[10d] This was accomplished by the spontaneous sequestration of proto-organelles, in this case, semipermeable polymersomes,

generating a unique biomimetic platform reminiscent of the spatial organization in eukaryotic cells. Indeed, this work presented the importance of the proto-organelles for spatial ordering of incompatible enzymes to prevent catalytic cross-talks and proteolytic deactivation. Additionally, the robust nature of this hierarchical system was highlighted in a co-culture experiment where the stability of such subcompartmentalized protocell was maintained in the same medium as living cells.

Another category of coacervate in the aqueous two-phase system (ATPS), which has also been adapted to mimic cells.^[36] The most common type of ATPS comprise two aqueous polymers, such as poly(ethyl glycol) (PEG) and dextran (Figure 4b). Such system prevails due to their biocompatibility and relatively easy preparation. When the concentrations of both PEG and dextran are sufficiently high, macrophase separation occurs.^[35] The partitioning of compounds between two phases endows the formed system with the ability of spatial separation and enrichment of molecules. Due to the difference in hydrophobicity in PEG and dextran-rich phases, proteins accumulate in dextran-rich phase and denatured proteins in PEG-rich phase (as the exposed hydrophobic areas in denatured proteins match more hydrophobic PEG). Moreover, the partitioning process is dependent on the size of solutes. Similar to complex coacervate systems, the stability of ATPS coacervate can be drastically increased after membrane formation, such as using polymers or lipids (Figure 4d), thus avoiding the aggregation due to the electrostatic repulsion generated from the membrane.^[37]

2.4. Polymersomes

Polymersomes are self-assembled copolymeric vesicles, usually made of amphiphilic copolymers.^[38] They are capable of displaying various properties dictated by the chemical structure of their components such as varying size, shape, response to pH and temperature, amphiphilicity, modular assembly, chain packing, and biofunctionalization. The behavior and properties of polymers are comparable to those of lipids where a balance between the hydrophilic and hydrophobic portions dictates assembly into micellar, rod-like, or lamellar structures. Their tunable properties and biofunctionality make them very interesting candidates for drug delivery, nano/microreactor, and cellular mimics.^[39] Polymersomes have been successfully used to encapsulate enzymes, and other functional bio-macromolecules, for tandem processes where a series of segregated enzymes can work together across defined boundaries.^[40] Polymersomes, a “soft” colloid, possess a great degree of functionality, owing to their compartmentalized structure. Indeed, polymer membranes have been presented as an interesting synthetic mimic of the cell membrane. Block copolymer (BCP) membranes display the same amphiphilic character as lipids, however, are more stable and chemically versatile. Moreover, the plethora of functionalization approaches for polymer membrane endow polymersomes beneficial properties such as the ability to tune surface chemistry and membrane permeability.^[38,41] Tunable permeability, which enables in- and efflux of substrates and products, paving the way for inter and intracompartmental communication, has been realized in different

approaches. Indeed, permeability toward molecule size can be controlled by tuning polymer packing in the membrane. Exemplary of this is the polymersomes self-assembled from PEGylated polycationic and PEGylated polyanionic peptides (PICsomes) with loosely packed membrane, which permits free diffusion of small molecules.^[42] Polymersomes permeability can be engineered to be permanently permeable through the insertion of membrane proteins such as OmpF,^[43] Aquaporin Z,^[44] or melittin.^[45] Another widely used approach is the on-demand control of permeability, which was achieved through the design of membranes that comprise stimulus-responsive moieties. Such permeability can be switched either on or off upon trigger such as pH,^[46] temperature,^[47] redox,^[48] or light.^[49] For example, redox-responsive polymeric nanoparticles reported by the group of Palivan were loaded with various cargo,^[48] such as fluorogenic substrate of lipase or protein ion channel. Such modification enabled the triggered rupture and release of cargo through the addition of disulfide reducing agent. This level of control allowed biobehaviors, namely, signaling cascade and communication. Such biomimetic communications will be discussed in Section 5.

Recently, polymersomes that encapsulate enzymatic cargos have been developed—they were shown to be capable of regulating cascade reactions and can be applied in cellular therapies as a kind of “synthetic organelles.”^[50] Enzymatic cargos can be encapsulated during polymersome formation through two main approaches, namely thin film rehydration and solvent switch.^[38] Thin film rehydration involves dehydration of polymer solution into a dry polymer film followed by rehydration with aqueous solution, similar to that for preparing liposomes (Section 2.1).^[51] Water-soluble payload can be dissolved in rehydration aqueous solution to be encapsulated; however, this could limit the encapsulation of fragile hydrophobic cargo. Solvent switch on the other hand could be used to encapsulate both hydrophilic and hydrophobic enzymes in the same polymersomes.^[40] Due to their versatility and ability to compartmentalize functional cargo, polymersomes are excellent candidates for the development of systems that impart a higher degree functional complexity such as a multistep enzymatic reaction occurring in multiple successive compartments.^[40,52]

Spherical polymersomes can undergo an osmotic induced shape transformation process, forming tubes, or bowl-shaped structures (stomatocytes).^[53] Of particular interest are stomatocytes, which possess dual compartments and are able to encapsulate enzymes in their cavity. Furthermore, due to their unique asymmetric shape, they were presented as motile particles^[54] (will be discussed in detail in Section 4). Polymersomes can be shape-transformed into stomatocytes via several approaches—all of which involve the introduction of osmotic imbalance over the membrane, leading to an outflow of solvent from the polymersome lumen and thus, volume reduction and consequent shape transformation (Figure 5). Most of the reported approaches of polymersome shape transformation utilize a large amount of organic solvent, making the process unsuitable for the encapsulation of sensitive enzymes. To overcome this complication, Abdelmohsen et al. developed a mild method for the shape transformation and protein encapsulation.^[54] Another case of asymmetric polymersomes with a bud has been shown by Battaglia and co-workers^[55] They formed

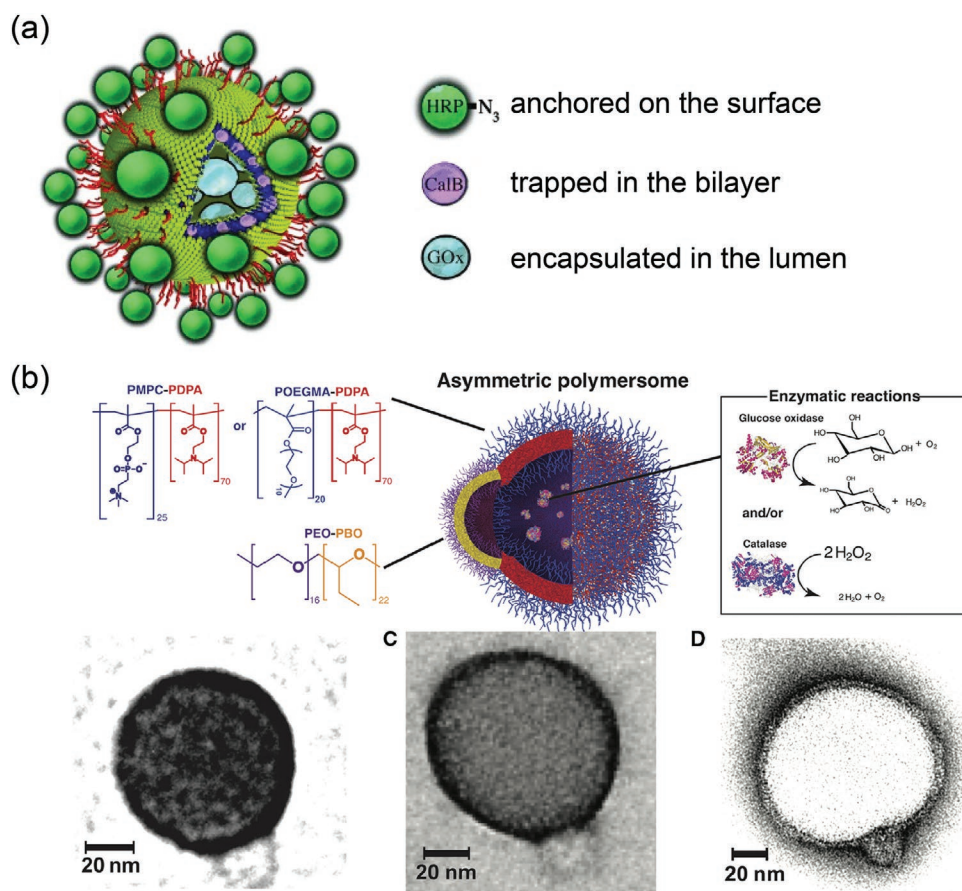


Figure 5. a) Formation of bowl-shaped stomatocytes from polymersomes. Tunable opening size allows the enzymes trapped inside aqueous cavity. Reproduced with permissions.^[40] Copyright 2009, Wiley-VCH. b) Schematic representation and TEM images of a chemotactic polymersome using a combination of membrane topology formed by PEO-PBO copolymers mixed with either PMPC-PDPA or POEGMA-PDPA copolymers. Reproduced with permissions.^[55] Copyright 2017, American Association for the Advancement of Science (AAAS).

one polymersome with two different copolymers (poly(ethylene oxide)-poly(butylene oxide) (PEO-PBO) with either poly[(2-methacryloyl)ethyl phosphorylcholine]-poly[2-(diisopropylamino)ethyl methacrylate] (PMOC-PDPA) or poly[oligo(ethylene glycol) methyl methacrylate]-poly[2-(diisopropylamino)ethyl methacrylate] (POEGMA-PDPA). The lateral segregation of polymers due to molecular weight and miscibility mismatch led to a patchy topology and eventually full coarsening and asymmetric polymersomes.^[56] With the higher permeability of the budded domain (PEO-PBO) and enzyme encapsulation in the lumen, the outflow of product was faster around the budded domain which created a local gradient (Figure 5). This local gradient can be used to induce chemotaxis of polymersomes that will be discussed further in Section 4.

2.5. Capsosomes and Polymeric Capsules

Multicompartmentalization in life can enhance metabolic efficiency and allow incompatible reactions to run in parallel while avoiding cross-talks.^[57] A class of such multicompartmentalized soft systems have been developed over the past decades, for example, vesosome (liposome(s) in liposome),^[58]

proteinosome(s) in proteinosome,^[59] polymersome(s) in polymersome^[60] and polymersome(s) in polymer capsules.^[9b] Interestingly, one class of structures that captures the advantages of both lipids and polymers are capsosomes.^[58] They comprise small liposomes within LbL polymeric capsules.^[61] In this case, the high stability of the polymer capsule and high permeability of the liposomes are combined. In order to fabricate such capsosomes, liposomes are first absorbed on a precursor polylysine layer that is predeposited on a spherical silica template. Subsequently, a separation polymer layer and liposomes are assembled on the previous layer in an alternating manner (Figure 6a). After the deposition of the final layer, the formed template is then dissolved without degrading neither the liposomes nor encapsulated proteins.^[61] During this assembly procedure, up to 160 000 liposomes can be incorporated into one capsule.^[10b] This large amount of liposomal subcompartments gives an incredible loading efficiency while keeping all loaded cargos separated. This separated space allowed spatial organization of enzymes and tandem and parallel enzymatic reactions at the same time (details in Section 3). Stability of the encapsulated liposomes can be enhanced by modifying the polymer layer with, for example, cholesterol to improve the anchoring of liposome on polymer layers. Moreover, the permeability of

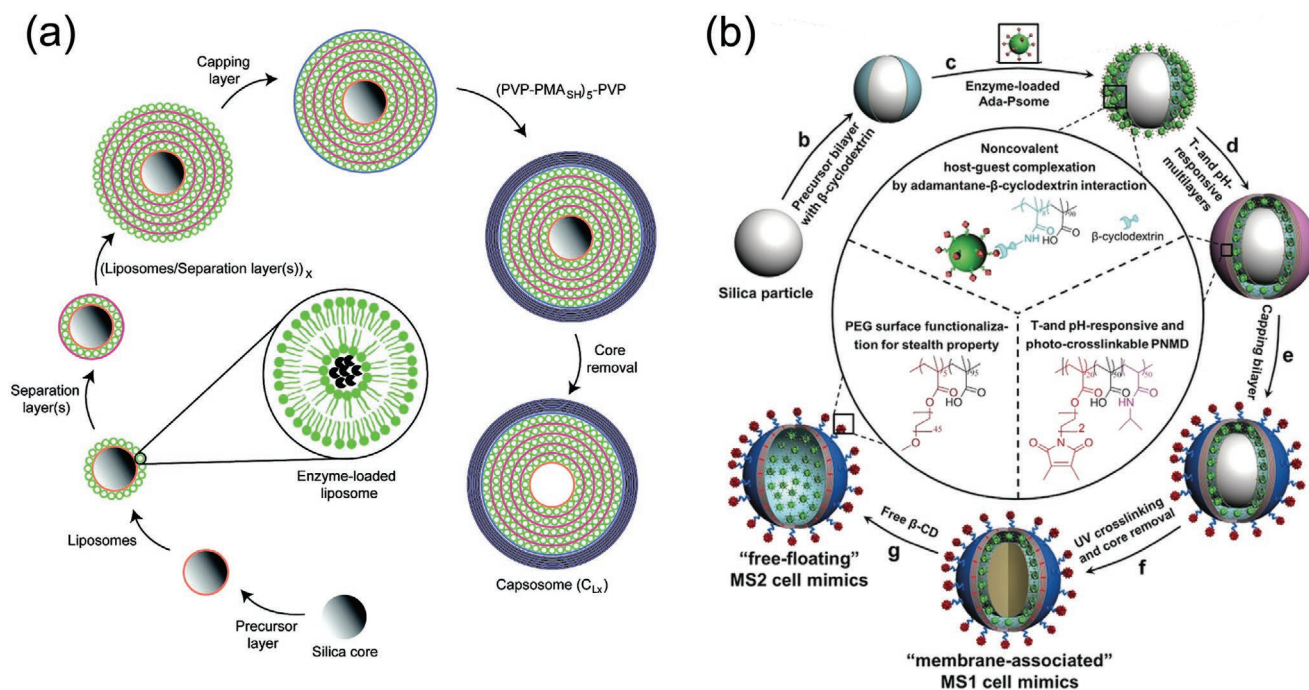


Figure 6. Schematic illustration of a) capsosome and b) polymer capsule assembly. a) A silica core as template for layer-by-layer (LbL) assembly. Separation polymer layer(s) and liposomes were alternatively deposited on silica template followed by adsorption of a polymer capping layer and core removal. Reproduced with permissions.^[10b] Copyright 2010, American Chemical Society. b) Responsive nanosized polymeric capsules were attached to silica template with precursor bilayer through β -cyclodextrin (β -CD) and adamantyl interaction followed by LbL assembly of polymer layers. Polymeric capsules were crosslinked after the adsorption of capping layer. Finally, core was removed to generate empty lumen. Reproduced with permissions.^[47] Copyright 2017, Wiley-VCH.

liposomal subcompartments can be triggered by the addition of surfactant or temperature responsiveness to induce cargo release or enzymatic cascade.^[6,61]

Similar to capsosomes are LbL polymeric capsules; they are a class of compartments that are successfully utilized in the field of biomimicry.^[47] LbL polymeric capsules are constructed through the sequential deposition of oppositely charged polymers/particles onto a template that can be sacrificed later to generate empty lumen.^[62] The assembly of LbL polymeric capsules can be achieved through several approaches and has been well covered in excellent reviews recently.^[63] The alternate deposition of polymers allows control over surface physiochemistry (e.g., charge), film thickness, and thus capsule permeability, which facilitates tunable entry and exit of payload and/or their products. Moreover, it is possible to have the entire assembly process in water, which helps to preserve bioactivity of some fragile biomolecules that are sensitive to organic solvents.^[63b] The abundance of polymer also makes it possible to incorporate stimuli-responsiveness such as pH and temperature in polymer capsules.^[38] For example, Voit and co-workers reported a polymeric capsule comprised of a copolymer PNIPAAm-*b*-poly(methacrylic acid) (PMAA)-*co*-3,4-dimethyl maleic imidobutyl methacrylate (PNIPAAm-*b*-PMAA-*co*-DMIBM) which responds to both pH and temperature (Figure 6b).^[47] To prevent the disintegration of polymeric capsules after polymer segments change their hydrophobicity, the capsules were first crosslinked through UV irradiation, where an enzymatic cascade was encapsulated, and the enzyme activity was shown to be controlled by temperature and pH due to the tunable permeability toward substrate.

3. Soft Compartments as Artificial Life-Like Biochemical Reactors

Cellular life is an unprecedented example of biochemical elegance, which provides cells with the energy required to maintain essential activities. Central to this is metabolism, which is a key biochemical activity, responsible for harnessing external source of energies (fuel) and converting them into another form of energy essential to cells' survival. Indeed, mediated by biochemical processes, cells are able to physically respond to chemical triggers, which can lead to, for example, motility or changes of cells' shape. The biochemical processes that make them possible have led to the development of synthetic soft systems able to undertake complex (biochemical) reactions. Indeed, as previously mentioned, compartmentalization strategies play a significant role in perfecting such biochemical reactions in biological systems by, for example, optimizing catalysts concentration while allowing communication with external niches—thus leading to all kinds of adaptive behaviors. The previous section of this review has described the most significant artificial soft systems that were designed to mimic life-like compartments. Therefore, this section will mainly focus on the combination of different synthetic soft compartments and artificial biochemical reactions to show active behaviors reminiscent of those observed in biology, toward the energy generation for cellular adaptive behaviors. We classify biochemical reactors reported so far into two categories according to their functions, namely kinetic enhancement and modulation.

3.1. The Kinetic Enhancement of Enzymatic Reaction

Cellular machineries are complex and allow reactions to proceed with unsurpassed efficiency and specificity. Biological catalysts, enzymes, ensure low activation energy barrier while allowing efficient conversion of substrates. Cells deploy various mechanisms to ensure such efficiency, such as substrate channeling and metabolon formation. Such natural process has informed us on the importance of spatial organization of enzymes in: i) reducing lag times by consecutive conversion of intermediate products; ii) regulating kinetics and thermodynamics of enzymatic processes by optimizing substrate-enzyme binding processes. Translating this information toward enzymatic kinetic enhancement in cell-like soft systems is an emerging topic, which has been demonstrated by several groups. Mann's group showed enhanced ATP-dependent enzymatic reaction in a membrane-less nucleotide/peptide coacervate.^[64] ATP was utilized as a building block for their coacervate protocell model. Such ATP-rich coacervate phase permitted efficient occurrence of ATP-catalyzed enzymatic reactions in such confined and crowded environment. Indeed, hexokinase (HK), an enzyme that requires ATP for its function, displayed two-time faster kinetics when it was sequestered in the coacervate phase. HK sequestration which led to high local enzyme concentration was one factor contributing to the enhanced activity. Another factor was hydrophobic modulation of HK which was involved the interaction between hydrophobic motif of HK and hydrophobic droplet interior. This hydrophobic modulation resembled mitochondria-bound HK in brain cells which had a positive impact on the enhanced activity.^[65]

Similarly, Keating's group showed an enhancement of ribozyme rate of catalysis when it was encapsulated in ATPS membrane-less coacervate system.^[66] Such ATPS-based coacervate comprised PEG and dextran whereas, ribozyme was partitioned into the dextran-rich phase due to the similar hydrophobicity between dextran and ribozyme.^[35] The partitioning of ribozyme was found to be dependent on the length of the RNA strand, allowing control of the rate of selective uptake and/or exclusion. The enrichment of ribozyme in the coacervate phase led to a 70-fold kinetic enhancement. The kinetic enhancement was attributed to enrichment (≈ 100 -fold concentration in RNA-enriched dextran-rich phase) as catalysis rate scaled with local ribozyme concentration. Due to the limited stability of such ATPS coacervate system, the same group continued to develop it further so that better stability can be achieved. PEG and dextran aqueous interfaces were stabilized using nanoscale liposomes ($d \approx 130$ nm), forming a Pickering type emulsion with dextran-rich phase dispersed in continuous PEG-rich phase.^[37] The additional layer of liposomes at the emulsion interface provided extra stability while allowing the in-/out-transport of RNA and DNA across the liposomal layer. Additionally, the ribozyme cleavage reaction enhancement maintained at the same level after liposomal stabilization. This system provided a stable interior, allowed in and out transport of substrate and product and maintained enzymatic reaction enhancement due to enzyme enrichment, which makes it a good candidate as protocellular reactor.

Another example of utilizing liposomes as subcompartments in protocell models are the capsosomes. As mentioned in Section 2, the spatial organization of liposomes in such

protocells facilitates biochemical processes without destructive cross-talk. In such hierarchical system, Hosta-Rigau et al. carried out an enzyme cascade comprising uricase and horseradish peroxidase (HRP) enzymes, whereas, both enzymes were loaded separately in two populations of liposomes before they assembled into capsosomes and became liposomal subcompartments. Assembling polymer capsules without enzyme-loaded liposomes but exposed to free enzyme solution showed almost zero enzymatic conversion since enzymes were not assembled into capsules in the absence of liposomal subcompartments. Subcompartmentalized enzymes showed enzymatic turnover and steadily increased conversion over time.^[6] Additionally, a third reaction involving ascorbate oxidase (AO) was performed parallel in liposomal subcompartments to the aforementioned uricase-HRP enzyme cascade. The ability to perform multiple enzyme reactions in the subcompartments of a concentric system is reminiscent of organisms with enzymatic reactions performing in the middle space of their double membranes (e.g., mitochondria).

Similarly, a coacervate protocell with hierarchical subcompartmentalization (polymersome-in-coacervate protocell) has been demonstrated by Abdelmohsen and co-workers^[10d] The structure and properties have been discussed in Section 2.3. Polymersomes, preloaded with glucose oxidase (GOx) and/or HRP, as proto-organelle were encapsulated in the interior of coacervate protocells. Two different spatial layouts of enzymes were obtained—GOx and HRP were in one coacervate but two populations of polymersomes, or GOx and HRP were co-encapsulated in one population of polymersomes and coacervate. As expected, the kinetic enhancement in co-encapsulation surpassed separate encapsulation since GOx and HRP stayed in proximity in one polymersome in the case of co-encapsulation and prevented intermediate substrate hydrogen peroxide from diffusing over polymeric membrane (Figure 7a). Moreover, the cascade was still performed successfully in the case of separate encapsulation, illustrating the transmembrane and cross-interior transport of intermediate from one polymersome to another polymersome, which endowed such protocells with the ability of interorganelle communication. To mimic cells, it is also important to preserve structural integrity and bioactivity during both the assembly and encapsulation process. To do this, van Hest and co-workers showed a functional polymersome system with enhanced activity that maintained its structural integrity upon its loading with multiple enzymes.^[67] When polymersomes were loaded with enzymes in aqueous lumen, a 100-fold increase of the activity was observed compared to free enzymes in bulk after enzyme concentration in both case was corrected to same level, which suggested enzyme enrichment was not the reason of enzymatic enhancement. A mechanism involving polymer-enzyme interaction were therefore proposed—this hypothesized interaction either absorbed more enzymes than calculated inside the polymersomal lumen or made the enzyme active site more exposed and accessible toward substrate, which gives a glimpse of the complexity that has been restored in cell-like systems. In the same system, three enzymes, *Candida antarctica* lipase B (CALB), GOx, and HRP were separated in three different domains. Every enzyme was positioned in a different region within such system (CALB in external solution, GOx in the aqueous lumen of

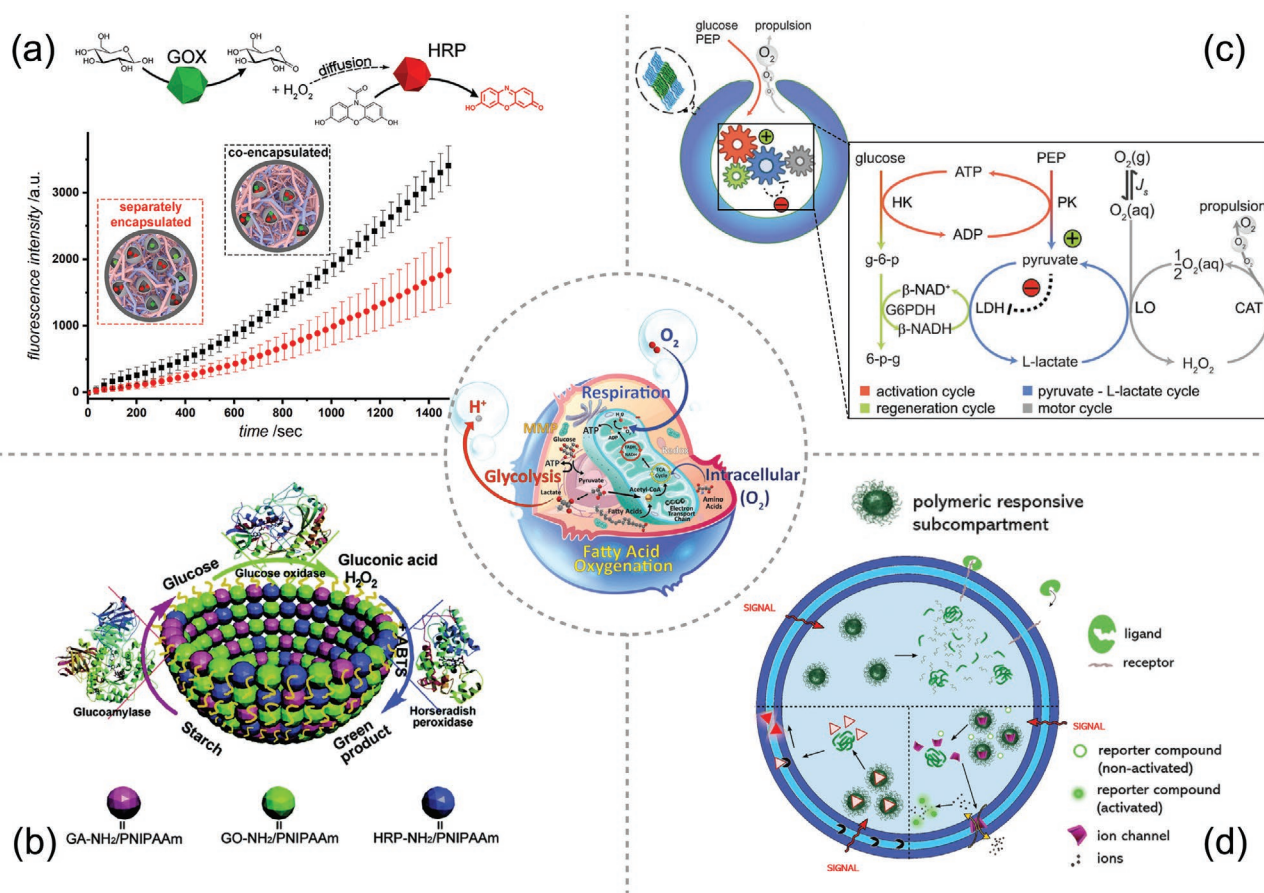


Figure 7. Schematic representation of soft compartments as artificial life-like biochemical reactors. a) A two-step enzymatic reaction in a multicompartimentalized polymersome-in-coacervate system. b) A proteinosome membrane-mediated three-stage cascade reaction in water. c) A compartmentalized metabolic network confined in a stomatocyte. d) A modular multicompartiment system composed of reduction sensitive subcompartments for triggered enzymatic activity and ion channel recruitment, encapsulated within polymeric giant unilamellar vesicles. a) Reproduced with permission.^[10d] Copyright 2019, American Chemical Society. b) Reproduced with permission.^[68a] Copyright 2014, Springer Nature. c) Reproduced with permission.^[68b] Copyright 2016, American Chemical Society. d) Reproduced with permission.^[48] Copyright 2019, Wiley-VCH.

polymersome, HRP in the hydrophilic part of polymersomal membrane) and a cascade reaction was smoothly performed upon the addition of substrate in external solution to initiate the tandem reaction.

3.2. Modulation of Enzyme Activity

Enzyme activity is modulated by various factors, including enzyme conformation and structure, dynamics and substrate/active site accessibility. Modulating enzyme activity is critical to regulating enzymatic networks in biology. Attempting to replicate such behaviors in the lab, several methods have been developed, such as spatial organization of enzymes, responsive substrate entry upon tunable membrane permeability, and incorporation of positive and negative feedback routes.^[69] One example of the role of spatial organization in modulating the rate of enzymatic reactions has been presented by Huang et al.^[68a] They incorporated a functional enzymatic cascade comprising three enzymes: gluco amylase (GA), GOx, and HRP, in proteinosomes.^[68a] These three enzymes were conjugated to PNIPAAm to form amphiphilic protein-polymer

nanoconjugates, as the building blocks of the membrane of proteinosomes. Using starch as a substrate, a significant variation of activity was observed upon translocation of intermediate products glucose and hydrogen peroxide around the proteinosomes (Figure 7b). When either GOx or HRP was moved from membrane into the aqueous lumen of the proteinosomes, the reaction rate remained constant as three enzymes all present in the membrane. The semipermeability of membrane plays a role in it—substrates for both GOx and HRP are small enough to diffuse over the proteinosome membrane, therefore relocation of either enzyme had no impact on the overall cascade reaction rate. As expected, however, the reaction rate was reduced by $\approx 90\%$ when GA was translocated in the lumen. This was attributed to the diffusion-limited uptake of GA substrate, starch, which cannot diffuse across the proteinosome membrane. Moreover, the proteinosomes have shown a membrane thermo-responsive behavior, as PNIPAAm possesses a lower critical solution temperature (LCST) at 33 °C. Peroxide myoglobin was encapsulated in proteinosomes to show temperature modulated reaction.^[10a] The reaction rate increased with elevated temperature until 33 °C, after which a drastic drop of the reaction rate was observed.

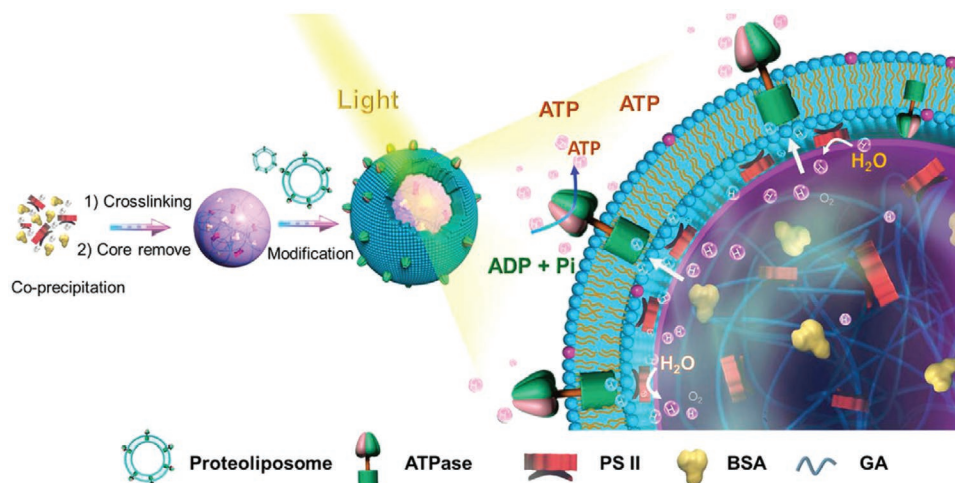


Figure 8. Schematic Representation of light-driven ATP synthesis: Coassembly of CFoF1-ATPase and PSII. Reprinted with permission.^[70] Copyright 2015, American Chemical Society.

By increasing or decreasing the temperature, a high degree of control over the permeability of the proteinosome membrane toward substrate was achieved, therefore a switch of activity of encapsulated peroxidase myoglobin, which also paves the way to actively control the cellular communicative behaviors (details in Section 5).

A metabolic pathway comprising a network of six enzymes with regulatory feedback was sequestered inside the aqueous cavity of stomatocytes.^[68b] This enzymatic network was operated far from equilibrium and able to convert chemical fuel (glucose) into kinetic energy as output (Figure 7c). The metabolic network, starting with an ATP-mediated reaction cycle containing HK and pyruvate kinase (PK), used ATP as an internal regulator to modulate reaction rate in a second circle comprising a self-inhibitory enzyme at high substrate concentration. Furthermore, the output of this compartmentalized out-of-equilibrium network was sustained even at very low concentration of fuel ($<5 \times 10^{-3}$ M), which was due to the built-in modulation regulatory machinery. Similarly, metabolic mimicry has been performed in a multicompartimentalized polymersome-in-polymer capsule.^[47] Utilizing an enzyme cascade comprising GOx and myoglobin, Voit and co-workers demonstrated biomimetic metabolism through a process communication between subcompartmentalized polymersomes. Moreover, the authors showed the ability to control this behavior by using external stimuli such as pH and temperature. To do this, polymersomes (as artificial organelles) were made from PEG-(poly(2-(diethylamino)ethyl methacrylate)-*stat*-2-hydroxy-4-(methacryloyloxy) benzophenone (PEG-PDEAEMA-*stat*-BMA) and polymer capsules were made from PNIPAAm-*b*-PMAA-*co*-DMIBM so that their permeability can be tuned using pH and temperature, therefore modulating the activity (Figure 7d). It is worth mentioning that the spatial positioning of enzymes, by increasing or decreasing the diffusometric barriers, played a role in controlling enzymatic activity. The close proximity of two kinds of enzymes was shown to benefit cascade activity. This kind of behavior is indeed expected and, in the future, can be utilized toward various applications requiring control over enzyme activity.

Another example is to reconstruct photosynthesis, also known as light-induced electron transfer within polymerosomes/liposomes. Basically, photosynthesis converts light into chemical energy (ATP production) with two enzymes involved—photosystem II (PSII), an enzyme oxidizes water and produces oxygen and protons and FoF1 ATP synthase (ATPase), a rotary motor protein hydrolyses ADP to ATP in the presence of phosphate and proton gradient.^[70] PSII was co-precipitated together with BSA on calcium carbonate spherical templates, followed by protein crosslinking and core removal to form a PSII–BSA capsule. ATPase reconstituted liposomes were subsequently coated on the surface of the capsule (Figure 8). This whole core–shell structure is reminiscent of thylakoid membranes present inside chloroplasts. ATP production was achieved in such a system and switched on and off by the exposure to visible light.

The aforementioned representative examples provide insights in designing artificial cell models with biological enzymatic reactions, toward the substantive outputs (e.g., kinetic output such as movement; chemical output such as signaling molecule or energy molecule production) that can be further utilized. Moreover, the modulation over outputs via (multi)compartmentalization and various types of tunable permeability enables more advanced control over these cell mimetic systems. This is a significant step toward mimicry of more complicated life-like activities.

4. Motility Mimicry

Nature offers numerous examples of the motility behavior of natural microscopic life, such as cells or bacteria which move through liquids by different motion styles, including swimming, gliding, twitching or floating.^[71] Many of them follow a tactic rule, i.e., sensing gradients and then moving forward/backward, which enables the realization of diverse cellular functions by accessing to the favorable environments, as a special form of adaptability. Inspired by the natural swimmers, many synthetic cell-like structures with different functions were

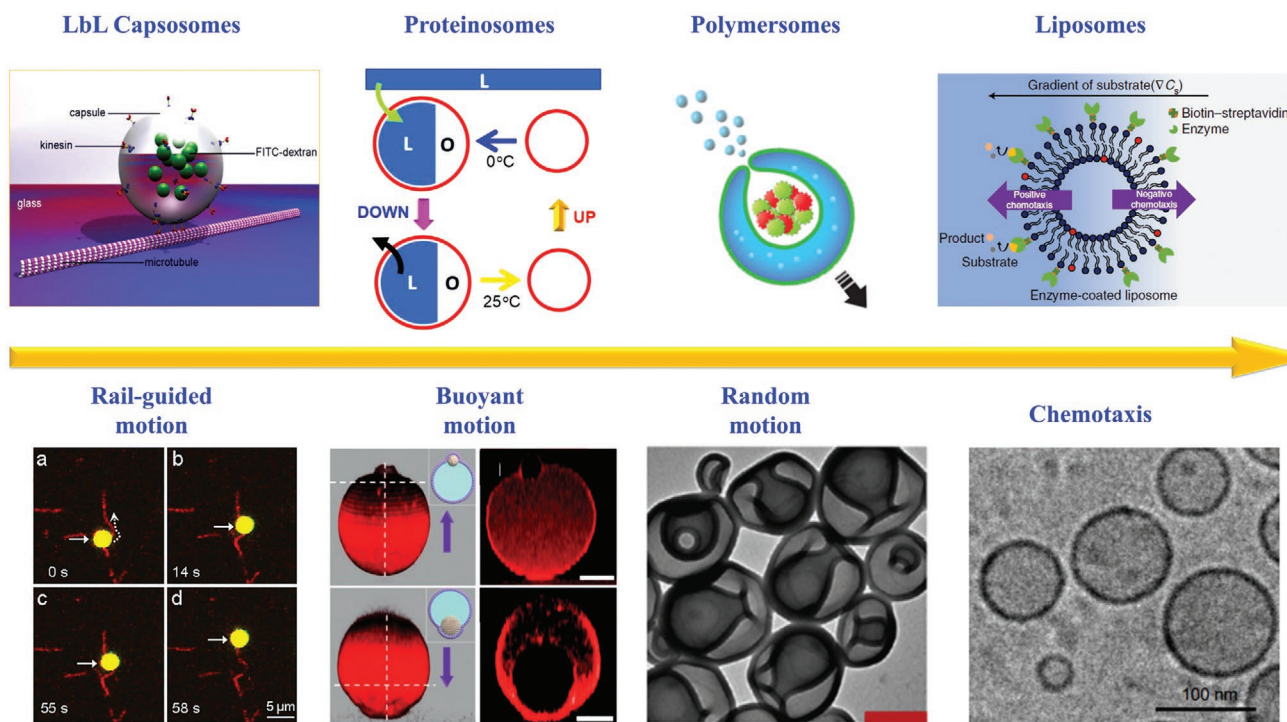


Figure 9. The mimicry of cellular motility behaviors based on soft systems, including representative rail-guided motion, buoyant motion, random motion, and chemotaxis. The examples are not limited in any certain type of artificial model. Rail-guided motion (Figures in First column): Reproduced with permission.^[72] Copyright 2008, Elsevier B.V. Buoyant motion (Figures in Second column): Reproduced with permission.^[73] Copyright 2019, Wiley-VCH. Random motion (Figures in Third column): Reproduced with permission.^[54] Copyright 2016, American Chemical Society. Chemotaxis (Figures in Fourth column): Reproduced with permission.^[74] Copyright 2019, Springer Nature.

created, following an idea of constructing the structure first, then endowed with the function of biochemical reactions for the final generation of power to trigger the motion. The development of cellular motility mimicry basically followed four basic steps, such as guided motion of capsules, then buoyant motility and random motion of artificial cells, and all of them are toward the final goal of chemotaxis of artificial cells. Herein, we will discuss the recent progress on the motility behavior of artificial protocell models based on the soft systems, focusing on these four motility styles (Figure 9).

4.1. Guided/Buoyant Motion

Various models were built up toward the final target for mimicking chemotaxis. To our best knowledge, the pioneering report on capsule motion is published in 2009 by Li and co-workers^[72] In this work, the authors fabricated polymersome based microcarriers using a template-assisted LbL assembly technique, with poly(allyl amine) and poly(styrene sulfonate); they modified the capsules with kinesin, which is an enzyme that triggers the motion of capsules along the microtubules formed from polymerizing of global protein α, β -tubulin heterodimers, in presence of ATP as fuel. Furthermore, they systematically investigated the velocity and other parameters influencing motility.^[75] However, the capsules are confined on the surface of the protein tubules for motion within a limited route, without the ability to move freely in the surroundings.

While, considering linear motion, buoyancy would be a simple force to trigger the motion of artificial cells. Based on this idea, Wang et al. developed a facile method to produce pure natural lipase-coated oil globules in a water system that are capable of seeking substrate upward and settling down for digestion by adjusting their buoyancy through temperature-controlled enzyme reactions,^[73] with similar mechanism to the examples mentioned in Section 3. In this case, the authors prepared stabilized aqueous suspensions of lipase-coated oil droplets comprising a mixture of lipid triglyceride substrate (tributyrin) and low-density polydimethylsiloxane (PDMS) and initiated or impeded lipase-mediated consumption of the droplets by judicious changes in temperature to control the rate of in situ substrate hydrolysis. A spontaneous uptake of the oil into the atrophied globules occurred, from the surrounding free tributyrin droplets or a floating layer of triglyceride substrate at low temperature. Therefore, enzyme activity in the oil droplets is sustained under the conditions of high reactivity when the net exchange associated with the rate of substrate uptake and the rate of lipase-mediated consumption reaches a steady state. Significantly, the lipase-mediated decrease in density of the lipid/PDMS globules results in an increase in buoyancy, which can be subsequently offset by additional uptake of substrate uptake in the floating PDMS globules such that the globules exhibited reversible vertical movement in a water column. Furthermore, the lipase-coated lipid/PDMS droplets were encapsulated within the water-filled interior of semipermeable protein-polymer microcapsules as a step toward constructing a model

protocell containing energy-rich subcompartments capable of activating a buoyant force. Taken together, these results highlight opportunities for the regulation of autonomic behavior in enzyme-powered oil globules and provide a new platform for increasing the functionality and energization of synthetic protocells.

Besides proteinosome-based artificial cells, colloidosome-based models were also utilized to explore the buoyant motility. For example, Mann's group designed a catalase-containing organoclay/DNA semipermeable microcapsule, which in the presence of hydrogen peroxide exhibits enzyme-powered oxygen gas bubble-dependent buoyancy.^[76] In that work, the velocity of giant protocell models could reach up to 40 mm s⁻¹ and can be offset by competing GOx-mediated consumption of the entrapped bubbles, which mimicked gas vesicle-mediated buoyancy of certain bacteria and archaea.^[77] Subsequently, the authors utilized the phenomena of artificial phagocytosis,^[78] to fabricate a similar bubble-triggered buoyant artificial cells. In this case, the magnetic iron oxide Pickering emulsion droplets could be manipulated to encapsulate another silica-based colloidosome community containing catalase and hydrogen peroxide (H₂O₂), leading to the ingestion of the colloidosomes within the droplets, where oxygen bubbles were generated *in situ* and increased the buoyancy of the whole system to trigger the motion. Both above works provide new insights into the mimicry of protocell model with buoyant motility, however, in the following work, improving this system by realizing both the 2D and 3D motion is of utmost importance to fully mimic the cellular motion behaviors.

4.2. Random Motion

To realize the mimicry of cellular 2D or 3D motion, many groups exerted efforts in this field. For example, He's group developed LbL capsosomes with the free motion style of artificial cell models, based on the catalytic reaction of hydrogen peroxide. Briefly, the obtained artificial cell models were coated by a microcontact printing for the asymmetric layer of Pt nanoparticles (PtNPs) on one side of the capsules, which catalyzes the decomposition of hydrogen peroxide fuel, thus leading to the local generation of oxygen bubbles at one side of the hollow capsules to trigger the motion.^[79] Besides, the authors also replaced PtNPs with catalase^[73] or utilized light^[80] as the driving power to improve the biocompatibility, and further investigated the motility behavior for the controlled drug delivery and release, which offered another platform for the mimicry of cellular motility.

Besides capsosome-based artificial cell models, polymers, the bottom-up assembly from block copolymers (Section 2.4), could be nice candidates for the fabrication of artificial cells.^[81] van Hest and co-workers pioneered in this field by the successful fabrication of stomatocyte artificial cell models in 2012,^[82] using polystyrene-*block*-poly(ethylene glycol) (PS-*b*-PEG) block copolymer as the building block, which consists of a flexible glassy membrane that can fold inward under osmotic pressure due to the presence of plasticizing organic solvent in both inner and outer compartments. By this kinetically manipulation, the asymmetry stomatocytes can be used to dynamically encapsulate various catalysts in the inner compartment, thus achieving

nanoscale motion in the presence of appropriate fuel,^[53] and the authors also hypothesized that fuel concentration determined mechanisms for the motion, i.e., self-diffusiophoresis at low concentration and bubble propulsion at high concentration of hydrogen peroxide in this case.^[83] Due to the unique nozzle of the structure stomatocyte, accurate designs can be realized by modulating the amount of organic solution to quench the glassy polymer branches, thus leading to the adjustment of nozzle size. In this way, the speed of the catalytic reaction can be tuned, which in turn controlled the motion speed of the motors. Therefore, different batches of motors with different motion speeds could be obtained. However, how to control the speed of one batch of motors in the solution remains a challenge. In order to fulfill this idea, thermosensitive polymer brushes, based on PNIPAAm, were introduced into the system to generate a hydrophobic layer on top of the small opening of the stomatocytes, which could close the aperture and prevent easy access of fuel into the nanomotor. Due to the lack of fuel, the propelling movement of these stomatocytes ceased. This is when the temperature is higher than the LCST of PNIPAAm; when the temperature is lower than LCST, the motion can be recovered. Therefore, the PNIPAAm brushes serve as the valve on the stomatocytes, with switch on and off the motion.^[84] In this case, the controlled motion is closer to cellular motion, as cellular motion can move or cease on demand. Although these motors mimicked well with the cellular motion, toward the goal of mimicking chemotaxis, further advanced investigations are still necessary.

4.3. Chemotaxis

To investigate the chemotaxis using artificial cells, Battaglia and co-workers reported Janus polymersomes (composed of PEO-*co*-PBO mixed with either PMPC-*co*-PDPA or PEOGMA-*co*-PDPA) that exhibited attractive chemotaxis driven by enzymatic conversion of glucose.^[55] In this case, the soft nanoswimmers are sensitive to glucose, and provided an enhanced penetration of brain with the help of the targeting molecule (LRP-1, low-density lipoprotein receptor-related protein 1). Besides, these nano-sized soft models have the potential for the motility mimicry of bacterial with other functions.^[85]

Another recent example is from Sen's group, in this case, liposomes embedded with acid phosphatase and urease were fabricated first, with the following motility mimicry of artificial cells. The diffusive motility of ATPase tagged vesicles in the absence and presence of substrate ATP was studied by fluorescence correlation spectroscopy.^[86] The authors found that the diffusion coefficient of the active vesicles would increase, when the enzymatic turnover number increased, which is consistent with previous results.^[87] Besides, for this protocell model, the motility is closely correlated with the ATPase activity for different substrates. Although these vesicles showed quite slow enhanced motility, they still served as membrane-bound protocell models that move by transducing chemical energy into mechanical motion; in the meanwhile, they also serve as models for motile living cells, to shed light on the interaction between active membrane dynamics and cellular movement.^[86] Moreover, the authors successfully demonstrated the positive

and negative chemotaxis using catalase and urease coated liposomes, respectively,^[74] showing the autonomous motion of protocell models, when they interacted with concentration gradients of either substrates or products in enzyme catalysis or Hofmeister salts, thus providing new insights into the chemotaxis swarming behavior.

5. Mimicry of Cellular Communication

Cells have ubiquitous communications, such as receiving a complex combination of signals that simultaneously trigger many different signaling pathways as responses, like quorum sensing, or even fleeing/approaching, which may come from the surrounding atmosphere or other cells, e.g., one type of motility-based collective behavior. Therefore, to mimic cellular communications, cell communities are necessary to be build up at a population level with signal transfer, since the communication behavior would be significant for the collective cells to survive, rather than an individual cell. Based on these ideas, in this section, we are going to discuss the current progress in the newly built artificial cell communities, i.e., intercellular communications, including their representative communication between different communities of artificial cells, and artificial cells with natural living cells.

Considering the structures discussed in Section 2, the systems, like liposomes, proteinosomes, polymersomes, and coacervates, differ strongly in their properties, thereby in their communicative behaviors with other artificial cell models or natural cells. The building blocks for the artificial cell models would not only behave as scaffolds, but also provide additional functions to the systems. For instance, lipid bilayers that separate internal and external aqueous solutions are desirable, because such liposomes are better able to exchange molecules with the surrounding niches and can more easily accommodate components that confer molecular specificity, such as ion channels or other membrane proteins. While, polymers, with the pH-, photo-, or temperature-responsive properties, etc., could offer more opportunities for tuning the membrane permeability and properties, therefore leading to the controlled release or receiving of chemicals;^[38] this would be similar to liposomes embedded with ion channels,^[88] which could facilitate the signal transduction without the direct contact of artificial cell models. Furthermore, to utilize the membrane functions during the communication process, proteinosomes would be good candidates, due to the intrinsic abilities of proteins in catalytic reactions or molecular recognition, which could be employed as the signal processor. Additionally, on account of the excellent capture/release and reversible assembly of coacervates, it would be quite interesting to explore them as signal receptor. Based on these properties, in the following section, we are going to discuss some representative examples using different systems for mimicking communicative behaviors.

5.1. Chemical Communications between Artificial Cells

There are several representative forms for the communication styles of artificial cells, such as the direct physical contact

and the release of chemical signals to the surrounding niches (**Figure 10**). For the first type, Huang's group provide a good demonstration of coordinated membrane fusion of proteinosomes by direct contact.^[89] In this case, the proteinosome fusion was realized by dynamic Schiff base covalent interchange and was accelerated in the presence of encapsulated glucose oxidase and glucose or inhibited with cinnamyl aldehyde due to enzyme mediated decrease in pH or competitive covalent binding, respectively.^[89] This example offered a direct contact example, demonstrating the possibilities of developing interacting microcompartmentalized soft colloidal communications between different synthetic protocell communities based on tuning the membrane properties. Subsequently, another example of such a direct contact induced communication was designed, demonstrating an artificial predatory system between coacervates and proteinosomes.^[90] In this work, the interaction between the oppositely charged coacervates and proteinosomes led to the engulfment of the proteinosome (prey) by the protease-loaded coacervates (predator), which is also based on the surface properties. Besides, the proteinosome payload was firstly trafficked into the coacervate droplets, and then the acquisition of material (e.g., DNA and dextran) carried by the proteinosome was realized during the engulfment process, thus leading to the following re-killing of the predator. Based on these mechanisms, another tit-for-tat system was designed,^[91] to demonstrate an example of protocell communication through antagonistic enzyme-mediated interactions. These communication cases were realized by the membrane charges of proteinosomes and the nice capture properties of coacervates. Furthermore, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors were also utilized for mediating the communication between different lipid vesicle communities, with the regulation on the genetically coded networks only after contact and fusion.^[92] Additionally, optical tweezers were recently used to actively manipulate liposomes to contact and then communicate, toward mimicking the communication within cellular networks.^[93]

For the second type of signal transduction, it would be quite significant for the release and receiving of chemicals through different compartments, thus making the membrane permeability playing an important role, as mentioned in Section 2. Taking polymersomes as an example, unlike natural semipermeable membranes, conventional polymersomes are subjected to severe membrane permeability issues due to the hydrophobicity and macromolecular nature of thick bilayers (as discussed in Section 2.4), thus making it difficult to transport substances across the membrane to the external niches for communication. Therefore, active methods are necessary to achieve the tunable permeation of chemicals during the mimicry of communicative behaviors. Basically, there are several well-established methods for each type of compartments. Such as polymersomes, due to the designability and controllability of the building blocks, can be orchestrated into multiresponsive hosts to facilitate the chemical transport, such as pH-, photo-, redox-, enzyme-, or temperature-responsive systems, which could be referred to several previous representative papers.^[95] Similarly, according to current progress, most of these methods could also be applied to proteinosomes.^[22,96] Additionally, regrading to liposomes, some other approaches have been performed, such

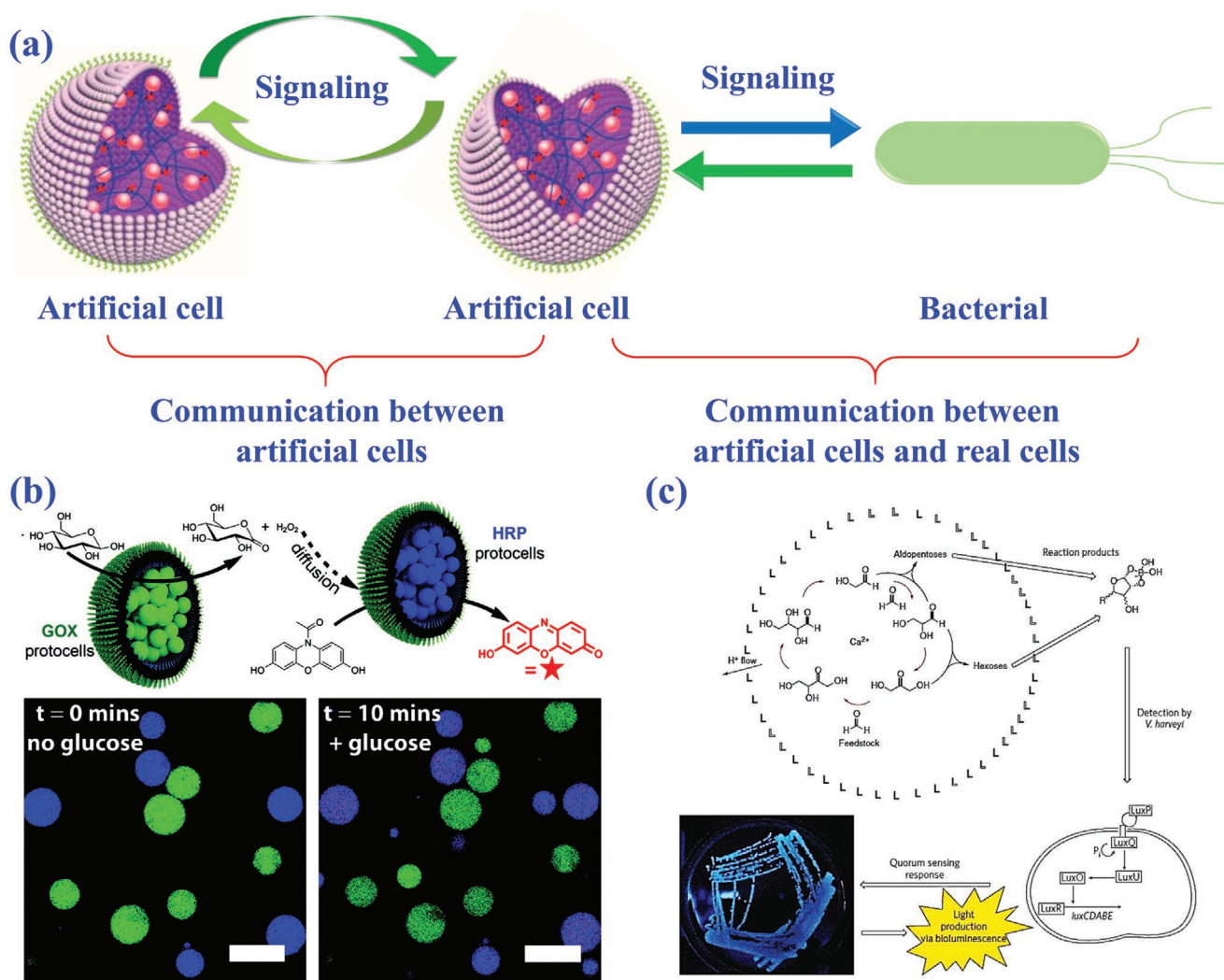


Figure 10. a) Scheme showing the signaling communication between artificial cells and artificial cells/bacterial. b) One representative example of communicative behavior between coacervate-based artificial cells. Adapted with permission.^[34] Copyright 2017, American Chemical Society. c) A representative example showing the communication between an artificial cell and *Vibrio harveyi*. Reproduced with permissions.^[94] Copyright 2009, Springer Nature.

as by the modification of temperature-responsive polymers,^[97] permeabilization with a mild detergent,^[98] embedding ion channels^[48] or by assembling leaky membranes.^[99] All these achievements are helpful to actively adjust the permeability of different compartments for the mass transfer during the communication process. For example, Liu and co-workers designed polymerosomes exhibiting photoswitchable and reversible bilayer permeability from poly(ethylene oxide)-*b*-PSPA (PEO-*b*-PSPA) diblock copolymers.^[49] In this work, to mimic the cellular transport of amino acids by taking advantage of light-mediated microcapsule permeability changes, BQCy-1 probe was encapsulated in the internal aqueous cavity, only after being irradiated with 405 nm laser light, the encapsulated BQCy-1 gave off efficient emission, which was induced by the increased membrane permeability of cysteine, thus mimicking the signal transfer from outside to the inside of artificial cells through an actively tunable design. Besides light-responsive systems, thermo-responsive building block (PNIPAAm) was introduced to glucose oxidase to construct proteinosome, for the gene-directed binary

chemical communication between protocell communities made of liposomes and proteinosomes, where the release of probes could be potentially adjusted by temperature, thus controlling the communication process.^[100] Similarly, redox-responsive systems were also designed for mimicking the cellular communication.^[48] In this work, GUVs were adopted as the nest for various subcompartments (micelles, polymerosomes, and nanoparticles), when reducing agent was added, this reductive milieu in the lumen would induce the disassembly of sensitive compartments to release signal chemicals to other nonresponsive compartments for next reaction, thus successfully mimicking the signal pathways of artificial cells. Moreover, van Hest's group showed intercommunications in a polymer-stabilized polysaccharide coacervate system (as discussed in Section 3), which employed the nice capture properties of coacervate, thus providing insights in designing protocells communication by signal sensing mechanism.^[34] Albeit the fast advancements in this field, to deeply mimic the communicative behaviors between artificial cell communities, more advanced artificial

cells are still needed, such as the ones that could sense the environment, synthesize and discharge a molecular response by using locally available material and then target specific cells or other community for dual responses; of course, sometimes they could be modulated by designed external stimuli.

5.2. Communications between Artificial Cells and Living Organisms

All living organisms interact actively with their surroundings to obtain better survival through the coordination of collective behaviors. In order to investigate the interaction between artificial cells and living organism, new artificial cells and novel bridges between artificial cells and living systems are necessary to be explored (Figure 10). Generally, there are two kinds of communication mechanisms, which is similar as those for artificial cells to communicate, including: i) direct contact to induce mass transfer and chemical reactions, ii) a chemical sensing manner, by chemicals diffused and transferred between artificial cells and living systems,^[101] therefore, leading to positive or negative feedbacks, such as collaboration between artificial cells and living cells, or antibacterial/cancer killing effect.

For the first type, recently, Zhao et al. reported an example by designing a positively charged thermo-sensitive proteinosome, with loaded L-arginine modified chitosan oligosaccharide as an antimicrobial in the hydrogel-based core domain (Figure 11). Subsequently, due to the changes of hydrophobicity and electrostatic interactions tuned by the temperature and ionic strength, a programmed interaction between living *Escherichia coli* and constructed proteinosomes was realized, including capturing, aggregation induced self-suicide (negative response from bacterial), and releasing of dead bacterial for the renewal of proteinosomes.^[102] The mechanism was dependent

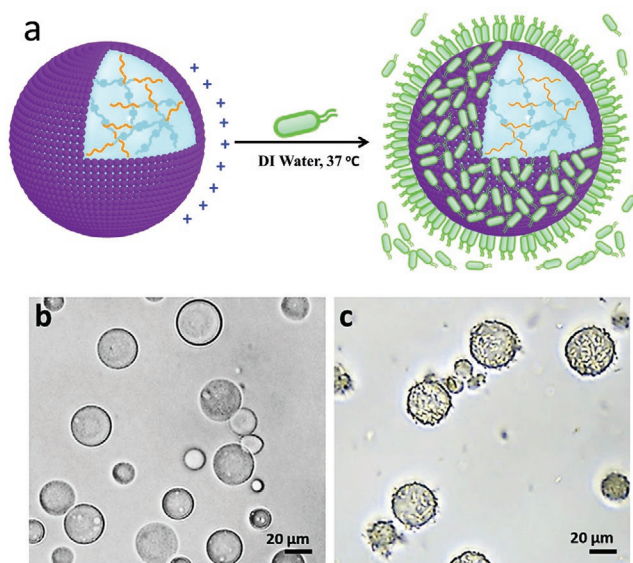


Figure 11. a) Scheme showing the capturing of *E. coli* by a hydrogel loaded proteinosome, based on the surface charges. Optical images showing hydrogel-loaded proteinosomes with b) and without c) *E. coli* attachment. Reproduced with permission.^[102] Copyright 2019, Royal Society of Chemistry.

on the direct contact of the proteinosomes and *E. coli*, due to the hydrophobicity and electrostatic interaction.

For the second type, to our best knowledge, one representative example is based on a liposome capable of initiating a quorum sensing response in *Vibrio harveyi* by means of the autocatalytic sugar-synthesizing formose reaction.^[94] This example showed the design of building up the link between artificial cells and live bacterial. Besides, Mansy group reported the idea of engineering *E. coli* through alternative means by targeting the sensory pathways of *E. coli*. In this case, the artificial cells function as chemical translators, which could sense molecules that *E. coli* alone could not sense; as a feedback, the artificial cells release a molecule that *E. coli* can naturally respond to, thus functionalize as the translation of an unrecognized chemical message into a recognized chemical message. This example provides new opportunities in engineering cellular behavior without exploiting genetically modified organisms.^[103] Similarly, Simmel and co-workers prepared some water-in-oil emulsion droplets, with initially hosting small bacterial consortia, to study their response to small diffusible inducer molecules, which was successfully realized, because the inducers could permeate through the surrounding oil phase, thus reaching other droplets. Based on this idea, the authors further prepared these droplets in confined microchannel, with the capability for sending out signals to surrounding bacterial, which offered a good example bridging the artificial cell with bacterial, with clear signal pathways,^[104] the same concept was reported by Stano group as well.^[105] However, this is only one-way communication, i.e., signals sent from liposome based artificial cells to bacterial, with no direct feedback to artificial cells. It would be necessary to explore the both-side communications. Bearing this in mind, Mansy group reported artificial cells that were able to sense and synthesize quorum signaling molecules that can chemically communicate with many different bacterial, such as *Vibrio fischeri*, *V. harveyi*, *E. coli*, and *Pseudomonas aeruginosa*.^[106] Notably, in this work, the extent to which artificial cells could imitate natural cells was quantified by a type of cellular Turing test, which provide new insights in justifying how life-like artificial cells are, thus giving a pioneering guidance in the development of this field. Subsequently, another representative work is from Tan group, which also demonstrated a sophisticated two-way feedback response between artificial cells and bacteria.^[107]

These works demonstrated the fundamental mimicry of cellular communications, including single or binary responses, however, compared with the complicated and sophisticated cellular signaling, there is still a long way to go. Currently, the models are mostly communicating with bacterial, it would be significant to investigate the communication or interaction between artificial models and mammal cells as well, toward the controlling or commanding on the collective behaviors of human cells through artificial cells, not only for cellular mimicry, but also toward the potential clinical applications.

5.3. Conclusions and Outlook

The past decades have witnessed a great progress in the emerging field of biomimicry—that is, as defined in this review,

mimicking cellular behaviors via the “bottom-up” construction of cell-mimetic soft compartments. In order to construct such artificial cytomimetics, scientists from various disciplines have set out to engineer a diverse array of bio-inspired structural mimics, such as liposomes, proteinosomes, coacervates, polymersomes, and capsosomes. As alluded throughout this review, such soft architectures with unique physicochemical properties have advanced biomimetic processes in various aspects. The construction of artificial cells with biocatalytic function, even up to the level of complexity that mimics a rudimentary metabolism has added to the sophistication of artificial cell systems. Recently, important advancements have been the incorporation of motility and communication, which can be triggered by signaling molecules and/or energy molecules generated in the systems. Owing to the current efforts and progress in the field of biomimicry, engineering synthetic cells for the study and the orchestration of higher-order biological processes such as signaling and collective behaviors has become feasible. These developments have contributed greatly not only in understanding fundamental principles of living systems but also in learning on how to think about constructing materials in the way as nature does. Although we have taken a significant step forward in the field, some challenges remain in the pursuit of a fully autonomous artificial cell. For example, most artificial systems incorporate only one or two biomimetic processes mentioned in this review. Considering the complexity of living systems, integration of multiple biological processes and functions in one entity should be further explored, which would also enable future technological advancements.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

L.W. and Sd.S. contributed equally to this work. L.W. and S.S. planned the manuscript. L.W. and Sd.S. drafted the manuscript. L.A., X.H., J.H., and S.S. commented and revised the manuscript. All authors gave final approval for publication.

Keywords

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