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Polymeric Artificial Cells: from Interfacial Membranization to Cytomimetic Architecture Engineering

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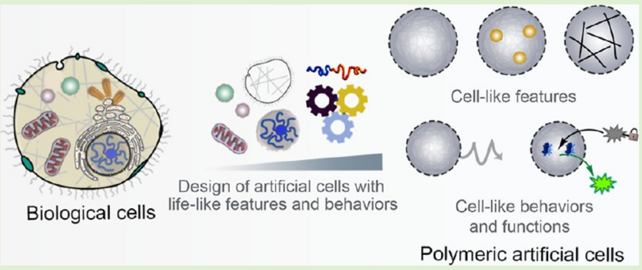
ABSTRACT: Artificial cells emulating the structure and function of living systems have attracted tremendous research attention. By integrating concepts and techniques from chemistry, materials science, and biochemistry, researchers have assembled functional building modules into advanced materials capable of exhibiting life-like behaviors. Recent advances have led to the creation of synthetic cells that mimic key characteristics of living cells. Polymer-based systems attract enormous interest due to their chemical versatility, robustness, and programmability. These attributes allow the construction of artificial cells with precise modulation of physicochemical properties, architecture and functionality. Representative polymeric artificial cells have demonstrated essential hallmarks of life, including membranization, integration of suborganelles, and formation of cytoskeletal frameworks. In this Review, we highlight recent advances in the design, assembly, and functionalization of polymer-based artificial cells, emphasizing how the intrinsic tunability and multifunctionality of polymeric materials enable the recreation and extension of life-like structures, dynamic behaviors, and biological functions.



1. INTRODUCTION

Inspired by nature, there is an emergent pursuit in the field of synthetic biology associated with engineering complex artificial systems embedded with biological features that can perform predesigned functions.[1−5] The design and construction of artificial cells has drawn a myriad of interest from the scientific community. Engineering artificial cells aims to recapitulate several hallmark features of biological cells, such as compartmentalization, molecular crowding, selective permeability, and the ability to perform various biochemical reactions and complex information processing.[6−11] For instance, compartmentalization is a prevalent strategy utilized by nature cells to orchestrate a myriad of biochemical reactions, execute fundamental cellular processes and display complex behaviors.[12,13] Intracellular molecular crowding enables the concentration and spatial distribution of biomacromolecules that allow control over the efficiency and selectivity of biochemical reactions.[14,15] Fabricating synthetic cells exhibiting these key characteristics of living systems is an exciting way for scientists to create smart, complex and biomimetic systems.[16−19] It deepens our understanding of the fundamental pathways and organization principles underlying the characters and properties of living cells. Such life-like materials and systems also open transformative avenues for applications in various fields, including biosensing, microreactor, drug delivery, and synthetic biocomputation.[16,20−24] Key structural and functional elements of living cells—such as lipid bilayers, membranized organelles, and cytoskeletal architectures—serve as blueprints for the design of synthetic analogs.[7] Reconstructing these features within artificial systems has proven essential for achieving life-like behavior and for exploring cell-mimicking functionalities in simplified, controllable platforms.[25,26] The creation of synthetic cells aims to replicate the structure, behavior and function of living cells with a minimal model.[27,28] This is usually realized either by a top-down approach (e.g., genome-editing of a natural organism) or by a bottom-up pathway (e.g., assembling nonliving, biomimetic components into integrated proto-cellular entities).[29] The focus of this Review is the bottom-up approach to constructing synthetic cells, which involves assembling structural and functional modules into integrated systems that can show cell-like properties.

Among various classes of building blocks toward fabricating artificial cells, polymer-based materials attract enormous interest due to their unique set of advantages.[25] Lipid-based systems, such as liposomes and giant unilamellar vesicles (GUVs), are widely employed as fundamental building blocks for constructing artificial cells and organelles due to the phospholipid compositions of their membrane that are

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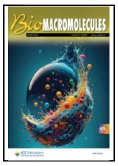

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comparable to those of natural cells. However, the inherent instability of phospholipid membranes under harsh conditions limits their advanced applications, prompting the exploration of more robust alternatives. Integrating the advantages of lipid and polymeric systems represents a promising strategy to enhance the structural and functional complexity of synthetic cells. Nevertheless, the incompatibility between the hydrophobic polymer segments and phospholipid tails poses a significant challenge in building and regulating stable hybrid artificial cells. Unlike lipid-based systems that are limited by low mechanical stability and limited chemical tunability, polymeric materials can be precisely engineered at the molecular level to control parameters such as composition, chain length, hydrophilicity, charge density, and functionality.[1,30] This molecular programmability enables the design of various polymeric systems that self-assemble into a wide range of architectures, such as polymersomes, proteinosomes, or coacervates, each with distinct compartmentalization features, life-like behaviors, and functional output emulating living systems.[31] For instance, the use of amphiphilic block copolymers and assembly of polymersomes permits the fine-tuning of membrane features (e.g., thickness and membrane permeability), enabling across-membrane transportation in a selective manner, which is critical to control and support complex reaction networks akin to those in the cytoplasm.[25] Proteinosomes, composed of amphiphilic protein−polymer conjugates, form robust and semipermeable membranes that enable selective exchange of molecules while maintaining structural integrity, thus providing a versatile platform for encapsulating and organizing biochemical reactions. Their inherent biocompatibility and ability to integrate enzymatic or functional protein domains make them ideal for constructing dynamic, responsive systems that mimic key aspects of living cells.[10,32] Polymeric coacervates, formed through liquid−liquid phase separation of oppositely charged or associative polymers, create membraneless, dynamic, and condensed compartments that mimic the cytoplasmic organization of living cells. Their ability to selectively concentrate active biomolecules and enhance the efficiency of enzyme-catalyzed reactions makes them powerful models for studying primitive cellular processes and constructing functional synthetic cells.[27,33] The vast library of synthetic and bioinspired polymers offers the flexibility to incorporate stimuli-responsive segments, introducing responsive behaviors. This enables polymeric artificial cells to undergo morphological or functional transformations in response to external cues such as light, pH, or redox conditions, thereby reproducing the dynamic adaptability of living systems.[34] Importantly, polymeric systems can be facilely endowed with specific chemical functionalities that facilitate the integration of biomimetic motifs, such as peptide sequences, polysaccharides, or nucleic acid analogs. This is critical for polymeric artificial systems for reproducing biological signaling or metabolic pathways via specific molecular recognition, selective binding, or catalysis.[25] Polymeric materials also provide a unique opportunity to incorporate functional modules (e.g., emissive, photothermal, therapeutic elements) directly into the artificial cell framework, extending their potential applications into areas such as biosensing, energy conversion, and smart drug delivery.[35−37] In addition, the scalability and reproducibility of polymer synthesis ensure consistent material quality and performance, an essential requirement for translating artificial cell technologies into practical biotechnological applications. Collectively, engineering polymeric artificial cells provides a versatile means to bridge the gap between engineerable synthetic systems and biological concepts, offering a modular and programmable approach to construct artificial systems that can not only replicate but also transcend the functional complexity of natural life forms. Recent studies have demonstrated a wide array of polymer-based artificial cells that emulate various life-like features—ranging from interfacial membranization and hierarchical subcompartmentalization to the integration of cytoskeletal mimics and adaptive response behavior.[38−41] Their ability to integrate structural complexity with molecular adaptability positions polymer-based artificial cells as a cornerstone for advancing the Frontier of bottom-up synthetic biology, enabling a deeper understanding of cellular organization, the creation of life-mimicking systems, and the design of emergent therapeutic, diagnostic, and environmental technologies.

In this Review, we will discuss the up-to-date progress in the design and assembly of polymer material-based artificial cells, mainly covering the literature published from 2010, which demonstrated the key structural characteristics and dynamic functionalities of living systems. For this review, we mainly discuss polymeric systems that are referred to as synthetic polymers, polypeptides, and polymer−nanoparticle hybrids. Pure lipid systems were excluded from this review unless incorporated into polymer hybrids. We will first outline the fundamental design principles that underlie the engineering of minimal polymeric synthetic cells, highlighting the importance of membrane-bound architectures and the crucial role of internal macromolecular crowding in facilitating biochemical reactions. Particular emphasis will then be placed on the hierarchical design and integration of suborganelles within polymeric artificial cells, which is essential for mimicking the compartmentalized organization and functional coordination in natural cells. Advances in polymer chemistry have enabled the fabrication of multicompartmental systems, such as vesicle-in-vesicle, coacervate-in-vesicle, and hybrid proteinosome architectures, that exhibit spatially controlled reaction environments, directional molecular transport, and emergent collective behaviors. We will then focus on the construction and regulation of internal cytoskeleton-like frameworks within polymeric artificial cells, highlighting recent strategies for introducing polymeric filaments, peptide-based networks, and supramolecular scaffolds that provide structural support, facilitate molecular transport, and drive morphological transformation. These developments are pivotal for replicating cellular mechanics, shape maintenance, and dynamic processes such as division and motility. Furthermore, we will explore the emergence of life-like behaviors in polymeric artificial cells, including energy dissipation, adaptive response to stimuli, signal transduction, and intercellular communication, which collectively bridge the gap between static molecular assemblies and dynamic living entities. Finally, we will conclude with a forward-looking perspective on how the synergistic integration of polymer science and artificial cell engineering can lead to creating the next generation of life-like materials and systems capable of autonomous sensing, self-regulation, and evolution. Such life-like systems not only offer a powerful platform for exploring the fundamental principles of life's organization but also hold transformative potential for applications in biomedicine, environmental sensing, soft robotics, and advanced material design. To maintain consistency throughout this review, we adopt a standardized terminology: protocell refers to a minimal synthetic compartment designed to emulate selected cellular features; synthetic cell denotes a higher-level construct integrating multiple regulatory or communication functions; artificial organelle describes a functional subcompartment within a larger host structure; and polymersomes (including *p*-GUVs) refer specifically to polymer-based vesicular architectures. These terms are used uniformly in this review to avoid ambiguity.

2. POLYMER-BASED INTEGRATED SYNTHETIC CELLS

2.1. Membrane-Bound Synthetic Cells. Compartmentalization is one of the defining hallmarks of life, enabling living cells to segregate biochemical reactions, maintain concentration gradients, and preserve homeostasis within dynamically changing and crowded environments.[42] In fact, researchers have reached a growing consensus about the concept of artificial cells and have explored several coping strategies for them, including (1) structural homeostasis, achieved through autonomous self-maintenance mechanisms that preserve integrity under physiological conditions and across applications; (2) implementation of biomimetic processes, such as growth, division, and stimulus-responsive differentiation pathways; (3) selective interfacial permeability engineered into membranes to enable controlled interactions with the surrounding environment; and (4) life-like metabolism supported by self-regulating or controlled biochemical networks. Early models, such as membrane-free protocells, which consist of minimal polymeric compartments such as coacervates and emulsion droplets, have provided valuable insights into how primitive compartments could spontaneously form and sustain elementary biochemical functions. These minimal compartments, composed of simple polymers, exhibit essential catalytic or encapsulation properties.[33] However, the lack of a defined interfacial boundary in these systems often results in uncontrolled molecular diffusion, limited structural stability, and an inability to coordinate multiple biochemical processes simultaneously.[27] To date, extensive research has been conducted to investigate the role of membrane-bound compartments in modulating both the properties of the internal microenvironment and the interface. Polymer-based membranes, as a central category, have been engineered with diverse material compositions and structural properties to fulfill specific functional requirements. These membranes vary considerably in key parameters, including polymeric compositions (e.g., PEG, protein, dextran), thickness, and permeability, which collectively determine their properties and capacity toward various payloads (including small-molecule drugs, proteins, nucleic acids, and nanoparticles). The introduction of an interfacial membrane thus represents a transformative milestone in synthetic cell research, offering not only physical containment but also regulatory complexity that more faithfully recapitulates the functional logic of living cells. Membrane-bound synthetic cells possess selectively permeable boundaries that enable the controlled transport of molecules, spatial organization of biochemical pathways, and maintenance of nonequilibrium states essential for life-like activity.[29,43,44] Rather than serving merely as passive enclosures, these membranes act as active, dynamic interfaces capable of communicating with the external environment through selective gating, ligand recognition, and stimuli-responsive transformations.[45−47] By rationally engineering membrane composition using amphiphilic block copolymers, protein−polymer conjugates, or hybrid assemblies, it would be facile to precisely tailor key parameters such as thickness, elasticity, permeability, and responsiveness. This molecular programmability endows polymeric artificial cells with exceptional mechanical robustness and adaptive capabilities, allowing them to function under diverse conditions while maintaining biological relevance.[48−52]

The presence of a defined membrane further facilitates hierarchical organization within synthetic cells, enabling the encapsulation of distinct subcompartments, coacervate domains, or enzyme cascades in a spatially coordinated manner.[3] Such a structural arrangement not only enhances biochemical efficiency but also allows the mimicry of advanced eukaryotic features, including organelle-like segregation and spatiotemporal regulation of metabolism.[53,54] Moreover, membrane-bound systems provide an ideal framework for integrating cytoskeletal elements or membrane-associated protein machinery, bridging structural organization with functional dynamics. For instance, coupling polymeric membranes with reconstituted cytoskeletal networks can induce morphological transformations, shape adaptability, or even motility, which are difficult to achieve in membrane-free architectures.[55,56] Another significant advantage of membrane-bound architectures lies in their stability and functional adaptability. Polymeric membranes, in particular, exhibit superior mechanical integrity, chemical resistance, and long-term durability under both physiological and extreme conditions, making them ideal for sustained studies and translational biomedical applications.[57,58] Furthermore, the modular nature of polymer chemistry allows for the facile incorporation of biofunctional moieties, such as receptors, enzymes, peptides, or nucleic acids, directly into the membrane interface.[59−61] These functional components impart the ability to sense, recognize, and catalyze specific molecular events, transforming synthetic membranes from passive boundaries into active participants in cellular mimicry. Such programmable versatility enables artificial cells to perform complex, life-like tasks such as molecular sensing and controlled release of encapsulated species.[47,62]

2.1.1. Polymersome-Based Biomimicry Systems. Self-assembly of amphiphilic polymers in aqueous phase, forming a hollow vesicular structure surrounded by a bilayer membrane, is a common approach to yield membranized compartments (with nano or micro size).[63] The physical and chemical characteristics of vesicle membrane, such as mechanical stability, permeability, and lateral mobility, are dictated by the features of constituent building blocks, such as molecular weight, charge, amphiphilicity, and flexibility.[64,65] These significant properties of membranes are critical determinants when applying polymeric vesicles in the fields of biomimetic modeling and biomedical applications.[1,66] Liposomes fabricated from relatively low molecular weight components often display thin membranes (3−5 nm) with compromised stability and limited control over their membrane permeability.[67,68] In light of this, the self-assembly of block copolymers to form polymeric giant unilamellar vesicles (*p*-GUVs) has recently gained arising research interest, due to their superior mechanical strength, enhanced chemical stability, and highly tunable membrane properties. This makes *p*-GUVs a robust and adaptable platform for biomimicry in synthetic biology. The versatility of synthetic chemistry has enabled the rational design of tailored monomers and the synthesis of desired building blocks in polymeric systems. And controlled polymerization techniques-such as atom transfer radical polymerization (ATRP), reversible addition−fragmen-

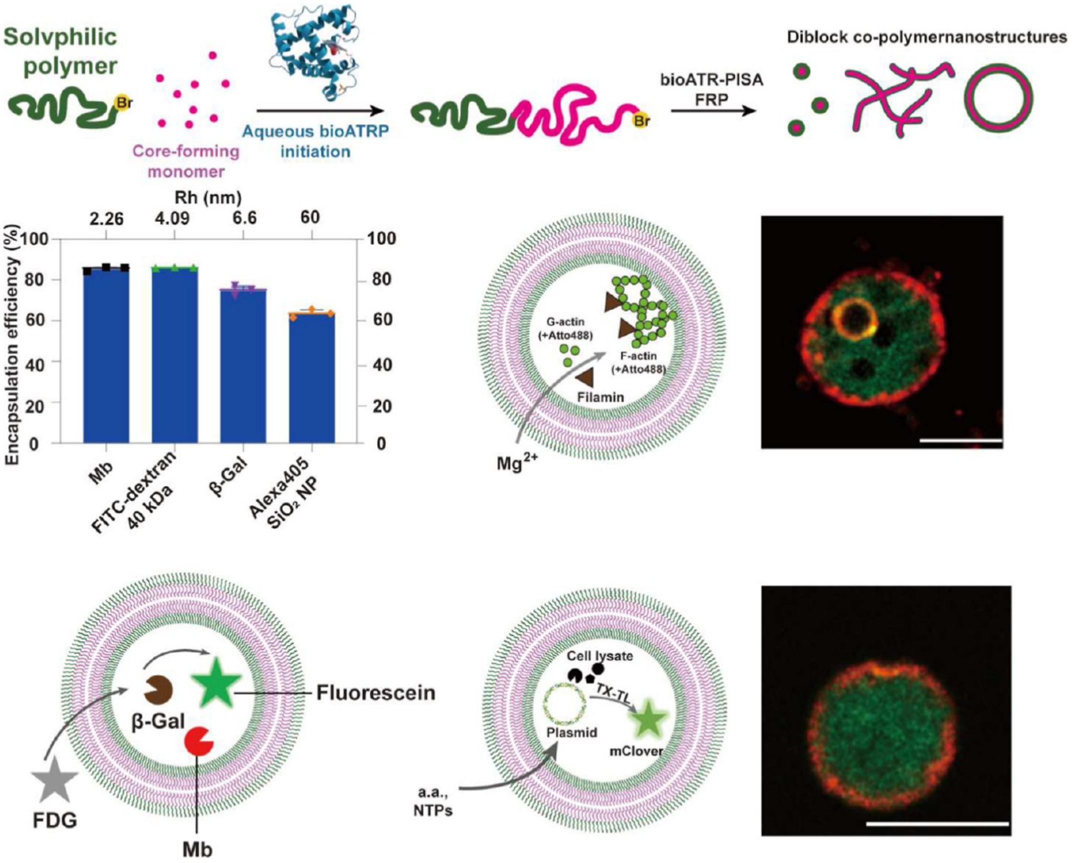

Figure 1. Combining biocatalysis with the PISA method enables self-assembly into *p*-GUVs within an aqueous phase. The assembled vesicles exhibit enhanced encapsulation efficiency for active molecules, serving as microreactors capable of mimicking the life-like processing of living cells. Adapted with permission under a Creative Commons CC BY license from ref 76. Copyright 2023 Springer Nature.

tation chain transfer (RAFT), and ring-opening polymerization (ROP), allow precise synthesis of a wide variety of amphiphilic block copolymers (BCPs).[69−71] These techniques facilitate the production of well-defined BCPs with controlled molecular weights, tunable hydrophilic/hydrophobic ratios, tailored architectures, and incorporation of specific functional groups.[72,73] The self-assembly of BCPs, driven by the phase separation between hydrophobic segments and hydrophilic coronas, can result in a diverse array of structures, including bilayer vesicles, micelles, and inverse mesophase with customized morphological and properties. Block copolymers with an appropriate hydrophilic/hydrophobic volume fraction can assemble into polymersomes featuring membrane thicknesses typically ranging from 5 to 20 nm, depending on the polymer molecular weight, chain architecture, and interfacial packing constraints.[30] This architectural feature of polymersomes allows the encapsulation of hydrophilic agents within their aqueous lumen and hydrophobic species within the membrane pocket. Nanoscale polymersomes are regarded as promising candidates for the design of drug delivery carriers or artificial organelles, while polymeric giant unilamellar vesicles with micrometer size serve as attractive models for engineering artificial cells.

One of the effective strategies utilized to prepare polymeric giant unilamellar vesicles (*p*-GUVs) is polymerization-induced self-assembly (PISA), which is characterized by the in situ synthesis and self-assembly of amphiphilic block copolymers in an aqueous medium.[74] The process typically begins with a hydrophilic macroinitiator or chain-transfer agent, which facilitates the polymerization of a hydrophobic monomer. As the hydrophobic block extends and reaches a critical chain length, it triggers self-assembly due to the decreasing solvation level. The gradual growth of the hydrophobic segment reduces the hydrophilic-to-hydrophobic ratio (f), inducing morphological evolution from spherical micelles to worm-like structures and finally to vesicles. Morphological outcomes are predominantly governed by the properties of the hydrophobic block and the hydrophilic volume fraction f. Vesicular structures typically form within $f = 0.25{-}0.45$, while cylindrical and spherical micelles prevail at $0.4 < f < 0.5$ and $f > 0.5$, respectively.[75] This precise control over the self-assembly pathway enables the tailored fabrication of polymeric vesicles with defined size, membrane properties, and encapsulation capacity, which overcome limitations such as complexity in preparation and inefficient encapsulation of functional molecules in conventional approaches. For instance, the Bruns' group combined biocatalysis with polymer science to fabricate polymer vesicles via a bioPISA technique. Their approach employed myoglobin (Mb) as a biocatalyst to initiate atom transfer radical polymerization (ATRP) in aqueous solution. This facilitated chain extension of a poly(ethylene glycol) methyl ether 2-bromoisobutyrate (mPEG-Br) macroinitiator with the monomer 2-hydroxypropyl methacrylate (HPMA), which induced a polymerization-induced self-assembly (PISA) process that ultimately leads to vesicle formation (Figure 1).[76] The morphology and size of vesicle formation can be precisely tuned by adjusting the monomer-to-initiator ratio and monomer concentration. At ratios of the

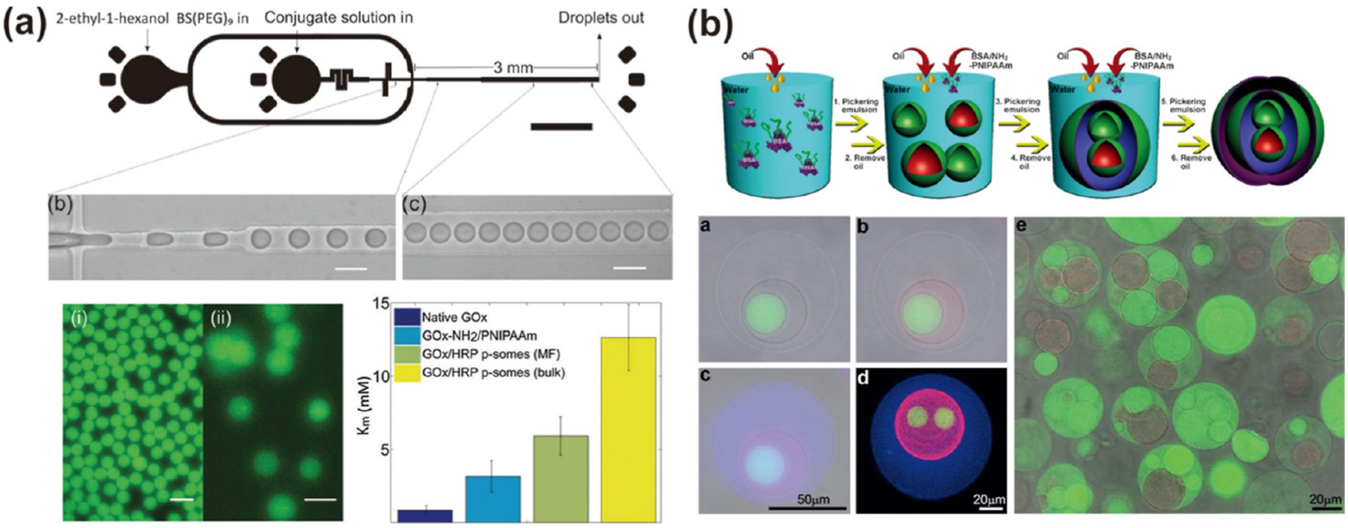

Figure 2. (a) Preparation of proteinosomes using microfluidics with homogeneous size and higher enzymatic activity. Adapted with permission under a Creative Commons CC BY license from ref 87. Copyright 2018 Royal Society of Chemistry. (b) Proteinosomes in multilayer chambers were prepared in Pickering emulsions by continuously removing the oil phase and adding the oil phase again and BSA-PNIPAAm. Adapted with permission from ref 90. Copyright 2016 Wiley.

monomer-to-initiator between 250 and 400, the self-assembly resulted in the formation of giant unilamellar vesicles (GUVs) with diameters ranging from hundreds of nanometers to several micrometers and membrane thicknesses of 50 ± 11 nm. These GUVs demonstrate high loading efficiency for proteins and other bioactive molecules, outperforming conventional encapsulation strategies. The GUVs also served as programmable artificial microcompartments and biomimicry microreactors capable of modulating cellular processes. Via coencapsulation of different enzymes (e.g., β-galactosidase or glucose oxidase) with Mb enabled enzymatic cascade reactions within a confined microenvironment, mimicking cellular bioreactions. Additionally, G-actin encapsulated in the GUVs underwent polymerizing into F-actin filaments in the aqueous lumen upon stimulation with Mg^{2+}, leading to the formation of actin-based subcompartments. The vesicles also supported reconstitution of cellular processes through the encapsulation of cell lysates, facilitating information expression and transmission in a biomimetic manner.

Biological tissues form through cellular aggregation, enabling collective signal reception and transmission, which significantly enhances metabolic and functional efficiency.[77,78] GUVs self-assembled from amphiphilic block copolymers with superior chemical and mechanical stability, along with extensive functional versatility, are considered as appealing candidates for the formation of synthetic tissues. For instance, the Palivan group reported a DNA-cross-linking strategy to mediate self-assembly of polymeric GUVs (pGUVs) for constructing higher-order protocellular tissues.[79] pGUVs were conjugated with cholesterol-anchored single-stranded DNA, enabling hybridization-induced assembly into multicompartment architectures whose size and organization behaviors depended on DNA surface density. In addition, the pGUVs were also functionalized as "Sender" protocells and "Receiver" protocells. "Sender" protocells were fabricated by encapsulating pH-responsive microgels, serving as membrane-free Mg^{2+} storage compartments, which also incorporated ionophores for controlled cation release. "Receiver" protocells contained a Mg^{2+}-sensitive fluorescent dye for signal detection. Upon DNA-directed assembly, these structures exhibited coordinated responses to extracellular pH variations, inducing Mg^{2+} release and interprotocellular signaling. In addition, Mg^{2+}-induced polymerization of G-actin into filamentous actin networks induced the reconstitution of a dynamic cytoskeleton in the "Receiver" protocells. Their approaches enable the construction of communicative protocell networks with a higher ordered organization behaviors by tailoring membrane features and functionalized coatings, a viable method to construct adaptive and communicating protocells capable of exhibiting complex lifelike behaviors.

Overall, polymer-based giant unilamellar vesicles (GUVs) possess robust mechanical stability and tunable chemical properties. Their adjustable membrane architecture, controllable size, and strong stimulus-responsiveness make them attractive platforms for biomimetic studies as well as biomedical applications such as controlled drug delivery and targeted therapy. Nevertheless, several challenges still limit their broader use in constructing reproducible, high-throughput cellular models. These include the difficulty of precisely regulating membrane permeability, the relatively slow kinetics of stimulus responses, potential damage to encapsulated cargo caused by certain polymers or fabrication conditions, and substantial batch-to-batch variability during preparation.

2.1.2. Polymer-Based Proteinosomes.

Proteinosomes are a unique class of vesicular structures composed of protein-modified membranes that have recently attracted considerable attention as versatile platforms for engineering synthetic cells.[32,80,81] Protein-based assemblies play central roles in organizing metabolism in viruses and eukaryotic cells through highly ordered architectures. Harnessing this intrinsic functional specificity, proteinosomes have emerged as a promising candidate in biomimicking in the field of synthetic biology.[10] Recent progress demonstrates that proteins, either alone or conjugated with synthetic polymers, can be engineered into proteinosomes that mimic the compartmentalization feature and functional behaviors of natural systems.[82,83] The incorporation of synthetic polymers not only improves their mechanical stability but also introduces stimuli-responsiveness and dynamic behaviors. Combined with their inherent permeability, these features greatly expand the functional scope of proteinosomes, enabling applications as biocatalytic nanoreactors, therapeutic delivery vehicles, and programmable artificial cells.[25] Typically, proteinosomes consist of a monolayer membrane enriched with polymers on the exterior and proteins on the interior, surrounding an aqueous lumen. Because most proteins are hydrophilic, spontaneous vesicle formation into proteinosomes is challenging. This limitation has been overcome through the self-assembly of protein−polymer conjugation strategies, such as poly(*N*-isopropylacrylamide) (PNIPAM)−BSA conjugates and elastin-like polypeptides, which act as amphiphilic hybrids.[84−86] Various fabrication approaches have been established, including interfacial self-assembly, layer-by-layer deposition, extrusion, and microfluidics.[10] For example, Zhao and colleagues developed a straightforward strategy for fabricating proteinosomes through the coassembly of poly(*N*-isopropylacrylamide) (PNIPAM) and bovine serum albumin (BSA) in aqueous solution.[86] Heating the system above the lower critical solution temperature (LCST) of PNIPAM resulted in the formation of vesicular structures characterized by collapsed PNIPAM in the membrane walls and surface-localized BSA. Microfluidic technology has been utilized to fabricate proteinosomes with uniform structural characteristics, leveraging its ability to generate highly monodisperse droplets under controlled fluid dynamics. The deMello group employed a microfluidic strategy to construct proteinosomes, enabling the high-throughput generation of monodisperse, microscale water-in-oil droplets. These compartments were further stabilized by cross-linking with cationic conjugates of either bovine serum albumin/PNIPAAm (BSA-NH_2/PNIPAAm) or glucose oxidase/PNIPAAm (GOx-NH_2/PNIPAAm) (Figure 2a).[87] High-speed imaging at bright-field confirmed stable and reproducible droplet formation at the flow-focusing junction. For instance, droplets with an average diameter of 27.1 μm (±1.0 μm) and narrow distribution were produced with flow rates of 1.0 μL min^{-1} for the dispersed phase (BSA-NH_2/PNIPAAm) and 3.5 μL min^{-1} for the continuous phase. Additionally, functional enzymes such as glucose oxidase (GOx) can also be encapsulated within the proteinosomes via this microfluidic approach. Kinetic analysis between vesicles synthesized by microfluidic and conventional bulk methods revealed a lower Michaelis constant (K_m) in the microfluidic group, indicating enhanced enzymatic efficiency. However, both vesicular systems showed reduced enzymatic efficiency compared to free GOx, likely due to steric hindrance from intermolecular cross-linking within the membrane architecture that reduced substrate diffusion.

Pickering emulsion-based assembly is another alternative approach for the self-assembly of protein−polymer conjugates into proteinosomes. This method utilizes amphiphilic protein−polymer nanoconjugates as colloidal stabilizers that adsorb at the water−oil interface, forming mechanically robust, compartmentalized architectures through interfacial jamming and elastic film formation.[80,88] The resulting Pickering emulsion droplets exhibit high stability against coalescence and Ostwald ripening due to the dense packing and cross-linking of nanoconjugates at the interface.[89] This approach enables precise control over compartment size, membrane permeability, and surface functionality, making it particularly suitable for constructing biomimetic microcompartments with enhanced structural integrity and programmable catalytic activity. For instance, Liu et al. developed a recursive Pickering emulsion strategy to fabricate hierarchically organized, multicompartmentalized proteinosomes (Figure 2b).[90] The size of the resultant proteinosomes was precisely controlled by adjusting the concentration of protein−polymer nanoconjugates and the applied shear stress during emulsification. Hierarchical assembly was achieved through iterative Pickering emulsification, in which the initial proteinosomes served as stabilizers for subsequent emulsion droplets. By varying the sequence of protein-coupling steps, two or three distinct proteinosomes could be encapsulated within a larger compartment. Furthermore, the incorporation of different enzymes into individual subcompartments enabled the construction of chemically organized microarchitectures with well-defined hierarchical levels. Critically, the protein−polymer membrane was engineered via rational cross-linking using a combination of NHS-PEG9 ester and PEG-bis(*N*-succinimidyl) disulfide (NHS-PEG16-DS), resulting in robust multicompartmentalized structures with programmable release profiles. Upon exposure to a protease solution or the stepwise addition of the reducing agent tris(2-carboxyethyl) phosphine (TCEP) in the presence of protease, the proteinosomes underwent tunable, subcompartment-specific disassembly, characterized by the sequential disappearance of internal compartments.

The proteinaceous nature of proteinosomes allows them to incorporate enzymatic active sites or functional groups with high precision, enabling the construction of stimulus-responsive artificial cellular systems. Their inherent biocompatibility and biodegradability also make them valuable intermediates that bridge synthetic chemistry with biological applications. However, this same protein-based composition can compromise long-term structural stability and mechanical robustness. In addition, hybrid assemblies in which proteins co-organize with synthetic polymers often display strong path dependence, resulting in considerable batch-to-batch variability in the final structures. The intrinsic fragility of many proteins may further limit downstream functionalization and restrict the expansion of their practical applications.

2.1.3. Membrane-Bound Polymeric Coacervates. Membraneless coacervate microdroplets, formed through liquid−liquid phase separation (LLPS) of associative components, serve as significantly important protocell models for studying the origin of life.[61,91,92] These compartments efficiently concentrate biomolecules as proteins, polynucleotides, and RNA within a molecularly crowded interior, thereby facilitating life-like processes including metabolic reactions, gene expression, signaling cascades, and orthogonal biochemical networks.[36,93,94] Coacervates and condensates, while important for both synthetic and biological systems, are inherently unstable, tending to fuse, ripen, or dissolve under environmental stress.[95−97] The absence of a membrane allows coacervates to exhibit high permeability, enabling rapid molecular exchange and enhanced responsiveness to environmental changes.[98]

The trade-off between dynamic material exchange and selective compartmentalization underscores a key evolutionary challenge in the transition from simple phase-separated droplets to membrane-protected protocells.[27,99] Extensive studies have demonstrated successful interfacial stabilization of coacervate droplets with various components, including but not limited to amphiphilic molecules, block copolymer systems, engineered proteins, PEGylated polymers, functionalized nanoparticles, charged supramolecular assemblies, and polysaccharide derivatives.[43,51,58,100−104] These interfacial

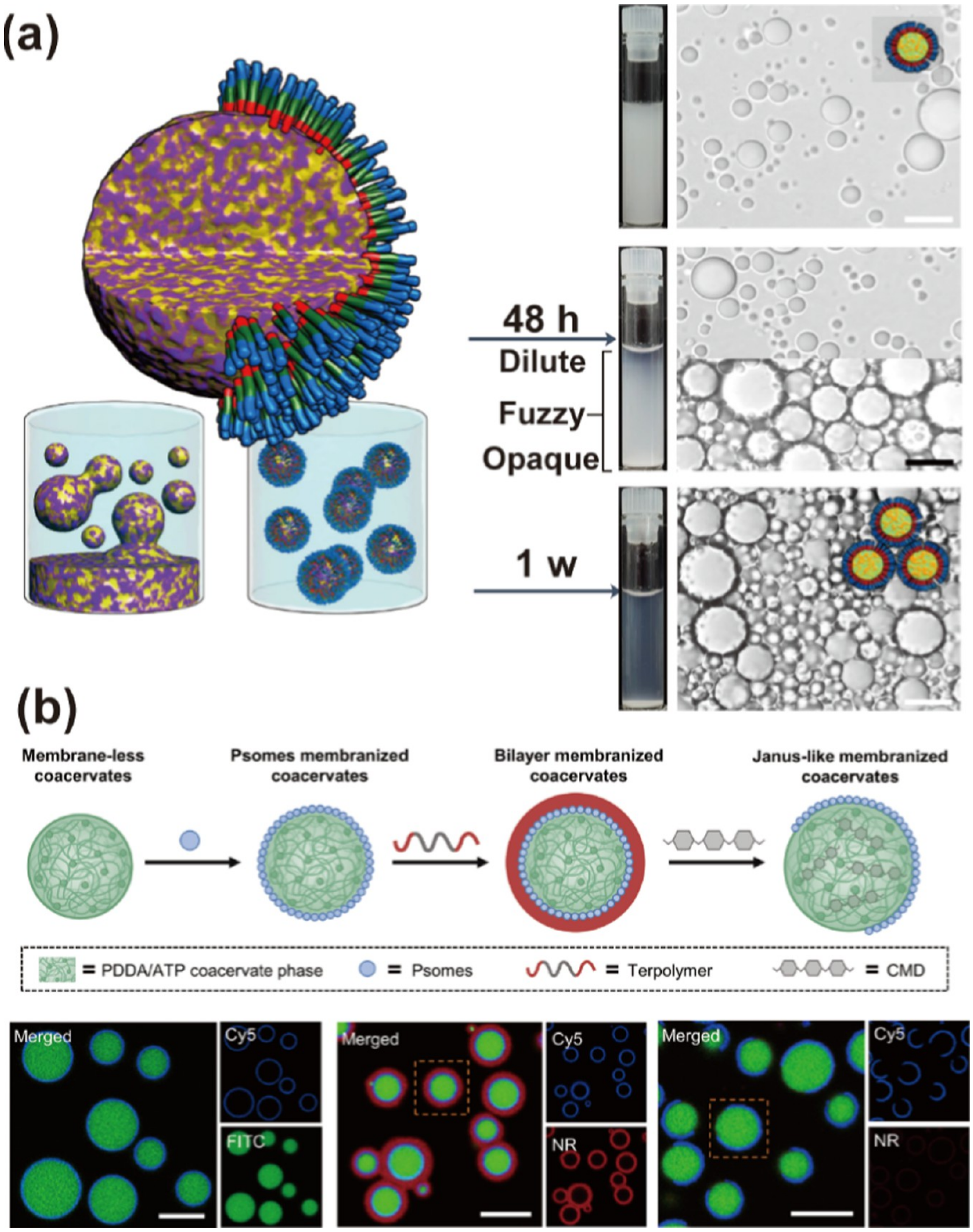

Figure 3. (a) Triblock copolymers ("condenophobic" fragments, "condenophilic" fragments, and self-association blocks) can be membranized on a wide variety of coacervates. Adapted with permission from ref 101. Copyright 2025 Springer Nature. (b) By adding CMD to the bilayer membrane formed by Psomes and triblock polymers, the membrane can be vanished, and Janus-like membranized coacervates can be formed. Adapted with permission from ref 100. Copyright 2025 American Chemical Society.

engineering strategies enable precise regulation of membrane properties encompassing dimensional control, selective permeability modulation, and surface functionality engineering, such as ligand conjugation and charge patterning.[3,27,39,45,105] For example, van Hest and co-workers developed an interfacial assembly strategy to prepare membranized complex coacervate droplets.[106] Complex coacervates were first fabricated by electrostatic interaction between carboxylate amylose (C-AM) and quaternized amylose (Q-AM), followed by interfacial stabilizing with a terpolymer comprising polyethylene glycol (PEG), poly(caprolactone-gradient-trimethylene carbonate, PCLgTMC), and poly(glutamic acid, pGlu). Interfacial assembly of a terpolymer monolayer was attributed to the strong interactions of PEG and pGlu with the continuous water phase and coacervate droplet, respectively, while the PCLgTMC domains provided the required flexibility for dynamic reorganization via hydrophobic chain association. The corresponding chain length of copolymers was ca. 16−18 nm, which was sufficient to span the all−water interface of the complex coacervates. Besides the charged polymers that were heavily utilized as membrane components of complex coacervates, protein-conjugates were also recognized as an appealing agent for interfacial stabilization. For instance, Huang et al. utilized PEGylated BSA as a protein−polymer membrane to stabilize complex coacervates from quaternary ammonium amylose and succinimidyl amylose.[51] Confocal 3D microscopy confirmed the successful membranization of the coacervate droplets by mPEG−BSA conjugates, resulting in hybrid protocells with continuous membranes and well-defined coacervate interiors. Electron microscopy further demonstrated significantly enhanced stability of the membranized coacervates, which maintained spherical integrity throughout drying, coating, and imaging procedures. Encapsulated functional molecules retained their activity within the coacervate phase, supporting the potential of these structures as functional protocells.

Existing stabilization methods are mostly system-specific and lack broad applicability. To overcome this, Jiang and coworkers presented a universal strategy for stabilizing synthetic coacervates and biomolecular condensates using condensate-amphiphilic block polymers (CAPs) (Figure 3a).[101] They designed CAPs with three components: a condenophilic block (phenylboronic acid and amidoamine) to anchor into the dense condensate phase, a condenophobic polyethylene glycol (PEG) block to extend into the dilute phase, and a self-association block (polycaprolactone-trimethylene carbonate, PCT) to promote membrane formation. A library of 24 CAPs was synthesized and tested on 15 representative condensate systems, ranging from small-molecule coacervates to protein-based condensates. Among them, triblock PEG−PCT-BMs, especially CAP23, showed broad and efficient stabilization, maintaining droplet individuality and preventing fusion for weeks to months. CAP membranes provided mechanical ultrastability, resisting fusion even under crowding, squeezing, or optical manipulation, while also ensuring physicochemical robustness across extreme pH, salinity, temperature, and organic solvent conditions. Importantly, CAPs preserved intradroplet fluidity while regulating permeability, acting as semipermeable barriers. Remarkably, CAPs such as CAP15 also induced spontaneous emulsification, producing stable droplet dispersions without external energy input, through interfacial instabilities similar to Rayleigh−Taylor patterns. By bridging the gap between system-specific stabilizers and universal needs, CAPs establish a versatile platform for droplet membranization. Their universality, ultrastability, and ability to trigger self-emulsification make them valuable tools for synthetic biology, protocell research, industrial formulations, and biomedical applications, while offering new insights into condensate regulation within living systems.

Membrane dynamics play a pivotal role in regulating biological processes within living systems, particularly in mediating communication between membrane-free and membrane-bound micro- or nanocompartments across intra- and extracellular environments.[47,102] Controlled membrane disassembly—triggered by external stimuli such as molecular cues, concentration changes, light irradiation, or enzymatic activity—enables the selective cleavage of dynamic bonds within the membrane.[100,102,107] This process allows the modulation of guest molecule encapsulation and release within the coacervate phase, thereby facilitating adaptive responses to changing environmental conditions.[108] Appelhans and colleagues introduced a promising strategy for the controlled demembranization of membranized coacervate droplets.[100] While their earlier studies demonstrated the transition of coacervates into giant vesicles, their subsequent work focused on achieving targeted demembranization of membranized coacervates, thereby imparting additional functionality and dynamic reconfigurability in response to external or biological stimuli.[109] In their system, membrane-less coacervates (MLCs) were first generated by mixing aqueous solutions of poly(diallyldimethylammonium chloride) (PDDA, 100−200 kDa) and adenosine 5′-triphosphate (ATP) at a PDDA/ATP weight ratio of 1:6. The resulting positively charged MLCs were then coated with a membrane by introducing poly(methacrylic acid)-*b*-poly(2-(methylthio)ethyl methacrylate)-*b*-poly(ethylene glycol) (PMAA-*b*-PMTEMA-*b*-PEG, 13.6 kDa). Electrostatic attraction between the terpolymer and the coacervate surface facilitated the formation of a stabilizing membrane. Subsequent addition of carboxymethyl dextran (CMD), a hydrophilic anionic polysaccharide, induced gradual membrane dissociation and dispersion into the dilute phase. This demembranization process is driven by electrostatic interactions between CMD and the cationic PDDA redistributed within both the membrane and the condensed coacervate matrix. To further enhance structural complexity, the authors incorporated photo-cross-linked polymersomes (Psomes) into the membranization process of MLCs (Figure 3b).[100] Upon subsequent demembranization, this approach yielded "Janus-like" coacervates with asymmetric membrane characteristics. The dynamic and reversible membranization−demembranization cycle endows these systems with tunable structural and functional adaptability, underscoring their great potential for applications in synthetic biology, systems biology, and biotechnology. Despite recent progress in assembling complex, dynamic, and higher-order membrane-based coacervate systems, key structural and functional limitations remain. In many cases, the membranes that coat coacervate droplets are discontinuous and patch-like, exhibiting structural defects and insufficient thickness. Such compromised integrity promotes leakage of encapsulated cargo and makes the droplets highly susceptible to osmotic stresses arising from changes in ionic strength. Moreover, the weak interfacial interactions between the membrane and the coacervate core led to poor membrane stability and short lifetimes. The limited compositional diversity and functional tunability of current membrane systems further constrain the development of advanced biomimetic features, such as selective transmembrane transport and integrated signal transduction in artificial cell constructs.

2.2. Integration of Suborganelles Inside Polymeric Artificial Cells.

The integration of suborganelles within polymeric artificial cells represents a crucial step toward achieving higher-order complexity and functionality reminiscent of natural eukaryotic systems.[42,110,111] In living cells, compartmentalization into specialized organelles enables the segregation and coordination of diverse biochemical processes, ensuring spatial and temporal regulation of metabolism, signaling, and energy transduction.[112,113] Emulating this organizational hierarchy within polymeric artificial cells allows multiple reactions to occur simultaneously yet independently, reducing undesired crosstalk and enhancing overall efficiency. By encapsulating functional subcompartments, such as coacervate droplets, proteinosomes, or enzymatic nanoreactors, within a polymeric membrane, it becomes possible to establish reaction cascades, create chemical gradients, and mimic organelle-like behaviors such as selective transport or dynamic communication.[1,5] This hierarchical structuring not only endows synthetic cells with emergent properties, including self-regulation and adaptive response, but also provides a versatile platform for studying fundamental principles of cellular organization and for engineering advanced

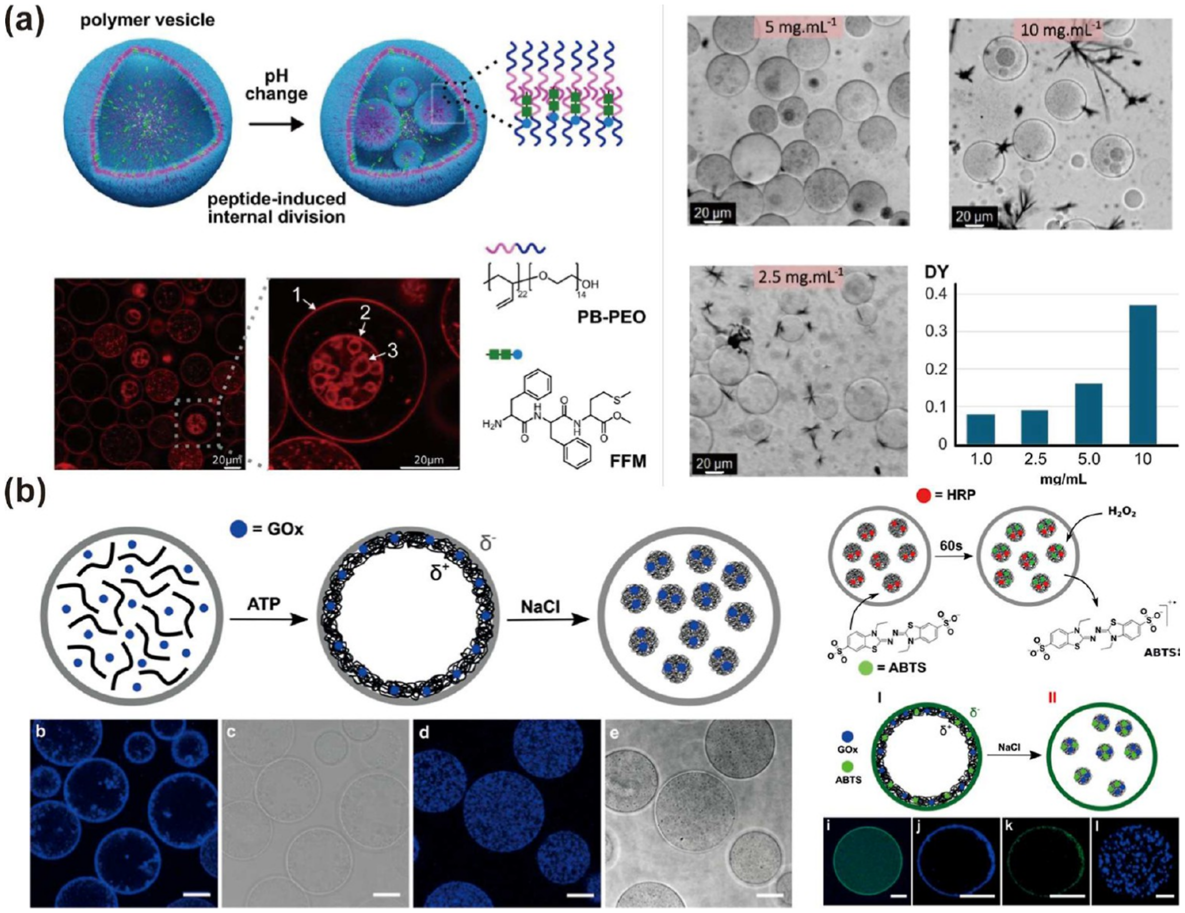

Figure 4. (a) pH-responsive short peptides are encapsulated into polymersome membranes. Following pH adjustment, short peptides induce membrane invagination, leading to the formation of internal compartments. These compartments can subsequently be activated to give rise to daughter vesicles via inward budding again. Adapted with permission under a Creative Commons CC BY license from ref 127. Copyright 2024 Wiley. (b) Salt regulates the in situ formation of subcellular organelle structures within artificial cells. . Adapted with permission under a Creative Commons CC BY license from ref 80. Copyright 2019 Wiley.

biomimetic systems capable of performing complex, life-like tasks.[16,114]

2.2.1. Formation Mechanisms and Pathway Control. As the fundamental unit of life, the cell represents a remarkably precise system, in which compartmentalization creates spatially organized microenvironments for orchestrating biochemical reactions and signal transduction. Internal subcellular compartments further partition the cytoplasm, isolating and protecting vital components while dynamically regulating processes essential for maintaining homeostasis and responding to environmental cues.[7,38,115] Membraneless organelles (MLOs), such as *P*-bodies, Cajal bodies, and nuclear speckles, achieve spatial and temporal control of biochemical reactions through dynamic liquid−liquid phase separation.[116−118] In contrast, membrane-bound organelles, including mitochondria, peroxisomes, and endosomal−lysosomal compartments, are enclosed by lipid bilayers and rely on selective transport mechanisms to mediate molecular exchange across their membranes.[119] Drawing inspiration from these natural systems, contemporary bioengineering has advanced the creation of biomimetic, multilayered artificial cells via bottom-up assembly approaches.[29] Current architectural paradigms for micrometer-scale artificial organelles include spherical assemblies enclosed within semipermeable membranes composed of lipids or polymers, water-in-oil droplets, colloidal vesicles stabilized by inorganic nanoparticles, protein−polymer microencapsulated proteinosomes, and both membrane-bound and membraneless coacervate droplets.[59,120−122] On the nanometer scale, suborganellar structures such as polymersomes, micelles, nanoparticles, and liposomes have been successfully integrated into artificial cells to form simple yet hierarchical systems.[55,123,124] Beyond physical encapsulation, the in situ formation of suborganelles within artificial cells, spontaneously triggered by environmental stimuli, offers a more faithful mimicry of natural biological processes.[80,125,126]

For instance, Landfester and co-workers demonstrated that multicompartmentalized vesicle systems can arise through self-division (Figure 4a).[127] Using microfluidic techniques, they encapsulated pH-responsive tripeptides capable of assembling into fibers at pH > 7 within polymeric giant unilamellar vesicles (pGUVs). Upon protonation of amino groups, the FFM tripeptides became hydrophobic, inserted into the vesicle membrane, increased membrane tension, and ultimately induced inward budding, leading to the formation of daughter vesicles. By manipulating peptide localization either inside or outside the vesicles, the authors demonstrated that the division direction (inward or outward) depends on the orientation of tripeptide insertion. Remarkably, they observed multigenerational vesicle formation, in which primary vesicles produced smaller internal vesicles that subsequently generated second-generation compartments. Such dynamic adaptability underscores the potential of polymersomes as life-like systems capable of responding to external cues, advancing their applicability in biotechnological contexts where environmental responsiveness is essential. Furthermore, these findings provide intriguing implications for prebiotic chemistry, offering insights into how early protocellular systems may have evolved compartmentalized structures and dynamic functional behaviors.

Complementary strategies for constructing artificial organelles with sophisticated morphologies include the incorporation of coacervates into artificial cells, the encapsulation of preformed coacervates within synthetic membranes, and the in situ generation of subcellular, organelle-like structures.[59,94] For example, Mann and co-workers developed molecularly crowded, polyelectrolyte- and ribonucleotide-rich, membrane-free coacervate droplets that were transformed into membrane-bound, finely compartmentalized vesicles via a polyoxometalate-mediated surface templating process.[128] This coacervate-to-vesicle transition reorganized the microdroplets into three-tiered compartments comprising a semipermeable, negatively charged polyoxometalate−polyelectrolyte outer membrane, a submembrane coacervate layer, and an aqueous inner lumen. In contrast, in situ formation of subcellular organelles via liquid−liquid phase separation (LLPS) is often triggered by environmental stimuli such as changes in pH, enzymatic activity, light, or ionic strength.[41,125] As illustrated in Figure 4b, proteinosomes encapsulating polyelectrolytes were first prepared, after which membrane-permeable, oppositely charged small molecules (ATP) were introduced into the surrounding aqueous phase.[80] The diffusion of ATP across the protein−polymer membrane induced in situ complexation and electrostatically driven LLPS, giving rise to an internal coacervate phase. Spatial organization and reconfiguration of the encapsulated coacervate droplets were finely controlled by modulating electrostatic interactions—achieved through NaCl-mediated charge screening—between the coacervate phase and the surrounding proteinosome membrane. This process yielded nested hybrid protocells featuring spatially organized and chemically coupled enzymatic activity. During coacervate formation, enzymes such as glucose oxidase (GOx, Figure 4b) became enriched within the coacervate domains, resulting in a multilayered artificial cellular system containing enzyme-based functional organelles. The spatial arrangement of the coacervate phase was dictated by electrostatic interactions between its positively charged components and the anionic proteinosome membrane, producing two distinct structural configurations: (1) a thin interfacial coacervate layer beneath the membrane, or (2) discrete microdroplets dispersed within the aqueous lumen. This electrostatic control mechanism enabled precise regulation of glucose-stimulated GOx−HRP cascade reactions. Furthermore, the reaction dynamics could be modulated through structural transitions between membrane-proximal coacervate layers and lumen-dispersed microdroplets by tuning ionic strength. Increasing NaCl concentration disrupted the planar coacervate layer, promoting its disassembly and reorganization into discrete internal droplets. Importantly, this phase transformation strategy employed membrane-permeable ionic triggers, obviating the need for conventional drivers such as thermal or osmotic gradients. Owing to the inherent membrane permeability, encapsulated enzymes functioned efficiently as catalytic centers within these coacervate compartments. Altogether, this design exemplifies a versatile approach for achieving spatiotemporal control of enzymatic cascades through electrostatically gated compartmentalization, offering valuable insights into the dynamic regulation of biochemical processes in synthetic cell models.

Despite persistent challenges in achieving precise spatiotemporal control over (bio)chemical reagent distribution within multicompartmental architectures, emerging microfluidic platforms-particularly droplet-based systems-have revolutionized the precision synthesis of vesicles and protocells.[99,129] These technologies enable high-throughput fabrication of monodisperse compartments with programmable morphological parameters (size, geometry, and membrane composition) and tunable formation kinetics.[130] Moreover, microfluidic systems allow on-demand encapsulation and dynamic exchange of bioactive payloads or membrane constituents via continuous phase manipulation, ensuring high reproducibility across production batches.[131] Collectively, these capabilities establish a modular design paradigm for compartmentalized artificial cells, facilitating the systematic engineering of hierarchical structures with orthogonal reaction environments and stimulus-responsive behaviors. Such advances effectively address critical limitations associated with scaling the complexity of artificial cellular systems, particularly in replicating the multiscale organization and adaptive dynamics characteristic of living cells. Seo and coauthors presented a microfluidic approach to prepare semipermeable polymersomes comprising of amphiphilic triblock copolymer, capable of supporting externally triggered complex coacervation and cytoskeletal reconstitution within the vesicles.[132] Using a glass-capillary-based microfluidic device, they produced monodisperse water-in-oil-in-water ($W_1/O/W_2$) double emulsion droplets. Quantitative control over the three phases, combined with rapid solvent volatilization, yielded Pluronic-based polymersomes with uniform size and morphology. The system allowed coencapsulation of adenosine triphosphate (ATP, MW 507 Da) and poly(allylamine hydrochloride) (PAH) within the polymer vesicles. Due to the inherent proton permeability of the polymer membranes, electrostatic complexation between ATP and PAH at physiological pH (7.4) led to in situ formation of coacervate subcompartments, effectively mimicking organelle-like domains within the polymersomes.

2.2.2. Responsiveness and Adaptive Dynamics.

Intracellular organelles are specialized and functional compartments that enable eukaryotic cells to carry out complex biochemical processes, respond to external stimuli, and coordinate cascade reactions.[133−135] These microcompartments achieve spatiotemporal regulation by locally concentrating substrates or signaling molecules while shielding them from degradation or interference by cytoplasmic components, thereby enhancing reaction efficiency through selective diffusion.[136,137] Moreover, natural organelles display intrinsic stimulus-responsiveness, a defining feature of living systems, that underlies adaptive development, metabolic homeostasis, and signal transduction. Such adaptability, manifested through modulation of protein activity, molecular localization, and concentration gradients, represents a fundamental survival mechanism that allows cells to adjust dynamically to environmental and metabolic changes.[113,138,139] Inspired by these biological principles, artificial organelles have been designed to emulate the responsive behaviors of natural counterparts.[140−142] When exposed to stimuli such as variations in temperature, pH, light, or signaling molecules, these engineered compartments exhibit biomimetic functions that recapitulate the dynamics of living

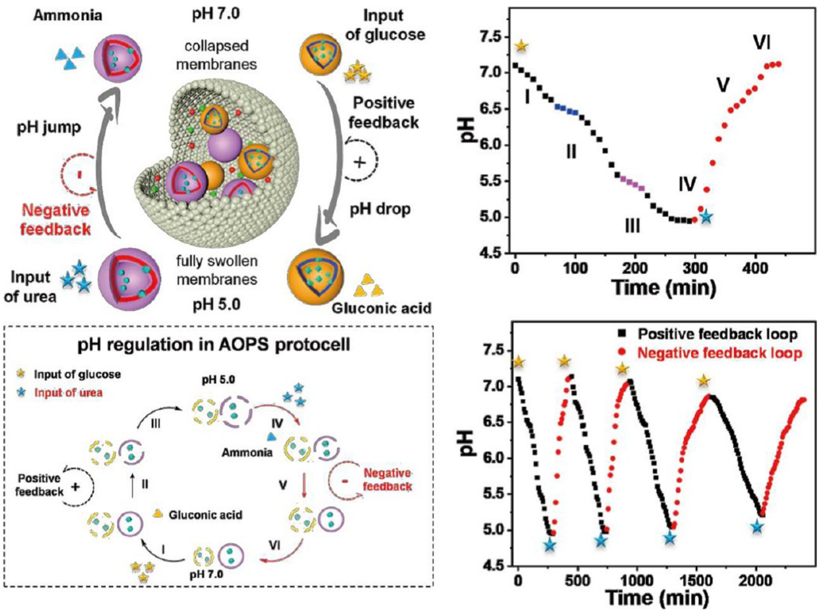

Figure 5. Up and down jumps in pH change the size and permeability of intramembrane polymersomes. Adapted with permission from ref 146. Copyright 2023 American Chemical Society.

cells. Incorporating such stimulus-responsive, organelle-like domains has thus become a key strategy for realizing life-like adaptability in artificial cellular systems. As an illustrative example, Qiao and co-workers constructed hierarchical protocells that exhibit modern cell-like compartmentalization and adaptive functionality.[126] They synthesized a bola-amphiphile containing a rigid azobenzene core flanked by two negatively charged glutamic acid groups [4,4′-glutamic acid azobenzene ($AzoGlu_2$)], serving as a photoswitchable component, and paired it with a pH-responsive cationic polyelectrolyte, diethylaminoethyl-dextran (DEAE-dextran). The carboxyl and amine groups of $AzoGlu_2$ and DEAE-dextran undergo pH-dependent protonation transitions, enabling coacervation only when $AzoGlu_2$ is in its trans-configuration. Semipermeable proteinosomes were fabricated from cross-linked monolayers of bovine serum albumin−poly(*N*-isopropylacrylamide) ($BSA-NH_2$/PNIPAAm) nanoconjugates, encapsulating DEAE-dextran inside, while trans-$AzoGlu_2$ (1:1 ratio) was added to the external medium. Owing to the selective permeability of the proteinosome membrane, $AzoGlu_2$ diffused inward while DEAE-dextran remained confined, leading to the formation of coacervate-based artificial organelles under suitable conditions. Increasing the pH above 7.5 induced deprotonation of $AzoGlu_2$, triggering coacervate dissociation. Leveraging this dynamic assembly process, the authors constructed a NOR logic gate responsive to ultraviolet (UV) light and urease: coacervate formation occurred only in the absence of both stimuli, whereas the presence of either or both inhibited organelle assembly. TAMRA-labeled ssDNA was recruited into and released from subcellular organelles through the light-controlled formation and dissolution of suborganelles. Following UV irradiation of the proteinosomes, the fluorescence intensity within the microdroplets gradually diminished and completely disappeared after 24 s, indicating the dissociation of coacervates and release of DNA. The compartmentalization of HRP enzymes within subcellular organelles further allows for the enzymatic conversion of Amplex Red to resorufin in the presence of H_2O_2. Compared to freely diffusing enzymes in bulk of proteinosomes, the reaction rate was significantly enhanced, owing to the local accumulation of HRP inside coacervates within proteinosomes. This work elegantly demonstrates programmable spatial organization and logical responsiveness within proteinosomes, offering a blueprint for integrating computation and adaptive behavior into synthetic cellular systems.

A hallmark of living cells is pH stability, supported by active self-regulatory and recovery processes that counteract external disturbances and re-establish equilibrium.[143−145] Appelhans and colleagues developed a binary enzyme-loaded polymersome-in-proteinosome system capable of pH feedback regulation and self-monitoring, exhibiting complex heterogeneous architecture with input-controlled pH changes mediated by antagonistic feedback loops, an essential feature for advanced protocell design (Figure 5).[146] The system incorporates antagonistic enzyme-loaded polymersomes: GOx-Psomes (A) and Urease-Psomes (B), along with catalase (which detoxifies H_2O_2 into O_2 and H_2O to protect enzymatic activity) and Dextran-FITC as a pH sensor, all coencapsulated within an artificial organelle-in-protocell system (AOPS). Upon addition of external fuels (glucose and urea), substrates diffuse into the compartment. Enzymatic conversion and production of gluconic acid induced a pH decrease of the environment (Step 1), triggering the swelling of polymersome that enhanced substrate influx via positive feedback. Conversely, the hydrolysis of urea raised the solution pH (Step 2), reversing the process through a negative feedback loop, ultimately restoring the system to its initial state. In the top-right panel of Figure 5, the coexistence of positive and negative feedback loops gives rise to distinct pH trajectories upon sequential substrate addition. When glucose is first introduced, the pH undergoes a transient decrease before reaching a new steady state. Subsequent addition of urea induces a pH increase, which likewise stabilizes over time. This alternating pH response can be sustained for at least four consecutive cycles, as shown in the bottom-right panel of Figure 5.

2.3. Formation of Cytoskeleton Structure Inside Polymeric Artificial Cells.

In living cells, cytoskeletal networks composed of actin filaments, microtubules, and

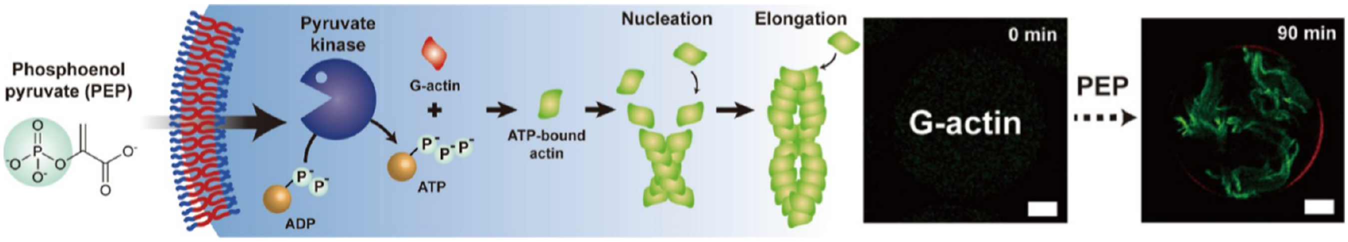

Figure 6. Encapsulation of Pyruvate kinase in polymersomes converts G-actin to ATP-bound actin by ATP consumption and assembles fibers as the cytoskeleton. Adapted with permission under a Creative Commons CC BY license from ref 132. Copyright 2022 Springer Nature.

intermediate filaments not only maintain cellular morphology but also facilitate essential processes such as intracellular transport, motility, and division.[147−149] Mimicking these dynamic structural frameworks within polymeric compartments offers a powerful strategy to bridge the gap between static artificial constructs and responsive, self-organizing systems.[150,151] By reconstituting natural filament-forming proteins or employing synthetic analogs, such as peptide amphiphiles, polymeric filaments, or DNA-based scaffolds, researchers have achieved controllable cytoskeletal assembly within polymersomes and coacervate-based protocells.[148,152−154] These internal architectures provide mechanical reinforcement, regulate the spatial organization of encapsulated components, and enable active deformation and shape transformation. Furthermore, coupling cytoskeletal dynamics with chemical energy inputs (e.g., ATP-driven polymerization) allows the realization of nonequilibrium behaviors akin to those observed in living cells.[107,155−157] Thus, the integration of cytoskeletal elements not only enhances the structural and functional sophistication of polymeric artificial cells but also lays the foundation for constructing adaptive, motile, and evolvable synthetic life-like systems.

2.3.1. Biomimetic Cytoskeletal Structures. As a structural framework fundamental to maintaining cell morphology, facilitating intracellular transport, providing mechanical support, and governing cell division, the cytoskeleton is indispensable to the organization and dynamics of living cells.[158,159] Accordingly, the biomimetic incorporation of cytoskeletal elements into artificial cells is a key step toward achieving cellular-level behaviors. In recent years, researchers have sought to reconstruct cytoskeletal functions using synthetic polymer networks, protein-based self-assemblies, and DNA nanostructures, thereby enabling preliminary control over directional transport, spatial organization of biochemical reactions, and morphological adaptation within artificial cellular models.[40,57,160−162] Incorporating cytoskeleton-like elements not only reinforces structural stability but also improves functional coordination across multiple compartments, laying the groundwork for advanced cellular behaviors such as polarization, motility, and division.[147,148] Consequently, the biomimetic design of cytoskeletal frameworks has emerged as a central strategy in transforming artificial cells from static chemical assemblies into dynamic, life-like systems.

Cytoskeletal reconstitution has been successfully achieved across a variety of synthetic cellular models, including polymersomes, proteinosomes, liposomes, coacervates, and colloidal vesicles.[37,79,163,164] Among these, vesicular architectures are particularly advantageous for cytoskeletal integration due to their inherent compartmentalization and semipermeability. A general approach involves three key steps: (1) membrane assembly of the protocell scaffold; (2) encapsulation of cytoskeletal precursors through passive diffusion or in situ synthesis; and (3) stimuli-induced polymerization, triggered by environmental cues such as pH, temperature, light, ionic strength, or enzymatic activity. The resulting cytoskeletal networks typically arise from supramolecular polymerization processes, including ATP-driven actin filament growth and GTP-mediated tubulin assembly.

A notable example is illustrated by Seo and Lee, who employed a microfluidic strategy to fabricate semipermeable polymersomes capable of external signal−driven coacervation and in situ cytoskeletal reconstitution (Figure 6).[132] Using a glass-capillary microfluidic device, they generated water-in-oil-in-water ($W_1/O/W_2$) double emulsion droplets composed of amphiphilic triblock copolymers. Within these vesicles, ATP and poly(allylamine hydrochloride) (PAH) were coencapsulated, taking advantage of the membrane's proton permeability to facilitate in situ formation of complex coacervate subcompartments at physiological pH (7.4). Subsequently, globular actin (G-actin) was introduced to assemble an artificial cell cortex within the polymersomes through a signal-triggered enzymatic reaction. When phosphoenolpyruvate (PEP) was added to the external medium, it initiated a pyruvate kinase (PyK)−catalyzed reaction that promoted actin filament elongation, effectively reconstructing cytoskeletal dynamics inside the polymersomes. Enzymatic regulation has become an increasingly powerful approach in cytoskeletal engineering, owing to its biochemical specificity and catalytic precision, which enable fine spatiotemporal control of polymerization kinetics. This strategy allows for the formation of highly ordered cytoskeletal architectures that closely emulate the dynamism of living cells. In a representative study, Wang and colleagues developed a multicompartment protocell platform by encapsulating alkaline phosphatase-loaded polymersomes (ALP-Psomes) within proteinosomes, forming an integrated system termed ALP-AOPs.[55] These inner ALP-Psomes function as artificial organelles (AOs), catalyzing the dephosphorylation of oligopeptides, which subsequently self-assemble into micrometer-scale nanofibers. Through the activity of membrane-integrated and membrane-associated ALP, a customizable artificial cytoskeleton was established within the multicompartment structure via enzyme-induced self-assembly. By controlling reaction time or substrate concentration, the cytoskeletal network could be dynamically tuned, leading to a series of morphological transitions in the artificial cell—from spherical integrity to swelling and ultimately rupture. This exemplifies how engineered cytoskeletal systems can actively regulate cellular stability, morphology, and mechanical adaptability in synthetic cells. Overall, these advances underscore the critical role of biomimetic cytoskeletal design in enabling artificial cells to transition from

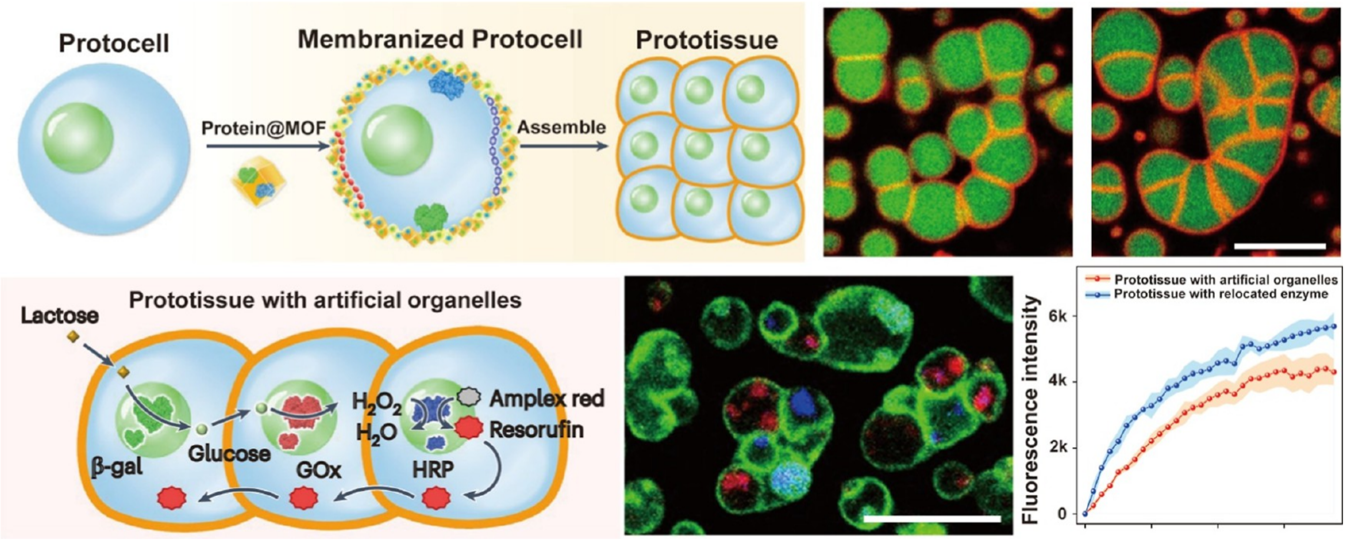

Figure 7. MOF-stabilized coacervate protoplast model capable of integrating proteins and constructing multicompartmental artificial cellular tissue-like structures, enabling retrograde communication and enzymatic cascade reactions. Adapted with permission from ref 3. Copyright 2025 Springer Nature.

simple compartmentalized microreactors toward adaptive, morphogenetic entities capable of performing life-like behaviors.

2.3.2. Mechanical Stabilization and Tunability. Intracellular cytoskeleton structures are dynamic and adaptive structural networks, serving as the primary architectural framework that supports the function of living cells. Composed predominantly of microtubules, actin filaments, and intermediate filaments, the cellular cytoskeleton governs cellular morphology, mechanical integrity, and spatial organization.[35,165,166] Through continuous assembly−disassembly cycles, it mediates essential cellular functions including motility, division, intracellular transport, and signal transduction, while enabling cells to interact dynamically with their environment. These behaviors are precisely regulated through ATP/GTP-driven polymerization, cross-linking proteins, and motor protein activity, collectively imparting both rigidity and flexibility.[163] Emulating such dynamic and reversible cytoskeletal control represents one of the foremost challenges in synthetic biology. Yet it is crucial for constructing artificial cells capable of adaptive, life-like behaviors, such as morphological plasticity, directed movement, and controlled molecular trafficking. To this end, recent efforts have focused on developing stimulus-responsive synthetic cytoskeletons employing programmable polymers, DNA-based nanostructures, and engineered protein assemblies, combined with precise spatiotemporal regulatory strategies. For example, in a water-in-oil emulsion system, Kerstin et al. utilized nucleoli to direct the assembly of a DNA−polymer hybrid cytoskeleton within liposomal vesicles, thereby enabling autonomous cargo transport.[37] External cues such as ATP or RNase H were used to dynamically modulate this transport function, illustrating how biochemical inputs can drive structural reconfiguration. The core design principle relies on the programmable modulation of network topology and mechanical properties through controlled conformational transitions, reversible assembly, or selective dissociation of scaffold components.[153] Such processes can be triggered by external physical stimuli (light, temperature, electric fields) or intrinsic chemical signals (pH variations, chemical fuels), providing an avenue for the dynamic tuning of cytoskeletal mechanics. Building on these principles, Novosedlik and co-workers developed a strategy to construct an artificial cytoskeleton that provides mechanical reinforcement to coacervate-based artificial cells.[56] By modulating the hydrophobicity of cytoskeletal building blocks, the researchers demonstrated precise control over their localization, either supporting the coacervate membrane or residing within the lumen, thus imparting distinct mechanical behaviors reminiscent of natural cells. In their design, coacervates were first stabilized with a terpolymer forming a semipermeable membrane, which mimics the cytoplasmic crowding and boundary functions of living cells. They then introduced a fibrous network of polydiacetylenes (PDA) as a cytoskeletal analog. These PDA fibers, functionalized with carboxylate−dibenzocyclooctyne (DBCO) or azide terminal groups (PDA-M and PDA-L, respectively), formed bundled nanofiber structures that localized selectively depending on surface hydrophobicity: (1) DBCO-terminated PDA (more hydrophobic) preferentially anchored to the membrane, supporting surface rigidity; (2) Azide-terminated PDA (less hydrophobic) localized within the cytoplasm, reinforcing the internal network. Comparative deformation studies revealed that coacervates containing internal cytoskeletal networks exhibited significantly enhanced mechanical resilience, analogous to HeLa cells, whereas those with membrane-anchored cytoskeletons were less effective in maintaining shape stability under stress. This finding highlights the critical role of internal cytoskeletal organization in preserving cellular morphology and mechanical integrity. Collectively, these studies underscore the central importance of cytoskeletal architecture in imparting mechanical stability, deformability, and responsiveness to both living and synthetic cells. Reconstructing such adaptive mechanical networks using self-assembling, stimulus-responsive polymers enable the emulation of dynamic reorganization and feedback-regulated mechanics characteristic of natural cytoskeletons. Achieving these capabilities marks a pivotal step toward engineering programmable artificial cells with tunable structure−function relationships, bridging the gap between static molecular assemblies and dynamic, life-mimetic systems.

3. LIFE-LIKE BEHAVIORS AND FUNCTIONS

3.1. Microreactors. The compartmentalized architecture of living cells allows precise regulation of diverse biochemical processes through orchestrated networks of chemical and enzymatic reactions.[124,167,168] We further distinguish between two functional categories of polymer-based compartments. Microreactors primarily serve as confined catalytic environments, facilitating reactions through concentration effects or selective partitioning. In contrast, cell-mimicking systems incorporate additional layers of regulation, such as gated transport, signaling, spatial organization, or intercompartment communication, that more closely resemble biological cellular behavior. This distinction is important for evaluating design principles and contextualizing recent advances in polymer-based synthetic biology. The hierarchical organization has inspired the development of membrane-bound synthetic cellular systems, among which polymer-based biomimetic microreactors (PBMs) have emerged as a powerful platform.[91,169] These artificial cells combine structural robustness, tunable physicochemical properties, and long-term stability, thereby enabling the execution of complex, life-like metabolic transformations within confined microenvironments.[53,170−172] Among them, polymersomes and proteinosomes, characterized by their hollow, aqueous interiors, provide an ideal milieu for sustained enzymatic activity, while complex coacervates offer intrinsically crowded, macromolecule-rich environments that closely mimic the cytoplasmic conditions of natural cells.[60,62,83] Integrating these systems into multilayered, hierarchically organized architectures enables the reconstruction of essential features of cellular metabolism with high spatial and temporal fidelity.[26,33,59] In a representative example, Qiao's group designed biomimetic cell models by integrating ZIF-8 metal−organic framework (MOF) nanoparticles with PDDA/PAA-based droplet assemblies (Figure 7).[3] These hybrid structures emulate fundamental cellular functions such as compartmentalization, multistage catalysis, and signal transduction, providing a programmable platform for spatiotemporal control of biochemical reactions. By encapsulating smaller Prot/FA subdroplets within the artificial cytoplasm as functional organelle analogs, the system enabled compartmentalized enzymatic cascades. Furthermore, salt-induced electrostatic screening promoted the self-assembly of MOF-coated protocells into tissue-like superstructures, facilitating substrate channeling across adjacent compartments. This design demonstrates how porous MOF nanoparticles can be organized into semipermeable, multilayered membrane frameworks that function as cell- and tissue-like catalytic systems, offering a promising blueprint for constructing artificial microreactors with lifelike metabolic behavior. Following the same reaction pathway, whether by encapsulating enzymes within subcellular organelles or immobilizing them on membranes, it is observed that reaction rates in MOF membranes are faster than in multicompartmental prototissue systems due to lower diffusion restrictions on MOF membranes. Complementarily, stimuli-responsive regulation of membrane permeability provides a powerful means to dynamically modulate reaction processes within artificial cells.[10,173−176] Landfester's research group developed a photoswitchable polymersome platform based on polybutadiene-*b*-polyethylene glycol (PB-*b*-PEO) vesicles fabricated via droplet microfluidics.[125] While these vesicles are inherently impermeable, surface functionalization with photoresponsive furan−maleimide derivatives enables light-controlled isomerization, thus reversibly tuning membrane permeability. Upon irradiation, increased permeability facilitates substrate diffusion (for molecules <4 kDa) and allows enzymatic reactions to proceed within the vesicle lumen. When loaded with β-galactosidase (β-Gal), the vesicles act as light-gated microreactors, catalyzing the hydrolysis of galactose-conjugated precursors and producing a quantifiable fluorescent output. Upon UV irradiation, the increased membrane permeability allowed glycoside substrates to diffuse into the vesicles and react with encapsulated enzymes, resulting in fluorescence emission. CLSM confirmed the appearance of distinct green fluorescence in the artificial cell matrix after 540 s. Compared to the nonirradiated control, the reaction efficiency was significantly enhanced under UV exposure. This system exemplifies a stimulus-responsive, adaptive microreactor design that captures key attributes of biological cells—selective transport, signal responsiveness, and metabolic control—and establishes a versatile framework for next-generation artificial cell engineering.

3.2. Communication. In synthetic multicompartment systems, communication can occur through several mechanistically distinct modes. Diffusion-based exchange relies on passive molecular flux across permeable interfaces, enabling gradient-driven exchange of small solutes. In contrast, gated transport requires an active or stimulus-responsive trigger, such as pH, light, or conformational switching, to transiently open selective pathways for molecular transfer. Contact-mediated signaling operates only when two compartments directly touch, permitting localized molecular transfer or interfacial reaction events. Finally, structured 'tissue-like' assemblies involve spatially organized clusters of compartments in which communication is coordinated across a higher-order architecture, enabling emergent collective behaviors that mimic cellular tissues. These distinct modes reflect increasing levels of specificity, localization, and hierarchical control.[177,178] Cells achieve this through a combination of membrane-mediated transport, receptor signaling, and mechanosensitive feedback, all of which operate within a highly crowded intracellular milieu.[179−181] Replicating such information processing within synthetic systems represents a central objective of bottom-up synthetic biology.[29,121,182] By employing minimal, nonliving components, researchers aim to reconstruct the fundamental principles of signal sensing, transduction, and response in programmable, life-like systems.[183−187] A representative strategy involves mimicking intracellular biomolecular condensate dynamics using polymer-based hybrid protocells. In one such design, coacervate droplets encapsulated within polymersomes were engineered to simulate signal-responsive condensate formation. By loading poly-L-lysine (PLL) inside the polymersomes and introducing light-triggered adenosine triphosphate (ATP) influx, researchers achieved photoinduced ATP−PLL coacervation, recapitulating the stimulus-responsive assembly of biomolecular condensates observed in living cells. This design provides a compelling model for studying light-regulated, phase transition−driven communication processes within confined synthetic environments. Beyond intracellular mimicry, cell-to-cell communication in natural systems involves both direct contact and diffusional signaling across cellular communities.[188,189] Synthetic analogs of these processes are being realized using membrane-bound artificial cells such as polymersomes, proteinosomes, and coacervate-based protocells.[25] Compared with liposomes or membrane-free droplets, these polymeric architectures offer enhanced

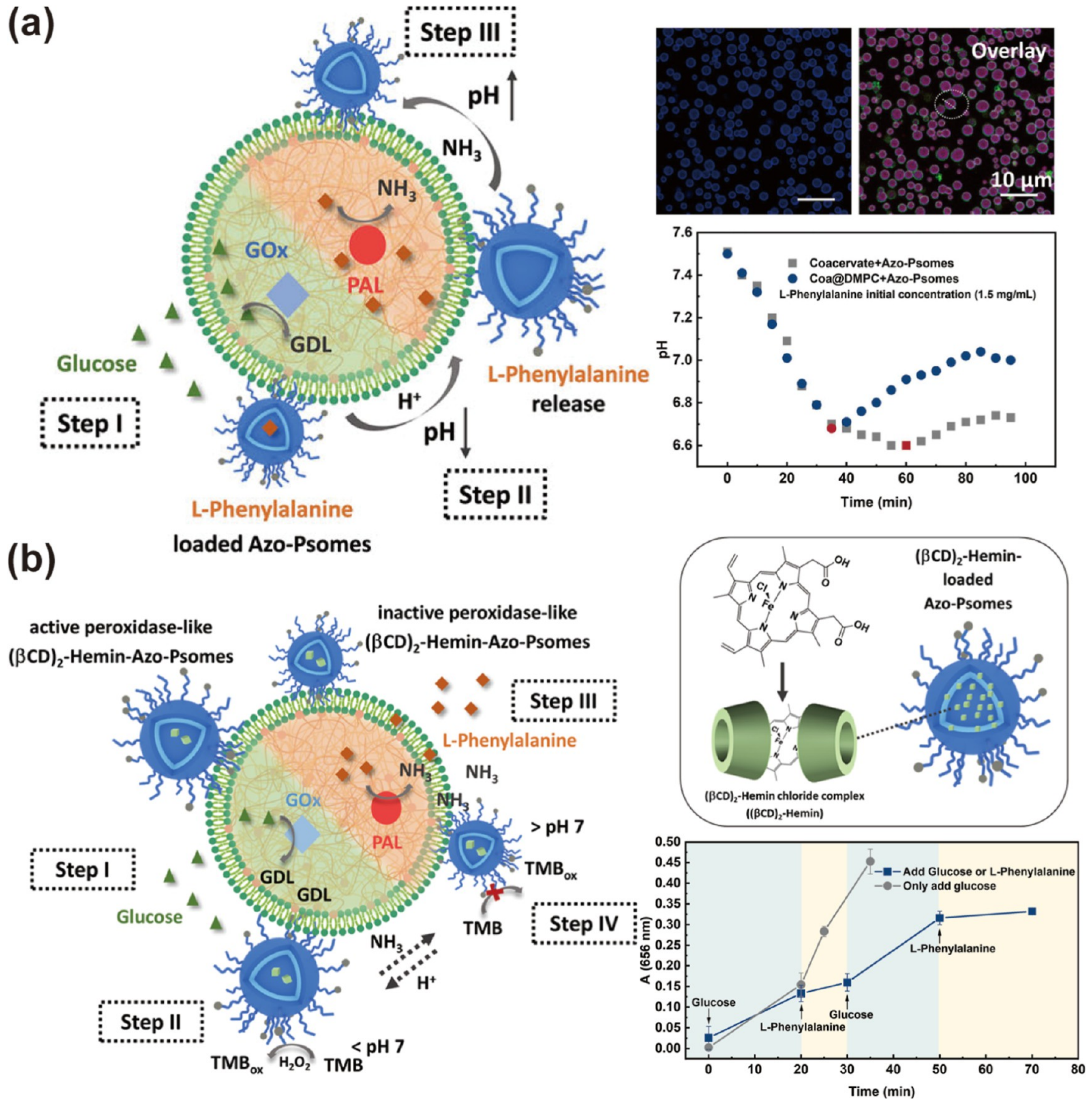

Figure 8. (a) pH modulation allows cellular communication between nano- and micrometer assemblies. (b) Within the artificial cell, cytoplasmic glucose oxidase (GOx) catalyzes glucose oxidation, acidifying the local environment. This pH decrease triggers swelling of the external nanovesicle and the subsequent release of encapsulated phenylalanine. Upon entry into the cell, phenylalanine is metabolized by cytoplasmic phenylalanine aminotransferase, raising the intracellular pH. As a result, the nanovesicle contracts, thereby terminating phenylalanine release. Adapted with permission under a Creative Commons CC BY license from ref 108. Copyright 2025 Wiley.

mechanical stability, chemical versatility, and tunable permeability, enabling the controlled exchange of chemical signals between compartments.[38,190−192] For instance, Qiao and coworkers developed a membranized coacervate system to model intercellular communication and organization.[102] Liquid-like protamine−folic acid coacervates were coated with rigid polysaccharide membranes using tetramethyl rhodamine−dextran isothiocyanate (TRITC−dextran, 500 kDa), producing structured, tissue-like protocell assemblies. Upon centrifugation, the membranized droplets compacted into a dense, coacervate superstructure resembling primitive tissues. Enzymatic compartmentalization further endowed the system with cascade signal transduction: β-galactosidase (β-Gal) converted lactose to glucose, which diffused to glucose oxidase (GOx)−containing droplets to generate hydrogen peroxide, subsequently activating horseradish peroxidase (HRP)−mediated oxidation of Amplex Red to resorufin. The localized increase in red fluorescence confirmed chemical communication between neighboring protocells, effectively mimicking metabolic cooperation within multicellular assemblies. Polymer-based com-

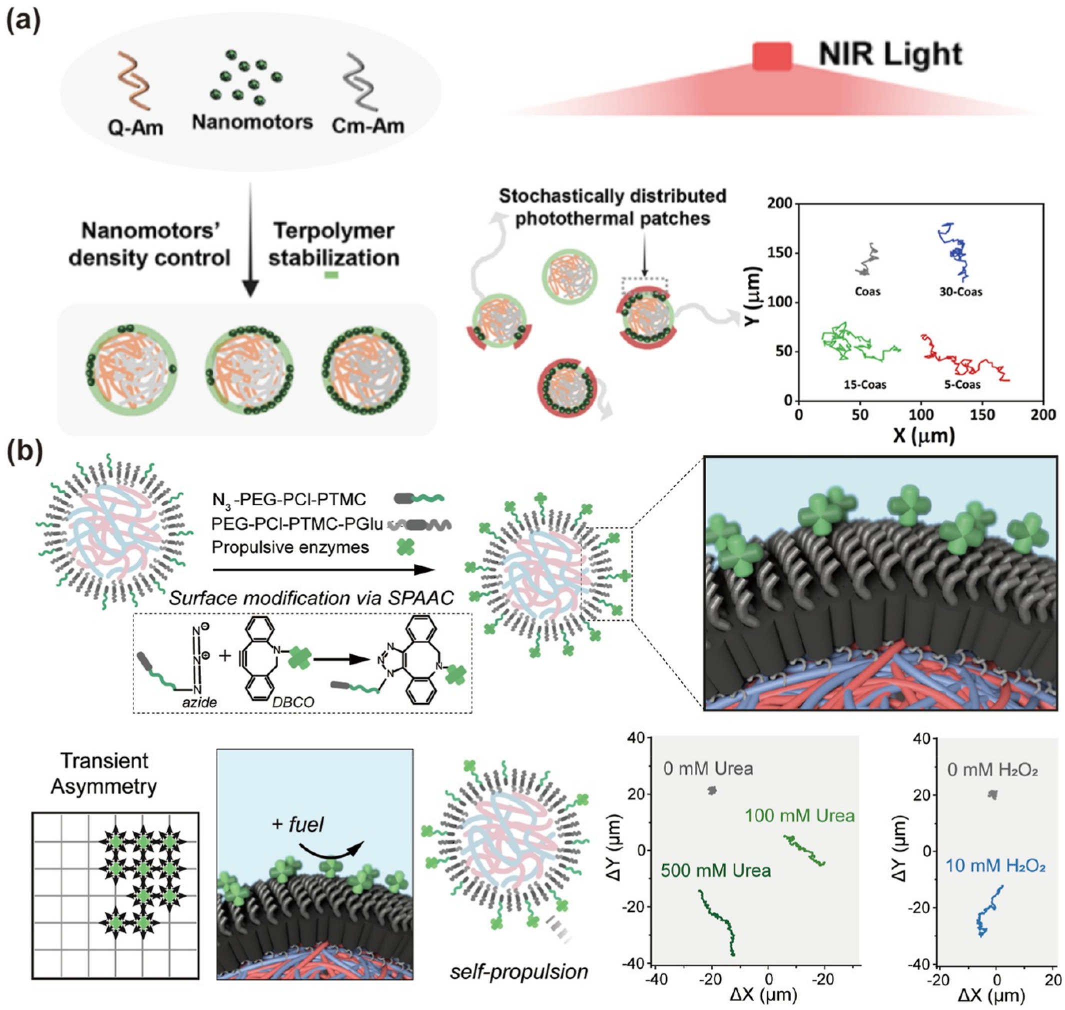

Figure 9. (a) Nanomotors and terpolymer-stabilized coacervate protocells exhibit light-driven mobility capabilities. Adapted with permission under a Creative Commons CC BY license from ref 202. Copyright 2025 American Chemical Society. (b) Enzyme motor-driven motility of polymer-membraned coacervate. Adapted with permission under a Creative Commons CC BY license from ref 203. Copyright 2021 Springer Nature.

partments offer several unique advantages over lipid vesicles with respect to communication. While lipid membranes provide biological relevance and high permeability selectivity, polymers introduce far greater tunability in mechanical rigidity, membrane thickness, permeability, and chemical functionality. The inherent modularity of polymers enables programmable responsiveness (e.g., thermal, redox, photoswitchable), integration of functional monomers, and the construction of nonequilibrium or anisotropic structures that are difficult to realize with lipids. Moreover, the enhanced mechanical robustness of polymers allows the formation of stable tissue-like assemblies and long-lived intercompartment contacts, supporting communication behaviors that extend beyond those typically achievable in lipid systems.

In a complementary approach, Huang et al. established hierarchical communication between micrometer- and nanometer-scale artificial cells through noncovalent, azobenzene-mediated self-assembly (Figure 8a).[108] Here, azobenzene-functionalized polymersomes spontaneously inserted into coacervate membranes, forming pH-responsive composite protocells. The coacervate phase encapsulated glucose oxidase (GOx) and L-phenylalanine ammonia lyase (PAL), enabling reciprocal chemical signaling. Glucose addition lowered the pH through GOx-mediated oxidation, prompting vesicle swelling and release of phenylalanine. The liberated phenylalanine was subsequently metabolized by PAL to generate ammonia, which reversed the pH shift, completing a feedback communication loop. By comparing pH changes between membrane-bound coacervate systems and membraneless coacervates, it is observed that membrane-bound coacervates exhibit a significant pH rebound, whereas membraneless coacervates show only a minor rebound. And Incorporation of peroxidase-mimetic $(\beta CD)_2$−Hemin within the polymersomes further allowed modulation of TMB oxidation kinetics through glucose−phenylalanine−pH coupling (Figure 8b). Under acidic conditions, vesicle swelling enables substrates from the solution to contact the encapsulated enzymes and initiate the reaction. In contrast, under alkaline conditions, vesicle contraction leads to low reaction efficiency. This pH-dependent behavior can be utilized to regulate reaction progression: adding glucose sustains continuous reaction, whereas adding phenylalanine raises the pH and slows the reaction rate. By alternately introducing glucose and phenylalanine, spatiotemporal control of the reaction rate can be achieved. This interpopulation signaling mechanism demonstrates programmable enzymatic reactivity governed by spatiotemporal control, reflecting a critical step toward collective metabolic behavior in artificial cellular communities.

Together, these advances illustrate how polymer-based artificial cells can be engineered to communicate, coordinate, and collectively respond to external stimuli—hallmarks of living systems. By integrating membranization, molecular transport, and reaction feedback, synthetic protocells are evolving from isolated chemical reactors into interactive microsystems capable of information processing, environmental adaptation, and cooperative functionality.

3.3. Motility. Cellular motility, a fundamental characteristic of living systems, governs critical biological processes including embryonic development, tissue repair, and immune surveillance.[193−195] In multicellular organisms, cellular movement occurs through a variety of mechanisms, including microtubule-driven flagellar or ciliary propulsion, cytoskeletal actomyosin remodeling, and passive hemodynamic transport.[196,197] The precise regulation of motility ensures physiological homeostasis, whereas its dysregulation contributes to pathological conditions such as tumor metastasis, neurodevelopmental disorders, and vascular dysfunction.[198] Reconstructing such adaptive motility within synthetic systems represents a central goal of bottom-up synthetic biology, bridging the gap between structural biomimicry and functional dynamism. Recent progress in artificial motile cells has been achieved by incorporating programmable molecular components, including reconstituted cytoskeletal proteins, stimuli-responsive polymers, and synthetic molecular motors, enabling life-like propulsion behaviors under external stimuli.[163,199−201] For example, Sun and co-workers presented light-driven motile artificial cells by growing gold nanoparticles (AuNPs) in situ on the surface of polymer stomatocytes, thereby creating plasmonic nanomotor-based protocells (Figure 9a).[202] The incorporation of AuNPs onto a terpolymer membrane not only enhanced membrane robustness but also endowed the system with photoresponsive motility. By modulating the surface density of AuNPs, the researchers tuned the membrane morphology from discrete speckles to continuous plaques, with the degree of surface asymmetry directly governing motility. Under infrared irradiation, protocells with the lowest nanomotor concentration (denoted as 5-Coas) exhibited the most pronounced asymmetry and, consequently, the highest propulsion velocity. Moreover, the movement could be directionally guided by adjusting the light intensity, demonstrating how membrane functionalization can couple structural stability with controllable locomotion—a key step toward developing autonomous, motile cell mimics. In contrast to membrane-bound systems, membrane-free artificial cells—such as coacervates—can achieve motility through externally applied physical fields, including electric, magnetic, or acoustic stimuli. For membrane-enclosed artificial cells, however, movement typically relies on the integration of active or responsive components into the vesicle structure. These may include photosensitive molecules, catalytic enzymes, or functional nanoparticles, which generate self-propulsive forces via local chemical gradients or asymmetric interactions with the surrounding medium. A representative example is provided by van Hest and co-workers, who demonstrated enzyme-powered motility in coacervate-based protocells (Figure 9b).[203] By conjugating urease and catalase enzymes to the polymeric membrane via bioorthogonal click chemistry, they endowed the coacervates with self-propulsive capability driven by chemical fuel conversion. The fluidity of the membrane allowed lateral diffusion of enzymes, resulting in transient surface asymmetry essential for propulsion. Systematic analysis revealed that motility depended critically on both enzyme surface density and distribution asymmetry, with optimal movement achieved at intermediate enzyme coverage. This work illustrates how chemically fueled, dynamic asymmetry can drive autonomous movement, providing a robust platform for probing biophysical mechanisms of cellular motility and designing next-generation active materials. Together, these advances demonstrate how synthetic systems can transcend static biomimicry to emulate dynamic, adaptive locomotion, a hallmark of living matter. Through the integration of responsive polymeric architectures, catalytic energy transduction, and spatiotemporal asymmetry, artificial cells are evolving toward programmable motile entities capable of environmental navigation, targeted transport, and collective motion—laying the groundwork for intelligent microscale robotic systems and self-organizing biomimetic materials.

3.4. Growth and Division. The origin of life likely began with the spontaneous assembly of nonliving molecules into primitive compartments capable of rudimentary evolution-structures that gradually acquired functions resembling those of modern cells.[204] In contrast, contemporary living systems rely on highly organized molecular architectures that orchestrate the fundamental cycles of growth, replication, and division.[205−208] These processes underpin the persistence and evolution of life. In efforts to reconstruct such hallmarks of cellular behavior, researchers have developed bounded artificial compartments that recapitulate key stages of early evolution. These synthetic systems-endowed with metabolic, catalytic, and informational capabilities-have further been engineered to exhibit the critical traits of growth and division.[208,209] Such advances transform static molecular assemblies into dynamically sustained protocells, enriching the conceptual framework of "liveness" in synthetic biology and offering mechanistic insights into life's emergence and operation. Boekhoven and co-workers pioneered the construction of active progenitor systems using lipids with aliphatic chains or peptide amphiphiles containing aspartic acid residues, enabling the reversible division of vesicular protocells and the cyclic appearance and disappearance of coacervate droplets.[210−212] Similarly, Nakashima et al. developed an enzymatically driven model of active coacervate protocells capable of controlled growth. In their system, pyruvate kinase (PyK) catalyzed the conversion of phosphoenolpyruvate (PEP) to pyruvate, simultaneously generating ATP.[213] While lysine-rich protein K72 alone failed to form droplets in the presence of ADP, the enzymatically produced ATP triggered coacervation of K72, leading to the growth of coacervate droplets. The extent of this growth could be precisely regulated by adjusting the concentration of PEP fuel, demonstrating programmable control over droplet size and dynamics.

Liposome-based vesicles, owing to the intrinsic fluidity of their lipid membranes, can also undergo division through mechanical or osmotic instabilities. Changes in environmental osmotic pressure, for instance, can induce membrane deformation and the fission of parent vesicles into daughter compartments—an elementary mimicry of cellular reproduction.[205] More sophisticated biomimetic behavior has been achieved in multilayered synthetic cellular systems capable of sustained growth. Mann's groups engineered living−synthetic hybrid protocells by spatially coencapsulating *E. coli* and *Pseudomonas aeruginosa* (PAO1) within poly(diallyldimethylammonium chloride) (PDDA)/ATP coacervate microdroplets (Figure 10).[110] Upon in situ bacterial lysis,

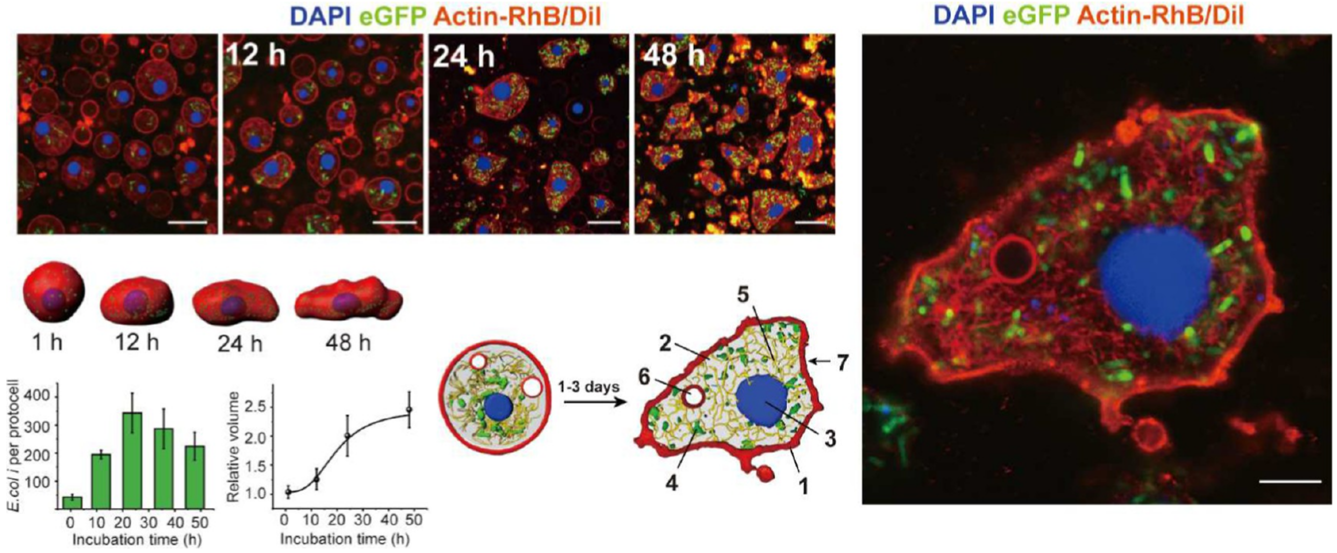

Figure 10. Assembly of live-cell-containing polymer coacervate system and its dynamic morphogenesis. Confocal micrograph of a nonspherical artificial cell showing life-like features, including 1: outer membrane, 2: crowded macromolecular cytoplasm, 3: DNA/Histone organelle, 4: encapsulated *E. coli* (mitochondria mimicking), 5: actin network, 6: spherical vacuoles, 7: amoeba-like morphology. Adapted with permission from ref 110. Copyright 2022 Springer Nature.

Table 1. Representative Membrane Types of Polymer-Based Microcompartments and Their Properties

membrane types	thickness	size	permeability	triggers	compatible cargos	applications	ref
PMOXA$_5$-*b*-PDMS$_{58}$-*b*-PMOXA$_5$/PDMS$_{65}$-*b*-heparin	11 nm	4−50 μm		DTT	protamin, heparin, lipase	triggered enzymatic reactions	42
TA-PEG/Au nanoparticles		∼30 μm	nanosized objects	light/Gox	Gox and guest protocell	protocell sorting	47
mPEG-BSA		5−25 μm	<10 kDa		Gox and HRP	microreactor for cascade reactions	51
PB-*b*-PEO	6 nm	10−20 μm			PAMO, CalB and ADH	multicompartment system for cascade reactions	53
BSA-NH$_2$/PNIPAAm conjugate	10 nm	10−200 μm		reducing agent TCEP	DNA/protease	cargo release control	90
PEG−PCT-BMs	15 nm	6−100 μm	<66 kDa				101
PNIPAM-*co*-MAA/BSA		∼70 μm	<80 kDa (25 °C) <30 kDa (47 °C)	temperature	Gox/AGL	thermoresponsive prototissues and microreactor	122
PB$_{22}$-*b*-PEO$_{12}$/Spiropyran		-	<4kDa	UV light	β-gal and alkaline phosphatase	light-activated microreactor	125
pluronic L121		∼120 μm	<500Da		actin, pyruvate kinase	enzymatic reactor	132
PBut$_{2.5}$-*b*-PEO$_{1.3}$	9.6 nm	micrometer	limited permeability	osmotic pressure	photodegradable dye	cargo release control	186

lipid membranes self-assembled around the coacervate cores, generating hierarchically organized protocells featuring: (i) an outer lipid bilayer derived from lysed cells, (ii) a multiphase, molecularly crowded coacervate interior, and (iii) an inner nucleus-like domain comprising phase-separated DNA/histone/carboxymethyl dextran complexes. These constructs exhibited sustained metabolic activity-ATP produced by residual viable *E. coli* powered actin polymerization into filamentous networks. Remarkably, during a 48 h incubation period, the protocells doubled in volume and underwent morphological transitions from spherical to amoeboid shapes, driven by endogenous metabolic processes. Reproducing the principle of division from pre-existing compartments remains a major challenge in synthetic cell research. Caire da Silva et al. addressed this by employing pH-responsive small molecules (FFM) to induce inward budding and division of polymer vesicles (polymersomes), forming daughter vesicles through controlled membrane remodeling. Complementarily, Landfester and colleagues developed a thermoresponsive approach to manipulate the division of giant unilamellar polymer vesicles (GUVs), systems typically limited by the low fluidity of high-molecular-weight polymers.[214] They incorporated a temperature-responsive block copolymer, poly(*N*,*N*-dimethylacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PDMA-*b*-PNIPAM), into poly(butadiene)-*b*-poly(ethylene oxide) (PBD-*b*-PEO) vesicles using a double-emulsion method. Cyclic temperature changes drove the reversible insertion of the responsive copolymer into the membrane, generating local heterogeneities and interfacial tension gradients. Relaxation of these stresses via budding and fission led to vesicle division. This strategy provided programmable control over division symmetry, frequency, and directionality. Collectively, these studies mark important progress toward constructing lifelike protocells capable of coordinated growth and division. Future efforts should focus on coupling divisional control with other essential cellular functions, such as metabolism, communication, and adaptive evolution, to establish self-sustaining synthetic cells that approach the complexity and autonomy of living systems.

4. CONCLUSION AND PERSPECTIVE

Over the past decades, remarkable advances have been made in the construction of artificial cells that emulate the structural and functional complexity of living systems. This Review has summarized the diverse methodologies for building such synthetic cellular architectures, including polymer vesicles, proteinosomes, and membrane-bound coacervates with membranized features, and highlighted recent progress in integrating life-like behaviors and functionalities within confined synthetic compartments (Table 1). Through rational molecular design, hierarchical self-assembly, and controlled compartmentalization, increasingly sophisticated systems can be achieved, which are capable of orchestrating biochemical reactions, information processing, and morphological transformations. While each platform offers unique advantages, their limitations, including membrane control, biochemical compatibility, and long-term stability, must be considered when designing functional artificial cell systems. These developments collectively mark a paradigm shift in bottom-up synthetic biology, from the simple structural imitation of cellular forms to the functional emulation of living behavior.

Polymeric artificial cells, as robust, tunable, and scalable analogs of biological compartments, have provided a versatile platform for exploring the principles governing life's organization and complex processing. Their chemical versatility enables precise control over critical parameters such as membrane permeability, elasticity, and responsiveness, thereby facilely allowing the incorporation of signaling modules and higher-ordered structures such as synthetic organelles and cytoskeletal mimetics. By integrating these features, polymeric protocells can reproduce key cellular behaviors—including selective molecular transport, directional communication, enzymatic reaction cascades, adaptive motility, and even regulated growth and division. These advances not only deepen our understanding of fundamental biological processes such as compartmentalization, homeostasis, and morphogenesis but also open routes toward creating fully programmable synthetic systems capable of performing designed biochemical tasks.

Despite exciting advances in emulating living systems with polymeric synthetic compartments, several key challenges remain. For instance, energy coupling and metabolic integration within synthetic compartments are still rudimentary, which usually rely on exogenously supplied fuels or stimuli, whereas the design of sustaining energy cycles is still limited. Information storage and processing within artificial cells are often realized by relatively simplified approaches, and integrating complex networks such as programmable nucleic acid circuits, peptide-based information processing, or dynamic feedback networks will be essential for developing systems that can sense and respond adaptively in a more life-like manner. Importantly, achieving coordinated population-level behavior, including communication, cooperation, and differentiation among multiple synthetic cells, represents the next Frontier toward multicellular synthetic systems. To overcome these challenges, future research will likely emphasize several directions: (1) self-sufficiency and sustainability metabolic systems to establish minimal yet functional networks that can continuously generate energy and functional modules, enabling persistent behaviors including growth and division; (2) multicomponent organization in a hierarchical and dynamic manner, such as the incorporation of dynamic subcompartments and cytoskeletal analogues to realize spatial and temporal control over biochemical processes, resembling adaptive eukaryotic intracellular organization; (3) programmable communication and intelligence of artificial cells (such as exchanging chemical or optical signals) in a collective manner, enabling the emergence of population-level functionality; (4) interfacing with biological systems to develop hybrid constructs that can communicate or integrate with living cells and tissues, opening pathways for biomedical applications such as targeted drug delivery and immune modulation. Through the continuous integration of molecular precision, dynamic functionality, and evolutionary potential, engineering polymer-based artificial cells hold the potential of gaining deeper insights into the fundamental principles and organization pathways of living systems, revolutionizing our approach to biomimicry engineering and developing innovative solutions and technologies that leverage cell-like properties for practical purposes.

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The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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