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Recent advances in membranized coacervates as functional compartments: synthetic strategies and mechanisms for prototissues

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Compartmentalization, a defining feature of living cells, has inspired the engineering of functional microcompartments that organize reactions and transport in cell-mimetic ways. Among current strategies, bottom-up approaches have attracted particular attention because they assemble synthetic compartments from nonliving building blocks such as polymers, peptides, and lipids. Herein, we review recent advances in complex coacervates as versatile platforms for protocells, with an emphasis on multiphase organization, key physicochemical properties, and structure–function relationships that govern assembly, stability, and regulated exchange. We further highlight major formation and functionalization routes, boundary reinforcement (membranes or membrane-like shells), stimulus-triggered droplet-to-vesicle transitions, and hierarchical assembly into prototissues, which enable controlled encapsulation, intercompartmental communication, and collective functions, such as distributed catalysis and homeostatic regulation. Finally, we discuss biomedical opportunities of coacervate-based compartments and outline future directions toward robust, programmable, and life-like artificial systems that sharpen design rules at the interface of synthetic constructs and living matter.

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1. Introduction

Living cells are the fundamental structural and functional units of life.[1] Since Schleiden and Schwann formally proposed the cell theory in 1839, cell and molecular biology have primarily addressed fundamental questions of cellular organization, dynamics, and regulation.[2] However, natural cells often become fragile when removed from their native environments and exposed to nonphysiological conditions. Moreover, their intrinsic complexity and heterogeneity can limit precise manipulation, standardization, and reproducibility. These challenges have driven the development of bottom-up synthetic biology and biomimetic materials that aim to construct minimalistic and programmable assemblies capable of reproducing selected cell-like functions. Consequently, artificial systems now enable the creation of customized, modular, cell-sized compartments with controlled composition, tunable molecular exchange, and improved experimental accessibility.

Building on these advances, the term artificial cells refers to engineered, cell-sized compartments that reproduce selected cellular features, such as compartmentalization, selective transport, information exchange, or energy transduction, in a controllable and testable manner, without attempting to replicate the full complexity of living cells.[3] Importantly, artificial cells do not necessarily require lipid membranes. Many biologically relevant cellular compartments are membraneless, such as biomolecular condensates, and prebiotic protocell models have also been widely explored without a classical lipid bilayer.[4] Conceptually, artificial-cell construction can be broadly divided into top-down and bottom-up strategies. The top-down approach derives simplified cellular systems from living cells or microorganisms through genome reduction, rewriting, or replacement, exemplified by genome-minimization and synthetic-genome installation efforts such as minimal *Mycoplasma*-derived chassis.[5] In contrast, the bottom-up approach constructs artificial cells *de novo* by assembling biomolecules or inorganic materials through chemical and physical processes to form functional microvesicles or microcompartments.[6]

Based on their constituent materials, artificial cells can be categorized into several major classes, including lipid or polymer vesicles,[7–12] hydrogels,[13,14] and condensate droplets,[15–18] among others, each exhibiting distinct physicochemical properties.[19] In this work, we use condensates as a general term for assemblies formed through liquid–liquid phase

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separation (LLPS), complex coacervates as a major subclass produced *via* associative electrostatic complexation, and condensed-phase droplets as a descriptive term for droplet-like phase-separated compartments. Among these systems, complex coacervates have attracted particular interest because they can be engineered with membranes and further assembled into higher-order prototissue-like architectures. Although current artificial cells remain far simpler than natural cells, the objective is not to reproduce their full complexity but to reconstruct selected cellular structures and functions in a controllable, programmable, and experimentally accessible manner, while progressively advancing functional integration and spatiotemporal regulation.

Building on these design principles, artificial-cell models provide powerful platforms for hypothesis-driven studies of cellular functions and prebiotic processes, while also offering engineering blueprints for therapeutic microdevices. In fundamental research, artificial cells can be manipulated to recapitulate selected systemic activities of natural biological cells,[20] enabling researchers to explore plausible pathways for the emergence of life[21,22] and to test whether chemical engineering strategies can reproduce specific cellular functions.[23] Among these functions, the ability to sustain energy transduction is a defining hallmark of living systems and remains a key objective in artificial-cell research.[24] Adenosine triphosphate (ATP) serves as the universal energy currency in nearly all living organisms, driving intracellular biochemical reactions. Consequently, ATP-centered energy transduction is a central design element in synthetic cellular systems. For example, an intracellular light-driven ATP-generation system has been demonstrated by reconstituting thylakoid fragments with phosphatidylcholine and cholesterol to form proteoliposome-like structures capable of photophosphorylation.[25]

Beyond single-cell mimics, artificial-cell engineering has also advanced the bottom-up construction of three-dimensional (3D) biomimetic prototissue systems. Prototissues consist of interconnected networks of artificial cells that emulate aspects of biological tissues and can exhibit bio-inspired behaviors, such as collective responses and chemical communication.[26,27] Although prototissue research remains at a relatively early stage, interest in interconnected artificial-cell assemblies has grown rapidly in recent years, driven by their potential for both fundamental biological studies and biomedical applications.[28,29]

In parallel with these fundamental advances, artificial cells are increasingly being explored for healthcare applications, where engineered systems can perform partial, task-specific cellular functions relevant to mammalian therapeutics.[30] For instance, platelet-mimicking artificial cells with key platelet-like attributes, including flexibility, hollow interiors, and disc-like morphology, have been developed.[31] Under *in vitro* physiological flow conditions, these platelet-mimicking systems exhibit enhanced surface binding and site-selective adhesion compared with spherical or rigid disc-shaped controls, thereby promoting platelet aggregation and partially recapitulating hemostatic function. Collectively, these studies illustrate how programmable compartment design can translate structural features into physiologically relevant functions.

In this Review, we first define key compartmental hallmarks and clarify the conceptual hierarchy linking condensed phases and coacervates, engineered protocells, and higher order prototissues. We structure the discussion across three levels: (i) condensed phases (including complex coacervates) as material compartments, (ii) protocells as engineered units incorporating additional regulatory or functional elements, and (iii) prototissues as higher-order assemblies that enable communication and distributed functions. We then focus on coacervate-based compartments, highlighting multiphase architectures and boundary engineering strategies, such as membranization or membrane-like shells, that regulate transport and stability. Next, we discuss hierarchical assembly into functional prototissues and distributed microreactor networks capable of chemical communication and cooperative cascade reactions. Finally, we outline key challenges and future opportunities that may guide the rational design of increasingly robust and programmable artificial cellular systems.

2. Characteristics of functional compartments

The concept of tissue engineering was first articulated by Robert Langer and Joseph P. Vacanti in 1993, emphasizing the integration of biology and engineering to fabricate three-dimensional functional tissue substitutes.[32] Conventional tissue-engineering approaches have since enabled the creation of clinically relevant tissue constructs by combining living cells with predesigned scaffolds and bioreactor-assisted maturation. Typically, cells obtained from patients or donors are cultured and expanded within engineered scaffolds, followed by tissue maturation in bioreactors that mimic key *in vivo* cues. Despite these advances, this paradigm faces significant challenges in regenerating complex tissues composed of multiple cell types and intricate microarchitectures. A central limitation lies in achieving precise spatiotemporal control over cell differentiation, proliferation, and intercellular interactions. Furthermore, even with emerging strategies, including bioreactor optimization, *in situ* tissue engineering, and particle-assisted tissue engineering, accurate regulation of cellular behaviors, such as communication, migration, and scaffold-responsive phenotypes, remains difficult to achieve.[33,34]

In native tissues, cells are not merely embedded in a matrix but are actively interconnected and able to communicate through signals. This observation motivates two complementary directions: constructing prototissues using artificial cells and integrating life-like artificial cells with conventional tissue engineering to develop hybrid or fully synthetic tissues. Because artificial cells can interact with natural cells, they can provide programmable, high-resolution guidance that is difficult to achieve with conventional scaffold materials alone.[35] Moreover, compared with single artificial cells, prototissues can exhibit higher-order behaviors such as networked signaling, distributed catalysis, and collective regulation, which are central to tissue-level emulation.[36] However, due to current

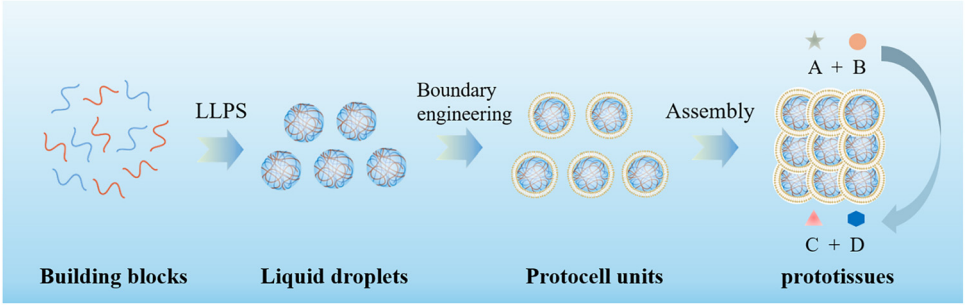

Fig. 1 Complex coacervate droplets form protocell units (including multiphase/core–shell compartments) that can be reinforced by membranization or shell engineering, then assembled into prototissues supporting intercompartmental communication, distributed catalysis, and homeostatic regulation.

limitations in artificial-cell design and robustness, prototissues mediated by artificial cells often lack structural integrity, thus requiring additional supporting frameworks in tissue models. Nevertheless, with continued progress in artificial-cell research and the improvement of functional performance, artificial-cell-enabled strategies are increasingly positioned to support higher-level control over tissue maturation.[37] Overall, prototissue engineering is an inherently multidisciplinary field and holds immense potential for biomedical applications.

In this section, we discuss the complex coacervates, their membrane formation and their use as a multiphase organization for higher order assembly of prototissues and their functional characteristics (Section 2.1 and Fig. 1). This framework highlights why coacervate systems that form higher order assembly of prototissues are increasingly being explored as an alternative strategy for achieving tissue-level functionality and biomimicry.

2.1. Colony formation

A variety of methods have been developed to construct biomimetic prototissues, including artificial-cell self-assembly,[38] microfluidics,[39] and 3D printing,[40,41] among others. From the perspective of architectural control, these approaches generally yield two types of assemblies: amorphous prototissues and structured prototissues.[42]

Amorphous prototissues typically arise from non-programmed mixing of artificial cells, such as complex coacervate droplets, decorated with specific surface motifs. Here, assembly is largely driven by intermolecular interactions, producing clusters or "colonies" without precise spatial or temporal control. For example, lectin–glycan interactions have been exploited to mimic cell–cell adhesion, where multivalent lectins bridge glycan-functionalized droplets to induce aggregation or deformation.[43] While these approaches can capture the basic principles of colony formation, they rarely reproduce the highly organized, heterotypic arrangements characteristic of native tissues, and therefore often display limited biomimetic fidelity.

By contrast, structured prototissues focus on programmable geometry and connectivity, offering a path toward more tissue-like architectures. Centimeter-scale synthetic tissues with thousands of interconnected compartments have been realized by assembling independent 3D-printed building blocks, allowing predefined patterns and functional integration.[40] Droplet-network strategies similarly provide programmable interfaces between compartments for controlled molecular exchange.[44,45] In addition, phospholipid-based assemblies with tubular, conical, spherical, or wedge-shaped stacked architectures have been used as modular building blocks, creating a continuum from individual phospholipid molecules and artificial organelles to functionalized coacervate cells and fully programmed prototissues.[46] Together, these approaches highlight how the principles of colony formation in complex coacervates can be extended and guided to produce higher-order, tissue-mimetic networks. By controlling droplet interactions, connectivity, and spatial organization, researchers can move beyond simple clusters to engineered prototissues capable of coordinated, emergent behaviors.

2.2. Budding and budding-like morphogenesis

Budding-like morphogenesis is a fundamental strategy in biology for generating new structures or releasing cargo through localized protrusions.[46–48] In synthetic systems, similar behaviors can be harnessed to drive the formation of higher-order assemblies, including prototissues. In artificial compartments, budding can be engineered by coupling membrane mechanics, phase separation, and reaction-driven interfacial remodeling.[49]

For example, giant vesicles containing a PEG/dextran aqueous two-phase system (ATPS) along with multicomponent lipid mixtures that support coexisting membrane domains can be induced to form budding-like protrusions under osmotic modulation.[50] Beyond vesicular systems, non-vesicular compartments such as coacervate droplets can also exhibit budding-like behaviors when reactions remodel their interfaces and promote membrane-like boundary formation. In one case, a reactive coacervate system formed *via* electrostatic complexation undergoes a chemically triggered, self-destructive transition upon exposure to formaldehyde. This drives multilevel self-assembly, producing budding-like protrusions and membrane-like structures that resemble early steps of compartmental organization.[51]

These examples illustrate that budding can arise from distinct driving forces, physical mechanisms such as osmotic and

domain coupling, or chemical mechanisms such as reaction-enabled interfacial remodeling. Importantly, in complex coacervate systems, such budding-like events can serve as nucleation points for higher-order organization, enabling droplets to connect, aggregate, and assemble into prototissue-like networks. By exploiting these processes, researchers can guide coacervate colonies toward programmable, tissue-mimetic architectures, bridging single-droplet behavior with emergent multicompartment functionality.

2.3. Life-like properties

In multicellular organisms, tissue-level functions emerge not from isolated cells but from organized networks of compartments that exchange matter and information, collectively giving rise to higher-order behaviors such as signal transduction, force generation, and stimulus responsiveness.[52] Similarly, biomimetic prototissues assembled from complex coacervate droplets provide a versatile platform to study how compartment architecture and intercompartmental coupling can generate coordinated, tissue-like functions.

At the level of coacervate-based prototissues, several life-like features can be recapitulated. These include modular compartmentalization, allowing discrete functional units to coexist within a network; intercompartmental communication, enabling chemical or physical signals to propagate across droplets; collective homeostasis through encapsulation or sequestration of biomolecules; and droplet or vesicle fusion, which facilitates material exchange, remodeling, and dynamic reorganization. Together, these properties illustrate how simple coacervate compartments can be engineered to mimic the emergent behaviors of living tissues, bridging single-droplet functionality with network-level complexity.

2.3.1. Modular compartmentalization. Compartmentalization is a central organizational principle of life, allowing biological systems to spatially segregate molecules and reactions, maintain local microenvironments, and coordinate complex biochemical processes.[53] In protocells and prototissues, this principle is translated into modular compartments that serve as discrete, addressable ''building blocks.'' These compartments can be individually designed, assembled into higher-order structures, functionally modified, or replaced, providing unparalleled control over spatial organization and enabling systematic exploration of emergent behaviors.

For instance, researchers have developed robust, self-supporting millimeter-scale prototissue fibers composed of large populations of vesicles, with controllable lengths and diameters.[54] These handleable modular fibers demonstrate how discrete compartments can be organized into coherent networks, bridging the gap between single-droplet functionality and tissue-level architecture. By assembling protocell compartments in defined arrangements, it becomes possible to study how spatial patterning, connectivity, and compartmental hierarchy influence collective behaviors such as coordinated signal transmission, distributed catalysis, and dynamic morphological remodeling. Furthermore, compartmentalization provides a practical platform for engineering modular synthetic systems. By controlling the composition, size, and connectivity of individual protocell units, researchers can fine-tune the reaction kinetics, control local chemical environments, and implement selective material exchange, all while preserving the modularity needed for scalable, reconfigured prototissues. In this way, compartmentalization not only reproduces a fundamental aspect of cellular life, but also enables programmable, experimentally accessible pathways toward building complex, life-like networks from simple synthetic units.

2.3.2. Intercompartmental communication and signal propagation. A defining feature of prototissues is their ability to transmit signals between individual artificial cells, enabling the assembly to emulate dynamic regulation observed in living tissues. In these systems, discrete reaction steps can be confined within separate compartments, such as coacervate droplets or vesicles, while intermediate chemical species are transferred between compartments to implement network-level communication.

To date, three primary mechanisms for intercompartmental signal transmission have been demonstrated. Coacervate architectures introduce a first paradigm: interface-engineered transport across inter-droplet junctions, such as in droplet interface bilayers (DIBs), which enforce connectivity and enable controlled exchange at the network level.[44,45] The effective signaling range in prototissues depends on both the network topology and the transport kinetics: pore-mediated exchange is generally suited for local, short-range coupling, while small-molecule diffusion or interface-mediated transport can support longer-range propagation, particularly when relay pathways are carefully designed.

The second involves molecular exchange through α-hemolysin (αHL) protein pores, which allow controlled transfer of reaction intermediates between neighboring compartments.[55] For example, a dual-droplet module incorporating a Zn^{2+}-sensitive αHL-4H pore enables precise regulation of reactions within multicompartment lipid-bilayer structures, while also allowing on-demand release of contents into the surrounding environment.[56]

The third mechanism relies on direct diffusion of small, membrane-permeable molecules, which has been the most extensively studied approach. A representative case uses hydrogen peroxide (H_2O_2) as a diffusible signaling intermediate: thermo-responsive proteinosome-based prototissues gate substrate diffusion to modulate a glucose oxidase (GOx)/horseradish peroxidase (HRP) cascade. In the swollen state (25 °C), glucose and Amplex Red diffuse freely, enabling efficient conversion to fluorescent resorufin, whereas in the contracted state (47 °C), restricted diffusion significantly suppresses the cascade rate, illustrating how morphological changes can dynamically control chemical communication.[57] Collectively, these strategies demonstrate how intercompartmental communication can be programmed and tuned, allowing synthetic prototissues to exhibit emergent behaviors reminiscent of tissue-level signaling, including cascade reactions, conditional responses, and distributed regulation across a multicompartment network.

2.3.3. Encapsulation and collective homeostatic regulation. Beyond intercompartmental communication, living tissues are characterized by protective mechanisms that maintain a stable internal environment, buffering against external perturbations such as pH fluctuations, a hallmark of cellular organization. Synthetic prototissues can recapitulate this feature by combining artificial cells with stimulus-responsive permeability to achieve collective homeostasis.

For example, spherical prototissues have been constructed from complementary populations of artificial cells that stabilize a local pH microenvironment and protect internal contents from external pH changes.[58] One population consisted of urease-loaded cells, which produce alkaline products and become permeable only under acidic conditions, while the other contained GOx-loaded cells, which generate acidic products and open only at high pH. When assembled into a single spherical prototissue, these complementary populations coordinate substrate transport within a defined pH window, implementing self-regulated buffering through a feedback loop between chemical production and compartment permeability. This approach highlights how modular encapsulation within protocell networks can transform simple droplet assemblies into higher-order, life-like systems capable of autonomous regulation and homeostatic control, bridging single-compartment behavior with emergent tissue-level functionality.

2.3.4. Droplet/vesicle fusion and coalescence. Fusion of droplets or vesicles, the coalescence of condensed-phase entities into larger structures, is a fundamental process in biology, underlying critical events such as vesicle–plasma membrane fusion during secretion and neurotransmission, membrane merging during fertilization, and information transfer through secreted microbial droplets. In synthetic systems, droplet and vesicle fusion can be harnessed to create higher-order assemblies with emergent functionality, bridging single-compartment behaviors to network-level organization. For example, giant vesicles encapsulating DNA-amplification reagents serve as a model for studying controlled fusion events. By lowering the pH, individual vesicles are induced to associate and fuse, forming larger coupled assemblies that enable coordinated biochemical reactions across multiple compartments.[51] This fusion not only increases the effective volume and connectivity of the system but also facilitates material exchange, signal propagation, and synchronized activity, mimicking the cooperative behaviors observed in living tissues.

In the context of coacervate-based prototissues, droplet fusion provides a complementary route for structural and functional integration. Budding, interfacial remodeling, and coalescence collectively allow discrete droplets to form interconnected networks, promoting emergent properties such as distributed catalysis, collective signaling, and dynamic remodeling of compartmental architecture. By controlling the timing, location, and extent of fusion, researchers can program coacervate assemblies to achieve tissue-like connectivity and responsiveness, making fusion a key strategy for building complex, life-mimetic prototissues.

3. Biomimetic cells based on condensed phase droplets

LLPS has emerged as a central mechanism for organizing the intracellular environment, enabling cells to create dynamic, membraneless compartments that concentrate and regulate biomolecules.[59–61] By selectively partitioning proteins, nucleic acids, and other biomolecules into liquid-like condensates, cells form discrete microenvironments that can arise from relatively simple building blocks, including ATP, oligopeptides, and polynucleotides.[62]

These biomolecular condensates serve multiple life-like functions. Within the dense phase, biomolecules are concentrated, creating locally enriched reaction hubs that can generate chemical gradients and support controlled molecular exchange with the surrounding medium.[63–65] Through such dynamic partitioning, cells achieve spatiotemporal regulation of biochemical reactions, allowing precise coordination of signaling, catalysis, and metabolic pathways.[66–68]

Importantly, multiphase condensates expand the functional repertoire of these condensates. Droplet-within-droplet architectures enable hierarchical compartmentalization, where partitioning rules dictate the localization and kinetics of reactions, effectively linking compartment structure to functional outputs.[69,70] These systems not only recapitulate cytoplasm-like physicochemical properties, such as viscosity, ionic strength, and dynamic fluidity, but also provide programmable microenvironments that can emulate key aspects of cellular organization. Overall, LLPS-derived condensed-phase materials offer a versatile platform for constructing biomimetic cells, where droplet composition, phase behavior, and dynamic exchange can be systematically tuned to replicate the structure–function relationships inherent in living cells. By harnessing these properties, researchers can create synthetic compartments that bridge the gap between simple chemical systems and life-like cellular behaviors, forming the foundation for engineered protocells and higher-order prototissues.

Multiphase core–shell coacervate droplets can emerge spontaneously when oppositely charged components form coacervates with distinct critical salt concentrations (where the core coacervate is enclosed by an outer layer, Fig. 2a), yielding coexisting phases with unequal densities and partial immiscibility.[71] Introducing a third component further increases architectural complexity (Fig. 2b), an effect attributed to unequal phase densities and inter-coacervate immiscibility arising from differences in critical salt concentrations. Similarly, core–shell peptide coacervates assembled from oppositely charged decapeptides produce concentration gradients across phases, enabling phase-selective RNA partitioning.[72] Specifically, differences in peptide concentration endow this structure with unique functions: single-stranded RNA (ssRNA) preferentially accumulates in the inner phase, while double-stranded RNA (dsRNA) preferentially accumulates in the outer phase, a phase-specific RNA distribution resembling certain activities in natural cells (Fig. 2c).

Despite their ability to mimic cytoplasm-like environments, coacervate-based artificial cells often suffer from limited

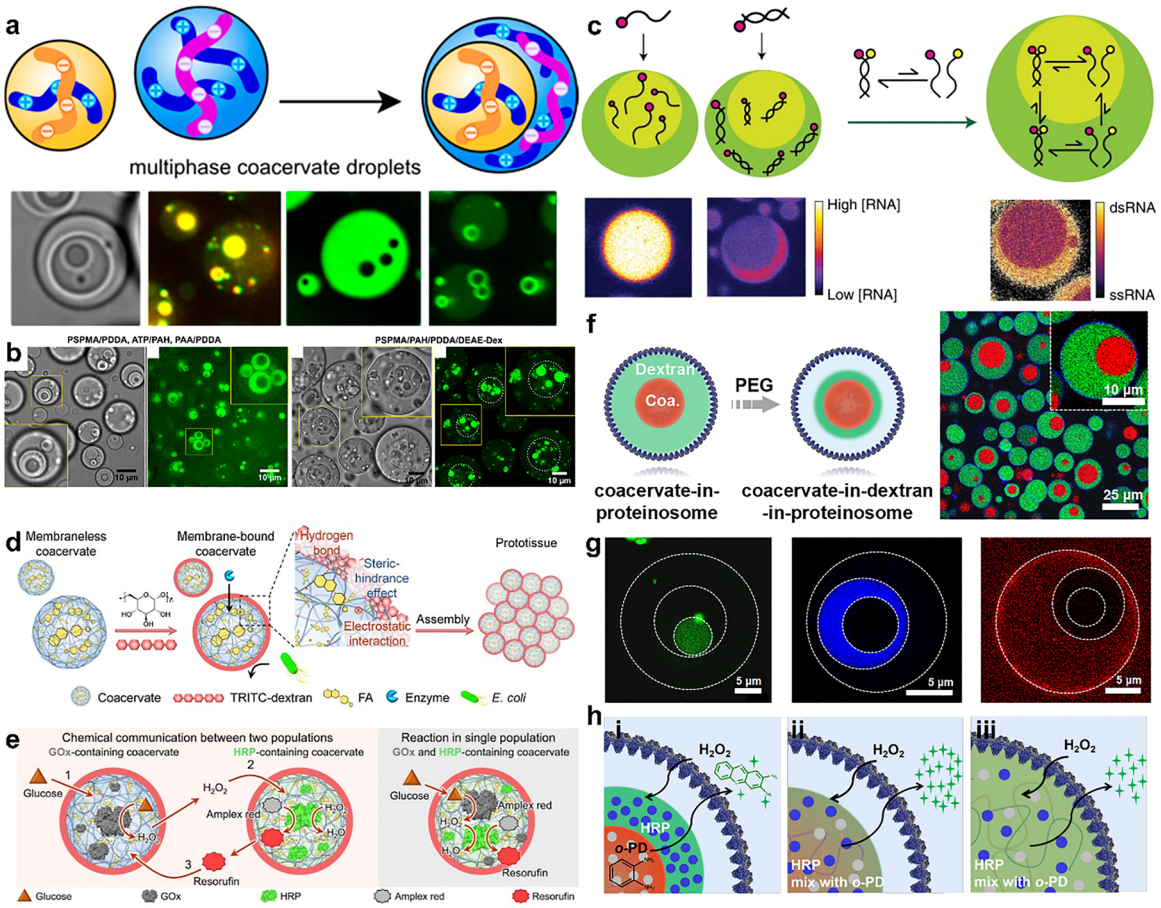

Fig. 2 Multiphase coacervates and boundary engineering for protocell functions. (a) and (b) Multiphase, core–shell coacervate droplets formed by mixing coacervate phases with distinct critical salt concentrations; representative confocal images show three coexisting coacervate phases. Reproduced with permission from ref. 71 Copyright 2020, American Chemical Society. (c) Phase-selective RNA partitioning in multiphase coacervates assembled from arginine (R_{10}), lysine (K_{10}), and aspartic acid (D_{10}). Reproduced with permission from ref. 72 Copyright 2022, Springer Nature. (d) and (e) Dextran-coated coacervate droplets forming a stabilizing membrane-like shell that supports population-to-population signaling and a compartmentalized cascade reaction. Reproduced with permission from ref. 78 Copyright 2023, American Chemical Society. (f) Proteinosome-based nested three-phase architecture (coacervate–dextran phase–proteinosome) with representative confocal images. (g) Spatial localization of DNA, horseradish peroxidase (HRP), and PEGylated GOx (PEGyGOx) in the three phases. (h) Schematic illustrating how spatial phase partitioning regulates enzymatic reaction rates. Reproduced with permission from ref. 79 Copyright 2022, Elsevier.

molecular selectivity and poor structural robustness, largely because membraneless droplets lack an enclosing boundary that can gate transport and stabilize compartment identity. In addition, without a physical interface constraint, coacervate phases can be prone to fusion, coalescence, or dissociation.[73] To address these challenges, inspired by natural cell membranes and cell walls, researchers have introduced diverse membrane-forming elements, including amphiphilic molecules,[74] copolymers,[75] proteins,[76] PEGylated macromolecules[77] or nanoparticles,[18] and charged assemblies. A protective polysaccharide coating strategy deposits a dextran-based shell onto arginine (Arg)/folic acid (FA) coacervate droplets (Fig. 2d).[78] This outer membrane exhibits strong stability: individual coacervates retain their size for 20 min, and multiple coacervates coexist for hours without fusion. Such stabilization enabled the design of a dual-population signaling system relying on H_2O_2 diffusion (Fig. 2e). Mechanistically, the dextran-defined boundary stabilizes the internal coacervate phase and expands the design space for programmable structures and functions. Building on this concept, a multiphase LLPS system was engineered within proteinosomes by introducing polyethylene glycol (PEG) to generate coacervate/dextran/PEG phases (Fig. 2f).[79] These nested compartments protect encapsulated payloads and enforce spatial localization of biomacromolecules (Fig. 2g): DNA resides in the coacervate phase, HRP in the dextran phase, and PEGyGOx in the PEG phase. This design enables controllable enzymatic reactions (Fig. 2h). The work provides a proof-of-concept for constructing multicompartmental artificial eukaryotic cells with spatial organization (mimicking nuclear-cytoplasmic segregation) and programmable functions. Beyond polymeric and polysaccharide shells, recent work has advanced membranized coacervates by coupling coacervate droplets with lipid-bilayer wrapping or related membrane reinforcement, improving resilience and providing an additional handle for transport gating.[80,81]

Beyond using membrane-forming components to construct an outer membrane, another feasible strategy for building advanced artificial cells involves triggering the reorganization of membraneless coacervates into membrane-bound vesicles, *via* the introduction of external components (surfactants, biomolecules, or nanoparticles) or external/internal stimuli (salts,

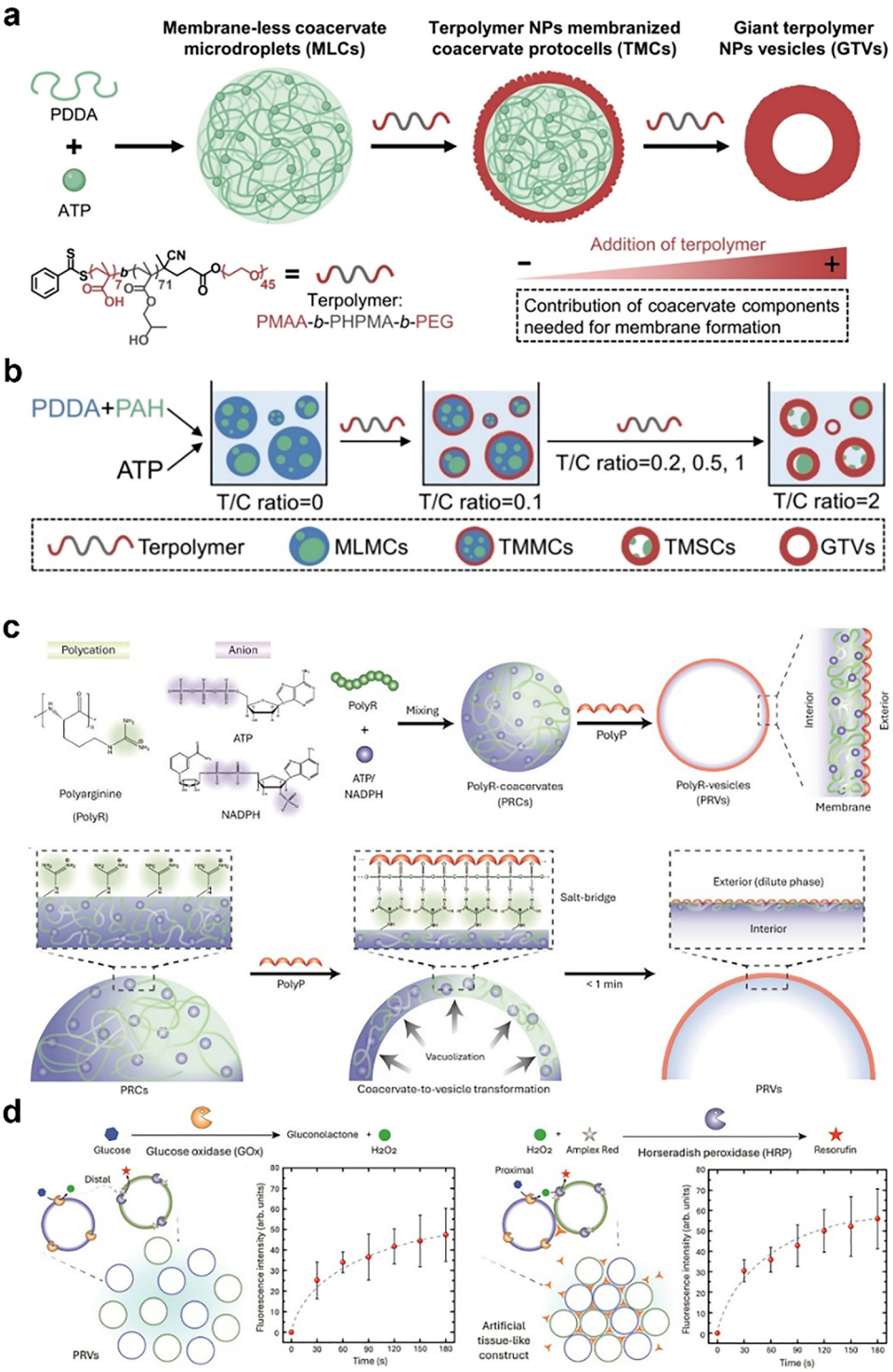

Fig. 3 Stimulus-triggered droplet-to-vesicle transformations and hierarchical assembly. (a) and (b) Continuous, stimulus-inducible reconfiguration of multilayer complexes (MLCs) into multicompartment structures containing porous membrane networks (*e.g.*, TMCs and GTVs; see the main text for definitions), mediated by multiphase condensation layers. Reproduced with permission from ref. 84 Copyright 2024, Wiley. (c) PolyR condensate design and transition from droplets to membrane-defined vesicles upon polyphosphate (PolyP) addition. (d) Comparison of cascade enzymatic reactions in dispersed vesicles (left) *versus* tissue-like clusters (right). Reproduced with permission from ref. 85 Copyright 2025, American Chemical Society.

temperature, pH, light, biological reactions).[82,83] Continuous addition of an anionic triblock copolymer induces structural reconfiguration in PDDA/ATP membraneless coacervate droplets (MLCs), yielding stable multicompartmental protocells with coacervate phases and porous membrane networks (Fig. 3a).[84] Coupling incompatible PDDA/ATP and PAH/ATP coacervate phases further generates membrane-enclosed coacervates/vesicles (TMMCs, TMSCs, GTVs) with distinct diffusion compartments (Fig. 3b). Polyphosphate addition triggers arginine-rich peptide coacervates to transform from droplets into membrane-defined vesicles (Fig. 3c).[85] This transition is driven by salt bridges between arginine guanidinium groups and phosphate moieties, rather than classical electrostatic complexation or osmotic effects. With the stabilized membrane

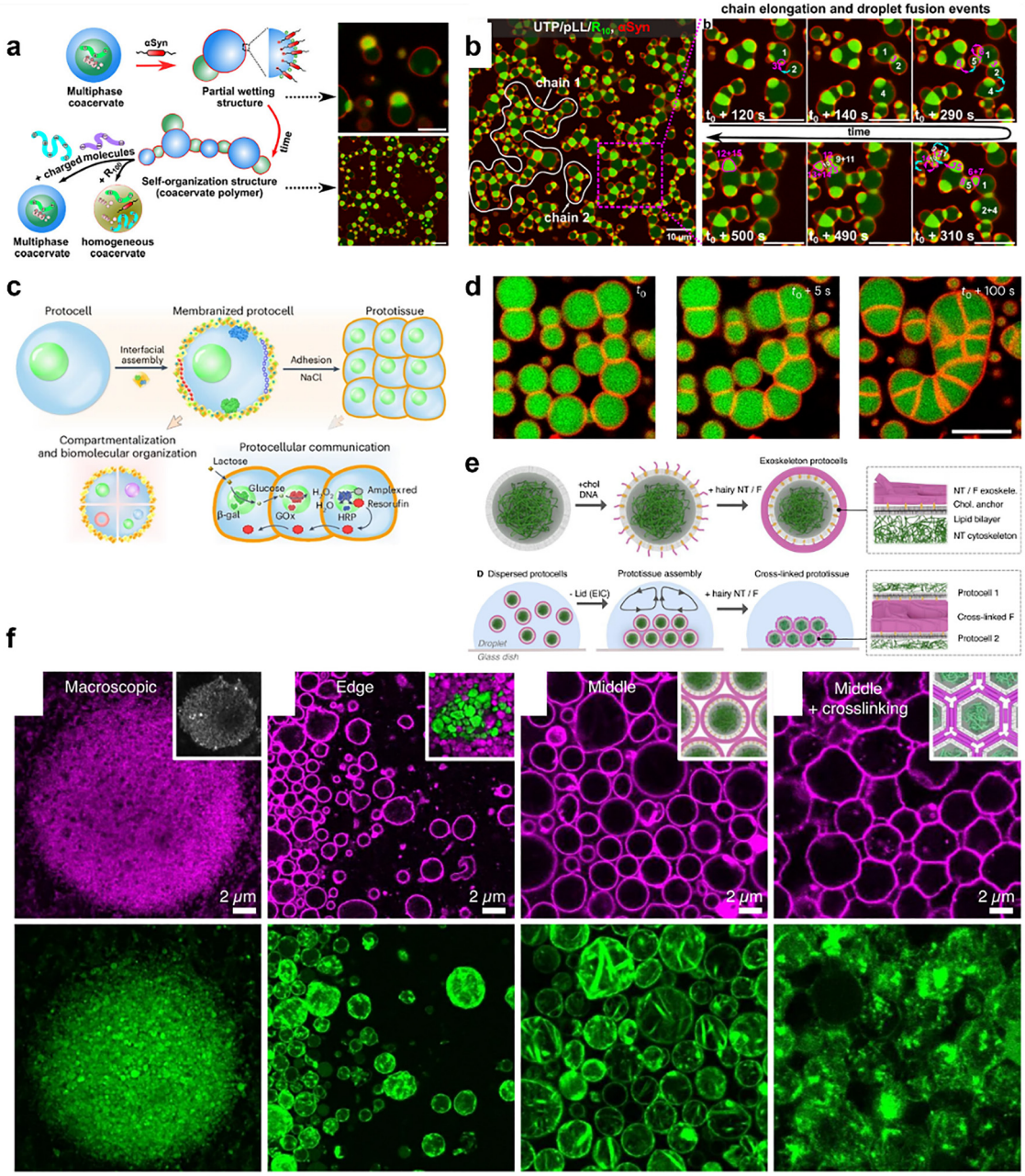

Fig. 4 Hierarchical assembly of coacervate-based protocells into prototissues and ECM-like stabilization. (a) and (b) Interfacial-protein-regulated self-organization of multiphase coacervate droplets, showing α-synuclein (αSyn)-mediated higher-order chaining/networking with representative confocal images. Reproduced with permission from ref. 86 Copyright 2025, American Chemical Society. (c) Design and formation of MOF-coated coacervate protocells, including internal sub-compartmentalization and inter-protocell communication. (d) Confocal images showing adhesion-driven organization of MOF-coated protocells into tissue-like assemblies. Reproduced with permission from ref. 87 Copyright 2025, Springer Nature. (e) DNA building blocks forming fiber frameworks for protocell/prototissue construction. (f) Representative schematic of DNA-fiber-crosslinked prototissues. Reproduced with permission from ref. 90 Copyright 2023, Springer Nature.

as a scaffold, a cationic clustering agent (DEAE-DEX) can be used to assemble negatively charged A-PRVs and neutral/negatively charged N-PRVs into heterogeneous, tissue-like clusters (Fig. 3d). Compared with isolated vesicles, these prototissues exhibited substantially faster cascaded enzymatic reactions, highlighting how structural stabilization and hierarchical assembly can enhance collective functionality.

4. Functionalized prototissue construction *via* biomimetic cells

Prototissues are ordered three-dimensional networks formed by interconnected artificial cells. They enable the simulation of life systems at a higher organizational level and exhibit biomimetic functions, including collective behavior and signal communication, that are of great significance in both scientific research and biomedical applications.[28,29] However, reconstructing prototissues is markedly more challenging than constructing individual artificial cells, and research in this area remains in its early stages. Nevertheless, driven by increasing application prospects, interest has surged in building interconnected artificial-cell assemblies that approximate tissue-like architectures and functions.

Interfacial proteins can drive hierarchical self-organization of multiphase coacervates into higher-order architectures; for example, αSyn addition can expel the core phase and promote chain-/network-like assemblies (Fig. 4a and b).[86] Such coacervate superstructures primarily encode connectivity, whereas biochemical programmability requires additional functional elements. MOF-coated coacervate protocells combine core–shell condensates with a metal–organic framework (MOF) nanoparticle boundary to introduce transport control and improve structural robustness.[87] In these protocells, a liquid PDDA/PAA condensate phase mimics the cytoplasm and encapsulates Prot/FA condensate microdroplets as organelle-like sub-compartments, while ZIF-8 nanoparticles assemble on the surface to form a membrane-like layer (Fig. 4c). Adding NaCl to the system screens electrostatic repulsion between the MOF membranes, promoting adhesion of protocells into stable tissue-like structures (Fig. 4d). Importantly, this modular prototissue format supports cascade catalysis simply by loading different enzymes into distinct protocell populations, thereby enabling distributed reaction networks at the tissue scale. Self-sorting coacervate/protocell networks that exhibit superstructural ordering have also been demonstrated, providing an alternative route to architecturally defined, coacervate-centered assemblies.[88,89]

Despite these advances, most coacervate-based prototissue assemblies still underrepresent a central element of living tissues: the extracellular matrix (ECM). The ECM is not only a structural scaffold but also a mechanochemical and biochemical microenvironment composed of fibrous networks and hydrated matrices that regulate cell adhesion, migration, and signaling. Therefore, incorporating ECM-mimetic architectures is essential for improving tissue-level integrity and controllability. DNA building blocks can self-assemble into fiber networks anchored on vesicle membranes, enabling alignment and crosslinking to generate cytoskeleton-/exoskeleton-mimetic architectures (Fig. 4e).[90] By adjusting the Mg^{2+} concentration, they formed DNA fibers, which were anchored onto the outer membrane of giant unilamellar vesicles (GUVs) to construct an exoskeleton-like structure. Magnetic microbeads guided fiber alignment, and complementary hybridization between fibers crosslinked the protocells. This ECM-inspired crosslinking remodels prototissue morphology (from spherical to honeycomb-like; Fig. 4f), increases inter-protocell contact length, and suppresses protocell mobility, collectively stabilizing the tissue-like architecture and enhancing structural programmability.

5. Microreactors

Prototissues can be viewed as modular microreactor networks, where spatially separated compartments cooperate to execute multistep reactions and transmit chemical signals. Here, "network-level" refers to the fact that different reaction functions are partitioned into separate modules, and system behavior emerges from module-to-module transport and communication. To demonstrate this concept and quantitatively evaluate module-to-module communication, a multilevel reaction system was implemented using prototissue fibers as programmable input/output units.[54] GOx-containing fibers served as input microreactors: in the presence of glucose, these fibers generated the diffusible signal H_2O_2. HRP-containing fibers served as output microreactors, sensing H_2O_2 from the input modules and catalyzing the oxidation of Amplex Red to resorufin, producing a red fluorescent readout. Overall, this modular cascade converts a chemical substrate cue into a diffusible intermediate and then into an optical signal, thereby demonstrating that compartmentalized prototissues can operate as distributed microreactor systems.

6. Conclusions and future perspectives

This work surveys bottom-up strategies for constructing artificial cells, with a particular emphasis on coacervate/condensed-phase droplets, and highlights how these building blocks can be assembled into functional prototissues. Artificial cells aim to reproduce selected cellular structures and functions, whereas prototissues integrate artificial cells into three-dimensional networks to recapitulate collective behaviors and intercellular communication at the tissue level. Across the studies discussed, three recurring functional themes emerge: compartmentalization, signal transduction (*e.g.*, small-molecule diffusion and transmembrane protein pores), and dynamic responsiveness (*e.g.*, temperature- or pH-triggered regulation). Together, these capabilities position droplet-based artificial cells and prototissues as tractable models for probing life-like organization and as engineering platforms with potential in drug delivery and tissue engineering.

Looking forward, progress will be shaped by four directions. First, increasing functional integration beyond single-function

compartments by incorporating genetic circuits, refined metabolic pathways, or energy-coupled reaction networks. Second, improving stability and controllability by engineering boundaries (membranes or ECM-like frameworks), tuning material exchange, and preventing undesired fusion or dissociation. Third, strengthening tissue-level biomimicry by introducing extracellular-matrix analogues, mechanical programmability, and vascular-like transport architectures to support long-range exchange. Fourth, accelerating translation toward biomedical and technological applications, including smart drug-producing microfactories, responsive therapeutics, and tissue-repair constructs.

Conflicts of interest

The authors declare no competing financial interest.

Data availability

No primary research results, software or code have been included, and no new data were generated or analyzed as part of this review.

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