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Shaping synthetic cells through cytoskeleton-condensate-membrane interactions

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Abstract

Constructing synthetic cells is fascinating both from the standpoints of understanding cellular machinery and developing bioinspired materials for targeted applications. The ability to impart dynamic shape and structure to such rationally designed assemblies, mimicking cellular morphogenesis, is still in its early stages. In this review, we discuss the interactions between three basic molecular assemblies that have shown promise in shaping synthetic cells: deformable membranes, phase-separated condensates, and the dynamic cytoskeleton. The interplay between these components facilitates compartmentalization, force generation, and dynamic shape changes. We particularly discuss the role of condensates as a versatile intermediary to link the cytoskeleton to the membrane. We propose that hybrid systems of these components present versatile platforms toward the eventual structuring and morphogenesis of artificial cells.

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Synthetic cells, Biomolecular condensates, Cytoskeleton, Membranes, Bottom-up biology, Cellular morphogenesis.

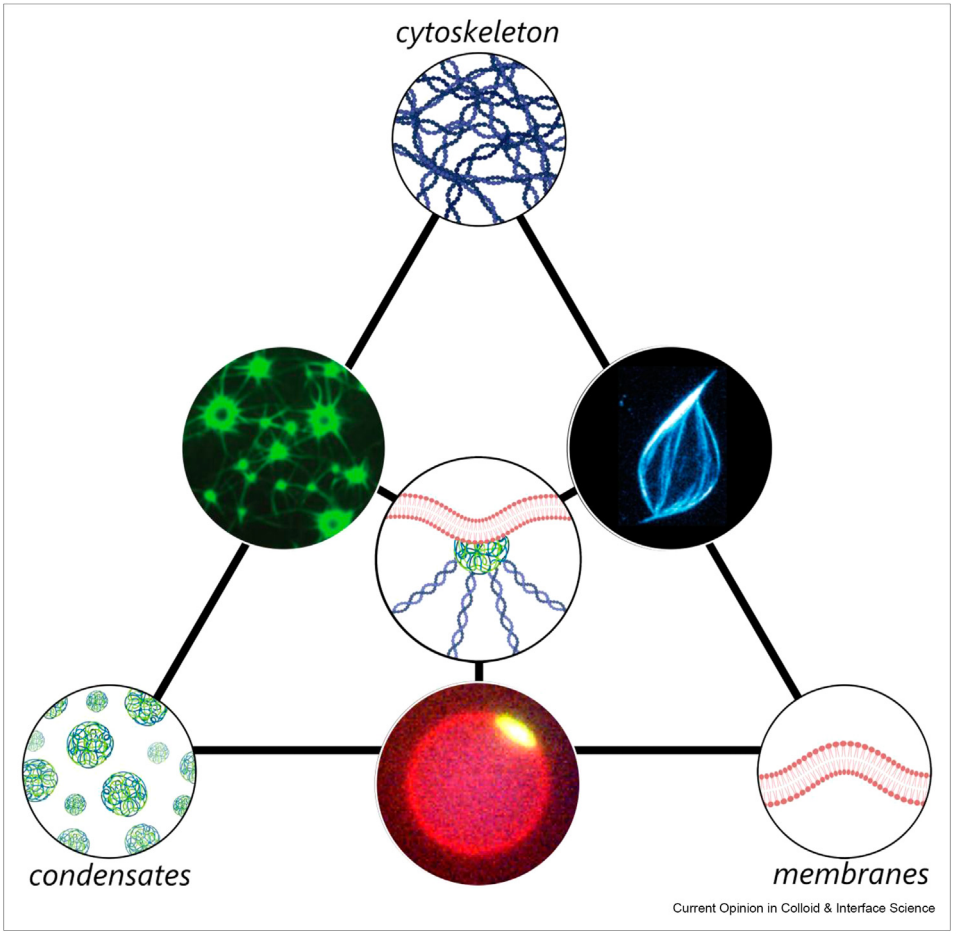
Introduction

Living cells are extremely complex, which makes unraveling their emergent behavior indeed a difficult task. The plethora of molecular interactions that take place within the micron-sized cellular confinements is truly astonishing. Aside from studying cells as whole units, in what other ways can we decode their dynamic behavior at the molecular level? An effective alternative is to use purified cellular components and understand their behavior by using simplified *in vitro* setups. Such a strategy often also provides better control over the experimental parameters and easier manipulation of the system compared to *in vivo* studies. *In vitro* design of molecular reactions, particularly within cell-like enclosed confinements, has given rise to the idea of building synthetic cells.

Using the wealth of information on individual biomolecules, synthetic biologists are keen on designing and building artificial mimics of natural cells using minimal components [1,2]. While *in vivo* genetic engineering and novel biomolecular pathways to reprogram organisms also fall under the broad umbrella of synthetic biology [3,4], this review specifically focuses on the bottom-up creation of cell-like entities from purified as well as synthetic biomolecules. The idea underlying the construction of synthetic cells is crucial in unpinning the fundamentals of biological systems with a lucrative application point-of-view, for example, in the field of molecular biosensing [5], metabolic engineering [6], and therapeutics, such as tumor suppression [7] and nanovesicles for drug delivery [4,8–10]. The scope for synthetic biology remains wide open: it encompasses both *a posteriori* entities made from and incorporating natural and synthetic components, as well as *a priori* entities without clear-cut biological analogs. From the many different modules – growth, metabolism, transcription-translation, division, etc. – currently being investigated [11–13], how to control and regulate synthetic cell morphology is one of the key challenges [14]. This review focuses on different strategies to impart a dynamic structure and shape upon synthetic cells. In particular, we highlight the recent use of three distinct biological components – membranes, biomolecular condensates, and the cytoskeleton – to achieve controlled morphological changes (Figure 1).

Membranes are vital components of cells. In fact, every living cell has a ~5 nm thin membranous boundary composed of a lipid bilayer with proteins and other elements embedded within. In addition, several

Figure 1



Utilizing cytoskeleton-condensate-membrane interactions to shape synthetic cells. A schematic expressing the interplay between active cytoskeleton, liquid condensates, and deformable membranes. Each side gives a representative example of an *in vitro* reconstitution of the pair in consideration, while the center presents a schematic of an *in vitro* reconstitution of the three elements together. Appropriate dynamics between these three elements could help in structuring and functionalizing synthetic cells. Images adapted from the following references – cytoskeleton-condensate: [41], used under license from John Wiley and Sons; cytoskeleton-membrane: [48], used under license from the American Association for the Advancement of Science; condensate-membrane: [59], used with permission from the American Chemical Society and under Creative Commons License. Image created using BioRender.com.

membrane-bound compartments — nuclei, mitochondria, chloroplasts, etc. — are crucial for intracellular biochemistry and organization. The amphiphilic nature of lipids, *i.e.*, a hydrophilic headgroup linked to hydrophobic tails, is responsible for their self-assembly, driven by hydrophobic interactions, into three-dimensional vesicular structures [15,16]. The self-assembly of lipids and other natural, as well as synthetic amphiphiles, has been extensively utilized in bottom-up synthetic biology to form several different kinds of vesicles such as liposomes, polymersomes, and proteinosomes [17]. Micron-sized liposomes, also known as Giant Unilamellar Vesicles (GUVs), are particularly useful and popular scaffolds used for designing synthetic cells. This is mainly due to their suitable size (>1 μm) for visual inspection, standardized preparation methods, biocompatibility, ability to integrate transmembrane/membrane-interacting proteins, semi-permeable nature enabling transmembrane transport, and the low bending rigidity ($\sim$10–20 k_bT [18], where k_b is the Boltzmann constant and T is the temperature) making them suitable for shaping and structuring.

Along with membrane-bound organelles, another layer of subcompartmentalization has become evident over the last decade: membraneless organelles [19,20]. Also known as biomolecular condensates (and simply referred to as condensates in this review), they are formed as a result of liquid–liquid phase separation (LLPS). LLPS is a consequence of the dissolved macromolecules having a higher affinity for each other than for solvent molecules. This results in free energy

minimization manifested by the segregation of the involved macromolecules into two phases in equilibrium with each other: a condensed phase with a high concentration and a dilute phase with a much lower concentration [21]. Typically, condensates exhibit liquid-like properties: they form spherical droplets owing to their interfacial tension, they deform and flow under shear forces, and they undergo continuous internal diffusive rearrangement [21]. Numerous condensates have been found inside cells (nucleolus, Cajal bodies, stress granules, etc.) and have been identified to play diverse roles, ranging from selective enrichment of molecules and enhanced reaction kinetics to providing protective environments, acting as organizational hubs, concentration buffering, and more [22,23]. The ability of condensates to physically manipulate their local environment primarily through interfacial tension forces ($\sim$0.1–100 pN μm^{-1}) and assist in mechanical work is only just beginning to be explored [24]. These unique properties of the phase-separated structures could play a particularly useful role in building synthetic cells.

The cytoskeleton, a highly dynamic proteinaceous scaffold, is responsible for cell mechanics, cellular motility, cell division, and intracellular transport. The cytoskeleton consists of three major proteins: actin microfilaments, microtubules, and intermediate filaments, with septins now being recognized as the fourth component [25]. The cytoskeletal elements, along with their numerous partner proteins, continuously (re) organize within the cytoplasm and provide a mechanical response against external forces. Actin and tubulin monomers polymerize at the expense of energy-rich nucleoside triphosphates to form polar filaments. Motor proteins (myosin in case of actin; kinesin and dynein in case of microtubules) migrate along these filaments and give rise to individual (e.g. cargo transport) or collective (e.g. contractile ring) motion and intracellular forces [26]. Septins are involved in a variety of membrane-remodeling processes, including cell division, cell motility, and cellular compartmentalization [25]. Encapsulation of purified cytoskeletal elements inside liposomes has shown substantial progress in recent years [27], and we will discuss them in the context of designing minimal, semi- or fully synthetic machineries capable of inducing dynamic morphologies.

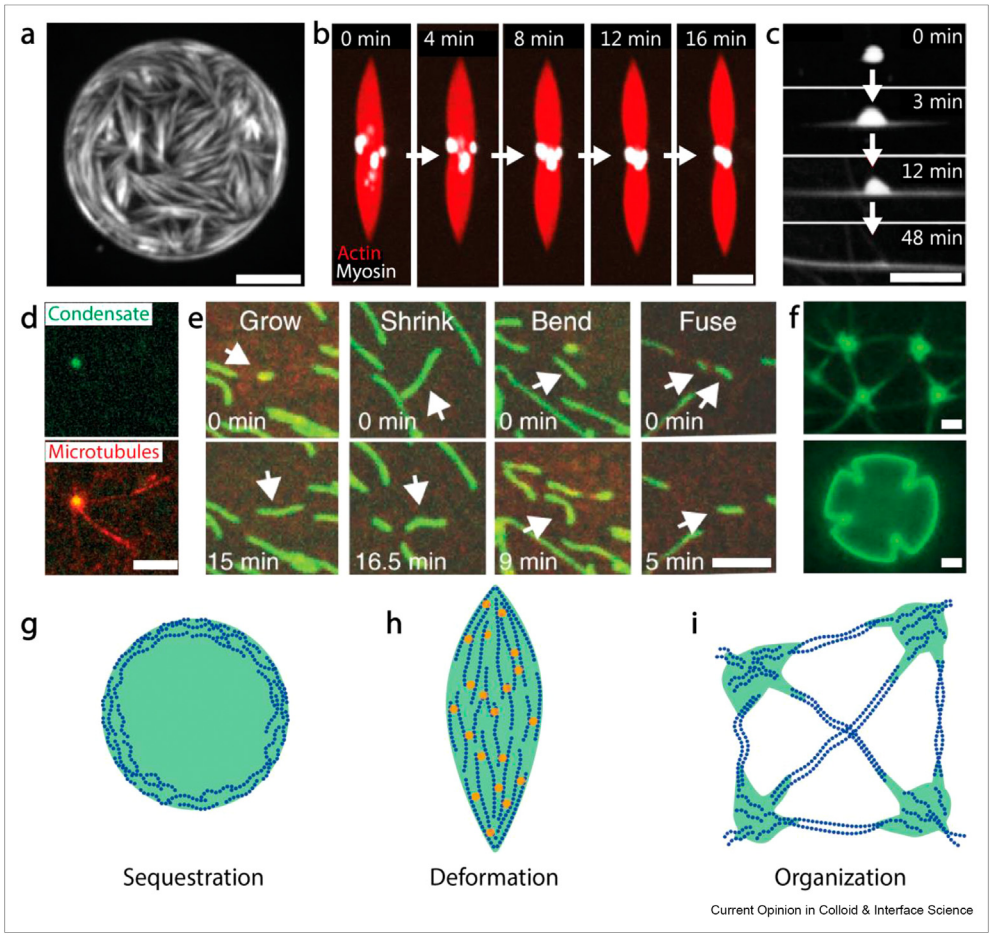
While each of these three elements — cytoskeleton, condensates, and membranes — is important on its own, the interplay between them is even more vital to the functioning of the cell. The interactions between the cytoskeleton and the cell membrane have already been extensively investigated, while the role of condensates in cytoskeletal and membrane dynamics is rapidly emerging. In this review, we discuss recent advancements that have led to a better understanding of the dynamic interactions between these three biological components. We begin with a closer look at the specific pairs, cytoskeleton-condensate, cytoskeleton-membrane, and condensate-membrane, followed by discussing the three-way interactions involving all the components, as illustrated in Figure 1. We particularly focus on the *in vitro* reconstitution approach, relevant to the bottom-up creation of synthetic cells, and emphasize the impact condensates can have in shaping and structuring them. We finally conclude that a hybrid combination of these elements and the resulting interactions can be aptly utilized in order to shape future synthetic cells.

Cytoskeleton–condensate interactions

Let us start with the behavior of cytoskeletal polymers in phase-separated environments, a newly discovered topic that is quickly gaining attention [28]. Like many other biomolecules, cytoskeletal proteins also have a tendency to be sequestered and concentrate inside condensates. What sets them apart, however, is their ability to undergo polymerization/depolymerization and form hierarchically assembled structures such as bundles and networks. This can exert internal/interfacial forces and deform the energetically favorable spherical shape of condensates. Furthermore, many cytoskeleton-associated regulatory proteins (plectin, tau, and epsin are just a few examples) contain intrinsically disordered regions [29], increasing their propensity to undergo LLPS. Actin-binding proteins like N-WASP and cortactin (both act as nucleation promoting factors), for example, have been shown to become enriched within condensates [30,31].

One of the first *in vitro* findings on actin-condensate dynamics demonstrated that actin filaments predominantly assembled at the surface of liquid droplets (Figure 2a) [32]. The condensates were made by the complex coacervation of polylysine (positively charged) and polyglutamic acid (negatively charged), two synthetic polypeptides. The spontaneous partitioning of actin at the interface substantially (approximately 50-fold) increased the rate of actin polymerization [32]. In another study, actin filaments partitioned inside RNA/protein condensates that were initially isotropic in shape. However, owing to the anisotropic nature of the filaments, this led to the shape transformation of the condensates from spheres to tactoids (spindle-shaped droplets) [33]. This happened in a concentration- and size-dependent manner with deformations observed in droplets of a few hundred nanometers in diameter. In addition to its interactions with synthetic condensates, actin is also capable of forming liquid droplets together with naturally occurring actin-binding proteins, such as filamin, a bundling protein [34]. Similar to the previous example, the formed condensates displayed a tactoid shape as a result of the orientational ordering of actin filaments [35]. Moreover, the addition of myosin motors to the system resulted in a surprising further observation: rod-like myosin clusters migrated to the midplane

Figure 2



Cytoskeleton-condensate interactions. (a) Actin partitions and polymerizes on the surface of polylysine/polyglutamic acid condensates with enhanced kinetics. (b) Actin filaments (red) and filamin form spindle-shaped condensates. Myosin motors (white) migrate toward the condensate midplane and promote droplet deformation, ultimately bisecting the liquid droplet into two equal halves. (c) Tubulin monomers partition inside tau condensates and polymerize in the presence of GTP. The growing microtubules dramatically alter the spherical shape of tau droplets through a wetting process. (d) Tubulin-binding protein TPX2 co-condensates with tubulin and preferentially localizes on pre-existing microtubules, acting as a nucleating site for branching. (e) FtsZ monomers partition into polylysine/RNA liquid droplets (green). The rapid GTP-dependent filament turnover brings about a variety of condensate transformations such as growth, shrinkage, bending, fusion, and even division. (f) FtsZ monomers localize at the surface of polylysine/GTP coacervates and eventually form a network of FtsZ bundles interconnecting the individual condensates. The mechanical properties of the condensates are altered as seen through their fracture under mechanical stress. (g–i) Sketches depicting three key cytoskeleton (in blue)-condensate (in green) interactions: sequestration (g), deformations (h), and organization (i), in terms of providing nucleation or branching points, network formation, etc. All scale bars 5 μm. Images adapted from the following references – a: [32], used under license from Elsevier; b: [35], used with permission from the National Academy of Sciences; c: [37], reproduced under a Creative Commons BY-NC-ND License; d: [38], re-used and adapted under a Creative Commons Attribution 4.0 International License; e: [40], used under license from Springer Nature; f: [41], used under license from John Wiley and Sons.

of the liquid droplet, leading to active deformation of the tactoids into two identical spindle-shaped actin liquid droplets joined by myosin puncta (Figure 2b). *In vitro* reconstitution of postsynaptic densities (protein-dense lamina located beneath the postsynaptic membrane) from neuronal cells was also shown to form condensates enriched in cortactin, an actin-binding protein and actin monomers [31]. This eventually promoted the formation of actin filaments and bundles, confirming that condensates can play a role in triggering actin assembly.

Similar to actin, tubulin has also been studied in phase-separated environments, with dramatic effects on the condensate morphology. One of the first studies of tubulin-condensate interactions was reported using an evolutionarily conserved BuGZ protein, which formed condensates *in vitro* and concentrated tubulin [36]. Subsequent *in vitro* studies revealed that tubulin monomers readily partitioned inside condensates formed by tau, a tubulin-binding protein found in neuronal cells. Interestingly, concomitant with the growth of microtubule bundles, tau droplets severely

deformed, wetting the microtubule surface as the bundles grew in length (Figure 2c) [37]. Likewise, TPX2 protein, a spindle microtubule regulator, was observed to form liquid droplets together with tubulin. Notably, these TPX2-tubulin droplets preferentially localized on pre-existing microtubules, suggesting a mechanism for microtubule branching (Figure 2d) [38]. Parallel examples are also found in prokaryotes, especially in the case of FtsZ, a tubulin homolog involved in bacterial cell division [39]. FtsZ was shown to partition inside RNA/polylysine condensates. Analogous to the case of tau condensates, FtsZ polymerization actively deformed the liquid droplets. The rapid turnover of FtsZ filaments in the presence of a high concentration of GTP notably led to diverse morphological changes such as growth, shrinkage, bending, fusion, and division of these hybrid structures (Figure 2e) [40]. In another study, FtsZ was shown to partition on the surface of polylysine/GTP condensates. Since FtsZ polymerizes by hydrolyzing GTP, the condensates functioned as a fuel reservoir and dissolved over time. A network of FtsZ bundles emerged when GTP was present in a sufficient quantity, interconnecting the individual condensates. Furthermore, these FtsZ-coated condensates exhibited solid-like behavior, as they fractured under mechanical pressure, forming flower-like structures (Figure 2f) [41].

All these examples clearly indicate that cytoskeletal biochemistry is not only compatible with LLPS environments, but it further leads to enhanced cytoskeleton capabilities in terms of increased reaction rates and spatiotemporal regulation. Condensates can thus provide architectural scaffolds and serve as reaction hubs for organizing and orchestrating the cytoskeletal response, as sketched in Figure 2g–i. Depending on the physical and chemical nature of the condensates, cytoskeletal partitioning and activity can be controlled, as evident from the given examples, and sketches of surface localization (Figure 2g), shape deformation (Figure 2h), and provision of branching, as well as nucleation points (Figure 2i). Interestingly, once cytoskeletal elements self-assemble into dynamic, micro-sized, self-assembled structures, they can bring about diverse morphological changes to condensates, forcing them into nonspherical, stressed configurations. These out-of-equilibrium states could be potentially useful to form force-generating structures within synthetic cells.

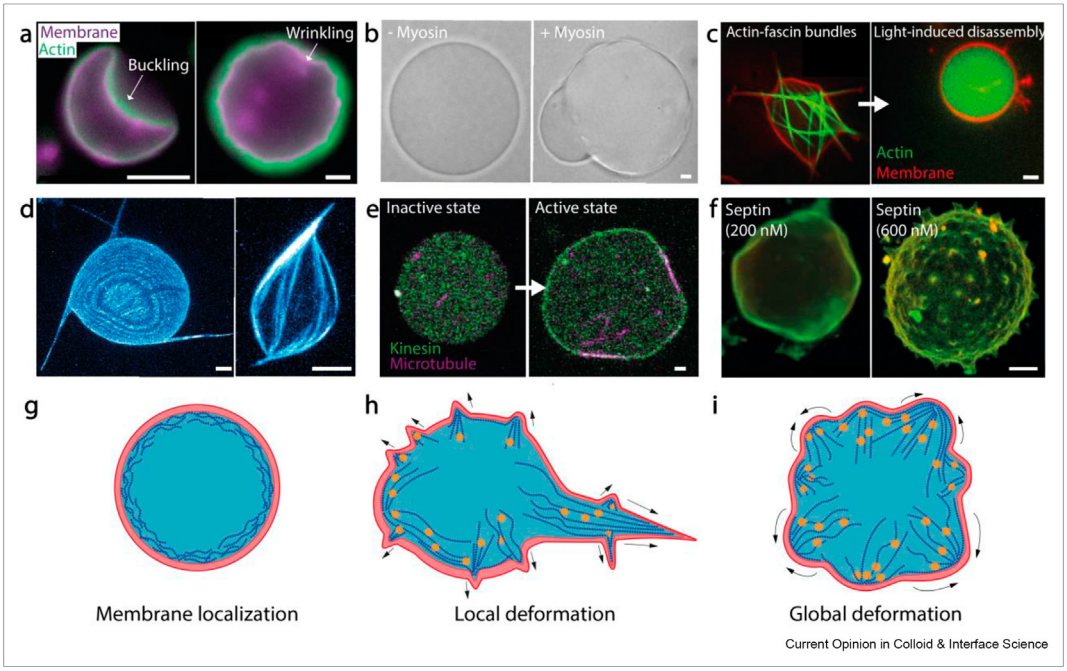
Cytoskeleton–membrane interactions

Just as the scaffolding beams are necessary to support the walls of a house, the cytoskeletal polymers can be roughly seen as the 'dynamic beams' that support the 'flexible walls' represented by the cell membrane. To unveil the role of the cytoskeleton in cellular morphogenesis, understanding the interaction of involved proteins with membranes is necessary. For this, purified protein systems have been extensively reconstituted both within and on the outer surface of GUVs, and this section reports the latest progress in this regard.

Caging multiple cytoskeletal proteins and further coupling them to the membrane in a natural/synthetic manner is a challenging task. Using natural linkers, *i.e.*, membrane/membrane-interacting proteins, further adds to the complexity, and their use is not always feasible. Alternatively, synthetic linkers in the form of strong binding partners, such as streptavidin-biotin and histidine-nickel couplings, are easier tools to direct proteins onto a membrane. For instance, by doping liposomes with biotinylated lipids, streptavidin-tagged actin regulatory proteins could be readily recruited to the outer leaflet of the membrane [42]. These recruited complexes eventually led to actin localization at the membrane and subsequent polymerization induced tubes and spike-like inward protrusions in osmotically-deflated 'floppy' liposomes [42]. It should be noted that the osmotic pressure difference across the liposomal membrane affects membrane tension: a liposome in a hypertonic environment will be deflated and will have a lower membrane tension compared to a liposome placed in a hypotonic environment. A recent investigation reinforced the role of membrane tension in supporting the deformations caused by a membrane-bound actin network. In nondeflated liposomes, capping protein- (regulator of actin filament elongation) bound actin filaments induced symmetric dumbbell-shaped deformations, whereas liposomes did not become deformed in the absence of capping protein [43]. However, in deflated liposomes, even in the absence of capping proteins, actin filaments alone induced symmetric dumbbell-shaped deformation to a similar extent as they did in the presence of capping protein, suggesting the interplay between membrane tension and actin network dynamics governs cell shape. In addition to the membrane tension, the actin coat thickness also influences the extent and the way a vesicle will deform. In osmotically deflated actin-coated liposomes, the membrane underwent irreversible buckling or wrinkling, depending on the actin shell thickness (Figure 3a) [44]. For thinner actin shells, membrane buckling was predominant, whereas globular wrinkling was observed in the case of thicker actin shells.

An alternate strategy to direct proteins to a membrane is by tagging them with a polyhistidine moiety and using nickel-containing lipids (e.g. nickel-nitrilotriacetic acid, Ni-NTA). A fine example is the recent use of histidine-tagged anilline proteins to reconstitute a minimal actomyosin cortex at the membrane, where sufficient membrane tethering and motor activity resulted in bleb-like membrane deformations, reminiscent of the blebbing motility of cells (Figure 3b) [45]. Interestingly, blebbing could also be inhibited either by increasing the density of membrane/cytoskeleton linkers or by

Figure 3



Cytoskeleton-membrane interactions. (**a**) Actin (green) shell coated on the outer side induces either buckling or wrinkling of osmotically deflated liposomes (magenta), depending on the actin shell thickness. (**b**) Actomyosin cortex, anchored to the membrane by anillin through Ni-NTA-His bonding, induces bleb-like deformations only in the presence of myosin. (**c**) Liposome exhibiting prominent filopodia-like membrane protrusions, induced by actin-fascin bundles (left). The spherical shape of the liposome is restored upon actin disassembly induced by light (right). (**d**) Encapsulation of microtubule-kinesin system inside liposomes induces filopodia-like membrane deformations. The topological constraints and vesicle deformability lead to dynamic, shape-changing vesicles, with smaller vesicles (right) deforming more than bigger ones (left). (**e**) A microtubule-kinesin system caged inside liposomes (left) triggered local membrane deformations (right) in response to the kinesin motor activity coupled to the membrane via DNA-based linkers. (**f**) Septin filaments localize on liposomes consisting of phosphatidylinositol-4,5-bisphosphate lipids, flattening the membrane locally at low concentrations (left) and inducing spike-like membrane protrusions uniformly across the liposome surface at high concentrations (right). (**g-i**) Sketches showing the three prominent cytoskeletons (in blue)-membrane (in red) interactions: localization at the inner leaflet (g), local deformations (h), and global deformations (i). All scale bars 5 μm. Images adapted from the following references – a: [44], used under license from the Royal Society of Chemistry; b: [45] under a Creative Commons License; c: [47], re-used and adapted under a Creative Commons CC-BY license; d: [48], used under license from the American Association for the Advancement of Science; e: [51], used under license from the American Association for the Advancement of Science; f: [53], re-used and adapted under a Creative Commons Attribution 4.0 International License.

reducing the myosin concentration, highlighting the importance of membrane linkage, as well as motor activity for inducing membrane deformation. In another recent use of histidine-nickel coupling, encapsulation of capping proteins with other actin-binding proteins — profilin, Arp2/3, and the His-tagged VCA domain of N-WASP — directed the protein complex to the lipid membrane. As a consequence of actin polymerization at the membrane, interesting topological deformations were observed in response to variations of capping protein concentration: lower concentrations of capping protein-induced star-like clusters on the membrane while higher concentrations promoted fission events [46].

Membrane deformations can also be induced by actin cross-linking and bundling proteins. As recently demonstrated, fascin-induced actin bundles formed prominent spiky filopodia-like protrusions in GUVs (Figure 3c, left panel) [47]. Upon light-induced disassembly of actin bundles, the spherical conformation of the liposome was restored within few minutes (Figure 3c, right panel).

Microtubules have also been effectively explored in *in vitro* settings to deform vesicles. Similar to myosin motors sliding along actin filaments, kinesin motors glide over microtubules to generate active extensile stresses. Microtubules confined in GUVs, along with ATP-fueled kinesin motor clusters, demonstrated remarkable active topological dynamics [48]. The microtubules were adsorbed to the membrane through depletion interactions [49], and upon osmotic deflation, these vesicles showed periodic shape fluctuations characterized by a change in ellipticity and the formation of filipodia-like protrusions. The extent of topological deformations was found to be inversely proportional to the vesicle size, with smaller vesicles showing more deviation from sphericity and vice versa (Figure 3d) [48]. By deviating from the conventional usage of cellular

machinery, an interesting study demonstrated the use of hydrostatic pressure to control the polymerization of tubulin encapsulated inside GUVs, leading to membrane deformations in the absence of motor proteins [50]. At lower pressure (0.1 MPa), tubulin polymerization induced prominent outward protrusions. These protrusions shrank rapidly at higher pressure (60 MPa), making it a reversible process. Lastly, using a totally synthetic route, DNA was used as an actuator to trigger microtubule-assisted shape changes. When engaged, the DNA linker transmitted the force generated by the motor to the membrane, resulting in a local flattening of the liposomal membrane (Figure 3e) [51]. Furthermore, this DNA 'clutch' could be rendered photosensitive in order to control the shape-changing behavior through exposure to light.

The fourth cytoskeletal element, septin, is also involved in membrane remodeling in cells and can bring about structural changes to vesicles through interactions with specific lipids [52], as demonstrated in a recent study. Similar to actin-binding proteins, the extent of deformation was concentration-dependent: at low concentrations, septin interactions led to the local flattening of the liposomal membrane indicating an alteration in the mechanical property of the septin-bound membrane (Figure 3f, left panel). At higher concentrations, septin developed evenly spaced, pronounced spike-like protrusions across the entire membrane (Figure 3f, right panel) [53].

Based on the above observations, it is evident that cytoskeleton–membrane interactions, as sketched in Figure 3g–i, are very effective in bringing about active local (Figure 3h), as well as global (Figure 3i) membrane deformations. A key parameter that seems to play a crucial role in enhancing cytoskeleton-based morphological changes is the reduction of the membrane tension of vesicles, often achieved through osmosis. Localization of the cytoskeletal filaments to the membrane is naturally facilitated by specific proteins acting as linkers. However, from the point of view of synthetic cells, a variety of synthetic membrane connectors can be used as replacements for natural proteins, partly simplifying the design process. Thus, condensates can be interesting choices to position the two elements in close proximity to each other. The next section discusses the interactions of condensates with membranes and how condensates can act as linkers between the cytoskeleton and the membrane.

Condensate–membrane interactions and further involvement of the cytoskeleton

The previous two sections have looked at the roles the cytoskeleton can play in phase-separated and membranous environments to shape synthetic cells, but what about the interactions between condensates and membranes, where the cytoskeleton is less of a participant? While relatively less breadth of research has looked at this emerging field, we expect these interactions to play crucial roles in shaping synthetic cells. This section will focus on recent studies on condensates interacting with membranes. Moreover, we also describe relevant examples where the cytoskeleton is recruited to this hybrid interface, and the three elements coexist to form a basic structuring unit.

In principle, when a condensation trigger is met, condensates can be expected to be distributed randomly within confinement. However, many MOs within cells have designated locations [19,20], which has led to a body of recent work that looks into how this ordering and structure can be guided, and in this particular context, how condensates interact with membranous components of the cell [54–58]. This primarily uses *in vitro* reconstitution of analogs of cellular systems owing to the complexity of cellular machinery. A recent work explored using a liposomal membrane as support for condensation [59], as depicted in (Figure 4a). To do so, liposomes were prepared using a mix of lipids involving a small amount of highly negatively charged lipids (phosphatidylinositol (3,4,5)-triphosphate). Strong electrostatic interactions between the overall negatively charged membrane and positively charged polylysine/ATP condensates promoted the adhesion of the formed condensates onto the liposome membrane, which could still migrate along the membrane. More notable, however, was that cholesterol-tagged RNA/spermine condensates wetted uncharged membranes [59] (Figure 4a, left panel), in opposition to the general tendency of the condensates to maintain their spherical shape; this wetting was due to the interaction of cholesterol with the membrane. Liposomes with sticky condensate patches were observed to adhere to each other at these patches (Figure 4a, right panel). This hints toward a possible role of the involved interactions to form multiparticle assemblies.

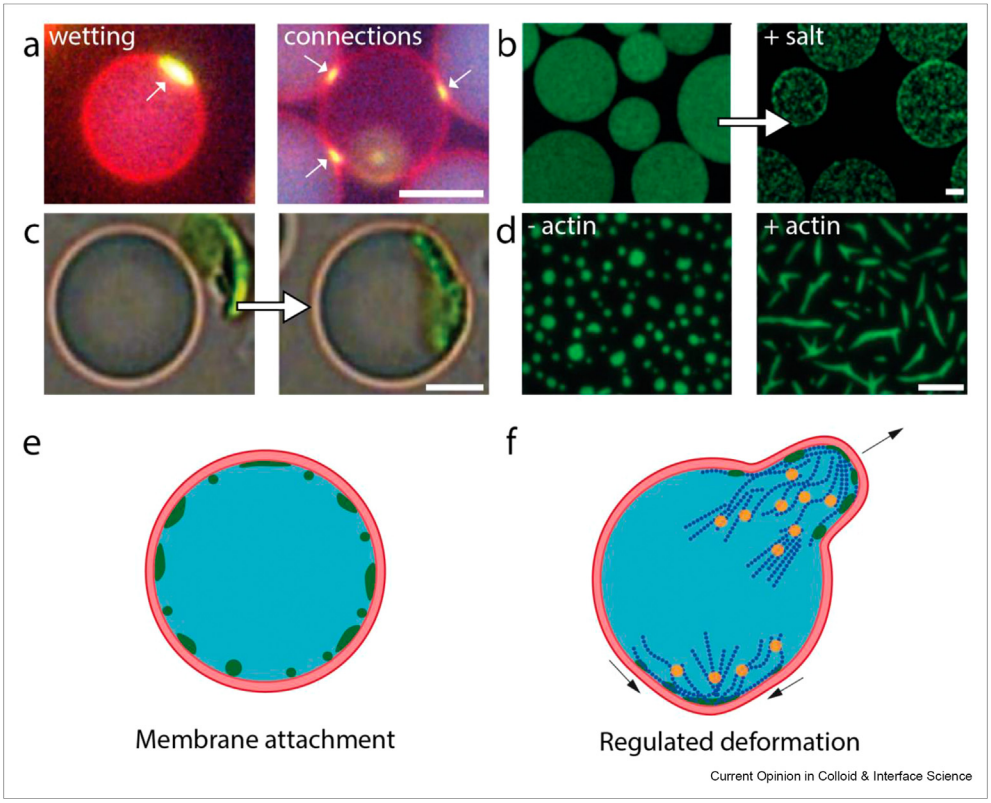
Such condensate–membrane interactions are certainly not only limited to liposomes, though: using proteinosomes as the containers, positively charged condensates were similarly adsorbed to the negatively charged protein-based membrane [60] (Figure 4b). The study further showed that modulation of the salt content could additionally have strong effects on the eventual spatial arrangement of the condensates: added salt desorbed the condensates from the membrane owing to the screening of the electrostatic interactions, resulting in the desorbed condensates assuming a spherical shape, as confirmed by fluorescence imaging. An equally promising and interesting result in this study was that the condensates could be used as chemical reactors, enhancing the catalytic activity of enzymes once

localized and sequestered at the protein-condensate membrane. The reversible switching of the condensation trigger proves promising here in that enzymatic or other reactive activity can thus become modulated through the adsorption and desorption process.

Another study, illustrated in (Figure 4c), looked at the interaction between synthetic condensates and chloroplasts to create a photosynthetic protocell [61]. Again, using a charge-based attraction between positively charged condensates and the negatively charged chloroplast membrane, chloroplasts harvested from plants could be rapidly trapped within the liquid droplets. This endocytic step occurs within seconds and is reversible, with the release of the chloroplasts achievable simply by dissolving the condensate. The photosynthetic capabilities of the chloroplasts were preserved, thus confirming the biocompatible nature of these hybrid systems. While not a strictly condensate—membrane interaction akin to the others, this fine example illustrates the use of charge-attraction principles to localize and trap organelles and other cellular components.

A natural progression from solely looking at the interactions of condensates and membranes alone is the recruitment and local action of the cytoskeletal elements. One such example is found in cellular interstitial spaces in vertebrates, known as tight junctions. A recent study showed that these tight junctions are filled with a variety of proteins that phase separate and subsequently enrich as well as localize adhesion receptors, transcription factors, and cytoskeletal adaptors [62]. This further leads to the recruitment of actin, providing more rigidity and structure to the cells. This and a few other *in vivo*

Figure 4



Condensate–Membrane interactions. (a) Cholesterol-RNA/spermine condensates wet at the liposome boundary due to cholesterol-mediated membrane anchoring, as indicated by the arrow (left panel). Multiple liposomes can be connected to each other through the condensate patches at the membrane, as indicated by arrows (right panel). (b) Reversible interaction of condensates with the membranous boundary of proteinosomes, modulated by tuning the ionic concentration. (c) Negatively charged membranous chloroplasts (green) can become rapidly engulfed by positively charged condensates (gray sphere), creating a kind of prototype photosynthetic cell. (d) The recruitment of actin to condensates localized at a membrane leads to anisotropic shape deformation of condensates, countering the interfacial tension that normally promotes their spherical shape. (e and f) The condensate (in green)-membrane (in red) interactions can be largely classified as membrane attachment interactions (e), where the condensates adsorb to the membrane, and regulated deformations (f), which can involve cytoskeleton (in blue) recruitment to induce a shape deformation in the membrane. All scale bars 5 μm. Images adapted from the following references – a: [59], used with permission from the American Chemical Society and under Creative Commons License; b: [60], used under Creative Commons License; c: [61], published by the Royal Society of Chemistry and used under Creative Commons License; d: [56] used under license from the publisher.

studies, along with *in vitro* reconstitutions of the involved set-ups, have looked at the interactions of the actin cytoskeleton with membrane-interacting condensates. One such example is where actin-binding protein complexes, such as N-WASP, NcK, and the transmembrane protein nephrin, were shown to form condensates at the membrane, and subsequently promoting the growth of actin at those sites [30]. The stoichiometric ratios of the condensate components had a direct effect on the membrane dwelling time of actin recruitment proteins: the lengthening of their dwelling time increased their overall activity in recruiting actin to the surface [30]. A similar study revealed that a phosphorylated state of the transmembrane LAT protein (linker for activation of T cells) promoted condensation with adaptor proteins Grb2 and Sos1 at the membrane [56]. As can be seen in (Figure 4d), these condensates recruited actin, leading to localized actin polymerization at these condensates and inducing shape changes to them, similar to as seen in (Figure 2c) and e. In the absence of actin, the shape assumed was more strongly interfacial tension-driven.

In general, condensate–membrane interactions can be grouped under two categories: condensate attachment to a membrane, as depicted in (Figure 4e), and cytoskeletal recruitment to condensates in order to induce a regulated deformation, as shown in (Figure 4f). These previously illustrated examples, however, only begin to scratch the surface of the possible interactions between condensates and membranes. Another recent work has shown that condensate–membrane interactions can, by themselves, induce membrane deformations: a condensate attaching to the inside of a membrane can lead to invagination (L.P. Bergeron-Sandoval et al., bioRxiv doi: 10.1101/145664), which is striking considering that deformations in the membrane for locomotion and endocytosis, for example, are commonly assisted by the cytoskeleton. This condensation is driven by the LLPS of proteins at the membrane interface, reducing the interfacial tension and the associated energy cost of membrane deformation. This is an important finding, as it hints at the role of condensation in performing mechanical work at membranous interfaces and how the membrane itself is readily deformable through components apart from the cytoskeleton.

What applications could these condensate–membrane interactions, both with and without the involvement of the cytoskeleton, lead toward? We can, first of all, look at the membrane as a scaffold to build condensate sites, with a useful option of their reversible dispersal into the cytoplasm. The binary switch-like ability of condensation can further provide options for tuning and triggering catalytic and enzymatic activity: this can be incorporated into a feedback loop, much like in living cells, where the reactions become actuated based on the environment. These membrane-bound condensate sites can additionally act as docking stations to which the cytoskeleton attaches. By tuning the interfacial properties of the condensates, their eventual distribution on the membrane wall can additionally be directed, which, once the cytoskeleton is recruited, can further be tuned to achieve desired and specific shapes and cellular morphology. These local sites can also prove useful for incorporating membrane-bound organelles and other elements in desired positions.

Conclusions

We are still at the initial stages of the monumental and exciting prospect of building autonomous, functional synthetic cells. Out of the numerous functional modules that synthetic biologists are seeking to build in a bottom-up manner, our review has focused, in general, on the shaping and structuring of synthetic cells and with specific emphasis on the possible ways of imparting dynamic morphological changes in them. We propose that the usage of three bioassemblies could be highly useful to attain this goal: flexible, deformable membranes that define the container boundaries; condensates that can spatiotemporally organize the internal biochemistry; and the cytoskeleton that governs active force generation, both locally and globally. Backed by a variety of recent studies, we propose that hybrid combinations of these elements could give us a versatile platform for shaping synthetic cells.

Recent discoveries have opened up new directions toward achieving and guiding cellular organization, in particular the discovery of biomolecular condensates. The studies referred to in this review suggest that condensates can have a direct effect on the cytoskeletal self-assembly within the cellular interior and at the membrane. Condensates can assist cytoskeleton-mediated force generation by providing better control over the localization of deformations, both in space and time, while LLPS can offer conducive environments for enhanced self-assembly and reaction kinetics. Being able to guide and direct the process of condensation can be beneficial from structuring point of view: by encoding the instructions for condensation within a system, whether through stoichiometry or through the identity of the materials used, one can guide the self-assembly process by positioning distinct elements in predefined locations, sequestering specific molecules/supramolecular assemblies, and regulating signaling cascades. Further, condensate–membrane interactions can be engineered as reversible, stimuli-dependent processes. Thus, condensates can act as an ideal ‘glue’ to recruit the cytoskeleton to the right position and at the right time. We would like to note that, while phase separation of membrane lipids can also spatially segregate molecules at the membrane, it inevitably leads to asymmetry in the membrane itself. Condensates, in principle, can decouple these two processes, i.e., maintain membrane

homogeneity while spatially organizing reactions at the membrane. Furthermore, by being a tunable physical linker between the membrane and cytosolic components, use of membrane-bound condensates may turn out to be a more versatile strategy.

Additionally, condensates themselves can act as thermodynamic machines and may thus contribute to morphogenesis [24]. The material and mechanical properties of condensates are manifested in their viscoelastic behavior and interfacial interactions, which can lead to a net mechanical output on the surrounding components. Biomolecular condensates could thus themselves perform mechanical work on soft interfaces, such as membranous boundaries and cytoskeletal networks. Although we do not yet have a thorough understanding of these interactions, we may observe more and more utilization of condensates to control the morphogenesis of vesicles. The suggested trio of elements offers fine control in the context of directed force generation and other scenarios such as polarity establishment and division.

In the rapidly changing interdisciplinary field of synthetic biology, newer methodologies, like microfluidics and DNA nanotechnology, are playing increasingly important roles. For example, the encapsulation efficiency and overall control over vesicle production can be significantly improved using newly developed microfluidic techniques [63,64]. At the same time, tuning of key physical parameters proves highly beneficial to accentuate shape changes in vesicles. The most effective parameter seems to be the membrane tension: osmotically reducing the tension to yield floppier vesicles greatly facilitates membrane deformation by cytoskeletal forces, and we expect to see extensive use of this strategy in the future. Other physical effects such as depletion interactions (for example, to enhance cytoskeleton proximity to membranes) and electrostatic attraction (for example, to establish reversible condensate–membrane interactions) can be helpful when strategizing the construction of a synthetic cell.

In the long term, research in shaping synthetic cells may act as a stepping-stone for emerging technologies that can lead to multifunctional dynamic materials, ones that can reconfigure and adapt in response to stimuli. One direction for progress could lie in the realm of synthetic tissues: vesicles with a functional cytoskeleton-condensate system could act as basic units to explore the collective behavior of multicellular systems. Alternatively, condensates could be used to link and communicate between individual units to form tissue-like artificial entities with desired properties (such as density, resilience, and texture). Lastly, such minimal morphogenetic systems can have far-reaching applications. The versatile nature of these systems can be utilized for advances, which can include, but are not limited to, drug delivery for therapeutics, screening of pharmaceutical compounds, and synthetic and novel vaccine platforms. The integration of these technologies into fundamental sciences can generate a wealth of opportunities with potentially strong positive impacts on human development.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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