Studying actin-induced cell shape changes using Giant Unilamellar Vesicles and reconstituted actin networks

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Cell shape changes that are fuelled by the dynamics of the actomyosin cytoskeleton control cellular processes such as motility and division. However, the mechanisms of interplay between cell membranes and actomyosin are complicated to decipher in the complex environment of the cytoplasm. Using biomimetic systems offers an alternative approach to studying cell shape changes in assays with controlled biochemical composition. Biomimetic systems allow quantitative experiments that can help to build physical models describing the processes of cell shape changes. This article reviews works in which actin networks are reconstructed inside or outside cell-sized Giant Unilamellar Vesicles (GUVs), which are models of cell membranes. We show how various actin networks affect the shape and mechanics of GUVs and how some cell shape changes can be reproduced in vitro using these minimal systems.

Introduction

Many biological processes rely on changes in cellular shape. Global changes, for example, in the case of motility and division, and local changes for exo/endocytosis and filopodia formation. The actin cytoskeleton plays a crucial role in such shape changes [1]. It generates polymerization-induced forces deforming the lipid bilayers surrounding the cell and most of its internal compartments by dynamically switching globular 42 kDa actin monomers (G-actin) to polymerized filaments (F-actin). In the cell, many Actin Binding Proteins (ABPs) control actin assemblies dynamics and structure in at least six manners:

I. By influencing the ability of G-actin to form filaments, such as profilin [2];
II. By severing actin filaments or preventing their elongation, such as capping proteins (CP);
III. By nucleating filaments, such as the Actin related protein or Arp2/3 complex [3] and formins. Arp2/3 is a protein complex that, when activated, can nucleate a new filament on the side of a pre-existing filament and thus generate branched networks. Arp2/3 is rich in the lamellipodium of migrating cells, and the polymerization of Arp2/3-nucleated networks generates forces that push the cell membrane forward. Formins nucleate the elongation of bundles of parallel actin filaments [4];
IV. By regulating the nucletation of filaments by activating the nucleators, for example, WASP (for Wiskott–Aldrich syndrome protein), that regulate F-actin nucleation by activating the Arp2/3 complex [5]. These types of proteins are known as Nucleation Promoting Factors (NPF);
V. By building higher-order actin-based structures. Such structures are achieved through both cross-linking filaments such as α-actinin [6] and fascin [7] and linking filaments with membranes as proteins of the ezrin-radixin-moesin family [8];
VI. By exerting forces on F-actin. Proteins of the myosin family exert these forces by walking along F-actin using the energy of ATP hydrolysis [9]. Myosin II forms minifilaments that create tension in actin networks by pulling on F-actin.

Within the cell, the diversity of these ABPs leads to the formation and cohabitation of several types of actin-based structures, such as the cortex, stress fibers, or filopodia [1], that reorganize both spatially and temporally in response to internal and external stimuli. Studying these actin-based structures can be problematic in the complex environment of the cell interior, which contains hundreds of proteins potentially interacting with actin. However, a bottom-up strategy based on biomimetic systems can decipher how actin-based structures such as the cortex or filopodia appear, the minimal set of components required for their formation, and their mechanical effects on cell shape. Model systems made of lipid membranes and actin networks reconstituted from purified proteins are helpful to answer these questions since they allow the in vitro reconstitution of cellular actin-based structures [10]. Therefore, it is possible to study the physical mechanisms of the formation and maintenance of these structures.

Among membrane models that study the effect of actin dynamics on lipid membranes, Giant Unilamellar Vesicles (GUVs), also called liposomes, are of practical interest as these spheres made of a nanometer-thick bilayer are the size of living cells and can be easily imaged by optical microscopy. Actin can be coupled either to the inside or outside leaflet of the GUVs membrane. In these two configurations, GUV membranes appear flat at the scale of actin networks mesh size, roughly tens of nanometers [11]. Note that the confinement of actin structures can affect membrane shape. Another critical difference is that the inside configuration imposes a finite amount of proteins, contrary to the outside configuration, where this supply is enormous. In this mini-review, we present how actin affects GUVs. In particular, we highlight how these biomimetic systems reproduce in vitro some steps of cellular shape changes, thus allowing for the deciphering of their physical mechanisms. We also show how these experiments pave the way for the design of synthetic soft nanomachines inspired by living cells.

We present results by ascending order of complexity in the actin machinery. In most cases, this also corresponds to chronological order. In section I, we present experiments with actin filaments polymerized without nucleators. Then, the same type of filaments in the presence of crosslinkers (section II) and myosin II (section III). Finally, we present examples where nucleators trigger actin polymerization specifically at the GUVs membrane. In each section, we distinguish between assays based on the ‘inside’ or ‘outside’ configuration, which can be associated with different preparation techniques (Table 1).

### Actin filaments formed without NPFs

The most straightforward assays correspond to actin filaments polymerized without NPFs that are artificially coupled to the outer leaflet or encapsulated in the lumen of GUVs.

Helfer et al. have studied GUVs surrounded by a layer of F-actin connected to their membrane by biotin-streptavidin links (Figure 1A). Optical tweezer microrheology shows that this layer strengthens the GUV membrane. It reduces its transverse thermal fluctuations by increasing its bending rigidity, i.e. the energy to pay to change the curvature of the membrane, from 10 $k_B T$ for a lipid membrane to 100–1000 $k_B T$ when the same membrane is coated with actin filaments [12]. Studying in-plane fluctuations of beads attached to the GUV membrane, the authors show that, in the presence of actin filaments, these composite membranes also have a viscoelastic behavior with a 2D shear modulus on the order of $10^{-6} \text{N/m}$ [13]. In contrast, due to their fluidity, there is no elastic resistance to shearing in actin-free lipid membranes. Therefore, these studies show that the actin layer strengthens the GUV membrane by increasing its bending and shearing rigidities.

To encapsulate F-actin, GUVs are formed by electroformation (EF) [14] in a solution containing G-actin (Figure 1B). Then, polymerization is triggered inside the GUVs by Mg$^{2+}$ influx through ionophores inserted in their membrane [15]. Thanks to steric hindrance, PEGylated lipids and cholesterol limit the adsorption of F-actin to the lipid bilayer. In these experiments, F-actin spatial distribution is correlated to the GUV size: in GUVs smaller than 18 μm, F-actin forms a cortex close to the GUVs membrane, whereas in larger GUVs, actin appears as a ‘fuzzy’ homogeneous network. Such differences in actin organization are due to a competition between the GUV size and the persistence length ($l_p$) of F-actin, which is ~10 to 17 μm [16,17]. These results were among the first to establish that in a GUV larger than $l_p$, filaments can distribute anywhere,
| Article | In vs. | GUVs Preparation | Lipid composition | Protein mixture | Membrane attachment | Solicitation/Deformation |
|---------|--------|------------------|------------------|----------------|-------------------|------------------------|
| F-actin without NPFs | Helfer et al. [12] | Out | EF | DOPC PE-Biotin 5% | F-actin (15% biotinylated) with streptavidin | Biotin-streptavidin Microhoreology; actin increases the membrane bending rigidity and adds a viscoelastic response |
| | Helfer et al. [13] | | | | | |
| | Limozin and Sackmann [19] | In | EF | DMPC DMPE-PEG 2.5% Cholesterol 17% | F-actin 2–10 μM α-actinin 0.1–1 μM filamin 100–300 nM | Non-specific Effect of confinement on F-actin morphology |
| | | | | | | |
| | Limozin et al. [15] | In | EF | DMPC DMPE-PEG 0–5% Cholesterol 0–37% | F-actin | Non-specific Effect of confinement on F-actin morphology |
| F-actin + crosslinkers | Tsai et al. [24] | In | Agarose gel swelling | DOPC PEG-PE 2.5–5% PE-Biotin 0–2.5% | F-actin 12 μM fascin 0.6–2.4 μM | Non-specific Actin protrusions |
| | Litschel et al. [25] | In | cDICE | POPC PE-Biotin 1% | G-actin 2–6 μM fascin 66–300 nM | Non-specific Ring-like formation of bundled actin |
| | Bashirzadeh et al. [20,26] | In | Modified cDICE | DOPC 70% Cholesterol 30% | G-actin 5 μM fascin 0.5–2.5 μM α-actinin 0.5–1.5 μM | Non-specific Crosslinked F-actin organization in confined space |
| | Tsai et al. [29] | In | Agarose gel swelling | DOPC 93.8% PEG-DOPE 5 PE-Biotin 1% | G-actin 24–95 μM Myosin 0.12–1.9 μM | Biotin-streptavidin Actin cortex detachment from membrane |
| | Takiguchi et al. [28] | In | IE | EPC | F-actin 50–200 μM myosin II 3.8–15 μM | Non-specific Crosslinking of F-actin by myosins |
| NPFs at membrane | Liu and Fletcher [35] | Out | EF | DPPC 17.2% DOPC 52.2% Cholesterol 30% Bodipy-TMR-PIP2 0.6% | actin 6 μM N-WASP 390 nM Arp2/3 150 nM | PIP2/N-WASP Effect of actin on membrane phase transition |
| | Liu et al. [38] | Out | EF | EPC 75% DOPS 20% PIP2 5% | actin 8.5 μM N-WASP 400 nM Arp2/3 160 nM | PIP2/N-WASP Filopodia-like protrusions emerging from dendritic networks |
| | Simon et al. [40,41] | Out | EF | EPC PE-Biotin 0.1% DGS-Ni 5% | actin 3 μM spVCA 350 nM profilin 3 μM Arp2/3 37 nM CP 0–25 nM | biotin-spVCA Inward and outward protrusions emerging from dendritic networks; Wrinkled or buckled deformations; Tubule stabilization by actin |
| | Kusters et al. [42] | | | | | |
| | Allard et al. [43] | | | | | |
| Continued | | | | | |
Table 1: Table summarizing the experimental details of all articles presented in the five sections of the text: inside or outside geometry, GUVs preparation techniques, lipid composition of the GUVs membrane, composition of the actin and actin-related proteins mixture, interaction of the actin structures with the GUVs membranes and effects of the actin on the membrane.

| Part 2 of 3 | Article | In vs. Out | GUVs Preparation | Lipid composition | Protein mixture | Membrane attachment | Solicitation/Deformation |
|------------|---------|------------|-------------------|-------------------|-----------------|---------------------|--------------------------|
| Pontani et al. [46] | In IE | EPC 58% DGS-Ni 5% Cholesterol 37% | G-actin 6.5 mM Arp2/3 0.12 mM Gelsolin 50 nM ADF-cofilin 2 mM Profilin 1 mM VCA-His 0.64 mM | Histidin-nickel | Mimic of the actin cortex |
| Wubshet et al. [47] | In Modified cDICE | DOPC 70% cholesterol 25% DGS-Ni 5% | Actin 5.3 μM Arp2/3 1 μM His-VCA 0.5 μM fascin 10–50% | Histidin-nickel | Protrusion only in the presence of fascin reveals competition with Arp2/3 for G-actin |
| Murrell et al. [48] | In IE | EPC 53% Cholesterol 37% DGS-Ni 10% | G-actin 13 mM Arp2/3 0.24 mM gelsolin 0.1 mM ADF-cofilin 4 mM VCA-His 2.2 mM | Histidin-nickel | Viscous dissipation slows down GUV spreading on adhesive substrates |
| Campillo et al. [50] Guevorkian et al. [52] | In IE/EF | EPC 58–62% DGS-Ni 5–10% Cholesterol 37% PE-Biotin 0.1–1% | Actin 3–13 mM Arp2/3 0.24–0.45 mM VCA-His 2.2–7.8 mM Gelsolin 0–0.1 mM ADF-cofilin 0–4 mM profilin 0–10 mM | Histidin-nickel | Friction induced by membrane nanotube extrusion and lipid diffusion hindered by actin cortex |
| Myosin II + branched networks Carvalho et al. [55] | Out EF | PE-Biotin 0.1% | Actin 1–3 μM S-pVCA 100–160 nM profilin 3 μM Arp2/3 50 nM CP 10–50 nM | biotin-SpVCA | Peeling of actin cortex or GUV squeezing |
| Myosin II + branched networks Caorsi et al. [57] | Out EF | PE-Biotin 0.1–1% | SpVCA 350 nM profilin 3 μM Arp2/3 35 nM CP 20 nM 50 nM Myosin II | biotin-SpVCA | Myosin motors increase actin cortex tension |

Continued
whereas, in a smaller GUV, they stay at the periphery to minimize their bending energy \cite{15}. A detailed study of the mechanism by which confinement affects the structure and mechanics of actin filaments was done in droplets instead of GUVs by Claessens et al. \cite{18}.

| Article                      | In vs. Out GUVs Preparation | Lipid composition | Protein mixture | Membrane attachment | Sollicitation/Deformation |
|-----------------------------|-----------------------------|-------------------|-----------------|----------------------|---------------------------|
| Loiseau et al. \cite{59}    | In cDICE                    | EPC DGS-Ni 0.1–10% PE-Biotin 2.5% | actin 10 \(\mu\)M His-anilin 0.1–1 \(\mu\)M Myosin II 0.5–1 \(\mu\)M | Histidin-nickel           | Formation of blebs        |
| Dürre et al. \cite{61}      | In cDICE                    | EPC DGS-Ni 0.1–10% PE-Biotin 2.5% | actin 3 \(\mu\)M His-VCA 300 nM Arp2/3 300 nM Profilin 13.5 \(\mu\)M CP 20–180 nM Myosin II 5 \(\mu\)M | Histidin-nickel           | CP-induced membrane bulging in/out and fission under Myosin II activity |
| Bashirzadeh et al. \cite{30} | In Modified cDICE           | DOPC 70% Cholesterol 25% DGS-Ni 5% | actin 5 \(\mu\)M His-VCA 1 \(\mu\)M Arp 2/3 1 \(\mu\)M Myosin II 63 nM Fascin 1 \(\mu\)M \(\alpha\)-actinin 0.5 \(\mu\)M | Histidin-nickel           | Blebs formed by branched contractile actomyosin ring |

**Table 1** Table summarizing the experimental details of all articles presented in the five sections of the text: inside or outside geometry, GUVs preparation techniques, lipid composition of the GUVs membrane, composition of the actin and actin-related proteins mixture, interaction of the actin structures with the GUVs membranes and effects of the actin on the membrane

**Figure 1.** Schematics of biomimetic systems made of GUVs coupled to actin filaments formed without NPFs. GUVs coupled to F-actin in the outside (A) and inside (B) configurations; GUVs encapsulating F-actin in the presence of crosslinkers (C) \(\alpha\)-actinin; (D,E) fascin that induces bundles or rings in the lumen of the GUV (F) GUVs encapsulating F-actin in the presence of myosin II motors.
Actin filaments formed without NPFs in the presence of crosslinkers

In this section, we show how actin filaments polymerized without NPFs in the presence of crosslinkers modulate the structure of the actin network and, in some cases, the GUV shape (Figure 1C–E). To the best of our knowledge, no articles study filaments polymerized without NPFs and crosslinkers on the external membrane of GUVs; we thus discuss papers where filaments and crosslinkers are co-encapsulated. As in the case of section I, the competition between the elasticity of the membrane and that of actin-based structures controls actin organization inside GUVs. In the presence of crosslinkers, the elasticity of actin-based structures varies due to structural differences between crosslinkers, actin to crosslinker ratio (rCA), and the length of the filaments, resulting in a wide variety of actin structures.

When F-actin crosslinked by α-actinin is encapsulated in GUVs (Figure 2C), two different organizations appear depending on the GUV size. At temperatures below 15°C, at which polymerization dynamics are slowed down and bundling formation favored, ring-like structures form in GUVs smaller than 12 μm, whereas spiderweb-like structures form in larger GUVs [19]. In addition, F-actin asters form at the periphery of GUVs larger than 16 μm at the same rCA of 1:10, and increasing the rCA does not affect actin organization [20]. However, bundles emerging from the asters are preferentially located at the GUV periphery to minimize bending energy. In the last article, GUVs were formed using an adapted version of the cDICE (continuous droplet interface crossing encapsulation) method [21,22].

In the presence of fascin (Figure 2D), F-actin forms bundles that are able to generate enough force to deform the membrane [23,24]. In GUVs smaller than 8 μm, stiff bundles (rCA = 0.2) form membrane protrusions, whereas soft bundles (rCA = 0.05) have lower bending energy and form ring or web-like organizations [24]. Confinement forces bundles to bend and accumulate at membranes if they are sufficiently long [25] (Figure 2E) or if membrane bending rigidity is high [24]. Moreover, increasing fascin concentration favors bundle to bundle attraction, increasing their overall persistence length and leading to membrane protrusions [26].

Lastly, simultaneous encapsulation of α-actinin and fascin still reveals an effect of the confinement over actin organization as previously described, as well as the competition between fascin and α-actinin for a finite amount of actin filaments, which results in α-actinin significantly impairing the protruding effect of fascin in these conditions [20,26].

Actin filaments formed without NPFs in the presence of myosins II

In this section, we discuss works that observe the effects of co-encapsulating F-actin formed without NPFs together with myosins II. Similar actomyosin networks in the outside configuration have never been reported.

Using the Inverted Emulsion (IE) method [27], Takiguchi et al. encapsulated F-actin and heavy-meromyosin (a fragment of myosin II) in GUVs [28]. The GUVs were of several tens of micrometers, much larger than ∅p. F-actin was homogenously distributed when encapsulated alone, and GUVs were always spherical. However, when heavy-meromyosin and F-actin were encapsulated, the resulting GUVs had non-spherical shapes. The author determined that when actin filaments are crosslinked by heavy-meromyosin, the resulting actin bundles are stiff enough to deform the lipid bilayer.

Other studies have encapsulated F-actin and bipolar myosin II filaments (Figure 2D), able to actively pull on the actin filaments [29]. The F-actin contained biotinylated actin monomers that can attach to the GUV membrane through biotin-streptavidin bonds. The GUVs were formed spontaneously during the hydration in the solution containing the proteins of a hybrid film of agarose and lipids. No external force such as an electric field or centrifugation was applied to form the GUVs. No membrane deformation was observed even if actin was attached via biotin-streptavidin bonds. Still, actin clusters were observed at high myosin II concentration, supposedly because myosin-induced forces ruptured actin-membrane links and condensation of actin filaments in one or few clusters.

To form contractile actomyosin rings, Bashirzadeh et al. [30] encapsulated actin filaments and myosin II in the presence of crosslinkers. They show that the probability of ring formation is higher when the fascin to α-actinin ratio is high and GUV size is smaller than 15 μm. Such conditions seem to impair the formation of protrusions induced by fascin and the formation of asters by α-actinin, as previously shown [20].
Figure 2. Schematics of biomimetic systems made of GUVs coupled to dynamic F-actin networks nucleated by the Arp2/3 complex.

In the outside configuration: reconstitution of actin-rich membrane domains (A), filopodia-like protrusions (B), filopodia-like protrusions and endocytic pits-like structures (C), global GUV deformation (D), and actin-coated nanotubes (E); In the inside configuration: reconstitution of Arp2/3-generated networks allows forming a cortex-like structure (F); studies on the effect of the cortex on GUV spreading (G) and on membrane dynamics by nanotube pulling experiments (H). Actomyosin networks coupled to the GUV surface (I) or GUVs doublets (J) show myosin-induced shape changes; Encapsulation of Arp2/3 generated actin networks comprising myosin II motors which then induce membrane protrusions (K,L).
Actin network polymerization with NPFs at the membrane

The next step in the reconstitution approach is to mimic the situation observed, for instance, in the cell lamellipodium, where NPFs are attached to the membrane, and actin dynamically polymerizes at the membrane.

Experiments in the ‘outside’ configuration are inspired by works mimicking in vitro the actin-induced propulsion of Listeria using beads coated with NPFs. When such beads are bathed either in cell extracts or in a minimal mixture of purified actin and regulatory proteins, they are propelled by the forces generated by actin polymerization at their surface. The process starts with filaments nucleation at the bead surface. The nucleation leads to the growth of an actin gel around the bead. As this gel grows from the bead surface, mechanical stresses are accumulated at the gel’s external surface and eventually lead to its rupture. At this point, the symmetry of the system is broken, and the bead is pushed forward by a cylindrical structure made of actin gel, sometimes referred to as an actin ‘comet’ [31]. This ‘bead assay’ was extended to liquid droplets [32] and GUVs coated by NPFs in cell extracts [33,34]. These last experiments have revealed the spatial distribution of polymerization-induced forces at the GUV surface during their propulsion by an actin ‘comet.’ Here, we focus on GUVs bathed in cocktails of pure proteins.

Liu and coworkers [35] used GUVs made of a ternary lipid mixture exhibiting a thermally controlled liquid–liquid phase separation. Below a critical temperature, their membrane segregates between liquid-ordered and liquid disordered domains; over this temperature, the membrane is homogeneous [36]. The actin network shifts the transition temperature of lipid phase separation and influences the spatial distribution of the induced domains (Figure 2A). Because cell membranes are close to phase separation [37], actin networks could contribute to regulating the spatial organization of cell membranes.

The same researchers also showed that branched actin networks polymerizing around GUVs form finger-like protrusions [38] (Figure 2B). In this experiment, there were no crosslinkers inducing bundles as in the articles presented in section II [24,26]. As discussed above, the formation of finger-like protrusion from branched actin networks stems from the balance between membrane and filaments elasticity. When few filaments push against a membrane, the energetic cost for membrane deformation favors gathering these filaments into a single bundle surrounded by a membrane nanotube. In this case, the energy for membrane deformation is reduced. Contrary to a single filament, the forces induced by the polymerization of several parallel filaments can overcome the force required to form such cylindrical membrane tubes, which depends on the membrane bending energy and tension [39]. These results show that membrane elasticity can shape the architecture of actin networks.

Simon et al.[40] showed two types of actin-induced membrane deformations on GUVs using branched Arp2/3-nucleated networks in the presence of profilin and capping protein. They observed membrane tubes pulled toward the GUV exterior and ‘spikes’ toward their interior (Figure 2C). The spikes are observed at low membrane tension and small mesh size of the actin network, while only tubes form for larger tension and mesh size. The tubes, created solely by the retrograde flow induced by actin polymerization from the membrane, share similarities with endocytic bulges even though no curvature-inducing proteins are present. The spikes contain a dendritic network comprising Arp2/3 and CP and are thus very different from the protrusions induced by bundled actin filaments observed in [38]. They form because local heterogeneities in the growth of the actin gel induce a normal pressure acting on the membrane. If such heterogeneity reaches a critical size, it leads to the formation of a spike. The results from [40] clearly show that the same polymerizing dendritic actin network can simultaneously deform membranes in both directions. These deformations in both directions were observed in the ‘inside’ geometry in 2018 in the article by Dürre et al. that will be presented below.

Using the same assay, Simon et al. [41] then investigated how both membrane tension and the presence of CP control shape changes of GUVs. The presence of CP favors membrane deformations for all values of the membrane tension, whereas, without CP, membrane deformations occur only at low membrane tension. In another study [42], these actin gel-covered GUVs were osmotically deflated, leading to global shape changes (Figure 2D). The actin network thickness controls the type of shape changes: for thicknesses higher than 1.5 μm, wrinkles with a wavelength between 5 and 25 μm appear, whereas smaller gel thicknesses lead to large-scale buckling, which results in crescent-shaped GUVs. Wrinkling is favored for thick cortices because buckling implies a strong local deformation of the actin gel, which costs more energy than the formation of wrinkles on all the vesicle surface.

Finally, the same assay was adapted to reconstitute dynamic actin networks at the surface of membrane nanotubes formed from GUVs (Figure 2E) [43]. The objective of this study was to decipher the largely
unknown mechanisms by which actin dynamics contribute to the remodeling of membrane nanotubes inside the cell [44]. During intracellular trafficking, nanotubes are pulled by molecular motors walking on microtubules at the velocity of one micrometer per second. Therefore, to investigate the mechanical stability of actin-coated nanotubes, they were submitted to elongation at this velocity. When the tubes are covered by actin sheaths thicker than 100 nm, they fully stabilize the tubes that cannot be deformed in the range of forces accessible to optical tweezers. Oppositely, thinner actin sheaths break during tube elongation. This breakage results in heterogenous tubes containing regions covered with actin and regions of bare membrane tubes. The membrane nanotubes are thicker under the actin coat. In contrast, regions devoid of actin have a smaller diameter that relaxes, expanding within minutes. These actin-free regions could provide enough time and the high curvature required for the binding of other proteins that act on tube stability. In addition, Atomic Force Microscopy (AFM) experiments directly probe the effect of the actin coat on tube mechanics and morphology [45] and validate the estimation of the actin sheath diameter presented.

Reassembling a dynamic actin network at the inner surface of a GUV (i.e. in the ‘inside’ configuration) to mimic the cell cortex was an experimental challenge. The process was achieved by preparing GUVs containing the actin machinery in non-polymerizing conditions using the IE technique [46]. A histidine-tagged NPF (VVCA-His) is specifically attached to the inner GUV membrane containing nickel lipids. The authors demonstrated that this NPF induces actin polymerization at the GUV membrane when the inside concentration of salts and ATP is tuned toward polymerization conditions using pores inserted in the membrane (Figure 2F). Membrane protrusions emerge when this type of cortex-containing GUVs is osmotically deflated [47].

The system developed in [46] was then used in several articles as a tool to investigate the mechanical role of the cell cortex in a biomimetic approach. First, [48] studied the spreading of the cortex-containing GUVs on adhesive substrates (Figure 2G). This article showed that a dense and connective actin cortex slows the early stages of the spreading, which highlights the role of viscous dissipation in the cortex at the onset of cell spreading.

The effect of the actin cortex on membrane dynamics has also been probed using membrane nanotube formation (Figure 2H). Nanotubes can be formed with optical tweezers from GUVs held in a micropipette in order to maintain a constant membrane tension. The nanotube length can then be varied to study membrane dynamics [49]. With this assay, [50] showed that the preparation technique of the GUVs, EF or IE, had a more significant effect on membrane dynamics than the presence of an actin cortex. Indeed, GUVs with the same lipid composition formed by these two techniques show no difference in their bending rigidity but a dramatic difference in their membrane friction. In the case of IE-formed GUVs, the membrane dynamics are very close to the ones observed on Giant Plasma Membrane Vesicles extracted from living cells [37]. Surprisingly, the actin network generates only an additional friction force, allowing for the reevaluation of membrane composition and membrane-cortex attachment roles on membrane dynamics. Nanotubes can also be formed by applying a hydrodynamic flow to a GUV attached to a microneedle [51]. With this assay, nanotubes are shorter on GUVs with an actin cortex at the same pulling force than their bare homologs [52], showing that such reconstituted actin cortices mechanically resist lipid movement. Finally, the cortex-containing GUVs have been used to demonstrate the role of actin on the scission of tubules induced by Shiga toxin towards the GUV interior [53]. In the presence of a cortex, these tubules are cut and float freely in the GUV lumen. The hypothesis for this scission is that actin induces nanodomains in the membrane, which could provoke tubule scission because of the energetic cost of domain coexistence, as proposed in [54].

**Myosin II effect on polymerized Arp2/3-nucleated actin networks**

Myosin II-induced contraction of dendritic actin networks at the external surface of GUVs leads to an increase in the mechanical tension in the network (Figure 2I). This results in either a global crush of the GUV or the peeling of the actin network that collapses on one side of the GUV. The first case is favored by actin-membrane solid attachment, high myosin II concentration, and network cohesiveness [55]. Peeling is a symmetry-breaking event happening when the built-up tension exceeds the critical stress, at which the actin network ruptures [56]. Quantifying the tension in the actomyosin network has been achieved by measuring the contact angle between GUV doublets linked by streptavidin-biotin links [57] (Figure 2I). When actin filaments polymerized without NPF are attached to the doublet surface, its tension remains constant. In contrast, the polymerization of an actin network increases the doublet tension. When myosin II is added, tension increases in both conditions: for
actin filaments polymerized without NPF, it strongly increases with myosin II whereas, for gels, polymerization and myosin-induced tension synergistically combine to establish a high tension in the actomyosin shell, as it is the case in living cells [58].

Inside GUVs, Loiseau et al. were the first to adapt the cDICE method [21] to form a contractile actomyosin cortex [59] using anillin as a crosslinker and membrane-cortex linker. In this assay, membrane protrusions resembling cellular blebs were observed (Figure 2K). Indeed, myosin II-induced stresses result in the contraction of the cortex and, therefore, lead to an inward pressure that counteracts the outward osmotic pressure. In regions where heterogeneities in the cortex lead to a weaker membrane-cortex attachment, this pressure induces the detachment of the membrane and its blebbing. This biomimetic assay shows in vitro the dependence of the blebbing process on the myosin II concentration and membrane-cortex attachment. Similar bleb-like protrusions were observed in the presence of α-actinin and fascin as crosslinkers [30].

Following [59], the same group showed how the actomyosin dynamics affected the spreading of these GUVs on adhesive substrates [60]. The active remodeling of the cortex provides GUVs with an excess membrane area that allows it to sustain the adhesion-induced increase in its membrane tension and prevents it from bursting. Finally, [61] showed that CP controls the shape of the actomyosin cortex (Figure 2L). At low CP concentrations, membrane protrusions towards the exterior appear, whereas concave regions are observed at higher concentrations (60–120 nM). The effect of motors on these membrane deformations is therefore also dependent on the CP concentration: formation of star-like clusters at low CP, actin-rich stable domains at intermediary CP, and fission of membrane protrusions at high CP.

**Perspectives**

- The studies presented here have shown the minimal components necessary to reconstitute in vitro some biological processes observed in cellular experiments. Based on such assays, it is possible to tune the experimental parameters as actin-membrane attachment, actin dynamics, or myosin-induced contractility to precisely decipher each process’s physical mechanisms.

- The works presented here show that some processes observed in the cell can be reproduced in vitro and mechanical models tested. Examples of such processes are blebbing, membrane tubulation or invagination, cell spreading, and the formation of contractile actomyosin rings that are able to induce membrane constriction.

- An essential aspect of future reconstitution studies would be the control of actomyosin dynamics in space and time. Recall that different actomyosin networks are simultaneously observed inside a living cell, and their dynamics are finely regulated. Therefore, a control on actin polymerization dynamics, motor activity, and membrane structure could be exerted using light-activated compounds (e.g. caged-ATP, blebbistatin), using DNA nanotechnology [62], or other external signals [63]. Such responsive systems would trigger the local assembly of artificial filopodia or blebs and allow for the investigation of how these different actin-based structures compete for actin-monomers or affect the shape of the GUVs.

- As recent studies on living cells have shown how actin networks are interconnected with other cytoskeletal players [64], another critical aspect for future work would be the reconstitution of these crosstalks. Biomimetic studies could play an essential role in investigating this coupling by using model systems; this potential can be seen in [65], where actin and keratin networks are co-encapsulated in GUVs. More generally, other players have to be coupled with cytoskeletal networks, as in [66], where the actin machinery is coupled to ATP production systems. These studies rely on the future development of cutting-edge encapsulation techniques, such as microfluidics [67].
Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author contribution
Authors contributed equally.

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Abbreviations
ABPs, actin binding proteins; CP, capping proteins; GUVs, giant unilamellar vesicles; IE, inverted emulsion; NPF, nucleation promoting factors.

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