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REVIEW ARTICLE

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Benefits and challenges of reconstituting the actin cortex

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Abstract

The cell's ability to change shape is a central feature in many cellular processes, including cytokinesis, motility, migration, and tissue formation. The cell constructs a network of contractile proteins underneath the cell membrane to form the cortex, and the reorganization of these components directly contributes to cellular shape changes. The desire to mimic these cell shape changes to aid in the creation of a synthetic cell has been increasing. Therefore, membrane-based reconstitution experiments have flourished, furthering our understanding of the minimal components the cell uses throughout these processes. Although biochemical approaches increased our understanding of actin, myosin II, and actin-associated proteins, using membrane-based reconstituted systems has further expanded our understanding of actin structures and functions because membrane–cortex interactions can be analyzed. In this review, we highlight the recent developments in membrane-based reconstitution techniques. We examine the current findings on the minimal components needed to recapitulate distinct actin structures and functions and how they relate to the cortex's impact on cellular mechanical properties. We also explore how co-processing of computational models with wet-lab experiments enhances our understanding of these properties. Finally, we emphasize the benefits and challenges inherent to membrane-based, reconstitution assays, ranging from the advantage of precise control over the system to the difficulty of integrating these findings into the complex cellular environment.

KEYWORDS
actin, actin crosslinkers, actin-membrane tethers, composite material, myosin II (myosin)

1 | INTRODUCTION

The actin cytoskeleton directly impacts many critical cell functions and shape changes, such as cell division, adhesion, migration, and polarity formation. To make these shape changes, the cell harnesses the contractility machinery, which is poised to sense and respond to mechanical cues from its environment (West-Foyle & Robinson, 2012). This machinery consists of actin, myosin II (also referred to here as "myosin" for simplicity; however, a multitude of other myosin paralogs exist that contribute to cortical function), and actin-associated proteins which construct large-scale assemblies underneath the cell membrane, forming the cortex. The cortex is built upon filamentous actin (F-actin), which forms complex structures and integrated networks to give the cell its shape. F-actin is polymerized from globular actin (G-actin). This polymerization is catalyzed by actin nucleators such as the Arp2/3 complex, which is commonly associated with the formation of branched actin networks when activated, or formin, which forms elongated parallel filaments (Svitkina, 2018). Actin nucleators are typically activated by nucleation-promoting factors.

Abbreviations: GUV, giant unilamellar vesicle; PIP2, phosphatidylinositol 4,5 bisphosphate; SLB, supported-lipid bilayer.

Specifically, Scar/WAVE, WASP, or N-WASP (proteins containing a VCA domain) activate the Arp2/3 complex to promote the formation of branched actin networks (Pantaloni et al., 2000). Similarly, the nucleation promoting factor WISH/DIP/SPIN90 also actives the Arp2/3 complex to promote the formation of de novo linear filaments (Cao et al., 2023; Wagner et al., 2013). Actin filaments can be lengthened by elongation factors such as Ena/VASP (Hüttelmaier et al., 1999), shortened by actin severing proteins such as cofilin and gelsolin (Blanchoin et al., 2000; Selden et al., 1998), or have elongation prevented by capping proteins (Hartwig & Kwiatkowski, 1991). Additionally, actin filaments can be crosslinked together or anchored to the membrane by actin crosslinkers such as α-actinins, filamins, fascin, talin/vinculin, and ezrin-radixin-moesin (ERM) proteins (Fehon et al., 2010; Kelley et al., 2020; Pollard, 2016). Actin polymerization can generate force to deform the cell membrane. Likewise, myosin, a force generating motor protein, can transmit force within the actin cortex linked to the membrane, therefore causing membrane deformation and cell shape changes (Vicente-Manzanares et al., 2009).

The field has had many advances in our understanding of the actin cortex through in vivo experiments and biochemical assays. However, within the past few decades, the field has worked to understand the complex structure, organization, and regulation of the actin cortex through in vitro reconstitution. A living cell is a highly complex system with many components interacting with each other, making it hard to decipher which cytoskeletal components are necessary for each function. In vitro reconstitution of the actin cortex on or within lipid membranes has allowed us to understand how each component of the cortex contributes to the formation of different structures and affects mechanical properties, while simultaneously determining the components necessary for the development of a synthetic cell (Baldauf et al., 2022; Kandiyoth & Michelot, 2023; Lopes Dos Santos & Campillo, 2022; Van de Cauter et al., 2023). While in vitro reconstitution has expanded our knowledge of actin and actin-associated proteins, there has also been a rise in co-processing wet-lab experiments with computational models to explain the observed biological phenomena and to further generate predictions that can be experimentally tested (Litschel et al., 2021; Saltini & Mulder, 2020; Wubshet et al., 2023).

Although there are many advantages to actin cortex reconstitution, many challenges in this bottom-up approach also exist. In cells, we often look at cortical actin structures that underlie the cell membrane; however, the addition of actin on or within a membrane structure does not cause actin accumulation near the membrane. Instead, actin will often uniformly distribute through its confined area (Abu Shah & Keren, 2014; Dürre et al., 2018; Limozin et al., 2003; Limozin & Sackmann, 2002; Tsai & Koenderink, 2015). Therefore, many approaches have been developed to localize actin to the membrane. These methods often use engineered recombinant biomolecules or crowding agents to achieve a cortical actin distribution. Using these methods deviates from the cellular mechanism, therefore limiting the system's biological relevance. Regardless of the method used to obtain a cortical actin distribution, reconstituted systems also lack many proteins that can contribute to the actin structure or mechanical property being mimicked. Thus, the question of how missing components would affect the system always persists. The addition of more components could change the actin cortex structure, affect localization of other components, or affect the mechanical properties of the reconstituted system; however, obtaining the additional components can be difficult and time consuming. In this review, we will examine the methods used to study actin and actin-associated proteins in reconstituted systems, summarize the discoveries from reconstitution experiments, explore computational modeling's roles in contributing to our understanding of the actin cortex, and assess the potential benefits and challenges of this reconstitution approach.

2 | MEMBRANE-BASED RECONSTITUTION EXPERIMENTS PROVIDE A MORE COMPLEX SCENARIO THAN MINIMAL PURIFIED BIOCHEMICAL ASSAYS AS MEMBRANE–CORTEX INTERACTIONS ARE INCORPORATED, ALLOWING FOR THE STUDY OF THE INTERPLAY BETWEEN MEMBRANE AND CORTEX REMODELING

Membrane-based, actin cortical reconstitution experiments have been performed on both quasi-2D bilayers and 3D mono- or bilayers. To create a quasi-2D actin cortex, actin and actin-associated components are placed in a chamber containing a supported-lipid bilayer (SLB). To recapitulate the 3D environment of the cell, giant unilamellar vesicles (GUVs), also known as cell-sized liposomes, are often utilized. Actin and actin-associated proteins can be encapsulated inside of the GUV, or the GUV can be put in a chamber containing actin components. Using fluorescent labels and microscopy techniques, the assembly of actin structures on the cortex can be analyzed. The use of these membrane-based reconstitution techniques provides a platform for analyzing the actin cortex's association with the cell membrane; however, each technique carries its distinct advantages and challenges.

SLBs are lipid bilayers that cover a glass or mica surface in which actin and actin-associated proteins can then be added to study actin interactions (Figure 1a). SLB development begins with the formulation of small unilamellar vesicles that are plated into a glass chamber, dehydrated, and rehydrated to ensure an even distribution of lipids across the chamber. The membrane can be prepared in various ways to localize actin to the membrane, and actin can be added to the chamber to create a minimal cortex. Proteins of interest can also be added to observe changes in actin localization or structure formation (Vogel, 2016).

The 2D environment of SLBs offers a unique advantage over the use of 3D environments because single molecule analysis and mechanistic studies are easier to visualize and quantify, due to the confined nature of SLBs. For example, actin's interactions with myosin motors and myosin's role in filament turnover have been studied to develop a better understanding of cortex growth and contraction (Murrell & Gardel, 2012; Sonal et al., 2019). Protein localization within the actin cortex can also be studied with

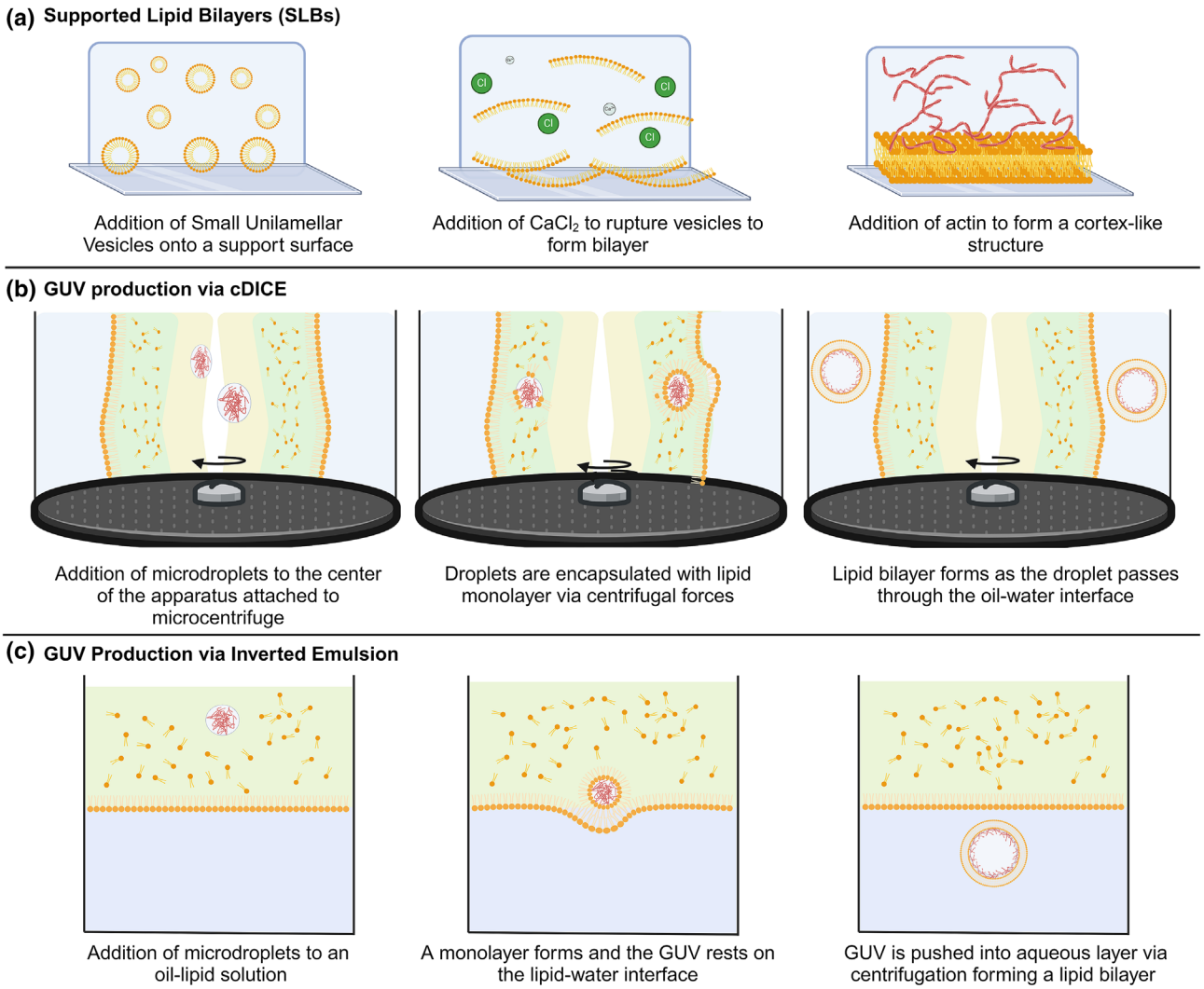

FIGURE 1 Schematic of methods for three different experimental setups used in cortex reconstitution assays. (a) Supported lipid bilayers (SLBs) form by adding monolayer vesicles to a glass or mica surface, which are broken apart with calcium chloride to form a lipid bilayer. Actin and actin-associated proteins can be added to form structures along the bilayer (Vogel, 2016). (b) cDICE consists of three layers: a decane layer (yellow) to help round and disperse droplets, an oil lipid layer (green) where droplets gain their first lipid layer, and an aqueous layer (blue) where final GUVs are deposited. Droplets are moved through each layer via a light centrifugal force (Abkarian et al., 2011). (c) The inverted emulsion method consists of an oil-lipid layer (green), resting on top of the aqueous layer (blue). Droplets are added to the oil-lipid layer where a lipid monolayer forms. Via centrifugal force, the droplet is pulled into the aqueous layer and forms a GUV (Yamada et al., 2014). Figure created using Biorender.

fluorescence microscopy and has been applied to several proteins such as VASP and Rho (Landino et al., 2021; Nast-Kolb et al., 2022). Additionally, through the study of protein–lipid binding kinetics, SLBs were determined to be useful in gaining thermodynamic insight into the lipid interface and actin network structures (Dobramysl et al., 2021; Gat et al., 2020; Nye & Groves, 2008). These additional thermodynamic and structural insights can be further applied to GUV studies or 3D computational models, as SLBs fail to account for the environmental factors of a 3D geometry. Additionally, membrane deformation, such as protrusions, can be analyzed with single molecule analysis techniques; however, they lack the advantage of a spherical shape to study the rapid diffusion and filament formation in a cell.

On the other hand, GUVs are synthetic liposomes, typically with a lipid bilayer, that can contain cortex proteins that self-assemble into cortex-like structures. Due to their 3D shape, GUVs better mimic a cellular environment and are ideal candidates for reconstitution experiments.

A variety of methods have been developed to create GUVs. One of the first techniques used to create GUVs was electroformation, which uses an electrical field to create a liposome (Mitov et al., 1993). Since then, it has been used to investigate the rheology, elasticity, and size of the actin cortex (Helfer et al., 2000, 2001a; Limozin et al., 2003). The addition of other actin-associated components within or on the GUVs has allowed for a deeper understanding of cortex formation (Liu et al., 2008; Simon et al., 2018). Continuous droplet

interface crossing encapsulation (cDICE) is a popular method of making GUVs with an actin cortex on the inside. In essence, using a light centrifugal force, droplets with proteins of interest are dragged through layers of lipid-in-oil dispersion, encapsulating the component while forming a vesicle (Figure 1b) (Abkarian et al., 2011). Several studies have used this technique to determine the minimal components necessary for the formation of different actin structures or cortical functions. There have been multiple modifications to the cDICE method, such as using a second chamber or creating microdroplets in the emulsion before addition to the centrifuge apparatus to create more monodisperse droplets (Bashirzadeh et al., 2022). cDICE and other modified versions are extremely useful when creating GUVs that encapsulate actin components. Inverted emulsion is another GUV formation technique that takes advantage of an oil–water interface (Figure 1c). By moving microdroplets with or without actin components through an interface boundary between oil and water, GUVs can form (Yamada et al., 2014). This technique is particularly useful when focusing on actin–lipid interface interactions. Through the combination of inverted emulsion and cDICE techniques, emulsion droplet interface crossing encapsulation (eDICE) was developed in which preformed droplets with all necessary components were pulled through a cDICE setup, rather than droplets formation occurring during the centrifugation process. eDICE allows for better control over GUV size, cortex component concentration, and actin polymerization time; therefore, this method is ideal for reconstitution experiments with low protein concentrations and fast time constraints (Baldauf et al., 2023). Additionally, eDICE can be used to make GUVs of different shapes and curvatures (Baldauf et al., 2023). Another method to create GUVs is agarose gel swelling in which lipids are dried onto a layer of agarose, and the addition of a swelling solution causes lipids to "peel" off the agarose and enter suspension to form a liposome (Tsai & Koenderink, 2015). Agarose gel swelling has similar advantages to eDICE with greater control over protein concentration, especially if the proteins are prone to aggregation; however, encapsulation efficiency and control over lipid composition between the inner and outer leaflet is limited (Van de Cauter et al., 2023).

Forming the cortex on the inside of a GUV naturally mimics a cellular environment and is thus useful for visualizing protrusions and actin organization within a 3D space (Luo et al., 2014; Sakamoto et al., 2023). However, once the GUV is formed, the droplet and its contents are essentially confined, making control over concentration, salinity, pH, and other factors very difficult, despite the aforementioned progress in GUV formation. Nonetheless, the addition of pores within the membrane can allow for the exchange of solutes between the inner and outer environments. GUVs with actin linked to the outside leaflet of the membrane can offer similar benefits to SLBs because forming networks outside the liposome allows for better control over the environment, including the easy addition of drugs to the surrounding solution (Simon et al., 2019; Wubshet et al., 2023). However, the GUV is surrounded by an "infinite" amount of protein unlike the amount of protein encapsulated in a cell, and its inverted nature allows protrusions to form more easily. As mentioned before, SLB and GUV experiments can be done simultaneously to collect data on systems in both 2D and 3D models.

Although less common than the use of SLBs and GUVs, two other techniques have been used to reconstitute the cellular cortex. Specifically, droplets have been formed through either water-in-oil or oil-in-water emulsions. Through the water-in-oil emulsion technique, water droplets containing actin components are placed in an oil phase to create an actin cortex on the inside of the aqueous droplet. Through the oil-in-water emulsion technique, oil droplets are placed in a water solution containing actin components to create an oil droplet with an actin cortex formed around it. These approaches are useful for analyzing single molecule behaviors as well as the thermodynamic favorability of different actin structures and self-assembly, as analyzing the free energy and entropic payoffs can be useful in understanding overall stability (Abu Shah & Keren, 2014; Claessens et al., 2006). Overall, the use of SLBs, GUVs, and other techniques to reconstitute the cortex have been developed to better our understanding of the actin cortex and its components; however, the diversity in techniques used can create issues in generating reproducible results.

3 | DIFFERENT MECHANISMS HAVE BEEN UTILIZED TO PROMOTE ACTIN ACCUMULATION AT THE MEMBRANE FOR THE FORMATION OF A MINIMAL ACTIN CORTEX

Cellular shape changes are driven by actin cortex reorganization. The cortex is formed by a network of filamentous actin that directly underlies the membrane. To study actin in a reconstituted system, it was essential to determine the cytoskeletal components necessary to achieve a cortically distributed actin network. When actin is encapsulated in GUVs, actin filaments often uniformly distribute throughout the GUV's lumen (Dürre et al., 2018; Limozin et al., 2003; Limozin & Sackmann, 2002; Tsai & Koenderink, 2015). Specifically, this happens in large GUVs (greater than 8 μm in diameter) especially as the GUV diameter approaches and/or exceeds the persistence length of F-actin. However, actin has a mild cortical distribution in small GUVs because the energetic consequences on the actin filament due to its bending energy are decreased when the actin filaments accumulate at the membrane (Häckl et al., 1998; Limozin et al., 2003). Therefore, the volume of confinement affects actin's distribution in GUVs. Changing the lipid composition, changing the ion valency, or adding different actin-associated proteins enables the formation of actin cortices in systems that would otherwise have uniformly distributed filamentous actin. In the presence of a zwitterionic lipid, actin filaments adsorb to the membrane, creating a cortical distribution. Since actin is negatively charged, the addition of positively charged lipids increases actin intensity at the membrane. This cortical distribution of actin was lost when the concentration of Mg^{2+} (divalent ion) was decreased or K^{+} (monovalent ion) was substituted because Mg^{2+} enhances weak interactions between actin and neutral lipids (Figure 2a) (Limozin et al., 2003). Similarly, SLBs created with different lipid compositions—neutral lipids

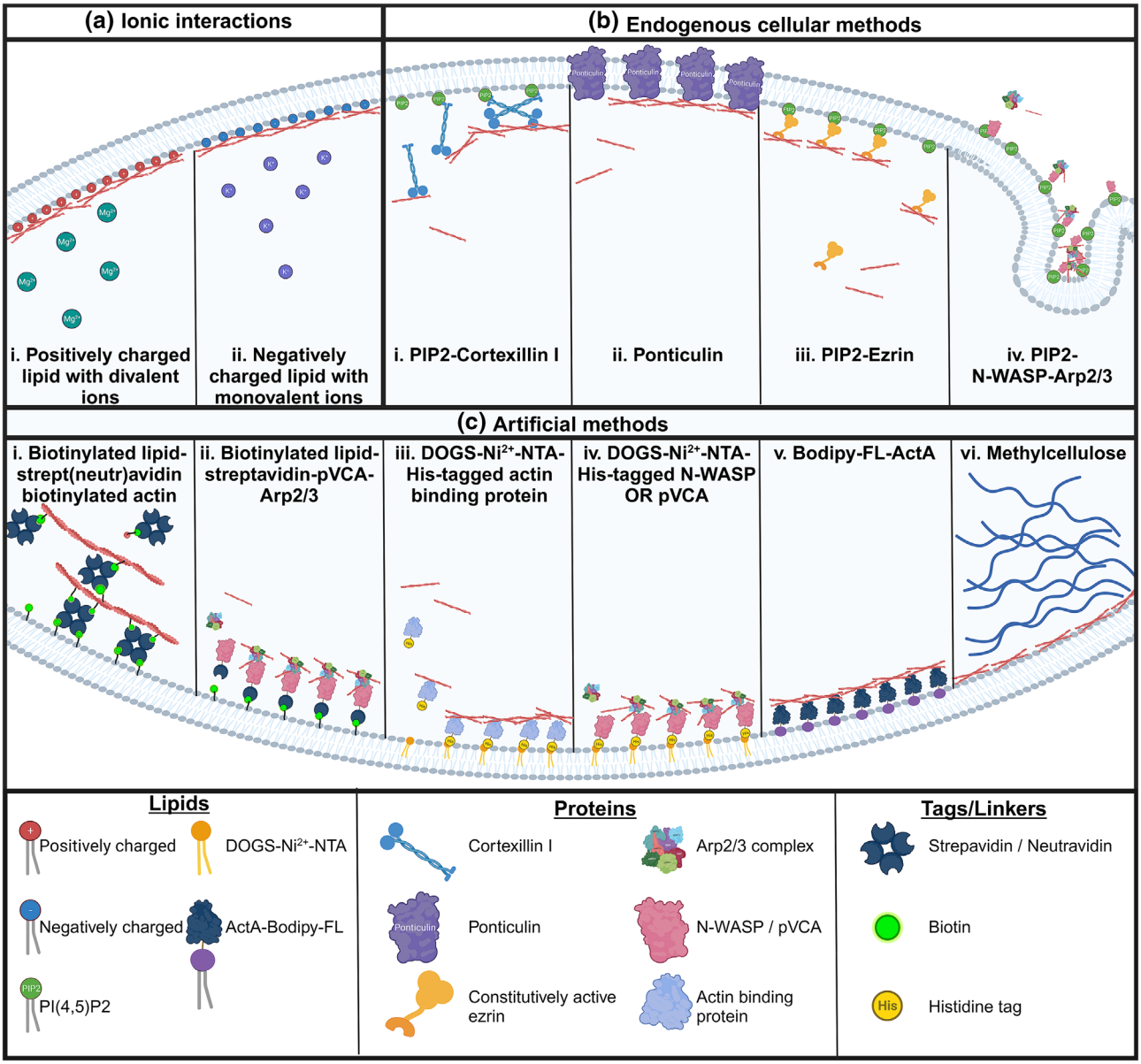

FIGURE 2 Methods for obtaining a cortical actin distribution. (a) Ionic interactions cause actin to adsorb to the membrane (Schroer et al., 2020). (i) Positively charged lipids coupled with a divalent ionic buffer causes actin–membrane adsorption. (ii) Negatively charged lipids coupled with a monovalently ionic buffer causes actin–membrane adsorption. (b) Endogenous proteins cause actin to accumulate at the membrane (i) Actin-crosslinker, cortexillin I, binds to PIP2 and actin (Luo et al., 2014). (ii) Ponticulin, integral membrane protein, has a high affinity for actin (Barfoot et al., 2008). (iii) Constitutively active ezrin binds to PIP2 and actin (Nöding et al., 2018). (iv) On the outside of a GUV membrane, N-WASP binds to PIP2 and activates the Arp2/3 complex to induce actin polymerization, forming a branched actin cortex that produces inward-directed membrane protrusions (Simon et al., 2019). (c) Artificial methods have been developed to cause actin to accumulate at the membrane. (i) Biotinylated G-actin monomers bind to biotinylated lipids via streptavidin or neutravidin (Helfer et al., 2000). (ii) Recombinant streptavidin-pVCA linked to a biotinylated lipid causes activation of the Arp2/3 complex which forms branched actin cortices (Carvalho et al., 2013). (iii) DOGS-Ni^{2+}-NTA can bind to His-tagged actin binding protein causing actin accumulation at the membrane (Loiseau et al., 2016; Murrell & Gardel, 2014; Nast-Kolb et al., 2022). (iv) DOGS-Ni^{2+}-NTA binds to His-tagged N-WASP or pVCA to form branched actin cortices (Pontani et al., 2009). (v) Conjugated lipid–protein complexes, such as Bodipy-FL-ActA, induce cortical actin localization (Abu Shah & Keren, 2014). (vi) Polymers, such as methylcellulose, push actin to the membrane (Murrell & Gardel, 2012). Figure created using Biorender.

only, 20% anionic lipids, or 20% cationic lipids—change actin–membrane interactions (Schroer et al., 2020). In the SLBs with neutral or negatively charged lipids in the presence of Mg^{2+}, actin localizes to the membrane; however, the substitution of Mg^{2+} for K^{+} decreased or eradicated actin localization at the membrane (Schroer et al., 2020). In SLBs with positively charged lipids, Mg^{2+} presence caused no actin–membrane interactions; however, K^{+} presence significantly increased actin localization at the membrane (Schroer et al., 2020).

Overall, lipid composition and ion valency changes actin's membrane binding ability by creating weak electrostatic interactions.

Likewise, actin crosslinkers can cause actin to localize to the cortex. The addition of phosphatidylinositol 4,5 bisphosphate (PIP2) and cortexillin I, a *Dictyostelium discoideum* actin crosslinking protein with binding sites for actin and PIP2, was sufficient to cause F-actin to accumulate at the membrane on the inside of GUVs (Figure 2b(i)) (Luo et al., 2014). The addition of a different *Dictyostelium* protein, ponticulin, a transmembrane protein with a high actin affinity (Luna et al., 1990), also was sufficient to cause actin accumulation at the membrane on SLBs made from l-α-phosphatidylcholine (Figure 2b(ii)) (Barfoot et al., 2008; Johnson et al., 2006). Similarly, the addition of constitutively active ezrin, a mammalian actin crosslinker that binds to PIP2 and F-actin, was sufficient to achieve a cortical actin distribution on SLBs (Figure 2b(iii)) (Nöding et al., 2018). These studies indicate that transmembrane actin crosslinkers or actin crosslinkers with binding sites for both lipids and actin are sufficient to induce an actin cortical distribution.

Since branched actin networks commonly form at the membrane, the next goal was to determine the components necessary to create cortically distributed, branched actin networks. In GUVs with phase-separated lipid domains (the lipid-ordered phase containing PIP2), adding purified N-WASP, the Arp2/3 complex, and actin to the outside of the GUVs caused the formation of branched F-actin network patches at the PIP2 containing lipid domains (Liu & Fletcher, 2006). In this system, N-WASP bound to and was activated by PIP2, thereby activating the Arp2/3 complex to nucleate actin filaments at the membrane and promoting the formation of a branched actin network on PIP2-containing domains. However, adding the same proteins to the outside of GUVs with a homogenous lipid composition resulted in protrusion formation at the membrane (Figure 2b(iv)) (Liu et al., 2008). Since decreasing actin filament length decreases actin's bundling ability, the addition of capping proteins during the assembly of the branched actin cortex decreased the number protrusions formed (Liu et al., 2008). The formation of a full branched actin cortex that does not result in any protrusion formation has yet to be achieved using naturally occurring proteins.

Additionally, other techniques have been developed to achieve a cortical actin distribution. These techniques often use engineered, recombinant actin, or actin-associated proteins that directly bind to the membrane through an artificial, biochemical linkage. Although these techniques cannot define the minimal components the cell uses to create an actin cortex, a substantial fraction of our understanding of actin and actin-associated proteins has originated from using engineered, recombinant biomolecules to biochemically link cortical components to the membrane. A variety of these membrane–cortex protein linkages have been developed from strong, biochemical linkages used for protein purification, protein-tagging, or immunoprecipitation. These protein–membrane linkage strategies have been used to create an actin–membrane association on SLBs or on the outside or inside surface of GUVs. The first engineered actin cortex used the biotin–streptavidin linkage. In this system, the membrane was synthesized with a small fraction of biotinylated lipids. The addition of biotinylated G-actin monomers with streptavidin or neutravidin caused actin to accumulate at the membrane (Figure 2c(i)) (Bashirzadeh et al., 2022; Heinemann et al., 2013; Helfer et al., 2000, 2001a, 2001b; Tsai et al., 2011). The streptavidin or neutravidin binds to both the biotinylated lipid and the biotinylated actin to create a biotinylated actin–streptavidin-biotinylated lipid linkage. However, this also allows for actin–actin binding as the streptavidin or neutravidin could link two actin monomers together. Using this system, the thickness of the actin shell can be increased by increasing the percentage of biotinylated lipids (Vogel, 2016). Since this linkage does not use any other actin-associated protein, changes in the actin cortex should only be related to the addition of other actin-associated proteins. Similarly, streptavidin was covalently linked to other actin-associated proteins to achieve a cortical actin distribution. Specifically, recombinant streptavidin-pVCA (the Arp2/3 complex activating domain of N-WASP), has been added to membranes containing biotinylated lipids to activate the Arp2/3 complex locally at the membrane and nucleate branched actin filaments (Figure 2c(ii)) (Caorsi et al., 2016; Carvalho et al., 2013; Simon et al., 2018, 2019). Although biotin–streptavidin-based linkages are capable of achieving cortical actin distributions, the bond between biotin and streptavidin is much stronger than what naturally occurs in cells (Schön et al., 2019).

Another common biochemical linkage used to create an actin cortex on membranes is the nickel–histidine interaction. Using membranes containing the lipid DOGS-Ni^{2+}-NTA, the addition of a His-tagged actin-associated protein causes actin to localize at the membrane. A commonly used technique to create a branched actin cortex utilizes nickelated lipids within the membrane that bind to either a 6–10xHis-tagged N-WASP or pVCA, which activates the Arp2/3 complex and nucleates the polymerization of branched actin filaments at the membrane (Figure 2c(iv)). This branched actin cortex is often similar in thickness to the actin cortex in cells (Pontani et al., 2009). Many other His-tagged proteins, such as ezrin (Köster et al., 2016; Schön et al., 2019), anillin (Loiseau et al., 2016), FimA2 (a constitutively active mutant of the actin crosslinker, fimbrin) (Murrell & Gardel, 2014), or VASP (Nast-Kolb et al., 2022), have been used to create a cortical actin distribution on membranes containing nickelated lipids (Figure 2c(iii)). This linkage is useful in mimicking cellular features because nickel–histidine linkages are reversible by the addition of imidazole or a chemical chelator, and DOGS-Ni^{2+}-NTA can diffuse freely through the membrane. However, differences have been reported for the stability of membrane adsorption between the nickelated lipid and His-tagged protein by various research groups. Some groups have observed stable membrane–protein linkages while others report desorption of the His-tagged protein away from the membrane. Differences in protein concentration and incubation time for protein–membrane binding could be the explanation for this disparity. Lower protein concentrations with longer incubation times result in the same membrane surface coverage as higher protein concentrations with short incubation times (Nye & Groves, 2008). When using high protein concentrations, the nickelated lipids only have a chance to monovalently chelate to one histidine residue, creating a weak interaction between the His-tagged protein and membrane; therefore,

the His-tagged protein can desorb from the membrane quickly. Using lower concentrations of His-tagged proteins allows multiple histidine residues within the tag to interact with the nickelated lipids, therefore increasing the His-tagged protein–membrane binding. Using His-tagged and streptavidin tags to cause actin or actin-associated protein to localize to the membrane is highly common. Additionally, using both tags on the same proteins can cause a cortical actin distribution on different lipid phases. For example, a streptavidin–pVCA-6xHis-tagged recombinant protein can cause actin to localize to both an ordered and/or disordered domain in a single GUV if the corresponding lipid (PEG-biotinylated lipid or Ni-NTA lipid) is present (Lopes dos Santos et al., 2023).

Other techniques have been designed to promote a cortical actin distribution. For instance, lipid-conjugated actin-associated proteins have been designed to create a stable, irreversible linkage between the membrane and cortex. Specifically, ActA (an actin binding protein from pathogenic bacteria *L. monocytogenes*) has been conjugated to the fluorescent lipid Bodipy-FL. ActA-Bodipy-FL causes F-actin from *Xenopus* egg extract to accumulate at the membrane (Figure 2c(v)) (Abu Shah & Keren, 2014). To create a significantly weaker protein–membrane interaction, crowding agents such as methylcellulose have been used to physically push actin to the membrane and to itself to mimic an actin cortex and actin bundling, respectively (Figure 2c(vi)) (Miyazaki et al., 2015; Murrell & Gardel, 2012, 2014). Overall, many methods have been developed to promote actin cortex formation on SLBs and in GUVs, including the use of naturally occurring proteins and the development of engineered membrane–protein linkages.

4 | RECONSTITUTION OF THE ACTIN CORTEX FURTHERS THE UNDERSTANDING OF THE MINIMAL COMPONENTS NECESSARY TO ACHIEVE DIFFERENT ACTIN STRUCTURES AND CELLULAR FUNCTIONS

Actin plays a fundamental role in driving a diverse range of cellular functions while interacting with many components and pathways. However, the complexity of the cellular environment makes it difficult to determine the minimal components necessary for each function. Membrane-based reconstitution assays have allowed for the determination of the minimal components necessary for the development of different membrane shape changes and the formation of different actin structures.

4.1 | Actin structures

Actin and actin-associated proteins assemble to form a variety of cellular structures, such as filopodia, lamellipodia, stress fibers, and the contractile ring. Each of these cellular structures are formed by distinct actin structures, such as parallel filaments, branched actin networks, actin asters, and ring-like structures. The actin crosslinker identity and concentration, confinement volume, and temperature can all affect which actin structures form.

4.1.1 | Actin bundles and filopodia formation

Filopodia are protrusions formed by bundles of parallel actin filaments that push out the cell membrane (Figure 3a(i)) (Blake & Gallop, 2023). Actin-associated proteins often form the actin bundles involved in filopodia development. For example, the addition of fascin, an actin crosslinker, and F-actin to the inside of GUVs caused the formation of stiff, parallel actin bundles which could lead to the formation of filopodia-like protrusions (Figure 3a(iv)) (Bashirzadeh et al., 2022; Honda et al., 1999; Tsai & Koenderink, 2015). Similarly, the addition of membrane-bound His-tagged VASP, an actin elongator, can cause the formation of actin bundles on SLBs containing nickelated lipids (Figure 3a(ii)) (Nast-Kolb et al., 2022). However, actin crosslinkers are not necessarily required in the formation of filopodia-like structures. Filopodia-like protrusions were observed in a reconstituted membrane system while reconstructing a branched actin cortex on the outside of GUVs. While the addition of N-WASP, the Arp2/3 complex, and actin created a reconstituted branched actin cortex on GUVs containing PIP2, membrane protrusions that mimicked filopodial dynamics (actin monomer addition occurring at the protrusion tip with no branched structures localized within the protrusion) were also observed (Figure 3a(iii)) (Liu et al., 2008). Despite the expectation of only creating a branched actin cortex, these protrusions resulted from actin polymerization forces from multiple individual filaments pushing on the membrane and causing a slight deformation. When the distance between two filaments pushing on the membrane is small enough, the membrane elasticity can cause the actin filaments to bundle together because membrane-induced filament bundling decreases the energy needed for creating a membrane deformation. Taken together, this demonstrates that the cell membrane itself is capable of guiding filopodia-like protrusions (Liu et al., 2008). Similarly, protrusions formed on the outside of GUVs when similar components (membrane-bound biotinylated pVCA replacing the N-WASP–PIP2 interaction) were added in the presence of capping proteins (Figure 3a(iii)). These protrusions were wider than what was observed in the previous experiments and had the Arp2/3 complex localized within the protrusion. Therefore, these protrusions resembled structures more similar to dendritic filopodia (Simon et al., 2019). The protrusions were concluded to form due to the uniform growth of the branched actin network, inducing a force on the membrane strong enough for protrusion formation. By reducing membrane tension (through hyperosmotic shock) or decreasing actin network thickness, protrusion formation increased, indicating that both the membrane and actin cortex contribute to this membrane deformation (Simon et al., 2019). Both narrow protrusions containing linear actin bundles and wide protrusion containing branched actin networks were observed again when a low concentration of streptavidin-tagged pVCA was bound to biotinylated lipids in the membrane; however, increasing the concentration of

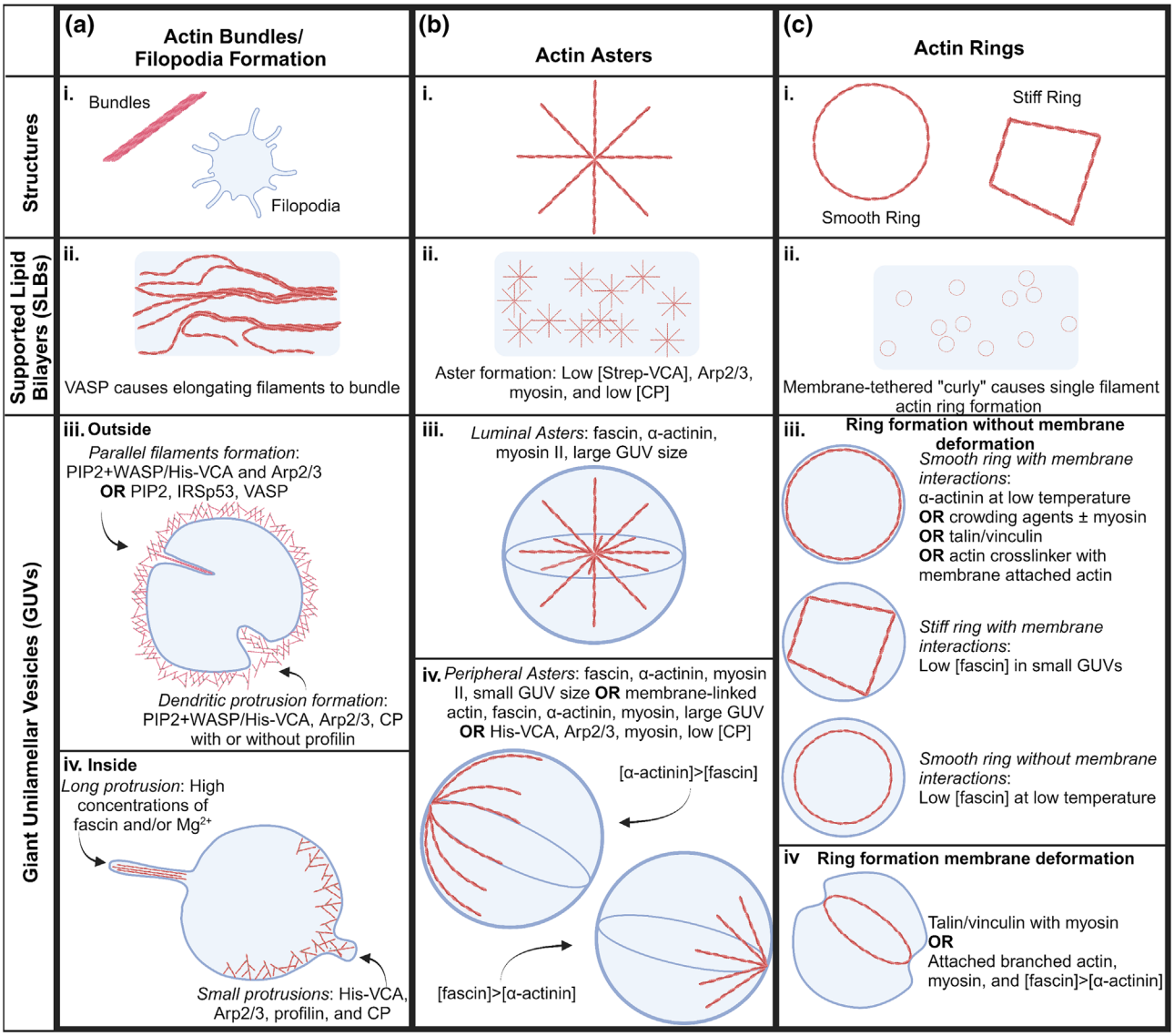

FIGURE 3 The minimal components necessary for the formation of different actin structures in membrane-based reconstitution assays. (a) Filopodia/protrusion formation. (i) Actin filaments align in parallel to form actin bundles that can push against a membrane to form filopodia-like protrusions (Blake & Gallop, 2023). (ii) His-VASP is biochemically linked to SLBs causes elongating filaments to bundle (Nast-Kolb et al., 2022). (iii) Filopodia-like protrusions can form on the outside of GUVs through bundled parallel filaments (Liu et al., 2008; Tsai et al., 2022) or a dendritic actin network (Simon et al., 2019). (iv) Small (Dürre et al., 2018) or long filopodia-like (Tsai & Koenderink, 2015) protrusions can form when actin and actin-associated proteins are encapsulated inside a GUV. (b) Aster formation. (i) Actin can form star-like shapes called asters. (ii) Asters formation has occurred on SLBs using streptavidin-tagged pVCA, Arp2/3, myosin, and low concentrations of capping proteins (Gat et al., 2020). (iii) Luminal asters can form on the inside of large GUVs in the presence of actin crosslinkers and myosin (Bashirzadeh et al., 2022). (iv) Peripheral asters (Bashirzadeh et al., 2021, 2022; Dürre et al., 2018) can form when actin and actin-associated proteins are incapsulated inside of GUVs to create asters with filaments aligning parallel to the membrane (*left*) or with filaments pushing into the GUV's lumen (*right*). (c) (i) Actin can form smooth, circular rings or stiff, rigid rings. (ii) Membrane-tethered "curly" causes actin to form 1 μm rings on SLBs (Palani et al., 2021). (iii) Smooth (Bashirzadeh et al., 2022; Limozin et al., 2003; Litschel et al., 2021; Miyazaki et al., 2015) and stiff (Bashirzadeh et al., 2022; Limozin et al., 2003) actin rings can form in the inside of GUVs and interact with the membrane; however, smooth rings have formed inside of GUVs without membrane interactions (Limozin et al., 2003). (iv) Actin rings capable of causing membrane deformations similar to cleavage furrow ingression have been created by adding myosin to membrane interacting actin rings (Bashirzadeh et al., 2022; Litschel et al., 2021). Figure created using Biorender.

pVCA eliminated the protrusions observed (Gat et al., 2020). This further reiterated that increasing actin network density decreases the likelihood of protrusion formations (Gat et al., 2020). When reconstituting the actin cortex on the inside of GUVs, the addition of membrane-linked His-pVCA, the Arp2/3 complex, profilin, and low concentrations of capping proteins cause the formation of small (~4 μm) protrusions (Figure 3a(iv)) (Dürre et al., 2018). Under the same conditions, larger protrusions formed when the branched cortex

was reconstituted on the outside of GUVs compared to the inside of GUVs because the force needed to generate membrane protrusions from the outside-in is lower than the force needed to generate membrane protrusions from the inside-out (Wubshet et al., 2021). Membrane tension works with actin polymerization to generate protrusions when the cortex is created on the outside of GUVs; however, membrane tension works against the same polymerization when the cortex is reconstituted on the inside of GUVs. Conversely, these longer protrusions have been argued to form when an actin cortex is presented on the outside of the GUVs because there is a near infinite supply of actin monomers available for polymerization (Kandiyoth & Michelot, 2023). In summary, the branched actin cortex can generate filopodia-like structures without the addition of proteins commonly involved in filopodia development.

Although components known to create branched actin networks can create protrusions on the membrane, the addition of higher concentrations of fascin (5–60 μM) to the inside of GUVs allowed for the formation of longer membrane protrusions. Longer protrusions are formed when the concentration of fascin is higher (Figure 3a(iv)) (Tsai & Koenderink, 2015). Increasing actin–membrane electrostatic interactions through the addition of Mg^{2+} to the inside of GUVs containing fascin increases the number of protrusions formed while decreasing protrusion length (Honda et al., 1999), demonstrating that actin–membrane interactions influence protrusion formation. When fascin is mixed with pVCA and the Arp2/3 complex on the inside of GUVs, protrusion formation decreases because fascin and the Arp2/3 complex compete for actin monomers (Wubshet et al., 2021). Protrusion formation can increase when both fascin and the Arp2/3 complex are present by increasing fascin concentration or decreasing the Arp2/3 complex concentration whether or not pVCA is linked to the membrane through nickel–histidine interactions. Overall, the interplay between different actin-associated proteins can affect protrusion formation. Furthermore, filopodia-like structures can be created through the addition of other actin-associated proteins. Using GUVs containing PIP2, IRSp53 (a membrane curvature sensing protein) localizes to PIP2 regions of the membrane, recruiting VASP, thereby causing actin elongation (Figure 3a(iii)) (Tsai et al., 2022). These actin structures form protrusions into the GUV's lumen when presented on the outside of GUVs. These protrusions form regardless of fascin presence, which again demonstrates that fascin is not necessary for the formation of filopodia-like structures. In summary, protrusions and filopodia-like structures have been reconstituted through the addition of a variety of actin-associated proteins; of course, the cell likely uses a combination of these mechanisms in parallel to form filopodia.

4.1.2 | Actin asters

Actin asters have been observed in cells treated with cytochalasin D (Verkhovsky et al., 1997), a small molecule that disrupts actin assembly, and during cellular adhesion (Fritzsche et al., 2017). Actin aster formation has also been related to lipid sorting at the membrane (Fritzsche et al., 2017; Gowrishankar et al., 2012; Köster et al., 2016). Asters can form within the GUVs in one of two ways: within the lumen with the aster's arms pointing out towards the membrane (luminal asters) or at the membrane (peripheral asters) with its arms either pushing along the membrane or pushing into the lumen (Figure 3b). In all sizes of GUVs, luminal asters form when fascin, α-actinin (an actin crosslinker that drives actin bundling at high concentrations and branched networks at low concentrations (Meyer & Aebi, 1990)), and myosin are present, especially when the concentration of fascin is higher than α-actinin (Figure 3b(iii)) (Bashirzadeh et al., 2022). However, the probability of luminal aster formation increases in larger GUVs. Luminal asters form even when α-actinin concentrations are higher than fascin concentrations; however, this only happens in large GUVs (>15 μm). The addition of the same components to small GUVs lead to a high probability of peripheral aster formation when α-actinin concentrations are higher than fascin concentrations (Figure 3b(iv)) (Bashirzadeh et al., 2021), and increasing the concentration of both crosslinkers simultaneously increases aster formation due to the confinement of these components to a smaller space. Biochemically linking actin to the membrane through biotin–streptavidin linkages causes the formation of peripheral actin asters when both fascin and α-actinin are present regardless of which actin crosslinker is more concentrated. However, higher α-actinin concentrations lead to the formation of peripheral asters with arms moving in parallel with the membrane while higher fascin concentrations lead to the formation of peripheral asters with arms pushing into the GUV's lumen (Figure 3b(iv)) (Bashirzadeh et al., 2022). Actin asters were also found when actin networks are nucleated at or accumulated at the membrane through the addition His-pVCA and the Arp2/3 complex or through the addition of His-ezrin, respectively. Specifically, asters were found in these systems when myosin and low concentrations of capping proteins were present in GUVs (Dürre et al., 2018) or on SLBs (Das et al., 2020; Köster et al., 2016). Aster formation has further been linked to the formation of filopodia by Gat et al. (2020). Decreasing membrane-bound, streptavidin-tagged pVCA in GUVs containing the Arp2/3 complex, profilin, capping proteins, and actin causes the formation of protrusions with small bases. Under the same condition on SLBs, smaller actin asters were formed at low pVCA concentrations; therefore, it was suggested that the protrusions with small bases could originate from aster formation (Figure 3b(ii)) (Gat et al., 2020). In general, aster formation in a reconstituted system has often relied on the addition of myosin to the system. To form an aster, myosin generates a contractile force on actin, forming an actin cluster which proceeds to grow outwards. Additionally, myosin also forms asters by processively moving along an actin filament towards the plus end and undergoing end-dwelling which results in the plus-end clustering of actin filaments (Baldauf et al., 2023). However, aster formation has been observed in adherent HeLa cells, and this aster formation was independent of myosin activity (Fritzsche et al., 2017).

4.1.3 | *Actin rings*

Actomyosin rings make up the contractile ring during cytokinesis. High interest in determining the components needed to create an

artificial cleavage furrow exists, in part, because of the desire to create a synthetic cell that can divide. Actin ring formation has also been observed in surface and membrane-bound systems. Actin by itself is capable of forming rings if actin is confined to a 2D surface through multivalent cation adsorption interactions (Sanchez et al., 2010). On SLBs, the addition of polymerized actin to a membrane with His-tagged-"curly" (the calponin-homology domain and unstructured domain of IQGAP proteins) caused tight, actin rings (1 μm diameter) to form (Figure 3c(iii)) (Palani et al., 2021). The addition of tropomyosin to this system increased the frequency of ring formation and caused the rings to form inward spirals. The addition of myosin resulted in these rings undergoing contraction. These rings are formed by a single actin filament that curves in on itself; therefore, it is hypothesized that curly increases actin filament flexibility. Actin rings can also form in a 3D environment. On the inside of small droplets, the addition of methylcellulose, a crowding agent, causes actin to spontaneously generate rings due to actin confinement (Figure 3c(iii)). This ring formation is enhanced with the addition of myosin (Miyazaki et al., 2015). When crowding agents are not present, the addition of α-actinin inside of GUVs leads to the formation of a ring that interacts with the membrane when the GUV is incubated at low temperatures where the speed of actin polymerization is decreased and the formation of actin bundles is favored (Figure 3c(iii)) (Limozin & Sackmann, 2002). The addition of low concentrations of fascin also leads to the formation of an actin ring; however, these rings are often not circular but are "stiff" or "rigid" (Figure 3c(ii)) (Bashirzadeh et al., 2022; Limozin & Sackmann, 2002; Litschel et al., 2021; Tsai & Koenderink, 2015). When a fascin-based actin ring forms at low temperatures, the ring has a significantly smaller diameter than the GUV and, therefore, was determined not to interact with the membrane (Figure 3c(iii)) (Bashirzadeh et al., 2022; Limozin & Sackmann, 2002; Litschel et al., 2021). Interestingly, the actin crosslinker dynacortin, anillin, and septin are sufficient to organize actin filaments into rings in purified actin binding assays, indicating that, under the right conditions, single actin crosslinkers can form rings (Kučera et al., 2021; Mavrakis et al., 2014; Robinson et al., 2002). The actin ring formed by anillin underwent constriction over time indicting that myosin is not essential for actin ring constriction (Kučera et al., 2021). In GUVs with diameters smaller than 15 μm, rings were likely to form when fascin and α-actinin were present if fascin concentrations were higher (Bashirzadeh et al., 2022). When biochemically linking actin to the membrane through biotin–streptavidin linkages, α-actinin or fascin could create ring-like structures in small GUVs (Figure 3c, iii) (Bashirzadeh et al., 2022; Litschel et al., 2021). However, talin/vinculin addition to GUVs with membrane-bound actin created rings 80% of the time (Figure 3c(iii)) (Litschel et al., 2021). The addition of myosin to the talin/vinculin rings creates a membrane deformation similar to the formation of the cleavage furrow (Figure 3c(iv)). However, this ring was not stabilized or properly positioned within the GUV, and therefore the ring slid across the membrane until it formed a condensate (Litschel et al., 2021). The addition of stabilizing factors may allow for the formation of a full cleavage furrow structure. The branched actin cortex could help stabilize the ring and help promote cleavage furrow-like deformations. When using membrane-bound His-pVCA to activate the Arp2/3 complex to create a branched actin cortex inside of GUVs, actin rings formed and constricted in the presence of low concentration of α-actinin, high concentrations of fascin, and myosin (Figure 3c(iv)). However, this contraction eventually led to bleb formation (Bashirzadeh et al., 2022). Overall, the probability of ring formation increases by restricting actin confinement or by linking actin to the membrane in the presence of specific actin crosslinkers.

4.2 | Non-protrusive membrane deformation

The actin cortex is highly involved in the formation of non-protrusive membrane deformation, such as endocytic vesicle formation during pinocytosis and the formation of blebs. Membrane deformations in which the membrane invaginates, like those found during endocytosis, have been reconstituted through the creation of an actin network. When creating an Arp2/3-branched actin cortex through membrane-bound biotinylated pVCA connected to the outside of GUVs in the presence of profilin and capping proteins, membrane "tubes" are observed pulling the GUV membrane out (Simon et al., 2019). These "tubes" formed with varying length depending on actin network thickness, illustrating that membrane bending proteins were not needed to create membrane shapes that mimic early endocytosis and were capable of being produced by actin polymerization alone. On the inside of a GUV with a branched actin cortex created through membrane-bound His-tagged pVCA and the Arp2/3 complex, the GUV's membrane was observed to have concave regions in the presence of profilin and high concentrations of capping proteins (Dürre et al., 2018). The addition of myosin to this system caused the invagination of the membrane. At capping protein concentrations of 60–120 nM, fission of the invaginated regions causes vesicles to form inside the GUVs, recapitulating the endocytic process. Another non-protrusive membrane deformation is a bleb which occurs when the actin cortex is pulled off the membrane due to high tension within the cortex. Blebs have been reconstituted with branched actin cortices on the inside of GUVs with α-actinin, fascin, and myosin. In this system, actin accumulated on one pole and then forms a small ring (like the ring observed during budding yeast cell division) while myosin constricted the membrane. This eventually led to the formation of a bleb (Bashirzadeh et al., 2022). Likewise, GUVs made with membrane-bound anillin, which causes actin to bind and accumulate at the membrane, formed bleb-like membrane protrusions in the presence of myosin (Loiseau et al., 2016). These experiments concluded that blebbing is dependent on myosin contraction and membrane–cortex attachment. Although actin can induce non-protrusive membrane deformations, actin is also capable of stabilizing induced membrane protrusions. When optical tweezers induced the formation of nanotubes in GUVs, the addition of a thick, branched actin sheath around the nanotube impeded the ability of the optical tweezers to further deform the nanotube. When thin, branched actin sheaths surround the nanotube, the force from the optical tweezers would crack the actin network during nanotube elongation causing actin patches to

form around the tube (Allard et al., 2021). In summary, actin can induce many different membrane deformations and stabilize induced membrane deformations.

4.3 | Actin–membrane interactions

As the actin cortex lies directly under the lipid membrane, the actin cortex and membrane interact with and affect each other. One way in which the membrane impacts actin is through its curvature as membrane curvature can change the localization of actin. In dumbbell-shaped GUVs that were created through membrane hemifusion, actin localizes at the neck region of the dumbbell when actin polymerization was promoted by His-VCA and the Arp2/3 complex; however, VCA by itself did not preferentially localize to the neck region of the dumbbell. Taken together, actin may sense the curvature of the membrane, though the mechanism of membrane-curvature sensing by actin is unknown and may result from either actin preferentially polymerizing at the neck regions or through lateral diffusion after polymerization (Baldauf et al., 2023). Likewise, membrane–actin linkage also affects actin localization. When myosin was added to actin adsorbed on the membrane, actomyosin clusters formed because myosin was able to bind to anti-parallel actin filaments and pull the filaments together into a cluster. However, biochemically linking the His-tagged constitutively active fimbrin mutant (His-FimA2) to the membrane, causing actin localization at the membrane, impeded cluster formation as actin was not as easily moved (Murrell & Gardel, 2014); therefore, actin–membrane linkages help to stabilize actin structures. Conversely, the actin cortex affects the localization of lipids and transmembrane proteins (Arumugam & Bassereau, 2015; Simons & Ikonen, 1997). When actin was bound to lipids or membrane-bound proteins, the diffusion rate of the lipid or protein decreased (Heinemann et al., 2013). The addition of myosin to this system caused actin to cluster with its associated lipid or protein; therefore, both lipid and transmembrane protein diffusion dynamics can depend on actin linkages. Additionally, actin can affect lipid domain formation. Lipid membranes can be created to have multiple lipid phases. These phases often homogenize in high temperatures and phase separate at low temperature. In phase separated GUVs containing PIP2, the addition of N-WASP, the Arp2/3 complex, and actin causes the formation of branched actin patches (Liu & Fletcher, 2006). An increase in temperature to homogenize the lipids followed by a return to low temperatures causes the phase-separated domains to localize to the same positions they were at previously (Liu & Fletcher, 2006). When the actin cortex was not present, the phase-separated domains formed randomly; therefore, actin affected the localization of the forming lipid domains. Actin's effects on membrane dynamics were further studied using SLBs by adhering an actin cortex to one lipid phase through biotin–streptavidin linkages. On phase-separated SLBs formed at low temperatures, an increase in temperature impeded lipid-phase homogenization (Honigmann et al., 2014). Therefore, actin modified the inherent properties of the membrane. When these SLBs were formed at low temperatures, homogenized at high temperatures, and then cooled while actin was added, the phase-separated domains formed based on actin localization. The size of the actin-linked phase-separated domain depended on the concentration of actin–membrane linkages. Increasing actin–membrane linkage would increase the actin-linked, lipid phase size. The addition of myosin to this system caused the actin-linked lipid phase to decrease in number and increase in size as myosin contraction clustered actin together and caused the fusion of the actin-linked lipid phase (Vogel et al., 2017). These studies concluded that myosin could move lipids throughout the membrane by pulling on F-actin. Myosin was also able to move membrane-bound, actin-binding proteins through the membrane and cluster these proteins into their own domain (Köster et al., 2016). However, actin polymerization through the activated Arp2/3 complex itself can cause lipid domain rearrangement as phase-separated GUVs that had membrane-localized actin polymerization resulted in fewer domains than phase-separated GUVs that have no localized actin polymerization (Lopes dos Santos et al., 2023). In summary, the membrane can affect the localization of actin, and actin can, in turn, affect lipid and transmembrane protein diffusion, lipid domain formation, and inherent membrane dynamics.

4.4 | Symmetry breaking

The actin cortex undergoes symmetry breaking events during embryo development and cell motility (Hawkins et al., 2011; Mullins, 2010). The first experiment in which symmetry breaking occurred in a reconstituted system was when actin polymerization was induced through the Arp2/3 complex on a bead coated with VCA with gelsolin and ADF/cofilin also present. In this system, the actin cortex underwent symmetry breaking through the release of elastic energy due to the actin network fracturing (van der Gucht et al., 2005). On the outside of GUVs, symmetry breaking was observed in three different scenarios (Carvalho et al., 2013). First, a membrane-localized, Arp2/3-generated branched actin network in the presence of capping proteins underwent a symmetry breaking event due to actin polymerization at the membrane (Carvalho et al., 2013; Lemière et al., 2015). The addition of capping proteins caused nucleation to occur at the membrane, causing new actin filaments to push on existing filaments and generate a force strong enough to induce a break in the actin network. This actin network fracture caused actin to cluster to one side of the liposome. Second, when actin filaments were tightly linked to the membrane through biotin–streptavidin linkages, the addition of active myosin caused a symmetry-breaking event by generating a break in the cortical actin layer. The actin cortex then underwent relaxation as the actin accumulated on one side of the membrane. Third, adding myosin to the membrane-localized, branched actin network in the presence of capping proteins caused myosin to again generate a force on the actin cortex strong enough to tear the cortex and cause a symmetry breaking event. Taken together, these observations indicate that causing a tear in the actin cortex through actin polymerization or myosin contraction is sufficient to cause symmetry breaking. This symmetry breaking can be inhibited by creating a thinner actin

meshwork even when myosin undergoes contraction (Caorsi et al., 2016). Symmetry breaking events have also occurred inside of GUVs. In the presence of high fascin and low α-actinin concentrations, membrane-localized, Arp2/3-generated branched actin networks form a ring on one pole of the membrane, which undergoes myosin contraction leading to actin clustering and the formation of a bleb (Bashirzadeh et al., 2022). Similarly, in droplets containing *Xenopus* egg extract with membrane-bound ActA, spontaneous symmetry breaking occurred at 22 °C; however, this symmetry breaking event was inhibited at 30 °C or upon removal of myosin from the system (Abu Shah & Keren, 2014). Increasing the concentration of the actin crosslinkers α-actinin or filamin increased the probability of symmetry breaking; therefore, symmetry breaking was determined to require myosin and sufficient actin network connectivity.

5 | MEMBRANE-BASED RECONSTITUTION ASSAYS PROVIDE THE ABILITY TO ACCESS MECHANICAL PROPERTIES OF CORTICES WITH PRECISELY DEFINED COMPOSITIONS

The actin cortex that underlies the cell membrane is considered the primary force that drives cell shape change and other mechanical events within the cell (Salbreux et al., 2012). Reconstituting the actin cortex in vitro offers more controlled conditions compared to in vivo scenarios. Bottom-up reconstitution of the cortex allows for the selected addition of choice components of the actin cortex at precise concentrations to a biomimetic cell membrane (Luo et al., 2014). The composition of these reconstituted cortices directly affects the mechanical properties of the cortex, such as cortical tension, membrane tension, and elasticity. The actin cortex relies on the interplay of myosin and other associated proteins to generate cellular mechanical events. Precise control over protein composition in the reconstituted system enables the investigation of whether a minimal cortex can replicate classical mechanical properties as well as helps to determine the specific proteins that contribute to these properties.

For context, terminology used to describe the biophysical properties of reconstituted actin cortices and cells has been specifically defined in the field. When performing either membrane-based reconstitution studies, two different types of tension are often referred to: membrane tension and cortical tension. In this context, the membrane tension is considered to be generated from forces acting upon the lipid bilayer alone (Pontes et al., 2017). Cortical tension then has been defined as being generated by contraction of the cortical cytoskeletal proteins, primarily actomyosin bundles (Winklbauer, 2015). For comparison, in living cells, the cortical tension is generally considered to be the energy cost for adding a unit of area and encompasses a broad range of molecular interactions that give rise to this parameter. Besides tension, the elastic moduli of GUVs or cells are often measured, and this refers to its resistance to elastic deformation, which generally occurs on short time-scales before the forces dissipate due to the cytoskeleton's viscoelastic properties, which emerge from the noncovalent bonds that hold the network together. The elastic modulus is also referred to as the effective Young's modulus and defines the ratio of stress to strain in an object in response to perturbation (Guz et al., 2014). Young's modulus is classically defined for a three-dimensional, homogenous material; elastic modulus or effective Young's modulus is used to define the analogous measurement for heterogeneous cells. Additionally, the actin cortex's flexural or bending modulus is another common measurement that denotes the resistance of a material to bending deformation; however, the shear modulus describes the resistance of a material to shear deformation. These physical measurements are important comparators when studying the effects of the inclusion of reconstituted cortices in in vitro biomimetic systems.

5.1 | Actin

Although actin is a critical component that directly contributes to many mechanical processes, the study of actin alone in a biomimetic, membrane-based system contributes to a deeper understanding of the mechanisms by which actin specifically affects mechanical properties. The addition of actin to a reconstituted membrane system has a significant effect on the mechanical properties of the system when studied through rheological experimentation (Helfer et al., 2000, 2001a, 2001b). In GUVs with actin bound to the outside of the membrane via biotin–streptavidin linkages, a significant decrease in thermal undulations was observed when compared to GUVs lacking actin, conferring a marked increase of the bending modulus and denoting an increase in stiffness of the GUV (Helfer et al., 2000). Under the same conditions, the addition of actin resulted in the GUV exhibiting a shear modulus in contrast to GUVs lacking actin which primarily have bending moduli (Helfer et al., 2000). The presence of actin networks also induced membrane buckling instability not seen in GUVs without actin (Helfer et al., 2001a). These actin-coated membranes exhibiting buckling instability resulted from the presence of a shear modulus and large bending modulus, consistent with their previous findings (Helfer et al., 2001a). The presence of actin is understood to provide viscoelastic properties to GUVs when compared to GUVs lacking actin, and viscous forces allows these properties to present (Helfer et al., 2001b; Murrell et al., 2011). Further study of the viscoelastic properties of actin-containing GUVs showed that spreading dynamics, governed by properties of adhesion and dissipation, were slowed compared to GUVs without actin. The viscous dissipation of the actin-containing GUVs reflected observed spreading dynamics in cells (Murrell et al., 2011). In GUVs containing an actin network formed by the inclusion of the Arp2/3 complex and VCA, membrane deformability as it relates to velocity of membrane extrusion measured by hydrodynamic tube pulling significantly decreased upon the inclusion of a cortical actin shell. The presence of an actin cortex changes the mechanism of dissipation, in which the lipid mobility is reduced by the presence of a "corral effect" caused by the actin filaments increasing membrane tension at the point of tube extrusion (Guevorkian et al., 2015). Taken altogether, reconstituted actin cortices provide viscoelastic properties to GUVs when compared to fluid membranes

lacking actin, resulting in a membrane that is more resistant to physical deformation due to the presence of a shear modulus and increased bending modulus and viscoelasticity, recapitulating a facet of classical cell membrane dynamics.

The utilization of in vitro reconstitution of the cell cortex allows for the study of distribution-dependent effects of actin on the mechanical properties of the cortex. In GUV preparation, actin concentration can be finely tuned to generate different types of actin cortices created by membrane-bound His-pVCA and the Arp2/3 complex (Baldauf et al., 2023). At low concentrations of actin, cortices either formed a "continuous" shell along the membrane, a "sparse" actin shell containing patches of bare membrane interrupted by long patches of actin, or a combination of the two. Upon an increase in actin concentration, the presence of GUVs with small patches of cortical actin were eliminated. Similarly, the Arp2/3 complex generated cortical actin density varies with GUV size. Small GUVs (radius smaller than 4 μm) exhibited sparse or actin-poor actin shells that diffuse along the membrane over time. In larger GUVs, actin uniformly distributes along the cortex, and this actin cortex undergoes little diffusion (Murrell et al., 2011). Bare liposomes and the actin-poor liposomes had similar spreading dynamics due to the elastic properties of the liposomal membrane. Conversely, actin-rich systems had a significant increase in resistance to membrane deformation and increased time needed for the GUV to spread. These GUVs adequately replicate the cell-spreading dynamics previously observed in eukaryotic cells. Similarly, the addition of an actin cortex to GUVs increases the area compressibility modulus, another measure of deformability (Schäfer et al., 2013). Additionally, strongly linking the cortex to the membrane further increased the GUVs' area compressibility modulus. Therefore, the addition of actin or membrane-bound actin decreases membrane deformability. Overall, cortical actin alone increases the bending modulus, stiffness, viscoelastic properties, and shear modulus of GUVs while simultaneously inhibiting GUVs' spreading dynamics.

5.2 | Myosin II

Myosin motors in the cortex drive contractility, which allows the cell to migrate, grow, divide, and dynamically change shape upon the induction of contractile stress on actin networks (Murrell et al., 2015). The presence of myosin in a reconstituted actin system is necessary to mimic other cellular processes. The addition of myosin to reconstituted actin cortices increases the disassembly rate of actin filaments (Carvalho et al., 2013; Murrell & Gardel, 2012; Sonal et al., 2019). In a network of cross-linked F-actin subjected to pulling by myosin filaments, the degree of myosin-mediated contraction was directly reflected in the extent of F-actin buckling and severing (Murrell & Gardel, 2012). Actin filament severing initiated the process of actin turnover by shortening actin filaments, causing filament dissociation. Therefore, the pool of available actin monomers increased and could be reassembled into new actin structures to contribute to changes in cell shape and dynamics. The presence of myosin in an actin cortex formed on SLBs was necessary to induce disassembly and redistribution of actin, forming a steady-state model of actin turnover (Sonal et al., 2019). Contractility and actin turnover function as two different mechanisms by which the cell increases cortical tension, which can drive symmetry-breaking events and cellular shape changes (Carvalho et al., 2013).

The addition of myosin to the membrane-based reconstituted actin cortex can greatly affect membrane or cortical tension. In oil-in-water droplets with actin attached to the membrane through biotin–streptavidin linkages, the addition of myosin caused rearrangements in the actin network, resulting in stiffer droplets (Ershov et al., 2012). To test how increasing membrane tension affects actin structure formation, GUVs with branched actin networks localized to the inner leaflet of the membrane through His-pVCA were adhered to a surface as they spread flat and ruptured (Sakamoto et al., 2023). As the GUVs underwent adhesion and spreading, membrane tension increased, leading to actin reorganization and indicating that membrane tension is capable of affecting actin structures. The addition of myosin to the system also increased pore opening rates as myosin dynamically reorganized the actin network and decreased network viscosity; therefore, the viscosity of the actin–myosin II cortex can affect membrane mechanics. Generally, the addition of myosin causes the actin network to rearrange; therefore, myosin affects the mechanical properties of membrane-based actin cortices.

5.3 | Addition of other actin-associated proteins

Continued advancements in membrane-based cortex reconstitution experiments necessitate the inclusion of additional actin-associated proteins to unravel the distinct roles each protein plays in developing cellular mechanical properties. The mechanical properties of the cell cortex can be modulated by actin crosslinkers. For example, the inclusion of α-actinin and actin within GUVs undergoing electrodeformation decreased the GUVs' deformability compared to GUVs containing a membrane-localized Arp2/3-generated actin cortex (Wubshet et al., 2023). In GUVs containing actin and purified *Dictyostelium* protein cortexillin I, increasing actin concentrations increased the GUVs' effective Young's modulus (Luo et al., 2014). The addition of an additional *Dictyostelium* actin crosslinker, dynacortin, further increased the GUVs' Young's modulus, denoting a decrease in deformability of the GUV. In this study, parallel "deconstitution analysis" was conducted in which the effective Young's modulus of wild type and mutant cells (*myoII* null and *myoII* null with *dynacortin* knockdown) was measured with and without latrunculin (actin depolymerizer) treatment to figure out where the "deconstituted" cells come close to the "reconstituted" GUVs. This intersection put an upper limit on the plasma membrane's contribution to the cell's elastic modulus, which was 2%–5% of the wildtype value. Taken together, the composite material of the underlying actin meshwork, complete with actin crosslinkers, myosin motors, and many other factors, enriches the strength of the membrane to prevent deformation and increase cortical stiffness and tension. The reconstitution of biomimetic actin

cortices in vitro provides a robust and relevant method for the study of these mechanisms.

6 | COPROCESSING COMPUTATIONAL MODELS WITH BIOCHEMICAL EXPERIMENTS PROVIDES EXPLANATIONS TO EXPERIMENTAL RESULTS WHILE SIMULTANEOUSLY ALLOWING FOR THE FORMULATION OF TESTABLE HYPOTHESES

With the surge in computing power and a deepened understanding of actin cortex structures and functions, computational modeling has become a useful method to both develop new testable hypotheses and rationalize observations in vitro. Models are now able to consider nuances in proteins structures due to advances in crystallography and cryo-electron microscopy, leading to more accurate models. In simulations, models typically consider a protein's conformation, flexibility, contours, and reactivity. Therefore, computational models are useful tools to test hypotheses that would otherwise be expensive and/or technically difficult to test in vitro.

When developing models representative of the cortex, the field often focuses on the formation of the actin network or actomyosin filaments and their respective properties. In developing robust computational models of the actin cortex, it is critical to precisely define specific actin properties. Since the actin cortex is a meshwork of actin and actin-associated proteins, defining the reactivity between components individually and as a network is essential in determining the contractility, movement, and deformability of the cortex. For example, modeling actin turnover and recycling in the cortex has been well studied and visualized using computational models. Myosin filaments were determined to be capable of producing enough force (20 pN) to break an actin filament into two, which helped propose a model for filament fragmentation, turnover, and coalescence. To do this, the model was designed to assume myosin heads as springs and actin filaments as a flexible rods that have a fixed spring constant (1 pN nm^{-1}) or fixed bending rigidity (60 nN μm^{-1}), respectively. These constants were obtained from previous experiments. Additionally, the simulation showed that ATP concentration affects myosin's ability to break actin filaments as increasing ATP concentration decreased myosin's ability to break actin filaments as sufficient curvature was not achieved. (Vogel et al., 2013). Likewise, filament turnover has been correlated to the cell cortex's ability to not collapse in on itself when undergoing contractile forces. When creating a model to explain why the cortex undergoes contraction and not expansion in in vitro models, the contractile forces outcompeted the expansile forces because expansile forces resulted in actin buckling, reducing the expansile force and leading to overall contraction of the system. However, adding actin filament turnover dynamics to the system by causing random filaments to disappear and be replaced with new filaments resulted in a pulsatile behavior in which the actin cortex contracts and then expands back, simulating how a cell could prevent cortex collapse (Belmonte et al., 2017).

Furthermore, when modeling larger networks, geometric constraints merit significant considerations in cortex formation and maintenance because actin structures can be greatly affected by cortex geometry. For example, a computational model designed to describe the viscoelastic properties of a reconstituted actin cortex was made. This model utilized the worm-like chain model, in which polymers (like actin) are assumed to be thin flexible rods, and combined it with a non-affine microsphere model, in which molecules subjected to shear force do not have the same deformations. Together, the continuum model designed was able to match experimental rheological data and accurately mimicked the viscoelastic mechanical properties seen in vitro (Unterberger, Schmoller, Bausch, et al., 2013; Unterberger, Schmoller, Wurm, et al., 2013). In a similar vein, a simple geometrical model made using analytical calculations explained how confinement affects actin polymerization. The mathematical model analyzed cortex formation in relation to compartment size and was able to explain why actin would or would not form a cortex when encapsulated in a GUV, which lead to a better understanding of cortical dynamics both from an experimental and conceptual standpoint (Pontani et al., 2009). Similarly, computational models have been developed to predict cortical tension using a 3D actin meshwork. This model created an actin cortex by visualizing actin and myosin filaments as rigid rods with actin crosslinked by crosslinkers represented as linear springs with a fixed length. When varying the actin lengths and computing cortical tension, actin filaments with "intermediate length" were able to create cortices with the highest cortical tension and established the maximum cortical tension that an actin cortex could produce, giving a defined limit for future models (Chugh et al., 2017). Overall, these studies emphasized the importance of giving defined geometric constraints when developing actin cortex models.

Computational and mathematical modeling has also been used to determine actin's role in complex cellular processes. When using analytical calculation and physical modeling, actin shell thickness determined by actin polymerization and depolymerization rates, myosin contraction, and the kinetic properties of actin crosslinkers that bind the actin cortex to the membrane were considered when attempting to model bleb-based cell motility (in which the actin cortex detaches from the plasma membrane to form a bleb and a new cortex grows within the bleb). Using a mix of their model and experimental micropipette aspiration data, the model was able to reproduce a variety of cell deformations that occur to cells during micropipette aspiration and lead to an understanding of how the viscoelastic properties of the cortex, rate of pressure changes during micropipette aspiration, membrane curvature, and contractile stress affect membrane detachment and bleb formation (Brugués et al., 2010). In another instance, a model of a single actin filament combined with a three-dimensional actin meshwork in which each actin filament was evenly space throughout a microsphere was designed to better model cortex elasticity in larger networks (Unterberger, Schmoller, Bausch, et al., 2013; Unterberger, Schmoller, Wurm, et al., 2013). This model incorporated both creep and relaxation behavior, as well as nonlinear behavior to explain the viscoelastic properties of the actin cortex and better represent data observed in vitro. Similarly, computational simulations were used to

determine which mechanism (actin buckling or actin polarity sorting by myosin plus-end dwelling) was dominant in causing actomyosin networks to undergo contraction compared to expansion (Wollrab et al., 2018). When modeling actomyosin contraction based on in vitro reconstitution experiments without crosslinkers, myosin plus-end dwelling was required for the system to undergo contraction. When crosslinkers were included in the model, polarity sorting was found to be the dominant mechanism for the majority myosin and crosslinker concentrations; however, at high concentrations of crosslinkers and low concentrations of myosin, local network contraction occurred mostly through actin filament buckling. Further, removing myosin end dwelling from the model inhibited contraction of the actomyosin system. Additionally, the model was adapted with parameters to fit an in vivo actin cortex (shorter actin filaments and a small actin network mesh size) and found that polarity sorting was the dominant mechanism of contraction as removing myosin plus-end dwelling from the model again inhibited contraction of the system and fewer conditions were able to contract through actin filament buckling alone.

Likewise, a model was designed to explain how the cell handles the excess plasma membrane that is present when a cell quickly transitions from a spread state to a rounded shape. When the cell undergoes this transition, electron micrographs have shown that the cell membrane forms a variety of bleb-like protrusions not seen through light microscopy. To describe this phenomena, three different models (a 2D Hamiltonian model, a 3D random seed and growth model, and a phase-field model) were used to describe different aspects of the bleb-like protrusions seen (Kapustina et al., 2016). The 2D Hamiltonian model was described using two layers of an actin cortex in which there is a layer of actin connected to the membrane and an actomyosin thick layer below. Both layers were modeled as beads and springs and explained how the membrane–cortex contact points (through crosslinkers) and amount of excess surface area can affect the shape of the bleb-like protrusions and the bending energy of each configuration. The 3D random seed and growth model was able to recapitulate the growth of bleb-like protrusions seen when the cell undergoes rounding and could explain the density and size of these protrusions. The 2D and 3D phase field model was able to combine the effects of the membrane, cortex, membrane–cortex connection, and the cytoplasm in the formation of these bleb-like protrusions. These different simulations together were able to describe different parts of the bleb-like protrusion formation and set the stage to develop more complex models going forward. Similarly, a model was developed that incorporated the cytoplasm, cortex, and cell membrane, which was able to determine how the elasticity of the cytoplasm mitigates pressure, and, thereby restricts the size of blebs (Strychalski & Guy, 2016). As shown by these collective efforts, introducing additional cellular properties to the defined network can further enhance our understanding of the role of different components have on the cortex while obeying fundamental rheology and energy balance laws.

Moreover, other proteins and cofactors can be added into computational models to further develop models and simulations of a complex cortex. For example, the incorporation of the Arp2/3 complex into a model with a node-based system demonstrated that mechanoresponsiveness of cortex components was required in the formation of a smooth contractile ring in order to explain observations in *Drosophila* development (Sharma et al., 2021). In a similar vein, a simple mathematical model that took actin assembly, reorganization of filaments by contractile flow, and actin turnover into consideration helped clarify the mechanism by which actin filaments align to promote tension in the membrane in the formation of a contractile ring. To do this, single molecule fluorescence tracking of formin in *C. elegans* cells combined with modeling proposed a mechanism in which filament assembly is directed by existing filament orientation rather than new filament orientation (Li & Munro, 2021). Formin has been further analyzed in computational models through the development of a mesoscale coarse-grained models and determined that the actin monomer binding site is revealed when tension is applied to the bind site's domain. This effect, in turn, increases the efficiency by which actin monomers are captured and increases polymerization rates (Bryant et al., 2017).

Furthermore, a coarse-grained molecular model analyzed the formation of Contractility Kits, which are macromolecular assemblies (condensates) of contractile proteins that form in the cytoplasm and are hypothesized to endow the cell with the ability to rapidly and synchronously deliver these proteins to the cortex when needed (Kothari et al., 2019; Plaza-Rodríguez et al., 2022). The model contained proteins known to form these Contractility Kits including *Dictyostelium* scaffolding proteins (IQGAP1 and IQGAP2), cortexillin I, and myosin. This model made many predictions about how different contractile proteins affect Contractility Kit formation and how the Contractility Kits control the response of the cell cortex to applied mechanical stresses, and, thereby, explaining the role of macromolecular assembly in cell cortex functions (Kothari et al., 2019; Plaza-Rodríguez et al., 2022). In the future, these models will be improved by adding additional proteins and cofactors to existing models to develop more complex cortical models which will aid in relating the cortex to other observed downstream effects and responses in the cell. Overall, the use of computational modeling in parallel with in vitro reconstitution experiments will further enhance our understanding of the actin cortex.

7 | RECONSTITUTION EXPERIMENTS INHERENTLY HAVE A MULTITUDE OF CHALLENGES ASSOCIATED WITH PERFORMING EXPERIMENTS AND NUMEROUS UNCERTAINTIES ABOUT THE RESULTS' CORRELATION WITH THE CELLULAR ENVIRONMENT

Reconstitution experiments have proven successful in enhancing our understanding of individual cytoskeletal components; however, these experiments have numerous technical challenges. Many reconstitution experiments utilize purified proteins which are either expensive to purchase or highly time consuming to produce. Since the cortex is a

complex and extensive network of proteins, numerous proteins must be obtained. As the field learns more about the cortex, the number of proteins needed for reconstitution experiments will increase. Similarly, the creation of GUVs can be challenging and encapsulation efficiency of proteins is often low (Baldauf et al., 2023); therefore, these experiments are often difficult to perform and require labor-intensive optimization. Consequently, the intrinsically difficult nature of mimicking a cellular cortex highlights the intricacies in advancing our understanding of the actin cortex through membrane-based reconstitution studies.

Furthermore, results from membrane-based reconstitution experiments can often be misconstrued or suggest more significance than warranted. As in all reconstitution experiments, the question remains about which components are missing. In these membrane-based reconstitution assays, often no extracellular environment, transmembrane proteins, membrane trafficking, or regulatory pathways are incorporated. All these factors have been shown to affect, or be affected by, the actin cortex. The inclusion of other biomolecules can affect actin structures, change the membrane or cortical mechanical properties, enhance or eradicate membrane deformation, or affect the localization of these components. As seen in the cellular environment, the cytoskeleton affects the regulation of other pathways and engages in extensive crosstalk (Kothari et al., 2019; Nguyen & Robinson, 2020, 2022) with various cellular functions such as metabolic processes (Balaban et al., 2023) and protein translation (Liu et al., 2022). Removing the crosstalk and complexity of the cellular environment through in vitro experimentation causes a separation between the cellular response and the mechanical response. Likewise, taking cytoskeletal components outside of the cell can cause the formation of structures that may not be fully biologically relevant as the formation of actin structures or membrane deformations can be enhanced or impeded when actin and other proteins are put in a more cell-like environment. Therefore, this reality challenges us to remain cognizant of the question: what do reconstitution experiments tell us about the biophysical and biological properties of a cell?

8 | CONCLUSIONS

In this review, we have summarized how membrane-based reconstitution experiments have contributed to our understanding of the actin cortex and cell shape changes while simultaneously considering the benefits and challenges of these experiments. Minimally, purified actin biochemical experiments have allowed for a great understanding of how each component functions individually. Adding a membrane to this system has increased the complexity of the conditions, allowing for us to recapitulate a more cell-like environment. Different membrane-based systems have been developed to study actin and its functions, such as SLBs, oil-in-water droplets, water-in-oil droplets, and GUVs. GUV formation and protein encapsulation has become easier and more efficient as more techniques are developed. These membrane-based reconstituted actin systems have deepened our knowledge of cortex formation and simultaneously provided a platform to development different techniques to create an engineered cortex. Likewise, these experiments have helped to determine the minimal components needed to form different actin structures or perform actin-associated cellular functions. Specifically, membrane-based reconstitution assays have enhanced our understanding of membrane and cortex mechanical properties. Simultaneously, computational and mathematical models have explained certain results and lead to further hypotheses that can be tested. In the future, there are multiple paths that can be taken to further understand basic biological principles and expand our ability to create a synthetic cell that can grow, move, and divide.

8.1 | Understanding the biological principles

The addition of other components to these membrane-based systems will likely further our understanding of how the cell performs its numerous functions using the actin cytoskeleton. Using naturally occurring proteins and lipids as much as possible will enhance our knowledge of how these cellular processes occur. Since there is widespread use of engineered cortices to recapitulate cell functions, additional components used by the cell will need to be incorporated to reconstitute these processes in a cell-like manner. Furthermore, additional proteins previously not associated with the actin cortex, but associated with other functions, including RNA translation, metabolism, and adhesion, have also recently been shown to affect cortex formation and cellular mechanical properties (Kothari et al., 2019; Liu et al., 2022; Nguyen & Robinson, 2020, 2022). Working to better understand these proteins in a reconstituted membrane-based system could be the key to mimicking cellular processes more accurately. However, the addition of more proteins to GUVs remains a challenge because most encapsulation methods can only encapsulate a few proteins inside the GUV. Additional techniques such as microfluidics are being developed and are used to increase encapsulation efficiency, but these techniques are not widely used at this time as the resources and tool needed for this method are not readily available or easily attainable (Van de Cauter et al., 2023).

Additionally, membrane-based reconstitution experiments often do not fully consider many biochemical and biophysical properties that cells utilize. First, accurate reconstitution of the actin cytoskeleton in a biological manner could be improved by correctly reconstituting the stoichiometries in the system. In the cited papers, wide ranges of concentrations for actin and actin-related proteins were seen. In a cellular system, the cell regulates the concentration of each protein to obtain its preferred stoichiometries for different protein complexes. When working to perform membrane-based reconstitution experiments, choosing protein concentrations that match the cellular system could allow for the reconstitution of additional cellular processes. It is noted that this could be difficult because the concentration of purified proteins would need to be high enough to be further diluted by additional proteins and buffer; therefore, finding ways to increase protein concentration when purifying proteins could result in membrane-based reconstitution experiments using protein concentrations that

more accurately represent cellular protein concentrations. Furthermore, membrane-based reconstitution could be improved through the regulation of actin polymerization and depolymerization. Actin-based cellular processes are highly dynamic, requiring both actin polymerization and depolymerization. Most membrane-based reconstitution experiments utilize filamentous actin or polymerize actin in the experiment; however, depolymerization or turnover is rarely considered. Computational models that include actin turnover dynamics often produce more cell-like results compared to reconstitution experiments as actin turnover is highly involved in many cellular processes. Overall, actin cortex reconstitution using membrane-based systems can be improved. Regardless, the combination of biochemistry, cellular biology, biophysics, and computational modeling together will be ideal to increase our understanding of biological principles.

8.2 | Developing a synthetic cell

One of the most common reasons used to explain the importance of actin cortex reconstitution studies was the aim of eventually creating a synthetic cell. Although naturally occurring proteins can be used to mimic cellular processes, the use of engineered, recombinant proteins could simplify the components needed in the development of a synthetic cell. As summarized previously, many cellular processes utilize the actin cortex have been successfully reconstituted in GUVs while other (specifically cell division) remain highly challenging (Baldauf et al., 2022). To create a synthetic cell, many different systems will have to be integrated together, including, but not limited to, the cytoskeleton, metabolism, DNA replication, transcription, and translation. Many of these processes have been reconstituted in GUVs individually (Martino & de Mello, 2016; Peters et al., 2014), but limited studies have been performed to reconstitute these processes simultaneously and cooperatively.

To begin to piece together different systems in a reconstituted experiment, actin cytoskeletal components and functional mitochondria were encapsulated in GUVs (Li et al., 2022). The addition of pyruvate, ADP, and inorganic phosphate to the system through porins inserted in the membrane allowed the mitochondria to generate ATP that the cytoskeletal components then used to create an actin meshwork. This meshwork then localized to the membrane upon addition of methylcellulose and changed the GUV's shape from spherical to oblong. Thus, the addition of organelles to GUVs could provide a path towards integrating different cellular systems with the actin cytoskeleton, bypassing the need to fully reconstitute each individual cellular process. As previously stated, the actin cortex structures and mechanical properties are greatly affected by the crosstalk between actin and its associated proteins with other cellular systems; therefore, integrating other systems with the actin cortex is essential when attempting to create a synthetic cell.

AUTHOR CONTRIBUTIONS

B.W. and D.R. conceived of the review. B.W., E.M., and R.J. reviewed the literature, drafted the text, and prepared figures. All authors edited the text.

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CONFLICT OF INTEREST STATEMENT

D.R. is exploring a start-up company. No other conflicts of interest need to be reported.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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