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Encapsulation of the cytoskeleton: towards mimicking the mechanics of a cell

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Abstract

The cytoskeleton of a cell controls all the aspects of cell shape changes and motility from its physiological functions for survival to reproduction to death. The structure and dynamics of the cytoskeletal components: actin, microtubules, intermediate filaments, and septins - recently regarded as the fourth member of the cytoskeleton family - are conserved during evolution. Such conserved and effective control over the mechanics of the cell makes the cytoskeletal components great candidates for *in vitro* reconstitution and bottom-up synthetic biology studies. Here, we review the recent efforts in reconstitution of the cytoskeleton in and on membrane-enclosed biomimetic systems and argue that co-reconstitution and synergistic interplay between cytoskeletal filaments might be indispensable for efficient mechanical functionality of active minimal cells. Further, mechanical equilibrium in adherent eukaryotic cells is achieved by the formation of integrin-based focal contacts with extracellular matrix (ECM) and the transmission of stresses generated by actomyosin contraction to ECM. Therefore, a minimal mimic of such balance of forces and quasi-static kinetics of the cell by bottom-up reconstitution requires a careful construction of contractile machineries and their link with adhesive contacts. In this review, in addition to cytoskeletal crosstalk, we provide a perspective on reconstruction of cell mechanical equilibrium by reconstitution of cortical actomyosin networks in lipid membrane vesicles adhered on compliant substrates and also discuss future perspective of this active research area.

Introduction

To what extent are we able to build membrane-enclosed active systems that resemble cell morphology and shape changes from scratch? The key factor of this question is how well we reconstitute and control its foundation, the cytoskeletal network. A major player of cell cytoskeleton is actin which, with the help of a variety of actin-binding proteins and motors, defines cell shape by polymerization/depolymerization, branching, crosslinking, bundling, and contraction. Networks of actin filaments have been reconstituted on patterned surfaces[1, 2], beads[2-4], supported lipid bilayers[5-8], droplets[9-11], and giant unilamellar vesicles (GUVs) [9, 12-14] forming different biomimetic structures. Microtubules are one of the most dynamic units of the cell cytoskeleton which have gained attention in bottom-up reconstitution studies for their role in active cell shape changes and polarity[15-17]. Dynamic microtubules and microtubule-based motors are the key players of active nematic systems[18-22], and are used in the design of molecular robots[23-25]. The third group of the cytoskeleton family are intermediate filaments which have gained attention in reconstitution of cytoskeleton for their major role in cell rigidity and providing mechanical support at adhesion sites[26-31]. Septins are considered the fourth cytoskeletal component as they function as filamentous scaffolds at the plasma membrane and regulate protein-membrane interactions[32-34]. Reconstitution studies are recently conducted to examine the role of septin structures in the mechanics of the cell. Although each of the cytoskeletal systems can be functionally reconstituted, crosstalk among cytoskeletal components is a key cellular feature enabling a robust mechanical response in cells. Cooperation of distinct networks of cytoskeletal components enables cells to resist mechanical stress to optimize their shape during almost every physiological process.

Besides using cytoskeletal elements to directly provide cell mechanical resistance and integrity, a dynamic cell also requires coordinated interaction with the ECM[35]. Cells adhere to their substratum by the formation of local contacts with ECM. Adhesion regulates the exchange of signals with the immediate microenvironment, cell shape changes, and migration. Active cytoskeleton networks govern such cell interactions with ECM through focal adhesions. Adhered artificial cells with reconstituted adhesion proteins to ECM have provided a better insight in the adhesion-induced reshaping of lipid membranes[9, 36]. The regulation of forces required for such shape changes and remodeling of the cytoskeleton could be studied by reconstitution of the cytoskeleton in adherent lipid vesicles[37].

Any internal or external transmission of forces requires congruous coordination and cooperation of constituent cytoskeletal components. A long list of coupling proteins works to mediate cytoskeletal rearrangements and architecture of the composite network. Complex and synergic behavior of the cytoskeleton raises a fundamental question of how many components are necessary for an artificial model to recapitulate a specific cytoskeletal function. A minimal set of coupled cytoskeletal components capable of exchanging information with the environment through a cell membrane-like boundary condition is likely essential for cytoskeleton-dependent cellular behavior. The sufficiency of the minimal yet required factors, however, is debatable and depends on the complexity of the expected outcome. For instance, monomeric actin polymerizes to form filaments in the presence of ATP and divalent magnesium ions while at least one actin binding factor is required for F-actin network formation, protrusion, or contraction. Creating a

biomimetic mechanically stable adherent cell requires the addition of actin-membrane and membrane-substrate binding factors. Actin cytoskeleton should team up with other cytoskeletal components to present a model with reconstituted cytoskeleton components for global cell deformation with structural integrity.

In this article, we first review the role of the four cytoskeletal components in cell mechanics and their dynamics *in vitro* with an emphasis on induction of shape changes in membrane-enclosed systems. Then we examine recent investigations in the adhesion of lipid membrane and cytoskeletal vesicles to their underlying substrates. Not covered here, the reader is referred to recent reviews addressing the pros and cons of different types of droplets and vesicles as common boundary conditions for encapsulated cytoskeleton[38-44]. One of the long-term goals we envision for the field is to observe shape changes of adherent cytoskeletal liposomes under controlled contraction. Finally, we discuss the importance of cytoskeletal crosstalk in the design of artificial bio-machines. Such composite networks of reconstituted cytoskeleton can improve the design of protocells and synthetic cells, cytoskeletal nematics as active systems of self-assembly, and actin- and microtubule-based soft robots.

Steady state dynamics of actin: protrusion, contraction, and remodeling

Actin networks are the mainstay of the cytoskeleton. Networks of actin filaments dynamically remodel to reshape cells. The lamellipodium protrudes by actin polymerization[45]. Actin remodeling and polymerization also governs inward pulling of the plasma membrane in endocytic and phagocytic sites[46, 47]. Besides dendritic actin networks, bundles of actin filaments also support integrity and motility of cells[48-51]. Actin crosslinking proteins such as filamins and α-actinin and actin-branching proteins such as Arp2/3 complex and membrane-associated nucleation promoting factors cooperate with regulators of actin elongation such as formins and capping proteins to organize actin network architecture and elasticity[52-54]. Actin disassemblers such as ADF/cofilin also help to regulate treadmilling of actin filaments to balance the rate of actin polymerization and disassembly[55]. Actin filaments are bundled by crosslinkers such as fascin. The mechanics of the cell membrane and therefore cell shape however is governed by the cell cortex, shell-like networks of crosslinked and branched actin bound to the membrane. Dynamic actin-membrane binding proteins such as ezrin, profilin, and cofilin [56, 57] couple the cell periphery to actin network to enable formation and regulation of the cortex. Myosin-driven contraction and protrusion of actin drive cell migration and predominately determine cell elastic properties and surface mechanics[53, 58-60]. During symmetry breaking, F-actin flow transforms myosin contraction into morphological processes of cell polarization to initiate motility[61]. Coarse-grained simulations have shown that contractility, bundling and polarity of actin cytoskeleton is a function of binding affinities of crosslinkers, motor proteins, and filament length[62]. By capturing fluctuation statistics of actin filaments and strain stiffening, these models show how crosslinker stiffness tunes the elasticity of the network[63].

To pave the way to understanding cell migration and division, reconstitution studies have recapitulated cell mechanics and shape changes governed by the complex dynamics of actin protrusion, cortex flow, and contraction[64, 65]. Despite being sufficient to generate

protrusion and contractility, biomimetic actin networks have not comprehensively explained the role of asymmetries in the constituent molecules behind such forces and global cell deformations.

The ability to control the internal structure and the choice of a functional membrane should be considered in the design of cytoskeletal synthetic cells. Lipid droplets and GUVs are two major platforms for this purpose. Moreover, enrichment of actin filament assembly in polymeric coacervates was recently observed[10] (Fig. 1A,B). In cytoskeletal GUVs, global shape changes of reconstituted actin is a result of trade-off between membrane tension and actin dynamics[66, 67]. Bundles of actin crosslinked with fascin deform liposomes that have small membrane bending rigidity and form filopodia-like protrusions while those bundles forming cortical rings and protrusion are suppressed in liposomes with high membrane rigidity[68]. Membrane itself is able to provide lateral forces to bring free-ends of a branched actin networks into parallel filaments and form fliopodia-like structures that protrude into the lumen of a GUV[69], and can also organize sites of actin assembly as shown by using a phase-separated membrane system[70] . The use of low and high concentrations of capping protein on polymerizing actin promotes protrusion and concave bending of the membrane respectively[71]. Interestingly, a branched network of polymerizing actin was recently shown to be sufficient for filopodia-like protrusions at low membrane tension and endocytic-like membrane deformations regardless of the tension[64] (Fig. 1C).

Beside the dynamics of protrusion, how local remodeling of actin architecture tunes stress production and dissipation in the cell, and regulates large-scale contractile network flow remains poorly understood. Cytoskeletal GUVs and droplets have been widely used in the pursuit of understanding actin network remodeling and contraction[72-74]. Droplet size-dependent localization of contractile actomyosin networks generated in *Xenopus* egg extracts lead to the emergence of symmetric and polar states in the lumen of water-in-oil droplets (Fig. 1D)[75]. Active fluid-like steady-state contraction in the droplets was shown to be independent of the density of the network and scales with the network turnover rate if the networks are not excessively branched or crosslinked[76] (Fig. 1E). Charge-induced coupling of contractile actomyosin to water-in-oil droplets induces stochastic oscillatory deformations at the lipid interface by the spontaneous formation of aster-like actin structures[77]. Cluster formation and the flow of minimal actomyosin cortices in such droplets can undergo vibrational states[65] (Fig. 1F). Global membrane deformations driven by actomyosin cortices can be explained by the balance between membrane tension and the contractile force, and anchoring density[78]. Deformation of the cortex itself depends on the network-membrane anchoring geometry and cortical tension buildup due to actomyosin contractility[73]. Cortex formation and membrane deformation induced by actomyosin contractility also depends on the concentration of capping protein[71]. These studies underscore the contractility and the mode of local network contraction are determined by the architecture of the network.

By an optimal design of cortical flows in reconstituted actin shells, actin polymerization and myosin-driven contraction can be studied simultaneously *in vitro*[79]. Such optimization can be achieved by maintaining the structural steady state of actin filaments protruding on the membrane on one end and being disassembled on the other end by myosin

contractility[80, 81]. These two major thrusts of cellular shape change are ATP-dependent. Thus, remodeling of actin vesicles under actin-generated forces can be temporally controlled by changing the amount of hydrolyzable ATP[12, 59, 60, 82]. Light-controlled shape changes by actin polymerization was achieved by encapsulation of photosynthetic artificial organelles for ATP synthesis in GUVs[83] (Fig. 1G). This also reflects the controllability of actin-driven forces by application of physical stimuli. At high actin concentrations comparable to those in the cell cytoplasm, GUV shape changes by actin polymerization can be externally controlled by alterations in osmotic pressure or application of light with no need for myosin motors[84] (Fig. 1 H,I). Another external inducer of actin remodeling and cell migration is electric field. Electrophoretic redistribution of membrane components has been shown to guide directionality of migrating cells in response to an electric field[85]. Application of an electric field on cells *in vitro* can perturb their polarity without affecting actomyosin-driven motility[86]. GUVs can be used as a platform to study the effect of electric field on phospholipid bilayer and cortical actin. A recent study showed that electric field-induced electrophoretic forces on actin filaments disrupt actin cortex reconstituted in GUVs and this attenuates the formation of pores on the membrane[87] (Fig. 1J). These all demonstrate the versatility of actin-containing liposomes and emulsion droplets for understanding and controlling actin network behaviour in response to external stimuli.

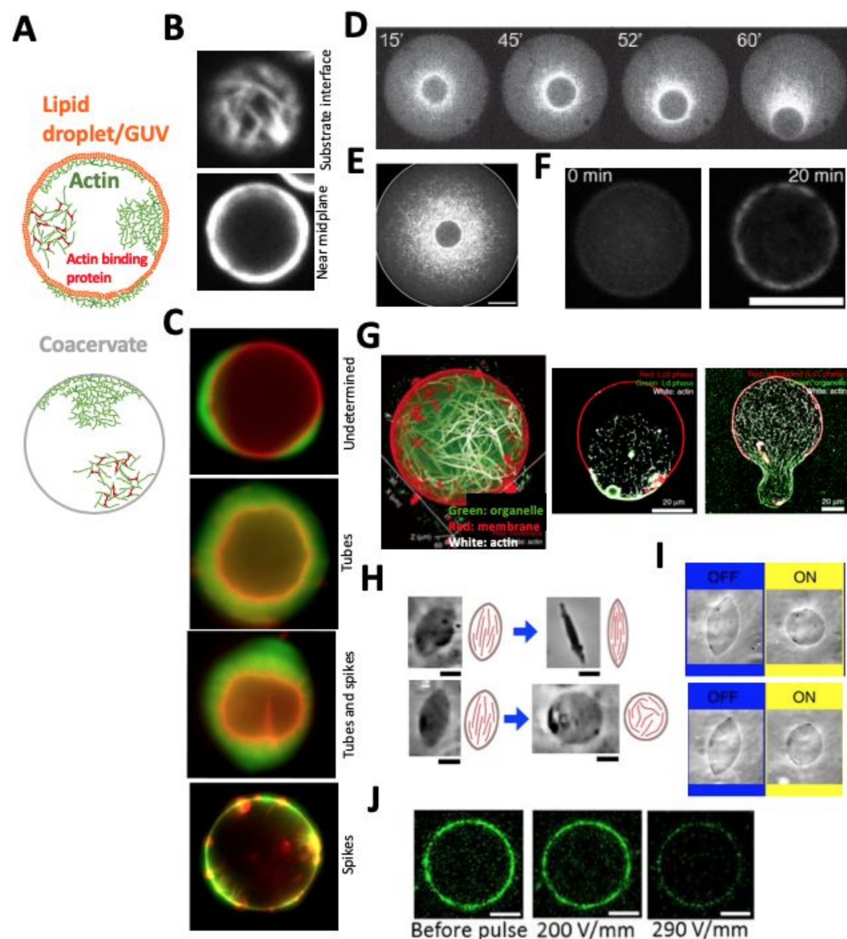


Figure 1. Vesicle- and droplet-based actin reconstitution. (A) A schematic representation. (B) Encapsulation of actin in adherent polypeptide coacervates. Actin partitions into coacervates, enhances its self-assembly, and localizes to coacervate periphery.[10] (C) Endocytic- and filopodia-like deformations by actin reconstituted on GUVs. Filopodia-like deformation events increase by application of hyper-osmotic shock.[64] (D) Time-lapse symmetry breaking of emulsion droplets by actomyosin contraction surrounding an aggregate. Symmetry breaking depends on droplet size.[75] (E) For a wide range of physiological conditions, myosin-driven contraction is density-independent in emulsion droplets and is proportional to the network turnover rate.[76] (F) Dynamic clusters of actin and actin cortex are formed at the equatorial plane of water-in-oil droplets in the presence of ATP. Flow-like motions in the clusters arise from vibrations within individual clusters.[65] (G) ATP synthesis and thereby actin polymerization can be optically controlled in GUVs by encapsulation of an engineered light-harvesting organelle containing ATP synthase (left). The growth of actin filaments can deform the shape of vesicles into teardrop (middle) or mushroom (right) shapes.[83] (H and I) High concentration of encapsulated actin can polymerize and deform GUVs into spindle shapes. The shape changes can be reversed and controlled by application of osmotic shock (H) or light (I).[84] (J) Application of high electric field pulses can break down actin cortex (green) in GUVs.[87]

Encapsulation of dynamic yet unstable systems: active microtubules and gliding microtubule on kinesins

Microtubules provide mechanical support for the cell. They shape mitotic spindles and are recognized as a critical cytoskeletal component for intracellular transport in cells, most

notably in neurons[88-93]. Microtubules provide long-range supports by reorganizing, pulling and pushing against loads[15]. To achieve this, they can manifest distinct mechanical behaviors by being elastic under low loads but strain-softening above a strain threshold[94]. Similar to actin, polymerization and crosslinking define the functional structure of microtubules[95]. However, their dynamic instability allows them to grow long and reorganize in short time scales. Reconstitution of microtubules in budding yeast lysates showed that its polymerization and dynamic instability are regulated by microtubule-associated proteins[96]. Microtubule motors such as kinesin and dynein transport cargo and can move microtubules if anchored or crosslinked to the substrate[19]. Microtubule and its motors also play a pivotal role in the tubulation of organelles and shaping eukaryotic cells[97].

Reconstitution of microtubules bound to GUV membranes demonstrated the capability of motor-less growing-shrinking microtubules in tubulating the membrane, sliding, and pulling it[16]. Microtubules can polymerize and cause protrusions in GUVs, and such growth-shrinkage of microtubules and therefore liposome deformations can be controlled by altering the hydrostatic pressure[98]. Kinesin-driven microtubule sliding (Fig. 2A) can self-organize into different structures in lipid-monolayered droplets depending on droplet size and motor activity[99] (Fig. 2B). Aggregation and polymerization of tubulin in these droplets is highly dependent on the composition of lipids[99]. Microtubules reconstituted into water-in-oil droplets with anchored kinesin motors have also demonstrated microtubule gliding behavior[100] (Fig. 2C).

Microtubule-motor systems are self-propelled active systems out of equilibrium[20, 101]. Methylcellulose-stabilized microtubule-kinesin networks show chiral active nematic behavior on glass surfaces[102]. The dynamics of microtubules sheared by motors has led to the emergence of synthetic active gels and emulsions featuring ATP dependent micro-motility and locomotion[20, 21, 103]. For instance, filopodia-like protrusions and oscillatory dynamic deformations in vesicles are generated via kinesin-driven microtubule nematics[104]. Sensor-actuator-based biomimetic micromachines can therefore be designed by modulating microtubule network dynamics. In this regard, vesicle shape changes were successfully triggered and controlled by strand of DNA-based signal molecules capable of engaging and disengaging kinesins anchored to liposome membrane[105] (Fig. 2D).

Apolar filaments: Keratins collapse, septins deform the membrane

Intermediate filaments have diameters slightly larger than F-actin (∼10 nm vs 6-8 nm), are dynamic and non-polar which self-assemble into complex bundles and networks[27, 28]. Besides their involvement in the localization of cell nucleus and mitochondria, intermediate filaments modulate cell mechanical properties and provide mechanical support for the plasma membrane by the formation of highly flexible and stretchable

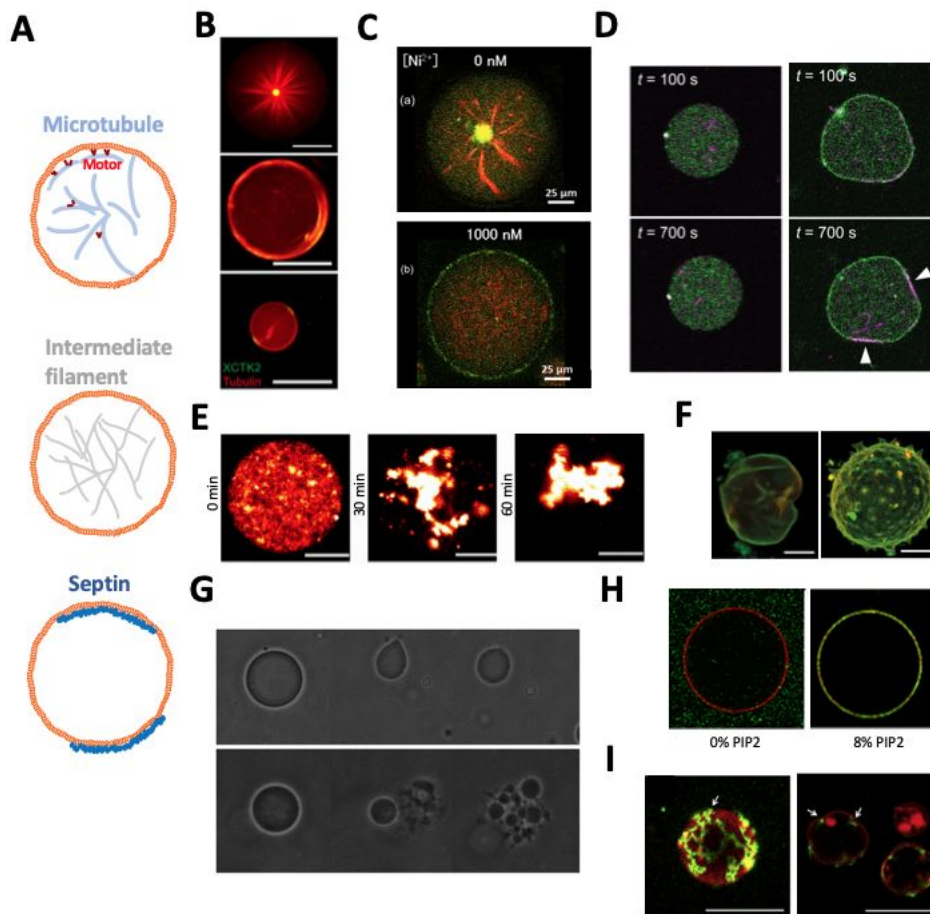


Figure 2. Vesicle- and droplet-based reconstitution of microtubules, intermediate filaments, and septins. (A) A schematic illustration. (B) Formation of astral (top), cortical (center), and ring-like (bottom) microtubule-kinesin complex in lipid-monolayer droplets depends on the droplet size. Scale bars, 20 µm.[99] (C) Microtubule (red)-kinesins (green) form aster-like aggregates in the lumen of emulsion droplets (top). They can be anchored to the inner surface of droplets by a Ni-NTA-His tag interaction (bottom).[100] (D) GUV shape changes by a microtubule (magenta)-kinesin (green)-based molecular robot in inactive (left) and active (right) state. A DNA-based clutch was used to couple microtubule-kinesin (actuator) to the lipid membrane.[105] (E) Keratin networks collapse in GUVs due to high self-affinity. Scale bars = 10 µm.[29] (F) Reconstitution of septin (green) outside PIP_2-containing GUVs (red) deforms the membrane (left), and forms spike-like deformations at higher concentrations (right). Scale bars, 10 µm.[106] (G) Shrinkage and reshaping of GUVs by SEPT5-SEPT6-SEPT7 complex outside GUVs (top). The complex induces dramatic shrinkage and formation of buds on PIP_2-containing GUVs, which leads to the formation of smaller vesicles (bottom).[107] (H) Septin (green) specifically binds to PIP_2 and was used as a reporter of PIP_2 incorporation on GUVs (red) formed by electroformation on platinum wires.[108] (I) smSEPT10 (green) forms organized structures (arrows) in PIP_2-containing GUVs (red) (top), and distorts GUV shape (bottom) at the sites of high smSEPT10 concentration (arrows). Scale bars, 30 µm.[109]

filaments compared to F-actin and microtubules[26, 110-112]. Among more than 70 types of intermediate filaments, keratin is expressed the most in the cell predominately in epithelial cells to control cell-cell and cell-ECM interactions at adhesion sites[26, 28]. The other key intermediate filament is vimentin that is well-known for its role in cell elasticity and migration, epithelial to mesenchymal transition, and cancer metastasis[113-116].

Vimentin filaments regulate actin dynamics and contractile force transmission, and orient cell traction stresses[117-119].

The reconstitution of keratin intermediate filaments into GUVs demonstrated their high self-affinity and cluster formation with time[29] (Fig. 2E). Encapsulation of actin in these keratin-containing GUVs formed a composite system without separation of the two networks where the actin network provided keratin with elastic resistance stabilizing the keratin network and preventing its collapse[29]. Encapsulated vimentin *in vitro* has never been characterized in details, however, inhibitory effect of vimentin filament assembly was observed when encapsulated in lipid vesicles[120]. Other *in vitro* studies of vimentin have demonstrated complex assembly of vimentin filaments triggered by salt, their high resistance to stress, and the exchange of subunits among vimentin filaments[119, 121, 122].

Septins, unconventional members of the cytoskeleton family, are GTP-binding proteins which form non-polar and stable filaments which assemble at the inner face of the plasma membrane and are involved in regulation of cell mechanics by interacting with the membrane and other cytoskeletal components[123-125]. By end-to-end polymerization of their subunits, they form complexes with distinct architecture[32]. Filamentous and bundled septin structures enhance cell rigidity, dynamically retract cell membrane during blebbing, and act as scaffolds to modulate membrane interaction with its binding proteins[32, 34]. Ring-like septin complexes play pivotal role as scaffold for myosin II and actomyosin rings of dividing cells and as diffusion barriers at the cytokinetic domain, the annulus of spermatozoon, and cell ciliary membrane[34, 126, 127]. Septin rings and cages are also required for the formation of phagosomes and traps during bacterial ingestion[33, 128, 129].

The *in vitro* reconstitution of septins has gained attention due to the recent discoveries in architecture and function of septin as a component of cell cytoskeleton (Fig. 2A). Septin could assemble into individual or networks of membrane-bound apolar filaments on vesicles and deform LUVs and GUVs (Fig. 2F) yet they do not impose a significant effect on membrane stiffness[106]. The higher concentrations of septin filaments on GUVs induced the formation of membrane spikes[106]. The architecture of septin subunit assemblies and lipid composition significantly influence the capability of septin filaments to deform GUVs[107]. SEPT5-SEPT6-SEPT7 filament complex could form small buds on POPC-containing GUVs but induced shrinkage of POPC/POPS-containing GUVs. The filaments significantly shrank POPC-PIP_2-containing GUVs and led to the formation of multiple buds[107] (Fig. 2G). PIP_2-containing GUVs are efficient platforms for septin reconstitution as septin specifically binds and interact with PIP_2[108, 130] (Fig. 2H). Both septins smSEPT5 and smSEPT10 from *Schistosoma mansoni*, a water-borne parasite, bind to PIP_2-containing GUVs where the latter could form cage-like structures on the vesicle membrane and deform it[109] (Fig. 2I). Another study showed that smSEPT10 also binds to DOPS-containing GUVs but not to those containing only DOPC[109]. These examples highlight the significance of intermediate and septin filaments in the mechanics of the cell and the versatility of lipid vesicles with different compositions for studying these apolar components of the cytoskeleton.

Recapitulation of mechanical equilibrium: Adhesion of cytoskeleton-encapsulating systems

Forces generated by actin cytoskeleton in the cell are transmitted to adhesion via integrin-actin linkage. Mediation of adhesion by myosin allows the regulation of cell polarity by substrate elastic properties which provides inhomogeneous contractile forces in cells[131]. Contractility-driven forces are transmitted to the cell membrane and ECM and therefore regulate membrane shape changes during cell polarization and movement. Molecular control of cortical homeostasis and cell polarization by a minimal network of encapsulated cytoskeleton may require a well-established assembly of actomyosin array bound to supported membrane. Time-dependent mechanical work generated by these networks requires an intimate coordination among the various components to equilibrate forces between each other internally and with substrate externally. Therefore, an ultimate minimal model of universal cell mechanics might require the presence and cooperation of entangled cytoskeletal components in synthetic cells.

In such a delicate mechanical equilibrium between adhesion-generated and internal forces, the lipid membranes and cytoskeletal biopolymers can influence each other's conformations. Dynamic deformations generated by actomyosin cortex in GUVs showed the necessity of active remodeling for strong adhesion to the substrate[37]. However, all cortex-free GUVs and those with cortex have been shown to spread and rupture within 2 minutes on rigid substrates[36, 37] (Fig. 3A and B). For an efficient mimic of cell adhesion, the choice of membrane ligand and adhesion receptor should also be carefully considered[132]. The reconstitution of integrin in liposomes could further enhance the adhesion and spreading of GUVs on compatible ECM-coated substrates[9] (Fig. 3C). However, an adhered GUV alone was shown to generate traction forces deforming soft substrates due to an increase in Laplace pressure[36] (Fig. 3D). A cortical network of actomyosin might significantly affect the generation of traction forces and tension buildup in GUVs. Contractility of substrate-anchored actomyosin networks depend highly on the network composition and architecture, and follow a sarcomeric-like mechanism which can in turn generate traction stresses and deform soft substrates[1, 133]. Such contractility on supported lipid bilayers changed the diffusive state of the membrane and retracted actin filaments and condensed them into foci[134]. Therefore, a minimal and stable contractile unit reshaping synthetic cells can potentially be achieved by cortical actomyosin GUVs adhered to a substrate with controlled stiffness[36] (Fig. 3A, D). The balance between adhesion and the shape of such active synthetic cells would determine the composition and concentration of these minimal set of proteins. Another prospective would be co-encapsulation of supportive cytoskeletal components such as microtubules with a cortical network of actin in adhered vesicles. If physically linked to the actin-membrane cortex, microtubules can internally adjust the morphology in response to external forces by providing mechanical support to the cortex.

Adhesion is not necessary for mechanical stability of cell in the absence of a central contractile unit. However, it is an essential unit for structural integrity and global deformation of migrating cells during power generation. A mimic of an adherent cell would ultimately require a direct link between the cytoskeleton and the units responsible for exchange of force with the environment, including to other 'cells'. This perhaps applies to biomimetics of cell-cell contact formation towards the reconstitution of prototissues[135-138].

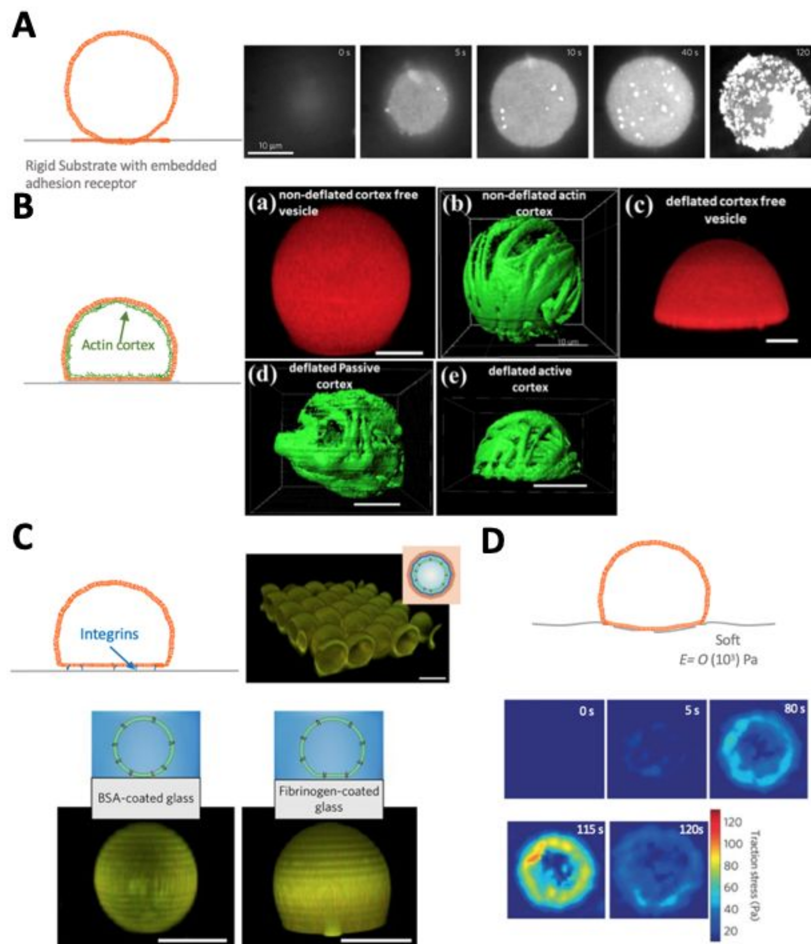


Figure 3. (A) Adherent cortex-free GUVs spread and eventually rupture on rigid substrates.[36] (B) Weakly bound cortex-free (a) and cortex-supported (b) GUVs eventually deflate on rigid substrates with strong adhesion (c and d) where the latter forms undefined shapes after deflation (d). Strongly adhered GUVs with myosin-driven contractile (active) cortices however, deform into spherical cap shapes (e) similar to those in (c).[37] (C) Droplet-stabilized GUVs with reconstituted integrin (left) were released into GUVs, and did not spread on BSA-coated glass (center). However, these integrin-containing GUVs spread well on fibrinogen-coated glass (right).[9] (D) Traction stress field of adherent GUVs on soft PAA gels changes with time. GUVs spread on the gel and tension builds within about 100 seconds before rupture.[36]

A direction for the future: cooperation and crosstalk

The studies of evolution have shown that biological phenotypes are a result of trade-off between natural selections for performing different tasks[139]. In the case of the cytoskeleton such trade-offs were achieved early in evolution and cytoskeletal components opted to use several yet conserved mechanisms to perform their multiple tasks. Indeed, there exists a close-knit cooperation and crosstalk between these components enabling cells to bear internal and external mechanical challenges. The mechanical control of the directionality of migrating cells is predominately a result of complex crosstalk between microtubules and actin networks with adaptable dynamics[35]. The interactions between actin filaments and microtubules have been long recognized[140,

[141], and bottom-up reconstitution of composite actin-microtubule networks require an assembly of proteins known to mediate actin-microtubule binding, interaction, and network plasticity[142, 143]. Plectin mediates cell mechanics and stress generation by crosslinking actin and microtubules while CLASP2 links microtubules and cortical actin at the periphery where its Abelson (a non-receptor tyrosine kinase)-dependent phosphorylation regulates cell protrusion[144, 145]. Beside CLASP2, a variety of cross-linkers such as MAP2c and Tau mediate actin-microtubule crosstalk during neurite formation and axon organization respectively[146, 147]. Key actin-microtubule coupling proteins including anillin, myosin X, and moesin mediate spindle and contractile ring organization[147, 148].

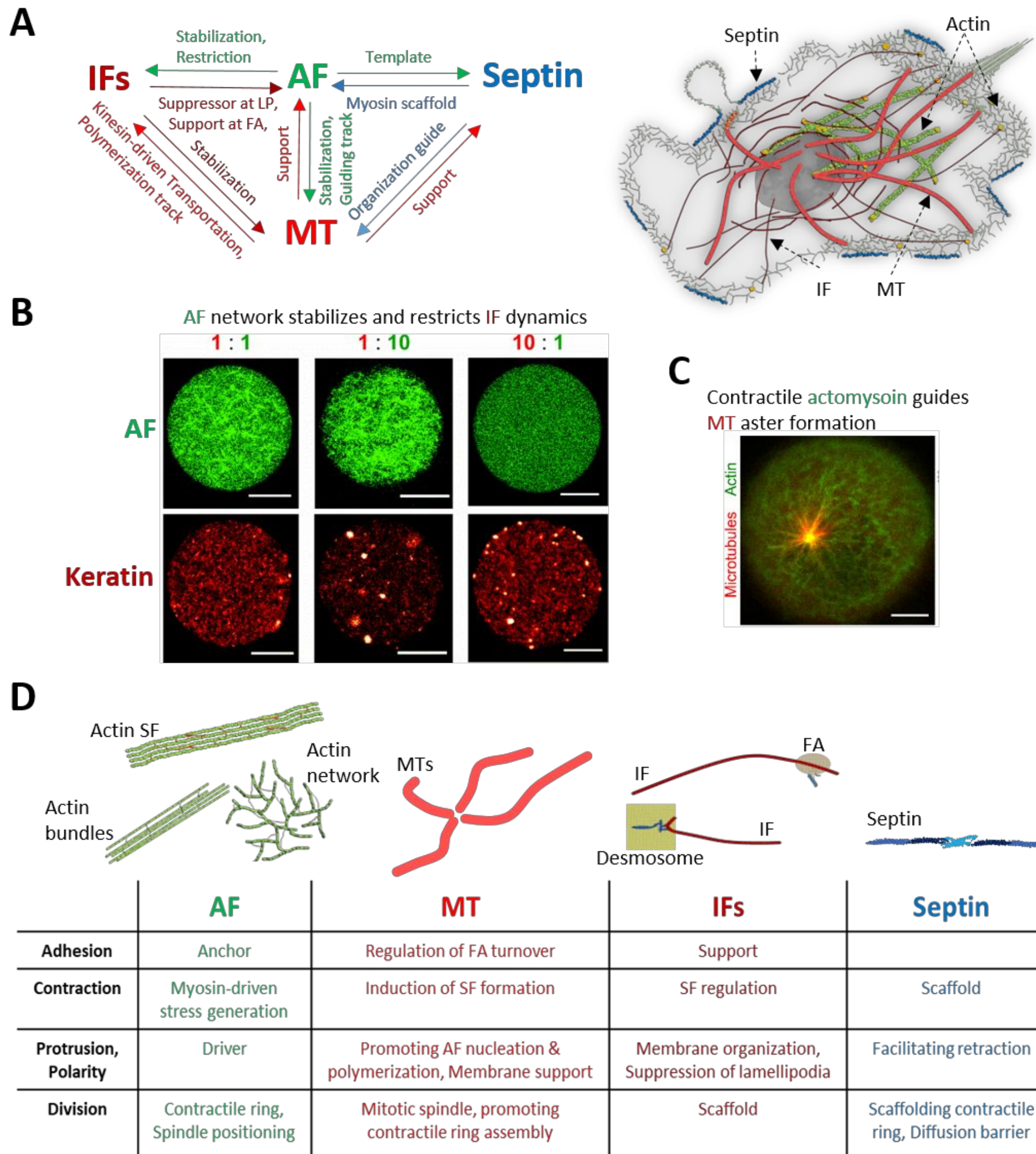
In vitro reconstitution systems can provide versatile platforms to study the role of individual coupling proteins in the dynamics of composite actin-microtubule networks and cell mechanics. However, the inclusion of intermediate filaments and septins in cytoskeletal biomimetic models of cells becomes indispensable when we realize that most types of cells require the interaction of multiple cytoskeletal components at key sites. Microtubules support actin networks at the periphery of migrating and dividing cells while they are teamed up with intermediate filaments at the adhesion sites where cell-generated forces are transmitted to the substrate as traction forces[92, 95, 148, 149]. Septins regulate reorganization of actin and microtubules and act as scaffold for myosin motors[124, 125, 150-153]. Actin networks act as template for septin filaments[154]. Septin filament formation itself depends on its interaction with microtubules[155]. Keratin filaments localize with actin cortex at the peripheral, apical and basolateral membrane of epithelial cells and assist actin networks in maintaining cell polarity[156-159]. The supportive role of each cytoskeletal component on the dynamics of other cytoskeletal components is summarized in Fig. 4A.

Actin co-encapsulation with keratin in GUVs showed the effect of their coupling in network properties and the supportive role of actin for stabilization of intermediate filaments[29] (Fig. 4B). Reconstitution of composite actin and microtubules can provide insights into cell's complex viscoelastic properties as well as cooperative dynamics and stress generation in the cell. Actin and microtubule networks dictate each other's dynamics *in vitro*[160]. Reconstituted contractile actin networks co-encapsulated with microtubules were shown to control the dynamics of microtubules and act as guiding tracks for microtubule aster mobility[160] (Fig 4C). Force generation of actin and microtubule networks is highly dependent on their distinct polymerization/depolymerization pathways, crosslinking proteins, and motors[35]. Actin binding and microtubule-associated proteins can physically link these two key cytoskeletal elements to couple their dynamics[149, 161]. Therefore, a careful assembly of the components is required to assess the effects of dynamics and formation of one network on the other. Cytoskeletal components deform membranes with distinct mechanisms. This indeed reflects the involvement of multiple cytoskeletal components in cell shape changes associated to polarization, protrusion, division, and death but the mechanisms behind how various cytoskeleton components cooperate remain largely unknown (Fig. 4D). A long-term goal for pursuing cytoskeletal reconstitution is the construction of a self-replicating and self-dividing or a motile synthetic cell[162]. Just as a natural eukaryotic cell uses microtubules for DNA segregation and actomyosin contractile machinery for cytokinesis, complex cell shape

changes are likely only possible with coordination of different cytoskeletal networks. An enclosed solution of cytoskeleton components in mechanical and thermal equilibrium with its surroundings will continue to be a valuable experimental system for studying the emergent mechanics and homeostasis of cellular systems. Co-encapsulating and crosslinking these components, and visualizing real-time network-network and network-membrane interactions will surely provide new biophysical insights.

One challenge associated with studying encapsulated cytoskeleton systems would be the design of experiments that enable us to simultaneously monitor and capture the interactions of different cytoskeleton components. While simultaneous imaging of multiple fluorescence channels is possible, they are limited to visualization of only a few proteins at a time. Synchronized visualization and tracking of each constituent in a spatial and temporally resolved manner seem inevitable to assess the effects of dynamics and formation of one network on the other. Stepwise complex assembly in bulk requires an experimental design with highly reproducible behaviour while enabling control over network mechanics and spatiotemporal dynamics. However, there are limited experimental approaches for introducing or activating components in an encapsulated system. Further development of strategies for controlling the assembly of encapsulated cytoskeletal networks would be highly desirable.

Finally, the self-organization of different cytoskeletal polymers is an ideal experimental system for studying composite active matter where it plays a rich emergent ordering behaviors[163]. Lyotropic suspensions of composite cytoskeletal networks can provide nematic systems with tunable network mechanics[164]. Importantly, the incorporation of membrane in this composite active matter is necessary to recapitulate cytoskeleton-membrane interaction and global shape changes, and this is also critical in the quest of cellular reconstitution of spatially organized processes[165]. The encapsulated cytoskeletal system should be a closed system that, ideally, would allow transport/transfer of energy and other molecular information across the membrane, rather than just passively confining the cytoskeletal components. In the case of cytoskeletal GUVs, the emergence of versatile flow-based techniques such as cDICE (continuous droplet interface crossing encapsulation) and one-pot droplet-destabilization techniques[166, 167] have facilitated the rapid production of encapsulated cytoskeleton in GUVs with high yield. These platforms will allow encapsulation of artificial organelles and cell-free expression of cytoskeleton-associated proteins to enable external control over network activity. Improvements in the design of microfluidic-assisted *in vitro* reconstitution platforms will continue to broaden our capability of synthetic cell design and cytoskeletal reconstitution.



	AF	MT	IFs	Septin
Adhesion	Anchor	Regulation of FA turnover	Support	
Contraction	Myosin-driven stress generation	Induction of SF formation	SF regulation	Scaffold
Protrusion, Polarity	Driver	Promoting AF nucleation & polymerization, Membrane support	Membrane organization, Suppression of lamellipodia	Facilitating retraction
Division	Contractile ring, Spindle positioning	Mitotic spindle, promoting contractile ring assembly	Scaffold	Scaffolding contractile ring, Diffusion barrier

Figure 4. (A) Crosstalk among cytoskeletal components.[160] (B) Actin (green) and keratin (red) can form composite networks in GUVs. Actin network stabilizes keratin network and prevents keratin collapse (see Fig. 2D). Scale bars, 10 µm. (C) Branched networks of actin (green) constrain microtubule (red) asters and potentially modulate aster mobility by myosin-driven contraction in droplets of *Xenopus* egg extracts. Scale bar, 10 µm.[29] (D) Cooperation of cytoskeletal components to modulate the mechanics of the cell. AF: actin filament, MT: microtubule, IF: intermediate filament, SF: stress fiber, FA: focal adhesion, LP: lamellipodium.

Conflicts of interest

There are no conflicts to declare.

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References

1. H. Ennomani, G. Letort, C. Guérin, J.-L. Martiel, W. Cao, F. Nédélec, M. Enrique, M. Théry and L. Blanchoin, *Current Biology*, 2016, **26**, 616-626.
2. P. Bieling, S. D. Hansen, O. Akin, C. C. Hayden, D. A. Fletcher and R. D. Mullins, *The EMBO journal*, 2018, **37**, 102-121.
3. A. Bernheim-Groswasser, S. Wiesner, R. M. Golsteyn, M.-F. Carlier and C. Sykes, *Nature*, 2002, **417**, 308.
4. O. Siton and A. Bernheim-Groswasser, *Journal of Biological Chemistry*, 2014, **289**, 31274-31286.
5. S. K. Vogel, F. Greiss, A. Khmelinskaia and P. Schwille, *Elife*, 2017, **6**, e24350.
6. K. A. Ganzinger, S. K. Vogel, J. Mücksch, P. Blumhardt and P. Schwille, *J Cell Sci*, 2019, **132**, jcs219899.
7. M. P. Murrell and M. L. Gardel, *Proceedings of the National Academy of Sciences*, 2012, **109**, 20820-20825.
8. I. Linsmeier, S. Banerjee, P. W. Oakes, W. Jung, T. Kim and M. P. Murrell, *Nature communications*, 2016, **7**, 12615.
9. M. Weiss, J. P. Frohnmayer, L. T. Benk, B. Haller, J.-W. Janiesch, T. Heitkamp, M. Börsch, R. B. Lira, R. Dimova and R. Lipowsky, *Nature materials*, 2018, **17**, 89.
10. P. M. McCall, S. Srivastava, S. L. Perry, D. R. Kovar, M. L. Gardel and M. V. Tirrell, *Biophysical journal*, 2018, **114**, 1636-1645.
11. E. A. Shah and K. Keren, *Elife*, 2014, **3**, e01433.
12. D. Merkle, N. Kahya and P. Schwille, *ChemBioChem*, 2008, **9**, 2673-2681.
13. F.-C. Tsai, B. r. Stuhrmann and G. H. Koenderink, *Langmuir*, 2011, **27**, 10061-10071.
14. K. Guevorkian, J. Manzi, L.-L. Pontani, F. Brochard-Wyart and C. Sykes, *Biophysical journal*, 2015, **109**, 2471-2479.
15. G. J. Brouhard and L. M. Rice, *Nature Reviews Molecular Cell Biology*, 2018, 1.
16. R. Rodríguez-García, V. A. Volkov, C.-Y. Chen, E. A. Katrukha, N. Olieric, A. Aher, I. Grigoriev, M. P. López, M. O. Steinmetz and L. C. Kapitein, *bioRxiv*, 2019, 590869.
17. A. Akhmanova and M. O. Steinmetz, *Nature reviews Molecular cell biology*, 2015, **16**, 711.
18. S. J. DeCamp, G. S. Redner, A. Baskaran, M. F. Hagan and Z. Dogic, *Nature materials*, 2015, **14**, 1110.
19. J. Prost, F. Jülicher and J.-F. Joanny, *Nature Physics*, 2015, **11**, 111.
20. A. Doostmohammadi, J. Ignés-Mullol, J. M. Yeomans and F. Sagués, *Nature communications*, 2018, **9**, 3246.
21. P. Guillamat, Ž. Kos, J. Hardoüin, J. Ignés-Mullol, M. Ravnik and F. Sagués, *Science advances*, 2018, **4**, eaao1470.
22. T. Sanchez, D. T. Chen, S. J. DeCamp, M. Heymann and Z. Dogic, *Nature*, 2012, **491**, 431.
23. H. Hess and J. L. Ross, *Chemical Society Reviews*, 2017, **46**, 5570-5587.
24. J. J. Keya, A. M. R. Kabir, D. Inoue, K. Sada, H. Hess, A. Kuzuya and A. Kakugo, *Scientific reports*, 2018, **8**.
25. P. Levi, *Frontiers in Neurorobotics*, 2015, **9**, 2.
26. S. Etienne-Manneville, *Annual review of cell and developmental biology*, 2018, **34**, 1-28.

27. N. T. Snider, N.-O. Ku and M. B. Omary, *Elife*, 2018, **7**, e35336.
28. J. T. Jacob, P. A. Coulombe, R. Kwan and M. B. Omary, *Cold Spring Harbor perspectives in biology*, 2018, **10**, a018275.
29. J. Deek, R. Maan, E. Loiseau and A. Bausch, *Soft matter*, 2018, **14**, 1897-1902.
30. J. Deek, F. Hecht, L. Rossetti, K. Wißmiller and A. R. Bausch, *Acta biomaterialia*, 2016, **43**, 218-229.
31. H. Atri, E. Bidram and D. Dunstan, *Materials*, 2015, **8**, 7472-7485.
32. K. Neubauer and B. Zieger, *Frontiers in cell and developmental biology*, 2017, **5**, 3.
33. A. A. Bridges and A. S. Gladfelter, *Journal of Biological Chemistry*, 2015, **290**, 17173-17180.
34. S. Mostowy and P. Cossart, *Nature reviews Molecular cell biology*, 2012, **13**, 183.
35. F. Burla, Y. Mulla, B. E. Vos, A. Aufderhorst-Roberts and G. H. Koenderink, *Nature Reviews Physics*, 2019, 1.
36. M. P. Murrell, R. Voituriez, J.-F. Joanny, P. Nassoy, C. Sykes and M. L. Gardel, *Nature Physics*, 2014, **10**, 163.
37. R. Maan, E. Loiseau and A. R. Bausch, *Biophysical journal*, 2018, **115**, 2395-2402.
38. E. Rideau, R. Dimova, P. Schwille, F. R. Wurm and K. Landfester, *Chemical Society Reviews*, 2018, **47**, 8572-8610.
39. Y. Mulla, A. Aufderhorst-Roberts and G. H. Koenderink, *Physical biology*, 2018, **15**, 041001.
40. K. A. Ganzinger and P. Schwille, *J Cell Sci*, 2019, **132**, jcs227488.
41. K. Kamiya and S. Takeuchi, *Journal of Materials Chemistry B*, 2017, **5**, 5911-5923.
42. P. Schwille, J. Spatz, K. Landfester, E. Bodenschatz, S. Herminghaus, V. Sourjik, T. J. Erb, P. Bastiaens, R. Lipowsky and A. Hyman, *Angewandte Chemie International Edition*, 2018, **57**, 13382-13392.
43. C. Martino and A. J. deMello, *Interface focus*, 2016, **6**, 20160011.
44. S. Majumder, N. Wubshet and A. P. Liu, *Journal of Micromechanics and Microengineering*, 2019, **29**, 083001.
45. K. Rottner and M. Schaks, *Current opinion in cell biology*, 2019, **56**, 53-63.
46. A. E. Carlsson, *Current opinion in cell biology*, 2018, **50**, 1-7.
47. D. Schlam, S. Grinstein and S. A. Freeman, in *Rho GTPases*, Springer, 2018, pp. 107-127.
48. N. Castaneda, T. Zheng, H. J. Rivera-Jacquez, H.-J. Lee, J. Hyun, A. Balaeff, Q. Huo and H. Kang, *The Journal of Physical Chemistry B*, 2018, **122**, 3826-3835.
49. J. Schnauß, T. Händler and J. Käs, *Polymers*, 2016, **8**, 274.
50. S. L. Freedman, C. Suarez, J. D. Winkelman, D. R. Kovar, G. A. Voth, A. R. Dinner and G. M. Hocky, *bioRxiv*, 2018, 493841.
51. I. Van Audenhove, M. Denert, C. Boucherie, L. Pieters, M. Cornelissen and J. Gettemans, *Journal of Biological Chemistry*, 2016, **291**, 9148-9160.
52. S. Espinoza-Sanchez, L. A. Metskas, S. Z. Chou, E. Rhoades and T. D. Pollard, *Proceedings of the National Academy of Sciences*, 2018, **115**, E8642-E8651.
53. M. Gardel, F. Nakamura, J. Hartwig, J. Crocker, T. Stossel and D. Weitz, *Proceedings of the National Academy of Sciences*, 2006, **103**, 1762-1767.
54. R. D. Mullins, P. Bieling and D. A. Fletcher, *Biophysical reviews*, 2018, **10**, 1537-1551.

55. A. Manhart, T. A. Icheva, C. Guerin, T. Klar, R. Boujemaa-Paterski, M. Thery, L. Blanchoin and A. Mogilner, *eLife*, 2019, **8**, e42413.
56. Y. Senju, M. Kalimeri, E. V. Koskela, P. Somerharju, H. Zhao, I. Vattulainen and P. Lappalainen, *Proceedings of the National Academy of Sciences*, 2017, **114**, E8977-E8986.
57. H. Nöding, M. Schön, C. Reinermann, N. Dörrer, A. Kürschner, B. Geil, I. Mey, C. Heussinger, A. Janshoff and C. Steinem, *The Journal of Physical Chemistry B*, 2018, **122**, 4537-4545.
58. A. G. Clark, O. Wartlick, G. Salbreux and E. K. Paluch, *Current Biology*, 2014, **24**, R484-R494.
59. L.-L. Pontani, J. Van der Gucht, G. Salbreux, J. Heuvingh, J.-F. Joanny and C. Sykes, *Biophysical journal*, 2009, **96**, 192-198.
60. J. Lemière, F. Valentino, C. Campillo and C. Sykes, *Biochimie*, 2016, **130**, 33-40.
61. P. T. Yam, C. A. Wilson, L. Ji, B. Hebert, E. L. Barnhart, N. A. Dye, P. W. Wiseman, G. Danuser and J. A. Theriot, *J Cell Biol*, 2007, **178**, 1207-1221.
62. S. L. Freedman, G. M. Hocky, S. Banerjee and A. R. Dinner, *Soft matter*, 2018, **14**, 7740-7747.
63. S. L. Freedman, S. Banerjee, G. M. Hocky and A. R. Dinner, *Biophysical journal*, 2017, **113**, 448-460.
64. C. Simon, R. Kusters, V. Caorsi, A. Allard, M. Abou-Ghali, J. Manzi, A. Di Cicco, D. Lévy, M. Lenz and J.-F. Joanny, *Nature Physics*, 2019, 1.
65. S. Vogel, C. Woelfer, D. A. Ramirez, R. J. Flassig, K. Sundmacher and P. Schwille, *bioRxiv*, 2018, 394700.
66. C. Simon, V. Caorsi, C. Campillo and C. Sykes, *Physical biology*, 2018, **15**, 065004.
67. A. Diz-Muñoz, D. A. Fletcher and O. D. Weiner, *Trends in cell biology*, 2013, **23**, 47-53.
68. F.-C. Tsai and G. H. Koenderink, *Soft Matter*, 2015, **11**, 8834-8847.
69. A. P. Liu, D. L. Richmond, L. Maibaum, S. Pronk, P. L. Geissler and D. A. Fletcher, *Nature physics*, 2008, **4**, 789.
70. A. P. Liu and D. A. Fletcher, *Biophysical journal*, 2006, **91**, 4064-4070.
71. K. Dürre, F. C. Keber, P. Bleicher, F. Brauns, C. J. Cyron, J. Faix and A. R. Bausch, *Nature communications*, 2018, **9**.
72. D. Hürtgen, S. K. Vogel and P. Schwille, *Advanced Biosystems*, 2019, 1800311.
73. K. Carvalho, F.-C. Tsai, E. Lees, R. Voituriez, G. H. Koenderink and C. Sykes, *Proceedings of the National Academy of Sciences*, 2013, **110**, 16456-16461.
74. T. H. Tan, M. Malik-Garbi, E. Abu-Shah, J. Li, A. Sharma, F. C. MacKintosh, K. Keren, C. F. Schmidt and N. Fakhri, *Science advances*, 2018, **4**, eaar2847.
75. N. Ierushalmi, M. Malik-Garbi, A. Manhart, E. Abu-Shah, B. L. Goode, A. Mogilner and K. Keren, *arXiv preprint arXiv:1907.10642*, 2019.
76. M. Malik-Garbi, N. Ierushalmi, S. Jansen, E. Abu-Shah, B. L. Goode, A. Mogilner and K. Keren, *Nature Physics*, 2019, 1.
77. Y. Nishigami, H. Ito, S. Sonobe and M. Ichikawa, *Scientific reports*, 2016, **6**, 18964.
78. E. Loiseau, J. A. Schneider, F. C. Keber, C. Pelzl, G. Massiera, G. Salbreux and A. R. Bausch, *Science Advances*, 2016, **2**, e1500465.

79. K. Carvalho, J. Lemière, F. Faqir, J. Manzi, L. Blanchoin, J. Plastino, T. Betz and C. Sykes, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2013, **368**, 20130005.
80. J. Plastino and L. Blanchoin, *J Cell Sci*, 2019, **132**, jcs219832.
81. D. R.-B. Aroush, N. Ofer, E. Abu-Shah, J. Allard, O. Krichevsky, A. Mogilner and K. Keren, *Current Biology*, 2017, **27**, 2963-2973. e2914.
82. J. Lemiere, K. Guevorkian, C. Campillo, C. Sykes and T. Betz, *Soft Matter*, 2013, **9**, 3181-3187.
83. K. Y. Lee, S.-J. Park, K. A. Lee, S.-H. Kim, H. Kim, Y. Meroz, L. Mahadevan, K.-H. Jung, T. K. Ahn and K. K. Parker, *Nature biotechnology*, 2018, **36**, 530.
84. S. Tanaka, K. Takiguchi and M. Hayashi, *Communications Physics*, 2018, **1**, 18.
85. G. M. Allen, A. Mogilner and J. A. Theriot, *Current Biology*, 2013, **23**, 560-568.
86. Y. Bashirzadeh, J. Poole, S. Qian and V. Maruthamuthu, *Bioelectromagnetics*, 2018, **39**, 289-298.
87. D. Perrier, A. Vahid, V. Kathavi, L. Stam, L. Rems, Y. Mulla, G. Koenderink, P. Boukany and M. Kreutzer, *bioRxiv*, 2018, 338566.
88. M. Harterink, S. L. Edwards, B. de Haan, K. W. Yau, S. van den Heuvel, L. C. Kapitein, K. G. Miller and C. C. Hoogenraad, *J Cell Sci*, 2018, **131**, jcs223107.
89. S. Forth and T. M. Kapoor, *J Cell Biol*, 2017, **216**, 1525-1531.
90. T. JA Craddock, S. R Hameroff, A. T Ayoub, M. Klobukowski and J. A Tuszynski, *Current topics in medicinal chemistry*, 2015, **15**, 523-533.
91. S. Hameroff and R. Penrose, *Mathematics and computers in simulation*, 1996, **40**, 453-480.
92. T. K. Akhshi, D. Wernike and A. Piekny, *Cytoskeleton*, 2014, **71**, 1-23.
93. L. C. Kapitein and C. C. Hoogenraad, *Neuron*, 2015, **87**, 492-506.
94. E. Memet, F. Hilitski, M. A. Morris, W. J. Schwenger, Z. Dogic and L. Mahadevan, *ELife*, 2018, **7**, e34695.
95. M. Dogterom and T. Surrey, *Current opinion in cell biology*, 2013, **25**, 23-29.
96. Z. J. Bergman, J. Wong, D. G. Drubin and G. Barnes, *J Cell Sci*, 2019, **132**, jcs219386.
97. K. Göpfrich, I. Platzman and J. P. Spatz, *Trends in biotechnology*, 2018, **36**, 938-951.
98. M. Hayashi, M. Nishiyama, Y. Kazayama, T. Toyota, Y. Harada and K. Takiguchi, *Langmuir*, 2016, **32**, 3794-3802.
99. H. Baumann and T. Surrey, *Journal of Biological Chemistry*, 2014, **289**, 22524-22535.
100. M. Tsuji, A. M. Rashedul Kabir, M. Ito, D. Inoue, K. Kokado, K. Sada and A. Kakugo, *Langmuir*, 2017, **33**, 12108-12113.
101. A. M. R. Kabir and A. Kakugo, *Polymer Journal*, 2018, 1.
102. K. Kim, N. Yoshinaga, S. Bhattacharyya, H. Nakazawa, M. Umetsu and W. Teizer, *Soft matter*, 2018, **14**, 3221-3231.
103. B. V. Hokmabad, K. A. Baldwin, C. Krüger, C. Bahr and C. C. Maass, *arXiv preprint arXiv:1810.07223*, 2018.
104. F. C. Keber, E. Loiseau, T. Sanchez, S. J. DeCamp, L. Giomi, M. J. Bowick, M. C. Marchetti, Z. Dogic and A. R. Bausch, *Science*, 2014, **345**, 1135-1139.
105. Y. Sato, Y. Hiratsuka, I. Kawamata, S. Murata and S.-i. M. Nomura, *Science Robotics*, 2017, **2**, 1-10.

106. A. Beber, C. Taveneau, M. Nania, F.-C. Tsai, A. Di Cicco, P. Bassereau, D. Lévy, J. T. Cabral, H. Isambert and S. Mangenot, *Nature communications*, 2019, **10**, 420.
107. D. C. Mendonca, J. N. Macedo, R. Itri, S. L. Guimaraes, F. L. B. da Silva, A. Cassago, R. C. Garratt, R. Portugal and A. P. Araujo, *bioRxiv*, 2019, 569251.
108. A. Beber, M. Alqabandi, C. Prévost, F. Viars, D. Lévy, P. Bassereau, A. Bertin and S. Mangenot, *Cytoskeleton*, 2018.
109. A. E. Zeraik, M. Staykova, M. G. Fontes, I. Nemuraitė, R. Quinlan, A. P. U. Araújo and R. DeMarco, *Biochimie*, 2016, **131**, 96-105.
110. L. Kreplak, H. Bär, J. Leterrier, H. Herrmann and U. Aebi, *Journal of molecular biology*, 2005, **354**, 569-577.
111. B. Nöding and S. Köster, *Physical review letters*, 2012, **108**, 088101.
112. J. Block, V. Schroeder, P. Pawelzyk, N. Willenbacher and S. Köster, *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 2015, **1853**, 3053-3064.
113. C.-Y. Liu, H.-H. Lin, M.-J. Tang and Y.-K. Wang, *Oncotarget*, 2015, **6**, 15966.
114. A. M. Richardson, L. S. Havel, A. E. Koyen, J. M. Konen, J. Shupe, W. Wiles, W. D. Martin, H. E. Grossniklaus, G. Sica and M. Gilbert-Ross, *Clinical Cancer Research*, 2018, **24**, 420-432.
115. Z. Gan, L. Ding, C. J. Burckhardt, J. Lowery, A. Zaritsky, K. Sitterley, A. Mota, N. Costigliola, C. G. Starker and D. F. Voytas, *Cell systems*, 2016, **3**, 252-263. e258.
116. M. Mendez, D. Restle and P. Janmey, *Biophysical journal*, 2014, **107**, 314-323.
117. D. D. Tang, G. Liao and B. D. Gerlach, *Journal of Engineering and Science in Medical Diagnostics and Therapy*, 2019, **2**, 010801.
118. Y. Jiu, J. Peränen, N. Schaible, F. Cheng, J. E. Eriksson, R. Krishnan and P. Lappalainen, *J Cell Sci*, 2017, **130**, 892-902.
119. N. Costigliola, L. Ding, C. J. Burckhardt, S. J. Han, E. Gutierrez, A. Mota, A. Groisman, T. J. Mitchison and G. Danuser, *Proceedings of the National Academy of Sciences*, 2017, **114**, 5195-5200.
120. G. Perides, A. Scherbarth, S. Kühn and P. Traub, *European journal of cell biology*, 1986, **41**, 313-325.
121. B. Nöding, H. Herrmann and S. Köster, *Biophysical journal*, 2014, **107**, 2923-2931.
122. C. G. Lopez, O. Saldanha, A. Aufderhorst-Roberts, C. Martinez-Torres, M. Kuijs, G. H. Koenderink, S. Köster and K. Huber, *Soft matter*, 2018, **14**, 8445-8454.
123. A. Bertin, M. A. McMurray, L. Thai, G. Garcia III, V. Votin, P. Grob, T. Allyn, J. Thorner and E. Nogales, *Journal of molecular biology*, 2010, **404**, 711-731.
124. J. R. Bowen, D. Hwang, X. Bai, D. Roy and E. T. Spiliotis, *The Journal of cell biology*, 2011, **194**, 187-197.
125. E. Joo, M. C. Surka and W. S. Trimble, *Developmental cell*, 2007, **13**, 677-690.
126. J. Marquardt, X. Chen and E. Bi, *Cytoskeleton*, 2019, **76**, 7-14.
127. Q. Hu, L. Milenkovic, H. Jin, M. P. Scott, M. V. Nachury, E. T. Spiliotis and W. J. Nelson, *Science*, 2010, **329**, 436-439.
128. Y.-W. Huang, M. Yan, R. F. Collins, J. E. DiCiccio, S. Grinstein and W. S. Trimble, *Molecular biology of the cell*, 2008, **19**, 1717-1726.
129. S. Mostowy, M. Bonazzi, M. A. Hamon, T. N. Tham, A. Mallet, M. Lelek, E. Gouin, C. Demangel, R. Brosch and C. Zimmer, *Cell host & microbe*, 2010, **8**, 433-444.

130. Y. Tanaka-Takiguchi, M. Kinoshita and K. Takiguchi, *Current Biology*, 2009, **19**, 140-145.
131. S. Banerjee and M. C. Marchetti, *EPL (Europhysics Letters)*, 2011, **96**, 28003.
132. S. M. Bartelt, E. Chervyachkova, J. Ricken and S. V. Wegner, *Advanced Biosystems*, 2019, 1800333.
133. T. Thoresen, M. Lenz and M. L. Gardel, *Biophysical journal*, 2011, **100**, 2698-2705.
134. F. Heinemann, S. K. Vogel and P. Schwille, *Biophysical journal*, 2013, **104**, 1465-1475.
135. S. M. Bartelt, E. Chervyachkova, J. Ricken and S. V. Wegner, *Advanced Biosystems*, 2019, **3**, 1800333.
136. S. Villringer, J. Madl, T. Sych, C. Manner, A. Imberty and W. Römer, *Scientific reports*, 2018, **8**, 1932.
137. O. I. Sentürk, E. Chervyachkova, Y. Ji and S. V. Wegner, *Small*, 2019, 1901801.
138. P. Gobbo, A. J. Patil, M. Li, R. Harniman, W. H. Briscoe and S. Mann, *Nature materials*, 2018, **17**, 1145.
139. H. Sheftel, O. Shoval, A. Mayo and U. Alon, *Ecology and evolution*, 2013, **3**, 1471-1483.
140. L. M. Griffith and T. D. Pollard, *The Journal of cell biology*, 1978, **78**, 958-965.
141. L. Griffith and T. Pollard, *Journal of Biological Chemistry*, 1982, **257**, 9143-9151.
142. S. N. Ricketts, J. L. Ross and R. M. Robertson-Anderson, *Biophysical journal*, 2018, **115**, 1055-1067.
143. S. N. Ricketts, M. L. Francis, L. Farhadi, M. J. Rust, M. Das, J. L. Ross and R. M. Robertson-Anderson, *Scientific Reports*, 2019, **9**, 12831.
144. G. Wiche, *Journal of cell science*, 1998, **111**, 2477-2486.
145. U. Engel, Y. Zhan, J. B. Long, S. N. Boyle, B. A. Ballif, K. Dorey, S. P. Gygi, A. J. Koleske and D. VanVactor, *Cytoskeleton*, 2014, **71**, 195-209.
146. Y. C. Fontela, H. Kadavath, J. Biernat, D. Riedel, E. Mandelkow and M. Zweckstetter, *Nature communications*, 2017, **8**, 1981.
147. K. Melková, V. Zapletal, S. Narasimhan, S. Jansen, J. Hritz, R. Škrabana, M. Zweckstetter, M. Ringkjøbing Jensen, M. Blackledge and L. Žídek, *Biomolecules*, 2019, **9**, 105.
148. M. Dogterom and G. H. Koenderink, *Nature Reviews Molecular Cell Biology*, 2018, 1.
149. F. Huber, A. Boire, M. P. Lopez and G. H. Koenderink, *Current opinion in cell biology*, 2015, **32**, 39-47.
150. B. E. Kremer, T. Haystead and I. G. Macara, *Molecular biology of the cell*, 2005, **16**, 4648-4659.
151. E. T. Spiliotis, *J Cell Sci*, 2018, **131**, jcs207555.
152. D. Bogorodskaya and L. A. Ligon, *bioRxiv*, 2019, 594515.
153. J. Hu, X. Bai, J. R. Bowen, L. Dolat, F. Korobova, W. Yu, P. W. Baas, T. Svitkina, G. Gallo and E. T. Spiliotis, *Current Biology*, 2012, **22**, 1109-1115.
154. M. Kinoshita, C. M. Field, M. L. Coughlin, A. F. Straight and T. J. Mitchison, *Developmental cell*, 2002, **3**, 791-802.
155. K.-i. Nagata, A. Kawajiri, S. Matsui, M. Takagishi, T. Shiromizu, N. Saitoh, I. Izawa, T. Kiyono, T. J. Itoh and H. Hotani, *Journal of Biological Chemistry*, 2003, **278**, 18538-18543.
156. A. S. Oriolo, F. A. Wald, V. P. Ramsauer and P. J. Salas, *Experimental cell research*, 2007, **313**, 2255-2264.
157. M. B. Omary, N.-O. Ku, P. Strnad and S. Hanada, *The Journal of clinical investigation*, 2009, **119**, 1794-1805.

158. R. Windoffer, A. Kölsch, S. Wöll and R. E. Leube, *The Journal of cell biology*, 2006, **173**, 341-348.
159. A. Kölsch, R. Windoffer and R. E. Leube, *Cell motility and the cytoskeleton*, 2009, **66**, 976-985.
160. A. Colin, P. Singaravelu, M. Théry, L. Blanchoin and Z. Gueroui, *Current Biology*, 2018, **28**, 2647-2656. e2644.
161. L. Peris, M. Bisbal, J. Martinez-Hernandez, Y. Saoudi, J. Jonckheere, M. Rolland, M. Sebastien, J. Brocard, E. Denarier and C. Bosc, *Nature communications*, 2018, **9**, 3775.
162. P. Schwille, *J Cell Biol*, 2015, **210**, 687-690.
163. L. Farhadi, C. Fermino Do Rosario, E. Debold, A. Baskaran and J. L. Ross, *Frontiers in Physics*, 2018, **6**, 75.
164. R. Zhang, N. Kumar, J. L. Ross, M. L. Gardel and J. J. de Pablo, *Proceedings of the National Academy of Sciences*, 2018, **115**, E124-E133.
165. A. P. Liu and D. A. Fletcher, *Nature Reviews Molecular Cell Biology*, 2009, **10**, 644.
166. M. Abkarian, E. Loiseau and G. Massiera, *Soft Matter*, 2011, **7**, 4610-4614.
167. K. Göpfrich, B. Haller, O. Staufer, Y. Dreher, U. Mersdorf, I. Platzman and J. P. Spatz, *ACS synthetic biology*, 2019.