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PLASMA MEMBRANE SHAPING

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CHAPTER

25

Physical principles of cellular membrane shapes

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25.1 Introduction

The main building block of the biological membranes is the lipid bilayer with embedded or attached proteins and carbohydrates (Cevc & Marsh, 1987; Israelachvili, 2011). Each membrane component, lipids and proteins, endows the membrane with a tendency to prefer a particular local shape, which is quantified by the local spontaneous curvature (Fig. 25.1). Some membrane components, such as specific lipids and specialized membrane proteins, possess well-defined intrinsic spontaneous curvature. However, in general, any molecular complex that is adsorbed to or embedded into the membrane and is not symmetric on the two bilayer leaflets necessarily introduces a spontaneous curvature. Cells can utilize such specialized molecular components and supra-molecular complexes to bend the plasma membrane into different shapes (McMahon & Gallop, 2005), which may be beneficial for the biological function of the cell. In addition, the cells can control their shape using the forces exerted on the membrane by the cytoskeleton. In this pedagogical chapter, we will aim to cover the basic mechanisms that determine cellular membrane shapes and shape dynamics, explaining the physical principles behind them. There are several reviews on this subject, for further reading (Alimohamadi & Rangamani, 2018; Bassereau & Sens, 2019; Deserno, 2015; Jarsch et al., 2016; Mouritsen, 1987; Seifert, 1997; Simunovic et al., 2015; Zimmerberg & Kozlov, 2006).

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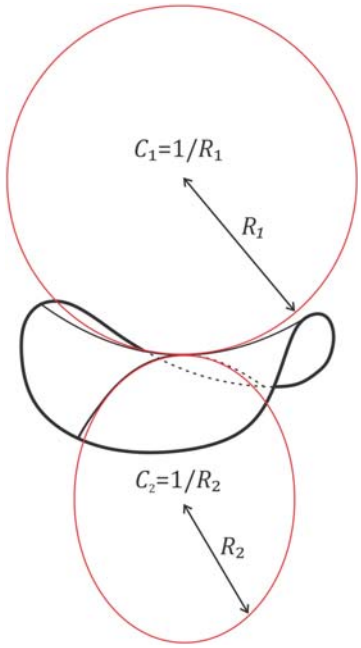

FIGURE 25.1 Schematic of the two principal curvatures (C_1 and C_2) of a saddle-like surface with anisotropic curvature. The first principal curvature is negative ($C_1 < 0$) and the second positive ($C_2 > 0$).

The introductory section describes the continuum theory of membrane shapes, through their curvature, and how they can be manipulated by "passive" membrane components. By passive, we mean that these components endow the membrane with a certain spontaneous shape, which minimizes the membrane's free (elastic) energy. In the second section, we describe how "active" processes in the cytoskeleton can produce protrusive and contractile forces that shape the cell. By active, we mean processes that rely on the consumption of ATP. Note that this division between passive and active processes can be blurred, as the passive processes may also involve chemical energy consumption: for example, a curved membrane protein may become membrane-bound only after phosphorylation, and therefore energy is involved in its recruitment to the cell membrane (Yamamoto et al., 2018).

This chapter serves as a short introduction to the physical principles that drive membrane shape deformations. The interested reader is encouraged to follow the bibliography to go deeper into the theoretical physics description of membrane elasticity. The mathematical description given here is meant only to demonstrate and emphasize that a quantitative treatment of membrane shapes exists and can be used to shed light on these complex biological processes.

25.2 Theory and mechanisms of passive membrane shaping

The local membrane shape can be described by the two principal membrane curvatures C_1 and C_2 (see Fig. 25.1), while the intrinsic shape of the membrane constituents (see Fig. 25.2)

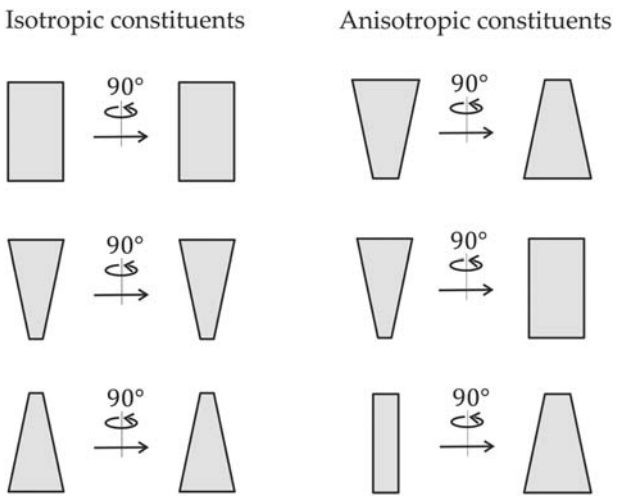

FIGURE 25.2 Schematic of isotropic ($C_{1m} = C_{2m}$) and anisotropic ($C_{1m} \neq C_{2m}$) shapes of membrane constituents. Front and side views of constituents are shown.

may be characterized by the corresponding intrinsic principal curvatures or other types of geometrical parameters (Iglič et al., 2015). Each membrane constituent may locally rotate with respect to the membrane (Kralj-Iglič et al., 1999). If the two principal curvatures of the membrane at a given location are equal ($C_1 = C_2$), the local membrane curvature is considered *isotropic*, while if they differ ($C_1 \neq C_2$), it is considered *anisotropic* (Fournier, 1996; Kralj-Iglič et al., 1996; Mesarec, Drab, et al., 2021; Mesarec, Iglič, et al., 2021) (see Fig. 25.1). Likewise, if the intrinsic principal curvatures of a membrane constituent are equal ($C_{1m} = C_{2m}$), the constituent is considered isotropic, while if they differ ($C_{1m} \neq C_{2m}$), it is considered anisotropic (Fournier, 1996; Kralj-Iglič et al., 1996, 1999, 2002; Mesarec, Drab, et al., 2021; Mesarec, Iglič, et al., 2021; Walani et al., 2014) (see Fig. 25.2).

Biological membranes might possess a certain degree of in-plane orientational ordering, especially in membrane regions with high and anisotropic curvature (Fournier, 1996; Fournier & Galatola, 1998; Kralj-Iglič et al., 1999, 2000, 2006; Kumar et al., 2019; Mahapatra et al., 2021; Mesarec et al., 2021a,b). By orientational ordering, we mean that the membrane components have some preferred direction or alignment in the plane of the membrane. In general, the anisotropic membrane components may individually rotate in the membrane plane due to thermal fluctuation. However, it is still possible that they maintain a preferred average orientation (direction) if the anisotropic membrane curvature ($C_1 \neq C_2$) is large enough to overcome the orientational disorder due to thermal fluctuations (Fournier, 1996; Fournier & Galatola, 1998; Kralj-Iglič et al., 1999, 2000, 2006). An example of a membrane region with very high anisotropic curvature is the membrane neck connecting the daughter vesicle to the parent cell (Kralj-Iglič et al., 1999, 2006). Direct nearest-neighbor interactions between anisotropic membrane components, due to steric excluded volume effect and van der Waals forces, may further hinder the influence of rotation due to thermal fluctuations and facilitate the average orientational ordering of anisotropic membrane components (Fournier & Galatola, 1998; Kralj-Iglič et al., 2006).

If the anisotropic molecules are strongly attached to the membrane surface, their freedom of rotation due to thermal fluctuation may be considerably reduced, and therefore, their orientational ordering is more pronounced. For example, orientational ordering may occur because of membrane-attached anisotropic rod-like BAR domains (Baumgart et al., 2011; Kabaso et al., 2010; Lee et al., 2021; Mesarec et al., 2016; Perutková et al., 2010) (Fig. 25.3). In regions with high concentrations of BAR domains, the rotation of an individual BAR domain becomes restricted due to direct/steric interaction with neighboring BAR domains (Jarin et al., 2021; Mesarec et al., 2016). BAR superfamily domains are able to induce the local bending of the membrane if the binding energy of the domain is larger than the energy required to bend the membrane (Zimmerberg & McLaughlin, 2004). BAR domains are important for cell migration, cell division, and membrane trafficking (Frost et al., 2009; Mesarec et al., 2016; Suetsugu et al., 2014).

The interaction between different proteins and the lipid bilayer component of the membrane may result in membrane deformations and changes in the topology of the cell or vesicle. This effect can be named "protein-mediated membrane remodeling" (Ayton & Voth, 2010; Davtyan et al., 2016). BAR proteins-induced membrane remodeling can, among others, result in tubulation and vesiculation of liposomes (Ayton et al., 2009; Mesarec et al., 2016).

Orientational ordering in biological membranes may also occur due to the asymmetry of the two flexible hydrocarbon chains of lipids or due to the anisotropic shape of the lipid head group (Fournier & Galatola, 1998; Kralj-Iglič et al., 2000, 2006; Perutková et al., 2011) (Fig. 25.4). Furthermore, tilt and hexatic orientational ordering may be developed by the tails of lipid molecules that may tilt relative to the surface normal (Helfrich & Prost, 1988; Lubensky & Prost, 1992; Smith et al., 1988). The orientational in-plane order in hexatic membranes with short-range positional order and long-range bond orientation order has also been observed experimentally (Bernchou et al., 2009). Within a statistical-mechanical approach, it has been indicated that in highly curved anisotropic membrane regions, the

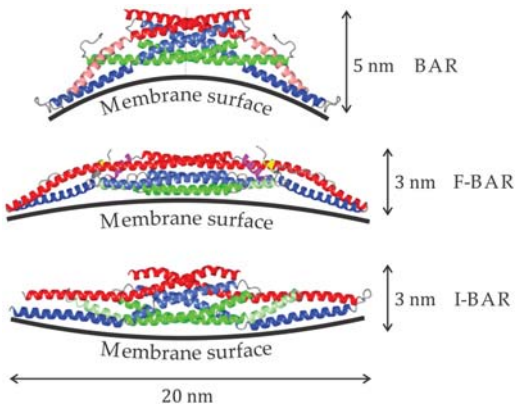

FIGURE 25.3 Schematic of the BAR superfamily domains shown along with their curvature preferences. Source: *Adapted from Mesarec, L., Góźdź, W., Kralj-Iglič, V., Kralj, S., & Iglič, A. (2016). Closed membrane shapes with attached BAR domains subject to external force of actin filaments*. Colloids and Surfaces. B, Biointerfaces, *141, 132–140. https://doi.org/10.1016/j.colsurfb.2016.01.010* and *http://bar-superfamily.org/*.

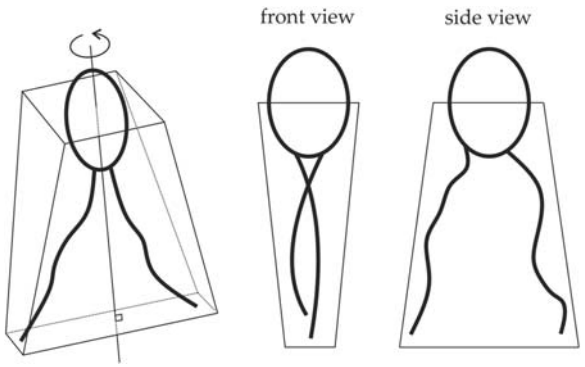

FIGURE 25.4 Different views of phospholipid molecules that have in general anisotropic shape. Source: *Adapted with permission from Mesarec, L., Góźdź, W., Iglič, A., Kralj-Iglič, V., Virga, E. G., & Kralj, S. (2019). Normal red blood cells' shape stabilized by membrane's in-plane ordering.* Scientific Reports, *9(1), 19742. https://doi.org/10.1038/s41598-019-56128-0.*

average orientation of lipid molecules cannot be neglected in spite of their rapid thermal rotational movement (Fournier & Galatola, 1998; Kralj-Iglič et al., 2006; Mesarec et al., 2019; Mesarec, Drab, et al., 2021; Mesarec, Iglič, et al., 2021; Penič et al., 2020).

To theoretically study membrane shapes with highly curved anisotropic regions, such as membrane tubular protrusions, a model considering deviatoric elasticity was proposed within a continuum approach (Fischer, 1992, 1993), introducing the spontaneous membrane warp as a parameter. However, because the existence of the membrane nanostructures was at that time not yet widely acknowledged and biological membranes were considered locally nearly flat, it was assumed that the value of spontaneous lipid bilayer warp is negligible (Fischer, 1992, 1993). In 1996, the deviatoric elasticity model was proposed, which takes into account also the anisotropic properties of membrane components (Fournier, 1996; Kralj-Iglič et al., 1996). Deviatoric membrane free energy was derived from an energy of a single-constituent applying the methods of statistical physics (Fournier, 1996; Kralj-Iglič et al., 1996, 1999, 2006). In general, membrane constituents in the deviatoric elasticity model can be considered isotropic or anisotropic (Baumgart et al., 2011; Fournier, 1996; Fournier & Galatola, 1998; Kralj-Iglič et al., 1996, 1999, 2000, 2002, 2006; Lubensky & Prost, 1992; Mahapatra et al., 2021; Mesarec et al., 2021a,b; Walani et al., 2014) (Fig. 25.2). Deviatoric elasticity model can theoretically explain (without additional external force) stable shapes of cells and vesicles possessing strongly anisotropically curved regions, such as shapes with thin tubular protrusions (Bobrovska et al., 2013; Fournier, 1996; Kabaso et al., 2012; Kralj-Iglič et al., 2000, 2002; Kumar et al., 2019; Mahapatra et al., 2021; Mesarec et al., 2017, 2021a,b; Ramakrishnan et al., 2010) and narrow saddle-like necks (Fournier, 1996; Iglič et al., 2007; Kralj-Iglič et al., 1999, 2006).

In all these examples, the cell achieves its desired shape by producing a sufficient amount of the curvature-producing component and ensure that it is concentrated at the right location on the membrane. Only at high enough densities and/or strong enough direct nearest-neighbor interactions can these passive components induce large shape changes, and it is, therefore, a challenge for the cells to maintain control over the locations

where these components aggregate. In addition, strong direct interactions or binding between these components, to aggregate them and induce a large shape change, may require energy (but not always, see, for example, Kralj-Iglič et al., 2000). The mechanisms allowing the cell to have precise control over the locations and timing of such membrane aggregates, and the shapes that they induce, are not fully known.

25.2.1 Bending energy of a membrane with curved, in general anisotropic, components (nanodomains with rigid or flexible inclusions)

Each membrane element adapts its shape such that it fits as much as possible to the local shape of the membrane (Fošnarič et al., 2005, 2006; Kralj-Iglič et al., 1999). The energy which is needed for the deformation of the membrane around the rigid, and in general anisotropic, inclusion molecule (in our case the rigid core of a membrane nanodomain) (Fig. 25.2), embedded in the lipid monolayer or bilayer (Fig. 25.5A), is actually the energy of the deformation of the surrounding lipid molecules which adjust their shape and organization to the intrinsic shape imposed by the rigid membrane inclusion (Fig. 25.5A). The deformations of the lipids around the rigid inclusion (for example, around the protein embedded in the lipid bilayer) are assumed to be constrained to the close vicinity of the inclusion and include the stretching and tilt of the lipid tails, as well as splay, twist, bending and saddle deformations (Fošnarič et al., 2005, 2006). We assume the existence of these local microscopic deformations of the lipids in the vicinity of the membrane-embedded rigid molecules (rigid inclusions). The free energy perturbation of the lipid bilayer,

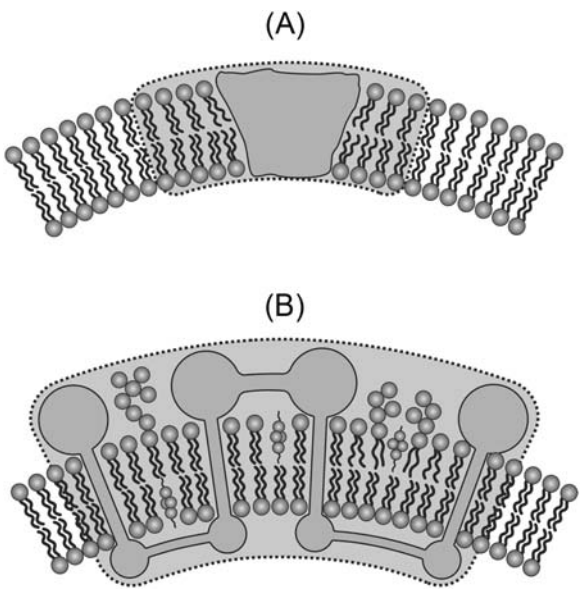

FIGURE 25.5 Schematic of flexible membrane nanodomain (shadow region) with membrane-embedded rigid (A) and flexible (B) inclusion.

induced by the rigid inclusion (the rigid core of nanodomain as presented in Fig. 25.5A), can be expressed as (Fošnarič et al., 2005, 2006; Kralj-Iglič et al., 1999):

$$w = (2K_1 + K_2)(H - H_m)^2 - K_2\left(D^2 - 2DD_m\cos(2\omega) + D_m^2\right)$$

and is defined as the energy of a flexible membrane nanodomain (composed of rigid inclusion and surrounding deformed lipids) induced by rigid (in general) anisotropic inclusion (Fig. 25.5A). Here $H = (C_1 + C_2)/2$ is the local mean curvature of the membrane, $D = |C_1 - C_2|/2$ is the local curvature deviator of the membrane, $H_m = (C_{1m} + C_{2m})/2$ and $D_m = |C_{1m} - C_{2m}|/2$ are the intrinsic mean curvature and intrinsic curvature deviator of the membrane nanodomain which depends on the shape of the membrane inclusion (characterized by its principle spontaneous curvatures C_{1m}, C_{2m}) (Fošnarič et al., 2005, 2006; Kralj-Iglič et al., 1999), K_1 and K_2 are the bending moduli and the angle ω describes the orientation of the nanodomain in the plane of the membrane. From stability analysis, it follows that $K_1 > -K_2/2$ and $K_2 < 0$ (Iglič et al., 2007). If the membrane nanodomain is isotropic (i.e., $D_m = 0$), the expression for the nanodomain energy transforms (up to the constant terms independent of H and D) into the well-known Helfrich (Helfrich, 1974) type of energy expression (Iglič et al., 2005):

$$w_H = \frac{k_c}{2}(2H - C_0)^2 + k_G(C_1C_2), \tag{25.1}$$

where $k_c = K_1$, $k_G = K_2$ and the spontaneous curvature C_0 is the function of parameters K_1, K_2 and H_m.

If the principal systems of the actual local membrane curvature tensor and the intrinsic nanodomain curvature tensor are assumed to coincide everywhere on the membrane surface, so that omega $\omega = 0$, the expression for nanodomain energy transforms into (Iglič et al., 2005; Kralj-Iglič et al., 2002):

$$w_0 = (2K_1 + K_2)(H - H_m)^2 - K_2(D - D_m)^2,$$

where $K_2 < 0$. The limiting expression w_0 was later applied in many other works for calculating membrane shapes in the presence of curved and anisotropic membrane components (Bobrovska et al., 2013; Mahapatra et al., 2021; Mesarec et al., 2017; Mesarec, Drab, et al., 2021; Mesarec, Iglič, et al., 2021; Walani et al., 2014).

These expressions for the energy of flexible membrane nanodomain induced by rigid inclusions (Fig. 25.5A) can also be applied for the calculations of the energy of flexible nanodomains involving the bending of the flexible inclusion (Fig. 25.5B), as occurs for example in the case of membrane-bound flexible chain-like proteins.

25.2.2 Spherical and tubular membrane shapes due to aggregates of passive curved anisotropic components

We now demonstrate several typical membrane shapes that can be obtained when closed vesicles contain curved (anisotropic) membrane components. These membrane components are considered to be freely diffusing on the membrane, and when aggregated, they maintain a membrane domain that allows them to deform until the overall bending

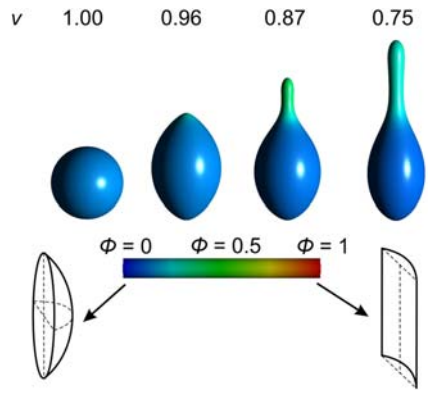

FIGURE 25.6 Equilibrium vesicle shapes calculated for two-component membrane containing anisotropic and isotropic flexible nanodomains and different values of the vesicle reduced volume v. The mixing entropy is considered in minimization of the membrane free energy. The red color denotes the highest possible local relative area density of anisotropic nanodomains. Source: *Adapted with permission from Mesarec, L., Góźdź, W., Kralj, S., Fošnarič, M., Penič, S., Kralj-Iglič, V., & Iglič, A. (2017). On the role of external force of actin filaments in the formation of tubular protrusions of closed membrane shapes with anisotropic membrane components.* European Biophysics Journal, *46 (8), 705–718. https://doi.org/10.1007/s00249-017-1212-z.*

energy is minimized. In addition to the characteristics of the curved, in general anisotropic, components and their membrane density, there is another control parameter: the cell/vesicle reduced volume v. This parameter is the ratio between the vesicle volume and the volume of a sphere with the same membrane area and is always between 0 and 1. For a living cell, this parameter is related to the excess membrane area of the cell: when v is small, the vesicle has a large amount of excess membrane area and can undergo large shape deformations.

As an example, Fig. 25.6 shows the calculated axisymmetric vesicle shapes for a two-component membrane comprising both anisotropic and isotropic nanodomains. For reduced volume $v = 1$ the vesicle can only have a spherical shape. In this spherical shape, both types of nanodomains are homogeneously mixed throughout the surface (Fig. 25.6). It can be seen in Fig. 25.6 that for the lower values of v the anisotropic nanodomains start to accumulate into a separate, more tubular protrusion region. The lateral segregation of the isotropic and anisotropic nanodomains is relatively weak due to the strong opposing effect of the mixing entropy.

Fig. 25.7 shows the equilibrium vesicle shape containing two types of isotropic nanodomains, i.e., highly curved and slightly curved isotropic nanodomains. It can be seen in the figure that the highly curved isotropic nanodomains impose the formation of a highly curved membrane bud. A relatively strong degree of lateral segregation of both types of curved nanodomains can be observed in this figure.

Fig. 25.8 presents the effect of anisotropic saddle-like nanodomains, which favor the formation of an anisotropic neck connecting the two parts of the vesicle. A high degree of

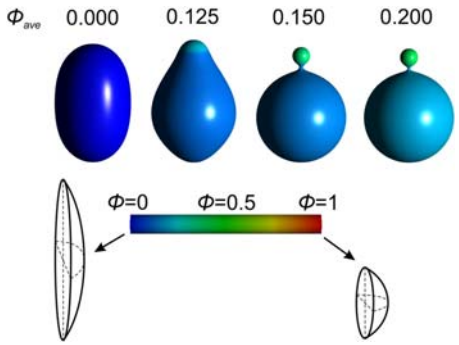

FIGURE 25.7 Equilibrium vesicles shapes with a two-component membrane composed of highly and slightly curved flexible isotropic nanodomains calculated for different values of the average relative area density of highly curved isotropic nanodomains (ϕ_{ave}). The red color denotes the highest possible local relative area density of highly curved isotropic nanodomains (Kralj-Iglič et al., 2020). Source*: Adapted with permission from Kralj-Iglič, V., Pocsfalvi, G., Mesarec, L., Šuštar, V., Hägerstrand, H., & Iglič, A. (2020). Minimizing isotropic and deviatoric membrane energy – An unifying formation mechanism of different cellular membrane nanovesicle types.* PLoS One, *15(12), e0244796. https://doi.org/10.1371/journal.pone.0244796*.

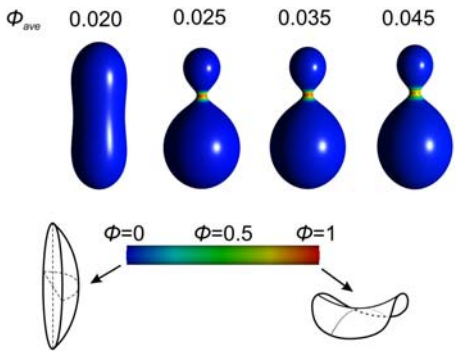

FIGURE 25.8 Equilibrium vesicles shapes calculated for different values of the average relative area density of flexible anisotropic saddle-like nanodomains (ϕ_{ave}). Besides saddle-like nanodomains, the membrane also contains isotropic nanodomains. The red color indicates the highest possible local relative area density of saddle-like nanodomains. Source*: Adapted with permission from Kralj-Iglič, V., Pocsfalvi, G., Mesarec, L., Šuštar, V., Hägerstrand, H., & Iglič, A. (2020). Minimizing isotropic and deviatoric membrane energy – An unifying formation mechanism of different cellular membrane nanovesicle types.* PLoS One, *15(12), e0244796. https://doi.org/10.1371/journal.pone.0244796*.

lateral segregation can be seen. The anisotropic saddle-like nanodomains are accumulated in the neck region, thereby stabilizing it.

These examples show the equilibrium configurations, which minimize the overall free energy of the system, including the entropy contribution. With respect to cell shapes, these examples provide us with the spectrum of steady-state shapes that cells may utilize, using passively curved, in general anisotropic, membrane components/nanodomains.

25.2.3 Pushing and pulling: mechanisms of active membrane shaping by the cytoskeleton

In addition to passive membrane components that shape the membrane, shape changes can be achieved by exerting forces on the membrane from the cytoskeleton. There are two main forces that the cytoskeleton can exert on the membrane: (1) pushing the membrane outwards, creating a local protrusion, and (2) pulling the membrane inwards into the cell. These forces, of protrusion and contraction, involve the consumption of chemical energy (ATP), and we, therefore, denote them as "active." We first describe briefly the origin of these cytoskeletal forces and then discuss how cells can utilize them to form specific shapes.

We will focus in this section on describing the membrane shapes arising from the protrusive forces exerted by the cytoskeleton as these are dominant in determining the cell shape. Contractile forces are mostly dominant during the cell division stage, where they are effective in the form of a contractile ring that "cuts" the cell into two daughter cells. Contractile forces are therefore dominant during a short duration of the cell cycle but play a less dominant role regarding the steady-state shape of the cell surface. Contractile forces do play an important role by dynamically shaping the cells during cell motility, such as in bleb-based cell migration, and affect the global shape of cells within confluent cellular tissues (Hannezo et al., 2014).

The most common method for the cytoskeleton to exert a pushing force on the membrane is by having actin filaments polymerize near the membrane. The actin filaments have a polarized structure, with fast-growing (barbed) ends where monomers readily get incorporated to elongate the filaments and a depolymerizing (pointed) end where the filaments have a tendency to destabilize (Pollard & Cooper, 2009; Salbreux et al., 2012). When the growing ends of the filaments are close to the membrane, the process of incorporation of additional monomers acts as a local pressure that pushes equally the membrane and the filaments in opposite directions (Carlsson, 2001; Liu et al., 2008; Mogilner & Oster, 2003) (Fig. 25.9A). The cell, therefore, has many membrane-bound proteins that direct and recruit the actin polymerization to the membrane (Membrane Actin Polymerization Factor—MAPF, or Nucleation promoting factor (NPF)) (Welch & Mullins, 2002). Typical examples of MAPF/NPF are (1) the class of proteins that initiate new branches to grow on existing filaments (such as the WAVE complex) (Derivery & Gautreau, 2010; Stradal et al., 2004), and (2) proteins that act a "processive cappers" (or "elongation factors"), which are bound to the growing ends, promoting their elongation and protecting them from capping (such as Formins or VASP (Chesarone & Goode, 2009; Krause et al., 2003)).

The growth of a branched actin network near the membrane, due to MAPF/NPF of class I, typically produces a local isotropic pressure, pushing the membrane outwards. The structures formed by the forces of the branched actin are mostly found in the form of flattened membrane protrusions (Fig. 25.9B), such as ruffles and lamellipodia (Chhabra & Higgs, 2007). The MAPF/NPF of class II tends to form elongated bundles of actin, which are more typically found in finger-like protrusions (Chhabra & Higgs, 2007) (Fig. 25.9C), such as filopodia, microvilli, and stereocilia.

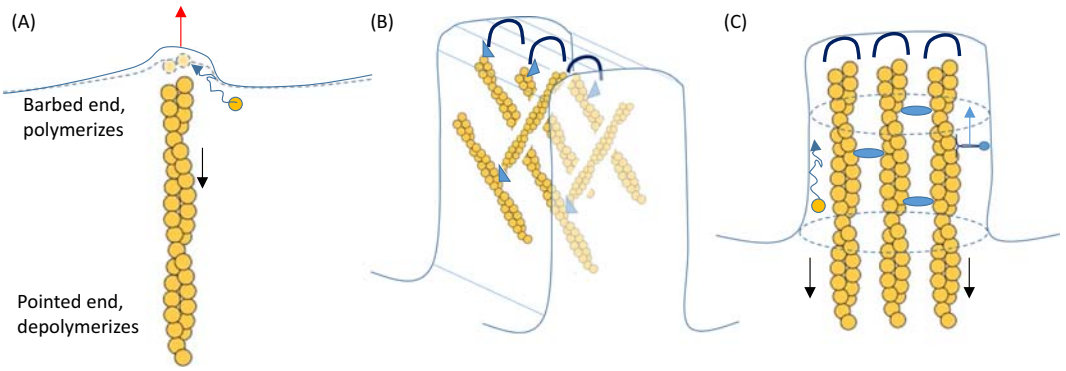

FIGURE 25.9 (A) Illustration of the "thermal-ratchet" process of polymerization of an actin filament (orange) by incorporation of monomers at the barbed end, near the membrane (blue curve). This polymerization occurs when a monomer diffuses between the membrane and the filament due to membrane and filament fluctuations (dashed line). Following the incorporation of new monomers, there is a pressure that exerts a protrusive force on the membrane (red arrow) and retrograde flow of the filament (black arrow). (B) Illustration of the growth of a branched actin network near the membrane, due to MAPF of class I (black arcs), usually producing flattened membrane protrusions. Blue triangles denote the Arp2/3-induced branching points. (C) Illustration of the finger-like protrusions formed by MAPF of class II. The blue ovals denote crosslinking proteins that bundle the actin, and the blue circle-tipped object denotes a tip-directed myosin motor carrying a cargo molecule, actively translocating along the surface of the actin bundle (blue arrow).

25.3 Patterning the actin polymerization on the membrane

Polymerizing the actin near the membrane, and inducing protrusive forces, does not yet specify the types of shapes that this process can produce. Clearly, if the actin filaments polymerize uniformly at the membrane, it amounts to a uniform pressure, which by itself does not produce any particular shape (Carlsson, 2018). To deform the membrane, the cell has to confine the polymerization of actin to specific locations. The localized actin polymerization can naturally push the membrane outwards but can also pull the membrane inwards if the membrane is bound to the actin filaments that flow away from nearby membrane edges (Carlsson & Bayly, 2014; Motahari & Carlsson, 2019). Since the actin is recruited to the membrane by the MAPF, the cell has to produce precise patterns of these proteins on the membrane to initiate the required shape deformation. There are various mechanisms that initiate pattern-formation that spontaneously break the uniform distribution of the MAPF, as we now describe.

One mechanism is in the form of reaction-diffusion (RD) models (Holmes & Edelstein-Keshet, 2012, 2016). Here, a combination of several biochemical factors that are diffusing in the membrane and the cytoplasm have non-linear interactions among them, which produce positive (and negative) feedback. The simplest of such models include positive feedback whereby, for example, the actin either enhances itself through the non-linear branching reaction or by recruitment of other factors that enhance polymerization. At the

same time, the polymerized actin can induce the accumulation of an inhibitor molecule on the membrane that acts to inhibit the polymerization. In other models, the RD dynamics cause patterns to form on the cell membrane independently of the actin polymerization itself (Arai et al., 2010), and the actin is recruited to follow these patterns by MAPF that are activated by one of the reaction components. Combined, these feedbacks can spontaneously cause the organization of the MAPF to form patterns, such as localized circular aggregates or linear stripes, as well as dynamic patterns, such as wave-like propagating patterns. Since the actin polymerization follows the location of the MAPF, the resulting pattern of the pressure that is applied to the membrane deforms it into specific shapes: for example, driving the cell forward during polarized migration (Bhattacharya et al., 2020; Campbell & Bagchi, 2018; Flemming et al., 2020; Rappel & Edelstein-Keshet, 2017; Taniguchi et al., 2013), driving the formation of dorsal ruffles (Bernitt et al., 2017) or into various cup-shaped structures that resemble macropinocytosis (Saito & Sawai, 2021). These theoretical studies of cellular pattern formation necessarily contain a small part of the complex biochemistry of the cell but serve to demonstrate the range of membrane patterns and their dynamics that the cell can exhibit.

Note that RD models can produce non-uniform patterns of passive curved membrane components (as we discussed in the previous sections), which will also result in shape deformations due to their spontaneous curvature, independent of active cytoskeletal forces. These models become richer if there is feedback between the membrane shape and/or changes in the local membrane tension (Diz-Muñoz et al., 2016), and the dynamics of the RD components, as was recently explored (Tamemoto & Noguchi, 2020, 2021; Wu & Liu, 2021).

In these examples, the membrane shape is deformed by the pattern of the MAPF due to the resulting actin protrusive forces, but there is no direct feedback between the membrane shape and the RD patterns that determine the MAPF organization. However, such feedback can readily arise if the MAPF, or their membrane-bound protein complex, has spontaneous curvature. There are today several known examples of membrane proteins with spontaneous curvature that are directly associated with MAPF (Begemann et al., 2019; Disanza et al., 2013; Kühn et al., 2015; Vaggi et al., 2011), or of MAPF proteins that seem to possess spontaneous curvature directly (Gaertner et al., 2021; Pipathsouk et al., 2021). The feedback between the membrane shape and the MAPF organization, due to the MAPF's curvature, was shown theoretically to produce numerous patterns (Gov, 2018). In general, convex-shaped MAPF (protruding outwards) that induce actin protrusive forces can result in the spontaneous formation of protrusions as they tend to aggregate at the highly curved tips (Fig. 25.10). On the other hand, concave-shaped MAPF (protruding inwards) that induce protrusive forces tend to have a self-straightening effect, adding a restoring force that acts to maintain the membrane in its flat shape (Fig. 25.10).

25.3.1 Lamellipodia and filopodia driven by curved MAPF

Recently it became possible to explore the three-dimensional, unconstrained shapes of membranes that are deformed by curved MAPF, which exert local protrusive forces that represent the pressure due to branched actin polymerization (Fošnarič et al., 2019).

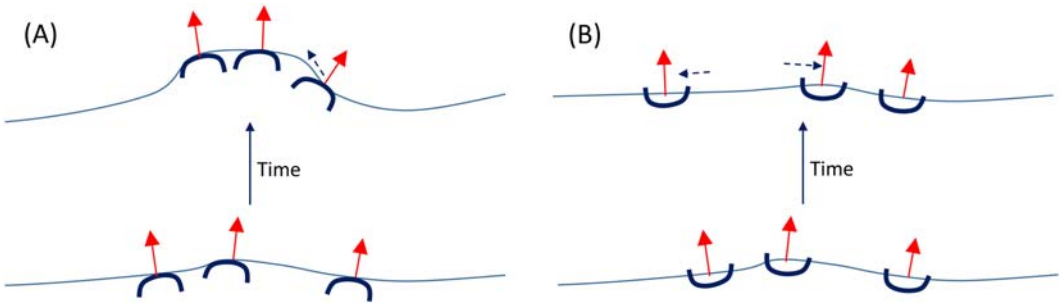

FIGURE 25.10 (A) Illustration of the positive feedback between curved (convex) MAPF and the membrane shape, which can destabilize the uniform membrane and induce the initiation of protrusions. At an early time (lower image), a small fluctuation in the membrane shape of the local density of the curved MAPF results in a growing protrusion since each MAPF induces a local protrusive force (red arrows). The growing protrusion tip attracts more curved MAPF (dashed arrow), since it has a shape that best fits their spontaneous curvature. (B) For curved (concave) MAPF, there is a negative feedback between their local density, force, and membrane shape, resulting in the stabilization of the uniform state and decay of fluctuations in shape and density.

Highly-curved, isotropic convex MAPF, which induces a local active force that is normally pointing outwards, spontaneously form flat, lamellipodia-like membrane protrusions. We can understand this spontaneous formation as follows (Fig. 25.11): The highly curved MAPF spontaneously forms small spherical buds on the membrane surface (Fig. 25.11A). However, the actin protrusive force acts isotropically outwards and acts to break up such localized aggregates. Therefore, the active MAPF forms ring-like or arc-like aggregates (Fig. 25.11B and C), where the forces act collectively to stretch the membrane and maintain the highly curved edge. Such an aggregate satisfies the large spontaneous (intrinsic) curvature of the proteins, at least along one direction. This calculation suggests that the width of the sheet-like ruffles and lamellipodia in cells (Fig. 25.11D–F) should be of the order of the spontaneous (intrinsic) radius of curvature of the curved proteins at the leading edge of the membrane. This prediction needs further investigation but is plausible, given that the radius of the membrane curvature at the leading edge of lamellipodia is of order ~ 50 nm (Pipathsouk et al., 2021), and should therefore be sensed by I-BAR proteins such as IRSp53 (Prévost et al., 2015).

Less highly curved MAPF are observed to produce elongated, filopodia-like protrusions (Fig. 25.12A). These elongated protrusions initiate when the protrusive force of actin polymerization overcomes the restoring force of the membrane, arising from tension and bending energies. When membrane tension is low, the restoring force arises from the bending energy cost of pulling a cylindrical membrane tube from the background flat cell surface (Fošnarič et al., 2019; Graziano et al., 2019). The bending energy of the tube is calculated using Helfrich's model (Eq. 25.1), from which the restoring force of the membrane tube is derived. This force balance is illustrated in Fig. 25.12B, and predicts that the protrusion radius is given by the relation: $R \sim \left(\frac{2\kappa a}{F}\right)^{1/3}$, where κ is the bending modulus of the membrane, and a is the area of a MAPF, and F is the protrusive force due to actin polymerization per MAPF. Using $\kappa \sim 10 k_B T$ and the measured force density of actin polymerization $F/a \sim 1\, nN/\mu m^2$ (Footer et al., 2007), we obtain $R \sim 50\, nm$, which is similar to the lower bound of the observed values (Mattila & Lappalainen, 2008). Cells with inhibited branched

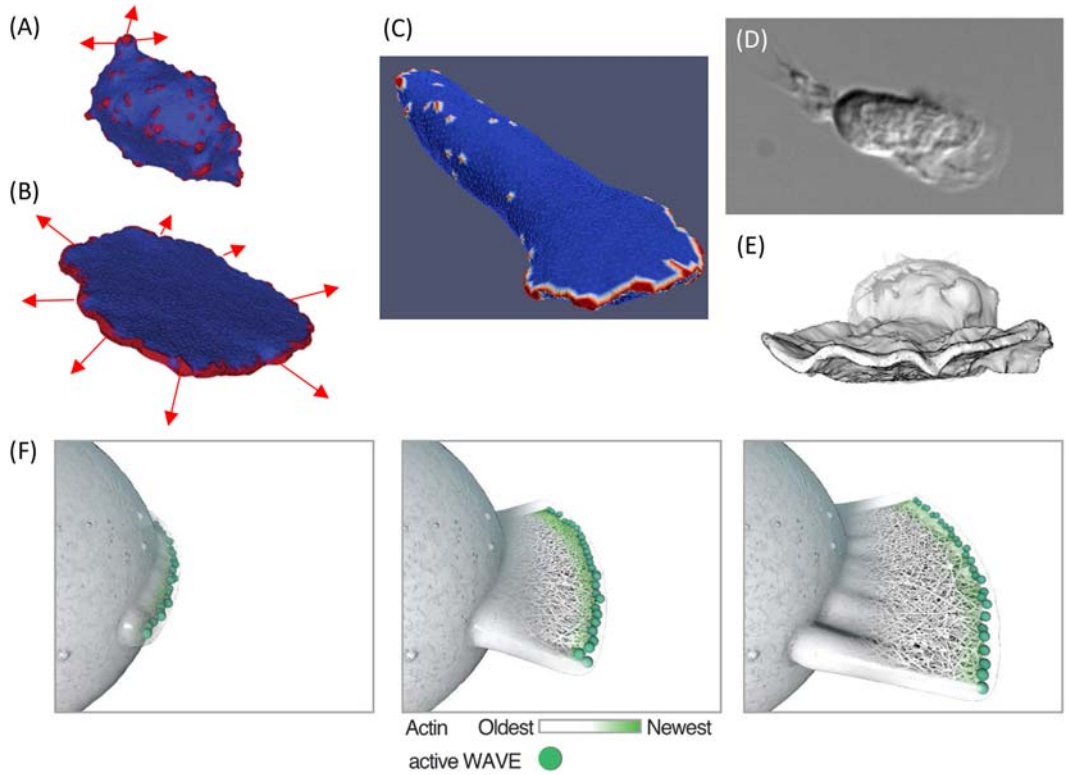

FIGURE 25.11 (A) Simulation of the vesicle shape containing passive, convex MAPF (Fošnarič et al., 2019). The red nodes denote the MAPF (Fošnarič et al., 2019), while the blue the bare membrane. The red arrows illustrate how active forces act to destabilize such spherical aggregates while they stabilize pancake-like (B) and arc-like geometries. The active forces act collectively to stretch the membrane and maintain the highly curved edge, which satisfies the spontaneous curvature of the proteins, at least along one direction. This calculation suggests that the flat geometry of sheet-like ruffles and lamellipodia in cells (Fig. 25.11D,E) may arise from this mechanism, as illustrated in (D) (Fritz-Laylin et al., 2017). Source: *(A–C) Adapted with permission from Fošnarič, M., Penič, S., Iglič, A., Kralj-Iglič, V., Drab, M., & Gov, N. S. (2019). Theoretical study of vesicle shapes driven by coupling curved proteins and active cytoskeletal forces.* Soft Matter, *15(26), 5319–5330. (D) Adapted with permission from Graziano, B. R., Town, J. P., Sitarska, E., Nagy, T. L., Fošnarič, M., Penič, S., Iglič, A., Kralj-Iglič, V., Gov, N. S., & Diz-Muñoz, A. (2019). Cell confinement reveals a branched-actin independent circuit for neutrophil polarity.* PLoS Biology, *17(10), e3000457. (E,F) Adapted with permission from Fritz-Laylin, L. K., Riel-Mehan, M., Chen, B.-C., Lord, S. J., Goddard, T. D., Ferrin, T. E., Nicholson-Dykstra, S. M., Higgs, H., Johnson, G. T., & Betzig, E. (2017). Actin-based protrusions of migrating neutrophils are intrinsically lamellar and facilitate direction changes.* Elife, *6, e26990.*

actin nucleation exhibit long protrusions that are similar to the shapes calculated for low curvature active MAPF (Fig. 25.12C and D). Note that the actin bundle inside finger-like protrusions can be rather rigid, and it may therefore determine the width of the protrusions beyond the simple calculation, as discussed below.

Note that actin filaments nucleated from the branched network can also serve to nucleate bundles of filaments in the presence of proteins that crosslink (Khurana & George, 2011)

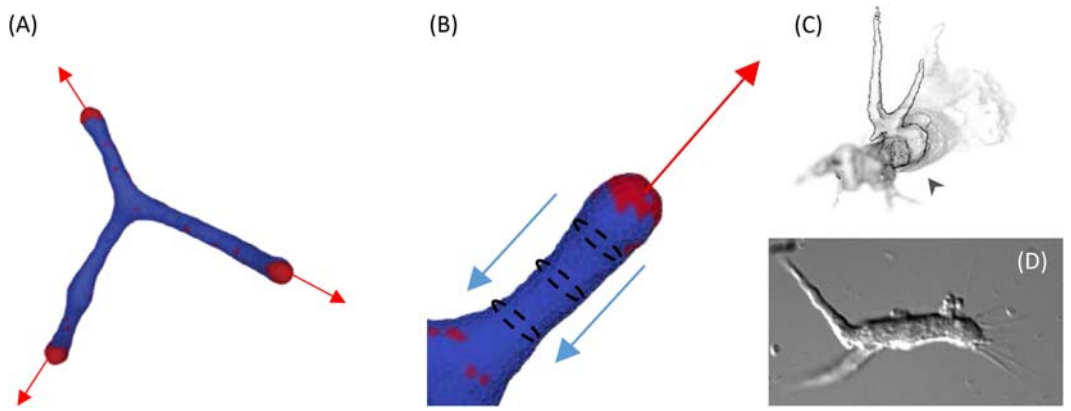

FIGURE 25.12 (A) Simulations of flat active MAPF (red) extending finger-like membrane protrusions (bare membrane in blue) due to active protrusive forces (red arrows). (B) Force balance between the active force at the tip (red arrow) and the restoring force (blue arrows) due to bending the membrane into a cylindrical shape (indicated by the dashed circles). (C,D) Similar-looking protrusions in cells where the branched actin is inhibited. Source: *(A,B) Adapted with permission from Fošnarič, M., Penič, S., Iglič, A., Kralj-Iglič, V., Drab, M., & Gov, N. S. (2019). Theoretical study of vesicle shapes driven by coupling curved proteins and active cytoskeletal forces.* Soft Matter, *15 (26), 5319–5330. (C) Adapted with permission from Fritz-Laylin, L. K., Riel-Mehan, M., Chen, B.-C., Lord, S. J., Goddard, T. D., Ferrin, T. E., Nicholson-Dykstra, S. M., Higgs, H., Johnson, G. T., & Betzig, E. (2017). Actin-based protrusions of migrating neutrophils are intrinsically lamellar and facilitate direction changes.* Elife, *6, e26990. (D) Adapted with permission from Graziano, B. R., Town, J. P., Sitarska, E., Nagy, T. L., Fošnarič, M., Penič, S., Iglič, A., Kralj-Iglič, V., Gov, N. S., & Diz-Muñoz, A. (2019). Cell confinement reveals a branched-actin independent circuit for neutrophil polarity.* PLoS Biology, *17(10), e3000457.*

parallel actin filaments (Isaac et al., 2013; Yang & Svitkina, 2011). Such bundles can also be initiated by "processive cappers," such as formins, and form the core of finger-like, cylindrical protrusions, such as filopodia, microvilli, and stereocilia. The dynamics within finger-like protrusions is complex (Faix & Rottner, 2006; Leijnse et al., 2015; Mattila & Lappalainen, 2008), with many myosin molecular motors involved in transporting various molecular cargo proteins from the protrusion base to its tip (Howard et al., 2011), where actin polymerization occurs (Fig. 25.9C). These molecular cargo proteins maintain the polymerization at the tip and the crosslinking (bundling) of the newly formed filaments. The resulting length and width of such protrusions, during their growth and at the final steady-state, is determined by the rates of actin polymerization and depolymerization, the myosin-driven flux of cargo proteins to the tip, and the resulting balance of forces on the cell membrane (Orly et al., 2014). Highly specialized protrusions, such as the stereocilia of the cochlear hair-cells, require many types of molecular motors to maintain their shapes (Rzadzinska et al., 2004), which are essential for their functioning during the hearing (Lin et al., 2005; McGrath et al., 2017). For these protrusions, the shape is highly regulated on the scale of the individual stereocilia protrusion (Naoz et al., 2008; Sakaguchi et al., 2008), and for their overall unique staircase organization on the cell surface (Orly et al., 2015).

The entire range of possible shapes and dynamics that can arise from the coupling of curved MAPF of different curvatures that recruit the actin cytoskeleton is still being

explored theoretically (Sadhu et al., 2021) and experimentally (Gaertner et al., 2021; Mancinelli et al., 2021; Pipathsouk et al., 2021).

25.4 Conclusions

In this chapter, we introduced the physical mechanisms that allow cells to deform their lipid membrane to achieve various dynamic or stable shapes. The cell controls its shape using its protein components, which adsorb to the membrane and elicit the shape changes. We did not describe the biochemical details of each protein component that deforms the membrane but rather focused on the physics of bending the membrane into various shapes by considering the energy and forces involved in this process. We kept the description mostly in non-technical terms and referred the interested reader to the original papers for more technical details. While many mechanisms that produce specific membrane shapes in cells have been already exposed, there are still fundamental processes that involve cellular membrane dynamics, which we do not fully understand, from cell migration to endocytosis. Whatever will be the biochemical and biophysical mechanisms that will be unraveled in the future, it is important to realize that the membrane shapes will be governed by the same basic energies and forces, which we have described earlier.

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PLASMA MEMBRANE SHAPING

Edited by Dr. Shiro Suetsugu

Plasma Membrane Shaping summarizes current knowledge on how cells shape their membrane. Organized in four sections, the book opens with a broad overview of the plasma membrane, its composition, usual shapes and substructures, Actin/WASP/arp2/3 structures, BAR domains, and Ankyrin repeat domains, dynamin, and phospholipid signaling. Other sections cover the shaping of the plasma membrane for transport processes, discussions on exosomes, microvesicles, and endosomes, clathrin-coated pits, caveolae, and other endocytic pits, membrane deformation for cell movement, and some of the most current dry and wet lab research techniques to investigate cellular membrane shaping.

This is an ideal resource for new researchers coming into this area as well as for graduate students. The methods section will be of interest to both microscopists and computer scientists dedicated to the visualization, data collection, and analysis of plasma membrane shaping experiments.

Key features

- Covers membrane shaping for both cytosis and cell movement
- Includes dry and wet lab research methods of plasma membrane shaping
- Describes the molecular machinery involved with protein and lipid balance in the plasma membrane
- Presents the coordination of cellular structures involved in cell deformation and motion

About the editor

Dr. Shiro Suetsugu is currently a professor at Nara Institute of Science and Technology, Japan. He received his Ph.D. and M.Sc. in cell biology from the Department of Biochemistry and Biophysics, Graduate school of Sciences, The University of Tokyo. His research is focused on the cellular plasma membrane and its essential role of distinguishing the inside and the outside of the cells. He and his team focus on the mechanisms connecting the membrane to the cytoskeleton, and the membrane-binding proteins connecting the membrane to the intracellular and intercellular signaling for varieties of cellular functions, including proliferation and morphological changes. They pioneered the membrane shaping protein of the plasma membrane and proposed the mechanisms of the membrane shaping. Notably, they contributed to the mechanisms of the concept of membrane shaping by the proteins having the BAR domains including the I-BAR domain. Throughout his career, Dr. Suetsugu received many international and national awards such as FEBS Letters Young Group Leader Award, the Young Scientists' Prize by the Minister of Education, Culture, Sports, Science and Technology, Young Investigator Award, the Japanese Biochemical Society, and the 2015 Kazato Prize.

About the cover

The image is the filopodia of the cells expressing the GFP-tagged MIM I-BAR domain, adapted from Nishimura et al. (2021).

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