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Review Article

Close, but not too close: a mesoscopic description of (a)symmetry and membrane shaping mechanisms

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Biomembranes are fundamental to our understanding of the cell, the basic building block of all life. An intriguing aspect of membranes is their ability to assume a variety of shapes, which is crucial for cell function. Here, we review various membrane shaping mechanisms with special focus on the current understanding of how local curvature and local rigidity induced by membrane proteins leads to emerging forces and consequently large-scale membrane deformations. We also argue that describing the interaction of rigid proteins with membranes purely in terms of local membrane curvature is incomplete and that changes in the membrane rigidity moduli must also be considered.

Introduction

A fascinating aspect of cellular membranes is their ability to adopt a variety of shapes, a feature manifested in the rich repository of morphologies in cellular organelles [1] and essential for vital cellular processes such as endocytosis, cell migration, signaling, cell division, and cellular respiration. Genetic mutations disturbing membrane architectures are implicated in many diseases such as Parkinson's Disease and liver dysfunction [2,3]. Moreover, structures with highly curved membrane shape offer many applications for biotechnological design, for instance, for the developments of non-viral vectors [4]. Therefore, investigation of the mechanisms that control membrane shape is of particular importance for understanding both cell function, and for optimizing numerous biomedical and nanotechnological applications.

Membrane shapes have been explored under simplified and controlled conditions using biomimetic systems such as lipid bilayers and vesicles [5–7]. Such model systems have been the subject of numerous experimental, theoretical, and simulation studies during the past decades, which has given us a comprehensive picture of the equilibrium architecture of simple fluid bilayers. At a molecular scale, model systems have contributed immensely to our understanding of the coupling between proteins and membrane shape by providing quantitative data, which can be used for testing theoretical models [8–10]. Using these assays, theoretical predictions on the ability of proteins to generate and sense membrane curvature, have been measured using advanced bio-manipulation in combination with molecular imaging [11–14]. These simple model systems have also allowed us to quantify and discover dynamics of lipid domains creating asymmetry in membranes, and how such asymmetry can be coupled to membrane shape and protein-induced curvature [15–18]. At larger scales, model systems and theoretical predictions have provided an understanding of how, and under which conditions, a spherical vesicle transforms into dumbbell shapes, stomatocytes, and, more generally, multispherical structures. These shapes can be explained through a macroscopic picture merely as an interplay between membrane bending elasticity, membrane tension, and osmotic pressure [19].

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However, cellular membranes are far more complex and evidence is emerging for many molecular mechanisms which govern membrane shape. They are heterogeneous, and composed of a myriad of proteins and lipid species [20]. The protein area coverage can be as high as 30.000 proteins per μm^2 in the plasma membrane or up to 50% area coverage for internal subcellular membranes [21]. Thus, to understand cellular membrane organization, it is essential to understand how proteins are organized on membrane surfaces and how their organization affects membrane conformations. Proteins or different lipid species may be introduced to model membranes to better approximate cellular membranes. These entities, through molecular interactions, change the membrane elasticity at the nanoscale, which can lead to large-scale membrane shape remodeling. Although, the shapes of complex membranes can be similar to those described above (multispherical structures), they often exhibit different shapes with many exciting emerging phenomena, such as phase separation, protein clustering, membrane-mediated interactions, and curvature instability, in which membrane thermodynamics becomes important [22].

The purpose of this minireview is to highlight the importance of nanoscopic membrane inhomogeneity and trans-bilayer asymmetry in formation of membrane shapes and present various strategies for addressing the challenges in investigating biomembrane shapes with varying spatial and temporal extent. Finally, we sketch possible routes for future developments to finally provide a comprehensive picture of the mechanisms leading to the many interesting shapes discovered in cellular membranes.

Membrane shaping mechanisms at the mesoscale

Helfrich Hamiltonian, a function that relates membrane geometry to elastic energy, provides a steadfast description of simple lipid bilayer shapes on large scales (large compared with the membrane thickness) [23,24]. Within this formulation, supported by experimental data, a spherical vesicle can deform into a variety of shapes. By the simplest form of transbilayer asymmetry, i.e., osmotic pressure difference, three families of vesicle shapes can develop: stomatocytes (large osmotic difference), discoid (oblate), and prolate (Figure 1A) [25]. An important transbilayer symmetry-breaking parameter is the spontaneous curvature of membranes, omnipresent in cellular and artificial membranes, that leads to the formation of a plethora of membrane morphologies (Figure 1B) [19].

Spontaneous membrane curvature is the propensity of a membrane, or a segment of a membrane, to bend and is different from the intrinsic curvature of individual lipid molecules [26]. An excellent way to understand spontaneous membrane curvature at the molecular level, beyond a single lipid, is through the lateral pressure profile across the membrane (for more information see Figure 2; also see [26,27]). This profile shows the distribution of lateral stresses across the thickness of the bilayer in which the first moment is proportional to spontaneous membrane curvature. Therefore, independent of the shape of individual molecular constituents, e.g., lipids, a symmetric membrane has zero spontaneous curvature. However, transbilayer asymmetry (asymmetry between the two monolayers), can lead to a non-zero curvature that can be induced in a variety of ways (Figure 2B). For example, by an asymmetry of chemical composition, asymmetric ionic conditions, molecular adhesion, or embedding of proteins. Such spontaneous membrane curvature could be large, small, positive, or negative depending on the type and the level of asymmetry [27,28].

The above discussion assumes homogeneity of the membrane since the spontaneous curvature is laterally uniform. However, the nonzero spontaneous curvature can originate from biomolecular impurities (inclusions) (Figure 3A), that are free to diffuse laterally. If the inclusions are distributed uniformly across large distances, they can be considered as an effective mean curvature in which the previous description still applies. However, there are meso- and molecular scale mechanisms (see below) that could lead to a non-uniform inclusion density that even persist at large scale (Figure 3B). The possibility of lateral inclusion inhomogeneities leads to a plethora of emergent behaviors depending on the character of the inclusions and their interactions with the membrane. The simplest form of inclusions is the one that only induces (local) curvature and is non- or weakly interacting. A membrane decorated by these inclusions can be described by position-dependent spontaneous curvature. Theoretical and simulation results show that the entropic contribution from lateral inclusion distribution makes the membrane effectively softer, and the degree of softening depends on the inclusion concentration and local curvature (Figure 3C) [29,30]. Therefore, above a certain concentration threshold, the membrane will undergo large surface undulation [31] that leads to protein segregation into curved regions, and consequently, membrane vesiculations (Figure 3B). This behavior can explain membrane remodeling by a non-bilayer forming lipid such as ganglioside GM1 [6]. It must be noted that in this mechanism the inclusions do not modify the local rigidity directly, but the softening (curvature instability) is instead a result of the

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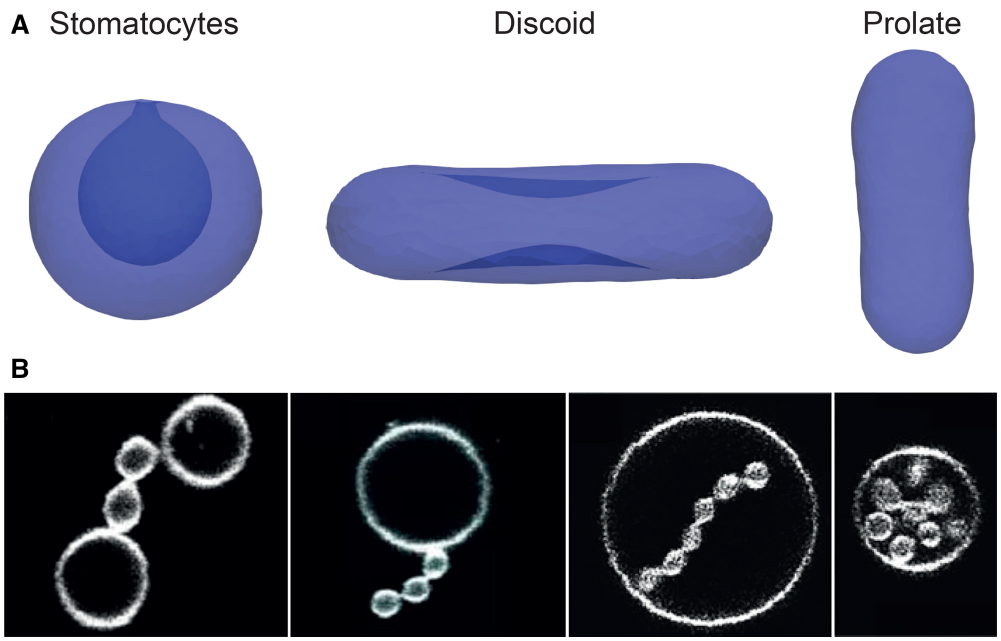

Figure 1. Vesicle deformation by transbilayer asymmetry.

(**A**) A spherical vesicle transforms into stomatocytes, oblates (discoid), and prolates through the change of reduced volume or osmotic pressure. (**B**) Multispherical structures as a result of an interplay between membrane bending elasticity, spontaneous curvature, membrane tension, and osmotic pressure. Figure adapted from [19].

cooperative action of curvature-active proteins on an undulating membrane. Therefore this behavior cannot be captured in single protein investigations or on a non-fluctuating elastic surface.

Large and rigid proteins and protein complexes prefer (impose) a well-defined membrane shape that can only be uniquely defined by Gaussian and mean curvatures. For instance, as it is depicted in Figure 4A, an infinite number of local shapes can be defined that all have identical mean curvature. In the framework of elastic energy-geometry, this means that the inclusions, in addition to inducing local membrane curvature, locally change the bending rigidity and the Gaussian modulus (Figure 4B) [13,32,33]. Such nanoscopic lateral inhomogeneities are important for large-scale membrane shape descriptions. For instance, it can lead to emergence of long-range membrane-mediated interactions like curvature mediated and Casimir-like forces, resulting in protein clustering and consequently membrane remodeling even at low concentrations as it has been shown for Shiga and cholera toxins [22,33–35]. Additionally, nanoscopic inhomogeneity in membrane rigidity can explain the tendency of certain proteins to be recruited by a narrow range of membrane curvatures, which is often referred to as membrane curvature sensing [13]. Other interesting inclusion types are the one that break in-plane rotational membrane symmetry (anisotropic proteins), e.g., such as BAR protein family, FtsZ, and dynamin A, by imposing different curvature in different directions on the bound membrane (Figure 4B) [8,36–38]. Membrane containing anisotropic inclusions can exhibit macroscopic quasi-order phases and become elongated vesicles even in absence of any direct interactions between the inclusions. The inclusions can also phase segregate through attractive curvature-mediated interactions [22,39].

As mentioned above, curvature inducing inclusions can form clusters and therefore, induce high curvature membrane deformation in a specific region of the membrane. Another mechanism behind clustering of inclusions is phase separation. Curvature inducing inclusion are recruited by different phases and even by phase boundaries of liquid ordered and disordered phases. Through this mechanism phase-induced enrichment of proteins and lipids at specific locations can assist in shaping and even contribute to fission of membrane-necks in model membranes [40]. Viral proteins have been found to be recruited, through helix insertion, to phase boundaries established in phase separated GUVs which could serve as a critical function in budding of viruses [41]. Membrane curvature can also efficiently lead to lipid sorting as shown in nanotubes extracted from GUVs which were held close to the miscibility transition temperature leading to pronounced lipid sorting between the

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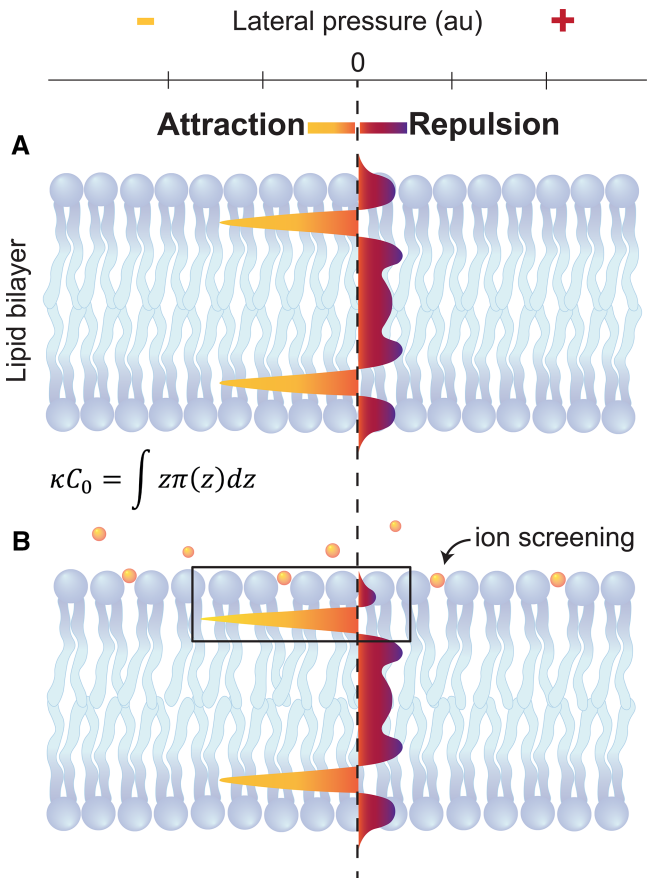

Figure 2. Lateral pressure profile of lipid bilayer.

The lateral pressure profile is the distribution of lateral stresses across the width of a lipid bilayer. It is composed of repulsive pressure components from interactions between the lipid molecules and a cohesive hydrophobic tension that favors segregation of the lipid chains from water. (**A**) The first moment of this profile is proportional to membrane spontaneous curvature. (**B**) A schematic view of how asymmetry can be induced in the profile which leads to non-zero spontaneous membrane curvature.

curved tube and the GUV [16]. Along these same lines it has been shown how curvature could control membrane phase boundaries and dynamically sort lipids and proteins in a GUV-tube model system [42]. Together these examples shed light on how membrane shapes are intimately coupled to proteins and lipids in model systems. However, investigation of membrane shapes in biological systems remains difficult primarily due to the transient nature and small scale of membrane shapes and due to the smallness of the putative nanoscopic lipid domains which are thought to be present in the membranes of living cells. Model systems in combination with advanced optical tools therefore provide an effective platform to isolate these interactions between proteins and lipid phases and hence are indispensable for gaining a deeper understanding of membrane shapes in cells.

One of the well-established features of cellular membranes is an asymmetry in the number and composition of constituent lipids and proteins between the two leaflets [20]. In recent years, several theoretical, simulation, and experimental efforts have explored the consequences (or implications) of this asymmetry. For instance, it is found that such asymmetry could affect the distribution of cholesterol in distinct manners [18,43–45], generating a mismatch in the lateral tension of the two leaflets, even for vanishing bilayer tension, leading to a multitude of behaviors such as a discontinuous increase in the bending rigidity [17,46], generation of highly ordered domains in the compressed leaflet, stress-induced flip-flop and bilayer instability (transient) to form globular micelles [47]. However, it is not fully clear yet how the composition mismatch affects membrane remodeling and shaping processes, despite the fact that the increase in bending rigidity, reorganization of lipids in different monolayers, and the ability of cholesterol (and other lipids) to flip-flop, can potentially change the behavior of

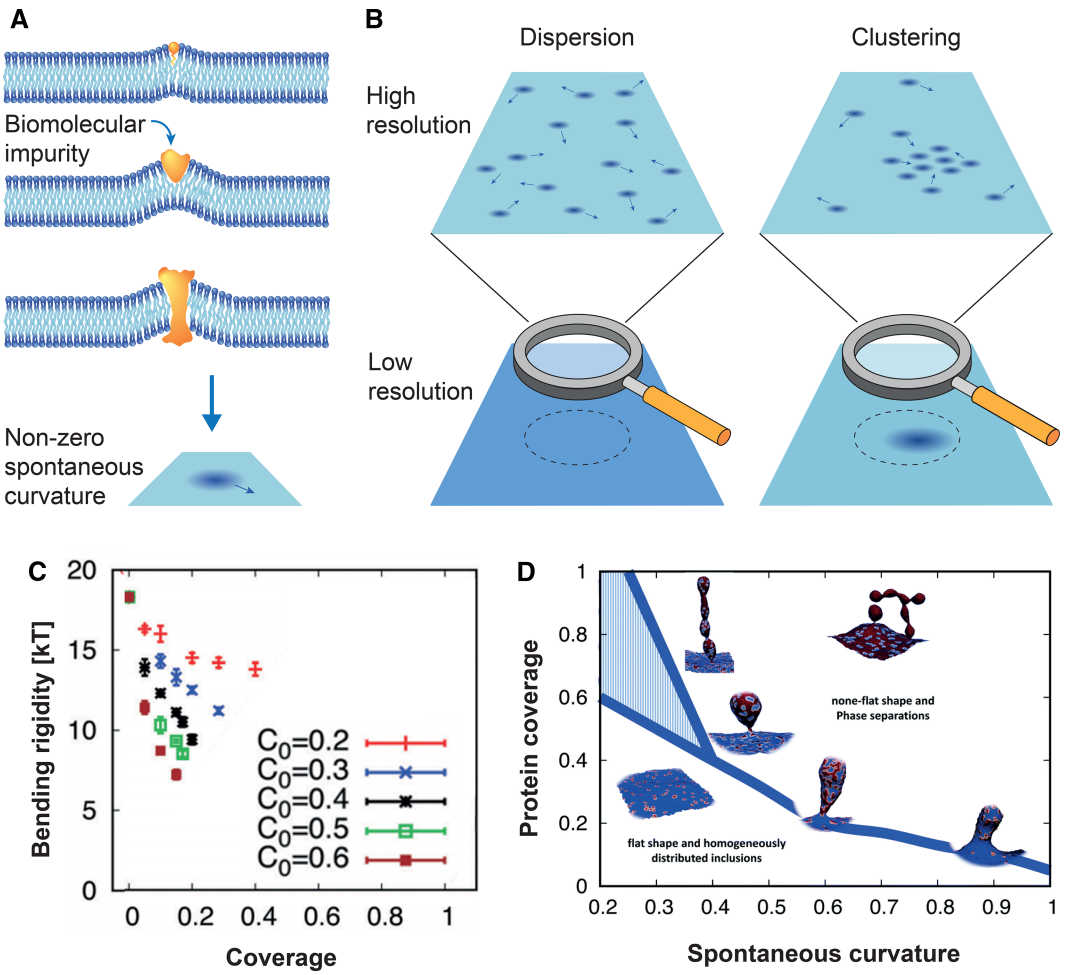

Figure 3. Non-uniform density of curvature-active inclusions can persists at large scale.

(**A**) The source of the nonzero spontaneous curvature can be some biomolecular impurities (inclusion). (**B**) The effective inclusion distribution could become non-uniform at even macroscopic scales through several possibilities for protein clustering. (**C**) As the concentration of curvature inducing inclusions increases, the effective bending rigidity decreases. (**D**) Above a certain concentration threshold, the membrane undergoes large surface undulation, that leads to protein segregation into the curved regions, and consequently, membrane vesiculations. (**C**,**D**) adapted from [29].

a membrane response to bending stress. We could envision that in the coming years more findings will shed light on this puzzle.

The above discussion indicates that the concepts of spontaneous membrane curvature and local curvature must be considered with care for complex membranes such as cellular membranes. Particularly rigid membrane proteins prefer a well-defined local shape that cannot be described by a single parameter, i.e. curvature. Three or four parameters, as depicted in Figure 4B, can provide a better approximation that can also unify the concepts of scaffolding and curvature sensing into one picture within the thermo-elastic description of membrane shape. Additionally, membrane remodeling processes must be considered as a collective phenomenon. As an example to illustrate this we can look at molecular crowding which can induce membrane bending above certain densities whereas the single molecules would not change the membrane shape on their own. Therefore, investigations at a length scale (mesoscale) a bit larger than a single biomolecule up to the length scale at which the discreetness of molecules is still relevant (5–100 nm) is essential for our understanding of complex membrane shapes. This is also supported by numerous exploratory methods that have been expanded, in recent years, to focus on these relevant length scales.

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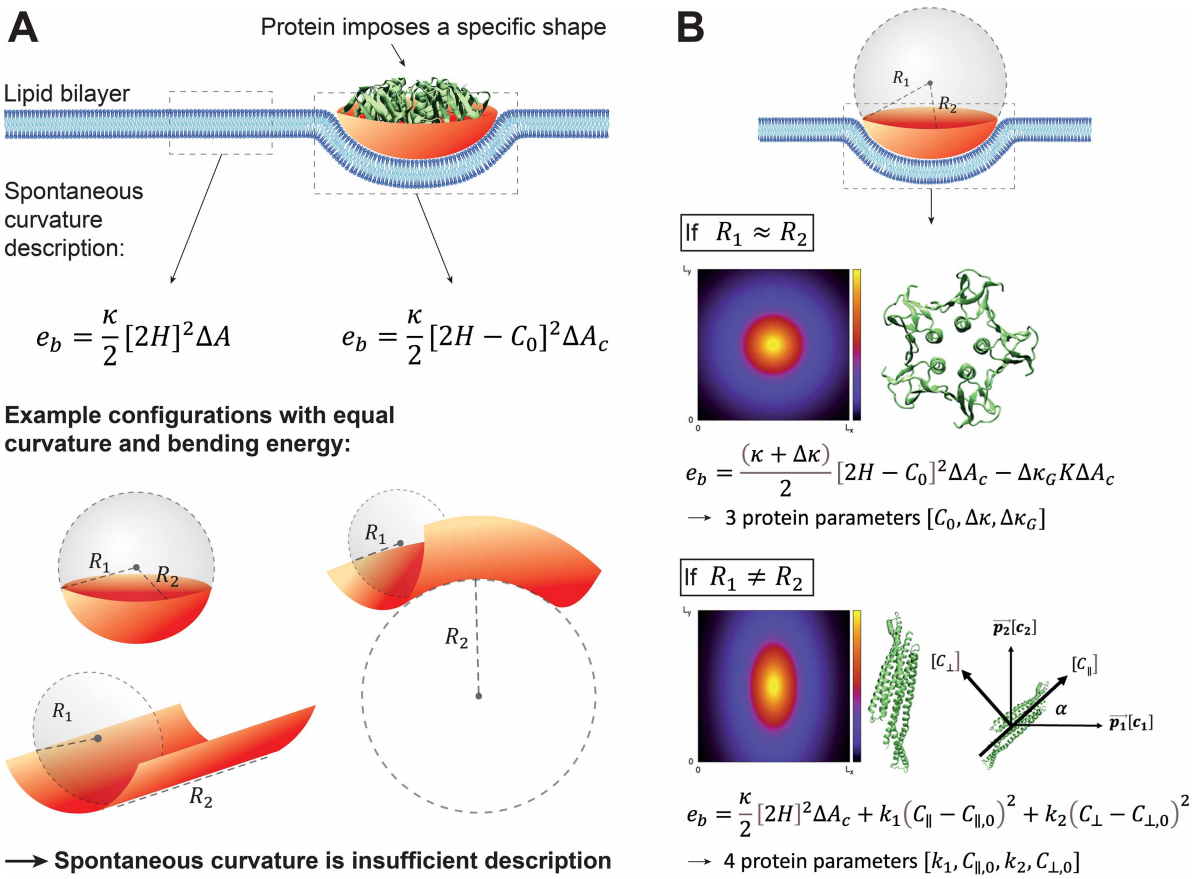

Figure 4. Describing large and rigid proteins purely in terms of local membrane curvature is incomplete.

(**A**) Large and rigid proteins and protein complexes often prefer or impose a well-defined shape on membranes. The bending energy of a membrane segment containing a rigid protein cannot be constructed by only considering spontaneous membrane curvature since there is an infinite amount of membrane shapes with the same, mean curvature and consequently the same bending energy. (**B**) A better description of the interaction of these proteins can be obtained by a three-parameter model for isotropic proteins, and a four-parameter model for anisotropic proteins. In all the equations, e_b is the bending energy of the considered segment of the membrane, κ is the membrane bending rigidity, H is the mean curvature, K is the gaussian curvature, δA is the area of the considered segment of membrane, $C_\parallel$ ($C_\perp$) is membrane curvature in the direction parallel (perpendicular) to the longest axis of the protein. (C_0, $\Delta\kappa$,$\Delta\kappa_G$) are model parameters for isotropic proteins and (k_1, k_2, $C_{\parallel,0}$, $C_{\perp,0}$) are model parameters for anisotropic proteins.

State-of-the-art techniques for exploring membrane shape

During the last few decades significant progress has been made in developing new assays for generation and detection of membrane shapes in both model membranes and living systems. New imaging platforms allow imaging below the optical diffraction limit whereas new developments in material fabrication have facilitated production of nanostructured substrates with interesting morphologies on which cells can be plated. These advances have allowed quantitative measurements of curvature affinity of proteins for both tubular, spherical and even negative gaussian curvatures [14,48].

Substantial efforts have been put into developing model membrane systems and assays to generate and detect membrane shapes. Model membranes have long been used to study lipid and protein dynamics and the complexity of these model systems has gradually increased in complexity and novelty to either enhance the biological relevance by mimicking plasma membrane or exaggerating biological effects such as lipid phase separation to study such phenomena in isolation. Both experimentally and theoretically a spectrum of systems varying from various geometries and cell-like systems, like supported lipid bilayers to cells have been used to facilitate biophysical investigation of membranes (Figure 5)

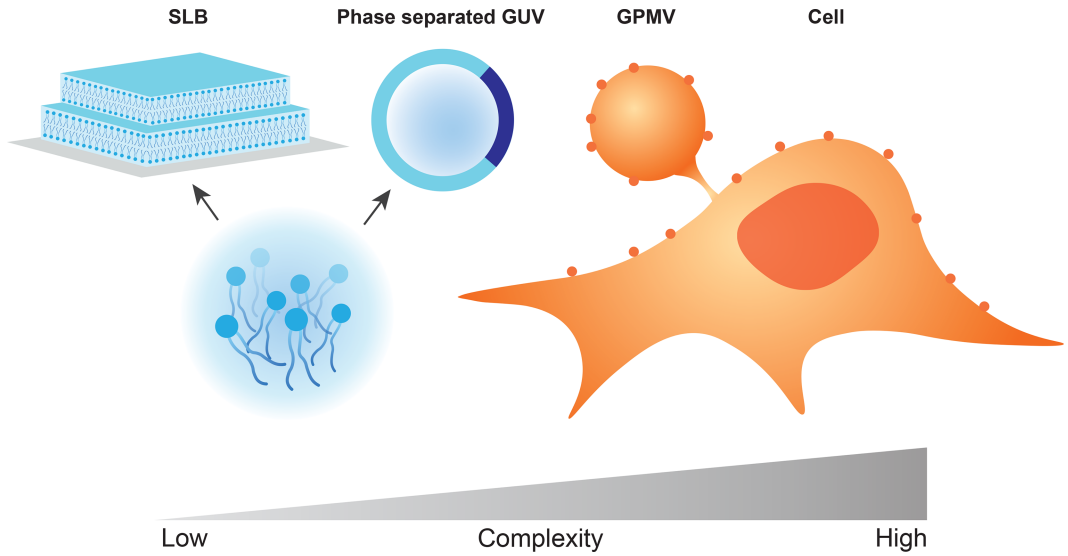

Figure 5. Model membrane systems presented in increasing complexity.

From left to right, illustrations represent four types of model membranes used in experimental investigations of membrane properties. (left) Lipids (bottom) can be used to make supported lipid bilayers (SLB) and giant unilamellar vesicles (GUVs). The latter an excellent platform to induce phase separation and explore for example protein sorting or membrane bending in 3D. (right) Giant plasma membrane vesicles (GPMVs) can be extracted from cell membranes and serve as a complex native-like membrane yet without the added complexity of i.e. the cytoskeleton and elaborate machinery governing the cell function.

3D Biomimetic systems such as GUVs remain an important experimental system for studying membrane shapes (Figure 5) as they allow researchers to insert membrane proteins into vesicles as well as create and reconstitute artificial membrane shaping agents. GUVs are, therefore, well-suited for controlling and introducing membrane asymmetry via lipid composition and protein insertions and for investigating membrane curvature effects. Lipid asymmetry in GUVs can be established simply by using electric swelling as the formation method which clearly result in large number of long nanotubes [7]. Asymmetric ionic solutions across GUV membranes also result in spontaneous membrane curvature leading to similar nanotubes [49]. Membrane tension was also shown in a GUV system to play an important role as a master regulator of membrane bending induced by N-BAR [50] or I-BAR [10]. However, GUVs are limited when it comes to studying native transmembrane proteins, as such proteins become randomly oriented in the membrane upon insertion, which does not properly reflect the inward-out orientation in biological membranes. This can be a major drawback when investigating e.g. the effect of protein crowding on membrane shapes for highly asymmetric proteins, as random inward–outward distribution will counteract any crowding effect on the transmembrane pressure profile. A promising method for obtaining vesicles with correctly oriented proteins is the extraction and isolation of giant plasma membrane vesicles (GPMVs) from cells. These membranes, harvested directly from cells, not only provide controlled conditions for transmembrane protein orientation, but also have the same lipid and and protein complexity as the plasma membrane, and therefore constitute a more complex, and native-like membrane system [13,51,52]. Recently, a new assay was developed which combines GPMVs with phase separated GUVs with the purpose of investigating phase affinity of membrane proteins, demonstrating the versatility possible when combining these two methods [53]. Yet limitations still exists with the GPMV platform, as it has recently been demonstrated that the permeability of GPMVs is much higher than for GUVs, which might significantly impact experimental studies. The origin of such permeability might stem from the invasive methods of extracting GPMVs from cell membranes [54]. However, simply mimicking the formation of spontaneous curvature and asymmetry in model membrane systems is not sufficient for gaining a quantitative understanding of membrane curvature generation, and therefore we discuss in the following how bio-manipulation tools allow for extended investigation of membrane shapes.

Optical bio-manipulation tools have been essential for quantitative investigation of membrane shapes in the above-mentioned model systems. An interesting aspect of membrane remodeling and curvature generation is

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the presence of free membrane edges. Such free edges can be examined using stacked SLBs [55] or using high precision thermoplasmonics to puncture 3D membranes, as shown in Figure 6A, which allows membrane edges to be studied both in GUVs and in live cells [56].These new systems have not been extensively used yet, but have shown how annexin proteins exhibit a curvature-generating effect and result in rolling of membranes [55,56]. Another technique which has been extensively used to generate high curvature membranes, is the use of optical tweezers to extract nanotubes (Figure 6B). Such nanotubes can, with the use of aspiration, be tuned to specific thicknesses by changing the tension in the membrane. This assay has therefore not only proven useful in examining simple curvature sorting or membrane associated proteins linked to membrane remodeling processes [13], but also specifically to quantify the curvature sorting in relation to minute changes in the nanotube radius or membrane curvature [12]. In addition, the nanotubes function as a platform for investigation of extended biological phenomena such as protein scaffolding and phase separation of curvature sensitive molecules [16,57,58].

Recent methods are now demonstrating how to induce curvature in cellular membranes via clever cell surface manipulation. It was recently demonstrated how cells can obtain various morphologies depending on the density of mucin bio-polymers on the surface [60], a study which elegantly showed how protein–protein crowding experiments previously conducted in GUVs [9,61] could be extended to living cells. Complementary to such experiments, focusing on extracellular membrane budding, nanostructured substrates provide an

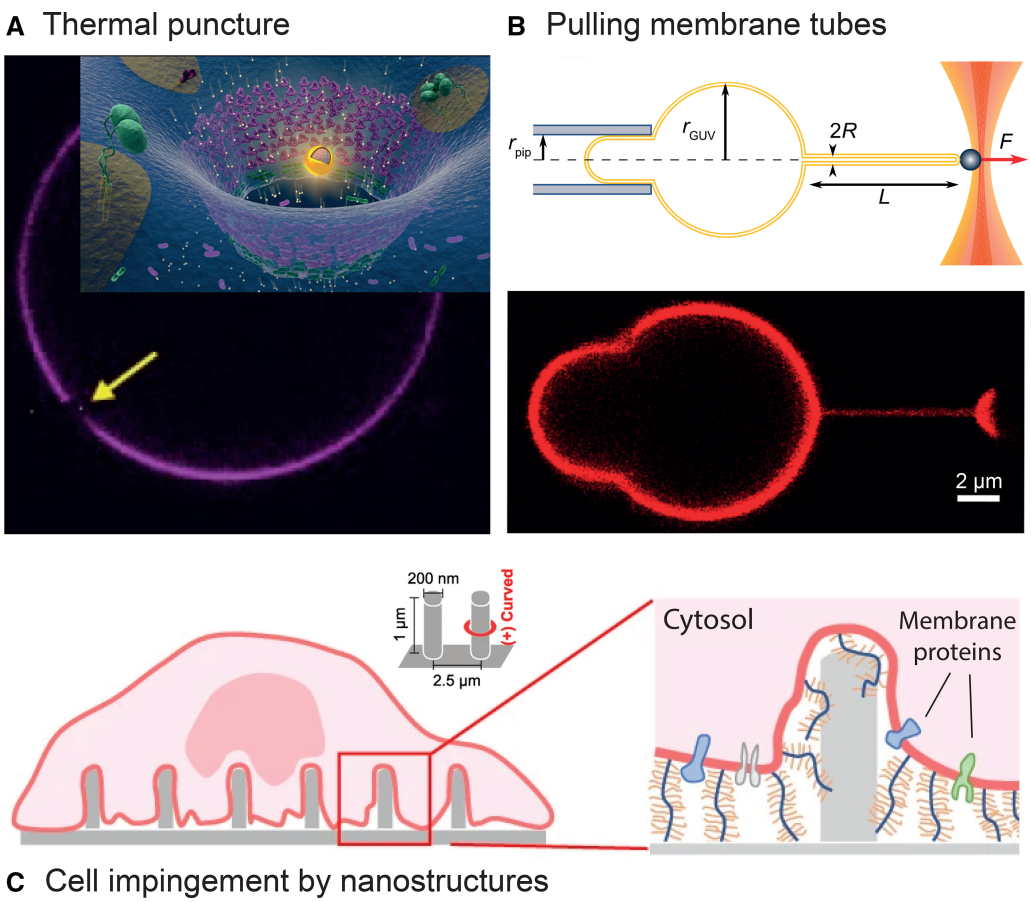

Figure 6. An advanced bio-manipulation toolbox drives research on membrane shaping mechanisms.
(**A**) Nanoscopic puncturing of a GUV using thermoplasmonic heating, creates membrane edge for investigating membrane remodeling processes at injury sites. Adapted from [56]. (**B**) Nanotubes pulled from a GUV with optical tweezers creates a platform for direct membrane probing and examination of curvature sorting of proteins. Reprinted from [59]. (**C**) Nanostructures creating curvatures by direct impingement of the cell membrane allows for in vivo investigations of protein dynamics in relation to membrane asymmetry. Adapted from [14].

excellent platform for investigation of intracellular membrane curvature effects. Such substrates have been developed to display a range of structures such as disks, spheres, rods, etc. [14,48]. Plating of living cells on such structures allows investigation of how intracellular proteins bind to membranes which are forced to wrap around these surface structures (Figure 6C). The combination of this assay with new development in protein expression, protein labeling, and fluorescent super-resolution microscopy opens up a unique door to explore protein dynamics in the intracellular environment of living cells.

The advances are not only concerning the development of model membranes and forced curvature generation, but also concerning the ways we can image and quantify membrane shapes and the physical forces driving membrane remodeling. Advanced imaging techniques, such as electron tomography and live cell imaging with super-resolution nanoscopy, have provided molecular scale information about the structure of cellular membranes. Stimulated emission by depletion (STED) microscopy can now be used to determine membrane tension without the need for invasive probing characteristic of other methods [62]. FRET imaging has recently been employed to detect changes in steric pressure at membrane surfaces, an important measure for membrane asymmetry effects [63]. In addition, a variety of crystallography techniques and cryo-EM have even provided insights into the atomistic details of membrane-shaping biomolecules [64,65]. The field is now heading toward combining all these new information to understand the shapes of membranes on all scales.

Theoretical models have also contributed eminently to our understanding of membrane shape at multiple scales. At large scales, theoretical calculations of membrane shape based on elastic energy (in particular Helfrich energy function), have provided (often) quantitative understanding of possible configurations of simple lipid bilayers that have been used for a qualitative description of cellular membrane shape [19,66,67]. At the molecular scale, concepts, such as the lipid shape model, wedging (insertion of amphipathic or hydrophobic domains), crowding (entropic repulsion between soluble domains), and scaffolding have provided a qualitative understanding of the mechanism that controls local membrane shape [27]. Additionally, curvature instability, membrane-mediated interactions, and even liquid order and liquid disorder phase coexistence have theoretical roots [29,68,69]. The rapid increase in computer power has enabled theoretical analyses of membrane organizations to take advantage of powerful numerical approaches to solve complex problems. These techniques are now developed to the point where a whole new field of study has emerged; computer simulation [70,71].

Computer simulations use theoretical models to describe the interactions between basic units, such as atoms, particles, or surface elements, and use computer power to evolve the configurations of complex systems. These techniques have been remarkably effective for exploring membrane shape remodeling at distinct scales [70–72]. Exciting examples include unraveling local membrane bending by protein complexes via atomistic and coarse-grained simulations, and protein clustering and the formation of large-scale membrane deformations by the cooperative action of proteins using mesoscopic simulations [32,33,73]. The field is now heading toward connecting distinct methods (multiscaling), to enable the modeling of realistic membranes with a full complexity at the level of cells and cell organelles [72,74]. Multiscale schemes are particularly important to bridge the gap between high-resolution simulations, which are often conducted on small system sizes, and their experimental counterparts. In the near future, we could expect highly sophisticated multiscaling methods, in particular, by adopting graphic processing computational algorithm and data driven methods [74–77]. Nevertheless, there are still several basic challenges that remain to be addressed. To name a few, atomistic and near atomistic simulations (for the major part) still relay on the simulation of segments of membranes by using periodic boundary conditions. This is, in particular, problematic for simulating asymmetric membranes [68] as for instance, in a closed membrane, where unequal stress could be released by membrane bending. While in a periodic boundary membrane, the periodicity, for small membrane bending, enforce the total membrane curvature to be zero. Another important challenge is how to extract bending capacity of membrane proteins, i.e., model parameters shown in Figure 4. Last but not least, developing a sufficiently accurate force field is still challenging. During the past few decades, state-of-the-art force fields have improved their ability to reproduce both single molecule conformations and certain collective behaviors, e.g., phase behaviors [78–80]. However, it is not clear whether the bending response of simulated membranes is equivalent to that of their experimental counterparts. For example, in both simulation and experiment, membrane bending rigidity is often determined by analyzing membrane shape fluctuations. Bending rigidity in the membrane shape fluctuations spectrum is a re-normalized quantity and changes mildly with system size which differs in simulations and experiments [31,81].

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Outlook

The shape of fluid lipid membranes has been extensively explored experimentally, theoretically, and through computer simulations. While exciting results have been obtained, many challenges remain in order to obtain a more complete understanding of how the complex shapes of cell membranes emerge.

We should note, for example, that most of our understanding is based on the assumption that lipid membranes have a spherical topology, and our understanding of membranes of higher genera remains extremely limited. Cellular membranes, particularly those of organelles, including mitochondria and the Golgi apparatus, exhibit high genus topological shapes (topological genus g is a measure of how many handles are attached to a sphere) [72,82]. Furthermore, living cell membranes are dynamic, highly affected, and driven by non-equilibrium processes, which may result in steady-state configurations that are far from equilibrium. However, it is not fully established how non-equilibrium processes affect membrane shape, and how such structures can be characterized and modeled [83,84]. Last but not least, many proteins, especially intrinsically disordered proteins, are not randomly dispersed in the cytoplasm but are found in membrane-less organelles, commonly known as biomolecular condensates. Often, these droplets interact with cellular membranes, and we have only recently realized their significance in shaping biomembranes [85–88].

Advances in computer simulation and experimental techniques will undoubtedly provide exciting results on membrane shapes in the coming year. However, there are major bottlenecks we need to overcome. On the computational side, while the inner elements of each technique need improvement, better techniques and tools (multiscaling schemes) to reach a higher level of spatiotemporal scales and, in particular, to include the effect of molecular activities and non-equilibrium processes, must be developed. Experimentally new platforms need to be developed in which the smallest shapes can be resolved in both time and space, ideally with molecular resolution. On the imaging side new systems which combine the strengths of two different types of super-resolution imaging techniques STED and STORM (stochastic optical reconstruction microscopy) have very recently shown unprecedented fluorescent imaging with near atomic scale resolution [89]. STED microscopy has also been combined with fluorescence correlation microscopy (FCS) to allow quantification of nanoscale dynamics of molecules in membranes at high temporal resolution [90]. New reconstitution assays also need to be invented which can be used to model the intracellular systems mimicking the Golgi and ER exhibiting a wealth of complex membrane shapes. We envision that the new progress in experiments and simulations will together be able to link the molecular origin leading to different membrane shapes.

Summary

- Mesoscale, a length scale in the range of 5–100 nm, is essential for our understanding of cellular membrane shapes.
- Describing the interaction of proteins with membranes purely in terms of local membrane curvature is incomplete.
- Membrane remodeling mechanisms must be considered as a collective phenomenon.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

GPMVs, giant plasma membrane vesicles; GUVs, giant unilamellar vesicles; SLB, supported lipid bilayers.

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