**THE MECHANICS AND THERMODYNAMICS OF TUBULE FORMATION IN BIOLOGICAL MEMBRANES**

A PREPRINT

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July 16, 2020

**ABSTRACT**

Membrane tubulation is an ubiquitous process that occurs at the plasma membrane and at the intracellular organelle membranes. These tubulation events are known to be mediated by the forces applied on the membrane by motor proteins and due to the interactions between membrane proteins binding onto the membrane. These experimental observations have been amply supported by mathematical modeling of membrane mechanics and have provided insights into the force-displacement relationships for tubulation. The advances in quantitative biophysical measurements of membrane-protein interactions and tubule formation have necessitated the need for advances in modeling that will account for the interplay of multiple physics that occur simultaneously. Here, we present a comprehensive review of experimental observations of tubule formation and provide context from the framework of continuum modeling. Finally, we explore the scope for future research in this area with an emphasis on iterative modeling and experimental measurements to enable us to expand our understanding of the tubulation processes in cells.

**Keywords** membrane tubule formation · membrane-protein interactions · membrane mechanics · thermodynamics

**Abbreviations** AC - anterograde carriers; BAR - Bin/Amphiphysin/Rvs; BDP - BAR domain protein; BFA - brefeldin A; BIN1 - Bridging Integrator 1; CICR - calcium-induced calcium release; ER - endoplasmic reticulum; ERES - ER exit site; ERGIC - ER-Golgi intermediate compartment; FBP17 - formin-binding protein 17; GFP - Green Fluorescent Proteins; GTPase - guanosine triphosphatase; GUV - Giant Unilamellar Vesicle; iPALM - interferometric photoactivated localization microscopy; LTCC - L-type Calcium Channel; PEC - Protrusion, Engorgement, and Consolidation; PSGL-1 - P-selectin glycoprotein ligand-1; RBC - red blood cell; RyRs - Ryanodine receptors; SR - sarcoplasmic reticulum; TC - transport carrier; T-tubules - Transverse tubules; wtENTH - wild-type epsin1 ENTH.

1 Introduction

The curvature generation capacity of biological membranes is critical for many cellular functions. In the past few decades, the study of curvature generation in cellular and synthetic systems has given us physical insights into the underpinnings of curvature generation in membranes (Figure 3) [1]. Many of these studies have revealed the quantitative relationships between protein density, applied force, and curvature generated [2] [3] [4].

The membrane deformations can broadly classified as buds, pearled structures, and tubes [5]. In this review, we focus on the formation of membrane tubes exclusively because of the broad application to membrane physiology. In
eukaryotic cells, we find numerous applications of tubular protrusions at the plasma membrane. A motile cell uses the actin-dense tubular structure, filopodia, to probe the environment during migration [6]. Filopodia also play a crucial role in neurite growth, the formation of dendritic spines, wound healing, cellular trafficking [7]. They also lead to cellularization in *Drosophila* embryo [8] and adhesion of epithelial cells during embryo development [9]. Tubular protrusions from the plasma membrane also aid in the trafficking of cargoes (through transport carriers) [10] and transport of ions through tubular cores (t-tubules) [11].

Furthermore, beyond the plasma membrane, organelle membranes, such as ER and the Golgi apparatus can generate complex and dynamic tubular protrusions [12, 13]. The generality of tube formation in vesicle based systems, protein crowding [14], liquid-liquid phase separation [15, 16], osmotic pressure [17], polymer binding [18] and even triblock copolymers [19] indicate that there are multiple ways to induce the compressive stresses associated with membrane tube formation.

A critical aspect of research in the area of membrane mechanics is the close interactions between theoretical developments and experimental observations. Indeed, for nearly five decades, the iterative development of theory, simulation, and experiment has resulted in a rich field. In that spirit, we review some key highlights of tube formation in different experimental systems (Section 2), the associated mechanical models to explain these observations (Section 3), and the thermodynamic underpinnings of tube formation (Section 4). We conclude with some critical open questions for future studies and suggest new interdisciplinary efforts in Section 5.

## 2 Experimental observations of membrane tubes

### 2.1 Tubular protrusion in cells and their myriad functions

Tubular membrane are ubiquitously found on the plasma membrane and intracellular organelles and these tubules are implicated in a variety of cellular functions including membrane trafficking, cell migration, signaling, and probing the extracellular environment. These tubular structures are found in all eukaryotic cells. We present a few examples of these tubules to elaborate on their detailed structure and function relationships (Figure 1).

#### 2.1.1 Tubular formation at the plasma membrane

**Filopodia:** Filopodia are finger-like cellular protrusions that play a crucial role in many cellular processes such as cell migration, axon, and dendrite formation in neural growth, wound healing, and adhesion to the extracellular matrix. This structure is mainly supported by a branch of actin filaments that also controls the length and elongation of the filopodia with the help of regulated polymerization and depolarization of actin monomers [20]. Filaments from the lamellipodial actin network can elongate and therefore filopodial protrusion happens with the help of ‘convergent elongation’ [21] process. The plasma membrane plays an important role in the formation of filopodia; the actin filament nucleating proteins (formins, Arp2/3, spire, etc.) bind to the membrane and induce polymerization of actin filaments, which eventually causes tubular protrusion [22]. Additionally, there are instances of membrane deformation in filopodial-like precursors in the dendritic spine where filopodial structure forms without an actin array [23]. Common to the spectrum of different scenarios that induce filopodial formation is the interaction of the plasma membrane with a variety of regulating proteins that play an important role in the initiation and the regulation of the filopodial geometry.

**Tubule formation during membrane trafficking:** Eukaryotic cells have multiple internal organelles, each of which has specific function. Proteins and lipids are transported from one compartment to another through the membrane-bound organelles, called transport carrier (TC) [24, 25]. TCs can be made of small vesicles, single tubes, or complex tubular membrane structures. In particular, the tubule-shaped TC can transport large cargo over longer distances when compared to vesicular TCs [10]. The mechanism of the tubular TC formation includes three basic steps— budding of the membrane loaded with the cargo from the donor membrane, tube elongation and tubular fission, and finally, fusion to the acceptor membrane [26]. Membrane scaffolding proteins help this tubular protrusion at the beginning from the donor site and subsequently support the elongation of the tube [27]. Similar tubular elements are responsible for the transport of cargoes through the endocytic pathway [28]. According to Sens *et al.* [27], membrane nanotubes *in vitro* can also be used to observe the role of membrane curvature in several processes such as membrane trafficking. Kwok and colleagues demonstrated the formation of tubular membrane protrusions in ternary mixtures of sphingomyelin, phosphatidylcholine and cholesterol. Kwok *et al.* [29] noted nearly four decades ago that there exists a possibility that membrane curvature and phase separation depend on lipid sorting.
Figure 1: Mechanisms of membrane tubulation in cell and intracellular organelles. (I) Actin-driven filopodial protrusion, (II) Tubular protrusion due to force generation caused by binding/unbinding of proteins to the membrane, (III) Tubular structure supported by microtubules in the cytoskeleton, (IV) Tubular shape transformation of the membrane due to steric effect of crowded proteins, (V) Spontaneous tubulation of membrane due to anisotropic intrinsic curvature induced by BDPs, (VI) Tubulation due to anchored motor protein or peptides, and (VII) Tubular TC during membrane trafficking in ERGIC.

2.1.2 Tubule formation in intracellular organelles

In Golgi-ER complexes ERGIC: In mammalian cells, protein cargo is transported from the ER to Golgi through a tubulovesicular cluster of the membrane, which is often called as ER-Golgi intermediate compartment (ERGIC) [30]. This tubular structure is extremely dynamic in nature and works as a mobile transport complex that delivers cargoes from the ER to Golgi [31]. The complexity of transport in ERGIC ranges from transport through a vesicle with a coat protein complex (COPI and COPII) [32] to the movement of the large carrier along microtubule with the help of TCs [24] and AC [33] that contains fusion protein from ERES. Microtubules in the cytoskeleton interact with the tubular membrane and regulate these dynamics with the help of motor proteins in the early secretory pathway [34, 35]. However, forces from the motor proteins alone are unable to overcome the initial energy barrier of tubular protrusion [36]; tubulation happens in the presence of GTPase and other curvature generating agents [37]. The ERGIC transport machinery also contains the SNARE-complex [38] and other tethering proteins [39] that help with transporting the multiprotein complex.

2.1.3 Select functions of tubules in whole cells

We focus on some select functions of tubular structures in whole cells based on some of our emerging research interests. While not exhaustive, these functions give us some context on how the shape of the membrane tubule is closely tied to cellular function.
Cardiac T-tubules: T-tubules are the tubular structures that present in the skeletal muscle cells and cardiac myocytes and play a major role in muscle contraction. In cardiac myocytes, these tubular structures invaginate from the sarcolemma and are organized along the z-disc surrounding the myofilaments. The t-tubules are organized in the close proximity to the SR and assist in the rapid entry of Ca$^{2+}$ from there to the z-discs through its tubular core. The LTCC on the membrane of the t-tubule stays in contact with RyRs of the SR membrane and forms dyads that help to stabilize the tubular structure. This spatial organization of the calcium handling units in the cardiac myocytes is thought to be important for the spatiotemporal dynamics of calcium in these cells.

The tubular morphology of t-tubules is found to be dynamic in nature and loss of tubules can occur in many disease states and can result in delayed kinetics of CICR. Even though the t-tubule structure dedifferentiates completely in vitro, the studies have confirmed that the tubular structure does not protrude as a result of forces applied to the membrane. Furthermore, these tubules are found absent in the stem cell regeneration for cardiac myocyte, which suggests that the mechanism of t-tubule formation is yet to be completely understood. Many studies suggest that the BDP BIN1 that attaches to the dyad is crucial to the formation of the tubular structure, indicating that curvature generating proteins play an important role in the formation of t-tubules.

In Neurons: Another excitable cell type, where the formation of tubules plays an important role is neurons. Neuronal precursors undergo a series of morphological changes through tubular protrusions in multiple stages before they develop into a mature neuron. Early stages in these steps include the formation and elongation of smaller length scale filopodial and lamellipodial structures. Many of the filopodial protrusions further elongate in a longer length scale with the help of actin-rich growth cone and form neurites. In subsequent stages, one of the neurites undergoes further rapid elongation and develops into the axon, whereas the remaining neurites become dendrites. The final stage consists of forming early dendritic spine (locations of synaptic contact) and axonal branches, which are protrusions at a smaller length scale. Neuritogenesis, the process of neurite formation, is largely an actin-driven membrane deformation and the process happens in coordination with the actin cytoskeleton and membrane scaffolding proteins. The tubular geometry along with their electric property efficiently transmits the signals received from synaptic input to other cells.

Membrane tubule formation is also important at the small length scale for neuronal function. Dendritic spines are small scale (Length~1–5 μm) protrusions along a dendrite that are sites of signal input from a neighboring neuron. Similar to t-tubule, the tubular structure in spines is also very dynamic in nature and changes both with age and excitatory stage. The early spines are made of long and highly motile filopodial structures that seek a synaptic partner. Eventually, the long filopodia develop into a dendritic spine if the synaptic pathway strengthens and firings of neurons occur. These spines undergo structural changes with afferent input and in many cases disappear from the old location. This remodeling of spine morphology, known as structural plasticity, causes strengthening and weakening of the structure of dendritic spines, which contributes to memory and learning.

The growth cone, as mentioned earlier, is the actin-rich filopodial structure that elongates from the early filopodial structure to mature neurite and often produces a neural circuit in the brain. The growth cone is very motile in nature and constitutes of three major structural regions—actin enriched peripheral domain often known as P-domain, central domain consisting of organelles and microtubule, and a transition domain where actin interacts with microtubules. The entire structure flows and elongates with the same rate of axon elongation with the help of PEC mechanism. Thus, the plasma membrane plays pivotal roles in the structure and motility of the growth cone by assisting actin polymerization, receptor trafficking, recycling and turnover of membrane surface area, and adhesion to the extracellular environment.

Development and Cellularization: Cellularization is the process that produces cell membranes for each nucleus in Drosophila embryo after they undergo mitotic division. During this nuclear division, the plasma membrane is covered with many finger-like small protrusions, known as microvilli. At the same time, many cleavages and furrows occur in the plasma membrane, which eventually propagate and form compartments. Microvilli contain much of the membrane that is required for furrow ingression in early cellularization. Additionally, the furrow canals contain proteins such as Myosin 2, Anillin, F-actin which actively control the compartmentalization process. Figard et al. stated that since pulling forces of furrow ingression induce high plasma membrane tension; this tension can be sufficient to limit and/or stall actin polymerization at microvillar tips. The interaction between the plasma membrane, trafficking machinery, and force generating machinery is thought to be critical for the process of cellularization. Taking these arguments into account, we note that microvilli unfolding depends on (a) interaction of the plasma membrane with BDPs; (b) interaction of the plasma membrane with actin filaments; and (c) membrane tension through regulation of furrow invagination and membrane trafficking.
2.2 Tubule formation using forces and membrane-protein interaction

In this section, we focus on how the observations of tubule formation in cells can be studied in experiments with reconstituted systems to identify the biophysical mechanisms involved. Synthetic and reconstituted systems such as GUVs are useful systems to study the biophysical interactions of membranes and curvature inducing components in a systematic manner. These systems also help to build iterative feedback between mathematical modeling and experimental observations [65].

2.2.1 Membrane forces and tubes

In a synthetic system using GUVs and optical tweezers, tubular protrusions can be generated by the forces exerted on membranes by motor proteins [66]. By using constant suction pressure, Shao and colleagues held an anti-CD162 or anti-CD45-coated bead at the micropipette tip. After the cell was moved toward the bead, a constant pulling force was applied on experimental system. Shao et al. [67] revealed that tubular protrusions can be generated when point force is applied on their tips. Similarly, Xu et al. [68] modeled the tube pulling force by examining the cell motion by piezoelectric stage holding the micropipette and reported that localized forces applied to the membrane are sufficient to generate tubules. Evans et al. [69] and Heinrich et al. [70] studied the detachment dynamics of P-selectin from PSGL-1 and observed an elastic-like deformation in the initial stage of tube formation.

Koster et al. [71] hypothesized a mechanism in which the individual motor proteins can dynamically form clusters of motor proteins and these can apply force to generate tubular protrusion. Due to the liquid nature of the bilayer, motor proteins can diffuse laterally on the vesicle and Klopfenstein et al. [72] argued that certain kinesin motor proteins can bind to lipids directly and they can induce dynamic preclustering mechanism. Koster et al. [71] measured these forces with optical tweezers and revealed that these forces depend on membrane parameters such as membrane tension and membrane bending rigidity. By varying the number of motors of the vesicle and by varying the properties of the membrane such as tension, Koster et al. [71] observed that multiple motors have to cooperate to generate tubular protrusion. These studies highlighted that the dynamic association of motor proteins with the cytoskeleton plays a significant role in the cellular context of formation of tubular protrusions and in control of the structure of intracellular membrane structures.

Another important environmental stressor known to form tubular protrusions is osmotic pressure. Sanborn and colleagues generated osmotic gradients by exposing giant vesicles to sucrose and concentration gradients. The induced osmotic gradient was either positive or negative; the negative osmotic gradient can be induced by entrapping pre-existing osmotic gradients by exposing giant vesicles to sucrose and concentration gradients. These studies highlighted that the dynamic association of motor proteins with the cytoskeleton plays a significant role in the cellular context of formation of tubular protrusions and in control of the structure of intracellular membrane structures.

There are several examples that illustrate the role of membrane tension and force in tubular protrusion formation (Figure 2). As cells store membrane in surface reservoirs of pits and protrusions, the formation of membrane reservoirs at the cell surface depends on membrane physical properties such as bending elasticity, membrane tension and membrane proteins such as BDPs [73].

2.2.2 Tubule formation from membrane-protein interaction

In this section, we focus on observations in reconstituted systems for curvature generation by proteins’ interaction with the bilayer. Several protein classes, such as motor proteins of the dynein and kinesin families can mediate the interactions of membranes with microtubules (Figure 2c) [74]. According to [75,76], in vivo and in vitro microtubule-based motor activity are both required in BFA-induced tubulation of Golgi membranes. Endophilin, amphiphysin, and the GTPase dynamin have a role in membrane fission events, and these proteins can directly bind to membranes with lipid binding domains. Such proteins can also generate tubular protrusions from liposomes in vitro [77,78,79]. More recently, Busch et al. [80] studied the curvature generating role of endocytic adaptor proteins Epsin1 and AP180 in the membrane. Stachowiak and colleagues studied tubular protrusion formations with protein densities on membrane surfaces by exposing GUVs to wtENTH. Stachowiak et al. [81] showed that tubular protrusions are generated by lateral pressure generated by collisions between bound proteins and steric congestion on cellular membranes.

Stachowiak and colleagues revealed that protein crowding on lipid domain surfaces forms a protein layer that buckles outward. This buckling bends the domain into stable buds and tubules spontaneously. Lipid domains can confine protein binding on vesicle surfaces and protein binding can generate buds and tubular protrusions by using two global parameters: domain size and membrane tension. Stachowiak et al. [14] demonstrated how steric interactions between
proteins and lipids can induce membrane bending. Coat proteins such as clathrin can generate bud protrusions and BDPs can generate tubular protrusions [82]. More recently, Stachowiak and colleagues showed that liquid-liquid phase separation on the surface of the membrane can lead to the formation of tubules [16].

Protein-induced membrane bending generates the curvature of clathrin-coated pits and caveolae. During clathrin-mediated endocytosis, epsin family proteins can insert amphipathic helices in the cytoplasmic membrane leaflet [14]. The structured clathrin coat concentrates epsins leading to membrane budding [3]. It was also hypothesized that caveolins deform the bilayer through application of steric pressure [83]. To explore the interaction between protein-lipid domain interactions in membrane protrusions, Stachowiak and colleagues generated a model system using GUVs and revealed that domains can concentrate protein binding interactions, which can lead to the formation of buds and tubular protrusions. Stachowiak et al. [14] observed that tubular protrusion formation depends on the presence of fluid-phase lipids in the domain and requires a high density of protein attachment. These experiments led to a quantitative observation that tubule length has a linear relationship with vesicle diameter and a specific protein structure is not a requisite for tubular protrusion formation.

As curvatures that are required to generate tubular protrusions also emerge through mechanical forces from localized activities of motor proteins, and cytoskeleton [19], and reconstitution, localization, and crowding of membrane proteins affect the tubular protrusion formation [84]. Kamioka et al. [85] established the FBP17-dynamin interaction and the role of FBP17 in tubular protrusion formation during dynamin-mediated endocytosis. Snead et al. [86] demonstrated membrane fission by dynamin and revealed the tubular protrusion formation upon exposure to curvature-inducing proteins. Roux et al. [87] revealed that tubular protrusions and complex tubular networks can be generated from GUVs by microtubules and ATP. Moreover, Roux and colleagues demonstrated that bundles of tubes can be generated if tubes connect at different points to the same microtubule and demonstrated that motor proteins are prerequisite elements for generating tubular protrusions [87]. Girard et al. [88] investigated the role of protein content in tubular protrusion formation during the reconstitution of membrane proteins into GUVs. As lipid domains are transformed into tubular protrusions by protein binding, Stachowiak and colleagues reported the synthesis and membrane behavior of a lipid-like molecule, DPIDA, and studied the role of lipid membrane composition in tubular protrusion formation. Stachowiak et al. [89] used lipid membrane composition with thermal treatment to control the structure of tubular protrusions and observed that steric interactions of surface-bound proteins can transform the lipid domains into tubular protrusions. Also, Leduc et al. [90] conducted experiments on dynamics of motors and tube growth, and observed tubular protrusions in vitro by kinesins that are in contact with GUVs and microtubules, establishing the role of membrane tension and motor density in tubular protrusion formation.
Figure 2: (a) Schematic of a growing tubular protrusion (brown) along a microtubule (green). The motors are attached to the membrane and they can be either bound (green and purple) or unbound (blue) from the microtubule. When bound motors far from the tip (green), they move with velocity $V_0$ and detach at a rate $k_d$. Unbound motors reattach to the tube at a rate $k_b$. The bound motors at the tip (red) detach at a rate $k_u$. The tube growth velocity is $V$. (b) Illustration of binding mechanism of F-BAR domain protein (grey) to a lipid bilayer (yellow) that generate membrane invagination. (c) Illustration of binding mechanism of I-BAR domain protein (purple) to a lipid bilayer (yellow) that generate membrane exvagination. (d) Tubular protrusion formation by forces that are exerted by cytoskeleton.
3 Mechanics of tube formation

The formation of tubular protrusions on membranes can be understood by considering the balance of forces on the membrane. We note that the mechanics approach is valuable for both equilibrium and dynamic configurations. The fundamental feature underlying many of these models is the elastic nature of the lipid bilayer. The lipid bilayer is a thin elastic sheet, fluid in plane but solid in bending. As a result, there have been significant advances in theoretical developments in the field of membrane mechanics [4, 5, 91, 92, 93, 94, 95, 96, 97]. We summarize them here in the context of membrane tube formation.

3.1 Helfrich energy for membrane mechanics

The Canham-Helfrich energy [98, 99] is commonly utilized for modeling the elastic bending energy of lipid bilayers in membrane mechanics. This model proposes that the strain energy of a lipid bilayer can be written as a function of the surface curvatures and the minimization of this energy will give us the equilibrium shapes of the membrane [99, 100, 101]. Subsequently, this model has been adapted for modeling the behavior of proteins that form spherical coats by inducing an isotropic spontaneous curvature [5]. The strain energy per unit area is given by

\[ w = \kappa (H - C)^2 + \kappa_G K \]  

where \( \kappa \) is the bending modulus, \( H \) is the mean curvature of the membrane (average of the two principal curvatures), \( C \) is the spontaneous curvature, \( \kappa_G \) is the Gaussian modulus, \( K \) is the Gaussian curvature (product of the two principal curvatures) and \( A \) is the total membrane surface area. We should point out that for Equation 1 and Equation 2, strain energy per unit area (\( w \)) and total energy of the membrane (\( W \)) differ from the classical Helfrich model [99] by a factor of 2, which was compensated by using the value of bending modulus to be twice that of the standard bending modulus typically measured in the literature.

3.2 Tubule formation using forces and tension

A classic result using the Helfrich energy for membrane tube dimensions and how they are related to the applied forces were presented in [4]. We briefly summarize it here to demonstrate the utility of mechanical models in predicting quantitative relationships between the applied force and the tubule radius. Derenyi et al. [4] studied the membrane pulling with the point force \( f \) and showed that the total membrane energy can be expressed as

\[ E = \pi \kappa L/r + 2\pi \sigma r L - f L \]  

where \( \sigma \) is the membrane tension, \( p \) is the transmembrane pressure, \( r \) is the radius of tubular protrusion and \( L \) is the length of tubular protrusion. Minimizing the energy of a tubular protrusion with respect to \( r \) and \( L \) yields

\[ \frac{\partial E}{\partial r} = -\pi \kappa L/r^2 + 2\pi \sigma L = 0 \]  

\[ \frac{\partial E}{\partial L} = \pi \kappa/r + 2\pi \sigma r - f = 0 \]

Therefore, the equilibrium tube radius is given by \( \sqrt{\frac{2\pi \sigma}{\kappa}} \) and the static force to hold the tube is \( 2\pi \sqrt{2\sigma \kappa} \).

Separately, the role of membrane tension and lipid flow was explored in substantial detail by Hochmuth and colleagues in a series of papers inspired by their experimental measurements. Considering that a majority of cellular membranes consist of two monolayers, Hochmuth et al. [102] formed a tubular protrusion from a cell body by using micropipette aspiration. Additionally, Hochmuth et al. [102] reported that tube pulling force is affected by membrane surface viscosity, the slip viscosity between the two monolayers, and the viscosity between membrane and cytoskeleton.

Leduc and colleagues studied a biomimetic system which involves GUVs, kinesin-1 motors and microtubules in the presence of ATP. Leduc et al. [103] presented both theoretical and experimental results based on fluorescence microscopy that elucidate the dynamics of membrane tube formation, growth and stalling. The results demonstrate that
molecular motors are able to pull membrane tubes without the aid of other proteins, and that tube formation should depend on both motor protein density and membrane tension. Evans [104] modeled a micropipette aspiration experiment for flaccid red cell and reported that minimum energy cell membrane contours is directly related to pipette suction force. Also, Hochmuth and colleagues formed tubular protrusion formations by pulling tubes from RBCs by using micropipette aspiration. They modeled the tube as a thin shell and demonstrated that there is an inverse relation between radius of tubular protrusion and axial tension [105, 106]. Furthermore, Waugh [107] conducted a micropipette aspiration experiment in which the lipid membrane bilayer was subjected to fluid drag and noted that osmotic properties of the vesicle interior do not have a contribution to tube formation.

3.3 Modeling the interaction of membranes with BDPs in tubule formation

Anisotropic components play a significant role in membrane morphology. Spherical buds require a fixed contact angle on the lipid membrane along a circular contact line [108]. On the other hand, for tubular protrusion forming proteins such as BDPs, the contact line with the planar membrane is highly anisotropic [108]. Anisotropic components generate a spontaneous deviatoric curvature, which aids in the formation of a cylinder [109].

To model a tube formation, we note that for a cylindrical shape (Figure 3c), unlike a spherical shape (Figure 3b), normal curvature along longitudinal axis is different from the normal curvature along the circumferential direction [110]. Spontaneous curvatures generated by tubule forming proteins, such as BDPs, are inherently anisotropic in nature.

It follows that the use of the isotropic spontaneous curvature model is insufficient for capturing the shapes of tubules and the relationship between tubule dimensions and protein densities on the membrane surface. To address this issue, a membrane strain energy density that captures the anisotropic curvature was proposed by many groups [5, 91, 97, 109, 111]. This modified Helfrich Model was used for modeling the behavior of proteins that form tubular protrusions and induce an anisotropic curvature. The energy per unit area in this case is written as

\[ w = \kappa (H - C)^2 + \kappa (D - D_0)^2 \]  

where \( H \) is the mean curvature as before and \( D \) captures the difference between the two principal curvatures. \( D_0 \) is the spontaneous deviatoric curvature. The total energy of the membrane is calculated as

\[ W = \int_A \kappa \left[ (H - C)^2 + (D - D_0)^2 \right] dA. \]  

There are several applications that use deviatoric curvature model to enhance our understanding of tube formations. Bobrovska et al. [92] and Alimohamadi et al. [91] modeled tube formation by using deviatoric curvature model to implement the effects of membrane elements and attached proteins with anisotropic properties. By using deviatoric curvature, Iglič and colleagues generated anisotropy bending energy model for anisotropic membranes. During their analysis of the stability of tubular protrusion formations, Iglič et al. [112, 113] used deviatoric curvature model and observed that anisotropic membrane components play an important role in stability of tubular protrusion formations. Also, Iglič et al. [114] and Kabaso et al. [115] studied deviatoric curvature model to demonstrate that BDPs generate and stabilize tubular protrusion formations.

BDPs have an intrinsic curvature that provides these proteins the ability to bind to the membrane surface and bend the membrane in what is known as the scaffold mechanism [97]. An additional mechanism that has been proposed for BDP induced tubulation is the amphipathic wedge mechanism, which proposes that curvature is induced as a buckling response to the insertion of amphipathic sequences into the leaflet of the bilayer [82]. The adhesion of F-BAR domain protein to the lipid bilayer induces positive curvature (Figure 2b), while the adhesion of the I-BAR domain protein to the lipid bilayer induces negative curvature (Figure 2c) [116]. These features can be captured by the curvature deviator model.

3.4 Current state of the art and future needs in dynamic measurements of tube formation in lipid membranes

Thus far, we have focused on the equilibrium aspects of membrane tubule formation. We now turn our attention to the dynamic measurements of tubule formation. Dynamic measurements of tube formation in lipid membranes
Figure 3: (a) $R_1$ and $R_2$ are principle radii of a hyperbolic paraboloid surface and $C_1$ and $C_2$ are principal curvatures of a hyperbolic paraboloid surface. (b) Principal curvatures of a sphere. (c) Principal curvatures of a tube.

Figure 3: (a) $R_1$ and $R_2$ are principle radii of a hyperbolic paraboloid surface and $C_1$ and $C_2$ are principal curvatures of a hyperbolic paraboloid surface. (b) Principal curvatures of a sphere. (c) Principal curvatures of a tube.

can be achieved using optical tweezers; such optical tweezers are used to characterize the mechanical properties of plasma membrane in terms of tether formation [117]. According to [117], compared to other tether formation techniques, optical tweezers provide noninvasive manipulation of cells with comparably great force resolution (~ 0.1 pN) and provide continuous monitoring of instantaneous tether force. Indeed, there is no dearth of data for dynamic measurements of tubule formation [93, 94, 95, 96, 102, 118, 119].

There are also have been several models of the dynamics of tubule protrusion. Simunovic and colleagues modeled the dynamics of tube formation by mimicking the tubular protrusion formation. This is done by pulling membrane nanotubes from GUVs using optical tweezers [95]. They constructed their model based on certain balance laws involving parameters such as external force, tube area, change in tube area, tube length, change in tube length, and membrane tension. Simunovic et al. [96] combined their model with *in vivo* and *in vitro* experiments and demonstrated that motors provide tube pulling force as well as frictional force for both tube formation and BAR domain scaffold.

Hochmuth and colleagues developed a thermodynamic analysis of tether formation process and they developed experiments which can be used to analyse neuronal growth cones. Hochmuth et al. [102] demonstrated that during dynamic measurements, membrane viscosity is one of the important considerations since it determines the rate of membrane deformation and it influences diffusion rate of particles in the surface plane [107]. Tian et al. [119] and Sorre et al. [118] examined the role of membrane curvature in lipid and protein sorting. Their models revealed that spontaneous
curvature in tube formations contributes to curvature sorting and this curvature sorting can be achieved by cooperative operation of lipids. Also, Sorre et al. [118] generated a model which demonstrates that curvature-induced lipid sorting is generated by the lipid cooperativity and can be influenced by lipid-clustering proteins.

Separately, based on experiments conducted in a multilamellar lipid system with osmotic pressure as a driver, Rangamani and colleagues developed a model including fluid drag, transmembrane pressure, and membrane tension along a tubular protrusion. The model predicted that the three stages during tubular protrusion are initiation, elongation, and termination. Based on experimental data Rangamani et al. [95] constructed a mathematical model that can predict the tubular protrusion growth. They reported that their force balance approach can explain the elongation phase of tubular protrusion and that confinement-based tubule growth system is regulated by osmotic pressure and drag.

Their simple force balance approach has also been used to explain the dynamics of elongation of acrosomes [93] and neurite retraction [94]. The applications of this model to different processes have revealed that the viscoelasticity of the membrane and membrane tension are significant factors in governing the dynamic behavior of membranes. In certain cases, model predictions were verified experimentally [94].

A comprehensive class of models incorporating surface lipid viscosity has also been developed [95, 120, 121, 122, 123]. Specifically, Rahimi et al. [121] built a continuum model that can explain the behavior of a bilayer configuration and its internal dissipative mechanisms. Using lipid hydrodynamics with shape dynamics and the Helfrich-Canham energy approach, they developed a dynamical model of tubular protrusion and generated dynamics by modeling the system as a constrained optimization problem. Their continuum model shows that the viscoelastic behavior of tube formation depends on membrane bending elasticity and inter-monolayer friction.

4 Thermodynamic considerations of tube formation

Thus far, we have discussed the mechanical considerations of tube formation in lipid bilayers. The applied forces and membrane-protein interactions are also influenced by thermodynamic considerations and will be briefly discussed in what follows.

4.1 Role of thermal fluctuations in tubule formation

Lipid bilayers are moderately soft compared to Boltzmann energy (k_B T) at physiological temperatures. As a result of this, they undergo shape undulations due to the thermal movement of the fluid molecules in the surrounding domain (Figure 4a). Experiments have reported the observations of membrane fluctuations in vesicles [124, 125, 126]. These undulations cause mechanical softening of the membrane [127] and can influence shape instabilities in the bilayer [128].

There are a series of theoretical studies [129, 130, 131] and Monte-Carlo simulations [132] that have reported that thermal fluctuations soften the membrane to a significant amount and also reduce local tension of the membrane. It was proposed that the effective bending rigidity in the presence of thermal fluctuations can be written as [130]

$$\kappa(T, \lambda, a) = \kappa_0 - \frac{3}{4\pi} k_B T \ln \frac{q_{\text{max}}}{q_{\text{min}}},$$

(8)

and the effective tension is given by

$$\sigma(T, a) \approx -\frac{3k_B T}{8}\left(q_{\text{max}}^2 - q_{\text{min}}^2\right),$$

(9)

where $q_{\text{min}}$ and $q_{\text{max}}$ are the magnitude of maximum and minimum wave numbers of the undulations.

It is worth mentioning that the equipartition of energy limits the energy of each undulation mode. Thus, the magnitude of the deflection correlates inversely with the square of the wavenumber of that particular mode of undulation. Further, the ratio of these wavenumbers correlated with the maximum and minimum size of the wavelengths ($\lambda$) of the undulations as

$$\frac{q_{\text{max}}}{q_{\text{min}}} = \frac{\lambda_{\text{max}}}{\lambda_{\text{min}}}. $$

(10)

The highest value of the wavelength ($\lambda_{\text{max}}$) is of the order of the size of the membrane ($L$), whereas the least value of it scales with the diameter of the lipid molecules ($a$).

Considering Equation 8 and the fact that thermal softening is directly correlated with the size of the domain, the role of fluctuations can become prominent on a larger length scale. In contrast, for a lower length scale, the effect of thermal fluctuation will be negligible. Thus, the persistent length $\xi$ below which the membrane behaves as a rigid surface is given by [131, 133]
The changes in physical properties of the membrane resulting from the effect of thermal fluctuations can facilitate shape instabilities, many of which lead to the formation of tubular protrusions \[128\]. For low surface tension membranes, the shape undulation generates a negative tension and thus inserts a compression in the plane of the membrane. As a result of this compression, the membrane undergoes a buckling instability resulting in the formation of a tubule out of the plane (Figure 4b). Such tubular structures have been observed in many experiments \[134, 135\]. Furthermore, the shape undulations alter the binding probability of the molecules from the surrounding fluid \[136\], which confer additional surface area on the membrane and impose compressive stresses that support tubulation.

The coupling between shape fluctuations and membrane-protein interactions can result in the clustering of proteins on the membrane surface due to in-plane attraction \[137\]. These protein clusters can lead to tubulation of the membrane by means of a steric effect \[14\] or by spontaneous tubulation \[138\].

4.2 Thermodynamics of protein binding, aggregation, and phase separation

The coupling between membrane mechanics and the thermodynamics of the membrane protein interactions results in thermophysical phenomena such as aggregation of proteins, separation of protein and lipid phase, binding and unbinding of proteins to the membrane, etc. Proteins that do not interact with one another prefer a homogeneous distribution in the lipid bilayer to maximize the entropy of the system \[139\]. However, proteins that interact with each other can experience a net attraction force among themselves and form a cluster \[15\]. Additionally, due to the difference in chemical composition from the lipid, the protein-coated region forms a separate phase on the lipid bilayer. The unbalanced force in the transition region induces line tension that drives formation of a cluster of the same phase energetically favorable \[140, 141, 142, 143\].

Theoretically, the effect of aggregation can be modeled by incorporating an aggregation potential in addition to the membrane bending energy \[144, 145, 146\]. Furthermore, binding and unbinding of proteins to the membrane and adhesion of the proteins on the membrane can decrease the free energy of the system and are energetically favorable \[147\]. Each of these thermophysical phenomena influences membrane bending and is conversely dependent on membrane curvature created by bending (Figure 4c). Veksler and Gov \[148\] presented a detailed theoretical model of filopodial protrusion where they considered the effect of protein adhesion, the force due to actin polymerization, and membrane tension separately on aggregation. They observed that force due to polymerization increases the critical temperature, whereas, adhesion strength and tension decrease the critical temperature for transition.

Curvature plays a significant role in aggregation and phase segregation. We discussed in Section 3.3 how BDPs insert anisotropic curvature to the membrane and induce spontaneous tubulation. Further, BDPs are the flexible rod-like proteins that undergo elastic deformation in addition to inducing membrane curvature. The energy for elastic bending of the BDPs is thus dependent on the membrane curvature and hence minimizes the total energy with a preferable distribution \[113\]. Moreover, the anisotropic curvatures of proteins also induce an orientation entropy in the system. A series of studies \[92, 112, 113, 149, 150\] modeled the thermodynamics of BDP interaction with the membrane by considering the energy of bending of both the membrane and the BDPs along with the entropy for configuration and orientation of BDPs. These studies suggest that BDP undergoes curvature induced aggregation that eventually results in a tubular protrusion of the membrane, corresponding to the minimum energy state of the system and the orientational entropy favors this process \[150\].

Another thermodynamic effect that influences tubulation of the membrane is protein crowding. Protein crowding is the phenomenon that is associated with a high concentration of proteins in the lipid bilayers. When such macromolecules adsorb onto the membrane, they tend to interact among themselves with steric effects, and often result in tubulation of membranes \[14, 151\]. Stachowiak et. al. \[14\] demonstrated this kind of tubular protrusion as membrane tension dominated, and proposes a physical model for the estimation of membrane tension (\( \lambda \)) as the function of protein-lipid binding energy (\( \Delta G \))

\[
\lambda \approx \frac{3\Delta GA_D}{A_P},
\] (12)

where \( A_D \) is the fractional area of protein domain and \( A_P \) is the fractional binding area of protein. They further assumed that membranes with crowded proteins undergo area dilation under this high tension. Such crowded proteins can collide with each other and generate a lateral pressure \[152\] that can lead to tubulation. This crowding pressure, when sufficiently large, can also facilitate membrane fission \[153, 154\]. Durgane and Copic \[155\] theoretically modeled the curvature generation due to crowding pressure and estimated a spontaneous curvature as a function of
Figure 4: (a) A fluctuating membrane with deflection $\varepsilon$ from the equilibrium position, (b) Membrane tubulation under compressive force caused by thermal fluctuation, (c) Tubulation due to active forces – binding and unbinding of proteins, pulling of actin, microtubules and motor proteins in cytoskeleton, and force induced by anchored and tethered proteins.

difference of crowding pressure between two monolayers, given by

$$C_0 = \frac{\delta p_C}{\ell},$$

where $\delta p_C$ is the difference between crowding pressure between two leaflets of lipid bilayer and $\ell$ is the modified length scale evaluated as the ratio of the projected area proteins to their height. Further, the crowding pressure is modeled in the same fashion as thermodynamic gas pressure, which encounters the effect of collision between the proteins. This process of curvature generation with a crowding pressure is also considered as one of the basic thermodynamic mechanisms of curvature generation in protein-lipid interface [156][157].
5 Future perspectives and open questions

In the previous sections, we have elaborated on how cell-based experiments, model systems, and mechanical models have focused on the problem of membrane tubulation. Here, we discuss certain new avenues for this area of research and how we might be able to bridge some of the gaps between mechanics and cell biology.

From a modeling standpoint, we need to develop models that take multiple physics into account. We are seeing an increase in extensions of models of membrane bending that go beyond the classical descriptors of spontaneous curvature, and include other features such as lipid viscosity and protein diffusion [95, 120, 123, 158]. However, these models need to be brought closer to the experimental observations. A challenge that lies ahead is the development of numerical methods that are robust [159, 160]. An additional opportunity lies in bridging molecular dynamics simulations to continuum mechanics simulations to build a truly multiscale model [161].

From an experimental standpoint, increasing the resolution of quantitative measurements in time and space in GUV based systems (e.g. protein density, tubule radius, surface coverage) would provide invaluable data to constrain the free parameters in model development process. Of course, as discussed earlier, dynamic measurements of the tubule formation process are critical for informing the relevant timescales in the models.

The next opportunity, in our view, lies in the gaps between models built for synthetic or purified systems and models for cellular processes. For instance, Shengel et al. [162] built an iPALM, a simultaneous multiphase interferometry that provides both molecular specification and resolution of cellular nanoarchitecture. Thus, there is an opportunity for the modeling community to interact more closely to work with large experimental data sets to identify the key physics underlying these processes. Finally, we would like to iterate that there are many opportunities that call for truly interdisciplinary collaborations with open science approaches that can help us gain more insight into the fundamental process of tube formation in cellular membranes.

Acknowledgments

The authors would like to thank their many collaborators in the field of membrane mechanics for discussing ideas and the organizers of the International Symposium on Cell Surface Macromolecules 2020 for engaging discussions. They would also like to acknowledge Haleh Alimohamadi, Elizabeth Heyde, Matt Akamatsu, and Ali Behzadan for providing their critical comments and feedback for the manuscript. This work was supported by NIH R01GM132106 to P.R.

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