Theoretical and Applied Mechanics Letters 12 (2022) 100321

Contents lists available at ScienceDirect

Theoretical and Applied Mechanics Letters

journal homepage: www.elsevier.com/locate/taml

Review

Simulation and prediction of membrane fusion dynamics

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ARTICLE INFO

Article history:
Received 30 November 2021
Accepted 27 December 2021
Available online 8 January 2022

Keywords:
Membrane fusion
Hemifusion intermediates
Fusion pathway
Regulation
Helfrich model

ABSTRACT

Membrane fusion is an important process by which biological membranes perform their life activities. Simulations show that the membrane fusion process happens mainly through three pathways, where the Stalk-Pore hypothesis, in which two membranes come into close contact to form a stalk to a hemifusion intermediate, and then the fusion pore opens to achieve completely fusion, is widely accepted, and there exist two free energy barriers that break the current structural steady state for lipid rearrangement. Factors of lipid composition, mechanical environment, protein and ion have regulatory roles in the membrane fusion process by effecting membrane curvature structurally and the free energy barriers from energetic perspective. Meanwhile, many theoretical models, represented by the Helfrich model, have been proposed to predict the membrane fusion process. In this paper, we review the research process of membrane fusion and mainly introduce the dynamics of membrane fusion, regulation factors and typical theoretical models.

1. Introduction

Biological membranes, presented in eukaryotic cells to compartmentalize organelles or extracellular microenvironment, are dynamic structures composed of double (single) lipid layer, membrane proteins and small amounts of sugars and thus play an important role in cellular activities [1]. When two separated lipid bilayer membranes come in close proximity, they merge into a single lipid bilayer membrane and a pore is formed, leading to the mass exchange inside and outside the membrane, this process is called membrane fusion. Membrane fusion is key in the evolution of biological membranes and widely presented in the biological activities of eukaryotic cells. For example, fusions of lysosomes with endosomes [2] and of intracellular transport vesicles with organelle membranes [3] are observed during intracellular mass transportation. In the process of endocytosis or exocytosis, membrane fusion takes place in the transmission of intercellular signals [4] and the release of neurotransmitters [5–7]. When a virus infects a cell, the virus with a lipid bilayer envelope invades the cell body by fusing the viral membrane with that of the target cell [8,9], and this process is further facilitated by the fusion protein of viral envelope [10]. In addition, membrane fusion also exists in cell-cell fusion [11], as exemplified in the fusion of sperm with oocytes in fertilization [12] and the virus-mediated intercellular fusion between infected cells and neighboring uninfected cells [13]. Thus, it is crucial to examine the dynamics of membrane fusion and its regulatory mechanism for understanding its biological function.

Biological membranes are mainly composed of lipids and membrane proteins, where the lipids form the main structure of the membrane. This is why the membrane fusion was assumed to be dominated by the related behaviors of liposomes in essence in those pioneering works [14,15]. Based on this assumption, a protein-free bilayer membrane system was first constructed artificially to decipher the membrane fusion process theoretically and experimentally [16–18], followed by investigating the viral fusion [19], intracellular fusion [20–22] and developmental cell fusion [23]. Among them, a series of experimental measurements were conducted for the fusion induced by influenza virus hemagglutinin (HA) [24–27], inhibited by lysozyme lipids between cell and organelle membranes [20], mediated by fluorescently-labeled SNARE proteins [21,22], or driven by EFF-1 transmembrane protein for cell fusion [23]. Meanwhile, numerical simulation methods are also widely used in elucidating the membrane fusion

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https://doi.org/10.1016/j.taml.2022.100321

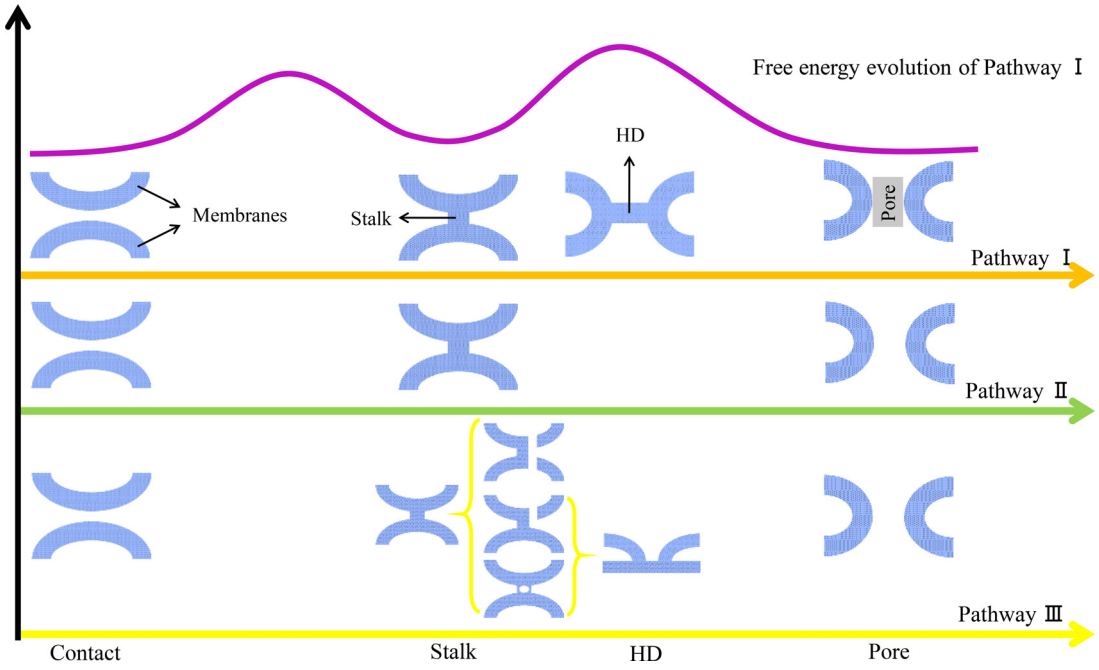

Fig. 1. Three pathways of membrane fusion and the free energy evolution of Pathway I. *Orange, green, and yellow arrows* denote Pathways I, II, and III, respectively. *Blue shadows* refer to the lipid bilayer, and *purple line* denotes the free energy evolution of two vesicle fusion *via* Pathway I.

processes, because it is hard to visualize the structural changes during membrane fusion *via* experimental means. For example, Monte Carlo simulation [28] was applied for the evolution of stalk structure during membrane fusion, Brownian dynamics (BD) simulation [29] for the fusion process of two self-assembled vesicles, dissipative particle dynamics (DPD) simulation for the protein-mediated membrane fusion process [30] and the effect of membrane tension on membrane fusion [31], and molecular dynamics (MD) simulation for the protein-promoted membrane fusion [32,33] and the structural evolution of membrane fusion process [34,35]. In this review, we mainly summary the progresses in membrane fusion process using numerical simulation as well as theoretical prediction.

2. Fusion dynamics of lipid bilayer membrane

Membrane fusion is a time-lapsed process and presents structural evolution with time. A body of simulations indicate that the membrane fusion process mainly consists of three stages: contact, hemifusion, and fusion pore [36,37]. Here the contact stage refers to the bending of the middle part of the two lipid bilayer membranes close to form a stalk structure, the hemifusion one denotes the fusion of the two lipid bilayer membranes into a single lipid bilayer membrane in the middle part, so-called the hemifusion diaphragm (HD) structure, and the fusion pore one terms the formation of a pore in HD structure to achieve complete fusion of the two lipid bilayer membranes. Membrane fusion processes between two vesicles or one vesicle and one planar membrane is observed using fluorescent labeling methods [38,39], and HD structure formation in two-vesicle fusion is visualized by confocal fluorescence microscopy [40] and cryoelectron microscopy [41]. Experimental evidence from monitoring membrane currents and membrane capacitance suggests that, once the fusion pore is open, it may also be reversibly closed [42]. In brief, membrane fusion is a highly dynamic process with rearrangements of lipid molecules.

To date, three main membrane fusion pathways are proposed [43] (Fig. 1). The first pathway, known as the "Stalk-Pore hypothesis" which was first conjectured theoretically [16] and gradually confirmed in numerical simulations [35,44], is widely accepted. In this fusion pathway, two lipid bilayers first come close together to form a stalk structure, and then the middle part of the two membranes fuse into a bilayer due to lipid rearrangement, *i.e.*, the HD structure, which eventually forms a hole in the middle of the HD structure leading to the complete fusion of the two lipid bilayers [35]. The second is similar to the "Stalk-Pore hypothesis", but in this pathway, the HD structure is unstable and the two lipid bilayers fuse rapidly upon approach, transferring directly from the stalk structure to the complete fusion state. This pathway was first proposed in theoretical predictions [45,46] and has been uncovered using MD simulations to favor this direct fusion pathway when the POPC content in the membrane is increased [47]. As a specialized case of the first fusion pathway, the second pathway is also generally accepted. The third is based on Brownian dynamics simulations [29] and Monte Carlo simulations [28]. In these simulations, a few uncommon fusion pathways were found to exist in addition to the two former pathways. During the fusion of two membranes, one of the membranes is ruptured into a pore outside the stalk structure [28,35,48] and these lipids tend to self-assemble and repair to form a diaphragm structure, which is finally ruptured to complete the fusion [28]; or, both membranes are ruptured into a pore outside the stalk structure and the two pores align to be encircled by the stalk structure to form a fusion pore to complete the fusion. In addition, the first pathway may form a structure with an intermediate compartment after the stalk structure, which gradually evolves into a diaphragm structure in the third pathway and is subsequently ruptured to complete the fusion. Among these fusion pathways, the first two pathways are widely accepted, with most simulations showing fusion processes in the first pathway [30-33,35,44,50-54], while the other pathways are considered as specialized cases in the membrane fusion.

During membrane fusion, changes in membrane structure are usually accompanied by the alterations in energy (Fig. 1). MD simulations indicate that the rearranging of lipid molecules for the formation of stalk structure need to overcome the first free energy barrier and the formation of the fusion pore in the HD structure correspond to the second one [55]. DPD simulations for

fusing the vesicles with planar membranes present the values of those free energy barriers between 8 k_BT and 15 k_BT [56]. After crossing over each energy barrier, the entire membrane system tends to maintain a steady-state structure with localized minimum energy [57]. While both experiment [40] and continuum model simulation [58] consider HD structure as a sub-steady state, the simulations of the free energy evolution in Fig. 1 show that the HD structure was not stable probably due to the insufficient size [55, 57]. In addition, MD simulations show that the membrane curvature also affects the values of the free energy barriers and the steady-state energy values. For example, using coarse-grained (CG) MD simulations to predict those membrane fusion process of two planar membranes, one planar membrane and one vesicle, and two vesicles, and to calculate the evolution of the free energy during membrane fusion, it was found that the larger the membrane curvature the lower the values of free energy barriers and steady-state energy [57]. Meanwhile, the composition of lipid bilayer as well as their symmetry features can also affect the energy evolution during membrane fusion, as exemplified in the fusion process of two vesicles predicted using MD simulations where an increase in cholesterol and stalk asymmetry enhances the free energy barrier [59]. A recent simulation study shows that disordered lipid tails and lipids with negative intrinsic curvature promote stalk formation and that lipids with anionic headgroups increase the free energy barrier for stalk formation [60]. Thus, membrane fusion dynamics is associated with intrinsic fusion pathways and energy landscapes that vary with distinct membrane systems.

3. Regulatory factors in membrane fusion

Several regulatory factors are reported in the membrane fusion process, such as lipid compositions, mechanics, proteins and ions, which structurally lead to changes in membrane curvature and thus affect the membrane fusion process [61]. Also, from the viewpoint of free energy, these factors regulate the fusion by affecting the membrane features to alter the level of the energy barriers or by applying an external force to break the barriers.

Lipid composition and structure regulate the membrane fusion process. For example, membrane fusion occurs spontaneously in membrane system containing PE and PC due to the presence of molecular thermal motion, when the two membranes are sufficiently close to each other by about 1-1.5 nm, as found using CG simulations [44]. In the case of elevated POPC content, the fusion is more inclined to a direct process without HD structure [47]. For those charged lipids, high transmembrane voltages are formed on both sides of the membrane to induce lipid membrane pore formation together with an accompanying risk of HD edge pore leakage, whereas cholesterol presence enhances lipid accumulation to inhibit the leakage due to its smaller structure [48]. The disorder in lipid tails and the negative intrinsic curvature of lipid molecules reduce the free energy barrier for stalk formation, while lipids with anionic headgroups increase the free energy barrier for stalk formation [60]. In addition, other physical factors such as temperature [39], pH [39,62], and light sensitivity [63] also manipulate the structure and chemistry of lipid molecules, which in turn affects the membrane fusion process. Collectively, lipid components regulate the membrane fusion process by altering the physical properties of the membrane [64] or relying on the differences in the chemical features, chargeability, structure, and other characteristics of different lipid molecules.

Membrane fusion process is usually accompanied by mechanical regulation. On the one hand, several factors alter the mechanical environment of the membrane. For example, membrane tension is altered by varying the number of membrane lipids on a fixed-size membrane, and high membrane tension promotes membrane fusion in terms of area per lipid [31]. A membrane tension threshold exists for transition from the unfused to the fused state, confirming the existence of free energy barriers and their break-up when the membrane tension increases [31]. Due to the existence of a hemifused sub-stable state, it fails to fuse when the membrane tension is below the threshold value [31]. In addition, a few fusion processes present the form of peptide bunches by fused peptides, suggesting that the lipid rings around such bunches yield a non-vanishing edge energy or *line tension*, which is required to stabilize the stalk structure and drive the subsequent fusion process [50]. On the other hand, the membrane fusion is associated with changes in membrane mechanical properties. For example, lipids form low density zones to accommodate the curvature distribution in HD structures, causing high lateral tension to form unstable structures that lower the potential barriers and induce the formation of fusion pore [35]. And the steeper side wings exert higher lateral tension, which reduces the threshold pore size and implies reduction in the potential barrier and easy pore formation for fusion [51]. In fact, most of the *in vivo* mechanical factors are imposed through biomolecules, suggesting that biomolecules such as proteins play an important role in regulating the membrane fusion.

Proteins are crucial for membrane fusion processes, such as the role of actin and myosin in cytosolic vomiting [65] and viral peptide-induced membrane fusion [53,66]. Most typical examples are the influenza virus HA-induced membrane fusion [24–27] and the effects of proteins presented on lipid raft domain of the yeast cell vesicle membrane on homotypic fusion [67]. Membrane fusion process is usually accompanied by alterations in membrane curvature [61], implying that numerous membrane curvature proteins also have important effects on membrane fusion process. For example, IRTKS was found to be specifically induced during osteoblast fusion and interact with Tks5, suggesting that IRTKS plays a role in the formation of cell protrusions during fusion through its BAR domain [68]. In addition, shell proteins, such as clathrin, COPI and COPII, affect membrane curvature by polymerizing into curved structures [61]. During membrane fusion for intracellular transport, the COPII periplasmic complex promotes vesicle budding in the endoplasmic reticulum (ER) and the COPI periplasmic complex plays a similar role in the Golgi apparatus, where clathrin-coated vesicles mediate the transportation among cell surface, trans-Golgi and endosomes [69]. Generally, protein-mediated membrane fusion model can be classified into four types [52], *that is*, proteinaceous pore model [30], fence model [70], scaffold model [30,32], and amphiphilic peptide model [33,54] (Fig. 2). In the protein pore model, the protein fusion pore is composed of oligomeric transmembrane proteins with hydrophilic channels in the middle [52]. The initial size of the pore is similar to that of an ion channel, merging with lipids to induce fusion upon swelling [71]. The V0 subunit of the vesicular proton ATPase is considered to be a protein fusion pore, which is activated by calmodulin in a calcium-dependent way [72]. The fence model is similar to the protein pore model in that an oligomeric ring of transmembrane proteins forms at the onset of fusion. The differences between the protein pore model and the fence model lie in that the latter yields more dispersed distribution and there exists a lipid region at the center of the ring [52]. For example, the membrane fusion induced by Class I viral glycoproteins (*e.g.*, IFV, HIV, or EBOV) likely follows the fence model [73]. A typical example of the scaffold model is the SNARE protein [74], in which two chains are inserted into two lipid membranes for applying mechanical forces to bring them close together and trigger the membrane fusion process [32]. The amphiphilic peptide model is proposed for the fusions induced by viral fusion peptides [53] and SP-B proteins [33], based on the principle that amphiphilic peptides initiate local perturbations to overcome the potential barriers and thus activate fusion. In addition, the lipid composition of the membrane can affect the structure of the fused peptide and regulate the fusion process [64]. In addition to their effects of protein

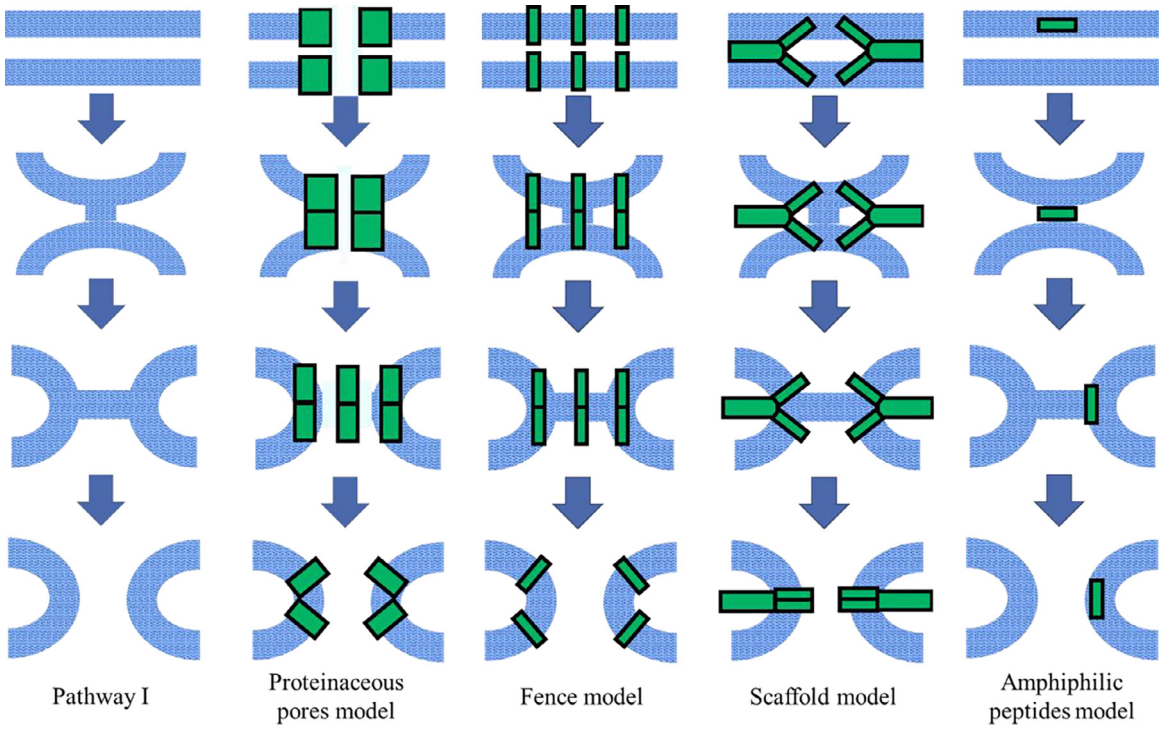

Fig. 2. Four types of models for protein-mediated membrane fusion. *Blue shadows* refer to the lipid bilayers, *green boxes* denote the proteins, *black lines* define the protein boundaries.

structures, these fusion peptides can manipulate membrane rigidity, polarity and heterogeneity, which in turn affects the membrane fusion process [75]. Membrane protein density also regulates the membrane fusion, suggesting that dense membrane proteins may prevent membrane fusion [76].

In addition to the above three factors, various ions also play a role in regulating the membrane fusion process. For example, calcium ions are able to destabilize the membrane and promote the formation of the pre-stalk transition state [77]. Observations in restoring intrinsic fusion ability by specific negative curvature molecules suggests that negative curvature itself is required for calcium ions to trigger the fusion [78]. Moreover, calcium or magnesium ions can induce the aggregation of PS vesicles and produce a complex of cations and PS at saturating concentrations, indicating that this complex is essential for the phase transition and fusion of PS vesicles [79]. A recent study has shown that calcium ions selectively bind membrane head groups into long-range lipid clusters in a lipid-specific way withnegative curvature of the membrane and lateral tension in the head group region, which finally speeds the fusion dynamics through reducing the energy barrier [80]. Taken together, membrane fusion process is strongly associated with lipid and protein composition as well as mechanical and chemical environment to which the membrane is exposed.

4. Theoretical predictions in membrane fusion

Membrane fusion process is accompanied by energy evolution, attracting attentions to quantify the energy landscape of membrane system. To address this issue, a physical model of the membrane fusion is required to include both the work proteins applied and the physicochemical changes in the lipid bilayer, where the deformation energy of the lipid bilayer could be sufficiently high and thus govern the rate of membrane fusion [81].

The deformation energy of the lipid bilayer is estimated using a *smectic A* liquid crystals similar to that of the lipid bilayer. Given that the membrane thickness is approximately 5 nm which is much smaller than the membrane transverse characteristic size and that the normal to the surface of the bulk liquid crystal coincides with the pointing vector, the deformation energy of the lipid bilayer, W_b, at a certain reference surface along the direction of the membrane thickness can be expressed, under the small deformation approximation of Hooke's law, as [81],

$$W_b = \int \frac{B}{2}(C_1 + C_2 - C_0)^2 \mathrm{d}S, \tag{1}$$

where B is the modulus of membrane expansion with respect to the reference surface, C_1 and C_2 are the principal curvatures of the reference surface, respectively, and C_0 is the spontaneous curvature of the lipid bilayer. Here the energy expression Eq. (1) is often referred to as the Helfrich functional [82]. This classical energy functional Eq. (1) has been effectively used to analyze a large number of membrane processes and structures, such as assessing the plausibility of the red blood cell shape [83], estimating the effect of lipids on membrane bending energy [84], and calculating the deformation energy of endoplasmic reticulum sheets [85]. For example, the Helfrich continuum media model was used to estimate the free energy of the elastic part of the membrane fusion process or the membrane deformation energy, and to evaluate the evolution of the free energy during membrane fusion obtained by MD simulations [55,57].

It should be noted that the condition that the pointing vector coincides with the normal of the reference plane in the derivation of the Helfrich functional leads to quite limited applicability. Any internal structure of the membrane is excluded from consideration in the Helfrich functional and the membrane in the Helfrich model actually becomes an infinitely thin, structureless, elastic membrane. Nevertheless, the Helfrich functional is still workable with the relevant modifications and generalizations, mainly due to its effectiveness and simplicity [16,86].

One example is the Kozlov-Markin model, which adapts the Helfrich model for theoretical description of membrane fusion process. This model first treats membrane fusion as a multi-stage

process, the "Stalk-Pore hypothesis", and quantifies the energy of HD structures [16]. The Helfrich functional Eq. (1) is then used to calculate the bending energy of the contact and distal monolayer membranes, enabling to interpret the experimental observations between the evolution of the membrane system and the spontaneous curvature of the fused membrane monolayer [84,87]. In addition, a model that links molecular interactions to curvature stresses is also developed using Helfrich functional to decipher the role of local composition of membrane [88],

$$F_H = \frac{k_c}{2}(C_1 + C_2 - C_0)^2 + k_g C_1 C_2, \tag{2}$$

$$C_0^{mix} = f^A C_0^A + f^B C_0^B, \tag{3}$$

$$F_{H,l} = \sum_i f_i \int \frac{k_c}{2}(C - C_{0,i})^2 \mathrm{d}S \text{ (local)} \tag{4}$$

$$F_{H,g} = \int \frac{k_c}{2}\left(C - \sum_i f_i C_{0,i}\right)^2 \mathrm{d}S \text{ (global)} \tag{5}$$

where F_H is the free energy per unit area of lipid membrane, k_c is the bending modulus, k_g is the Gaussian modulus, A and B refer to any two lipids, $C_0{}^{mix}$ is the spontaneous curvature of A and B lipids after mixing, f^A and f^B is the composition of A and B lipids, $C_0{}^A$, $C_0{}^B$ is the spontaneous curvature of A and B lipids, i refers to different lipids, f_i denotes the composition of different lipids, and $C_{0,i}$ is the spontaneous curvature of different lipids. This model suggests that cholesterol presence in lipid membrane reduces the lateral pressure below the curved neutral surface, favoring positive membrane curvature, while unsaturated flexible acyl chains have null effect, and that hydrogen bonding between sphingolipids at sufficient concentrations leads to positive membrane curvature [88].

In addition to the generalizations and application of the Helfrich model, other theoretical models are also developed for elucidating membrane fusion processes. For example, the HD growth rate model, derived using the net thermodynamic driving force equation for expansion for HD structures, predicts HD growth rates that are dependent on tension and salt concentration in quantitative agreement with experimental measurements [89]. A string method that overcomes the difficulty of calculating transition paths in structural evolution is proposed and also applicable to MD simulations [49]. Collectively, these theoretical models provide an insight in deciphering the energy landscape in membrane fusion, together with those from numerical simulations and experimental measurements.

5. Other membrane pore dynamics

Pore formation existing in many biological processes is associated with membrane fusion, presenting other modes of membrane pore formation. For example, membrane pores are formed on the surface of red blood cells exposed to blood flow, facilitating the release of hemoglobin [90]. Charge imbalance or surface tension on both sides of the membrane, as well as involvement of small molecules, peptides and lipids, can induce monolayer membrane pore formation [91]. More specifically, fenestrae exist on the liver sinusoidal endothelial cells (LSEC) that penetrate the entire cell [92], with a diameter of approximately 100 nm. This specialized structure facilitates the mass exchange between the liver sinusoidal lumen and Disse space, which can be inhibited by lipid rafts and the cytoskeleton [93,94]. Unfortunately, the mechanisms for the formation of this large-sized penetrating pore remain unknown. Meanwhile, the vesicles synthesized or derived from cells with protein phase separation on their surface generate considerable compressive stress in the membrane plane, and this pressure drives the membrane to bend inward to form protein-lined membrane tubules [95]. These observations suggest a mechanism by which membrane tubules lining one side of a cell membrane can fuse with the other side of the cell membrane, finally forming a single cell penetration pore. In brief, whether and how membrane fusion affect physiological pore formation need more investigated.

6. Conclusion and prospective

Membrane fusion is extensively observed in biology, such as intracellular transport, endocytosis or exocytosis, virus invasion, and cell fusion, which is critical for biological functions. A widely-accepted fusion pathway, the "Stalk-Pore hypothesis", refers to two lipid bilayer membranes that approach each other to form a stalk and hemifusion diaphragm structure with a fusion pore open to achieve completely fusion. Since the fusion process requires lipids rearrangement to break up the current steady state, there exist two free energy barriers corresponding to the formation of the stalk structure and the opening of the fusion pore. External factors such as lipid composition, mechanical environment, protein action and ionic action can affect or break the energy barriers and thus regulate the fusion process. In this regard, various molecular simulation approaches are applicable to uncover the configurational pathways and the energy landscape in the membrane fusion dynamics.

Theoretical models are also proposed to estimate the membrane deformation energy during membrane fusion. To date, no universalized models are available to estimate the protein-mediated membrane fusion process mainly due to the simplified assumptions of the models and the complexity of the membrane fusion. Although the existence of HD structures is observed experimentally, how these structures are formed and what the subsequent dynamic is evolved at the molecular level is still not visualized explicitly yet. Moreover, majority of the current works is applying CG simulations to predict the structural changes during membrane fusion, which is far away from elaborating the structural evolution of large-size structures at all-atom level due to computational resource limitations. It is also noticed that those pores formed during membrane fusion are quite small, usually only a few nanometers before complete fusion. From the viewpoint of biological application, the mechanism for the formation of large-sized pore penetrating structures on a single cell like LSEC fenestrae remains unclear, calling for new numerical and theoretical approaches along this line.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Grants 32130061 and 12172366).

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