Membrane fusion based on the stalk model

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It is a commonly accepted assumption that membrane fusion involves an hour-glass-shaped local contact between two monolayers of opposing membranes, an intermediate structure called a stalk. The shape of the stalk is considered as an axisymmetrical surface of revolution in 3D space, with a planar geometry in the initial configuration. The total energy of the stalk is evaluated from the assumption that the stalk has a constant mean curvature. We analyze on this basis the energetic path of evolution of the stalk from hemifusion to complete fusion, adopting the radius of the stalk, the peel-off angle at the interstices and the stalk total energy as characteristic quantities. An extension of the original model is proposed, in which any geometrical feature of the stalks can be expressed in explicit form, by considering the stalks as nodoid surfaces.

Keywords: lipid membranes, stalk model, nodoid surfaces.

INTRODUCTION

Membrane fusion plays a vital role in cell physiology and life, and for this reason has attracted the interest of many researchers, resulting in many attempts to develop representative models of this process. The ability to fuse is shared by biological membranes, consisting of phospholipid bilayers with embedded and bound membrane proteins, and by artificially formed purely lipid membranes. Normally, the membranes are mechanically stable, due to powerful hydrophobic effect [38], which drives self-assembly of the lipid molecules into bilayer and maintains the bilayer integrity. Membrane fusion requires transient structural reorganization of at least some lipids (see [1, 4, 8, 22]). Experimental evidence points to the existence of so-called hemifusion structures [3, 4, 39], which are relatively long living intermediates appearing during the early stage of fusion. Membrane hemifusion is a possible pathway [32] to the complete fusion of membranes [4]. The modeling of the lipidic fusion intermediates has more than two decades history. Although, at the early stages, several different structures have been suggested, only one of them, called fusion stalk [9,15], is currently recognized to describe adequately the transition stage of membrane fusion [4,11].

Fusion stalk is a local lipidic connection between the proximal (contacting) monolayers of the fusing membranes. At the stage of stalk formation, the distal monolayers of the membranes are still separated, and the achieved state is referred to as hemifusion. A physical model of the fusion process, based on this hypothetical intermediate and referred to as the stalk model has been developed in a series of contributions [4, 14, 20, 24, 25] and further modified by Siegel [34, 35] and Kuzmin et al. [18]. A number of important predictions of the stalk hypothesis have been verified experimentally for fusion of protein-free lipid bilayers and for some examples of biological fusion. The hypothesis suggests that merger of the proximal monolayers of the membranes precedes merger of their distal monolayers. Indeed, the existence of a distinct hemifusion stage has been documented for different experimental systems based on protein-free bilayers [3-5,19,33] and for fusion of biological membranes [6, 12, 21, 29, 37].

Current theories associate the initiation of hemifusion with the formation of a contact zone between the membranes in which the two proximal monolayers are connected by a stalk-shaped neck. The stalk then expands and a region is formed in which the two distal monolayers form a single bilayer. In general, the energetic cost of the splay of the lipid chains in the stalk prohibits its spontaneous expansion. However, the presence of additional, external forces (e.g., pressure, surface tension gradients, electrostatic

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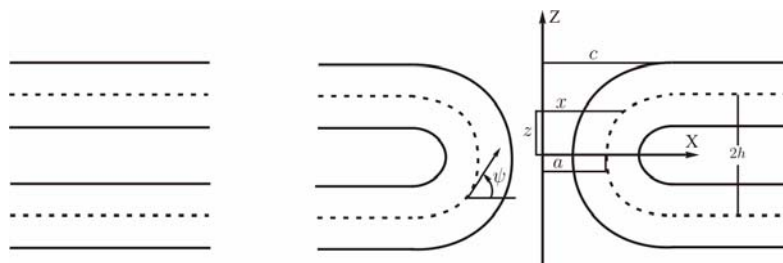**Fig.1.** Schematic view of the process of fusion via stalk formation.

Fig. 2. Steps in membrane fusion. Solid lines represent hydrophilic surfaces, dotted lines hydrophobic surfaces (left). Geometric parameters of the stalk (right).

effects) can lead to expansion of the stalk into a hemifusion region and to the growth of this zone. Clear evidence for the existence of these two distinct prefusion stages, stalk formation and hemifusion, was found for poly(ethylene glycol)-mediated fusion of vesicles [19]. Looking at a much smaller scales than continuum models do, molecular dynamics simulations allow to study the biological processes of lipid vesicle fusion in atomic details, as reported in [13].

A few words related to energetic aspects are in order, since a few factors may influence the energy required to form intermediate structures. A potential effect of membrane lateral tension on membrane fusion was assessed by two-dimensional simulations of the contact of monolayers in [2]. In [7], an energetically feasible structure of pre-stalk intermediates has been advanced, called point-like protrusion. The role of the Gaussian curvature elastic energy of the intermediate structures on membrane fusion has been highlighted in [36]. The role of membrane edges due to tilt deformations as a mechanism for the strong reduction of the energy of fusion stalks has been analyzed in [28]. The energetics of formation of vesicle intermediates was analyzed in [23], where the authors calculate the effects of membrane curvature and osmotic stress, basing on the stalk assumption. A new structure of the fusion stalk has been presented in [17], the deformations of the two membrane monolayers not only involve bending as in [15, 25,34], but also a tilt of the hydrocarbon chains. The tilt constitutes an additional degree of freedom and allows the monolayers to relax the overall energy. This view contributes to resolve partially the energy challenge (sometimes coined

energy crisis in the relevant literature, see [17]) inherent to the fact that membrane configurations at the intermediate stages of fusion require high energy barriers.
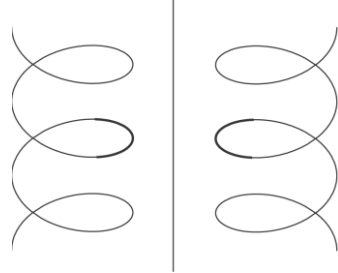
THE STALK MODEL

The fusion process involves the successive steps of membrane aggregation, a destabilization nucleating at a point defect inducing a highly localized rearrangement of the two bilayers, further inducing a mixing of the components of the two bilayers, resulting in either hemifusion (Fig. 1, middle) or full fusion (Fig. 1, right).

A connection between membranes involves local contacts between two phospholipid bilayers in their aqueous environment, which is difficult due to the hydrophobic nature of the interior part of the membranes (each bilayer has a trans and a cis-monolayer, Fig.2). The required connection between the two membranes in order for fusion to occur involves an hourglass-shaped local contact between two monolayers of opposing membranes, an intermediate structure called a stalk in the original model developed in [15].

The mechanical basis of the model relies on the calculation of the shape of the stalk, taken as an axisymmetrical surface of revolution in 3D space (Fig. 2), with a planar geometry in the initial configuration. The neutral surface is represented as a dotted line. There x and z are the coordinates of the contour, the parameter a is the shortest distance separating the neutral surface from the axis of revolution (the neck of the stalk), c is the distance from the axis of revolution to the point where the stalk branches become horizontal (the width of the stalk), $2h$ is

the distance separating the two neutral surfaces, and the angle between the neutral surface and the horizontal line is ψ.

The contour of the stalk surface is given from the slope ψ. The bending energy of the stalk depends on the principal and the spontaneous curvatures. Assuming that the curvature of the stalk is constant, the total energy of the stalk is calculated versus parameter a, and is found to be negative, hence promoting hemifusion (the presence of spontaneous curvature in the monolayers favors hemifusion). We analyze the path of evolution of a stalk from hemifusion to complete fusion, versus the radius of the stalk, the peel-off angle at the interstices, and the stalk total energy. The present contribution proposes an extension of the model suggested in [26], by considering explicitly a subclass of the Delaunay surfaces, the so called nodoid surfaces. Following the argumentation developed in [10] in the case of beaded nerve fibers (the surfaces

there are unduloids), we elaborate a model in which any geometrical characteristic of the stalks can be expressed in explicit form, by considering the stalks as nodoid surfaces Fig. 3.

A novel description of the shape of the stalk is next exposed.

THE MATHEMATICAL DESCRIPTION

The sum of the principal curvatures is given by the classical relation

$$c_p(x) + c_m(x) = c_{\text{stalk}} \qquad (1)$$

with the two principal curvatures expressed versus the angle $\psi(x)$ as

$$c_p(x) = \frac{\sin \psi(x)}{x}, \qquad c_m(x) = \cos \psi(x)\frac{\mathrm{d}\psi}{\mathrm{d}x}\cdot \quad (2)$$

The slope of the at any point along the contour of the stalk surface is determined from

$$\frac{\mathrm{d}z}{\mathrm{d}x} = \tan \psi(x) = \frac{x c_p}{\sqrt{1 - x^2 c_p^2}}\cdot \qquad (3)$$
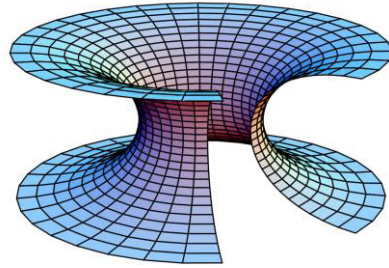

Fig. 3. The profile curves of the nodoid (left, solid parts) generating under revolution the stalk surface and a 3D-view of the open part of the stalk (right).

Previous relations then lead after straightforward calculations to the equations

$$x c_p = \frac{1}{2}c_{\text{stalk}}x + \frac{a}{x}(1 - \frac{1}{2}c_{\text{stalk}}a) \qquad (4)$$

$$\frac{\mathrm{d}z}{\mathrm{d}x} = \{[\frac{1}{2}c_{\text{stalk}}x + \frac{a}{x}(1 - \frac{1}{2}c_{\text{stalk}}a)]^{-2} - 1\}^{-1/2}\cdot \quad (5)$$

Next, due to the relations we have also

$$c = \sqrt{a^2 - \frac{2a}{c_{\text{stalk}}}} \qquad \text{or} \qquad \frac{c}{a} = \sqrt{1 - \frac{2}{a c_{\text{stalk}}}}\cdot(6)$$

From all above it results that the contour of the stalk surface is given as the integral

$$\frac{z}{a} = \int_1^{x/a} \{[\frac{1}{2}a c_{\text{stalk}}t + \frac{1}{t}(1 - \frac{1}{2}a c_{\text{stalk}})]^{-2} - 1\}^{-1/2}\mathrm{d}t \qquad (7)$$

and when $c_{\text{stalk}} = \mathbb{h} = \text{const}$ the stalk is called stress free. The surface specified by the equation (7) is a constant mean curvature (CMC) surface. CMC surfaces of revolution were classified long time ago by the French geometer Delaunay and were described in analytical form in [30, 31]. Differentiating $c_p(x)$ and taking into account the

expression for $c_m(x)$ one gets the equation

$$\frac{\mathrm{d}c_p(x)}{\mathrm{d}x} = \frac{c_m(x) - c_p(x)}{x}\cdot \qquad (8)$$

This equation and the CMC condition

$$H = \frac{c_m(x) + c_p(x)}{2} = \mathbb{h} = \text{const} \qquad (9)$$

yields

$$\frac{\mathrm{d}c_p(x)}{\mathrm{d}x} = \frac{2(\mathbb{h} - c_p(x))}{x} \qquad (10)$$

and therefore

$$c_p(x) = \mathbb{h} + \frac{\mathbb{b}}{x^2} \qquad (11)$$

where $\mathbb{b}$ is the integration constant.

By taking into account the geometrical relation $c_p(x) = \frac{\sin \psi(x)}{x}$ one finds immediately

$$\sin \psi(x) = \mathbb{h}x + \frac{\mathbb{b}}{x} \qquad (12)$$

and this is exactly the Gauss map of the surface. From the two obvious geometrical conditions

$$\mathbb{h}a + \frac{\mathbb{b}}{a} = 1, \qquad \mathbb{h}c + \frac{\mathbb{b}}{c} = 0 \quad (13)$$

it follows that

$$\mathbb{h} = \frac{a}{a^2 - c^2}, \qquad \mathbb{b} = -\frac{ac^2}{a^2 - c^2}. \quad (14)$$

Finally, the integration of the slope equation

$$\frac{\mathrm{d}z}{\mathrm{d}x} = \tan \psi(x) \quad (15)$$

gives the profile curve

$$z = \int \tan \psi(x)\mathrm{d}x = a \int_a^x \frac{(x^2 - c^2)\mathrm{d}x}{\sqrt{(x^2 - a^2)(c^4 - a^2 x^2)}}. \quad (16)$$

A parameterization of the contour can be done using the elliptic functions, i.e.,

$$x(u) = \frac{c^2}{a}\mathrm{dn}(u, k), \qquad k = \frac{\sqrt{c^4 - a^4}}{c^2}$$

$$z(u) = \frac{c^2}{a}E(\mathrm{am}(u, k), k) - aF(\mathrm{am}(u, k), k) \quad (17)$$

in which $F(\mathrm{am}(u,k), k)$, $E(\mathrm{am}(u, k), k)$ denote the incomplete elliptic integral of the first and second kind respectively, that depend on their argument in the first slot and the elliptic modulus in the second slot. Plots of the meridional sections of the cell fusion resulting from those expressions are shown in Fig. 3 (left) and a 3D view of the stalk is pictured on the right.

GEOMETRIC AND ENERGETIC ASPECTS

Having the explicit parameterization (17) of the profile curves it is a simple matter to write down the parametrization of the relevant part of the stalk surfaces in the form

$$\tilde{\mathbf{x}} = (x(u)\cos v, x(u)\sin v, z(u) - z(K(k))), \quad v \in (0, 2\pi). \quad (18)$$

With this at hand it is easy to find also coefficients E, F, and G of the first fundamental form of the surface (18) via the formulas

$$E = \tilde{\mathbf{x}}_u \cdot \tilde{\mathbf{x}}_u = \frac{(a^2 - c^2)^2 \mathrm{dn}^2(u, k)}{a^2}$$

$$F = \tilde{\mathbf{x}}_u \cdot \tilde{\mathbf{x}}_v = 0$$

$$G = \tilde{\mathbf{x}}_v \cdot \tilde{\mathbf{x}}_v = \frac{c^4 \mathrm{dn}^2(u, k)}{a^2}.$$

These coefficients are necessary to find out the infinitesimal element $\mathrm{d}A = \sqrt{EG - F^2}\,\mathrm{d}u \wedge \mathrm{d}v$ of the surface area

$$\mathrm{d}A = \frac{c^2(c^2 - a^2)\mathrm{dn}^2(u, k)}{a^2}\mathrm{d}u \wedge \mathrm{d}v, \quad u \in \left\{\frac{K(k)}{2}, \frac{3K(k)}{2}\right\}.$$

Integrating over the whole surface of the neck one easily finds that the energy of the stalk is given by the formula

$$W_s = \frac{1}{2}\bar{k}\left[(c_{\mathrm{stalk}} - \mathbb{h})^2 - \mathbb{h}^2\right] A = 2\pi\bar{k}\frac{c^2(c^2 - a^2)}{a^2}E(t, k)\big|_{K(k)/2}^{K(k)} \quad (20)$$

in which $\bar{k}$ is the bending module and $E(K(k), k)$ and $E(K(k)/2, k)$ denote the omplete elliptic integrals of the second kind.

Having the explicid parametrization (18) of the stalk one can find also its height *2h* (see Fig. 2) by the formula

$$h = z(K(k)/2) - z(K(k)). \quad (21)$$

This allows to plot the dimensionless normalized distance *h/a* which depends only on a single parameter c (i.e., $\mathbb{h}$) and to compare it with the approximate function presented in [26]. The result is depicted as curve 1 in Fig. 4.
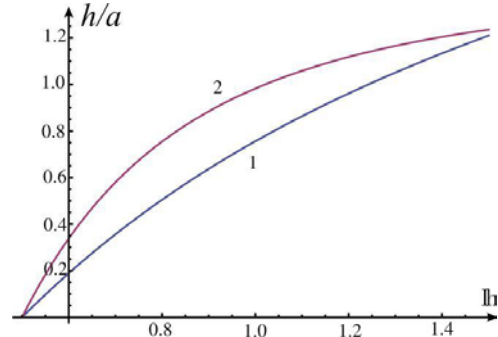

Fig. 4. The first curve is produced via the analytical result (21) and the second one is ploted by making use of the approximation formula presented in [26].

It should be noted that the resulting plot in Fig. 4 has an universal character which is applicable to any spontaneous curvature.

CONCLUSIONS

Fusion involves drastic although local changes in the initial membrane structure. The membrane configurations emerging at the intermediate stages of fusion require input of energy and, hence, represent energy barriers the membranes have to overcome on the way to the new fused state. Those energy barriers are essential determinants of the fusion rate. The free energy of fusion stalks has been calculated by different approaches.

E.g., Kuzmin et al. [18] suggested a theoretical model that includes, besides bending, a tilt of the lipid molecules. The model starts from preformed nipples that decrease the local distance of two fusing membranes and requires an extraordinary high energy to form a stalk out of two apposed, planar bilayers. The geometry of this model however is predefined.

Markin and Albanesi [26] postulated a stress

65

free stalk. The key point of their model is the optimization of the cross-sectional shape of the stalks neck in terms of its bending energy. Relying on numerics they did not recognize that this is a constant mean curvature surface which is the main point of the present study.

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СТЕБЛОВИ МОДЕЛ НА СЛИВАНЕ НА МЕМБРАНИ

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(Резюме)

Съвременното схващане относно сливането на мембраните е, че при този процес формата на двете локални контактни повърхнини на участващите   мембрани   е подобна на формата на пясъчен часовник, която се оприличава със стебло. Формата на стеблото се разглежда като аксиално симетрична повърхнина в тримерното пространство, докато първоначалната конфигурация е с равнинна геометрия. Пълната енергия на стъблото е пресметната като следствие на предположението, че стъблото има постоянна средна кривина. На тази основа е анализирана и   еволюцията на енергетичния път на стъблото от полусливане до пълно сливане, отчитайки радиалното отстояние на стъблото до оста на симетрия   като функция на ъгъла който сключва тангентата с абсцисата   в   точките от профилната крива. Представено е разширение на съществуващия модел, в които геометричните характеристики на стъблото са представени в явна форма и отчитайки, че то   има формата на нодоид,   характеристиките на мембраната и заобикалящата я среда.