Adhesion of Lipid Vesicles

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Abstract
We present elastic theory of lipid bilayer membranes and show how it can be used to determine the shapes of free vesicles as well as their adhesion. Firstly, we introduce vesicles adhering to flat substrates, followed by adhesion of rigid particles to a membrane. In the last section vesicle-vesicle adhesion will be discussed as an important aspect of cell biology.

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1 Introduction

In cell biology, a vesicle is a relatively small compartment formed by a closed membrane. It represents a well-defined model for studying certain physical aspects of biological cells. These cells consist of inhomogeneous interior, where the nucleus and the organelles are embedded in a complex fluid. Cells are bounded by the cell membrane, which is a complex lipid bilayer decorated by proteins polysaccharides and a cytoskeleton [1]. Despite the fact that vesicles lack the complexity of a cell, their overall shape is reminiscent on some types of biological cells, such as erythrocytes.

The main constituent of the membrane is the lipid bilayer. Lipid molecules consist of hydrophilic dipolar head with hydrophobic chains attached to them. In a bilayer, these chains are shielded from the aqueous environment. Bilayers are formed by self-aggregation if lipids are dissolved in aqueous solution. Once a vesicle is formed, it can be studied using videomicroscopy. Such observations have revealed a variety of different shapes and despite the fact that vesicles lack the complexity of cells, some shapes are reminiscent on red blood cells [1].

Biological cells and vesicles are rarely found in isolation; instead, they tend to stick to other cells or non-cellular components of their environment. In this paper we discuss shapes of free vesicles, as well as adhesion of vesicles on both flat and curved rigid as well as free surface.

2 Curvature model

To understand the variety of shapes of vesicles from a mechanical perspective, one needs to identify an appropriate energy model. The minimization of this energy functional then gives the equilibrium shape of the vesicle. For a symmetric membrane, the chemical composition and environment of both monolayers is identical. Therefore, the flat conformation is the state with the lowest energy. On the contrary, for a closed configuration, which is non-flat, the energy model has to be guided by the physical principles and properties of closed bilayer membranes.

2.1 Minimal model

Lipid bilayers are about 4 nm thick and a vesicle is about $10^3 - 10^4$ times larger [1]. These facts allow us to describe the membrane as a two dimensional surface $R(s_1, s_2)$ in a three-dimensional space. Any such surface can be characterized by the local radii of curvature $R_1$ and $R_2$ (Fig. 1) which form the mean curvature [2]

$$H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$

and the Gaussian curvature

$$K = \frac{1}{R_1 R_2}.$$  

The basic assumption of the curvature model is that the density of the elastic energy of bent is given by

$$f = \frac{\kappa}{2} (2H)^2 + \kappa_G K$$

where $\kappa$ and $\kappa_G$ are the ordinary and the Gaussian bending rigidity [2] (a typical value for phospholipid membranes is $10^{-19} J$ [1]).

An open edge of a membrane, which is exposed to water costs a lot of energy. That is why free membranes patches do not exist. Furthermore, we can expect that the topology of a vesicle does not change since this would imply that the membrane forms transient edges. Therefore, we only consider closed membranes, and their energy reads

$$F_\kappa = \frac{\kappa}{2} \int_A (2H)^2 dA + \kappa_G \int_A K dA$$
Figure 1: Local radii of curvature $R_1$ and $R_2$ of a membrane patch [1].

where $A$ is the surface of the vesicle. Due to the Gauss-Bonnet theorem [2]

$$\int K dA = 4\pi(1 - g) \quad (5)$$

where $g$ is the number of handles, the integral of the Gaussian curvature depends only on the topology of the vesicle. Since the change of topology (e.g. from spherical to toroidal shape) would require a membrane to form open edges for some period of time the transition between different topological shapes represents a huge energy barrier. Therefore, the topology of a vesicle is assumed fixed and we can omit this term in further calculations.

Since a membrane is a two-dimensional fluid, it cannot withstand in-plane shear stress. In addition to that, the solubility of the phospholipid bilayer is extremely low. We can conclude that there is no exchange of material between the membrane and the surrounding solution. In addition, due to small compressibility of the membrane it can be considered locally incompressible. Therefore, for a closed vesicle, the total surface area $A$ is fixed.

In experiments, one would control the osmotic conditions of the environment. The membrane is permeable for water and therefore, the volume changes up to the point where there is no more osmotic pressure difference between the interior of the vesicle and the environment. Thus the vesicle shape must be determined at a prescribed volume $V$ [1].

We have now established a minimal curvature model of vesicle:

$$F_k = \frac{\kappa}{2} \int_A (2H)^2 dA + \Sigma A + PV, \quad (6)$$

which gives the shape of a vesicle for a given surface $A$ and volume $V$. In this model, we introduced $\Sigma$ and $P$ as the Lagrange multipliers corresponding to surface tension and pressure, respectively. The minimization of this potential can be done numerically by solving Euler-Lagrange equations [3] or using some other numerical recipes as implemented in, e.g. Surface Evolver [4].

The elastic energy is conventionally represented in a dimensionless form based on a characteristic length scale $R_s$ defined by the radius of a sphere such that its area is equal to the area of a single vesicle $A$ ($R_s = \sqrt{A/4\pi}$). It turns out that the stationary shapes of vesicles obtained by minimizing the energy depend only on one parameter, the so-called reduced volume:

$$v = \frac{V}{A^{3/2}}, \quad (7)$$

whereas the energy is measured in the units of $8\pi\kappa$, which is the bending energy of a sphere with radius $R_s$ [5].

With the curvature model, we obtain the basic shapes of the vesicles as shown in Fig. 2 and 3. As it can be seen in these figures, we roughly get three different classes of vesicles: prolate, oblate, and stomatocytes. The variational method [1] showed that at small volumes the stomatocyte shape is the state with the lowest energy. The transition to the oblate
shape takes place at $v \approx 0.591$, though the results shown in Fig. 3 suggest that transition occurs at smaller volumes. The transition between the oblate and the prolate shape is less obvious. However, according to variational method the transition should occur at $v \approx 0.65$. Based on Figs. 2 and 3 we can also conclude that the vesicles exhibit an invagination when the reduced volume is small enough. The calculations show that the threshold volume for the invagination to appear is $v \approx 0.85$.

![Diagram](image1)

**Figure 2:** *Equilibrium shapes at zero spontaneous curvature for different values of the reduced volume, calculated numerically by solving Euler-Lagrange equations [1].*

![Diagram](image2)

**Figure 3:** *Equilibrium vesicle shapes at zero spontaneous curvature for different values of the reduced volume ($v = 0.53, 0.64, 0.8, 0.96$) calculated with Surface Evolver. It can clearly be seen that at small volumes vesicles exhibit a much more pronounced invagination, however, there is still no transition from oblate to stomatocyte at $v = 0.53$, despite the fact that Euler-Lagrange equations predict it at $v \approx 0.59$.***
2.2 Refined model

It is obvious that the minimal model captures the most important aspects of vesicle shapes since all these theoretical shapes have been previously found experimentally. However, there is still something missing, because minimization of Eq. 6 does not lead to all shapes observed experimentally [1]. This suggests that the model is incomplete. The missing part in the model is that we cannot treat the membrane as a structureless two-dimensional surface. It turns out that the key aspect is its bilayer character. The two monolayers are tightly coupled, but the exchange of molecules between them is very slow. Therefore, the difference between the number of molecules in the monolayers is conserved. From the number difference we can derive the area difference between monolayers:

\[ \Delta A_0 = \Delta N a_0, \]  

where \( a_0 \) is the equilibrium area per molecule and \( \Delta N \) is the number difference of molecules in the monolayers. The actual area difference can be expressed as an integral of the mean curvature

\[ \Delta A = 2h \oint_A H dA \]  

with \( h \) being the distance between the neutral surfaces of the two monolayers. If the area of each molecule is conserved, the area difference is conserved too, which introduces a new constraint to the curvature model. It turns out that this constraint is too hard and that instead of it we have to add to the energy functional an area difference elasticity [1]:

\[ F_{ADE} = \frac{\alpha \pi \kappa}{2h^2 A} (\Delta A - \Delta A_0)^2, \]  

where \( \alpha \) has a numerical value close to 1 and \( \kappa \) is the ordinary bending rigidity. If we construct the new energy functional

\[ F = F_\kappa + F_{ADE} \]  

we get all the variety of shapes like pears and starfish (Fig. 4). However, beside on the volume \( v \) the shape now depends on one more parameter

\[ m_0 = \frac{\Delta A_0}{2h} \left( \frac{4\pi}{A} \right)^{3/2} \]  

which is a scaled number difference of lipid molecules in the two layers. It was also suggested by Helfrich that the refined model should include a spontaneous curvature, replacing the \((2H)\) in 6 with \((2H - C_0)\) [1]. However, that model would give the same shapes as ADE model.

Thermal Fluctuations The model described in the previous chapter is independent of temperature. However, microscopy revealed that the vesicles exhibit visible fluctuations at finite temperature. To some extent, these thermal fluctuations have also been investigated in the context of vesicle shapes. The influence of fluctuations highly depends on the size of vesicle as well as on the rigidity of the membrane. It has been shown that fluctuations can be calculated perturbatively in the case of weak fluctuations (low temperature, small vesicle, high rigidity) [3], whereas in the regime of strong fluctuations calculation is much more demanding and non-trivial.

3 Adhesion

Until now, only free vesicles have been discussed. However, far more interesting and lifelike are vesicles interacting with other objects. The simplest case of the kind is adhesion of vesicles to a flat rigid substrate. Experiments have shown that there are two complementary
aspects of this problem, a macroscopic one (the overall shape of vesicle) and a microscopic one (fluctuations in the vicinity of the substrate) [3, 1].

We will not deal with the microscopic aspects as they are related to fluctuations, which have already been ignored in the previous section. In principle, we could combine all the forces that bind the vesicle to the substrate in an effective potential and then transform it into an adhesion strength. However, due to thermal fluctuation this potential is very complex and would make computation much more demanding [1].

From the macroscopic point of view, adhesion is much simpler, as we only have to know the value of adhesion strength, which can be measured in the lab. Adhesion strength or so-called contact potential is a macroscopic result of the forces which origin in the van der Waals interaction between the molecules of both bodies as well as in electrostatic forces and molecule bridging, where individual molecules locally interact with each others. The minimal model for computation would be to add the adhesion energy to the existing energy functional. We assume that the adhesion energy is proportional to the contact area $A_c$

$$F_a = -\Gamma A_c$$

where $\Gamma$ is the adhesion strength (contact potential). With this model we introduced the reduced adhesion strength

$$\gamma = \frac{\Gamma A}{4\pi K}$$

Depending on the value of $\gamma$, we can speak of weak and strong adhesion regimes.

**Weak adhesion** is of order $\gamma \approx 1$. It turns out that a threshold value $\gamma_{\text{min}} > 0$ is required for adhesion to occur. If the adhesion strength is too weak, the gain in adhesion energy is too small to compensate for the cost of bending energy in deforming the shape of vesicle. Therefore the vesicle would not adhere to the substrate. For adhesion strength $\gamma > \gamma_{\text{min}}$ adhesion occurs and the contact area $A_c$ increases with increasing $\Gamma$. The exact value of the threshold depends on the reduced volume.

**Strong adhesion** usually occurs when $\gamma \gg 1$. This is the regime, where the bending energy becomes irrelevant and the shape is determined by desire to maximize the adhesion area. The optimal shape in this case is a spherical cap with an effective contact angle $\Psi_{\text{eff}}$ determined by the geometrical constraints of fixed vesicle area and volume (see Fig. 5).

Using *Surface Evolver*, we have calculated weak adhesion of vesicles in two dimensional space (Fig. 6). These figures serve as an illustration of a proposed three dimensional shape, which is much more demanding to calculate. One can observe that in the weak regime vesicles tend to maintain their original shape as bending to accommodate to a flat surface.
would cost too much energy. However, at larger adhesion constant the vesicles become flatter and the adhesion surface is maximized.

Fig. 7 shows a phase diagram of adhering vesicles. It can be seen that for vesicles of large reduced volumes the adhesion threshold is rather high, because vesicle cannot be deformed substantially due to high volume-surface ratio. As volume decreases the adhesion threshold decreases as well. The minimum is reached at $v \approx 0.5$ which is the volume of erythrocytes. When the reduced volume approaches 0, the adhesion threshold again increases. Vesicles with small reduced volume cannot accommodate to the flat surface, because they would form very sharp edges, which cost a lot of bending energy.
3.1 Adhesion of rigid particles to a membrane

Now that we understand the basic model of adhesion, we can extend it to the more complex geometries. In cell biology, vesicles are used for transport between cells or cell organelles. This kind of transport is passive and is driven by generic physical interactions, such as sufficiently strong adhesion between transported particles and the membrane. In this case, the particles adhere to a membrane and as the membrane is not rigid it can envelop the particle and thereby deform on a scale that is large compared to its thickness. The passage of particle into the cell and subsequent fission of the membrane is known as endocytosis (see Fig. 8) and is a crucial process in cell development. The well-known and studied examples of endocytosis include the passage of viruses from their host cells as well as certain gene transfection systems. All these biological examples are complemented by physically oriented experiments on the adsorption of micrometer-sized beads onto model lipid bilayers [6]. For these non-flat bilayers, it is unfortunately difficult to extract detailed information about the membrane shape close to contact, where the bending contribution of the membrane becomes more important. Therefore, it is desirable to have a better theoretical understanding of how physical parameters like bending stiffness, lateral tension, or adhesion strength control the shape of the particle-membrane complex and under which circumstances complete wrapping ensues.

Using an adapted curvature model described above, the shape of a complex formed when a particle adheres to a membrane has been studied [6, 7]. In order to simplify the calculations, wrapping was analyzed using a spherical colloid of fixed radius adsorbed on a deformable surface. However, in this case the area of the membrane is not fixed, but it changes according to the degree of wrapping. The model includes three terms, the first being the bending energy already present in previous chapters, the second one being the surface tension energy and the third one is adhesion energy:

\[
F = \int \left[ \frac{\kappa}{2} (2H)^2 + \sigma \right] dA - \Gamma A_c,
\]

where \( \sigma \) is the lateral tension. The characteristic length \( \lambda \) is defined by

\[
\lambda = \sqrt{\frac{\kappa}{\sigma}}
\]

typically of the order of a few 10 of nanometers. Deformations on a length scale smaller than \( \lambda \) are controlled by bending energy, whereas tension is predominant on larger scales [7].

The bending and tension energies of the adhesion part can easily be calculated, however energies of the free part of the membrane are more demanding to evaluate. The solution was obtained by solving the Euler-Lagrange equations numerically [7]. As we learned in the previous chapter, the adhesion does not occur if its strength is too low. In study [7] it was
shown that the transition from free to partially wrapped state is continuous and occurs at the threshold value of $\gamma_c = 2\kappa/a^2$ which surprisingly does not depend on tension. On the other hand, the partially wrapped and fully wrapped state are separated by an energy barrier and so this transition is discontinuous. The barrier predominantly stems from tension and is rather high. Therefore, it cannot be overcome by thermal fluctuations.

Furthermore, it was also discovered that the energy of the free section of membrane vanishes in the limit of full wrapping [7]. The reason for this behavior is that as the neck contracts, the membrane back-bending occurs on a length scale which is much smaller than $\lambda$ and is thus entirely dominated by the bending energy. In that case the membrane will assume the shape of a catenoid, which has a zero mean curvature and thus does not cost any bending energy. However, this theory breaks down when the membrane is no longer treated as an idealized surface. Another result of the wrapping scenario is also that colloid wrapping is extremely sensitive to the size of the particle. As long as tension is low enough, large particles can be wrapped much more easily than small ones. Fig. 9 shows a phase diagram of the three possible results of adhesion of a colloid to a vesicle.

![Figure 9: Phase diagram of colloid-vesicle complex. The white, light gray, and dark gray regions correspond to unbound, partially wrapped, and enveloped states, respectively [6].](image)

The Golgi apparatus is an organelle found in typical eukaryotic cells. Its primary function is to process and package macromolecules synthesised by the cell. The Golgi apparatus is composed of membrane-bound sacs known as cisternae (see Fig. 10). Surrounding the main cisternae are a number of spherical vesicles which have budded off from the cisternae. Vesicles from the endoplasmic reticulum fuse with the so called cis-Golgi network (the inner part of the organelle) and subsequently progress through the stack to the trans-Golgi network (the most outer part), where they are packaged and sent to the required destination [8]. The transport of the vesicles through the apparatus is driven by the adhesion process described above.

### 3.2 Vesicle-vesicle adhesion and rouleau formation

Another important aspect of adhering membranes is vesicle-vesicle adhesion. The analysis of adhesion of two vesicles can be extended to describe aggregation of vesicles and thus the formation of rouleaux. Despite the fact that formation of rouleaux has been known for over a century, it still represents an important research topic. The reason for the interest is that the rouleau formation represents one of the basic problems of cell biology and is used to study the membrane-membrane interaction. On the other hand, erythrocyte aggregation is interesting
because it can significantly influence blood viscosity. For example, some hemorheological
abnormalities which occur in patients suffering from diabetes mellitus can be related to
increased rouleau formation [10].

We proceed in a similar way as in the previous chapters. The basic model for calculation
is the refined curvature model

\[ F = F_{\kappa,1} + F_{ADE,1} + F_{\kappa,2} + F_{ADE,2} - \Gamma A_e, \]  \hspace{1cm} (17)

which includes the elastic energies of both vesicles and the adhesion energy. The equilibrium
shapes of this model can be found by one of the methods mentioned above.

Due to time consuming calculations this topic has not been studied very extensively. It
has been believed that axisymmetric aggregates are the states with lowest energy. However,
recent studies have shown [5] that these states are stable only at certain values of model
parameters. The study [5] concentrated on vesicles of reduced volume \( v = 0.6 \) and area
difference \( \Delta a = 1.04 \). A vesicle corresponding to these values is reminiscent on the discocyte
shape of normal human erythrocyte. The results have shown that if the dimensionless
adhesion strength \( \gamma \) is small but still larger than the threshold value for adhesion, the contact
zone between the two vesicles is planar and circular. In this case both vesicle adopt the same
shape and the doublet is axisymmetric. However, if the adhesion strength is increased, the
stable doublet consist of identical vesicles joined at a sigmoidal, S-shaped contact zone. This
dooublet is nonaxisymmetric (Fig. 11).

The external appearance of both doublets is quite different. In the sigmoidal doublet
vesicles do not sit directly on top of each other but are displaced alongside the contact
zone. Furthermore, the outer surface of the doublet is much less concave. With increasing
adhesion strength the displacement of vesicles decreases and the outer surface becomes more
convex. We get a similar behavior as in adhesion of vesicles to a rigid surface. The bending
energy becomes irrelevant and the contact area is maximized thus making vesicles adopt
the shape of spherical caps. For \( \gamma \to \infty \) there are two obvious solutions for a doublet, the
first one being a plan-convex spherical cap and the second a perfectly spherical doublet with
nonplanar contact. The latter was suggested for the first time 25 years ago [11]. However,
this shape only exists for reduced volumes \( v \leq 4/3^{3/2} \approx 0.77 \).
Figure 11: The flat-contact doublet ($v = 0.6$, $\Delta a = 1.04$, $\gamma = 3$) (left) is axysymmetric, where the symmetry axis is perpendicular to the contact zone and the sigmoid-contact doublet ($v = 0.6$, $\Delta a = 1.04$, $\gamma = 6$) (right) [5].

For the given parameters ($v = 0.6$ and $\Delta a = 1.04$) it was shown that vesicles are free for $\gamma \lesssim 0.3$. The transition from flat-contact to sigmoid-contact occurs at $\gamma \approx 4.1$. If the energy model is modified such that it does not include the area difference energy ($\Delta a$ is not constrained) the results are similar, but the sigmoid-contact is much more pronounced. In the latter case the vesicles are free for $\gamma \lesssim 0.4$ and sigmoid contact doublets are stable for $\gamma \gtrsim 3.5$. A few typical doublets for both cases are presented in Fig. 12.

Figure 12: Cross-section of free vesicles and doublets with $v = 0.6$ and $\Delta a = 1.04$ (left) and unconstrained $\Delta a$ (right) [5].

Experimental research on vesicle adhesion supports the recent theoretical results. The adhesion strength for erythrocytes in plasma and in dextran ranges from $1 \mu J/m^2$ to $10 \mu J/m^2$. Together with erythrocyte surface area $A \approx 140 \mu m^2$ and bending constant of membrane $\kappa \approx 10^{-19} J$ this gives a reduced adhesion strength $\gamma$ of a few $10^3$ [5]. This value is far beyond the flat-contact/sigmoid-contact transition described above. The vastly predominant doublet shape reported is indeed of the sigmoid-contact shape (Fig. 13). Furthermore, the comparison of shapes in Fig. 11 and experimental results suggest that, upon adhesion, some relative stretching of monolayers within a membrane does take place.

Figure 13: Sigmoid contact of erythrocyte doublet in dextran [5].

The sigmoid contact has not been observed only in doublets but also in multicellular
aggregates. The morphology of any two vesicles in these rouleaux is reminiscent of the sigmoid-contact doublet (Fig. 14) which suggests that the sigmoid contact is not limited to doublets.

Figure 14: External appearance of rouleau. The zig-zag arrangement of cells suggests a sigmoid-contact between them [11].

4 Conclusions

Lipid vesicles are a simple model of the complex biological structures. Despite the fact that the model is very simple and consists of only two free parameters, it can be used to predict a very broad range of shapes and structures found in real life. The structures, phenomena, and models discussed in this paper describe some of the basic processes of life, which can be used for further biological studies in cell biology as well as for understanding development of the different diseases and their treatment.

References