Budding and fission of a multiphase vesicle

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Abstract. We present a model of bi-phasic vesicles in the limit of large surface tension. In this regime, the vesicle is completely stretched and well described by two spherical caps with a fold, which concentrates the membrane stress. The conservation laws and geometric constraints restrict the space of possible shapes to a pair of solutions labeled by a parameter  \( \sigma \) given by line tension/pressure. For a given value of  \( \sigma \), the two solutions differ by the length of the interface between domains. For a critical value,  \( \sigma_c \), the two vesicle shapes become identical and no connected solution exists above this critical value. This model sheds new light on two proposed mechanisms (osmotic shocks and molecule absorption) to explain the budding and the fission in recent experiments.

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Introduction

The cell membrane is a bilayer made out of a mixture of lipid species. The membrane can be either the outer boundary of the cell or an interface separating different compartments inside the cell. This soft structure has many biological properties and also realizes intracellular traffic: a membrane vesicle buds from one compartment, travels through the cytosol and fuses with another compartment. Despite the fluidity of the lipid bilayer, the cellular membrane presents a lateral inhomogeneity due the formation of dynamical microdomains, called rafts [1]. These microdomains have been shown to be rich in cholesterol and sphingolipid [2]. In vivo, the rafts have not been directly observed but their size has been estimated to be between 20 and 700 nm [3]. A central question in membrane biology and biophysics is to understand how this spatial organization is used by the cell, in particular to favor interactions with proteins. Due to their size and specific composition, it has been argued that rafts play a role in protein docking, signaling, intracellular traffic [4] or virus budding [3].

Recently, a model system of Giant Unilamellar Vesicles (GUV) including sphingomyelin-cholesterol domains was developed [5]. These domains, which are supposed to reproduce the raft composition, are the result of a phase separation of the lipid species [6]. They are more structured than the surrounding classical liquid bilayer but remain in a liquid state. For this reason, they are called “liquid-ordered” domains whereas the classical membrane is called “liquid-disordered” domain. A large number of studies have focused on the thermodynamics of the liquid-ordered phases, in particular the effect of temperature or composition on domain formation [6,7]. Multi-phase vesicles are elegant and efficient tools to study the mechanical properties of microdomains. They can be used to understand how rafts bud and make daughter vesicles for intracellular traffic, but also how detergent addition can isolate rafts from the cell membrane. Recent experiments have shown that liquid-ordered domains can be separated from the initial vesicle by using tubular deformations [8], osmotic shocks [9,10] or absorption of external molecules like proteins or detergents [11,12]. Here, we develop a macroscopic theory for the last two situations. Our model describes the budding preceding the fission where the liquid-ordered domain lifts up from the liquid-disordered vesicle.

Budding and fission have already attracted many theoretical works for vesicles made by a mixture of lipids, segregated into two phases or not [13–19]. The models vary depending on the physical interactions involved but they are all based on the minimization of the bilayer energy. For a vesicle with one phase, the budding is induced by molecules whose shape is different from the main lipids of the membrane: if the molecules prefer a curved bilayer, they will favor the formation of a bud. These models rest on a continuous approach and are well adapted to describe the absorption of external molecules [20,21] or a mixture of lipids [13,22]. Even when the concentration of molecules is strongly inhomogeneous, this approach does not describe a true phase separation. For a bi-phasic vesicle, a line tension exists between the two phases, trying to reduce the interface length, and favoring budding [23]. Previous studies have mainly focused on two cases: a constant...
surface with a volume free to adjust \[24, 15\] and a constant surface with a fixed volume \[15, 25\]. In both cases, the shape of the vesicle is controlled by the geometric constraints and by the invagination length \(\zeta\), the ratio between the bending rigidity and the line tension. The main results of these numerical studies where elasticity plays the dominant role is the preferred budding and ejection of large domains as the line tension increases.

Here we focus our attention on multi-phase stretched vesicles, a situation often encountered in experiments, and not considered in the previous treatments \[15\]. In this case, osmotic-pressure effects dominate and stretch the bilayer. The volume can change but not at negligible cost. The vesicles can then be described by two spherical caps with an elastic junction. Our model is also suitable for a vesicle with a fixed volume, as long as the two-spherical-cap description remains valid. The variational procedure with surface constraints allows to find two solutions for any ratio \(\dot{\sigma} = \text{line tension/pressure}\) less than a critical value \(\dot{\sigma}_c\). The stable solution is the one observed experimentally. An osmotic shock increases the control parameter \(\dot{\sigma}\) and induces a budding, which may lead the system to a complete fission if \(\dot{\sigma}\) reaches the critical value. The case of detergents is slightly different since it requires an additive energy for molecular absorption in the membrane. As a large number of different detergents can be used to achieve the budding \[12\], we restrict on a common characteristic: the conical shape. Therefore, when detergent molecules are added in the membrane, they locally deform the bilayer. According to Leibler’s model \[20\], the deformation due to these molecules is taken into account via an elastic contribution simultaneously proportional to the average curvature and the molecule concentration. Stretched bilayers favor homogeneous concentration of molecules except at the interface between domains. We show that chemical inhomogeneities contribute to an effective line tension, not necessarily positive. Depending on the concentration, this effective line tension can induce a budding and a possible fission into two independent vesicles.

This paper is organized as follows. The next section is devoted to a detailed description of the model and defines the energies involved in the physics of the vesicle, such as the stretching of the bilayer, the bending of the vesicle or the absorption of molecules. Then, we explain how we can describe analytically the shape of stretched multi-domains vesicles below a critical parameter. Above this critical parameter, we predict a topological bifurcation. This explains the fission induced by a change of osmotic pressure. The last section extends this treatment to the absorption of molecules.

**Membrane description**

**Inhomogeneous lipid bilayer**

We consider an inhomogeneous vesicle made of two lipid phases: a “liquid-ordered” phase \((L_o)\) and a “liquid-disordered” one \((L_d)\). Both phases are in the liquid state but the \((L_o)\) domain is more structured than the \((L_d)\) phase for the following reasons: there exist specific interactions between molecules \[26\] and/or a possible optimization of biphilic space packing \[27\]. Steady morphologies and their out-of-plane deformations are commonly described by the Canham and Helfrich’s model with energy for each phase \(i\) given by

\[
F_i^n = \int_S \left[ 2\kappa_i H^2 + \kappa_G^{(i)} K + \Sigma_i \right] dS; \tag{1}
\]

\(H\) and \(K\) are, respectively, the mean and Gaussian curvature. The elastic bending rigidity \(\kappa_s\) and Gaussian rigidity \(\kappa_G^{(i)}\) are expected to be higher in the \(L_o\) phase. Typical values can be found for example in \[28\]: \(\kappa_{L_o} \simeq 20k_B T\) and \(\kappa_{L_d} \simeq 80k_B T\). Values of Gaussian moduli are notoriously more difficult to measure but a recent study mentions values of order \(\kappa_G^{(i)} = -0.83\kappa_s\) \[29\]. Although \(F_i^n\) is a surface integral, the Gaussian contribution to the energy is indeed a contour integral calculated at the interface between the two domains, according to the Gauze-Bonnet theorem. The most general Canham and Helfrich’s model also includes a spontaneous curvature, allowing a difference between the two leaflets. This difference can induce a difference of area between the two leaflets, which is not described by the spontaneous curvature \[30, 31\] but can induce a budding and a possible fission. In general, for multi-phases vesicles, the lipid bilayer is symmetric, with no spontaneous curvature \[32\]. Introducing the spontaneous curvature does not change the results of this paper so, for simplicity, we assume a symmetric bilayer. A difference of area between the two leaflets induces a lateral constraint; as the lifetime of the stretched vesicle we are considering here is larger than the flip-flops rate of the lipids (minutes compared to seconds), we assume that these constraints are relaxed. Then, we do not consider explicitly the difference of area here. Moreover, the description of molecule absorption treated in this paper can be extended to the case of a difference of area.

The last contribution in equation (1) is related to the possible extension of the membrane. For a stretched vesicle, this contribution is large compared to the elastic energy. Moreover, stretching the surface of the vesicle for more than 3% forms pores in the bilayer \[28\], not observed in the fission experiments. Therefore, the membrane surface can be considered as constant. This is taken into account by introducing the Lagrange multiplier \(\Sigma_i\).

The total energy of the two-domain vesicle includes the energy (Eq. (1)) of each phase plus two coupling terms. First, a sharp interface of vanishing thickness exists between the \(L_o\) and \(L_d\) phases. Any increase of its length requires an energy proportional to a line tension \(\sigma\). Second, the vesicle membrane is lightly permeable to the water but not to the ions or large molecules present in the surrounding water. This induces an osmotic pressure \(P\). The energy of the coupling terms is

\[
F_c = \sigma \int_C dl - \int_V PdV. \tag{2}
\]

The osmotic pressure normally varies with the enclosed volume. For a tense membrane, it is possible to assume