The forces that shape caveolae

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1 Introduction

Caveolae are Ω-shaped invaginations of the plasma membrane, found in many types of cells[1]. Caveolae are enriched in cholesterol, and have a membrane composition similar to that of lipid rafts[2]. In addition, caveolae show a high concentration of the protein caveolin, a hairpin-structured membrane protein possessing a hydrophobic domain (32 amino acids (AA)), flanked by two hydrophilic terminii (N-terminal: 101 AA, C-terminal: 43 AA)[3]. Both terminii of caveolin extend from the cytosolic side of the membrane, conferring a strong asymmetrical structure to the caveolar domain. The goal of the present chapter is to give an overview of the possible physical effects that can stem from both these characteristics of caveolae, namely the composition difference with the rest of the plasma membrane (“raft-aspect”) and their patent asymmetry (“hairy-aspect”).

Caveolae were first observed more than 50 years ago, but many of their properties and functions still remain unknown. Caveolae formation seems to require the presence of the protein caveolin[4] and is very dependent of the cholesterol level in the cell[5]. We also have evidence that caveolin is coupled to other types of membrane deformation (e.g. tubular structures in endothelial cells[6]). These are strong indications that at least some biological functions of caveolae rely heavily upon their biophysical properties. Plasma membranes typically resist bending, and the formation of membrane invaginations requires the action of mechanical forces on the membrane. Even though caveolae are very complex biochemical objects, they are bound to obey the laws of physics. We must therefore understand the origin of the forces at play in the formation of the invaginations if we are to understand how and why caveolae form. As we make progress towards this we may gain important insights into the biological functions of caveolae. Indeed, since caveolae are inherently coupled to the mechanical state of the plasma membrane, one may envision that the cell has taken advantage of this coupling, and may use caveolae as mechano-sensors or mechano-regulators for the plasma membrane. Before discussing these possibilities in the last section of this chapter (Section 6) we must first review some of the physical concepts behind the formation and structure of membrane domains and how this relates to the physical properties of membrane proteins.
The description we present relies on coarse-grained physical models where the molecular structure of the membrane and the proteins is only taken into account in an approximate way. This is justified by the fact that caveolae (of size $\sim 100\text{nm}$) are much larger than the size of the individual caveolin proteins and of the thickness of the plasma membrane ($\sim 5\text{nm}$). This physical description is based on the well-known properties of fluid bilayer membranes, described in Section 2. Following this, two different points of view are taken to describe the formation and invagination of caveolae. In Section 3, caveolae are regarded as membrane domains chemically immiscible in the plasma membrane. This neglects effects associated directly with the details of the domain composition. It is assumed there that the membrane phase separation into domains doesn’t depend on the mechanical properties of the membrane, although the domain shape might. This description somewhat overlook the importance of the protein caveolin in the formation of caveolae. In an attempt to get nearer to the complexity of biological membrane, theoretical physicists have studied the behaviour of membrane inclusions, and in particular how protein aggregation is coupled to membrane deformation and vice-versa. These models are briefly overviewed in Section 4, and applied to the particular case of caveolin aggregation in caveolar membranes in Section 5, by taking some account of the protein structure. The end of the chapter includes a discussion of what such a description can say about the “life” of caveolae at the plasma membrane of cells. Finally we speculate on further possible biological functions of caveolae.

2 Physical modeling of lipid membranes

Mathematical models of deformable, fluid membranes have been available for many years [7, 8], and have been successfully compared with experimental results, both on artificial[9] and biological[10] membranes. At the most fundamental level these theories rely on the single basic principle underlying statistical mechanics: that the probability of observing a given membrane deformation depends on the energy change involved in making this deformation[11]. The higher the energy, the less likely the deformation. Statistical mechanics tells us that the probability $p_i$ of an event $i$ is related to its energy\(^1\) $F_i$ according to

$$p_i \sim \exp \left[ -\frac{F_i}{k_B T_m} \right]$$

(1)

This probability compares the deformation energy $F_i$ to some energy source in the system. In passive systems, the only energy source comes from the thermal fluctuations, of energy $k_B T$ where $k_B$ is the Boltzmann constant, and $T$ is the temperature (in Kelvin). Biological systems are called active, because chemical energy, coming from e.g. ATP hydrolysis, can be harnessed by specific enzymes (molecular motors) to perform mechanical work. The cell membrane is generally the site of many active processes including, e.g. cytoskeleton polymerization and ion pumping. One may adopt the approach that these active processes provide an effective “membrane” temperature $T_m > T[13]$ and it is this that appears in Eq.(1).

\(^1\)This energy is written $F$ to remind us that it is a free energy and therefore includes changes in entropy, as well as internal and chemical energies[12]. Reactions that reduce the entropy of the system are disfavored in the same way as are those that involve a spontaneous increase in the energy by, e.g. disrupting chemical bonds. Strictly speaking Eq.(1) only holds for (sub)systems that are at equilibrium but this can often be a reasonable approximation for, e.g. small patches of membrane that can move and relax quickly, even though it may be inappropriate for the cell as a whole.
In practice, much information can be obtained by the study of membrane deformations that minimize the membrane energy (those having the higher probability to occur). One contribution to the membrane energy can arise from any change in the area of the cell, which must act against the tension in the membrane. This is reminiscent of the work required to deform a child’s balloon by, e.g. pinching a small patch of its surface between your fingers. As with any interface a lipid membrane bears a surface tension (denoted $\gamma$ throughout), which is the energy cost per unit area associated with decreasing the membrane area. However, while the surface tension observed at a water-air interface is of order $10^{-1} \text{J/m}^2$, and typically dominates any other type of deformation energy, the surface tension of lipid bilayers can be extremely low ($10^{-8} \text{J/m}^2$ in very floppy artificial systems, and $\sim 10^{-5} \text{J/m}^2$ for the plasma membrane). As the surface tension is low, other modes of deformation can also play an important role. One such is the energy associated with bending the membrane. A symmetrical bilayer membrane wants to be flat, so that both monolayers have the same structure. Bending the membrane one way or the other breaks this symmetry, and costs an energy which varies quadratically with the membrane curvature (deformation) $C$. This is fundamentally analogous to the fact that the energy of an ideal spring varies with the square of its extension, known as Hooke’s law and is ultimately the reason why any flexible material that is bent wants to spring back into its original shape. If the membrane is asymmetrical, and cell membranes are indeed rather asymmetrical, it may prefer a non-zero curvature. This means that the membrane energy might be minimized by, and the membrane therefore most happy with, a non-zero curvature. This curvature is called the spontaneous curvature $C_0$. In this case, the deformation energy is again quadratic, but now in the difference between the membrane’s (local) curvature and its spontaneous curvature. This can also be identified with a version of Hooke’s law. While the membrane tension tells us how much the energy increases when the membrane area is increased, the energy increase caused by a deviation from the preferred membrane curvature is controlled by the “bending rigidity”, conventionally denoted $\kappa$. Adding the curvature energy to the energy of membrane tension, the total energy of a patch of membrane of area $S$, with a curvature $C$ is

$$F = \gamma S + \frac{1}{2} \kappa S (C - C_0)^2$$  \hspace{1cm} (2)$$

Typical value for the bending rigidity of biomembranes\cite{10} is $\kappa^2 = 20k_BT$.

In this chapter, we will be mostly concerned by the flask-shaped membrane deformations mimicking the caveolae Fig.1. For simplicity, we will assimilate the invagination to a spherical cap of constant curvature. In practice, there exist a membrane neck connecting the concave central cap to the flat surrounding membrane. Specialized proteins are likely to be present near the caveolae neck\cite{14}, and we don’t include it in the present models. From Eq.(2), the energy of a spherical membrane (with no spontaneous curvature), is $F_{\text{sphere}} = (\gamma S + 8\pi \kappa)$. The energy of large patches is dominated by membrane tension, and the energy of small patches by membrane rigidity. We can see right away that this has strong consequences for the stability of membrane invaginations in general, and of caveolae in particular. Indeed, small invagination all have the same energy ($\sim 8\pi \kappa$), which is dominated by the bending energy of the membrane. Large invaginations on the other hand, have an energy increasing with their size ($\sim \gamma S$), and are much less likely to be observed. The cross over size between small and large invagination in the physical sense corresponds to an area $S \sim 8\pi \kappa / \gamma$. Choosing a bending rigidity $\kappa \sim 20k_BT$ and a surface tension $\gamma \sim 10^{-5} \text{J/m}^2$, the crossover size corresponds to a sphere of radius $R \sim 120 \text{nm}$. The fact that this scale is close to the typical size

\footnote{It is convenient to measure energies in units of the thermal energy scale which, at room temperature is, $k_BT = 4 \times 10^{-21} \text{J}$. Thus 1kJ/mol = 0.4$k_BT$.}
scale of the invaginations is very encouraging for our physical approach. It indicates that even such
simple physical arguments can reveal a competition between different physical energies (and hence
forces) that could give rise to invaginations with roughly the observed size.

![Figure 1: Sketch of a spherical membrane cap. The membrane curvature $C$ is roughly constant over the
deformed area $S$ which has radius of curvature (the radius of the circle passing through the cap) $1/C$, as
shown. Inset: the bilayer structure of a lipid membrane](image)

Of course biological membranes have complexity that is not reflected in the seminal elastic model of
Eq.(2). In particular the complex lipid composition (up to 25 different lipid species), the inclusion
of a host of membrane proteins ($\sim 30\%$ of the whole genome), and the support of the membrane
cytoskeleton. The question of how to incorporate the two former features will be the subject of most
of what follows. The cytoskeleton provides a visco-elastic scaffold to the cell, and is able to exert
direct forces to the membrane. Cytoskeletal anchoring of the plasma membrane is crucial to the
membrane’s mechanical behaviour. The breaking of some anchoring sites upon cell deformation and
membrane extension is a major component of the energy cost of such deformations. In particular,
cytoskeleton anchoring can account for up to 75 \% of the measured tension of cell membranes[15].
Here we adopt the philosophy that the cytoskeleton acts to maintain the plasma membrane under
tension, but does not exert direct forces to pull flask-shaped invaginations from the membrane. In
fact we will find that a consistent physical explanation of invagination can be constructed without
the cytoskeleton playing any direct role in the formation of caveolae.

3 Caveolae as invaginated lipid rafts

Caveolae are one example of lipid domains in the cell membrane[2]. These domains and other lipid
“rafts” are characterized by a high concentration of cholesterol and saturated lipids. Although many
controversies exist regarding the size and lifetime of lipid rafts, the morphology of caveolae seems
much better defined. One likely reason for this is the stabilization of these membrane domains by
the protein caveolin, and in particular the fact that they are invaginated. In this section, we present
some general physical arguments concerning the behaviour of membrane domains in a flexible, fluid
lipid bilayer[16], without discussing the origin of their formation. Here, we assume that the phase
separation is driven by the chemical incompatibility between the raft and the non-raft phase,
regardless of the mechanical state of the membrane. In Physical terms, chemical incompatibility
can be accounted for by an energy cost of creating an interface between the two phases. Since the
membrane is in two dimension, the interface between the two phases is a line, and the immiscibility
parameter is a “line tension”, which we call $\sigma$. For a given domain size, the line energy is the
smallest when the interface is smallest, meaning that we can expect domains to be circular\(^3\) with a radius \(R = \sqrt{S/\pi}\) \((S\) is the domain area).

Figure 2: Invagination of a membrane domain (in red) due to chemical incompatibility. (a) A flat domain has a large line energy, due to a large interface with the surrounding membrane. (b) An invaginated domain as a small interface, but is accompanied with a bending energy. (c) Comparison of the energy of a flat domain (in red) and of an invaginated domain (in black). The invagination is favored for domains larger than a critical size, estimated to be of order 100 nm.

Fig.2 shows how the chemical incompatibility alone can have a strong influence of the domain shape. Indeed, a flat domain (Fig.2.a) has a large interface with its surrounding, costing a line energy of order \(\sigma R\). If the domain is large, this can be quite a big energy, and may cause the domain to bud off the membrane in an attempt to reduce the size of the interface to the membrane neck connecting the bud to the rest of the membrane. On the other hand, an invaginated domain costs the energy of bending the membrane into a spherical shape. The bending energy of a sphere is proportional to the bending rigidity of the membrane \(\kappa\), and it has the remarkable feature that it does not depend on the size of the sphere for a symmetrical domain (it is equal to \(8\pi\kappa\)). Since the line energy increases with the domain size, and the bending energy does not, there is a critical domain size for which we can expect the domain to spontaneously invaginate[16] (Fig.2.c). The domain size at which this occurs is of order \(R \sim 4\kappa/\sigma\). The actual value of the physical parameters \(\kappa\) and \(\sigma\) vary from membrane to membrane, but we have however a good idea of the order of magnitude of such parameters. The bending rigidity of biological membrane is typically of order \(\kappa \sim 20k_B T\). The line tension arises from unfavorable contacts at the molecular scale (the size of a lipid molecule, or the thickness of the bilayer). It is estimated to be of order \(\sigma \sim k_B T/nm\), although its actual value is very sensitive to the nature of the two phases in contact (in raft, it is a contact between liquid order and liquid disordered lipid phases). Using those numbers, the critical size for domain invagination is \(R = 80nm\) (corresponding to a spherical bud of radius 40nm). Caveolae are precisely in this range of size, which is a good indication that the physical phenomenon of membrane bending energy and raft line energy play a crucial role in caveolae formation and stability.

One possible picture of the formation of caveolae is the following[16] (see Fig.2). Imagine a given amount of caveolin, cholesterol, and raft-forming lipids (in particular sphingolipids), dispersed in the cell membrane. With time, these various components diffuse in the membrane and find each

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\(^3\)Unless the line tension is very small in which case the rafts are very small and their shape can fluctuate significantly. This signifies that they are only weakly phase separating from the non-raft membrane and are close to dissolving back into it.
other, forming growing membrane domains. Although the rate of this phase separation might be quite slow\cite{17}, domains should eventually grow to a large size if they are not perturbed by other dynamical phenomenon at the cell membrane\cite{4}, and even more so if the presence of caveolin promotes the phase separation. When domains grow beyond the critical size discussed above (Fig.2), There are at their lowest energy, and therefore most stable, when invaginated rather than flat.

Such a scenario is still qualitatively valid if the membrane supports a tension and the domain has a spontaneous curvature. In this case, however, the critical budding size depends upon the membrane tension. Indeed, work against membrane tension has to be performed to invaginate a domain, and this stabilizes the flat shape. As a result, this simple theory applied to caveolae would predict that the size of the invagination increases with membrane tension. However, caveolae have very similar sizes across several cell types, that can, presumably, bear different membrane tensions. As we will see in Section 5, this indicates that the structure of the protein caveolin might play a crucial role in controlling the size of the invaginated domains.

\section{4 Membrane Inclusions}

Membrane proteins have hydrophobic regions inserted within the lipid bilayer, and this insertion may perturb the bilayer structure. For example, a mismatch in thickness between the hydrophobic core of the protein and that of the bilayer has an associated energy cost. It implies either that some hydrophobic residues are left unshielded from contact with water, or that the membrane (or the protein) changes its thickness to obtain a good match\cite{18}. The consequence of hydrophobic mismatch may be protein clustering, as shown in Fig.3 a-b. Clustering may occur even in the absence of direct (specific) interaction between the proteins, as the result of an effective attraction mediated by the membrane. As shown in Fig.3 a-b, the membrane order is locally perturbed in the vicinity of the inclusion (the figure shows a local stretching of the lipid tails, to accommodate the hydrophobic thickness of the membrane). Bringing two inclusions together reduces the membrane area that needs to be perturbed, hence reduces the energy of membrane deformation. The result of this is an effective force that brings the proteins together.

An important phenomenon in the context of caveolae and their asymmetric membranes is when protein clustering is coupled to a change of membrane morphology. This can be expected for very asymmetric proteins, or for peripheral proteins that mostly extend on one side of the membrane. Such asymmetric membrane proteins can be thought of as imprinting a local spontaneous curvature (the $C_0$ term in Eq.(2)) to the neighboring membrane (Fig.3.c). The membrane is locally curved near the protein, which again leads to a frustration of the bilayer order. As it is the case for hydrophobic mismatch, some of the frustration can be released if the proteins aggregate. In this case however, the concentration of a large number of protein over a limited membrane area leads to a morphological change of the membrane, which adopts the preferred curvature of the proteins (Fig.3.d). As will be discussed in Section 5, this phenomenon, called “curvature instability” in the physics literature\cite{19}, is likely to play an important part in the formation of caveolae. The caveolin proteins found in caveolae are a very good candidate for such large morphological changes for two reasons. On the one hand, both their hydrophobic termini face the cytoplasm, which makes their

\footnote{Membrane recycling, endocytosis, and exocytosis might perturb domain growth, and may be invoked to explain the small size of the non-caveolae rafts observed in-vivo, see \cite{17}}
interaction with the neighboring membrane very asymmetric. A similar situation can to some extent be reproduced in artificial systems, by mixing bilayer forming lipids with hydrophilic polymers (typically polyethylene glycol) with a hydrophobic anchor attached. Lipid membranes with grafted, but mobile polymer chain, can be obtained that way, and it has been shown theoretically[20], and observed experimentally[21], that such membrane can exhibit a phase separation. Another reason to expect membrane deformation near caveolin proteins is the fact that they are known to form homo-oligomers of about 15 proteins. This is due to specific biochemical interactions between caveolin via a short section of the N-terminus cytoplasmic domain, quite close to the transmembrane domain[3]. As is shown in Section 5, this oligomerization may have a lot to do with the success of caveolin in aggregating and promoting membrane invagination. By concentrating the caveolin, oligomerization also concentrates the effect of their membrane asymmetry over a small membrane area, creating a large asymmetric pressure.

5 Caveolae as a thermodynamic phase separation of membrane proteins

Simple mixtures of two or more material components can reside in a variety of states or “phases”. The simplest of these is the mixed state when all components are evenly mixed throughout the system. However, if molecules of one component have a sufficiently large mutual attraction for one-another, or equivalently a sufficiently large repulsion from the remainder, they can “phase sep-
arate”. There can then be large regions that are rich in this component suspended in a background which contains relatively little of it. It is this effect that causes oil to de-mix from water at room temperature but it is a far more generic effect than is often realized. It is now clear that there are components in the plasma membranes of cells that phase separate, e.g. into caveolae. This is a slightly unusual phenomenon in that the phase separated domains are typically only 100nm across, rather than macroscopic in size, but the principle is the same.

There is now good evidence that caveolin proteins form homo-oligomers[3] containing approximately 15 molecules. While this is not a necessary feature for the generation of bending forces, which require only a molecular asymmetry between the two sides of the membrane, it may act to amplify those forces by increasing the density of interacting cytoplasmic domains, see Fig. 4.

Figure 4: Sketch indicating the origin of the forces that act to bend the membrane near an asymmetric membrane protein (left), or homo-oligomer thereof (center), with domains extending on one (cytoplasmic) side only. The cytoplasmic domains may be entirely disordered, resembling a random coil (as shown), or may contain some folded structure(s) forced into dense contact within the oligomer. In any case these domains exert forces on the membrane. Even disordered coils have their configurational entropy restricted by the presence of the membrane. This restriction is large for a planar membrane but is reduced if the interface bends away from the coils, leaving more room in which to fluctuate. This means that the proteins exert forces that give rise to bending moments (as shown, right).

It has been suggested[22] that the mechanism by which caveolin homo-oligomers form is reminiscent of micellisation on a membrane. Usual spherical surfactant micelles are formed by the aggregation of amphiphilic molecules which experience a mutual attraction between their hydrophobic tails[8]. Such micellar aggregates do not grow indefinitely because of the packing and stretching constraints of their tails. The same is true of caveolin. It has been determined that there is an attractive interaction between N-terminal segments of caveolin[3] and, as the aggregate grows, more and more of the caveolins enjoy such contacts. Eventually the repulsive forces between the greatly confined cytoplasmic domains is enough just to balance the force of attraction experienced by the next caveolin molecule that seeks to join the aggregate and, at this point, the optimal size has been reached. For caveolin oligomers this size appears to be ~ 15 molecules. Given the size of the cytoplasmic domains this indicates that the N-terminal attractive domains probably give rise to a substantial attraction, perhaps of the order of $10k_B T$[22]. This, in turn, results in substantial repulsive forces between the cytoplasmic domains in the oligomers which acts to ‘amplify’ the bending forces indicated in Fig.4.

In the same way that there exists a critical micelle concentration (cmc) in surfactant systems there is a similar concentration at which caveolin oligomers will start to form. For simplicity we denote this the cmc. Above this concentration there will be a few single caveolin molecules on the membrane at concentration equal to that of the cmc, with the remainder forming as many oligomers (micelles) as are required to incorporate all the caveolin. Given that caveolins experience a substantial attraction their cmc is probably so low that oligomers will always form at physiological
concentrations. However, there is another scale of self assembly which also, in its way, involves a concentration that resembles a cmc. This is a critical budding concentration (cbc) which lies above the cmc. Below the cbc all oligomers exist on a roughly flat membrane while above it the flat membrane supports oligomers at, or very close to the cbc, while the remainder of the oligomers form buds that each have an area fraction of oligomers $\phi^* > \phi_{cbc}$. As more oligomers are added to the membrane so more, but similar, buds are formed. We are able to establish the cbc by comparing the free energy of a membrane bearing buds to that which does not. In what follows we will implicitly assume that we are above the cbc and hence buds form.

A free inclusion, such as is sketched in Fig.4 (left & center panels), is one which isn’t anchored to any external structure such as the cytoskeleton. In this case it is further possible to prove that there can be no net overall force acting to move the membrane up or down, nor torques which tilt it left or right. This is a direct consequence of Newton’s third law: The membrane cannot experience a force (or torque) without another body or structure experiencing one that is equal and opposite. If there is no such structure there can be no such forces. This leaves the bending moment sketched in Fig.4 (right panel) as the dominant mechanism for local membrane deformation [23]. The inclusion pushes down on the membrane with its ‘arms’ and pulls up with its ‘body’ but isn’t connected to any other structure.

We propose to investigate how an asymmetric inclusion, such as the protein caveolin or an oligomer thereof, can generate a local curvature in the membrane and how this curved membrane can then provide an environment preferred by other identical curvature-sensitive caveolin molecules. While this approach has the advantage of providing a formal method for calculating the size of a caveolae bud directly from physical arguments it suffers from the limitation that several parameters are known only to within an order of magnitude. Its utility should therefore be understood in the following terms:
(i) It represents a check on whether invaginations of 100nm diameter might possibly be driven by the physical process that we describe. In particular, since we find that this is indeed plausible, it gives a mechanism for the formation of caveolae that explicitly does not involve cytoskeletal forces playing any significant role.
(ii) Such a model is then able to predict how the stability and size of the invaginations will vary with the control parameters, e.g. surface tension, membrane rigidity and the tendency of any inclusions to curve the membrane. We are then able to compare these predictions with experiments involving several mutant caveolins.

While an exact calculation of these bending forces is difficult there is one calculation that can at least give us an indication of the magnitude of these forces. It involves treating the cytoplasmic tails as random coils. In this case there are well established theories from the theory of polymers[24, 25] that allow these bending forces to be calculated exactly[20, 26, 23]. The basic principle is that the chains gain more configurational entropy if they live next to a convex surface than a flat one. This is formally equivalent to saying that each caveolin oligomer imprints a local spontaneous curvature $C_0$ on the membrane, which is of order $C_0 \sim f_0/\kappa$, where $f_0$ is a characteristic force exerted on the membrane by the cytoplasmic tails (‘arms’) of the caveolin oligomer, of order $10pN$[22].

One final physical ‘ingredient’ is required in order to complete our model for the formation of caveolae. It is necessary for us to include the effect of mixing $n$ caveolin oligomers, each of areas
s, and hence area fraction $\phi = ns/S$, on the surface of a caveolae. Simplistically we view the surface of the caveolae as being made up of two components, caveolin oligomers (with fraction $\phi$) surrounded by the rest of the caveolar membrane (with fraction $1 - \phi$). The energy of the membrane deformation is given by Eq.(2), where the spontaneous curvature of the bud increases with the density of caveolin, and is equal to $C_0\phi$. Assuming that these components have no interactions of longer range than $a$ then this is a classical two-component ideal mixture. The free energy of mixing of this fluid is well known$^{[22, 12]}$, contains similar contributions from the oligomer and non-oligomer membrane patches and has the natural feature that it is very costly to remove all of either component. Indeed the energy required to do this actually increases without bound as $\phi$ or $1 - \phi \to 0$. For inclusions that interact with one-another the interaction energy includes a contribution that scales like the density of oligomer-oligomer interactions ($\sim \phi^2$).

The formation of buds is controlled by a variety of physical processes that we have introduced above. These can be combined into a single equation for the free energy per caveolin oligomer on a caveolae of radius $R$ containing oligomers with area fraction $\phi$. This merely encodes mathematically all of the physical contributions to the energy discussed earlier in this section. These are:

(a) The existence of a spontaneous curvature, indicating that energy is gained when the bud, with curvature $1/R$ bends in response to the bending moments of the oligomers. This is the only effect which drives bending of the membrane.

(b) The energy cost of bending a membrane away from its preferred shape. If there are no oligomers the membrane would like to remain flat. When it forms a bud it needn’t always have a curvature exactly equal to its spontaneous curvature and this, similarly, costs bending energy.

(c) The energy cost of drawing the area of the bud away from the remainder of the cell membrane into the bud. This involves doing work against the membrane tension $\gamma$.

(d) Finally, the repulsion between caveolin oligomers and the mixing energy must be included.

The origin of all of these contributions is sketched in Fig.5.

The preferred caveolae state can be obtained by identifying the minimum of this free energy which, in turn, yields predictions for the preferred caveolae bulb radius $R^*$ and oligomer density $\phi^*$, see Fig.6. The invagination radius decreases with increasing caveolin density, so that the curvature of the bud approaches the spontaneous curvature of the caveolar membrane: $C^* = C_0\phi^*$.

These results follow from the same model for the bud as a nearly-closed sphere attached to the membrane by a small neck as was introduced in the previous section. We find that a physical description of caveolae can yield predictions for such observable quantities as the caveolae radius $R$ that are in good agreement with observations. In this model we find that the primary reason why caveolae form is because of the coupling between membrane curvature and protein density: proteins accumulate to a curved membrane (bud), stabilizing its shape and thereby attracting more proteins. If the formation of a curved membrane is too energetically costly because of a high surface tension, the domains are destabilized$^{[22]}$.

Also we can again return to make contact with caveolae as lipid rafts. The consensus seems to be that these are the only rafts the identity and stability of which isn’t controversial. They are large, comparatively easy to observe and are very stable. We take this as evidence that the particular membrane curvature of caveolae stabilizes the phase separation into buds, a mechanism which is entirely consistent with the results of our physical analysis of their stability. Further evidence for a coupling between caveolae and membrane curvature can be found in the fact that an increase
Figure 5: Sketch indicating the origin of the contributions to the total free energy. (a) Energy is gained by bending the membrane in response to the bending moments exerted by the caveolin oligomers. (b) There is an energy cost when the membrane is bent away from its preferred shape: flat in the absence of caveolin and at precisely the preferred (spontaneous) curvature when they are present. (c) There is an energy cost when the area of the sphere is removed from the cell membrane against surface tension. (d) There is an energy cost associated with both mixing of and interactions between inclusions on the sphere surface. The latter increases with the area fraction of buds.

of the number of caveolae can enhance other types of membrane deformation, namely endothelial capillary tubule formation[6].

Finally, our theory provides a framework with which to seek to understand caveolae formation in mutant caveolin systems[27]. Mutants lacking the mutually attractive domain of the N-terminus are still able to drive membrane invagination but with a much larger size $R \approx 1 \mu m$. This is consistent with the fact that the force exerted by isolated proteins should be an order of magnitude smaller than the force exerted by oligomers which results in a 10-fold increase of the bud radius. Other mutants lacking the mutually attractive C-terminus also form larger buds. We would understand this as being due to a weaker oligomer-oligomer attraction, resulting in a lower density of caveolin in caveolae and therefore larger buds.

The origin of the striated texture observed on the surface of caveolae[1], which superficially resembles tree bark, is still not understood. It may be that such structures are due to the spatial organization of caveolin oligomers on the surface of these “gnarly buds”. If this is the case it may not be immediately obvious how circularly symmetric oligomers can organize themselves into asymmetric phases consisting of long, linear structures. However, it is now known that caveolin proteins also interact via the distal regions of their C-termini tails[3]. This attraction, together with a purely physical, membrane mediated, longer range repulsion that it is possible to estimate[23] could lead to a phase separation of the caveolin oligomers with caveolin dense regions (stripes)
Figure 6: **Main figure:** The variation of the preferred radius of the caveolar bulb $R$ with surface tension of the membrane. The size of the caveolae reduces with increasing tension for small tensions but at larger tensions reaches a preferred value that is rather insensitive to tension. The two sketches of the caveolar bulb indicate this with darker shading representing higher density of caveolin. **Inset:** The variation of the critical budding concentration of inclusions, expressed as the area fraction $\phi_{cbc}$, and the area fraction of oligomers on buds, $\phi^*$. For all but the smallest tensions our simple two-component model predicts that the caveolae should be almost entirely covered with caveolin oligomers, at a concentration $\phi^*/s$ that is always above that of the surrounding membrane between caveolae $\phi_{cbc}/s$.

coexisting with a caveolin-poor surface. Such phenomenon have been studied in physically similar systems [28] and a reasonable conclusion would be that such structures may occur naturally as a result of a balance between a short ranged (C-terminal) attraction and a longer ranged (membrane deformation mediated) repulsion.

6 Caveolae and membrane tension: mechano-sensitivity and mechano-regulation

So far, we have two possible mechanisms by which invaginated membrane domains may form at the plasma membrane. The raft model of Section 3 tells us that a phase separation in the membrane promotes membrane curvature because of chemical immiscibility, the driving force is the domain line tension. The curvature instability model of Section 5, takes the point of view that membrane curvature promotes the phase separation because of the aggregation of proteins into patches of preferred curvature, the driving force is the protein spontaneous curvature. In both cases, the tendency to membrane invagination has to overcome the membrane tension, which favors a smooth, flat membrane, and in both case, increasing the membrane tension may lead to the flattening of the invagination. Fig.7 shows the effect of membrane tension on the energy of flat and invaginated
domains for the raft model. At low tension, chemical incompatibility and the asymmetry of the membrane conspire to promote domain invagination, the budded state is the most stable state. At large tension, budding the domain cost too much energy, and invaginated domains flatten. Flat caveolae have indeed been reported in the literature[29], although they appear much less common than their invaginated counterparts. Fig.7 shows that an additional complexity arises from the fact that there is an energy barrier between the flat and budded states. This means that intermediate states (such as the hemispherical state - $\beta = 0.5$ of Fig.7) are very unfavorable. This fact has two very important physical consequences. On the one hand, this means that the transition from invaginated to flat domains does not occur continuously. Instead, the domains will abruptly snap open has the membrane tension is raised to a sufficient value. We argue below that interesting biological functions for caveolae could stem from this physical fact. Furthermore, we have seen in Eq.(1) that states of high energy are exponentially unlikely, which means that the passage of an barrier $\Delta F$ requires an appropriate thermal fluctuation. It is a rare event which occurs only after a time proportional to $e^{\Delta F/k_B T_m}$. This means that the response of caveolae to a mechanical perturbation is sensitive to the time scale over which this perturbation occurs.

![Energy plot](image)

**Figure 7:** Variation of the energy of a domain as a function of the domain shape for different membrane tension. The shape is characterized by a single parameter $\beta$, which vanishes for flat domain and is equal to unity for fully budded domain (corresponding to a closed sphere, a state that can be attained only if the domain endocytoses). The energy plot shows the domain energy (in $k_B T$ unit) for a domain of size $R = 100 nm$, of line energy $\sigma = k_B T/ nm$, and of bending rigidity $\kappa = 20 k_B T$. The energy is shown for three different membrane tension. Under small tension (Red), the domain tend to bud to minimize contact with the surrounding phase, and the minimum of energy is for $\beta = 1$. As the membrane tension increases (Green), the energy of the budded domain increases with respect to the energy of the flat domain. At even higher tension, the flat domain becomes the minimum of energy. Flattening the invagination requires however to go over an energy barrier ($\Delta F$). The transition over an energy barrier (Inset) is activate by thermal fluctuations, and requires a time that increases exponentially with the height of the barrier (see text). The higher the membrane tension, the lesser the time need to operate the flattening of the invagination.

A new putative function of caveolae at the plasma membrane emerges from these physical considerations, in addition to their supposed role in cell signalling and cholesterol transport (to cite
only a few). Specifically, this is a possible role in cell mechano-sensitivity and mechano-regulation. Although direct experimental evidence of such role is lacking at this time, Fig.7 provides a clear picture of the effect membrane tension should have on caveolae. The one remaining question at this stage is the level of membrane tension required to affect the caveolae morphology. We are able to relate this tension to relevant physical parameters of the caveolar membrane, such as the line tension, bending rigidity, and spontaneous curvature[22, 30]. The expected values for these parameters leads to the identification of a characteristic membrane tension that can be observed in cells. However, these parameters are not known with sufficient precision at this time for the theory to produce quantitatively precise predictions.

We may however investigate some biological consequences of the disruption of caveolae at high membrane tension. In that respect, the scenari presented in Section 5 and Section 3 are somewhat different. If the very formation of the caveolin raft is coupled with the membrane curvature, which is what the thermodynamic model of Section 5 predicts, increasing the membrane tension will lead, not only to the flattening of the invagination, but also to the dispersion of the caveolin aggregate. One can relate this phenomenon to a putative function of caveolae in cell signaling, which is to hold signaling component inactive until they are released and activated by an appropriate stimulus[31]. The thermodynamical model of Section 5 suggests that an increase of the mechanical tension of the cell membrane could provide such stimulus. If, on the other hand, the phase separation leading to caveolin aggregates is independent of the shape of the membrane, the caveolin rafts will remain regardless of the membrane mechanical tension. Their morphology will however change upon tension increase, as is described Fig.7.

The morphological changes of caveolae with the tension of the plasma membrane provides the basis for mechanical regulation at the cell membrane. It is known that the tension of a cell increases if it is mechanically perturbed[32], and that cells have developed regulatory mechanisms to accommodate mechanical perturbations[33, 32]. Caveolae might play a role in this mechanism, and many pathologies associated to caveolin seem to involve the mechanical behaviour of the cell. One can cite their involvement in various muscular diseases[34], and defects in vascular relaxation and contractility in mice deficient of caveolin-1[4]. There is an increase of the number of caveolae in Duchenne Muscular Dystrophy[35], and such an increase has also been observed in cells subjected to long lasting shear stress[36]. Furthermore, there exist evidence that caveolin can contribute significantly to cell cycle regulation[37], and cell entry into mitosis can be inhibited by artificially maintaining a high level of caveolin prior to mitosis. One can argue on the basis of physical arguments that the coexistence of flat and invaginated membrane domains regulates the cell membrane tension[30]. Fig.8 illustrates the mechanism a perturbation of the cell membrane area can be buffered by flattening and invaginations of domains. By this mechanism caveolin expression could regulate and buffer the membrane tension of cells by controlling the number of caveolae at the cell membrane. Along with many other factors, this could be one reason why caveolin is down-regulated prior to mitosis[37], as a mean to release membrane tension by releasing the membrane area stored in caveolae, thereby assisting cell division. This is consistent with the fact that caveolin-1 knockout mice show an increased rate of cellular proliferation[4].

Compensatory pathways exist for cells lacking caveolin that do not exhibit caveolae at their plasma membranes [4]. In order to further investigate the possible role of caveolae in tension regulation one potentially promising line of research might be to perturb (e.g. mechanically) cells that do have caveolae, and to observe the effect of that perturbation on caveolae before any alternative
Figure 8: Sketch of the possible involvement of caveolae in mechanical regulation at the cell membrane. (Left) A mechanical perturbation is said to be positive if membrane area is taken out, and negative if it is put in. In the absence of invaginations, such perturbation would influence the membrane tension (visualized as a spring of various length), increasing it for positive perturbation, and decreasing it for negative perturbation. The presence of membrane invaginations, for which caveolae are a good candidate, allow to buffer the change in membrane tension, as positive perturbations flatten the invagination (P1) prior to tension increase (P2), and negative perturbation leads to more invagination (N1), before it starts affecting the tension (N2). (Right) Variation of the membrane tension with perturbation in the absence of invagination (dashed line), and with invagination (solid line). The membrane reservoir in the invagination helps keep the tension unaltered.

regulatory mechanisms can have a significant effect.

7 Conclusion

Fluid membranes can be described using physical laws. The membrane has some tension which resists the formation of invaginations of finite area. The area of each invagination must be removed from the rest of the cell membrane under tension in order to form a bud. The membrane has a rigidity which parameterizes the stiffness of the membrane. Patches (rafts) on the membrane which have a distinct chemical composition also experience a line tension that acts at the interface of the patch with the rest of the membrane. This line tension acts to minimize the length of the contact line and tends to either make the domain circular or to form a bud, with a much reduced contact region near the neck of the bud. Finally, membrane inclusions such as caveolin that are asymmetrically distributed in the membrane can always be expected to want to make the membrane curve. We argue that the predominantly random coil of the N-terminal section of caveolin acts to bend the membrane away from it and hence favors the formation of endo, rather than exo, buds. These physical ingredients can be combined in simple theories to understand when and if caveolae-like invaginations should be stable and if so at what length scales. The characteristic scales that arise from comparing surface tension and line tension with rigidity both are in the 100nm range and thereby give an early hint that a balance between the various physical effects listed above could be expected to give rise to invaginations similar to caveolae. We describe how this can be analyzed in
more detail leading to a theory for the stability of the buds under variation of surface tension. We find that buds of a single characteristic size stabilized by line tension can act as mechanical tension regulators and how these could very effectively buffer the cell’s tension in the physiological range.

References


