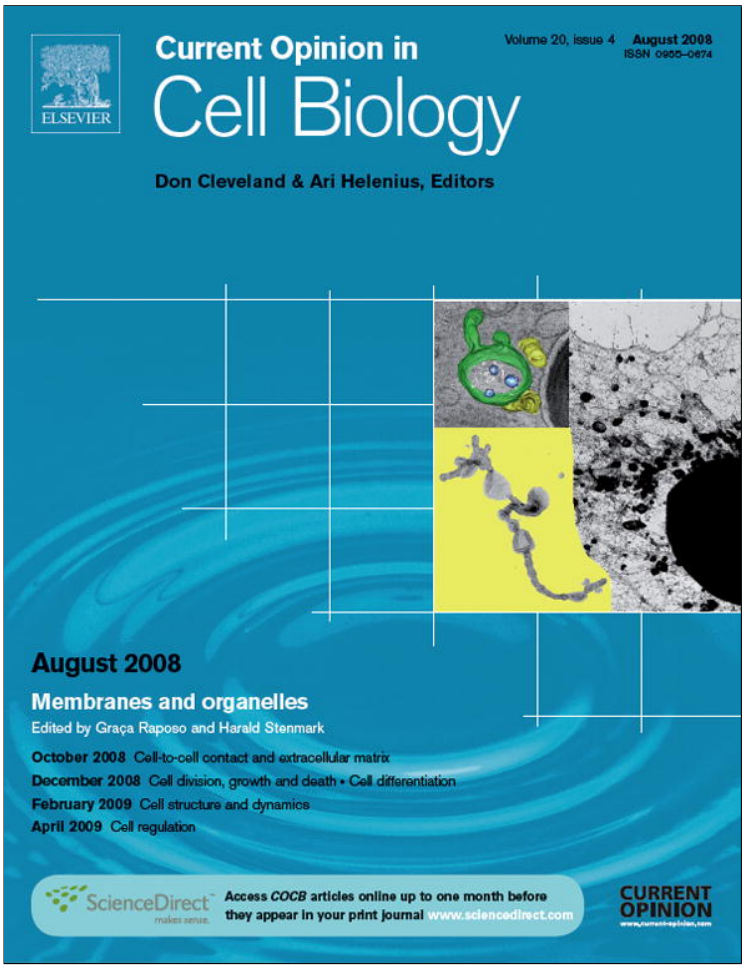

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Biophysical approaches to protein-induced membrane deformations in trafficking

Pierre Sens[1], Ludger Johannes[2] and Patricia Bassereau[3]

Membrane traffic requires membrane deformation to generate vesicles and tubules. Strong evidence suggests that assembly of curvature-active proteins can drive such membrane shape changes. Well-documented pathways often involve protein scaffolds, in particular coats (clathrin or COP). However, membrane curvature should, in principle, be influenced by any protein binding asymmetrically on a membrane; large membrane morphological changes could result from their aggregation. In the case of Shiga toxin or viral matrix proteins, tubules and buds appear to result from the cargo-driven formation of protein–lipid nanodomains, showing that collective protein behaviour is crucial in the process. We argue here that a combination of *in vitro* experiments on giant unilamellar vesicles and theoretical modelling based on statistical physics is ideally suited to tackle these collective effects.

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Introduction

Membrane deformation is essential in intracellular trafficking. When macromolecules are internalized in a cell, transported from one cell compartment to another or secreted from cells, they often have to bind to a membrane. These molecules concentrate in membrane patches that grow into buds or tubules and eventually detach by scission [1]. This budding event is frequently driven by the formation of complex 2D protein assemblies termed 'coats', such as clathrin, caveolae, COPI and COPII. These coats are found on several compartments, such as the plasma membrane, endosomes, *trans*-Golgi network, *cis*-Golgi and endoplasmic reticulum. Cargo is also thought to influence the formation of protein scaffolds such as clathrin [2,3•]. However, protein coats may not always be required for cargo internalization. At the plasma membrane, several examples of uptake processes have been described that are entirely clathrin and caveolae-independent [4••]. These modes of endocytosis are frequently used by viruses, toxins or bacteria [5,6], and also contribute to the uptake of cellular factors such as GPI-anchored proteins [4••]. We have recently reported that the binding of Shiga toxin, a bacterial toxin protein, to its cellular glycolipid receptor induces membrane deformation without needing contributions from other cell machinery [7••]. These results strongly suggest that cargo alone can sometimes trigger its own internalization by influencing membrane curvature. We will show other examples in this review of cargo-induced deformation. As the underlying mechanisms are very generic and may be involved in many aspects of cell traffic, it is crucial to propose a physical description of this phenomenon.

In recognition of its ubiquity, large efforts are underway to decipher the mechanisms leading to membrane deformation and budding. Most biological models of protein-induced membrane deformation focus on the molecular level and ask whether proteins of known structures can induce the deformation of membranes of given compositions. This approach overlooks a crucial aspect of protein-induced budding: *cooperativity* between protein molecules drives the formation of dense clusters and can generate strong membrane curvatures. In this review, we argue that a physical approach combined with experiments using model membranes [8] can address these issues in a manner that is complementary to classical cell biology, and we describe the generic mechanisms underlying the process of cargo-induced membrane deformation.

Protein-induced membrane deformations: biomimetic systems

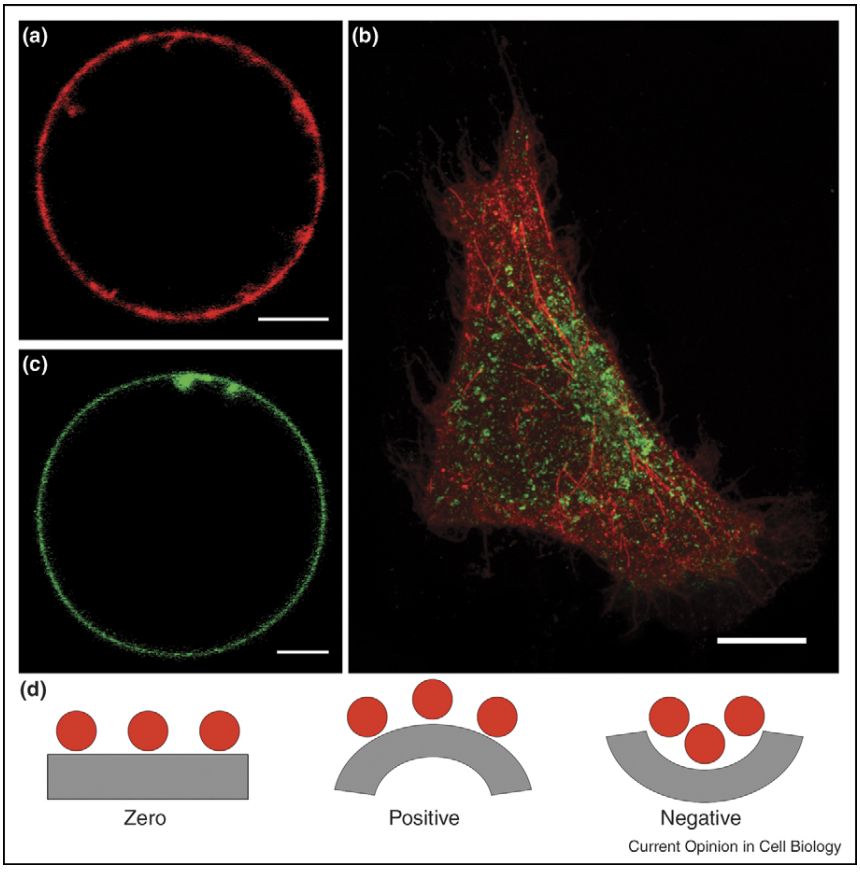
Physical parameters such as membrane tension or lateral membrane heterogeneity are expected to influence protein assemblies on cell membranes. These parameters, which are probably regulated in cells [9,10], can be controlled in biomimetic membrane systems. Reconstituting budding processes using liposomes with size, lipid composition and tension similar to cellular conditions is a very attractive approach to explore the mechanisms of protein-induced deformation. For this, giant unilamellar liposomes (also

called giant unilamellar vesicles (GUVs)) are good candidates (for an overview, see reference [11]). Their typical size of a few microns to 100 μm matches the range of organelle and cell dimensions. For three decades, physicists have used these model membranes to measure mechanical membrane properties, study global shape changes under diverse experimental conditions and test theoretical physical models. Stimulated by the raft hypothesis [12], GUVs containing lipid mixtures have been used to investigate domain formation. The complexity of GUV membranes in terms of lipid and protein composition has increased over the past years: asymmetric bilayers can be produced [13], and functional membrane proteins can be reconstituted [14,15].

GUVs have been used to study the mechanics of Shiga-toxin-induced membrane invaginations. This protein toxin can be efficiently internalized into cells in the absence of functional clathrin or caveolae [16,17]. Shiga toxin specifically binds to its cellular receptor – the glycosphingolipid globotriaosyl ceramide (Gb3) – on the plasma membrane. The interaction of the purified receptor-binding B-subunit of the toxin (termed STxB) with GUVs containing Gb3 leads to the formation of tubular invaginations [7••] (Figure 1a). These deformations mimic the invaginations observed *in vivo* when scission is inhibited by different means: energy depletion, inhibition of the scission factor dynamin or inhibition of actin polymerization [7••] (Figure 1b). The GUV experiments demonstrated that binding of STxB to its glycolipid receptor was sufficient to locally produce negative membrane curvature (Figure 1d). Shiga toxin appears to be able to induce the first step of its uptake into cells in a process that can be described as a cargo-induced membrane invagination reaction.

The budding of enveloped viruses is another interesting case where membrane deformation can be reproduced

Figure 1



Membrane deformations of GUVs and cells induced by proteins binding to their lipid ligand reconstituted in GUVs or in cell membranes. Proteins are in the bulk solution outside of the GUVs or of the cell. **(a)** B-subunit of the Shiga toxin (labelled in red) interacting with GUVs containing 5% Gb3 (from reference [7••]). **(b)** Formation of tubules in ATP-depleted HeLa cell incubated with STxB (red). The tubular structures do not co-localize with transferin receptors (green), classical markers for clathrin-dependent endocytosis (from reference [7••]). **(c)** Matrix proteins of VSV interacting with GUVs containing 10% negatively charged lipids (DOPS labelled in green) (from reference [19•]). **(d)** Definition of the membrane curvature sign. The red objects represent curvactants. Bars, 5 μm. Images: courtesy of Berland and Römer.

with GUVs. Matrix proteins (M proteins) are involved in the final budding steps where the virus acquires its membrane envelope. M proteins from vesicular stomatitis virus (VSV) have a positively charged N-terminal part and interact electrostatically with anionic lipids [18]. The incubation of VSV-M proteins with GUVs containing negatively charged lipids produces invaginations [19•] (Figure 1c). Noticeably, membrane curvature induced by VSV-M is negative both *in vivo* and *in vitro*. However, these invaginations are 1 order of magnitude longer than virus particles, which are 180 nm long, and their diameters are also larger (a few microns instead of 75 nm for the virus bud). These differences most probably are a result of constraints that *in vivo* are imposed by the concomitant presence of nucleocapsids. Invaginations on GUVs have also been reported for M proteins from Newcastle disease virus [20•]. In this case, membrane deformation also occurred in the absence of charged lipids, although charged lipids enhanced the spontaneous detachment of buds. The M protein examples again show that protein–lipid interactions can induce membrane deformations with negative curvature.

Interestingly, when Shiga toxin or M proteins adsorb on membranes, they rapidly cluster and form protein domains. Aggregation on membranes is also observed with non-biological compounds, such as colloids [21,22] or polymers [23]. Several theoretical explanations have been proposed to account for this membrane-mediated clustering phenomenon (see next Section 'Protein aggregation and membrane deformation'). This aggregation step appears crucial for subsequent function. Indeed, if proteins were to remain homogeneously distributed on membranes, they could only induce global shape changes as a consequence of asymmetric curvature modulation of the external membrane leaflet [24]. Such an effect has been illustrated by the action of enzymes modifying selectively homogeneously distributed lipids in the exoplasmic leaflet of GUV membranes [25]. In cells, global deformations would not be expected to contribute to membrane trafficking. Indeed, such a process would require large numbers of proteins (proportional to the cell size) and would probably be prevented by regulatory mechanisms [9]. On the contrary, protein-dense domains can produce local deformations and require a lower number of proteins (proportional to the domain size). For instance, it was found that enzymatic lipid modification within segregated domains [26] leads to localized deformations which can sometimes grow into buds and detach from the membrane.

In the following section, we will discuss possible mechanisms that can account for the formation of membrane deformations, with a special focus on cargo-induced invaginations.

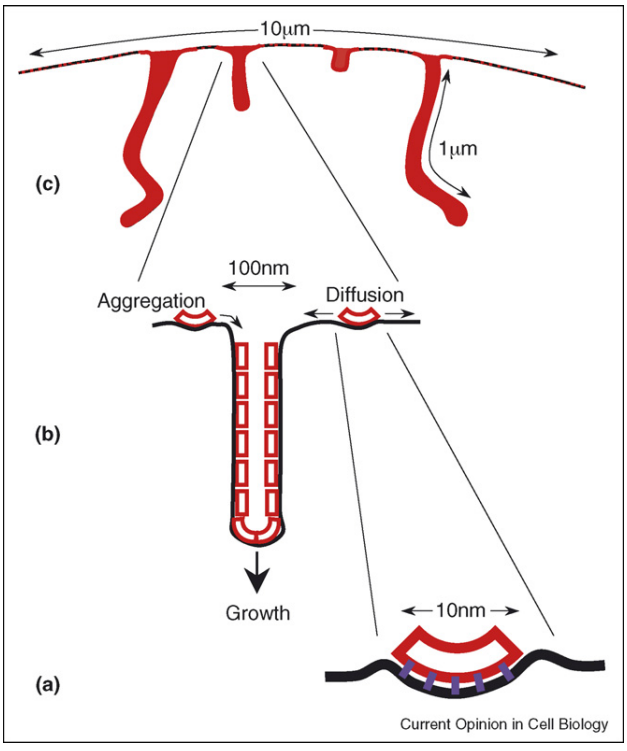
Modelling protein-induced membrane deformation

A complete picture of large-scale protein-induced membrane deformation needs to address at least three levels of physical description (Figure 2). At the molecular scale (1–10 nm), the properties of the lipids and the proteins and their interactions are crucial to understand how proteins can influence membrane composition and shape [27–30]. At the scale of a protein aggregate (0.1–1 μm), molecular interactions can be translated into physical couplings in terms of stresses or deformations. The optimal membrane shape can then be derived from the mechanical energy of deformation. At the scale of many aggregates (more than 1 μm—the whole cell), thermodynamics can be used to compute the kinetics of protein aggregation and the evolution of membrane morphology. The two larger scales do not crucially depend on molecular details, and obey general physical principles.

Membrane phase separation and protein aggregation

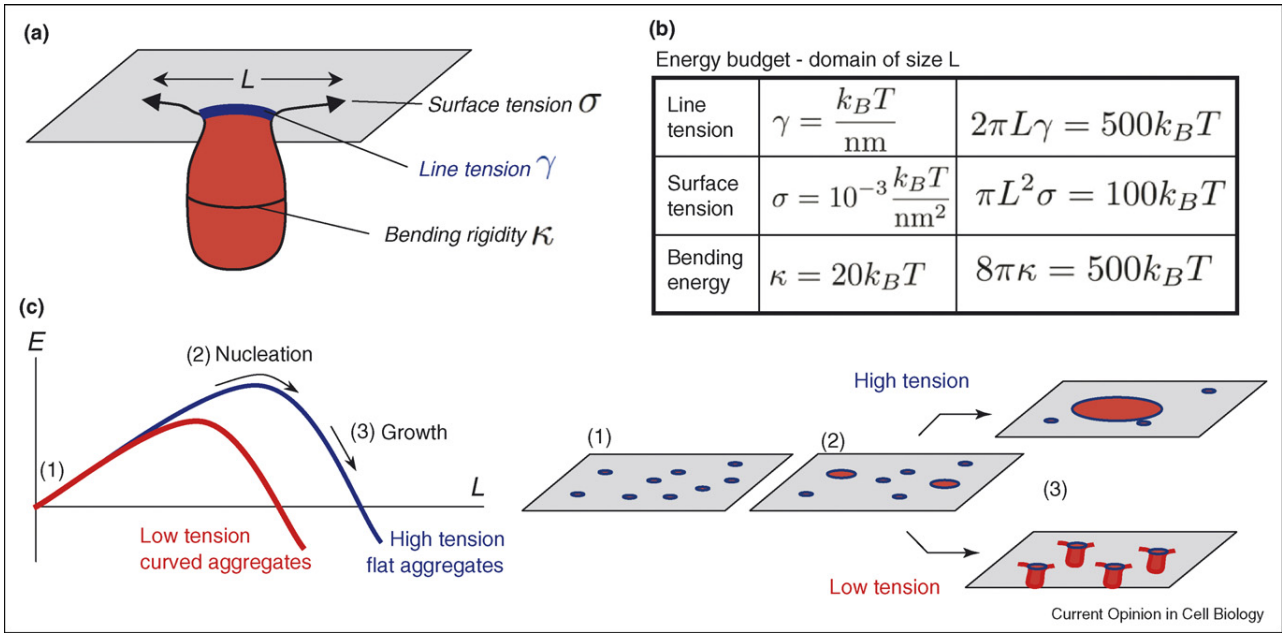
For membranes composed of a mixture of lipids, lowering the temperature can cause spontaneous phase separation [31]. Phase separation typically proceeds by the random

Figure 2



Levels of description of protein-induced deformations. **(a)** Membrane–protein interactions at the molecular level (~10 nm) couple to membrane deformation. **(b)** Invaginated protein clusters (with a curvature ~1/100 nm) are characterized by their energy of deformation, and their growth is controlled by protein diffusion. **(c)** At larger scales (~10 μm), thermodynamics controls cluster growth and membrane invagination.

Figure 3



Energy budget. **(a)** The energy of a protein aggregate involves an interfacial energy (line tension), and if the aggregate is curved, a bending energy and the energy of surface tension. **(b)** The energy budget of a spherical aggregate for typical values of the physical parameters. Energy values are given for L = 100 nm. For a spherical bud, the bending energy is independent of bud size. **(c)** The growth of protein aggregates is formally analogous to the diffusion in an energy landscape. The nucleation energy barrier controls the initial stage of protein segregation. Membrane tension controls the shape of the aggregate and influences its growth. Under high tension, protein clusters adopt a flat shape and grow to large lateral sizes.

nucleation of small membrane domains that grow by attracting more material of a given lipid phase. An energy barrier must be overcome to form a domain (Figure 3) and this nucleation energy determines the probability of domain formation from random fluctuations of the membrane composition. The typical energy scale for phase separation is the thermal energy k_BT where T is the temperature in Kelvin and k_B is the Boltzmann constant ($k_BT \sim 2.5$ kJ/mol at physiological temperature). The concentration of a given membrane component required for phase separation increases exponentially with the energy barrier, expressed in units of thermal energy. For comparison, the energy of ATP hydrolysis is of the order of 20 k_BT.

By favouring particular lipid compositions or ordering [19•,32,33], proteins can lower the domain nucleation energy and trigger the formation of lipid domains in otherwise homogenous membranes. The resulting domains are enriched in protein, as if proteins experience an effective, membrane-meditated, attraction.

Protein aggregation and membrane deformation

Protein-free lipid domains are generally flat and can reach large sizes (several micrometers, of the order of the system size) [34]. They sometimes show a slight curvature due to interfacial effects. Indeed, the domain's interface with the surrounding membrane costs an energy (the line tension, Figure 3), which is a significant part of the domain nucleation energy. Domain deformation can reduce the length of the domain interface and the resulting interfacial energy. In pure lipids, this effect is generally quite small and produces much smaller curvature than is observed for cellular transport intermediates (1/5 μm versus 1/100 nm) [35,36,37••,38].

Protein-induced phase separation is not necessarily coupled to membrane deformation. The addition of Shiga toxin triggers phase separation even when the membrane cannot undergo large-scale deformations (e.g. high tension, see below). When the membrane can be deformed, STxB aggregates into highly curved tubular domains, indicating a preference for high membrane curvature. In principle, asymmetrical binding of the protein to the membrane can explain this preferred or spontaneous curvature [28,29]. The spontaneous curvature is based on principles of symmetry and is not restricted to biomolecules. Polymers [35,39] or colloids [21] may also act as 'curvactants', deforming synthetic membranes into a variety of complex shapes. This phenomenon is qualitatively well understood. If one protein forces a given membrane curvature, it attracts more proteins that favour a similar curvature [40••]. This potentially long-ranged membrane-mediated interaction

between proteins might be an important factor in promoting the formation of protein domains [21,41–43].

Proteins-induced membrane invagination may show isotropic or anisotropic curvature (spherical or tubular shapes). The protein structure itself may lead to anisotropic spontaneous curvature. However, the optimal domain shape is not easily inferred from protein structure and is influenced by many factors. Proteins may create chiral order in the membrane (an ordering without mirror symmetry), which is frustrated in flat membranes and optimized in curved membranes, thus leading to membrane invagination or tubulation [44•]. Indeed, cargo-induced membrane tubules are often corkscrewed [7••], which is a likely signature of chiral effects. Cargo-induced budding probably results from a combination of these three effects (line tension, spontaneous curvature and chirality) and maybe contributions from other mechanisms. Structural modification of the proteins or alteration of the membrane composition will provide important insights into the molecular mechanisms responsible for membrane deformation [7••]. Quantitative analysis of the stresses involved in membrane deformation is another promising approach, and this goal can be achieved by varying the membrane tension.

Membrane tension

Membrane tension increases the energy required for deformation and must affect membrane budding [37••,42]. One can imagine two different responses to high membrane tension. If the driving force for protein aggregation mostly originates from the deformation of the membrane (spontaneous curvature effect [41]), protein aggregation will be inhibited by high tension [42]. If, as seems to be the case for Shiga [7••], other driving forces (line tension) bring the proteins together, high tension will prevent membrane deformation and constrain the protein aggregates to form flat clusters even if the proteins prefer a curved (i.e. tubular) shape [7••] (Figure 3). The effect of membrane tension on the shape of membrane domains is well documented theoretically [36,37••,42,44•], and membrane tension can be controlled in model membrane systems. Measuring the level of membrane tension needed to influence protein aggregation is a useful tool to probe the strength of the coupling between protein and membrane shape [42].

Conclusions and perspectives

Model membrane based reconstitution of protein-induced membrane deformations appears to be a very promising strategy that is complementary to *in vivo* cell biology approaches, to understand the underlying mechanisms.

As demonstrated by recent studies of cargo-induced membrane deformation, this approach allows the minimal requirements for membrane deformations to be identified and physical models can then be proposed. A range of biomimetic systems has been developed. In addition to submicron-size liposomes on which protein binding and deformation cannot be followed simultaneously, new procedures using membrane sheets with a negligible tension have recently been developed to detect membrane deformation induced by cytosolic proteins [45,46]. However, these geometries are quite different from those found in cells. GUVs have a great potential: their dimensions are cell-like and the kinetics of protein association and membrane deformation can be followed as in living cells. Physical parameters and membrane composition can be controlled and tuned, thus allowing a direct comparison with physical models. In the future, different types of protein assemblies (in particular. coat assemblies) producing deformations can be investigated. Furthermore, mechanical studies can be performed on GUVs, and mechanical characterization of the protein assemblies can contribute to a better description of the aggregation process.

Membrane curvature seems to be a crucial issue for protein binding (for instance, see references [27,47]). The diameter of membrane nanotubes pulled from GUVs can be precisely controlled [48] in the relevant range (10 to a few 100 nm), and have a length (typically 10 μm) perfectly suited for optical detection. In the coming years, they should provide a useful model of membrane geometry. In addition, the actin cytoskeleton, whose role has been well established in different endocytosis events [49], should be added to these model systems in the near future. With these experimental tools at hand, different classes of protein assemblies will be identified on the basis of complete physical characterizations at a mesoscopic scale. Eventually, the link between the molecular structure of the proteins and lipids and the membrane deformation that they induce will be clarified.

At the conceptual level, the most immediate challenge is to understand the kinetics of protein aggregation. Upon adsorption onto a membrane *in vitro*, some proteins drive the formation of thin and long tubules that resemble *in vivo* membrane carriers [7••], whereas others aggregate into big lumps with no obvious structure [19•,20•]. Understanding the difference between these two paths, which probably involves kinetics, will be a crucial step in the description of protein-induced membrane deformation.

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