LETTERS

Aggregation and vesiculation of membrane proteins by curvature-mediated interactions

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Membrane remodelling[1–5] plays an important role in cellular tasks such as endocytosis, vesiculation and protein sorting, and in the biogenesis of organelles such as the endoplasmic reticulum or the Golgi apparatus. It is well established that the remodelling process is aided by specialized proteins that can sense[4] as well as create[6] membrane curvature, and trigger tubulation[7–9] when added to synthetic liposomes. Because the energy needed for such large-scale changes in membrane geometry significantly exceeds the binding energy between individual proteins and between protein and membrane, cooperative action is essential. It has recently been suggested[10,11] that curvature-mediated attractive interactions could aid cooperation and complement the effects of specific binding events on membrane remodelling. But it is difficult to experimentally isolate curvature-mediated interactions from direct attractions between proteins. Moreover, approximate theories predict repulsion between isotropically curving proteins[12–15]. Here we use coarse-grained membrane simulations to show that curvature-inducing model proteins adsorbed on lipid bilayer membranes can experience attractive interactions that arise purely as a result of membrane curvature. We find that once a minimal local bending is realized, the effect robustly drives protein cluster formation and subsequent transformation into vesicles with radii that correlate with the local curvature imprint. Owing to its universal nature, curvature-mediated attraction can operate even between proteins lacking any specific interactions, such as newly synthesized and still immature membrane proteins in the endoplasmic reticulum.

Far from being a mere outer envelope, lipid bilayer membranes form the basis of many important cellular organelles, such as the endoplasmic reticulum, the Golgi apparatus, or the vesicular transport system. The biological function of these structures often depends on their highly intricate geometry, topology and dynamics, which are actively monitored by the cell. The necessary control is exercised, at least in part, by specialized membrane proteins. These must act cooperatively, as the following simple estimate of the energy requirements shows: at the continuum level, the elastic membrane behaviour is described by a local bending energy per unit area, $E = \frac{1}{2}\kappa(1/R_1 + 1/R_2)^2$, where R_1 and R_2 are the local curvature radii and κ is the bending modulus[16]. For typical phospholipid bilayers, $\kappa \approx 20 k_B T$, where $k_B T \approx 4.1 \times 10^{-21}\,\mathrm{J} \approx 0.6\,\mathrm{kcal\,mol^{-1}}$ is the thermal energy. Creating a spherical vesicle of radius R thus costs about $\frac{1}{2}\kappa(2/R)^2 \times 4\pi R^2 = 8\pi\kappa \approx 500 k_B T$, independent of its radius. This exceeds the typical interaction energy between proteins and also their binding strength to the bilayer by at least an order of magnitude[17].

This energy consideration and the very function of remodelling proteins suggest that specific binding might be complemented by a universal mode of interaction. Such a universal mode can arise because much of the free energy of binding associated with the adsorption of a membrane-curving protein onto a lipid bilayer is stored as elastic bending energy in the membrane. When two membrane-curving proteins approach one another, the bilayer deformations overlap long before any direct interaction occurs. The resulting change in stored bending energy is distance dependent, thus yielding a force. A recent simulation study explicitly posed the question of the cooperative interaction of many such domains[10], and it has been suggested that even without direct interactions membrane-curving proteins might cluster and subsequently tubulate in order to reduce the curvature energy[11]. As two such proteins could share the work needed to bend the membrane and thereby lower the stored elastic energy, one might intuitively expect that the resultant force between them is attractive. Yet existing experimental and theoretical work shows that the sign of the force is anything but obvious.

Experimental and theoretical approaches to quantification of curvature-mediated interactions have proven difficult and inconclusive. Aggregation of proteins[7–9] and colloids[18] has been observed, but the local geometry was not resolved and direct interactions could not be ruled out. Theoretical calculations require the membrane shape of lowest bending energy, but this calls for use of a fourth-order nonlinear partial differential (shape-)equation that can only be solved in very exceptional cases. Approximate linearized solutions for weakly perturbed membranes exist, and suggest that two proteins imposing isotropic curvatures repel[12–15] while attractions require anisotropic curvature imprints[19,20]; however, linearized solutions are not expected to remain valid for strong membrane deformations.

When both experiment and theory encounter difficulties, tailored computer simulations offer an alternative approach, with their unique ability to identify and separate individual contributions to the phenomenon or process of interest. A meaningful simulation of membrane vesiculation calls for model membranes extending in excess of 100 nm and simulation times of the order of milliseconds, which were until recently out of reach for conventional atomistic and many coarse-grained simulations. But these problems are overcome with our recently developed[21] coarse-grained model, which achieves efficient simulation owing to the elimination of explicit solvent molecules (see refs 22 and 23 for current reviews on coarse-grained membrane simulations in general, and ref. 24 for solvent-free models in particular). Like all coarse-grained approaches, the model eliminates atomistic detail and thus cannot be used to explore phenomena dependent on such detail. But on the length scales of tens to hundreds of nanometres relevant to our study, it faithfully reproduces all key properties of self-assembling fluid bilayers, in particular the bending elasticity[21,25]; it thus is a suitable tool for isolating and identifying curvature-mediated interactions between mutually non-interacting local membrane curvers.

Full technical details on the membrane model can be found in ref. 21 and the Supplementary Information. Briefly, it is built from model lipids approximated by three connected beads (see Fig. 1). Bilayer assembly ($\kappa \approx 12\, k_B T$) is triggered by effective tail attractions. The

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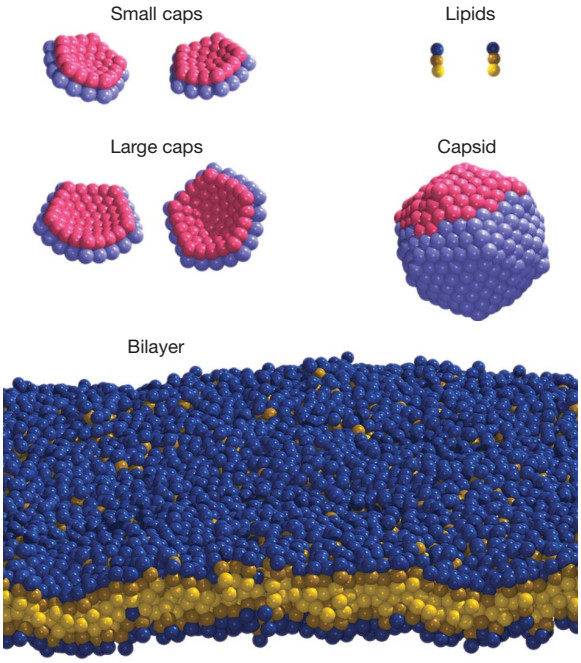

Figure 1 | Illustration of the individual entities used in the simulation. Three-bead lipids with one hydrophilic head-bead and two hydrophobic tail-beads form a flat fluid bilayer spanning the simulation box. Curved caps and full capsids are created from beads of the same size. Only the light blue ones attract the dark blue hydrophilic head-beads of the lipids. None of the beads of caps or capsids attract other caps or capsids.

bead diameter, σ, is set to about 1 nm, to yield an appropriate membrane thickness. The natural simulation timescale is $\tau \approx 15$ ns, based on lipid self-diffusion. The simplified curvature-inducing proteins are curved caps of two different sizes, corresponding to 10% and 16% of a sphere of radius 5.5σ. Their outer surface attracts the hydrophilic lipid beads, thus locally curving the membrane isotropically. The size and degree of deflection of these model particles are comparable to real proteins (see Supplementary Information for details). We also study complete spheres of radius 5σ with 75% of their surface rendered attractive to hydrophilic lipid beads, so we can probe both weak and strong curvature perturbations.

Placing 36 small caps onto a tensionless square membrane with a side length of $\sim 160\sigma \approx 160$ nm (46,080 lipids) results in only weak clustering for the entire $70{,}000\tau$ simulation time (see Supplementary Fig. 1), indicating that any mediated interaction is small compared to the thermal energy. In stark contrast, the large caps behave qualitatively differently (see Fig. 2 and Supplementary Video 1): after initial weak clustering with a protein–protein interaction energy of $\sim 1.3 k_B T$, most caps suddenly aggregate at $\sim 40{,}000\tau$ into a single, almost flat cluster that then rapidly vesiculates within the subsequent $30{,}000\tau \approx 0.5$ ms. Note that throughout this process, individual caps neither touch nor order in a crystalline-like fashion. When using yet larger caps, the aggregation proceeds more rapidly, the aggregates are denser, and the vesicle sizes are smaller because fewer proteins suffice to create them (see Supplementary Video 2). In cellular organelles, this curvature-mediated vesiculation mechanism has to compete against a residual bilayer tension that suppresses vesicle formation beyond a critical size.

The vesiculation pathway observed in our simulations differs fundamentally from scaffolding[3,6] schemes that require direct and specific protein contacts, such as clathrin-dependent endocytosis. Evidently, curvature-mediated interactions alone can induce aggregation and vesiculation (see Supplementary Information for a detailed discussion of the energetics). This effect has not been seen in approximate linearized continuum theories for isotropic membrane-curvers, possibly because the induced deformations are too strong to permit linearization (an approximation to which the sign of an interaction is known to be sensitive[26]). On the other hand, although the attractive interactions seen in our simulations weaken and ultimately vanish as the membrane curvature induced by protein adsorption diminishes, a crossover to the repulsive behaviour predicted by linearized continuum theories could not be identified with statistical significance. However, we note that in this regime other contributions can induce interactions (such as fluctuations[12], or depletion or tilt-mediated[27] forces) and compete with effects arising from the diminishing extent of membrane curvature. We also note that the interaction behaviour for small membrane deformation is only known for large separations, so it is not clear which forces one should expect for small deformations in our system.

Our final simulations use colloidal spheres that could represent viral capsids or nanoparticles. These spheres have a radius comparable to that characterizing the curved caps used before, and 75% of their surface is attractive to the hydrophilic lipid beads. Within the first $2{,}000\tau$ of placing 16 such capsids onto a tensionless square membrane of initial side-length 160σ (see Fig. 3), the membrane contracts to $\sim 140\sigma$ as it coats the attractive part of the colloidal spheres (Fig. 3a to Fig. 3b). After the initial contraction, clustering

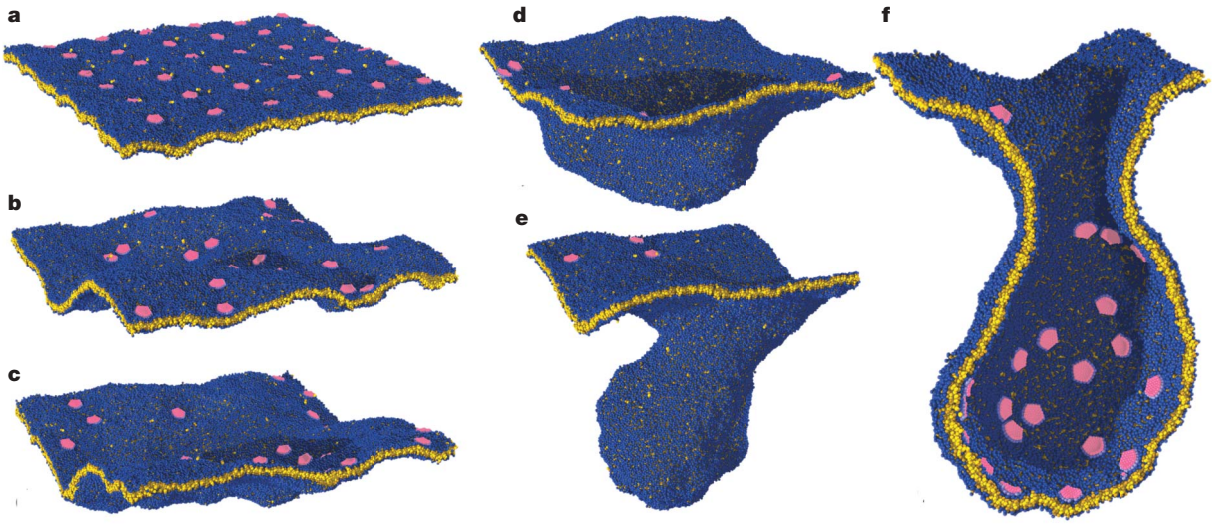

Figure 2 | Successive stages of a vesiculation event driven by 36 large caps on a membrane containing 46,080 lipids. The times of the simulation snapshots are: **a**, 0τ; **b**, $20{,}000\tau$; **c**, $40{,}000\tau$; **d**, $50{,}000\tau$; **e**, $60{,}000\tau$; and **f**, $70{,}000\tau$, the last corresponding to roughly 1 ms.

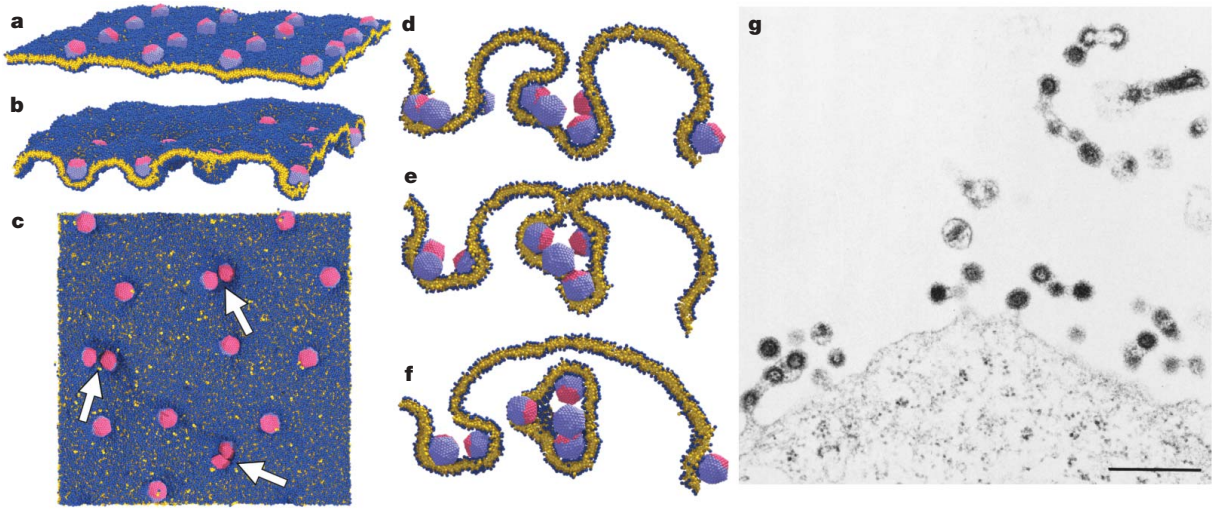

Figure 3 | Attraction and cooperative budding driven by 16 capsids on a membrane containing 40,960 lipids. a–f, A series of simulation snapshots. The times are: **a**, 0τ; **b**, 1,000τ; **c**, 7,000τ; **d**, 16,000τ; **e**, 17,000τ; and **f**, 18,000τ, the last corresponding to roughly 0.3 ms. The arrows in **c** point to formed capsid-pairs. The slices **d–f** indicate cooperative budding, a phenomenon also seen in the electron micrograph (**g**) of late domain mutated MPMV virions (scale bar, 500 nm; reprinted from ref. 28 with permission of authors and publisher; copyright 2003, The American Society for Microbiology).

sets in; it always starts with the formation of pairs (Fig. 3c), and is followed by subsequent tight vesiculation (Fig. 3d–f and Supplementary Video 3). The final multi-capsid structures closely resemble morphologies encountered in the cooperative budding of late domain mutated Mason-Pfizer monkey viruses (MPMV)[28], which lack individual budding activity (Fig. 3g).

To quantify the attraction that induces the spheres to form pairs, we placed two capsids on a membrane and fixed their separation d. As illustrated in Fig. 4, the constraining force needed to maintain this separation revealed a significant attraction, which is strongest around $d = 12\sigma$ and decays very weakly (for very short distances, capsids repel owing to direct contact). System size requirements and slow thermal shape fluctuations make it difficult to determine the interaction behaviour at large distances even for such strong deformers as these capsids, but we nevertheless obtain a total mutual binding energy of $\sim 10 k_B T \approx \kappa$. This value rules out aggregation due to fluctuation effects, and points instead to a true ground state curvature-mediated interaction.

The simulation snapshot in Fig. 4 illustrates that capsids significantly tilt towards each other. We also note the finding of a nonlinear geometry analysis that the net force between the capsids results from a competition between the force associated with the curvature along the direction joining the particles (which drives repulsion) and the force associated with the curvatures perpendicular to it (which drives attraction)[27,29]. Taken together, this information points towards a possible mechanism for capsid attraction: as the colloidal spheres approach each other they flatten the former curvature by tilting, thus enabling the attractive forces associated with the second curvature direction to take over. This effect may be supplemented by a slight 'peeling' of the membrane from the front and back of the capsids, as visible in Fig. 4. Although it is difficult to predict the outcome of this subtle balance analytically, our simulations clearly show that a sufficiently large curvature imprint will result in an overall attraction between membrane-adsorbed proteins. The universal nature of this effect renders it extremely robust, and suggests that cells take advantage of it. In fact, cellular membrane control might even require measures to prevent such omnipresent aggregation.

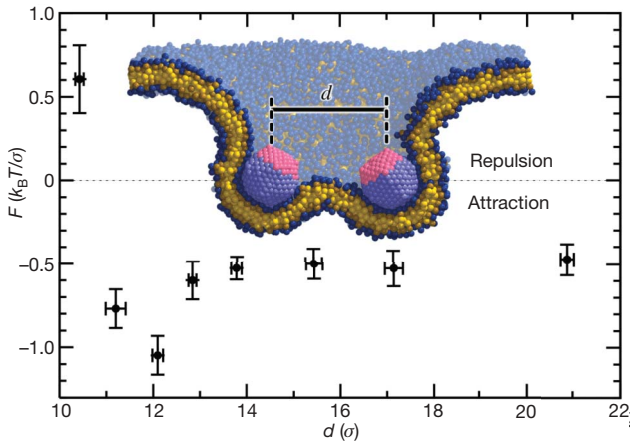

Figure 4 | Force versus distance for two capsids. Negative forces signify attraction. Vertical error bars, ±1 s.e.m.; horizontal error bars, ±1 s.d. Inset, a cross-sectional cut through the membrane profile for a separation of $d = 21\sigma$.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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