Theoretical study of vesicle shapes driven by coupling curved proteins and active cytoskeletal forces

Miha Fošnarič, Samo Penič, Aleš Iglič, Veronika Kralj-Iglič, Mitja Drab, and Nir Gov

Eukaryote cells have a flexible shape, which dynamically changes according to the function performed by the cell. One mechanism for deforming the cell membrane into the desired shape is through the expression of curved membrane proteins. Furthermore, these curved membrane proteins are often associated with the recruitment of the cytoskeleton, which then applies active forces that deform the membrane. This coupling between curvature and activity was previously explored theoretically in the linear limit of small deformations, and low dimensionality. Here we explore the unrestricted shapes of vesicles that contain active curved membrane proteins, in three-dimensions, using Monte-Carlo numerical simulations. The activity of the proteins is in the form of protrusive forces that push the membrane outward, as may arise from the cytoskeleton of the cell due to actin or microtubule polymerization occurring near the membrane. For proteins that have an isotropic convex shape, the additional protrusive force enhances their tendency to aggregate to flat pancake-shaped vesicles, where the curved proteins line the outer rim. This second transition is driven by the active forces, coupled to the spontaneous curvature, and the resulting configurations may shed light on the organization of the lamellipodia of adhered and motile cells.

Curved membrane proteins, for example membrane embedded proteins with non-zero intrinsic curvature, flexible nanodomains, or curved membrane-attached proteins, have been identified to play an important role in driving the formation of various membrane shapes. Coupling between non-homogeneous lateral distribution of membrane components and membrane shapes may be a general mechanism for the generation and stabilization of highly curved membrane structures, such as spherical buds, membrane necks, thin tubular or undulated membrane protrusions. In addition, it was found in many cellular processes that the curved proteins, or complexes containing the curved proteins, are able to recruit the cytoskeleton of the cell to produce additional protrusive forces, for example due to actin polymerization. Such protrusive forces are active, meaning that they consume energy (ATP) and maintain the system out of thermal equilibrium. The resulting steady-state configurations of the system may therefore differ from those in thermal equilibrium. Note that in cellular membranes such active forces can originate also from other sources, such as ion pumps.

Curved membrane proteins with a convex shape, such that they induce outwards bending of the membrane, that also recruit the cytoskeletal forces which push the membrane outward, can serve as efficient initiators of membrane protrusions. This mechanism was first suggested theoretically, and has since been found in experiments. This coupling of membrane curvature and recruitment of actin polymerization is therefore emerging as an efficient cellular mechanism for the production of actin-based protrusions. It also appears to be exploited by certain viruses during their budding from the infected cell. Previous studies of the coupling between curved membrane proteins and the cytoskeletal forces were mostly limited to the linear regime, and indicated that convex proteins can undergo phase separation and aggregation at lower concentrations (or higher temperatures) when the protrusive forces are present. We study here the membrane shapes and the aggregation properties of such systems using numerical simulations, which allow us to go beyond the linear deformations limit. We find that the presence of the active protrusive forces affects the phase-separation (budding) transition, as well as induces transitions into new classes of shapes that are not accessible in the equilibrium (passive) systems. The ability of active processes, associated with curved proteins, to lead to global shape transitions was previously found in numerical simulation, where activity was in the form of proteins with fluctuating spontaneous curvature.

1 Theoretical model

The energy of the membrane is expressed as the sum of contributions of membrane bending, direct interactions between membrane proteins and outward protrusive cytoskeletal forces,

$$W = W_b + W_d + W_F,$$  (1)

respectively.

For membrane bending energy the standard Helfrich expression is used,

$$W_b = \frac{K}{2} \int_A (C_1 + C_2 - C_0)^2 dA,$$  (2)

where the integral runs over the whole area $A$ of the membrane with bending stiffness $K$, $C_1$ and $C_2$ are principal curvatures and $C_0$ is the spontaneous curvature of the membrane. The proteins on the membrane are modeled as patches of the membrane with given spontaneous curvature $C_0$. On the patches occupied by the curved proteins we therefore set $C_0 = C_0$ and elsewhere we assume a symmetric membrane $C_0 = 0$.

For direct interactions between neighboring proteins we assume...
the step potential,
\[ W_d = -w \sum_{i<j} \mathcal{H}(r_0 - r_{ij}), \]
where \( w \) is a direct interaction constant, the sum runs over all protein-protein pairs, \( r_{ij} \) are their mutual in-plane distances, \( \mathcal{H}(r) \) is the Heaviside step function and \( r_0 \) is the range of the direct interaction.

Finally, the energy contribution of the local protrusive forces due to the cytoskeleton is
\[ W_F = -F \sum_i \hat{n}_i \cdot \vec{x}_i, \]
where \( F \) is the size of the force, the sum runs over all proteins, \( \hat{n}_i \) is the outwards facing normal to the membrane at the location of the protein \( i \) and \( \vec{x}_i \) is the position vector of the protein \( i \).

Note that the system with the active forces does not generally have global force balance, since the proteins are not symmetrically distributed on the surface of the vesicle. However, we are interested in shape changes, so disregard any center-of-mass motion.

2 Monte-Carlo simulations

The membrane is represented by a set of \( N \) vertices that are linked by tethers of variable length \( l \) to form a closed, dynamically triangulated, self-avoiding two-dimensional network of approximately \( 2N \) triangles and with the topology of a sphere. The lengths of the tethers can vary between a minimal value, \( l_{\text{min}} \), and a maximal value, \( l_{\text{max}} \). Self-avoidance of the network is ensured by choosing the appropriate values for \( l_{\text{max}} \) and the maximal displacement of the vertex \( s \) in a single updating step. In this work we used \( s/l_{\text{min}} = 0.15 \) and \( l_{\text{max}}/l_{\text{min}} = 1.7 \). The dynamically triangulated network acquires its lateral fluidity from a bond flip mechanism. A single bond flip involves the four vertices of two neighboring triangles. The tether connecting the two vertices in diagonal direction is cut and reestablished between the other two, previously unconnected, vertices. The treatment is within the Rouse description, as it ignores the effects of hydrodynamics.

The microstates of the membrane are sampled according to the Metropolis algorithm. The probability of accepting the change of the microstate due to vertex move or bond flip is \( \min[1, \exp(-\Delta E/kT)] \), where \( \Delta E \) is the energy change, \( k \) is the Boltzmann constant and \( T \) is absolute temperature. The energy for a given microstate is specified in Eq. \([1]\). The binding energy is discretized as described by Gompper and Kroll\([51,52]\).

In our Monte-Carlo simulations the whole membrane is the triangulated surface. To quantitatively analyse the demixing of the proteins in our system, we define the ensemble averaged mean cluster size as
\[ \langle N_{\text{vc}} \rangle = \left( \frac{\sum N_{\text{vc}}(i) N_{\text{cl}}(i)}{\sum N_{\text{vc}}(i)} \right), \]
where the angle brackets denote the canonical ensemble average. Inside the brackets, \( i.e. \) for a given microstate, \( N_{\text{vc}} \) is the mean cluster size and the sums run over all clusters of vertices representing proteins. In the sums, \( N_{\text{vc}}(i) \) is the number of vertices in cluster \( i \) and \( N_{\text{cl}}(i) \) is the number of clusters of size \( N_{\text{vc}}(i) \).

3 Results and discussion

Using our simulations we aim to improve the understanding of clustering of curved and active proteins on the membrane and how this process of demixing is coupled with membrane shape changes. Especially we are interested in the budding of curved protein clusters. We expect the demixing and budding to be enhanced by attractive direct interaction between the proteins and the additional membrane deformation induced by the protrusive (cytoskeletal) forces recruited by the proteins.

In our Monte-Carlo simulations the whole membrane is the triangulated surface. To quantitatively analyse the demixing of the proteins in our system, we define the ensemble averaged mean cluster size as
\[ \langle N_{\text{vc}} \rangle = \left( \frac{\sum N_{\text{vc}}(i) N_{\text{cl}}(i)}{\sum N_{\text{vc}}(i)} \right), \]
where the angle brackets denote the canonical ensemble average. Inside the brackets, \( i.e. \) for a given microstate, \( N_{\text{vc}} \) is the mean cluster size and the sums run over all clusters of vertices representing proteins. In the sums, \( N_{\text{vc}}(i) \) is the number of vertices in cluster \( i \) and \( N_{\text{cl}}(i) \) is the number of clusters of size \( N_{\text{vc}}(i) \).

3.1 Phase transition in thermal equilibrium

In Fig.\([1]\) we plot the cluster size distribution and snapshots of typical microstates of vesicles with curved proteins, in the absence of active protrusive forces. The system is in thermal equilibrium\([1]\) at different temperatures and densities (area coverage fraction, \( \rho = N_c/N \) of curved proteins).

At low average protein densities the equilibrium vesicle shapes remain quasi-spherical, with clusters that increase in size with decreasing temperature (in the far left column of Fig.\([1]\) the largest clusters are composed of 5 proteins at \( T/T_0 = 1.33 \) and of 8 proteins at \( T/T_0 = 0.63 \)). At higher average protein densities, cluster sizes increase and curved protein buds burst on the membrane.

At even larger average protein densities, the vesicle shapes deviate drastically from quasi-spherical and large necklace-like protein clusters often form. The size of these necklace-like clusters and the number of “beads” they contain increase with decreasing temperature.

\(^{1}\)Note that in some cases, for example when large necklace-like clusters form, thermal equilibrium is not always easily obtainable. However, after monitoring different measures for membrane shape change and demixing, we expect that the presented results correspond to the correct phase behaviour.
ing temperature. These necklace-like structures form since the isotropically curved proteins cannot form flat aggregates, due to their spontaneous curvature, and resemble aggregates calculated for membrane-adsorbed spherical particles. The theory of self-assembly of curved proteins can approximately explain observed necklace-like structures (see SI1 text), while anisotropic curved proteins may form aggregates with other geometries on vesicles. Necklace-like membrane protrusions have been observed in cellular membranes, under different conditions and in many in-vitro experiments.

We compare these simulation results with the prediction of the linear stability analysis (corresponding to the spinodal) of Gladnikoff et al. The linear stability analysis yields the critical thermal energy, \( kT^{(c)} \), below which the instability occurs and buds start to form:

\[
kT^{(c)} = 12w(1 - \rho/R_0) \left( 1 + \sqrt{\frac{I^2_{\text{min}}}{12w} \left( 1 - \frac{1}{\rho R_0} \right)} \right),
\]

where \( 1/R_0 \) is mean membrane curvature at the site of a curved membrane protein. In the following we approximate \( R_0 \) with the radius of a spherical vesicle with the same membrane area. In thermal equilibrium, in the absence of the protrusive forces \( F = 0 \), this simplifies to: \( T^{(c)} = 12w(1 - \rho/k) \), which is plotted in Fig. 1. It can be seen that the prediction of the linear stability analysis qualitatively agrees with our simulation results: above the critical temperature line the protein budding is weak and the cluster size distribution is highly peaked at the size of isolated proteins, while below it buds are larger (together with the corresponding membrane deformation) and the size distribution exhibits a secondary peak at aggregates containing 8 or more proteins (which is the number required to form the smallest closed spherical cluster of proteins). For a more quantitative description, \( T^{(c)} \) is defined from the simulations as the temperature where \( \langle N_{\text{vc}} \rangle \approx 2 \) (Eq. 5), which agrees quite well with the predicted budding transition line, as plotted in Figs. 1a,b. We find that the mean aggregate size collapses to a universal curve when the temperature is scaled by \( T^{(c)} \) (Fig. 2b). The agreement with Eq. 6 is also found as function of protein interaction strength (Fig. 2h), and as function of vesicle radius (Fig. S7). We conclude that the critical temperature for budding in the passive system agrees very well with the prediction of the linear stability model (Eq. 6).

The phase separation of the passive system was additionally approximated with a two-dimensional model of self-assembly of curved proteins (see SI1† for details). The total free energy of the model reads

\[
F = M \left[ \hat{x}_1 \tilde{\mu}_1 + kT \hat{x}_1 (\ln \hat{x}_1 - 1) \right] + M \sum_{i=1}^{\infty} \left[ x_i \tilde{\mu}_i + kT x_i \left( \ln \frac{x_i}{l} - 1 \right) - \mu M (\hat{x}_1 + \sum_{i=1}^{\infty} x_i) \right].
\]

Here, \( \hat{x}_1 \) and \( x_i \) are the number densities of nanodomains in the weakly curved region and highly curved aggregates, respectively. The energy contributions come not only from the free energies per nanodomain (\( \mu_i \) and \( \tilde{\mu}_i \)), but also from configurational entropy, while the Lagrange multiplier \( \mu \) assures a constant number of nanodomains in the system through a conservation relation. We minimize \( F \) with respect to the number densities, arriving at the equilibrium distributions for aggregate size (Eq. S10). This model predicts that the critical density beyond which the growth of aggregates is energetically favourable is given by (Eq. S11‌)

\[
\hat{x}_c \approx \exp \left( \frac{\Delta f - w}{kT} \right),
\]

where \( \Delta f = f_w - f_{sp} \) is the difference between the energy of a single protein nanodomain on the highly curved necklace-like aggregates (\( f_w \)) and on the weakly curved membrane region (\( f_{sp} \)). Comparison between the model phase transition and MC simulations of aggregate distributions shows good overall agreement, with large necklace-like aggregates appearing below the calculated transition line (Fig. 1).

### 3.2 Phase transitions in the presence of active protrusive forces

Next, we consider the effects of active protrusive forces on the system. In Fig. 3 we plot the typical shapes and aggregate size distribution as in Fig. 1 but with \( F = kT R_0/\mu \). We can see that the active protrusive forces promote demixing and budding of the convex curved proteins, such that the transition temperature \( T^{(c)} \) is shifted to higher temperatures and lower densities, as expected and predicted by the linear stability analysis (Eq. 6). A more dramatic effect of the active forces is seen in Fig. 3 at low temperatures, where below the budding transition there is now a second transition to a new class of shapes that was not seen in the equilibrium system (Fig. 1). Namely, below the red dashed curve we find that the vesicles change from deformed-spherical to flattened pancake-like shapes, where all or nearly all the proteins aggregate at the rim, forming one large cluster in the form of a closed

---

\[ \text{Eq. 2} \]

\[ \text{Eq. 5} \]

\[ \text{Eq. 6} \]
Fig. 1 Microstates of the vesicles in thermal equilibrium (absence of protrusive forces), for different average densities of curved proteins $\rho$ and relative temperatures $T/T_0$. The blue vertices represent the protein-free bilayer and have zero spontaneous curvature; red vertices denote the curved proteins and have spontaneous curvature $c_0$. In the corresponding cluster-size distributions, the $y$-axis is the ensemble averaged number of protein clusters of each size and the $x$-axis is the protein cluster size. Solid black line denotes the prediction of the critical temperature by the linear stability analysis (Eq. 6), while green points denote the line where $\langle N_c \rangle = 2$. The yellow line marks the critical boundary of the phase space below which the self-assembly theory predicts aggregate growth (for $r = 1.35l_{min}$, $R_0 = 10r$; Eq. [S1]).
Results for the equilibrium system with curved proteins ($c_0 = 1$) without active forces $F = 0$. (a) Contour plot of the ensemble averaged mean cluster size ($\langle N_c \rangle$) as function of protein density $\rho$ and relative temperature $T/T_0$. The prediction of the critical temperature $T^c$ by the linear stability analysis (Eq. 5) is shown (solid yellow line), as well as the points where $\langle N_c \rangle = 2$ (green). (b) Critical temperatures as predicted by the linear stability analysis (solid line) and from simulations ($\langle N_c \rangle = 2$, green points). Representative snapshots are added for the mixed phase (for $\rho = 0.05$, $T/T_0 = 4/3$) and the budded phase (for $\rho = 0.08$, $T/T_0 = 0.625$). (c) Mean cluster size ($\langle N_c \rangle$) as function of the temperature normalized by the critical temperature $T/T^c$, for $\rho = 5, 7.5, 8, 8.5, 9, 9.5, 10.5, 11, 11.5, 12, 12.5, 14, 15, 15.5, 17, 20$ and $25\%$. The horizontal dashed line indicates where $\langle N_c \rangle = 1$. INSET: $\langle N_c \rangle$ as a function of $T/T_0$ for three values of $\rho$. (d) $\langle N_c \rangle$ as a function of $T/T^c$ for two different values of the direct interaction constant $w$. The average protein density is $\rho = 9.5\%$ (INSET: the same but as a function of $T/T_0$).

The organization of the proteins into a circular cluster around the rim of a flat vesicle is highly effective in stretching out the flat membrane parts. We indeed find that these regions are almost devoid of proteins (Figs. 3, 5b), since these regions are energetically unfavorable for the curved proteins. The stretching of the membrane in these regions also acts to suppress aggregation of the curved proteins, and the rim aggregate is highly stable (see Supporting Movie S1†).

Below a critical density, we find that there are simply not enough proteins to form a continuous cluster around the rim of the flattened vesicle, and the system changes to a different class of shapes (Fig. 5c). In this regime the curved proteins form arc-like clusters that line the flattened ends of an elongated vesicle, and remain highly dynamic (see Supporting Movie S2†). Since the curved proteins are isotropic, the curvature of the rim of the flattened vesicles along circumferential direction is much smaller compared to $c_0$. The curved protein therefore tend to bend the rim and cause it to undulate along this direction (Fig. 5b). At large protein densities we find that the excess proteins crowd the rim and cause it to undergo buckling and curling, so as to be able to accommodate more curved proteins (Fig. 3). At the highest densities, the excess proteins extend from the rim cluster as spherical and necklace-like clusters (Fig. 5).

We can propose the following mechanism that drives the transition from deformed spherical vesicles with small buds to pancake-shapes in the presence of the active protrusive forces. When the proteins are isolated, or in very small clusters, the membrane is rather flat and the protrusive force promotes the aggregation of small clusters, since the active force is directed at the outwards normal at each protein, thereby enhancing the outwards deformation that the curved protein induces. However, for larger clusters that are highly curved, the active forces point in different directions which acts to inflate and deform the clusters (Fig. 5b). We can estimate the critical cluster size at which the in-plane

The error bars on the pancake-transition curve in Fig. 4 mark the region in which the transition occurs, i.e. the region between observed no-pancake states with lowest $T$ and pancake states with largest $T$ (Fig. 3).
Fig. 3 Same as on Fig. 1 but for the system with active protrusive forces $F = k T_0 / \ell_{\text{min}}$. Approximate temperatures below which a transition into a pancake-like shapes is observed are indicated (red).
are shown for the mixed phase (for $\rho$). Representative snapshots of the actin protrusive force (legend). The average protein density is $\langle N_c \rangle = 2$ (green) and approximate temperatures below which a transition into a pancake-like shapes is observed (red). (a) Contour plot of the ensemble averaged mean cluster size ($\langle N_c \rangle$) as a function of protein density $\rho$ and relative temperature $T / T_0$. The prediction of the critical temperature $T^{(c)}$ by the linear stability analysis (Eq. 5) as a function of temperature normalized by the critical temperature $T / T^{(c)}$, for $\rho = 5, 7.5, 8, 8.5, 9, 9.5, 10.5, 11, 11.5, 12, 12.5, 14, 15, 15.5, 17, 20$ and $25\%$. The horizontal dashed line indicates where $\langle N_c \rangle = 1$. INSET: $\langle N_c \rangle$ as a function of $T / T_0$ for three values of $\rho$. (c) Critical temperatures as predicted by the linear stability analysis (solid line) and from simulations (green points). Approximate temperatures below which a transition into a pancake shapes is observed in the simulations are shown in red. Representative snapshots are added for the mixed phase (for $\rho = 0.05$, $T / T_0 = 4/3$), the budded phase (for $\rho = 0.14$, $T / T_0 = 4/3$) and the pancake phase (for $\rho = 0.105$, $T / T_0 = 0.625$). (d) $\langle N_c \rangle$ as a function of temperature for three different values of the actin protrusive force (legend). The average protein density is $\rho = 9.5\%$. For $F = 1.5kT_{0}/l_{min}$, the snapshots of steady-state microstates are shown for $T / T_0 = 0.625$ (pancake), 1 (prolate) and 1.33 (quasi-spherical).

Fig. 4 Results for the system with active curved proteins ($c_0 = 1/l_{min}$, $F = 1kT_0/l_{min}$). (a) Contour plot of the ensemble averaged mean cluster size ($\langle N_c \rangle$) as a function of protein density $\rho$ and relative temperature $T / T_0$. The prediction of the critical temperature $T^{(c)}$ by the linear stability analysis (Eq. 5) as a function of temperature normalized by the critical temperature $T / T^{(c)}$, for $\rho = 5, 7.5, 8, 8.5, 9, 9.5, 10.5, 11, 11.5, 12, 12.5, 14, 15, 15.5, 17, 20$ and $25\%$. The horizontal dashed line indicates where $\langle N_c \rangle = 1$. INSET: $\langle N_c \rangle$ as a function of $T / T_0$ for three values of $\rho$. (b) Critical temperatures as predicted by the linear stability analysis (solid line) and from simulations (green points). Approximate temperatures below which a transition into a pancake-like shapes is observed are also indicated (red). (c) Critical temperatures as predicted by the linear stability analysis (solid line) and from simulations (green points). Approximate temperatures below which a transition into a pancake-like shapes is observed are also indicated (red). (d) $\langle N_c \rangle$ as a function of temperature for three different values of the actin protrusive force (legend). The average protein density is $\rho = 9.5\%$. For $F = 1.5kT_{0}/l_{min}$, the snapshots of steady-state microstates are shown for $T / T_0 = 0.625$ (pancake), 1 (prolate) and 1.33 (quasi-spherical).

Fig. 5 (a) Zoom-in of the lower left corner of Fig. 2 showing system snapshots at low protein densities. Approximate temperatures below which a transition into pancake-like shapes is observed are also indicated (red). (b) Zoom-in on the edge of the pancake shapes for $\rho = 10.5\%$, $T / T_0 = 0.625$ (LEFT) and $\rho = 15.5\%$, $T / T_0 = 0.625$ (RIGHT), to highlight their convoluted shape. The circle in the middle has radius $l_{max} = 1/c_0$ and indicates the length-scale of a single spherical bud of proteins.
tion curve, which marks the transition into such hydra-like shapes of a cluster of proteins at the protrusion’s tip. An approximate transition line is still well described by the linear stability expression 

\[ N_{cl} F = 2 \pi c_0^2 (1 - \cos(\theta)) \]

by calculating the phase diagram for flat active proteins, i.e., with proteins in driving the shape transition discussed above. We start exploring the role of the spontaneous curvature of the active proteins. This is shown in Fig. 7, where we change the spontaneous curvature of the active proteins from \( c_0 = 0 \) (flat proteins) to \( c_0 = \lambda_{min} \) (the spontaneous curvature used in the previous section). We find that as \( c_0 \) is increased, the multi-tube-like shapes transform continuously into shapes that have a single tube-like part and flattened arc-like clusters at the two tips. Above a critical spontaneous curvature there is a sharp transition into the flattened shapes with continuous rim cluster. From our simple estimates (Figs. 6, b), we predict that the critical cluster size that enables the pancake shape transition decreases with increasing \( c_0 \), while the tube-like shape transition does not depend on this parameter. We therefore expect that above a critical value of \( c_0 \) the pancake shape will dominate, as we observe (Fig. 7d).

### 3.3 Dependence on the spontaneous curvature of the active proteins

We next explore the role of the spontaneous curvature of the active proteins in driving the shape transition discussed above. We start by calculating the phase diagram for flat active proteins, i.e. with \( c_0 = 0 \). The result is shown in Fig. 7a. We find that the budding transition line is well still described by the linear stability expression (Eq. 6). At low temperatures we find a global shape transition from quasi-spherical vesicles to shapes with highly elongated protrusions, which are driven by the protrusive force provided by a cluster of proteins at the protrusion’s tip. An approximate transition curve, which marks the transition into such hydra-like shapes regime (Fig. 7a, b) can be obtained from locating the sharp change of the slope of the asphericity dependence on \( T \) (Fig. 7d).

This shape transition can be understood by calculating the conditions that allow a tube-like protrusion to start growing from the vesicle, driven by the protrusive force induced by a circular protein aggregate (Fig. 4f). This occurs when there is a force balance between the protrusive force provided by the protein cluster at the tip and the elastic restoring force due to membrane bending (as in tether pulling): 

\[ N_{cl} F \approx 2 \pi \kappa / \sqrt{N_{cl}} \]

From this force balance we derive the radius of the protrusions (details in the S1 Eq. S17): 

\[ R_c = \left( \frac{2 \pi c_0}{4 \pi} \right)^{1/3} \]

(where \( a \) is the average area per protein), which is in very good agreement with the simulated widths of the protrusions (Fig. S3). As the temperature decreases, the mean cluster size increases until it is larger than the threshold size (Fig. 6b) for the elongation of tube-like protrusions (Fig. 7b). In this phase the shapes are highly dynamic and unstable, with protrusions merging and growing (see Supporting Movie S3)

By fixing a low temperature and large enough density, we explore the dependence of the global vesicle shape transitions on the spontaneous curvature of the active proteins. This is shown in Fig. 7a, where we change the spontaneous curvature of the active proteins from \( c_0 = 0 \) (flat proteins) to \( c_0 = \lambda_{min} \) (the spontaneous curvature used in the previous section). We find that as \( c_0 \) is increased, the multi-tube-like shapes transform continuously into shapes that have a single tube-like part and flattened arc-like clusters at the two tips. Above a critical spontaneous curvature there is a sharp transition into the flattened shapes with continuous rim cluster. From our simple estimates (Figs. 6, b), we predict that the critical cluster size that enables the pancake shape transition decreases with increasing \( c_0 \), while the tube-like shape transition does not depend on this parameter. We therefore expect that above a critical value of \( c_0 \) the pancake shape will dominate, as we observe (Fig. 7d).

### 4 Conclusions

We have explored here the coupling of convex proteins (such as complexes that contain IRSp53) that recruit the protrusive force of the cytoskeleton (most commonly due to actin polymerization), using Monte-Carlo computer simulations. We found that the presence of the protrusive forces gives rise to the formation of protein aggregates and budding at a higher temperature and lower average protein density, compared to the passive system that is in thermal equilibrium (no active forces). This is in an agreement with analytic linear-stability analysis. The more robust aggregation and budding due to the recruited active forces of the cytoskeleton has important consequences for a variety of biological processes, such as budding of viruses and initiation of cellular protrusions.

---

Footnote 11: Deviation of the vesicle from quasi-spherical shapes can be characterized conveniently in terms of the asphericity. To this end, we make use of the Gyration tensor, whose components are defined for a discrete object through

\[ S_{ij} = \frac{1}{N} \sum_{k=1}^{N} r_{ik} r_{kj} \quad (i, j = 1, 2, 3). \]

Here, \( r_{ik} \) is the \( i \)-th Cartesian coordinate of the position vector \( \mathbf{r}_k \) of the \( k \)-th particle. The origin of the coordinate system is located at the center of mass and the sum runs over all particles of the object. From the principal moments (i.e., the eigenvalues) \( \lambda_1 \geq \lambda_2 \geq \lambda_3 \) of \( S_{ij} \) (calculated using the algorithm outlined by Smith), we obtain the asphericity of the object

\[ \text{Asph} = \left( \frac{\lambda_1 - \lambda_3}{\lambda_2 - \lambda_3} \right) + \left( \frac{\lambda_2 - \lambda_1}{\lambda_3 - \lambda_1} \right) + \left( \frac{\lambda_3 - \lambda_2}{\lambda_1 - \lambda_2} \right) \]

with \( (\cdot) \) denoting ensemble averages. We point out that a one-dimensional object (where \( \lambda_2 = \lambda_3 = 0 \) leads to \( A = 1 \), a two-dimensional axisymmetric disk (where \( \lambda_1 = \lambda_2 = 0 \)) entails \( A = 1/4 \), and a sphere (where \( \lambda_1 = \lambda_2 = \lambda_3 \)) gives rise to \( A = 0 \). The asphericity \( A \) has frequently been used in the past to characterize polymers and membranes.
Beyond the budding transition, we found a new and unexpected global shape transition that occurs only in the presence of the active forces. In this transition the spherical vesicle is transformed into a flat, pancake-like shape, with all the curved proteins and associated cytoskeleton forces along the circular rim. This structure resembles the lamellipodia and ruffles of spreading and motile cells (see Supporting Movies S1 and S2), where the actin polymerization is localized to the highly curved leading edge. Our results show that lamellipodia-like structures spontaneously form when convex proteins recruit the protrusive force of the cytoskeleton, on a closed membrane. Future studies could test these predictions, by exploring the spontaneous curvature properties of the actin nucleators at the leading edges of these cellular structures.

Let us note that flat oblate membrane shapes can be obtained without the presence of active protrusive forces, for vesicles with low volume to area ratios, However, in our simulations the volume of the vesicle is free to relax, and under such conditions we find that the active forces are essential for the curved proteins to drive the global flattened vesicle shape transition.

In addition, a variety of tubular and flattened shapes may be stabilized in the absence of active forces, due to the presence of anisotropic curved membrane proteins (or protein complexes). Tubular protrusions can occur due to accumulation of anisotropic membrane components, as was shown theoretically and supported by experiments. Vesicles with flattened edges can be stabilized by anisotropic (arc-like) proteins that form a cluster at the edge of the flattened regions. We also note that the edge of the disc-shaped vesicles that we obtained here are highly convoluted (Fig. 5b), due to the isotropic curvature of the proteins. Lamellipodia in cells may avoid this and maintain a smooth edge by using anisotropic proteins that recruit the actin polymerization. All of these considerations motivate the exploration of the vesicle shapes induced by coupling active forces with anisotropic membrane constituents in our future studies.

To conclude, our study highlights the rich variety of membrane shapes that may be induced by curved membrane proteins that recruit the active forces of the cytoskeleton. These include steady-state shapes that are not possible for the passive, equilibrium system. Future computer simulations could explore further the space of these non-equilibrium, and dynamic membrane shapes.

**Acknowledgements**

NSG is the incumbent of the Lee and William Abramowitz Professorial Chair of Biophysics. The study was supported by the grants No. P2-0232, P3-0388, J5-7098, J2-8166 and J2-8169 of Slovenian Research Agency (ARRS).
References

[1] J. Zimmerberg and M. M. Kozlov, Nature reviews Molecular cell biology, 2006, 7, 9.
[2] S. Leibler, Journal de Physique, 1986, 47, 507–516.
[3] J. Fournier, Physical review letters, 1996, 76, 4436.
[4] V. Kralj-Iglič, S. Svetina and B. Žekž, European biophysics journal, 1996, 24, 311–321.
[5] N. Bobrovksa, W. Gózdz, V. Kralj-Iglič and A. Iglič, PloS one, 2013, 8, e73941.
[6] M. Fošnarič, A. Iglič and S. May, Physical Review E, 2006, 74, 051503.
[7] T. Baumgart, B. R. Capraro, C. Zhu and S. L. Das, Annual review of physical chemistry, 2011, 62, 483–506.
[8] J. Gómez-Llobregat, F. Elias-Wolff and M. Lindén, Biophysical journal, 2016, 110, 197–204.
[9] A. Iglič, M. Lokar, B. Babnik, T. Slivnik, P. Veranič, H. Hägerstrand and V. Kralj-Iglič, Blood Cells, Molecules, and Diseases, 2007, 39, 14–23.
[10] A. Iglič, T. Slivnik and V. Kralj-Iglič, Journal of biomechanics, 2007, 40, 2492–2500.
[11] H. Noguchi, Scientific reports, 2016, 6, 20935.
[12] L. Mesarec, W. Gózdz, V. K. Iglič, S. Kralj and A. Iglič, Colloids and Surfaces B: Biointerfaces, 2016, 141, 132–140.
[13] V. Markin, Biophysical Journal, 1981, 36, 1–19.
[14] U. Seifert, Advances in physics, 1997, 46, 13–137.
[15] H. T. McMahon and J. L. Gallop, Nature, 2005, 438, 590.
[16] B. Božič, V. Kralj-Iglič and S. Svetina, Physical Review E, 2006, 73, 041915.
[17] N. Walani, J. Torres and A. Agrawal, Physical Review E, 2014, 89, 062715.
[18] M. Chabanon, J. C. Stachowiak and P. Rangamani, Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 2017, 9, e1386.
[19] W. Helfrich and J. Prost, Physical Review A, 1988, 38, 3065.
[20] U. Seifert, Physical review letters, 1993, 70, 1335.
[21] V. Kralj-Iglič, A. Iglič, H. Hägerstrand and P. Peterlin, Physical Review E, 2000, 61, 4230.
[22] M. Laradji and P. S. Kumar, Physical review letters, 2004, 93, 198105.
[23] J.-M. Allain and M. B. Amar, Physica A: Statistical Mechanics and its Applications, 2004, 337, 531–545.
[24] V. Kralj-Iglič, H. Hägerstrand, P. Veranič, K. Jezernik, B. Babnik, D. R. Gauger and A. Iglič, European Biophysics Journal, 2005, 34, 1066–1070.
[25] A. Iglič, H. Hägerstrand, P. Veranič, A. Plemenitaš and V. Kralji-Iglič, Journal of Theoretical Biology, 2006, 240, 368–373.
[26] V. Kralj-Iglič, S. Svetina and B. Žekž, European biophysics journal, 1996, 24, 311–321.
[27] S. Semrau, T. Idema, T. Schmidt and C. Storm, Biophysical Journal, 2009, 96, 4906–4915.
[28] L. Mesarec, W. Gózdz, S. Kralj, M. Fošnarič, S. Penič, V. Kralj-Iglič and A. Iglič, European Biophysics Journal, 2017, 46, 705–718.
[29] H. Noguchi, Soft matter, 2017, 13, 7771–7779.
[30] N. Gov, Phil. Trans. R. Soc. B, 2018, 373, 20170115.
[31] S. Saha, T. L. Nagy and O. D. Weiner, Phil. Trans. R. Soc. B, 2018, 373, 20170145.
[32] H. Alimohamadi, R. Vasan, J. Hassinger, J. Stachowiak and P. Rangamani, Biophysical Journal, 2018, 114, 600a.
[33] P. Girard, J. Prost and P. Bassereau, Physical review letters, 2005, 94, 088102.
[34] N. Gov, Physical review letters, 2004, 93, 268104.
[35] N. S. Gov and A. Gopinathan, Biophysical journal, 2006, 90, 454–469.
[36] H. Miki, H. Yamaguchi, S. Suetsugu and T. Takenawa, Nature, 2000, 408, 732–735.
[37] S. Krugmann, I. Jordens, K. Gevaert, M. Driessens, J. Vandekerckhove and A. Hall, Current Biology, 2001, 11, 1645–1655.
[38] P. K. Mattila, A. Pykäläinen, J. Saarikangas, V. O. Paavilainen, H. Vihinen, E. Jokitalo and P. Lappalainen, J Cell Biol, 2007, 176, 953–964.
[39] T. H. Millard, G. Bompard, M. Y. Heung, T. R. Dafforn, D. J. Scott, L. M. Machesky and K. Fütterer, The EMBO journal, 2005, 24, 240–250.
[40] A. Disanza, S. Mantoani, M. Hertzog, S. Gerboth, E. Frittoli, A. Steffen, K. Berhoerster, H.-J. Kreienkamp, F. Milanesi, P. P. Di Fiore et al., Nature cell biology, 2006, 8, 1337–1347.
[41] F. Vaggi, A. Disanza, F. Milanesi, P. P. Di Fiore, E. Menna, M. Matteoli, N. S. Gov, G. Scita and A. Ciliberto, PLoS computational biology, 2011, 7, e1002088.
[42] A. Disanza, S. Bisi, M. Winterhoff, F. Milanesi, D. S. Ushakov, D. Kast, P. Marighetti, G. Romet-Lemonne, H.-M. Müller, W. Nickel et al., The EMBO journal, 2013, 32, 2735–2750.
SUPPLEMENTAL INFORMATION

S1: A theoretical model of self-assembly of curved nanodomains in a two-component membrane

We use the theory of self-assembly to describe the accumulation of curved membrane nanodomains composed of lipids and proteins into spherical or necklace membrane protrusions. The curved nanodomains (of total number \( N \)) are initially distributed in the weakly curved spherical membrane surface of constant mean curvature \( H = 1/R_0 \). We assume that the nanodomains are laterally mobile over the membrane surface. For isotropic curved membrane protrusion of constant high mean curvature \( H = 1/r \). Here, \( r \) is the radius of curvature everywhere on the membrane protrusion which may be a sphere or necklace formation (see Fig. S1 and assume \( R_0 > r \).

\[ H = 1/r \]

**Fig. S1** Growth of necklace-like protrusions is energetically favorable when critical concentration \( \tilde{x} \) is surpassed.

For the sake of simplicity we assume that the free energy of a single flexible membrane nanodomain can be written in the form [V. Kralj-Iglič et al. Deviatoric elasticity as a possible physical mechanism explaining collapse of inorganic micro and nanotubes, Physics letters A, 2002]:

\[ f = \frac{\xi}{2} (H - H_0)^2 a_0. \]  \hspace{1cm} (S1)

where \( H_0 \) is the intrinsic mean curvature of an isotropic membrane nanodomain, \( \xi \) is the elastic constant and \( a_0 \) is the area per single nanodomain. In aggregates of curved flexible membrane nanodomains the local membrane bending constant is \( k_c = \frac{\xi}{4} \) and the membrane spontaneous curvature \( c_0 = 2H_0 \).

Curved flexible membrane nanodomains in aggregates interact with neighbouring membrane nanodomains. We denote the corresponding interaction energy per curved flexible membrane nanodomain (monomer) in an aggregate composed of \( i \) nanodomains as \( w(i) \) where we assume that the energy \( w(i) \) depends on the size of the aggregate composed of \( i \) nanodomains. The mean free energy per nanodomain in a curved aggregate (where \( H = D = 1/r \)) composed of \( i \) nanodomains can be written as:

\[ \mu_i = f_c - w(i), \]  \hspace{1cm} (S2)

where \( f_c = f(H=1/r) \) and \( w(i) > 0 \). We assume that in the weakly curved spherical regions of the membrane (having \( H=1/R_0 \)) the concentration of nanodomains is always below the critical aggregation concentration and therefore nanodomains cannot form two-dimensional flat aggregates. The mean energy per nanodomain in the weakly curved membrane regions is \( \tilde{\mu}_1 = \tilde{f}_c \), where \( \tilde{f}_c = f(H=1/R_0) \). The number density of curved proteins in the weakly curved membrane regions is

\[ \tilde{x}_1 = \frac{\tilde{N}_1}{M}, \]  \hspace{1cm} (S3)

where \( \tilde{N}_1 \) is the number of monomeric curved nanodomains in the weakly curved membrane regions and \( M \) is the number of lattice sites in the whole system. The distribution of highly curved aggregates in the membrane protrusions on the scale of number density is expressed as

\[ x_i = \frac{i\tilde{N}_i}{M}, \]  \hspace{1cm} (S4)

where \( \tilde{N}_i \) denotes the number of aggregates with aggregation number \( i \). The number densities \( \tilde{x}_1 \) and \( x_i \) must fulfil the conservation
The free energy $F$ of all nanodomains in or on the membrane can be written as:

$$F = M \sum_{i} \left[ \beta x_i kT \ln(\tilde{x}_i - 1) \right] + M \sum_{i} \left[ \beta x_i kT \left( \ln \frac{x_i}{c_0} - 1 \right) \right] - \mu M \tilde{x}_1 + \sum_{i} \left[ \beta x_i kT \right] .$$

where $\mu$ is the Lagrange parameter assuring conservation of protein concentrations. The above expression for the free energy also involves the contributions of configurational entropy. We minimize $F$ with respect to $\tilde{x}_1$ and $x_i$:

$$\frac{\partial F}{\partial \tilde{x}_1} = 0, \frac{\partial F}{\partial x_i} = 0, \quad i = 1, 2, 3, \ldots ,$$

which leads to equilibrium distributions:

$$\tilde{x}_1 = \exp \left( - \frac{f_{sp} - \mu}{kT} \right) ,$$

$$x_i = i \exp \left( - \frac{i}{kT} \left[ f_c - w - \mu \right] \right) ,$$

where we assumed for simplicity that $w(i) = w$ is independent of aggregate size. The quantity $\mu$ can be expressed from Eq. [S8] and substituted in Eq. [S9] to get:

$$x_i = i \left[ \tilde{x}_1 \exp \left( \frac{f_{sp} + w - f_c}{kT} \right) \right] .$$

We see that if the concentration $\tilde{x}_1$ is small, aggregate growth will not be favourable, since $x_1 > x_2 > x_3 \ldots$. Furthermore, $x_i$ can never exceed unity, leading to the maximal possible value of the number density of monomeric curved flexible nanodomains in the weakly curved parts of the membrane when $\tilde{x}_1$ approaches $\exp \left( (f_c - f_{sp} - w)/kT \right)$. The critical concentration is therefore

$$\tilde{x}_c \approx \exp \left( \frac{\Delta f - w}{kT} \right) ,$$

where $\Delta f = f_c - f_{sp}$ is the difference between the energy of a single nanodomain on the highly curved membrane protrusion and the energy of the single nanodomain in the weakly curved membrane region with:

$$\Delta f = \frac{\xi a_0}{2r} \left( \frac{1}{r} - 2H_0 \right) - \frac{\xi a_0}{2R_0} \left( \frac{1}{r} - 2H_0 \right) .$$

If $\tilde{x}_1$ is above $\tilde{x}_c$, the formation of a very long necklace membrane protrusions composed of curved membrane proteins is energetically favourable. It can be seen from Eq. [S12] that longitudinal growth of the necklace membrane protrusions is dependent on the energy difference $\Delta f$ (Eq. [S12]) and the strength of the direct interaction between nanodomains $w$. The critical concentration $\tilde{x}_c$ strongly depends on $H_0$.

In the approximation limit $R_0 \gg r$ we can rewrite Eq. [S12] as:

$$\Delta f \approx \frac{\xi a_0}{2r} \left( \frac{1}{r} - 2H_0 \right) = \frac{2k_c}{r} \left( \frac{1}{r} - c_0 \right) .$$

where $k_c$ and $c_0$ are the local bending constant and spontaneous curvature of aggregates of nanodomains, respectively. We may rewrite Eq. [S11]

$$\tilde{x}_c \approx \exp \left( 2 \frac{k_c}{kT} \frac{a_0}{r^2} \left( 1 - c_0 r \right) - \frac{w}{kT} \right) .$$

For $1 < c_0 r$ the value of $\Delta f$ is always negative. The theoretically predicted existence of necklace membrane protrusions (without application of the local forces) within the self-assembly theory is in line with our MC predictions.

Since the density of nanodomains in or on the membrane is defined with the conservation condition (Eq. [S5]), this also gives us the relation between normalized temperature $T/T_0$ and total curved nanodomains concentrations $\rho = N/M$. Using the parameters from the MC simulations, we may graph dependencies $x_i(i)$, as seen in Fig. [S2]. Above small concentrations and especially above $\tilde{x}_c$, aggregates start to form, where the peaks of the distributions are strongly dependent on the total protein concentration in the lattice. We see that the critical line beyond which aggregate growth is favourable agrees well with the results of MC simulations.
Fig. S2 Aggregate concentrations in dependence on number of nanodomains in the aggregate for different number of flexible nanodomains on the membrane.
The conditions that trigger the transition into the tubular-shapes (Fig.7) are given by the following force balance:

The force applied at the tip of the cylindrical protrusion by the cluster of active proteins is

\[ F_a = F \frac{\pi R^2}{a} \]  

(S15)

where \( F \) is the force per active protein, \( R \) is the radius of the cylinder, and \( a \) is the area of a protein on the membrane.

This is balanced by the restoring force of the membrane bending energy

\[ F_b = \kappa \frac{2\pi}{R} \]  

(S16)

with \( \kappa \) the bending modulus. The force balance gives the radius of the cylindrical protrusions in this phase of the vesicle shapes

\[ R_c = \left( \frac{2\kappa a}{F} \right)^{1/3} \]  

(S17)

The prediction of Eq. (S17) is in good agreement with simulations (see Fig. S3), where we took for \( a \) the area that corresponds to one vertex in a hexagonal mesh, \( a = \sqrt{3} l_0^2 / 2 \), where \( l_0 = (l_{\text{min}} + l_{\text{max}}) / 2 \).

In the phase of tubular shapes, there are several protrusions (typically 2-3) that pull in opposite directions to provide an overall force balance, and maintain the relative stability of this shape. Some fusions of protrusions do occur, especially for cases with a larger number of thinner protrusions, so that their number fluctuates.

**Fig. S3** Radius of cylindrical protrusions as a function of the \( \kappa / F \times l_{\text{min}} \) ratio for the system with almost flat active proteins with parameters \( c_0 = 1/(0.9 l_{\text{min}}) \), \( \rho = 11\% \), \( w = 1kT_0 \) and \( T/T_0 = 0.7 \) (see top-left hydra-like snapshot on Fig. 7d). Black solid curve is the prediction of Eq. (S17), while red dots are the results of the simulations with error bars indicating standard errors.
SI3: Cluster size dependence on the strength of the direct interaction for active system

See Fig. S4

**Fig. S4** Mean cluster size $\langle \bar{N}_v \rangle$ as a function of $T/T^{(c)}$ for two different values of the direct interaction constant (legend), for an active system with $F = 1 kT_0 / l_{\text{min}}$. The average protein density is $\rho = 9.5\%$. The graphs do not collapse, unlike in the passive system (Fig. 2d).
SI4: Testing for hysteresis of the pancake transition

See Fig. S5.

Fig. S5 Hysteresis test for the transition into the pancake shape (corresponding to the system shown in Figs. 3,4), showing ensemble averaged mean cluster size for active curved proteins as a function of temperature for two different initial states – above (blue) and below (red) pancake transition. Average protein density is \( \rho = 11\% \). Error bars denote standard deviations.
SI5: Cluster size dependence on the density

The activity-driven transition is clearly seen in Fig. 4b of the main text – in the mean cluster size $\langle N_{vc}\rangle$ as a function of temperature $T/T_0$ for different average densities of proteins $\rho$. Without the active protrusive force, $\langle N_{vc}\rangle$ monotonically increases with $\rho$ and decreases with $T$, while the protrusive force gives rise to the sharp transition into pancake-like shapes. The lower stability of the rim aggregate at high protein densities, that we already noticed in Fig.3, is manifested in the non-monotonic dependencies of $\langle N_{vc}\rangle$ on $\rho$ and $T$ (Fig. S6).

![Graph showing ensemble averaged mean cluster size as a function of average density of curved proteins with $c_0 = 1/l_{min}$. Results with active protrusive force $F = 1kT_0/l_{min}$ are shown for $T/T_0 = 0.625$ (solid) and without it for $T/T_0 = 0.4$ (dashed).]

**Fig. S6** Ensemble averaged mean cluster size as a function of the average density of curved proteins with $c_0 = 1/l_{min}$. Results with active protrusive force $F = 1kT_0/l_{min}$ are shown for $T/T_0 = 0.625$ (solid) and without it for $T/T_0 = 0.4$ (dashed).
SI6: Vesicle size dependence of the budding and pancake transition

The dependence of the pancake transition on the vesicle radius mirrors the effect on the overall cluster size distribution: a smaller vesicle has smaller protein clusters and a lower transition temperature (Fig. S7).

Fig. S7 Dependence of the budding (green) and pancake (red) transition curves as functions of the number of vertices composing the vesicle with $F = kT_0/l_{\text{min}}$, $c_0 = 1/l_{\text{min}}$, $\rho = 9.5\%$. Spherical vesicle with the same membrane area $A$ has the radius $R_0 \approx 0.35\sqrt{N}$ (in units of $l_{\text{min}}$). Black solid curve is the prediction for the budding transition line from the linear stability analysis.
SI7: Pressure difference dependence of the pancake transition

See Fig. S8.

(a) Difference of pressures between inside and outside of the vesicle $\Delta p = 0.01kT_0/l_{min}$

(b) Difference of pressures between inside and outside of the vesicle $\Delta p = 0.1kT_0/l_{min}$

Fig. S8 Pressurization snapshots: Same system as in Fig.3, but with a finite pressure difference $\Delta p = p_{\text{inside}} - p_{\text{outside}}$. At high pressure, the membrane tension inhibits the transition to the pancake shape.
MOVIES

**Movie S1**: Animation of snapshots in steady-state of the system with $\rho = 7\%$ of curved active proteins with $F = 1kT_0/l_{\text{min}}$, $c_0 = 1/l_{\text{min}}$, $w = 1kT_0$ at $T/T_0 = 0.6$ (see the last snapshot in the second line from below on Fig. 5a).

**Movie S2**: Animation of snapshots in steady-state of the system with $\rho = 5\%$ of curved active proteins with $F = 1kT_0/l_{\text{min}}$, $c_0 = 1/l_{\text{min}}$, $w = 1kT_0$ at $T/T_0 = 0.6$ (see the second snapshot in the second line from below on Fig. 5a).

**Movie S3**: Animation of snapshots in steady-state of the system with $\rho = 11\%$ of almost flat active proteins with $F = 1kT_0/l_{\text{min}}$, $c_0 = 1/(9l_{\text{min}})$, $w = 1kT_0$ at $T/T_0 = 0.7$ (see the top-left shape on Fig. 7d).