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Mechanical stability of membrane nanotubular protrusions influenced by attachment of flexible rod-like proteins

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ABSTRACT

It is indicated that nonhomogeneous lateral distribution of membrane attached and flexible rod-like proteins (MRPs) may stabilize nanotubular membrane protrusions. We have shown that curvature induced accumulation of MRPs in the nanotubular membrane protrusion and the corresponding reduction of the membrane free energy are possible if the decrease of the deviatoric free energy of MRPs in the nanotubular protrusions is large enough to overcome the increase of the free energy due to decrease of configurational entropy in the process of lateral sorting of MRPs. The decrease of isotropic curvature energy of MRPs in the region of membrane protrusion is usually not sufficient for substantial MRPs sorting and consequent stabilization of the nanotubular membrane protrusions.

1. Introduction

It has been shown that isotropic bending energy of lipids is not sufficient to stabilize tubular membrane protrusion and may also not contribute significantly to sorting of lipids between tubular membrane protrusions and parent membrane due to too strong decrease of configurational (mixing) entropy (Iglič et al., 2006; Tian and Baumgart, 2009). Very thin nanotubular protrusion of one component liposomes can be stabilized due to average orientational ordering of lipids in nanotubes (Kralj-Iglič et al., 2002) while in more complex bilayer membrane systems the lipid-lipid and lipid-protein direct interactions may result in formation of small membrane (in general anisotropic) nanodomains (clusters, inclusions) which can stabilize tubular membrane protrusion and also significantly contribute to lipid sorting (Kralj-Iglič et al., 2005; Iglič et al., 2006; Tian and Baumgart, 2009; Sorre et al., 2009).

The deformation of biological membranes can be also driven by proteins that are attached to the membrane surface (Iglič et al., 2007; Tian and Baumgart, 2009; Powel, 2009). A number of proteins have been identified that directly bind and deform biological membranes (Farsad and De Camilli, 2003; Bouma et al., 1999). The binding of proteins to the membrane surface, liposomes or lipoproteins may be driven by electrostatic forces and/or by penetration of the protein hydrophobic protrusions in the membrane bilayer (Farsad and De Camilli, 2003; Masuda et al., 2006). The proteins attached to the membrane surface may thus influence the elastic properties of membrane (Farsad and De Camilli, 2003; Zimmerberg and Kozlov, 2006; Iglič et al., 2007). In this work we shall analyze the influence of flexible membrane-attached 1-dimensional curved proteins (MRPs) on the mechanical stability of thin membrane tubular protrusions.

2. Theory

The membrane-attached proteins can be more or less rigid than cellular or lipid bilayer membranes (Nossal, 2001). In this work the membrane-attached proteins are considered as flexible elongated curved rod-like proteins having similar rigidity as a membrane bilayer. The limit of strong adhesion is assumed (Fig. 1).

The elastic (curvature) energy of MRP which is attached to the membrane surface is written in the form (Landau and Lifshitz, 1996):

$$E_1 = \frac{K_p L_0}{2}(C - C_p)^2, \quad (1)$$

where K_p is the flexural rigidity, C_p the intrinsic (spontaneous) curvature and L_0 the length of the protein. The curvature

$$C = H + D\cos(2\omega), \quad (2)$$

is the local membrane curvature seen by the MRP for a given rotation of the protein described by the angle ω between the normal plane in which the protein is lying and the plane of the first principal curvature $C_1 = 1/R_1$ (see Fig. 1), $D = (C_1 - C_2)/2$ and

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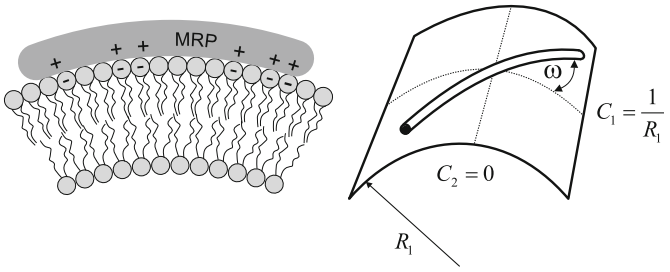

Fig. 1. Schematic figures of a flexible rod-like protein (MRP) strongly attached to a cylindrical membrane surface having $C_1 = 1/R_1$ and $C_2 = 0$, i.e. $H = D = 1/2R_1$. At a given value of the protein orientation angle ω the protein senses the curvature $C = (C_1+C_2)/2+((C_1-C_2)/2)\cos(2\omega)$ (see Eq. (2)).

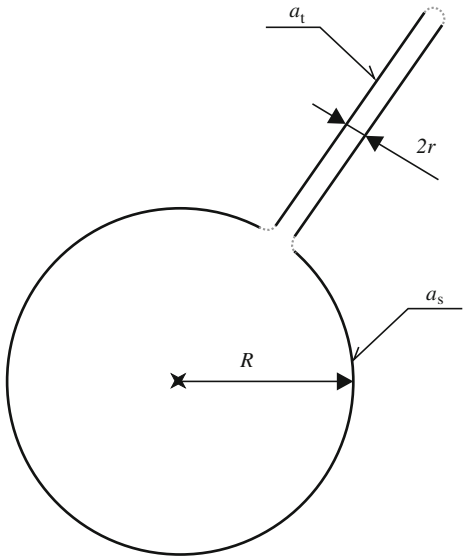

Fig. 2. Schematic model of the cell (vesicle) with tubular protrusion. One or more tubes with diameter $2r$ are growing from the cell (vesicle) with radius R. Due to simplification the model does not take into account membrane regions where the tubes attach to the spherical part and the curved top closing the tube (dashed gray lines).

$H = (C_1 + C_2)/2$ are the curvature deviator and the mean curvature at the given location on the membrane surface and C_1 and C_2 are the two principal curvatures, respectively.

If the rigidity of the MRPs is of the same order of magnitude or not much larger as the rigidity of the membrane, the shape of the membrane is the result of interplay between membrane and membrane-attached proteins. In this case the flexural rigidity of MRP is $K_pL_0 \sim 3k_c a_0^p \sim 10^{-35}\,\mathrm{J\,m^2}$ (Iglič et al., 2007), where a_0^p is the area of single attached protein and k_c the local membrane bending constant. In the case of lipid bilayers containing cholesterol the value of $k_c \simeq 10^{-19}$ J (Duwe et al., 1990 and our unpublished results).

In order to estimate the contribution of the MRPs to the total free energy of the bilayer membrane with tubular protrusions (Fig. 2) we shall first calculate the partition function of a single membrane attached protein:

$$Q = \frac{1}{\omega_0}\int_0^{2\pi} \exp\left(-\frac{E_1(\omega)}{kT}\right) \mathrm{d}\omega, \tag{3}$$

where different orientational states of the protein on the membrane surface were taken into account (see also Fig. 1), ω_0 is an arbitrary angle quantum, k is the Boltzmann constant and T is the absolute temperature. Combination of Eqs. (1)–(3) yields

$$Q = \frac{1}{\omega_0}\exp\left[-\frac{K_pL_0}{2kT}(H-C_p)^2\right] \cdot \int_0^{2\pi} \exp\left[\frac{K_pL_0}{2kT}(2D(C_p-H)\cos 2\omega - D^2\cos^2 2\omega)\right] \mathrm{d}\omega. \tag{4}$$

The free energy of the single MRP can be then obtained by the expression:

$$f_i = -kT\ln Q. \tag{5}$$

Our study is limited to a simple case of spherical cell (or spherical giant lipid vesicle) with tubular membrane protrusion(s) (Fig. 2), where MRPs (Fig. 1) can attach to the spherical part of the cell and also on the tubular protrusion(s). For nonzero flexural rigidity (K_p) the attached proteins have different energy on the spherical and on the tubular part of the cell. For the proteins which are attached on the spherical part of the cell with a constant mean curvature $H = 1/R$ and the curvature deviator $D=0$ (where R is radius of the spherical cell (vesicle), see Fig. 2) the free energy of the single MRP, derived by using Eqs. (4) and (5), is

$$f_{ps} = \frac{K_pL_0}{2}(H-C_p)^2. \tag{6}$$

For the proteins attached on the tubular membrane protrusions (where $H = D$, see also Figs. 1 and 2) Eqs. (4) and (5) yield

$$f_{pt} \cong \frac{K_pL_0}{2}(H-C_p)^2 + \frac{K_pL_0}{4}D^2 - kT\ln Q^{rot}, \tag{7}$$

where we omitted the constant term $-kT\ln(2\pi/\omega_0)$ and where $kT\ln Q^{rot} = \int_0^{2\pi}\exp[(K_pL_0D/kT)D(C_p-H)\cos 2\omega)]\mathrm{d}\omega$ (see Eq. (4)). The second term in Eq. (7) originates from $\int_0^{2\pi}\exp[-(K_pL_0D^2/2kT)\cos^2 2\omega]\mathrm{d}\omega$ in Eq. (4). Calculating $kT\ln Q^{rot}$ in Eq. (7) yields the following expression for the free energy of the single MRP on the tubular membrane protrusion (having $H = D$):

$$f_{pt} = \frac{K_pL_0}{2}(H-C_p)^2 + \frac{K_pL_0}{4}D^2 - kT\ln\left[I_0\left(\frac{K_pL_0D}{kT}(C_p-H)\right)\right], \tag{8}$$

where we again omitted the constant term $-kT\ln(2\pi/\omega_0)$.

Considering the configurational entropy of the MRPs we can write the normalized free energy of the MRPs for arbitrary shape of the cell membrane as

$$F = kT\int n\ln n\,\mathrm{d}a + kT\int(1-n)\ln(1-n)\mathrm{d}a + \int nf_i\,\mathrm{d}a = \int f\,\mathrm{d}a, \tag{9}$$

where $n(\varrho)$ is the fraction of the bilayer membrane area covered by attached proteins at given position $\varrho = (x,y,z)$, f_i is the free energy of the single MRP, a is the normalized (relative) area of the membrane (a=1) and $\mathrm{d}a$ is normalized area element. The integration is performed over the entire (normalized) area of the membrane surface. Definition of $n(\varrho)$ takes into account that the effective area occupied by single MRP (a_{ef}^p) allows the free rotation of MRP.

The fraction of the membrane area covered by MRPs $n(\varrho)$ varies over the membrane surface as a function of the membrane curvature. By taking into account the conservation equation for all attached proteins:

$$\int n\,\mathrm{d}a = \overline{n}, \tag{10}$$

where $\overline{n}$ is the average value of n, a functional is constructed:

$$\int(f+\lambda n)\,\mathrm{d}a = \int L(n)\,\mathrm{d}a, \tag{11}$$

where λ is the Lagrange parameter and energy density f is defined by Eq. (9). The variation is performed by solving the corresponding Euler equation $\partial L/\partial n = 0$ which gives the expression for the

function n:

$$n = \frac{\vartheta \exp(-f_i/kT)}{1+\vartheta \exp(-f_i/kT)}, \tag{12}$$

where $\vartheta = \exp(-\lambda)$. The parameter ϑ is determined from the condition Eq. (10).

In the following we shall continue with a simple case of spherical cell with tubular protrusion(s) (Fig. 2), relevant to discuss a possible physical mechanism which may explain the curvature induced accumulation of MRPs in the region of highly curved tubular membrane protusions and the contribution of these proteins to stabilize them. In the model the membrane is divided into two parts (Fig. 2), the spherical part with the relative area a_s and constant mean curvature $H=1/R$ ($D=0$) and the highly curved tubular part of the membrane protrusions with the constant mean curvature $H=D=1/2r$ (where r is the radius of tubular membrane protrusions), the relative area a_t and the fraction of the area covered by the attached proteins equal to n_t. The parameter ϑ can be then determined from the condition (see Eq. (10)):

$$n_s a_s + n_t a_t = \overline{n}, \tag{13}$$

where n_s is fraction of the area covered by MRPs on the spherical part. We should also take into account that $a_s + a_t = 1$. If the values of n_s and n_t are given by Eq. (12) it follows from Eq. (13):

$$\vartheta = [-\beta - (\beta^2 - 4\alpha\overline{n})^{1/2}]/2\alpha, \tag{14}$$

where $\alpha = \alpha_r\alpha_R(\overline{n} - a_s - a_t)$, $\beta = \overline{n}\alpha_r + \overline{n}\alpha_R - a_s\alpha_R - a_t\alpha_r$, $\alpha_R = \exp(-f_{ps}(H=1/R)/kT)$ and $\alpha_r = \exp(-f_{pt}(H=D=1/2r)/kT)$. In the above expressions the free energy of the single MRP (f_i) was calculated using expression (6) for the spherical part and expression (8) for the tubular part.

3. Results and discussion

For given values of model parameters we calculate the normalized free energy of the spherical cell (vesicle) with tubular protrusions of radius r as

$$F_m = a_t f_t + a_s f_s, \tag{15}$$

where $f_t = f(H=D=1/2r,\ n=n_t,\ f_i=f_{pt}) + w_b(H=D=1/2r)$ is the normalized energy area density on the tubular protrusions, while $f_s = f(H=1/R,\ D=0,\ n=n_s,\ f_i=f_{ps}) + w_b(H=1/R,\ D=0)$ is the normalized energy area density on the spherical part of the membrane. Here the expression for the energy area density due to attached proteins (f) follows from Eq. (9):

$$f = kTn\ln n + kT(1-n)\ln(1-n) + nf_i, \tag{16}$$

while the area density of the normalized bending energy of the bilayer membrane is (Kralj-Iglič et al., 2006)

$$w_b = \frac{k_c}{2}a_{ef}^p(2H)^2 + k_G a_{ef}^p(H^2 - D^2) - 2kT\frac{a_{ef}^p}{a_0^l}\ln\left[I_0\left(\frac{\xi}{kT}DD_m\right)\right]. \tag{17}$$

Parameter a_0^l is the area of a lipid molecule in the region of polar heads (Petrov and Derzhanski, 1976), ξ is effective interaction constant (Kralj-Iglič et al., 2002, 2006), $H^2 - D^2 = C_1C_2$ is the Gaussian curvature, $D_m \cong 10^8\ \mathrm{nm}^{-1}$ is the spontaneous curvature deviator (Kralj-Iglič et al., 2002), while k_c and k_G are the membrane local bending constant and the Gaussian saddle-splay constant, respectively (Evans and Skalak, 1980). For spherical part of the cell (vesicle) where $D = 0$ the deviatoric (i.e. the last) term in Eq. (17) is zero, thus Eq. (17) will turn into the expression for the area density of the normalized local bending energy of the membrane (Helfrich, 1974; Petrov and Derzhanski, 1976).

Fig. 3a shows the dependence of the normalized free energy of the spherical cell (vesicle) with a tubular protrusion (F_m) (Eq. (15)) on the radius of tubular protrusion (r) and spontaneous curvature of the attached proteins (C_p=1/8, 1/10, 1/12, 1/15 [nm^{-1}]). For comparison the corresponding normalized free energies of the spherical cell (vesicle) without the tubular protrusion (and with the area which equals to the area of the vesicle with protrusion) are also shown (dotted lines). The radius of such vesicle with re-merged (fused) protrusion is therefore $R_0 = R/\sqrt{a_s}$, while its normalized free energy is $F_s = f(H=1/R_0, D=0, n=\overline{n}, f_i=f_{ps}) + w_b(H=1/R_0, D=0)$. It is obvious that the energy of such spherical vesicle increases with C_p. It can be seen in Fig. 3a that there is a considerable free energy drawdown for small protrusion radii relative to the energy of a spherical vesicle without protrusion. Consequently for thin enough protrusion and large enough C_p it is not energetically

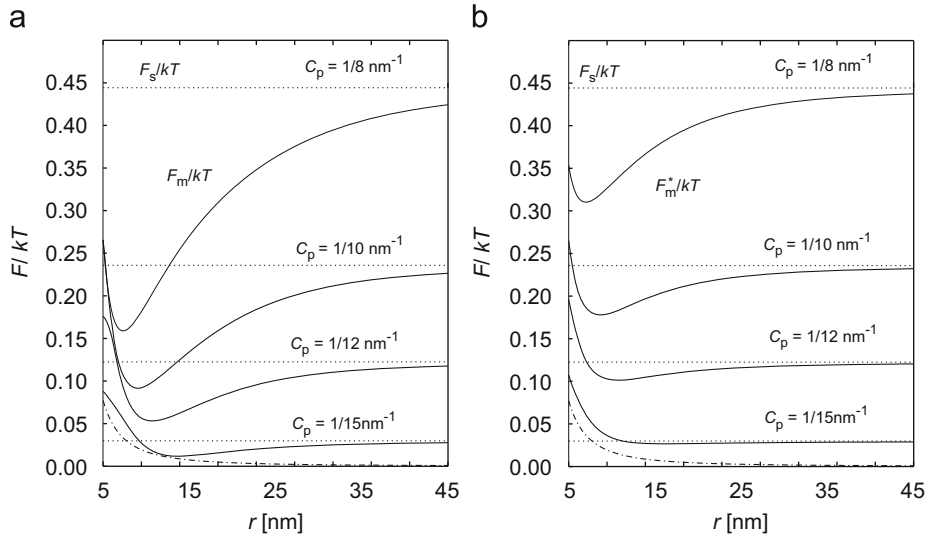

Fig. 3. (a) The normalized free energy of the spherical membrane surface with tubular protrusions (F_m) as a function of the radius of tubular protrusions (r) for different values of spontaneous curvature of attached proteins (C_p). (b) The free energy (F^*_m), computed by Eq. (8) without the last (deviatoric) term, as a function of r. For comparison to each $F_m(C_p)$ (continuous line) the corresponding free energy of spherical vesicle $F_s(C_p)$ (dotted line) is added in both panels. The dependency of the local bending energy (F_b) is also shown (dashed line down). The values of model parameters are $\overline{n} = 0.03$, a_s = 0.98, a_t = 0.02, $R = 20\,\mu\mathrm{m}$, $K_pL_0 = 10^{-35}\,\mathrm{J\,m^2}$, $k_ca_{ef}^p = 200kT\,\mathrm{nm^2}$, $k_G = -k_c$, $\xi = 12kT\,\mathrm{nm^2}$ (Kralj-Iglič et al., 2002) and $a_{ef}^p/a_0^l \simeq 20$.

favorable that tubular protrusion would re-merge (fuse) with the spherical vesicle. For comparison, the corresponding local bending energy $F_b = a_t w_b(H=D=1/2r) + a_s w_b(H=1/R, D=0)$ is also shown in Fig. 3.

Fig. 3b shows the free energy (F_m^*), computed by Eq. (8) without the last (deviatoric) term, as a function of r. One can see that the decrease in the free energy at small r is considerably larger when deviatoric term is taken into account (Fig. 3a).

The dependence of the fraction of the protrusion area covered by the attached proteins (n_t) (Eq. (12)) on the radius of protrusion (r) is presented in Fig. 4. The correspondence between Figs. 3a and 4 can be observed. The highest fraction of the protrusion area covered by MRPs corresponds to the lowest free energy (F_m). This effect may account for stabilization of tubular protrusion driven by accumulation of MRPs in the region of membrane tubular protrusions. It can be also seen in Fig. 3a that the highest effect on the lowering of free energy have proteins with $C_p = 1/8\,\mathrm{nm}^{-1}$ which corresponds to intrinsic curvature of some protein BAR domains (Peter et al., 2004). Moreover, for $C_p = 1/8$ and $1/10\,\mathrm{nm}^{-1}$ and appropriate values of radius of the tubular protrusion (r) the area fraction occupied by MRPs on the tubular membrane protrusions (n_t) may approach to unity, i.e. the proteins may occupy the whole tubular part of the cell (vesicle) membrane.

It should be stressed at this point that neglecting the deviatoric term (i.e. the last term) in Eq. (8) would considerably reduce the depth of the free energy minima (compare $F_m(r)$ in Fig. 3a and $F_m^*(r)$ in Fig. 3b). This indicates that the decrease of isotropic curvature energy of the MRPs (first term in Eq. (8)) in the region of membrane protrusion is for smaller C_p not large enough for substantial protein sorting and consequent stabilization of the nanotubular membrane protrusions. In this case only the decrease of the deviatoric free energy of the attached proteins with nonzero spontaneous curvature may overcome the increase of the free energy due to decrease of the configurational entropy as a consequence of lateral sorting of MRPs and thus stabilize the nanotubular membrane protrusion.

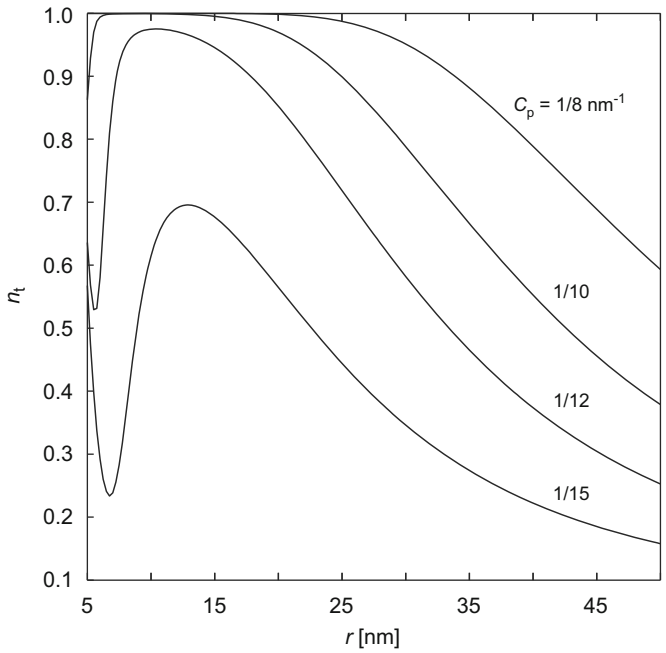

Fig. 4. The fraction of the area covered by the attached proteins (n_t) as a function of the radius of tubular protrusions (r) for different values of spontaneous curvature of the attached proteins (C_p). The values of the model parameters are the same as in Fig. 3.

The deviatoric term in Eq. (8) originates from the expression for the elastic energy of the attached MRP given by Eqs. (1) and (2) taking into account that the deformation of the protein attached to the anisotropic membrane surface (having $C_1 \neq C_2$) depends on its orientation angle ω (see Fig. 1). Neglecting the orientation dependent elastic (free) energy of the single attached MRP would omit from our theoretical description the influence of temperature dependent rotational movement of the attached proteins. Moreover, even in the limit of infinite temperature by neglecting Eq. (2) and replacing the curvature C in Eq. (1) by mean curvature $H = (C_1 + C_2)/2$ would lead to incorrect expression for the elastic (free) energy of a single attached MRP (see Eq. (8)). Similarly, also in the zero temperature limit such simplified expression for the elastic (free) energy of single attached MRP would not be correct for arbitrary value of C_p.

A possible experimental example of a system described in the present work may be membrane tethers of a giant unilamellar lipid vesicle (GUV) induced by attached spherical bead which is moved apart from the membrane by optical tweezers. Accumulation of MRPs in the region of GUV tubular protrusion (tether) could stabilize the tether so it may be stable also without or just with a very small external pulling force.

In cellular systems there are many examples of proteins whose attachment to the membrane surface has important physiological consequences. As for example FtsZ proteins in bacterias, which are short protofilaments constructing the Z ring incorporated in inner layer of the cell membrane (Osawa et al., 2008). Such proteins are proposed to have an important role in the initiation of the contractile ring in cells (Shlomovitz and Gov, 2008). Next examples of membrane attached proteins are for instance apolipoprotein B-100 and β_2−glycoprotein I (β_2−GPI) (Gamsjaeger et al., 2005). β_2−GPI (also known as apolipoprotein H) and apolipoprotein B-100 have elongated shape composed of distinct domains that are connected by linkers which allow them to change their orientation (Hammel et al., 2002; Moore et al., 1989; Di Scipio, 1992; Johs et al., 2006). Electrostatic character of β_2−GPI attachment to negatively charged membrane surface is attributed to large positively charged regions of the molecule (Balasubramanian and Scroit, 1998; Bouma et al., 1999; Hamdan et al., 2007). Atomic force microscopy has shown that the average height of β_2−GPI bound to supported lipid bilayers is approximately equal to the diameter of the elongated molecule which means that bound β_2−GPI has a horizontal-like orientation on the bilayer surface (Gamsjaeger et al., 2005; Hamdan et al., 2007) as schematically shown in Fig. 1.

In this work the effect of β_2−GPI attachment to the negatively charged membrane surface was tested experimentally in the suspension of negatively charged 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine(POPC)–cardiolipin–GUVs (Fig. 5) prepared by modified electroformation method (Angelova et al., 1992). It was previously indicated (Mathivet et al., 1996) but not also observed that GUVs prepared by the electroformation method are often connected by thin tubular membraneous structures. Later it was also shown that GUV nanotubular protrusion are usually invisible under the optical microscope; however, they could become visible if their diameter is sufficiently increased during shape transformation of GUVs (Kralj-Iglič et al., 2002). Fig. 5 shows that after addition of β_2−GPI into the suspension of GUVs (previously invisible) nanotubular connections between GUVs become visible. The effect is stronger if serum IgG (from a patient with antiphospholipid syndrome), containing antibodies against β_2−GPI (anti-β_2−GPI antibodies) are added along with β_2−GPI into the suspension of GUVs (Willems et al., 1996). This anti-β_2−GPI IgG antibodies target primarily membrane-attached β_2−GPI, can enhance β_2−GPI binding to negatively charged

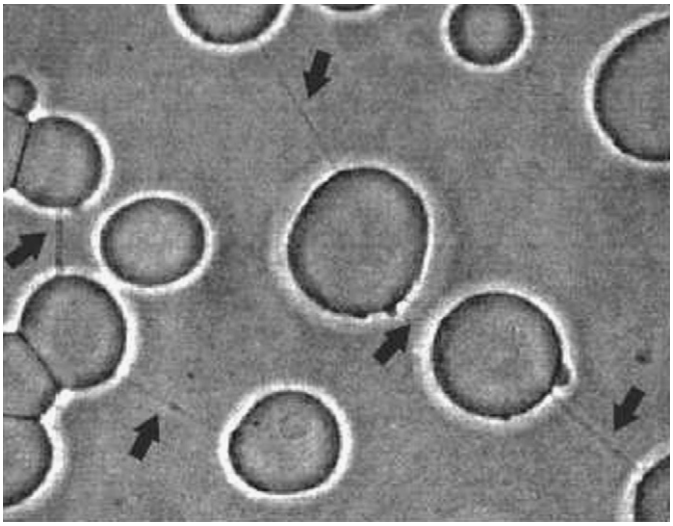

Fig. 5. Network of thin nanotubular connections (indicated by black arrows) between negatively charged POPC–cholesterol–cardiolipin giant unilamellar vesicles (GUVs) in the presence of β_2−GPI (100 mg/L) and serum IgG antibodies (75 mg/mL) from an antiphospholipid syndrome patient, containing antibodies against β_2−GPI. GUVs were observed under Zeiss Axiovert 200 Phase Contrast Microscope (Zeiss, Germany), magnification 1000x in 0.2 mol/L sucrose/glucose/PBS solution; pH 7.4; T = 37 °C; ionic strength 10 mmol/L. POPC:cholesterol:cardiolipin mass proportion in GUVs = 7:2:1.

membranes and can connect/dimerize two membrane attached β_2−GPI molecules. We suggest that in accordance with the described theoretical predictions (Fig. 4) β_2−GPI molecules are accumulated on the nanotubular connections and make them visible because of increased nanotube diameter. The stronger effect seen after addition of IgG antibodies may be explained by increased protein length(L_0) through dimerization of membrane attached β_2−GPI by anti-β_2−GPI IgG antibody (see also Eq. (8) for the role of L_0 in single MRP free energy).

Membrane tubular protrusions can be stable after their detachment from the mother (spherical) cell (Kralj-Iglič et al., 2005) or can change to the necklace-like shape which corresponds to maximal average mean curvature $\langle H\rangle = \frac{1}{A}\int H\,\mathrm{d}A$ of the detached vesicle at given area of the membrane surface A and a given volume enclosed by the vesicle membrane V as shown in Fig. 6. Initial tubular shape of the detached tubular membrane protrusion corresponds to the maximal possible average curvature deviator $\langle D\rangle = \frac{1}{A}\int D\,\mathrm{d}A$ and minimal possible $\langle H\rangle$ at given A and V as also shown in Fig. 6. The first and the last shape in Fig. 6 are obtained by the solution of the variational problem of the extreme average curvature deviator or extreme average mean curvature of the vesicle at given A and V (Iglič et al., 1999).

In the above described example of protein attachment to the membrane surface the domains of the proteins are assumed to be rigid while the flexibility of the protein is a consequence of the flexible linkers connecting the protein domains (units). In the limit of strong adhesion of the rigid attached proteins, the rigid domains may induce different local microscopic perturbations of the membrane shape around each of the attached rigid protein domain such as flexible rod-like protein schematically shown in Fig. 1 which might increase the local bending energy around the attached protein. In addition, the nonlocal bending energy of the membrane bilayer (Evans and Skalak, 1980 and Helfrich, 1974), which increases quadratically with the total number of membrane rigid domains of all MRPs, would also be increased (Iglič et al., 2007). Consequently, the attached protein having rigid domains may induce stronger membrane rigidification as flexible MRPs shown in Fig. 1.

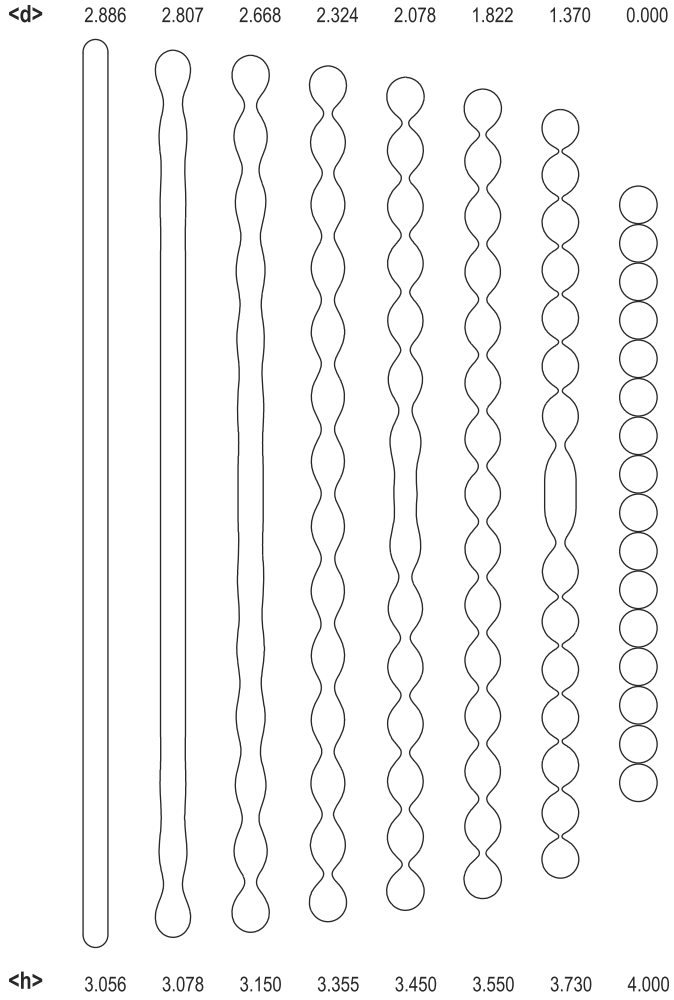

Fig. 6. The series of prolate vesicle shapes calculated by minimization of the local membrane bending energy (first two terms in Eq. (17)) as described by Iglič et al. (1999). The relative volume $v = 1/\sqrt{16}$. The corresponding values of $\langle h\rangle = R\langle H\rangle$ and $\langle d\rangle = R\langle D\rangle$ are also given. Here $R = \sqrt{A/4\pi}$, where A is the vesicle area.

In the present theoretical study we included also many other simplifications, as for example we did not take into account the role of the hydrophobic protrusion of the MRP like in the case of β_2−GPI (Bouma et al., 1999; Masuda et al., 2006; Iglič et al., 2007) which is embedded in the outer membrane layer and may increase the affinity of the attached proteins for highly curved membrane surfaces (Masuda et al., 2006) such as nanotubular membrane protrusions (Iglič et al., 2007). In addition we did not take into account the restrictions in packing and rotation of MRP at higher values of n_t where also direct interactions between MRPs become important. Instead the effective membrane area needed for the free rotation of a single attached protein (a_{ef}^{p}) was introduced. At high values of area density of MRPs, accumulated in the region of membrane nanotubular protrusions, MRPs may exhibit nematic ordering driven by orientation dependent curvature energy of MRP (Eqs. (1) and (2)) and direct interactions between MRPs.

Further, we assumed strong adhesion of MRPs to the membrane surface. In the case of binding (adhesion) of positively charged protein to negatively charged membrane surface (Fig. 2) the nature of attractive force is in large part electrostatic. To this end positively charged groups of attached protein may induce lipid demixing (Gamsjaeger et al., 2005), i.e. accumulation of negatively charged lipids (like phosphatidiylserine) below the

attached protein which may in some cases additionally facilitate the protein clustering predicted in Fig. 4 in order to reduce the line tension around lipid domains of negatively charged proteins.

In conclusion, it is shown that curvature induced accumulation of the MRPs in the nanotubular membrane protrusion and the corresponding decrease of the membrane free energy may in general occur if the decrease of the deviatoric free energy of the attached proteins in the nanotubular protrusions overcomes the increase of the free energy due to decrease of configurational entropy in the process of lateral redistribution of MRPs. The decrease of isotropic curvature energy of the MRPs (first term in Eq. (8)) in the region of membrane protrusion is usually not enough for distinct protein sorting and consequent stabilization of the nanotubular protrusions of the biological (lipid bilayer) membranes.

Conflict of interest statement

No conflict of interest.

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