ELSEVIER

Journal of Biomechanics 40 (2007) 579–585

JOURNAL OF BIOMECHANICS

www.elsevier.com/locate/jbiomech
www.JBiomech.com

On the role of anisotropy of membrane constituents in formation of a membrane neck during budding of a multicomponent membrane

Aleš Iglič[a,*], Blaž Babnik[a], Klemen Bohinc[a], Miha Fošnarič[a], Henry Hägerstrand[b], Veronika Kralj-Iglič[a,c]

[a]*Laboratory of Physics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia*
[b]*Department of Biology, Åbo Akademi University, Biocity, Åbo/Turku, Finland*
[c]*Institute of Biophysics, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia*

Accepted 9 February 2006

Abstract

The expression for the isotropic membrane bending energy was generalized for the case of a multicomponent membrane where the membrane constituents (single molecules or small complexes of molecules–membrane inclusions) were assumed to be anisotropic. Using this generalized expression for the membrane energy it was shown that the change of intrinsic shape of membrane components may induce first-order-like shape transitions leading to the formation of a membrane neck. The predicted discontinuous membrane shape transition and the concomitant lateral segregation of membrane components were applied to study membrane budding. Based on the results presented we conclude that the budding process might be driven by accumulation of anisotropic membrane components in the necks connecting the bud and the parent membrane, and by accumulation of isotropic (conical) membrane components on the bud. Both processes may strongly depend on the intrinsic shape of membrane components and on the direct interactions between them.

Keywords: Membrane biomechanics; Anisotropy; Membrane inclusions; Membrane energy

1. Introduction

In biological cells the budding process represents the first step in the formation of the small vesicles which are needed in transport of molecules through cell membrane and between cell organelles (Holthius et al., 2003). The budding process has also been observed in simpler artificial organic systems such as one-component or multicomponent lipid vesicles with or without protein inclusions (Sackmann, 1994; Holopainen et al., 2000; Staneva et al., 2005; Yamashita et al., 2002; Hanczyc and Szostak, 2004). The driving force for membrane budding can arise from different physical and biological mechanisms (Wiese et al., 1992; Lipowsky, 1992; Iglič et al., 1999; Kralj-Iglič et al., 1999; Yamashita et al., 2002; Hanczyc and Szostak, 2004; Peeters et al., 2005; Yin et al., 2005). In general, it goes beyond the simple mechanism of extension of a membrane but regulates the formation of a neck having a much higher (anisotropic) curvature than the bud (Kralj-Iglič et al., 1999; Huttner and Zimmerberg, 2001; Staneva et al., 2005). For example, it was observed that upon cholesterol depletion, vesicle biogenesis from the trans-Golgi network proceeds through formation of a membrane bud which does not develop a neck nor undergoes membrane fission (Wang et al., 2000). On the other hand, in some cases the tendency for formation of a very long neck connecting the daughter vesicle and the mother membrane was observed before fission (Gad et al., 1998; Huttner and Zimmerberg, 2001; Kralj-Iglič et al., 2001).

Recently, it has been indicated (Kralj-Iglič et al., 1999) that formation of the membrane neck during the

*Corresponding author. Tel.: +386 1 4768 825; fax: +386 1 4768 850.
E-mail address: ales.iglic@fe.uni-lj.si (A. Iglič).

0021-9290/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2006.02.006

budding process might be related to the anisotropic properties of membrane constituents. Accordingly, it has been shown that the formation of the membrane neck may be induced by lateral redistribution (diffusion) and accumulation of certain membrane components (lipids, proteins) in the highly curved and anisotropic region of the membrane neck which connects the daughter vesicle (bud) with the parent membrane (Sprong et al., 2000; Holthius et al., 2003). The formation of the membrane neck can thus be driven by coupling between the local membrane curvature and the local lateral molecular composition of the membrane (Kralj-Iglič et al., 1999; Shemesh et al., 2003).

In addition to the accumulation of anisotropic membrane components (domain formation) in highly curved regions of membrane necks, the budding process may also be driven by the formation of domains composed of membrane components with a preference for high spherical curvature. Such domains may be formed in the spherical regions of the buds/vesicles (Hägerstrand and Isomaa, 1994; Sackmann, 1994; Thiele et al., 1999; Laradji and Kumar, 2004; Hanczyc and Szostak, 2004; Staneva et al., 2005).

Accordingly it was recently observed that in multi-component unilamellar lipid vesicles, the membrane components can separate in coexisting domains with distinct composition and curvatures (Holopainen et al., 2000; Staneva et al., 2005). This process may resemble raft formation in cell membranes. The observed curvature-dependent domain sorting in lipid vesicles may result in membrane budding (fission) at domain boundaries (Laradji and Kumar, 2004; Staneva et al., 2005).

In this work, the role of coupling of the non-homogeneous lateral distribution of membrane constituents (inclusions) and the budding energetics is studied, in which the special attention is devoted to the influence of the anisotropic shape of membrane constituents (inclusions) and the direct interactions between them on neck formation during the budding process.

2. Theoretical model

For the sake of simplicity our theoretical analysis is limited to a case where buds and vesicles are depleted in membrane skeleton (Hägerstrand and Isomaa, 1994; Hägerstrand et al., 1999), therefore, the shear energy (Evans and Skalak, 1980; Iglič, 1997; Kosawada et al., 2005) of the membrane is neglected. We assume that the membrane constituents (single molecules or small complexes of molecules comprising a small number of molecules (Jacobson and Dietrich, 1999)—called membrane inclusions) as a result of their structure and local interactions energetically prefer a local geometry that is described by the two intrinsic principal curvatures ($C_{1m,i}$ and $C_{2m,i}$). The intrinsic principal curvatures are in general different ($C_{1m,i} \neq C_{2m,i}$) (Fig. 1). If they are identical ($C_{1m,i} = C_{2m,i}$), the in-plane orientation of the molecule or inclusion is immaterial. Such molecule or inclusion is called isotropic (Fig. 1). If $C_{1m,i} \neq C_{2m,i}$ the molecule or inclusion is called anisotropic (see Fig. 1). The small complex of molecules (inclusion) can have in general different intrinsic curvatures $C_{1m,i}$ and $C_{2m,i}$ from the intrinsic curvatures of the molecules which compose the inclusion (Fig. 2) (see also McMahon and Gallop, 2005).

The orientation of anisotropic molecule (inclusion) is important for its energy. It is assumed that anisotropic molecule (inclusion) will on the average spend more time

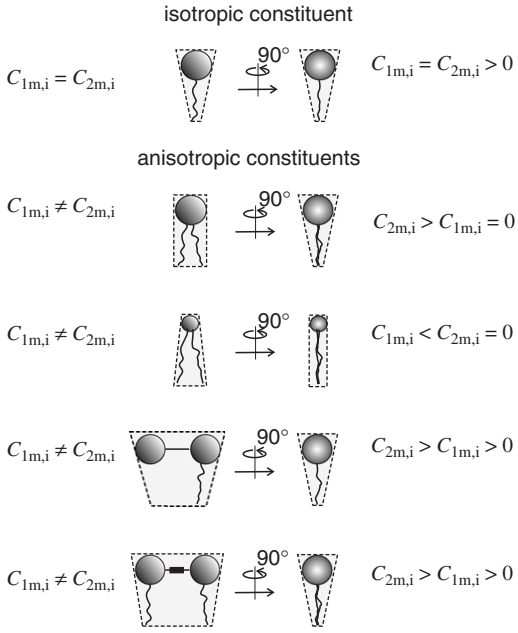

Fig. 1. Schematic representation of different intrinsic shapes of some membrane constituents. Front (left column) and side (right column) views are shown. Isotropic constituent: $D_{m,i} = (C_{1m,i} - C_{2m,i})/2 = 0$, anisotropic constituents: $D_{m,i} = (C_{1m,i} - C_{2m,i})/2 \neq 0$.

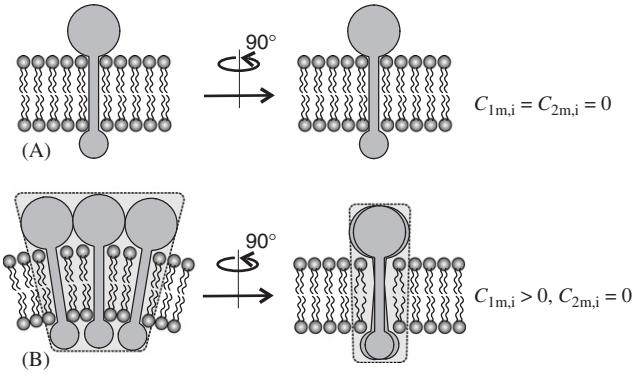

Fig. 2. Schematic presentation of the intrinsic shape of the membrane protein (A) and the membrane protein–lipid inclusion (B).

in the orientation that is energetically more favourable than in any other orientation. The free energy of a single membrane constituent (inclusion) of the ith kind can be written in the form (Kralj-Iglič et al., 1999; Fournier, 1996; Kralj-Iglič et al., 2002):

$$F_i = \frac{\xi_i}{2}(H - H_{m,i})^2 + \frac{\xi_i + \xi_i^\star}{4}(D^2 + D_{m,i}^2) - kT \ln\left(I_0\left(\frac{(\xi_i + \xi_i^\star)D_{m,i}D}{2kT}\right)\right), \quad (1)$$

where ξ_i and $\xi_i^\star$ are the interaction constants, $H = (C_1 + C_2)/2$ and $D = |C_1 - C_2|/2 = \sqrt{H^2 - C_1C_2}$ are the mean curvature and curvature deviator, and $H_{m,i}$ and $D_{m,i}$ are the intrinsic mean curvatures and intrinsic curvature deviators describing the intrinsic shape of the membrane constituents (inclusions) of the ith type, kT is the thermal energy and I_0 is the modified Bessel function.

Let us consider a single (sufficiently large) lipid layer of lateral area A and local mean and deviatoric curvatures H and D, respectively. The lipid layer contains three molecular (inclusion) species, N_1 molecules of type $i = 1$, N_2 molecules of type $i = 2$ and N_3 molecules of type $i = 3$. From the conservation of the overall number of constituents in the lipid layer (N), we express the number of molecules of first type N_1 as $N_1 = N - N_2 - N_3$. For the sake of simplicity we assume that all three species occupy the same lateral cross-sectional area $a = A/N$ per molecule (inclusion) on the lipid layer. The nature of the lipid bilayer allows its constituents to laterally redistribute. That is, in a non-homogeneously curved lipid layer, the molecules of all species are assumed to migrate towards their energetically preferred membrane regions so as to minimize the overall free energy. We describe this degree of freedom by the local compositions of the species, namely m_2, m_3 and $m_1 = 1 - m_2 - m_3$. In our model the local compositions m_1, m_2 and m_3 are equal to the local fractions of the membrane area covered by the molecules of type 1, 2 and 3, respectively. We denote the average compositions $\bar{m}_i = N_i/N$ as

$$\bar{m}_i = \frac{1}{A}\int_A m_i \, \mathrm{d}A. \quad (2)$$

We compose the free energy per molecule F of the lipid layer that contains the single-molecule energies (f_i), the corresponding configurational entropies (Kralj-Iglič et al., 1999, 2002) and also direct interactions between molecules (considered within the Bragg–Williams approximation) of the second type ($i = 2$)

$$\frac{F}{kT} = \frac{N}{A}\int_A \left\{\sum_{i=1}^{3}\left(m_i f_i + m_i \ln\frac{m_i}{\bar{m}_i} - m_i + \bar{m}_i\right) + \frac{wz_2}{2}m_2^2\right\}\mathrm{d}A, \quad (3)$$

where $f_i = F_i/kT$, w is the constant of direct interactions between molecules of type $i = 2$ (in units of kT) and z_2 is the corresponding coordination number. From this we can construct a Lagrangian of the form

$$L = \sum_{i=1}^{3}\left(m_i f_i + m_i \ln\frac{m_i}{\bar{m}_i} + \tilde{\lambda}_i(m_i - \bar{m}_i)\right) + \frac{wz_2}{2}m_2^2, \quad (4)$$

where $\tilde{\lambda}_i$ are the Lagrangian multipliers. Inserting into the Lagrangian the relations $m_1 = 1 - m_2 - m_3$ and $\bar{m}_1 = 1 - \bar{m}_2 - \bar{m}_3$, we then can eliminate one of the Lagrangian multipliers by defining $\lambda_2 = \tilde{\lambda}_2 - \tilde{\lambda}_1$ and $\lambda_3 = \tilde{\lambda}_3 - \tilde{\lambda}_1$. Using the Euler–Lagrange equations $\partial L/\partial m_2 = 0$ and $\partial L/\partial m_3 = 0$, we can derive an expression for composition m_3 in the form

$$m_3 = \bar{m}_3 \frac{m_2 \, \mathrm{e}^{f_2 - f_3 + wz_2 m_2}}{(1/A)\int m_2 \, \mathrm{e}^{f_2 - f_3 + wz_2 m_2}\,\mathrm{d}A}. \quad (5)$$

While m_1 can be obtained from $m_1 = 1 - m_2 - m_3$, the equation for m_2 is in general an integral equation that cannot be solved easily. However, for small direct interactions ($w \ll 1$) we can obtain (up to the first relevant term in w)

$$m_2 = m_2^0(1 - wz_2 m_2^0(1 - m_2^0)), \quad (6)$$

where

$$m_2^0 = \frac{\bar{m}_2 \, \mathrm{e}^{-f_2 - \lambda_2}}{\bar{m}_2 \, \mathrm{e}^{-f_2 - \lambda_2} + \bar{m}_3 \, \mathrm{e}^{-f_3 - \lambda_3} + \bar{m}_1 \mathrm{e}^{-f_1}} \quad (7)$$

is the value of m_2 for $w = 0$. Values for the Lagrangian multipliers λ_2 and λ_3 are obtained from constraints (2). In the above theoretical model of a three-component membrane all three molecular species can in general be anisotropic (by having a non-zero curvature deviator $D_{m,i}$).

3. Model predictions

3.1. Formation and stability of membrane neck

First, we shall study the influence of the anisotropic membrane components on the formation of the membrane neck for the special case of the undulated tubular membrane shape given by the function

$$r(z) = R_0 + u \cdot \sin(qz), \quad (8)$$

where r is the radial coordinate, z is the coordinate along the longitudinal axis, R_0 is the average radius, q is the wave number of the modulation and u is its amplitude. For the sake of simplicity we shall assume that the membrane contains only two molecular species, lipids ($i = 1$) and anisotropic inclusions ($i = 2$), while the third membrane component will not be considered, i.e. $m_3 = \bar{m}_3 = 0$. The lipid molecules which are in general anisotropic (Kralj-Iglič et al., 2002) are considered as isotropic. From the constraint for the surface area per

unit length (along the z-axis) $\int_A \mathrm{d}A = A$ the parameter R_0 can be expressed as a function of q and u:

$$R_0 = \frac{A - 2\pi u \int_0^l \sin qz \sqrt{1 + u^2 q^2 \cos^2 qz}\,\mathrm{d}z}{2\pi \int_0^l \sqrt{1 + u^2 q^2 \cos^2 qz}\,\mathrm{d}z}. \quad (9)$$

By using Eq. (8) the sum and the product of the main curvatures are calculated:

$$C_1 + C_2 = \frac{1 + u^2 q^2 + R_0 u q^2 \sin qz}{(R_0 + u \sin qz)(1 + u^2 q^2 \cos^2 qz)^{3/2}}, \quad (10)$$

$$C_1 C_2 = \frac{u q^2 \sin qz}{(R_0 + u \sin qz)(1 + u^2 q^2 \cos^2 qz)^2}. \quad (11)$$

From Eqs. (10) and (11) the average mean curvature $H = (C_1 + C_2)/2$ and the curvature deviator $D = \sqrt{H^2 - C_1 C_2}$ are calculated. The mean curvature and the curvature deviator derived from Eqs. (10) and (11) are then inserted into above equations. The Lagrange parameter λ_2 is calculated from constraint (2). Minimization of the free energy (Eq. (3)) with respect to the remaining two parameters q and u gives us values of q and u that correspond to the equilibrium shape having the minimal possible free energy of the membrane (F) for chosen values of model parameters. The minimization procedure was performed numerically.

The calculations were performed for membrane inclusions of saddle-like shape with $H_{m,2} = 0$ and $D_{m,2} \neq 0$ (Fig. 3). For small values of the intrinsic curvature deviator $D_{m,2}$, the tubular shape was shown to be energetically most favourable. With increasing $D_{m,2}$ a certain critical value of $D_{m,2}$ is reached where the tubular shape is changed discontinuously to an undulated shape with a narrow neck (Fig. 3). Fig. 3 shows a part of the calculated membrane shape for two values of the intrinsic curvature deviator $D_{m,2}$ above the critical value ($D_{m,2\mathrm{crit}} \approx 0.33\,\mathrm{nm}^{-1}$). As can be seen in Fig. 3, the saddle-like anisotropic inclusions accumulate in the energetically favourable saddle-like neck regions. With increasing values of the intrinsic curvature deviator of anisotropic inclusions ($D_{m,2}$), the neck becomes thinner.

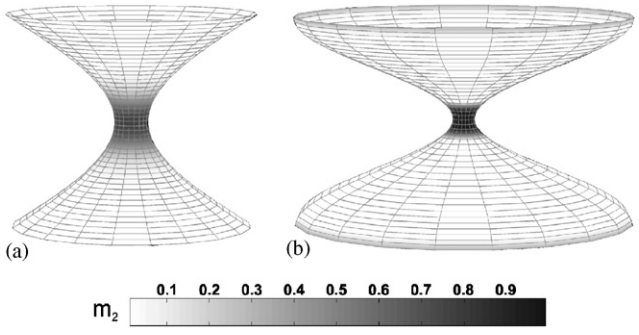

Fig. 3. Part of a membrane shape calculated for two values of $D_{m,2}$: $0.4\,\mathrm{nm}^{-1}$ (a) and $0.7\,\mathrm{nm}^{-1}$ (b). The shading of the surface of undulated tubular shape is proportional to the local fraction of the membrane surface occupied by the anisotropic inclusions of type 2 (m_2). The anisotropic inclusions are assumed to be distributed only in the outer layer of the membrane bilayer. The model parameters are $\xi_1 = \xi_1^\star = 12\,\mathrm{kT\,nm}^2$, $\xi_2 = \xi_2^\star = 120\,\mathrm{kT\,nm}^2$, $H_{m,2} = 0$, $\bar{m}_2 = 0.02$, $z_2 = 4$, $w = 0.1$, $l = 100$ nm, $A = 2\pi \cdot 4000\,\mathrm{nm}^2$.

3.2. Membrane budding

Based on the results presented in Fig. 3 we suggest that the anisotropic (saddle-like) membrane constituents (inclusions) with a preference for regions with saddle-curvatures may induce the formation of a membrane neck in the budding process. In addition to the accumulation of anisotropic membrane components in the membrane necks, the budding process may also be driven by the formation of domains composed of membrane components with a preference for high spherical curvature (Holopainen et al., 2000; Staneva et al., 2005). These may be formed in the spherical regions of the buds/vesicles. In accordance, it was observed that in a multicomponent membrane the membrane constituents may separate into coexisting domains with distinct composition, i.e. the raft formation takes place (Hägerstrand and Isomaa, 1994; Harder and Simons, 1997; Staneva et al., 2005).

To illustrate the role of the intrinsic shape of membrane constituents and their lateral distribution in membrane budding, we calculated the lateral distribution of the membrane components for the special case of an axisymmetric vesicle shape composed of a large mother cell (only partially shown in Fig. 4) and a small

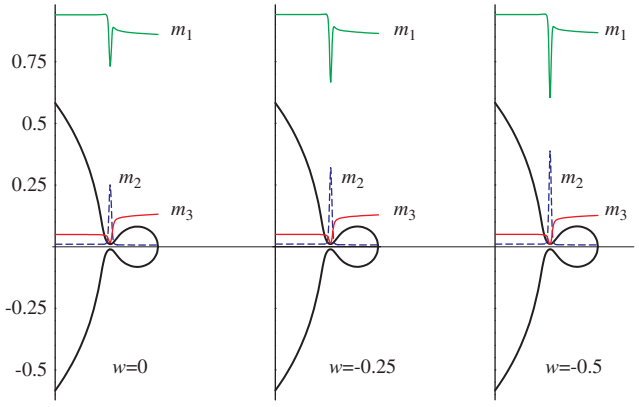

Fig. 4. Equilibrium lateral distribution of membrane components in the budding region of the bilayer membrane. Figure shows the space variation of the fractions of the membrane area occupied by the molecules of type i (m_i) (also called local compositions) for a three-component membrane (lipids: 1, anisotropic inclusions: 2, isotropic conical inclusions: 3) calculated for three values of the interaction constant describing the strength of direct interactions between anisotropic inclusions of type 2 (w). The values of m_i are dimensionless. The axisymmetric closed membrane shape has a relative average mean curvature of 1.075 and a relative volume 0.99. The values of the model parameters are $\xi_1 = 16\,\mathrm{kT\,nm}^2$, $H_{m,1} = D_{m,1} = 0$; $\xi_2 = 320\,\mathrm{kT\,nm}^2$, $H_{m,2} = 0$, $D_{m,2} = 0.3\,\mathrm{nm}^{-1}$, $\bar{m}_2 = 0.01$, $z_2 = 6$; $\xi_3 = 240\,\mathrm{kT\,nm}^2$, $H_{m,3} = 0.3\,\mathrm{nm}^{-1}$, $D_{m,3} = 0$, $\bar{m}_3 = 0.05$; $\xi_i^\star = 0$.

daughter vesicle connected by a thin neck (Fig. 4). The closed membrane shape given in Fig. 4 was calculated for an inclusion-free membrane, based on minimization of the Helfrich local bending energy as explained elsewhere (Iglič et al., 1999). We assume that the presence of the anisotropic inclusions in the membrane does not substantially affect the vesicle shape. This assumption is justified by our choice of membrane shape (Fig. 4) which is close to the limiting shape composed of two spherical vesicles connected by infinitesimally thin neck, i.e. a larger mother vesicle and a smaller daughter vesicle, and also by the choice of the small concentrations of membrane constituents (inclusions) of type 2 and 3.

The membrane is assumed to contain three molecular (inclusion) species; lipid molecules (type $i = 1$) which for the sake of simplicity are assumed to be isotropic (i.e. $D_{m,1} = 0$), anisotropic molecules (inclusions) of type $i = 2$ ($D_{m,2} \neq 0$) and isotropic conical molecules (inclusions) of type $i = 3$ ($D_{m,3} = 0, H_{m,3} > 0$) with a preference for a high spherical curvature. The molecules (inclusions) of type 2 and 3 are assumed to be distributed only in the outer layer of the membrane bilayer.

It can be seen in Fig. 4 that the anisotropic saddle-like membrane inclusions (m_2) are predominantly distributed in the region of the membrane neck, while the isotropic inclusions with a preference for a highly isotropically curved membrane (m_3) are predominantly accumulated on the spherical daughter vesicle (bud). Increase of m_2 with increasing interaction constant w indicates that the direct interactions between the inclusions may play an important role in the energetics of the budding process and clustering of membrane inclusions (i.e. raft formation).

4. Discussion and conclusion

The results presented in this paper may add to a better understanding of the mechanisms that are important for formation of highly curved membrane regions and for maintaining increased concentrations of certain membrane components in such regions, for example, in spherical parts of the vesicles (Thiele et al., 1999) and in vesicle necks (Holthius et al., 2003). In this work we propose that accumulation of some membrane components in membrane necks is coupled by in-plane ordering of these components in necks where the difference between the principal membrane curvatures (C_1 and C_2) is very large. Also, formation of the spherical part of the vesicles may be favoured by the specific intrinsic shape of membrane components. Small protein–phospholipid–cholesterol complexes (Thiele et al., 1999; Jacobson and Dietrich, 1999) are obvious candidates for such membrane components (inclusions). Anisotropic (lipid and cholesterol binding) proteins which form small anisotropic protein–lipid membrane inclusions (raft elements) may coalesce into larger domains (rafts) (Holthius et al., 2003) upon curvature-induced clustering in spherical parts of the vesicles (Thiele et al., 1999) as shown in Fig. 4.

Blocked biogenesis of synaptic-like vesicles after limited cholesterol depletion from the membrane protrusions indicates the importance of cholesterol (Thiele et al., 1999) as the interaction partner in the formation of small protein–phospholipid–cholesterol complexes (inclusions) and their clustering into larger rafts. The clustering of the membrane inclusions (raft elements) into larger rafts is probably additionally promoted by direct interaction between the inclusions (Jacobson and Dietrich, 1999), as presented also in Fig. 4. The short-range phospholipid (and cholesterol) mediated attractive interactions between membrane inclusions (Bohinc et al., 2003) may offer a possible explanation for the nature of such interactions. In the present work, the direct interactions between the anisotropic membrane constituents (inclusions) are considered within the Bragg–Williams approximation. Alternatively, the direct interactions between the membrane components can also be described by minimization of the boundary (line) tension between membrane domains (Lipowsky, 1992). However, considering the line tension assumes that the composition of the membrane domains is fixed in advance which is not in accordance with experimental observation (Hägerstrand and Isomaa, 1994) and does not allow study of the process of formation of membrane domains where the composition may be gradually changed.

In the past, an increased local concentrations of membrane components has also been predicted on highly curved tubular membrane protrusions (Kralj-Iglič et al., 2000; Corbeil et al., 2001). Coupling between the non-homogeneous lateral distribution of membrane components and the local anisotropic membrane curvature has also been recently indicated in Golgi bodies (Sprong et al., 2000; Iglič et al., 2004) in which some of the membrane components are concentrated mainly on the bulbous rims of the Golgi vesicles, where the difference between the two principal membrane curvatures (C_1 and C_2) is very high. Similar phenomena have also been observed in the photoreceptor discs (Corbeil et al., 2001) and flattened endovesicles of the erythrocyte membrane (Hägerstrand et al., 2004), indicating that the coupling between the non-homogeneous lateral distribution of membrane (generally anisotropic) components and specific membrane shapes may be a general mechanism of stabilization of highly curved membrane structures (flattened disc-like vesicles, spherical buds, necks, tubular protrusions) (Thiele et al., 1999; Hägerstrand et al., 2004; Markin, 1981; Iglič et al., 2004).

The predicted membrane shape changes due to the changed (increased) intrinsic curvature deviator of

anisotropic membrane components ($D_{m,i}$) can among other factors be applied to explain the membrane shape changes (inward and outward bending, budding) arising from conformational changes of membrane proteins induced by ligand binding or variation of ionic strength and pH (Gimsa and Ried, 1995).

In the above described theoretical consideration of the membrane budding we assumed that buds and released daughter vesicles are depleted in membrane skeleton. The presence of the membrane skeleton in the budding region would decrease the lateral mobility of the membrane constituents and thereby their ability to sort due to membrane curvature and/or direct interactions. In addition, the in-plane shear energy of the membrane skeleton would increase during the budding process (Hägerstrand et al., 1999) and the calculated shapes of membrane buds would be different from those presented in Fig. 4, especially in the vicinity of the neck region (Kosawada et al., 2005).

In predicting the equilibrium lateral distribution of membrane components in multicomponent membrane, we did not take into account different cross-sectional areas of membrane components (Shah, 1998). However, considering the different cross-sectional areas of membrane components, for example, within lattice statistics model (Manciu and Ruckenstein, 2002), would considerably increase the complexity of our theoretical model, but would not alter the basic conclusions of our work.

To conclude, based on the results presented in this work we suggest that the budding process might be driven by at least three different but complementary mechanisms: (1) by accumulation of anisotropic membrane components in the neck connecting the bud and the parent membrane; (2) by accumulation of isotropic conical membrane constituents (inclusions) in the region of the bud (i.e. daughter vesicle) (Holopainen et al., 2000; Sprong et al., 2001; Holthius et al., 2003) and (3) by a local increase of the area difference between the outer and inner lipid layer (Iglič and Hägerstrand, 1999; Staneva et al., 2005). Reports on the clustering of rafts and membrane proteins in highly curved membrane regions (invaginations) and vesicles ($H \approx \frac{1}{50}$ nm) (Hägerstrand and Isomaa, 1994; Harder and Simons, 1997) support these conclusions.

References

Bohinc, K., Kralj-Iglič, V., May, S., 2003. Interaction between two cylindrical inclusions in a symmetric lipid bilayer. Journal of Chemical Physics 119, 7435–7444.

Corbeil, D., Röper, K., Fargeas, C.A., Joester, A., Huttner, H.B., 2001. Prominin: a story of cholesterol, plasma membrane protrusions and human pathology. Traffic 2, 82–91.

Evans, E.A., Skalak, R., 1980. Mechanics and Thermodynamics of Biomembranes. CRC Press, Boca Raton, FL.

Fournier, L.B., 1996. Nontopological saddle-splay and curvature instabilities from anisotropic membrane inclusions. Physical Review Letters 76, 4436–4439.

Gad, H., Löw, P., Zotova, E., Brodin, L., Shupliakov, O., 1998. Dissociation between Ca^{2+}-triggered synaptic vesicle exocytosis and clathrin-mediated endocytosis at a central synapse. Neuron 21, 607–616.

Gimsa, J., Ried, C., 1995. Do band 3 protein conformation changes mediate shape changes of human erythrocyte? Molecular Membrane Biology 12, 247–254.

Hägerstrand, H., Isomaa, B., 1994. Lipid and protein composition of exovesicles released from human erythrocytes following treatment with amphiphiles. Biochimica et Biophysica Acta 1190, 409–415.

Hägerstrand, H., Kralj-Iglič, V., Bobrowska-Hägerstrand, M., Iglič, A., 1999. Membrane skeleton detachment in spherical and cylindrical microexovesicles. Bulletin of Mathematical Biology 61, 1019–1030.

Hägerstrand, H., Kralj-Iglič, V., Fošnarič, M., Bobrowska-Hägerstrand, M., Wróbel, A., Mrówczynska, L., Söderström, T., Iglič, A., 2004. Endovesicle formation and membrane perturbation induced by polyoxyethyleneglycolalkylethers in human erythrocytes. Biochimica et Biophysica Acta 1665, 191–200.

Hanczyc, M.M., Szostak, J.W., 2004. Replicating vesicles as models of primitive cell growth and division. Current Opinion in Chemical Biology 8, 660–664.

Harder, T., Simons, K., 1997. Caveolae, DIGs, and the dynamics of sphingolipid–cholesterol microdomains. Current Opinion in Cell Biology 9, 534–542.

Holopainen, J.M., Angelova, M.I., Kinnunen, P.K.J., 2000. Vectorial budding of vesicles by asymmetrical enzymatic formation of ceramide in giant liposomes. Biophysical Journal 78, 830–838.

Holthius, J.C., van Meer, G., Huitema, K., 2003. Lipid microdomains, lipid translocation and the organization of intracellular membrane transport (review). Molecular Membrane Biology 20, 231–241.

Huttner, W.B., Zimmerberg, J., 2001. Implications of lipid microdomains for membrane curvature, budding and fission. Current Opinion in Cell Biology 13, 478–484.

Iglič, A., 1997. A possible mechanism determining of stability of spiculated red blood cells. Journal of Biomechanics 30, 35–40.

Iglič, A., Hägerstrand, H., 1999. Amphiphile-induced spherical microexovesicles correspond to an extreme local area difference between two monolayers of the membrane bilayer. Medical and Biological Engineering and Computing 37, 125–129.

Iglič, A., Kralj-Iglič, V., Majhenc, J., 1999. Cylindrical shapes of closed lipid bilayer structures correspond to an extreme area difference between the two monolayers of the bilayer. Journal of Biomechanics 32, 1343–1347.

Iglič, A., Fošnarič, M., Hägerstrand, H., Kralj-Iglič, V., 2004. Coupling between vesicle shape and the non-homogeneous lateral distribution of membrane constituents in Golgi bodies. FEBS Letters 574 (1–3), 9–12.

Jacobson, K., Dietrich, C., 1999. Looking at lipid raft? Trends in Cell Biology 9, 87–91.

Kosawada, T., Inoue, K., Schmid-Schönbein, G.W., 2005. Mechanics of curved plasma membrane vesicles: resting shapes, membrane curvature, and in-plane shear elasticity. Journal of Biomechanical Engineering–Transactions of the ASME 127, 229–236.

Kralj-Iglič, V., Heinrich, V., Svetina, S., Zekš, B., 1999. Free energy of closed membrane with anisotropic inclusions. European Physical Journal B 10, 5–8.

Kralj-Iglič, V., Iglič, A., Hägerstrand, H., Peterlin, P., 2000. Stable tubular microexovesicles of the erythrocyte membrane induced by dimeric amphiphiles. Physical Review E 61, 4230–4234.

Kralj-Iglič, V., Iglič, A., Bobrowska-Hägerstrand, M., Hägerstrand, H., 2001. Tethers connecting daughter vesicles and parent red blood cell may be formed due to ordering of anisotropic membrane constituents. Colloids and Surfaces A 179, 57–64.

Kralj-Iglič, V., Iglič, A., Gomišček, G., Arrigler, A., Hägerstrand, H., 2002. Microtubes and nanotubes of phospholipid bilayer vesicles. Journal of Physics A: Mathematical and General 35, 1533–1549.

Laradji, M., Kumar, P.B.S., 2004. Dynamics of domain growth in self-assembled fluid vesicles. Physical Review Letters 93, 198105/1–4.

Lipowsky, R., 1992. Budding of membranes induced by intramembrane domains. Journal de Physique II (France) 2, 1825–1840.

Manciu, M., Ruckenstein, E., 2002. Lattice site exclusion effect on the double layer interaction. Langmuir 18, 5178–5185.

Markin, V.S., 1981. Lateral organization of membranes and cell shapes. Biophysical Journal 36, 1–19.

McMahon, H.T., Gallop, J.L., 2005. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438, 590–596.

Peeters, E.A.G., Oomens, C.W.J., Bouten, C.V.C., Bader, D.L., Baaijens, F.P.T., 2005. Mechanical and failure properties of single attached cells under compression. Journal of Biomechanics 38, 1685–1693.

Sackmann, E., 1994. Membrane bending energy concept of vesicle and cell shapes and shape transitions. FEBS Letters 346, 3–16.

Shah, D.O., 1998. Micelles, microemulsions, and monolayers: quarter century progress at the University of Florida. In: Shah, D.O. (Ed.), Micelles, Microemulsions, and Monolayers. Marcel Dekker, New York, pp. 1–52.

Shemesh, T., Luini, A., Malhotra, V., Burger, K.N.J., Kozlov, M.M., 2003. Prefission constriction of Golgi tubular carriers driven by local lipid metabolism: a theoretical model. Biophysical Journal 85, 3813–3827.

Sprong, H., van der Sluijs, P., van Meer, G., 2000. How proteins move lipids and lipids move proteins. Nature Reviews Molecular Cell Biology 2, 504–513.

Staneva, G., Seigneuret, M., Koumanov, K., Trugnan, G., Angelova, M.I., 2005. Detergents induce raft-like domains budding and fission from giant unilamellar heterogeneous vesicles. A direct microscopy observation. Chemistry and Physics of Lipids 136, 55–66.

Thiele, C., Hannah, M.J., Fahrenholz, F., Huttner, W.B., 1999. Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nature Cell Biology 2, 42–49.

Wang, Y., Thiele, C., Huttner, W.B., 2000. Cholesterol is required for the formation of regulated and constitutive secretory vesicles from trans Golgi network. Traffic 1, 952–962.

Wiese, W., Harbich, W., Helfrich, W., 1992. Budding of lipid bilayer vesicles and flat membranes. Journal of Physics Condensed Matter 4, 1647–1657.

Yamashita, Y., Masum, S.M., Tanaka, T., Tamba, Y., Yamazaki, M., 2002. Shape changes of giant unilamellar vesicles of phosphatidylcholine induced by a de novo designed peptide interacting with their membrane interface. Langmuir 18, 9638–9641.

Yin, Y., Chen, Y., Ni, D., Shi, H., Fan, Q., 2005. Shape equation and curvature bifurcation induced by inhomogeneous rigidities in cell membranes. Journal of Biomechanics 38, 1433–1440.