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ORIGINAL PAPER

Attachment of β_2-glycoprotein I to negatively charged liposomes may prevent the release of daughter vesicles from the parent membrane

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Abstract The temperature-induced budding of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol and POPC–POPG–cholesterol giant lipid vesicles in the presence of β_2-glycoprotein I (β_2-GPI) in the outer solution was studied experimentally and theoretically. The observed budding transition of vesicles was continuous which can be explained by taking into account the orientational ordering and direct interactions between oriented lipids. The attachment of positively charged β_2-GPI to the negatively charged outer surface of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol and POPC–POPG–cholesterol giant vesicles caused coalescence of the spheroidal membrane bud with the parent vesicle before the bud could detach from the parent vesicle, i.e. vesiculate. Theoretically, the protein-mediated attraction between the membrane of a bud and the parent membrane was described as an interaction between two electric double layers. It was shown that the specific spatial distribution of charge within β_2-GPI molecules attached to the negatively charged membrane surface may explain the observed attraction between like-charged membrane surfaces.

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Introduction

The budding of the bilayer membrane is a process, vitally important for cells (Greenwalt 2006; McMahon and Gallop 2005). Accordingly, it is of interest to understand mechanisms that are involved in the budding process (Miao et al. 1994; Sens and Turner 2004; Laradji and Kumar 2004; Božič et al. 2006; Iglič et al. 2007a, b; Sens and Gov 2007). An important role of membrane budding has been found in immune response and pathologic conditions (Farsad and De Camilli 2003; Greenwalt 2006). It was shown that membrane skeleton-detached, laterally mobile membrane lipids and integral membrane protein components or their small complexes (membrane nanodomains) may sort in highly curved spherical or tubular regions of cell membranes depending on their intrinsic shape and/or direct interactions between them (Farsad and De Camilli 2003; Huttner and Zimmerberg 2001; Holopainen et al. 2000; Hägerstrand et al. 2006; Iglič et al. 2007a). Clustering of membrane components or membrane nanodomains in highly curved membrane regions therefore plays an important role in generation and stabilization of spherical and tubular membrane protrusions (Farsad and De Camilli 2003; Huttner and Zimmerberg 2001; Iglič et al. 2007b).

To understand the basic physical properties of membrane budding, the budding process has been studied in a simple system of bilayer membrane vesicles composed of a single phospholipid species (Sackmann 1994; Lipowsky 1991; Miao et al. 1994) where changes of the temperature

may cause changes of the vesicle shapes and may in certain conditions induce formation of buds of the membrane bilayer (Käs and Sackmann 1991).

Certain proteins that are present in human plasma and are able to attach to membrane surface were found to mediate interactions between phospholipid-containing structures such as microvesicles and lipoproteins (Bevers et al. 2005; Distler 2005). It was shown that β_2-glycoprotein I (β_2-GPI) can attach to the negatively charged lipid bilayer surface (Asherson et al. 1989; Roubey et al. 2000) and can induce adhesion between negatively charged giant phospholipid vesicles containing cardiolipin (Ambrožič et al. 2006; Urbanija et al. 2007). β_2-GPI is also able to induce the agglutination of mitochondria (Schousboe 1979) whose membrane contains negatively charged cardiolipin and was found to bind to negatively charged (Khalil et al. 2006) low-density lipoprotein (LDL) nanoparticles (Polz and Kostner 1979), which contain the majority of cardiolipin in human plasma (Deguchi et al. 2000).

The majority of physiologic functions of β_2-GPI are associated with its binding to negatively charged lipids, such as phosphatidylserine and cardiolipin, demonstrated in the in vitro studies (Hagihara et al. 1995; Wang et al. 2002). The electrostatic interactions of β_2-GPI with negatively charged membrane phospholipids are considered crucial for its physiological and pathogenic roles. It was suggested that β_2-GPI may have an important function in blood coagulation, in the clearance of phosphatidylserine and cardiolipin-containing negatively charged apoptotic bodies from the circulation (Brighton et al. 1996; Balasubramanian and Schroit 1998) and in the membrane budding process (Urbanija et al. 2007). As β_2-GPI is a major autoantigen for the production of antiphospholipid antibodies, it may play an important role in many diseases including antiphospholipid syndrome (Bouma et al. 1999; Miyakis et al. 2004). Two subgroups of antiphospholipid antibodies represent the laboratory criteria for antiphospholipid syndrome: β_2-GPI dependent anticardiolipin antibodies (usually designed as anticardiolipin antibodies) and antibodies against β_2-GPI (Miyakis et al. 2006).

Our study was focused on the influence of β_2-GPI membrane binding on the budding transition of negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-phosphatidilcholine–cardiolipin–cholesterol (POPC–cardiolipin–cholesterol), POPC-1–palmitoyl-2-oleoyl-sn-glycero-phosphatidilserine–cholesterol (POPC–POPS–cholesterol) and POPC-1–palmitoyl-2-oleoyl-sn–glycero-phosphatidil-glycerol-cholesterol (POPC–POPG–cholesterol) giant vesicles. Using the negatively charged giant lipid vesicles we try to mimic the negatively charged surface of cells such as apoptotic cells and/or particles that have negatively charged lipids (phosphatidylserine or cardiolipin) in their outer membrane lipid layer.

In this work, the budding of negatively charged giant lipid vesicles was induced by increase of temperature and by binding of β_2-GPI to the outer lipid layer of the vesicle membrane (Fig. 1). In general, the binding of proteins to membrane surface may be driven by electrostatic forces and by penetration of protein hydrophobic protrusions in the membrane bilayer (Farsad and De Camilli 2003; Masuda et al. 2006). Positively charged proteins can bind to the negatively charged outer surface of cells, lipid vesicles or lipoproteins, where the electrostatic attractive forces may play a decisive role. In the case of β_2-GPI it was shown that the character of membrane–β_2-GPI interactions (binding) is at least partially of an electrostatic nature, i.e. it is governed by an electrostatic attraction between the positively charged fifth domain of β_2-GPI and negatively charged lipid headgroups (Willems et al. 1996; Gamsjaeger et al. 2005) (Fig. 1). Strong attractive interaction of highly positively charged fifth domain of β_2-GPI (Bouma et al. 1999) with the membrane surface is possible only if the membrane surface is negatively charged (Willems et al. 1996; Gamsjaeger et al. 2005). The strength of membrane–β_2-GPI interactions increases with increasing fraction of negatively charged lipids in the membrane, while it is suppressed by increased ionic strength in the solution (Gamsjaeger et al. 2005). The hydrophobic protrusion of membrane-attached β_2-GPI which is embedded in the outer membrane layer (Fig. 1) increases the strength of β_2-GPI membrane attachment (binding) (Bouma et al. 1999).

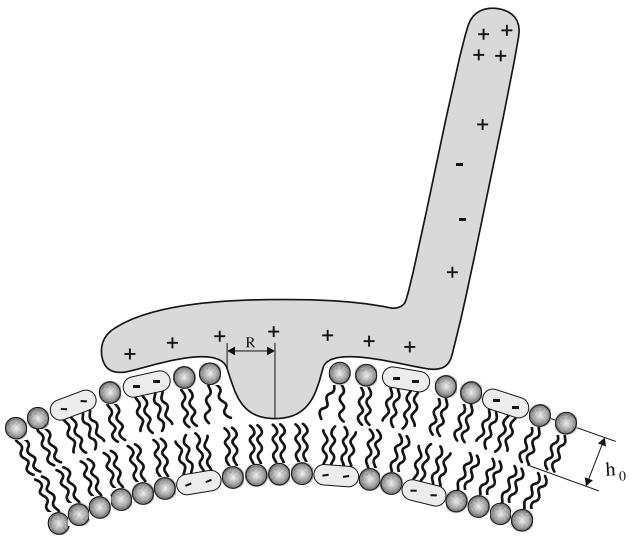

Fig. 1 Schematic figure of a positively charged β_2-GPI molecule attached to the outer surface of a negatively charged bilayer membrane of giant lipid vesicles containing negatively charged molecules of cardiolipin (bearing two negative unit charges each). The hydrophobic protrusion of the membrane attached β_2-GPI which is embedded in the outer lipid layer is also shown

In the present study, the attachment of β_2-GPI to the negatively charged outer surface of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol and POPC–POPG–cholesterol giant vesicles is studied experimentally and theoretically. Special attention is devoted to the budding process of the vesicle membrane which can be substantially influenced by the attachment of β_2-GPI. The presented results are discussed with respect to the general principles that govern the budding of biological membranes.

Material and methods

β_2-GPI

β_2-GPI (Hyphen BioMed, France) was aliquoted and stored at −70°C. In all experiments, the final concentration of β_2-GPI in solution containing giant lipid vesicles was 200 mg/L, which is the average physiological concentration of β_2-GPI in human plasma.

Giant lipid vesicles

Giant lipid vesicles (GVs) were prepared at room temperature (23°C) by modified electroformation method originally proposed by Angelova et al. (1992). The synthetic lipids cardiolipin (1,1′,2,2′-tetraoleoyl cardiolipin), POPC, POPG, POPC and cholesterol were purchased from Avanti Polar Lipids, Inc., Appropriate volumes of POPC, negatively charged phospholipid (either cardiolipin, POPS or POPG) and cholesterol, all dissolved in a 2:1 chloroform/methanol mixture, were combined in a glass jar and thoroughly mixed. For negatively charged giant lipid vesicles, POPC, cholesterol and negatively charged phospholipid (either cardiolipin, POPS or POPG) were mixed in the proportion of 2:2:3. Cholesterol was added to phospholipid mixture to increase the longevity of vesicles. Ten microliter of lipid mixture was applied to platinum electrodes. The solvent was allowed to evaporate in a low vacuum for 2 h. The coated electrodes were placed in the electroformation chamber which was then filled with 3 mL of 0.2 M sucrose solution. An AC electric current with an amplitude of 5 V and a frequency of 10 Hz was applied to the electrodes for 2 h, which was followed by 2.5 V and 5 Hz for 15 min, 2.5 V and 2.5 Hz for 15 min and finally 1 V and 1 Hz for 15 min. The content was rinsed out of the electroformation chamber with 5 mL of 0.2 M glucose and stored in a plastic test tube. The vesicles were left for sedimentation under gravity for 1 day at 4°C. About 200 to 400 mL of the sediment was collected from the bottom of the tube and used for a series of experiments. Before placing the vesicles into the observation chamber, the sample was gently mixed.

Observation

Vesicles were observed by a Zeiss Axiovert 200 inverted microscope with phase contrast optics (objective magnification 100×) and recorded by a Sony XC-77CE video camera. The solution containing vesicles was placed in an observation chamber made from cover glasses sealed with grease. The larger (bottom) cover glass was covered by two smaller (18 × 18 mm glasses), each having a small semicircular part removed at one side. Covering the bottom glass with two opposing smaller glasses thus formed a circular opening in the middle of the observation chamber. In all experiments the solution of vesicles (45 μL) was placed in the observation chamber. The solution containing β_2-GPI (5 μL) was added through the circular opening in the middle of the observation chamber. The osmolarity of the sample containing vesicles was 205 mosm/L (measured by a Knauf Semiosmometer). The observation chamber was mounted on a temperature-regulated microscope stage. The budding of the vesicles was induced by increasing the temperature and by binding of β_2-GPI to the outer surface of the membrane bilayer.

Experimental results

Figures 2 and 3 show the process of transformation of POPC–cardiolipin–cholesterol vesicle induced by increasing the temperature in the presence of β_2-GPI in the outer solution. Figure 2 shows the first stage of vesicle shape transformation where the shape of POPC–cardiolipin–cholesterol vesicle continuously changes from the initial pear-like shape to the limiting shape composed of larger spheroidal parent vesicle and spheroidal daughter vesicle which are connected by a thin neck. In the second stage the presence of β_2-GPI in the outer solution induces coalescence of spheroidal daughter bud with the parent vesicle membrane before it could detach from it (Fig. 3).

The same combined effect of increasing temperature and β_2-GPI on the budding process of giant lipid vesicles was observed also in the case of POPC–POPS–cholesterol (Fig. 4) and POPC–POPG–cholesterol giant vesicles (Fig. 5). Figures 4 and 5 thus show the second stage of temperature-induced vesicle budding in the presence of β_2-GPI where the spheroidal daughter bud is agglutinated to the membrane of the parent vesicle.

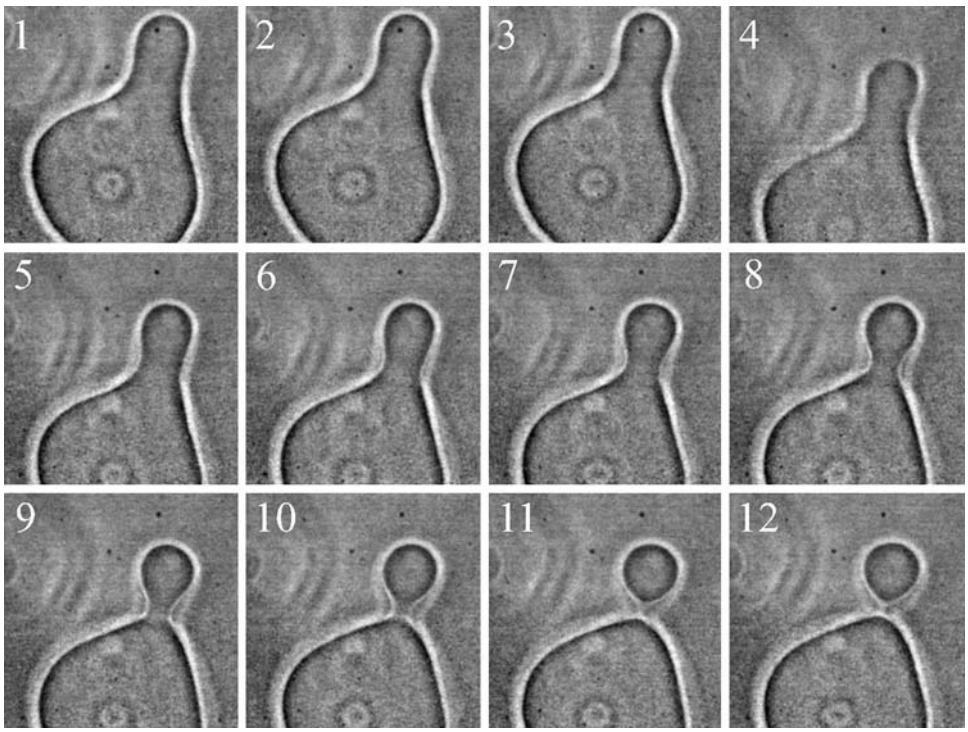

Fig. 2 Budding of POPC–cardiolipin–cholesterol vesicle induced by increasing the temperature in the presence of β_2-GPI in the outer solution: the first stage

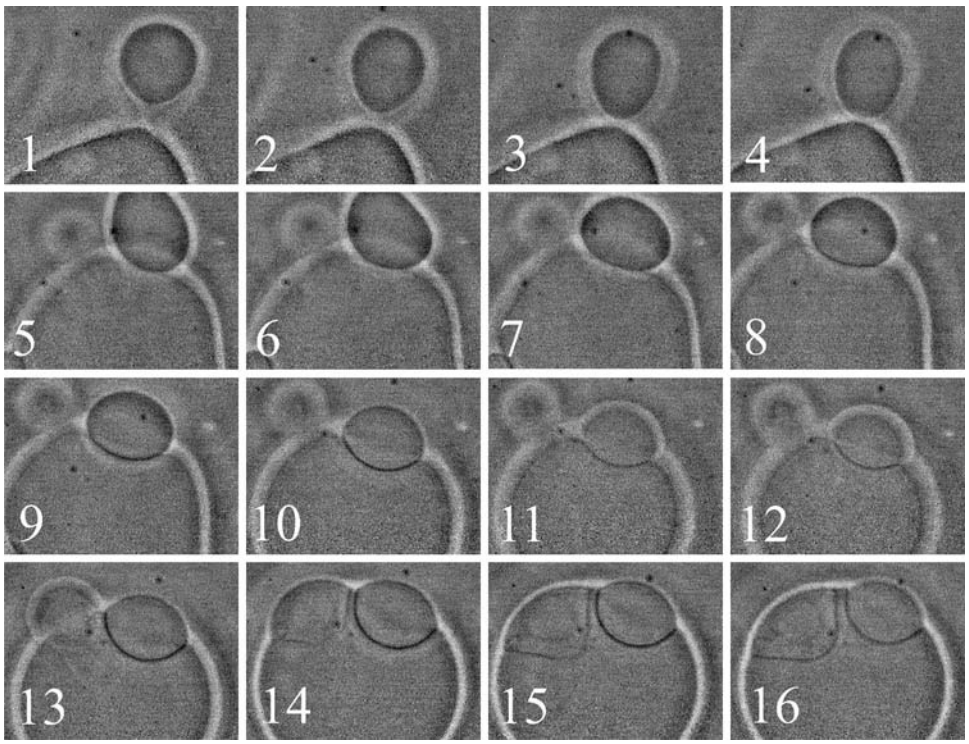

Fig. 3 Budding of POPC–cardiolipin–cholesterol vesicle induced by increasing the temperature in the presence of β_2-GPI in the solution: the second stage. Adhesion of the daughter vesicle to the outer surface of the mother membrane can be observed

Theoretical discussion

Budding transition

In the observed budding transition of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol and POPC–POPG–cholesterol vesicles the initial pear-like shape continuously (smoothly) transforms into a limiting shape composed of a larger spheroidal parent vesicle and a smaller spheroidal daughter vesicle which are connected by a thin neck (Fig. 2). In general, similar previously observed temperature-induced budding of liposomes was continuous or

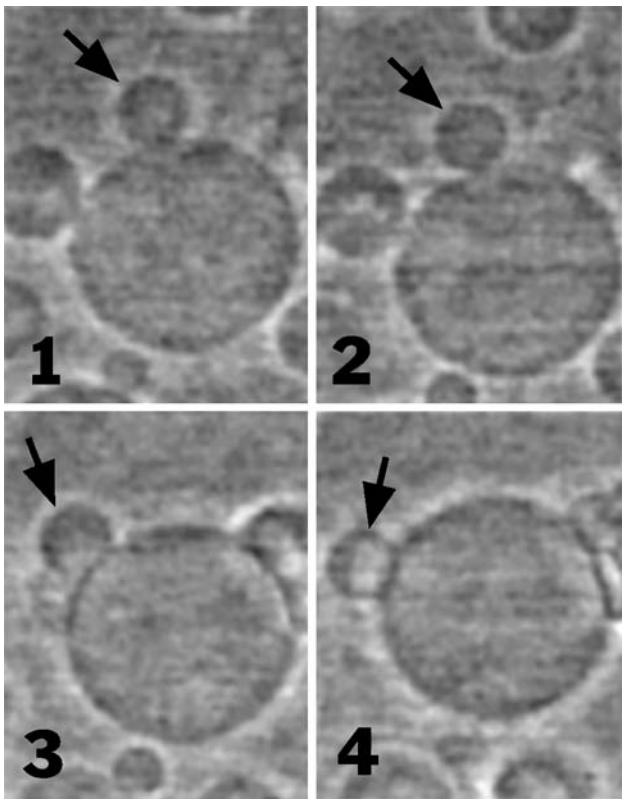

Fig. 4 Budding of POPC–POPS–cholesterol vesicle induced by increasing the temperature in the presence of β_2-GPI in the solution: the second stage. The *black arrows* show the daughter vesicle which is in the final phase adhered to the mother membrane

discontinuous (Käs and Sackmann 1991). It was observed that vesicles formed of charged lipids can undergo continuous transitions to an outside budded state via the pear shape, while in pure vesicles made from POPC or DMPC the budding seems to be discontinuous in the vicinity of limiting shapes (Käs and Sackmann 1991). The area-difference-elasticity (ADE) model, based on the minimization of the local and non-local bending energy (Evans and Skalak 1980; Stokke et al. 1986; Helfrich 1974), cannot explain the above-mentioned continuous shape transitions and/or discontinuity in the vicinity of limiting shapes (Miao et al. 1994). Namely, according to predictions of the ADE model the shape transition from the pear-like shape to the limiting shape composed of larger spheroidal parent vesicle and smaller spheroidal daughter vesicle is always discontinuous (Fig. 7a).

In accordance with the results of some previous studies (Kralj-Iglič et al. 2002, 2006) in this work the continuous transition and a discontinuity in the vicinity of the limiting shape are explained by taking into account the orientational ordering and direct interactions between oriented lipids. The intrinsic properties of membrane lipids and interactions between them are thereby assumed to influence the macroscopic features such as the equilibrium shape of the vesicle and/or the budding process of the membrane (Kralj-Iglič et al. 2006).

We assume that a single lipid molecule, due to its structure and local interactions, energetically prefers a local geometry that is described by the two intrinsic principal curvatures C_{1m} and C_{2m} (Kralj-Iglič et al. 2006). The intrinsic principal curvatures are in general not identical (Fig. 6). If they are identical ($C_{1m} = C_{2m}$), the in-plane orientation of the lipid molecule is immaterial. Such lipid molecule is called isotropic. If $C_{1m} \neq C_{2m}$ the lipid molecule is called anisotropic (Fig. 6). The orientation of such lipid molecule is important for its energy. It was assumed that the lipid molecule will spend on the average more time in the orientation that is energetically more favourable than in any other orientation (Kralj-Iglič et al. 2006).

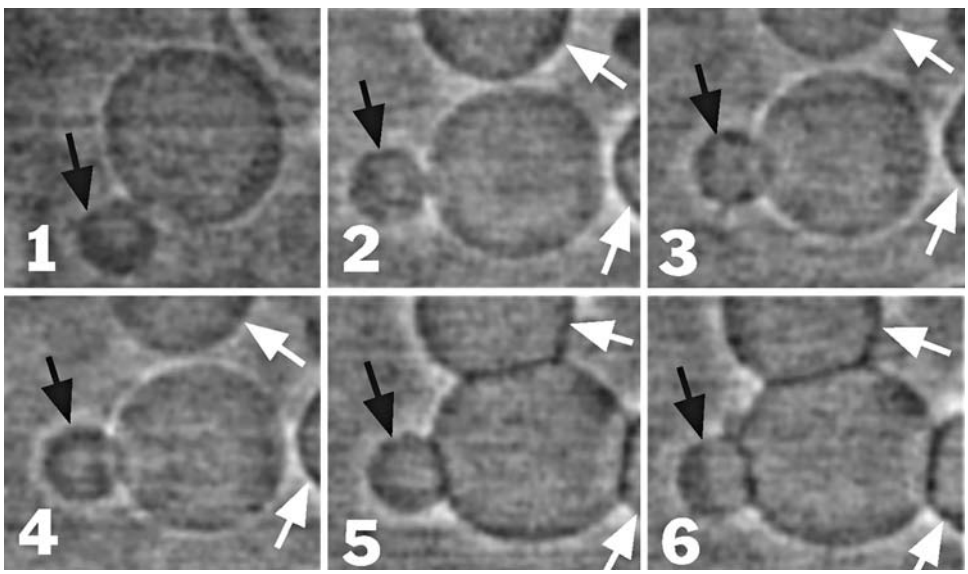

Fig. 5 Budding of POPC–POPG–cholesterol vesicle induced by increasing the temperature in the presence of β_2-GPI in the solution: the second stage. The *black arrows* show the daughter vesicle which is in the final phase adhered to the mother membrane, while *white arrows* show the two neighbouring vesicles which are also adhered to the observed vesicle

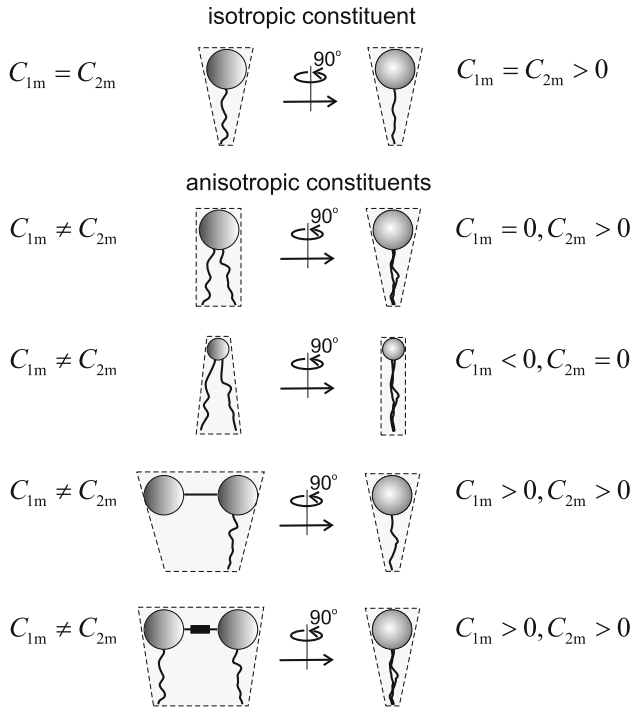

Fig. 6 Schematic representation of different intrinsic shapes of some isotropic and anisotropic membrane constituents. Front and side views are shown

Taking into account the orientational ordering and direct interactions between oriented lipids the expression for the free energy of a closed bilayer lipid vesicle F can be written in the form (Kralj-Iglič et al. 2006):

$$F = \frac{k_c}{2}\int (2H)^2 \mathrm{d}A + 2k_r A(\langle H\rangle - H_0)^2 - 2m_0 kT \int \ln\cosh\left(\frac{(\xi+\xi^*)D_m D}{2kT}\left(1+\tilde{k}/kT\right)\right)\mathrm{d}A. \quad (1)$$

The first term in Eq. 1 yields the local bending energy of a closed lipid bilayer vesicle (Helfrich 1973). The second term accounts for the nonlocal isotropic bending elasticity of the lipid bilayer membrane (Evans and Skalak 1980; Miao et al. 1994) while the third term accounts for the orientational ordering of lipid molecules which includes also the direct interaction contribution. Here $k_c = (3\xi + \xi^*)$ m_0 /4 is the local bending constant, $H = (C_1 + C_2)/2$ is the mean curvature of the membrane, $\langle H\rangle = \frac{1}{A}\int H\mathrm{d}A$ is average mean curvature, A is the membrane area, dA is the membrane area element, $D = (C_1 - C_2)/2$ is curvature deviator, C_1 and C_2 are two principal curvatures, C_{1m} and C_{2m} are intrinsic principal curvatures describing the intrinsic shape of the lipid molecules (see Fig. 6), $H_m = (C_{1m} + C_{2m})/2$ is intrinsic mean curvature, $D_m = (C_{1m} - C_{2m})/2$ is intrinsic curvature deviator, ξ and ξ^* are interaction constants, m_0 is the average area density of the lipid molecules, k_r is the nonlocal bending constant and H_0 determines the average mean curvature of the membrane under lowest possible stress. The constant $\tilde{k} \geq 0$ describes the direct interactions between locally oriented lipid molecules (Kralj-Iglič et al. 2006). The value $\tilde{k} = 0$ corresponds to the case when direct interactions are not taken into account.

The binding of β_2-GPI to the outer lipid monolayer results in an increase of its area which is the consequence of the incorporation of the hydrophobic part of the membrane bound β_2-GPI in the outer lipid monolayer (see Fig. 1). Consequently, the binding of β_2-GPI facilitates the observed budding of giant lipid vesicles induced by increase of the temperature.

For thin and not too strongly curved lipid bilayers the average mean curvature $\langle H\rangle$ is proportional to the area difference between the two membrane monolayers (ΔA): $\langle H\rangle = \Delta A/2A\delta$, where δ is the distance between the two monolayer neutral surfaces. Therefore, we can write the spontaneous average mean curvature H_0 as a function of the total number of membrane β_2-GPI molecules bound in the outer layer (M) as

$$H_0 = \overline{H}_0 + M\pi R^2/2A\delta, \quad (2)$$

where R is the average radius of the hydrophobic protrusion of the attached β_2-GPI which is embedded in the outer lipid layer (Fig. 1). The value of $\overline{H}_0$ increases with increasing temperature (Käs and Sackmann 1991).

The equilibrium configuration of the closed axisymmetric bilayer vesicles and the corresponding distribution of the quadrupolar ordering was determined by minimizing the membrane free energy F under relevant geometrical constraints as described in detail elsewhere (Iglič et al. 1999; Božič et al. 2006; Iglič and Kralj-Iglič 2006). We require that the normalized membrane area $a = A/4\pi R_s^2 = 1$ and normalized enclosed volume $v = 3V/4\pi R_s^3$ are fixed. For the sake of simplicity we considered only the pear-shaped axisymmetric vesicle shapes. The corresponding shape equation was solved as described in Kralj-Iglič et al. (2006).

The equilibrium vesicle shapes as a function of normalized spontaneous average mean curvature $h_0 = R_s H_0$ was calculated for given relative cell volume v. Figure 7 thus shows the equilibrium value of normalized average mean curvature $\langle h\rangle = R_s\langle H\rangle$ as a function of h_0. Case A corresponds to area-difference-elasticity (ADE) model, where the free energy consists of the local and nonlocal isotropic bending energy terms only (first two terms in Eq. 1), while in cases B–D we took into account also quadrupolar ordering of lipid molecules and the direct interactions between them ($\tilde{k}/kT \neq 0$).

Within so-called area-difference-elasticity (ADE) model the absolute minimum of the membrane free energy may be

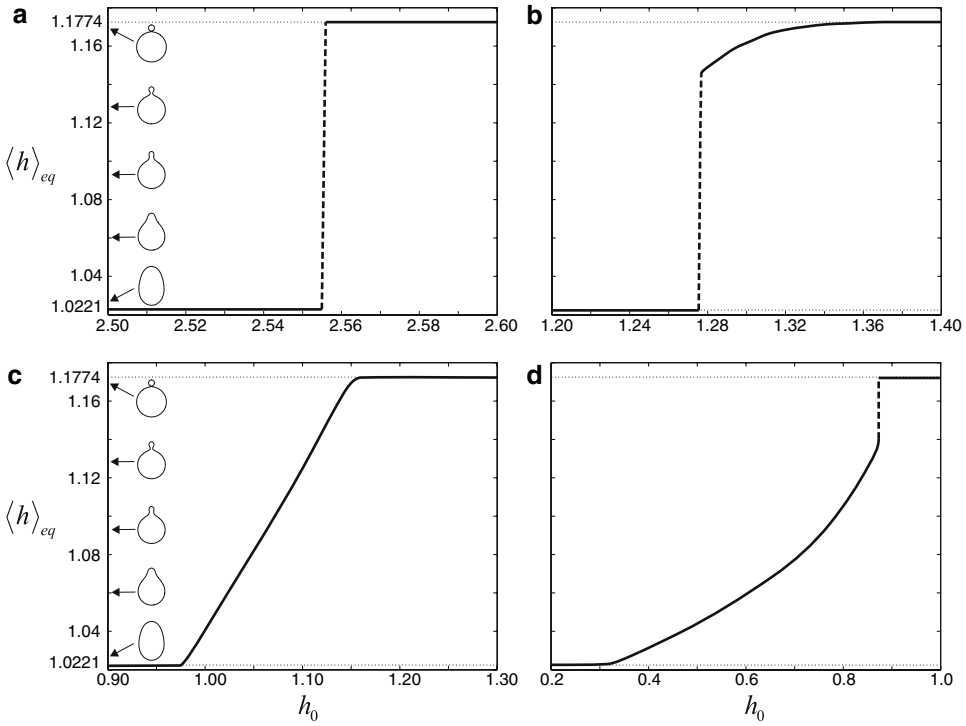

Fig. 7 Calculated normalized average mean curvature corresponding to the minimum of the membrane energy ($\langle h\rangle_{eq} = R_s \langle H\rangle_{eq}$) as a function of h_0 for pear-shaped axisymmetric vesicle shapes with a relative volume $v = 0.95$ and $k_r/k_c = 2$ (Hwang and Waugh 1997). **a** Corresponding dependency of $\langle h\rangle_{eq}$ within ADE model where the orientational ordering of interacting lipid molecules is not taken into account. Calculated dependencies in **b**–**d** take into account the orientational ordering of lipids and direct interactions between oriented lipids, where $(\xi + \xi^*) D_m/4kTR_s = 1.5 \times 10^{-4}$, $4kTR_s^2/(3\xi + \xi^*) = 7 \times 10^6$ (Kralj-Iglič et al. 2006) and $\tilde{k}/kT = 0.67$ (**b**), 0.8 (**c**) and 1.0 (**d**). Figure also shows some characteristic vesicle shapes corresponding to different values of $\langle h\rangle$

by an appropriate choice of the parameters k_r/k_c and H_0 shifted to the limit shape of pear-shaped vesicle (composed of the two spheres connected by an infinitesimal neck). However, a considerably higher value of k_r/k_c than the experimentally estimated one (Hwang and Waugh 1997) is needed to obtain this effect (Miao et al. 1994) or alternatively the values of H_0 should be larger than any $\langle h\rangle$ within the sequence of pear shapes (Fig. 7a). This gives a significant increase of the membrane free energy and of the lateral tension within the membrane. It seems unlikely that the vesicle would favour high lateral membrane tension within the membrane as it may develop processes to relax it, as for example transient pore formation (Raphael and Waugh 1996; Holopainen et al. 2002).

We have shown that a decrease of the free energy due to the orientational ordering of phospholipid molecules and direct interactions between oriented lipid molecules may complement the nonlocal isotropic bending in stabilizing pear vesicle shapes, including shapes with thin neck(s). It should be also noted that the calculated equilibrium vesicle shapes determined by minimization of the membrane free energy given by Eq. 1 can have also a wider neck (see Fig. 7c) corresponding to deep minima of the membrane free energy that would exceed the energies of thermal fluctuations. Minimization of the membrane free energy given by Eq. 1 predicts the continuous transition between pear-shaped vesicles (Fig. 7c) or discontinuity in the vicinity of limiting shapes composed of spherical parent cell and spherical daughter vesicle (Fig. 7d) which is not possible within the ADE model (Käs and Sackmann 1991; Miao et al. 1994) where the transition is always discontinuous (Fig. 7a).

To conclude, including the orientational ordering of lipids and their direct interactions in calculating the equilibrium vesicle shapes may render a minimum of the vesicle membrane free energy close to the shape with the narrow neck also for low values of h_0. Contrary to the predictions of ADE model, both, the continuous shape transitions and discontinuous shape transitions in the vicinity of limiting shapes are possible in accordance with experimental observations.

Coalescence of the bud with the parent vesicle

An important result of this work is that if β_2-GPI was present in the solution, the membrane bud coalesced with the mother vesicle before it could detach from it (Fig. 3). We suggest that β_2-GPI induced coalescence of spheroidal bud with the parent membrane is of electrostatic nature, i.e. it is governed by electrostatic attraction between the positively charged first domain on the tip of membrane-bound β_2-GPI and negatively charged lipid headgroups of either cardiolipin, POPS or POPG in the opposite membrane (see also Fig. 8).

β_2-GPI is a *J*-shaped molecule, composed of five domains (Fig. 1). The fifth and the first domains are predominantly positively charged. Besides, there is a surface exposed hydrophobic loop on the fifth domain. If the membrane is negatively charged (as in our case), the fifth

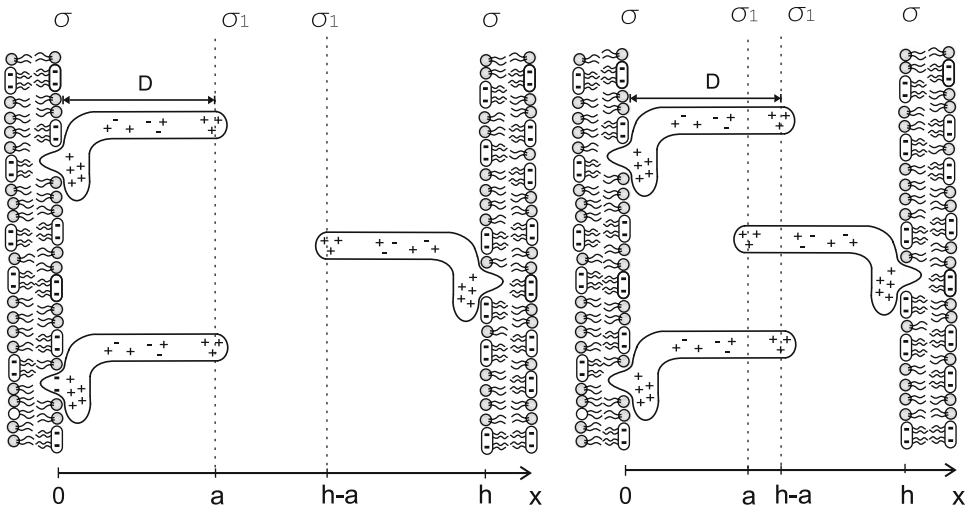

Fig. 8 Schematic presentation shows two different regimes regarding the distance between the lipid surfaces: $h \geq 2D$ (*left*) and $h < 2D$ (*right*)

domain binds strongly to the membrane surface (see Fig. 1) because of electrostatic attraction and also because of the insertion of hydrophobic loop into the membrane (Bouma et al. 1999; Schwarzenbacher et al. 1999).

In our theoretical model we consider two planar charged lipid (containing either cardiolipin, POPS or POPG) surfaces with electrolyte (salt) solution between the surfaces (Fig. 8). Because of positively charged first domains of membrane-bound β_2-GPI an apparent positively charged region approximately at the distance of β_2-GPI's length D away from each surface is created (Fig. 8). In the model the positive charge of the tips of the β_2-GPI molecules bound to both surfaces is represented by two charged surfaces (with the surface charge densities σ_1) at the distance D from the each of lipid surfaces (Fig. 8).

The electric charge distribution of both cardiolipin containing lipid surfaces is in the first approximation described by surface charge densities of both lipid surfaces (σ), where the contribution due to fifth domain of bound β_2-GPI molecules is neglected.

If the distance between the lipid surfaces (h) is larger than $2D$, the space between both lipid surfaces can be divided into three different regions $0 \leq x \leq a$, $a < x \leq (h-a)$ and $(h-a) < x \leq h$ (see Fig. 8). If $h \geq 2D$ the value of $a = D$, while in the case $h < 2D$ the value of a depends on h and is always smaller than D (Fig. 8).

It is well known that linearized Poisson–Boltzmann (PB) theory overestimates electrostatic free energies for lipid membranes. Nevertheless, to keep our model traceable we adopt the result of linearized PB theory, i.e. the electrostatic potential $\Psi(x)$ in the system was calculated from linearized PB (LPB) equation:

$$\nabla^2\phi = {\kappa_\mathrm{d}}^2\phi, \tag{3}$$

where $\phi = e_0\Psi/kT$ is dimensionless electrostatic potential and the Debye length $\kappa_d^{-1} = \sqrt{\epsilon_\mathrm{w}\epsilon_0 kT/(2n_0 N_\mathrm{A} e_0^2)}$. Here, ε_w is the dielectric constant of the aqueous solution, ε_0 is the permittivity of free space, n_0 is the ionic strength in the bulk solution (i.e. bulk salt concentration; assuming a 1:1 salt such as NaCl), N_A is Avogadro's number and e_0 is the unit charge. The bulk solution (outside the space between the membrane) provides a suitable reference for the electric potential (i.e. $y = 0$).

Due to the symmetry of the system we are searching for the solution of Eq. 3 only in the region $0 \leq x \leq h/2$ (see Fig. 8). The solution of Eq. 3 can be then written as

$$\phi = A\exp(-\kappa_\mathrm{d}x) + B\exp(\kappa_\mathrm{d}x), \quad 0 \leq x \leq a, \tag{4}$$

$$\phi = C\exp(-\kappa_\mathrm{d}x) + D\exp(\kappa_\mathrm{d}x), \quad a \leq x \leq h/2, \tag{5}$$

where the constants A, B, C and D were determined analytically from the boundary conditions $\frac{\mathrm{d}\phi}{\mathrm{d}x}(x=0) = -\sigma e_0/\epsilon_\mathrm{w}\epsilon_0 kT$, $\phi(x=a_-) = \phi(x=a_+)$, $\frac{\mathrm{d}\phi}{\mathrm{d}x}(x=a_-) = \frac{\mathrm{d}\phi}{\mathrm{d}x}(x=a_+) + \sigma_1 e_0/\epsilon_\mathrm{w}\epsilon_0 kT$ and $\frac{\mathrm{d}\phi}{\mathrm{d}x}(x=h/2) = 0$.

Including also the configurational entropy of the anions ($i = 1$) and cations ($i = 2$) of the salt dissolved in the water between both planar lipid surfaces, we can write the free energy of the system in the form (Kralj-Iglič and Iglič 1996; Evans and Wennerström 1999):

$$F/A = \int_0^h \left(\frac{1}{2}\epsilon_\mathrm{w}\epsilon_0 \left(\frac{\mathrm{d}\phi}{\mathrm{d}x}\frac{kT}{e_0} \right)^2 + kT\sum_{j=1}^{2}\left(n_j \ln\left(\frac{n_j}{n_0}\right) - (n_j - n_0) \right) \right) \mathrm{d}x, \tag{6}$$

where n_j are the number densities of anions ($i = 1$) and cations ($i = 2$) in the salt solution, n_0 is the number density of the anions and cations in the bulk solution (i.e. outside the space between the planar lipid surfaces). The bulk solution provides a suitable reference also for electric potential $\phi_\mathrm{bulk} = 0$ (see also Evans and Wennerström 1999).

Figure 9 shows the free energy (F) of the system as a function of the distance (h) between the two adjacent membrane surfaces with attached β_2-GPI. As it can be seen in Fig. 9 that for small values of σ_1 the free energy F increases with decreasing inter membrane distance h while for larger values of σ_1 the free energy decreases with decreasing h until the absolute minimum of F close to $h \cong D$ is reached.

The results presented in Fig. 9 reflect the fact that two adjacent membranes without bound β_2-GPI repel each other while for high enough concentration of membrane-bound β_2-GPI the force between two negatively charged membranes (containing either cardiolipin, POPS or POPG) becomes strongly attractive leading to the equilibrium distance at $h \cong D$. The origin of attractive interactions between two like-charged membrane surface is the electrostatic attraction between the positively charged first domain on the tip of the membrane-bound β_2-GPI and negatively charged opposite membrane (Fig. 8). The observed weak β_2-GPI-induced interaction between zwitterionic POPC–cholesterol vesicles indicates that the proposed attractive Coulomb (charge–charge) interactions between positively charged part of the first domain of membrane bound β_2-GPI and the negatively charged phospholipids of the adjacent membrane is not the only possible interaction responsible for β_2-GPI-mediated agglutination between vesicle membrane surfaces. In general, also the charge–dipole and dipole–dipole electrostatic interaction (Israelachvili 1997) between the lipid headgroup electric dipole moment and β_2-GPI may contribute

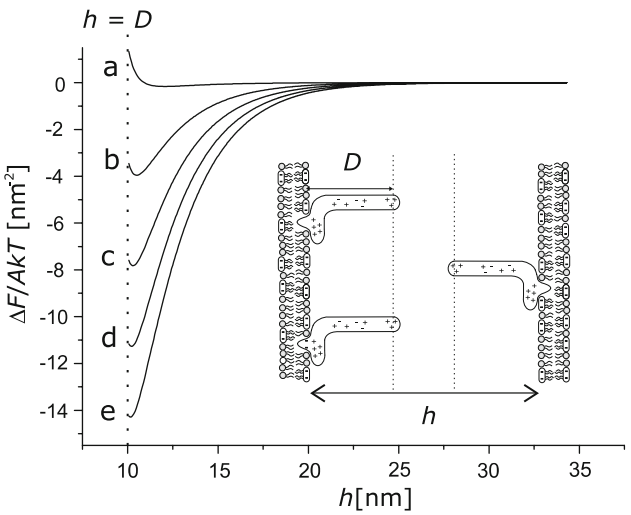

Fig. 9 Free energy $\triangle F = F - F(h \to \infty)$ as a function of x for five different values of σ_1: 0.002 As/m^2 (**a**), 0.003 As/m^2 (**b**), 0.004 As/m^2 (**c**), 0.005 As/m^2 (**d**), 0.006 As/m^2 (**e**). Length of the β_2-GPI molecule D = 10 nm. Values of surface charge density at lipid surface and buffer concentration are constant: $\sigma = -0.05$ As/m^2. Salt concentration in the bulk solution n_0/N_A = 15 mmol/l, where N_A is Avogadro's number

to the β_2-GPI-induced agglutination between like-charged membrane surfaces.

It was suggested that adhesion of the buds to the mother membrane due to β_2-GPI-mediated interactions between the bud and the mother cell membranes may take place also in vivo (Urbanija et al. 2007). According to this suggestion, membranes subjected to stronger mediating effect of proteins in the extracellular solution would shed into the circulation less microvesicles than membranes subjected to weaker mediating effect of extracellular proteins. As increased number of microvesicles was found in peripheral blood of patients with thrombosis (Diamant et al. 2004), compounds that mediate a short ranged attractive interaction between membranes are considered anticoagulant while compounds which impair the effect of adhesion mediators are considered procoagulant. Our results indicate an anticoagulant effect of β_2-GPI, which agrees with observations that a decrease in plasma β_2-GPI level (Brighton et al. 1996) and an increase in the level of prothrombogenic microvesicles (Dignat-George et al. 2004) was observed in patients with disseminated intravascular coagulation.

Conclusions

Effect of the membrane-attached proteins on the membrane elastic properties can have important physiological consequences. Some proteins that are present in human plasma and are able to attach to the membrane surface were found to mediate interactions between phospholipid-containing structures such as cell membranes, microvesicles and lipoproteins (Bevers et al. 2005; Distler 2005). Modifications of membrane bending rigidity due to the attached membrane proteins (Iglič et al. 2007) may affect the fluctuations of the membrane and in this way may additionally influence the adhesion process (Helfrich 1995).

In this work, we observed the coalescence of the spheroidal membrane bud with the parent vesicle before the bud could detach from parent vesicle membrane, i.e. vesiculate. It is proposed that the attachment of positively charged parts of β_2-GPI to the negatively charged outer surface of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol or POPC–POPG–cholesterol giant lipid vesicles may cause (at least partially) the observed adhesion of like-charged lipid surfaces. Theoretically, β_2-GPI-mediated interaction between the membrane of the bud and the membrane of the parent vesicle was explained by a specific spatial distribution of charge within β_2-GPI attached to the negatively charged membrane surfaces containing either cardiolipin, POPS or POPG.

The observed adhesion of phospholipid surfaces, mediated by β_2-GPI may play an important role in

microvesiculation of membranes and therefore in blood clot formation.

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References

Ambrožič A, Čučnik S, Tomšič M, Urbanija J, Lokar M, Babnik B, Rozman B, Iglič A, Kralj-Iglič V (2006) Interaction of giant phospholipid vesicles containing cardiolipin and cholesterol with ß2-glycoprotein-I and anti-ß2-glycoprotein-I antibodies. Autoimmunity Rev 6:10–15

Angelova MI, Soleau S, Meleard Ph, Faucon JF, Bothorel P (1992) Preparation of giant vesicles by external AC electric fields: kinetics and applications. Prog Colloid Polym Sci 89:127–131

Asherson RA, Khamashta MA, Ordi-Ros J, Derksen RH, Machin SJ, Barquinero J, Outt HH, Harris EN, Vilardell-Torres M, Hughes GR (1989) The "primary" antiphospholipid syndrome: major clinical and serological features. Medicine (Baltimore) 68:366–374

Balasubramanian K, Schroit AJ (1998) Characterization of phosphatidylserine-dependent beta2-glycoprotein I macriphage interactions: implications for apoptotic cell clearance by phagocytes. J Biol Chem 273:29272–29277

Bevers EM, Janssen MO, Comfurius P, Balasubramanian K, Schroit AJ, Zwaal RFA, Willems GM (2005) Quantitative determination of the binding of ß2- glycoprotein I and prothrombin to phosphatidylserine-exposing blood platelets. Biochem J 386:271–279

Božič B, Kralj-Iglič V, Svetina S (2006) Coupling between vesicle shape and lateral distribution of mobile membrane inclusions. Phys Rev E 73:041915/1–11

Bouma B, de Groot PG, van den Elsen JMH, Ravelli RBG, Schouten A, Simmelink JA, Derksen MJA, Kroon J, Gros P (1999) Adhesion mechanism of human β_2-glycoprotein I to phospholipids based on its crystal structure. EMBO J 18:5166–5174

Brighton TA, Hogg PJ, Dai YP, Murray BH, Chong BH, Chesterman CN (1996) Beta 2-glycoprotein I in thrombosis: evidence for a role as a natural anticoagulant. Br J Haematol 93:185–194

Deguchi H, Fernandez JA, Hackeng TM, Banka CL, Griffin JH (2000) Cardiolipin is a normal component of human plasma: a lipoproteins. Proc Natl Acad Sci (USA) 97:1743–1748

Diamant M, Tushuizen ME, Sturk A, Nieuwland R (2004) Cellular microparticles: new players in the field of vascular disease? Eur J Clin Invest 34:392–401

Dignat-George F, Camoin-Jau L, Sabatier F, Arnoux D, Anfosso F, Bardin N, Veit V, Combes V, Gentile S, Moal S, Sanmarco M, Sampol J (2004) Endothelial microparticles: a potential contribution to the thrombotic complications of the antiphospholipid syndrome. Thromb Haemost 91:667–673

Distler JHW, Pisetsky DS, Huber LC, Kalden JR, Gay S, Distler O (2005) Microparticles as regulators in inflammation. Arthritis Rheum 52:3337–3348

Evans EA, Skalak R (1980) Mechanics and thermodynamics of biomembranes. CRC Press, Boca Raton

Evans DF, Wennerström H (1999) The colloidal domain: where physics, chemistry, biology and technology meet, 2nd edn. Wiley, New York

Farsad K, De Camilli P (2003) Mechanisms of membrane deformation. Curr Opin Cell Biol 15:372–381

Gamsjaeger R, Johs A, Gries A, Gruber HJ, Romamnin C, Prassl R, Hinterdorfer P (2005) Membrane binding of β_2-glycoprotein I can be described by a two-state reaction model: an atomic force microscopy and surface plasmon resonance study. Biochem J 389:665–673

Greenwalt TJ (2006) The how and why of exocytic vesicles. Transfusion 46:143–152

Hägerstrand H, Mrowczynska L, Salzer U, Prohaska R, Michelsen KA, Kralj-Iglič V, Iglič A (2006) Curvature dependent lateral distribution of raft markers in the human erythrocyte membrane. Mol Membr Biol 23:277–288

Hagihara Y, Goto Y, Kato H, Yoshimura T (1995) Role of the N- and C-terminal domains of bovine beta 2-glycoprotein I in its interaction with cardiolipin. J Biochem (Tokyo) 118:129–136

Helfrich W (1973) Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch 28c:693–703

Helfrich W (1974) Blocked lipid exchange in bilayers and its possible influence on the shape of vesicles. Z Naturforsch 29c:510–515

Helfrich W (1995) Tension-induced mutual adhesion and a conjectured superstructure of lipid membranes. In: Lipowsky R, Sackmann E (eds) Structure and dynamics of membranes. Elsevier, Amsterdam, pp 691–721

Holopainen JM, Angelova MI, Kinnunen PKJ (2000) Vectorial budding of vesicles by asymmetrical enzymatic formation of ceramide in giant liposomes. Biophys J 78:830–838

Holopainen JM, Angelova MI, Söderlund T, Kinnunen PKJ (2002) Macroscopic consequences of the action of phospholipase C on giant unilamellar liposomes. Biophys J 83:932–943

Huttner WB, Zimmerberg J (2001) Implications of lipid microdomains for membrane curvature, buddding and fission. Curr Opin Cell Biol 13:478–484

Hwang WC, Waugh RA (1997) Energy of dissociation of lipid bilayer from the membrane skeleton of red blood cells. Biophys J 72:2669–2678

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. J Biomech 32:1343–1347

Iglič A, Kralj-Iglič V (2006) Budding of liposomes: role of intrinsic shape of membrane constituents. In: Leitmannova Liu A (eds) Advances in planar lipid bilayers and liposomes. Elsevier, Amsterdam, pp 253–279. http://www.sciencedirect.com/science/bookseries/15544516

Iglič A, Babnik B, Gimsa U, Kralj-Iglič V (2006) On the role of membrane anisotropy in the beading transition of undulated tubular membrane structures. J Phys A Math Gen 38:8527–8536

Iglič A, Babnik B, Fošnarič M, Hägerstrand H, Kralj-Iglič V (2007a) On the role of anisotropy of membrane constituents in formation of a membrane neck during budding of multicomponent membrane. J Biomech 40:579–585

Iglič A, Lokar M, Babnik B, Slivnik T, Veranič P, Hägerstrand H, Kralj-Iglič V (2007b) Possible role of flexible red blood cell membrane nanodomains in the growth and stability of membrane nanotubes. Blood Cells Mol Dis 39:14–23

Israelachvili JN (1997) Intermolecular and surface forces. Academic Press, London

Käs J, Sackmann E (1991) Shape transitions and shape stability of giant phospholipid vesicles in pure water induced by area-to-volume change. Biophys J 60:825–844

Khalil MF, Wagner WD, Goldberg IJ (2006) Molecular interactions leading to lipoprotein retention and the initiation of atherioscleroosis. Arterioscler Thromb Vasc Biol 24:2211–2218

Kralj-Iglič V, Iglič A (1996) A simple statistical mechanical approach to the free energy of the electric double layer including excluded volume effect. J Phys II (France) 6:477–491

Kralj-Iglič V, Iglič A, Gomišček G, Sevšek F, Arrigler V, Hägerstrand H (2002) Microtubes and nanotubes of a phospholipid bilayer membrane. J Phys A Math Gen 35:1533–1549

Kralj-Iglič V, Babnik B, Gauger DR, May S, Iglič A (2006) Quadrupolar ordering of phospholipid molecules in narrow necks of phospholipid vesicles. J Stat Phys 125:727–752

Laradji M, Kumar PBS (2004) Dynamics of domain growth in self-assembled fluid vesicles. Phys Rev Lett 93:198105/1–198105/4

Lipowsky R (1991) The conformation of membranes. Nature 349:475–481

Masuda M, Takeda S, Sone M, Ohki T, Mori H, Kamioka Y, Mochizuki N (2006) Endophilin BAR domain drives membrane curvature by two newly identified structure-based mechanisms. EMBO J 25:2889–2897

McMahon HT, Gallop JL (2005) Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438:590–596

Miao L, Seifert U, Wortis M, Döbereiner HG (1994), Budding transitions of fluid-bilayer vesicles: effect of area difference elasticity. Phys Rev E 49:5389–5407

Miyakis S, Giannakopoulos B, Krilis SA (2004) Beta 2 glycoprotein I-function in health and disease. Thromb Res 114:335–346

Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, De Groot PG, Koike T, Meroni PL, Shoenfeld Y, Tincani A, Vlachoyiannopolous PG, Krilis SA (2006) International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 4:295–306

Polz E, Kostner GM (1979) The binding of β_2-glycoprotein-I to human serum lipoproteins. FEBS Lett 102:183–186

Raphael RM, Waugh RE (1996) Accelerated interleaflet transport of phosphatidylcholine molecules in membranes under deformation. Biophys J 71:1374–1388

Roubey RAS (2000) Antiphospholipid syndrome: antibodies and antigenes. Curr Opin Hematol 7:316–320

Sackmann E (1994) Membrane bending energy concept of vesicle and cell shapes and shape transitions. FEBS Lett 346:3–16

Schousboe I (1979) Purification, characterization and indentification of an agglutinin in human serum. Biochim Biophys Acta 579:396–408

Schwarzenbacher R, Zeth K, Diederichs K, Gries A, Kostner GM, Laggner P, Prassl R (1999) Crystal structure of human β2-glycoprotein I: implications for phospholipid binding and the antiphospholipid syndrome. EMBO J 18:6228–6239

Sens P, Gov N (2007) Force balance and membrane shedding at the red-blood-cell surface. Phys Rev Lett 98:018102/1–018102/4

Sens P, Turner MS (2004) Theoretical model for the formation of caveolae and similar membrane invaginations. Biophys J 86:2049–2057

Stokke BT, Mikkelsen A, Elgsaeter A (1986) The human erythrocyte membrane skeleton may be an ionic gel. Eur Biophys J 13:203–218

Urbanija J, Tomšič N, Lokar M, Ambrožič A, Čučnik S, Rozman B, Kandušer M, Iglič A, Kralj-Iglič V (2007) Coalescence of phospholipid membranes as a possible origin of anticoagulant effect of serum proteins. Chem Phys Lipids 150:49–57

Wang F, Xia XF, Sui SF (2002) Human apolipoprotein H may have various orientations when attached to lipid layer. Biophys J 83:985–993

Willems GM, Janssen MP, Pelsers MAL, Comfurius P, Galli M, Zwaal RFA, Bevers EM (1996) Role of divalency in the high-affinity binding of anticardiolipin antibody-β_2-glycoprotein I complex to lipid membranes. Biochemistry 35:13833–13842