Cellular Engineering

Amphiphile-induced spherical microexovesicle corresponds to an extreme local area difference between two monolayers of the membrane bilayer

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Abstract—*It is shown that an increase of the area difference between the outer and the inner membrane lipid layers of the skeleton-free membrane segment as a result of exogenously added amphiphilic molecules results in budding of the segment. The process reaches its final point when the segment attains the shape of the local maximal area difference, corresponding to formation of a spherical microexovesicle.*

Keywords–*Vesiculation, Amphiphile, Membrane, Elastic energy*

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1 Introduction

THE HUMAN red blood cell (RBC) has no internal structure, therefore its shape at any given cell volume is determined solely by the membrane properties (ZARDA *et al.*, 1977; EVANS and SKALAK, 1980; MOHANDAS and EVANS, 1994; SEIFERT, 1997). The human RBC membrane is composed of two parts, the lipid bilayer and the continuous network of proteins, composing the membrane skeleton (STECK, 1989). At normal conditions the entire bilayer is underlaid with the skeleton (LIU *et al.*,1989; IGLIČ *et al.*,1995; SIKORSKI and BIALKOWSKA, 1996).

Following incubation of the normal discocytic RBC suspension with different water-soluble amphiphilic molecules it was observed that after RBCs had reached an extreme spiculated (echinocytic) or cup (stomatocytic) shape (SHEETZ and SINGER, 1974), microvesicles were released from the membrane (HÄGERSTRAND and ISOMAA, 1989). The echinocytogenic amphiphiles, which bind preferentially in the outer layer of the RBC membrane bilayer, induced release of microexovesicles (Fig. 1) while the stomatocytogenic amphiphiles which preferentialy bind in the inner layer of the RBC membrane bilayer induced release of the endovesicles. The amphiphile-induced RBC microexovesicles are in general, free of membrane skeleton (HÄGERSTRAND and ISOMAA, 1994).

To understand the RBC shape changes induced by amphiphiles in RBCs, the bilayer couple principle was proposed (SHEETZ and SINGER, 1974; EVANS, 1974). In terms of the bilayer couple model, by keeping the volume of the cell (V) and the area of the cell (A) constant, RBC shape changes result from the change in the difference between the outer and inner monolayer areas (ΔA) of the bilayer. Binding of the amphiphiles in the outer layer of the RBC membrane bilayer increases the area difference ΔA and causes the normal discocytic RBC shape to change towards the echinocytic shape; binding of the amphiphiles in the inner layer of the bilayer decreases ΔA and causes transformation of the discocytic shape into the stomatocytic shape (SHEETZ and SINGER, 1974; ISOMAA, *et al.*, 1987; SVETINA and ZEKŠ, 1989; SEIFERT, 1997; IGLIČ, *et al.*, 1998*a*).

Fig. 1 *Transmission electron micrograph showing spherical exovesicles of human red blood cells treated with 3-(dodecyldimethylammonio)-1-propanesulphonate at 37°C for 30 min at 263 μM concentration as previously reported (HÄGERSTRAND and ISOMAA, 1989, 1994). Bar represents 100 nm*

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The aim of this work is to analyse a possible physical mechanism for the formation of skeleton-free spherical microexovesicles induced by intercalation of amphiphilic molecules in the outer layer of the RBC membrane bilayer.

2 Proposed mechanism for the formation of spherical microexovesicles

2.1 *General description*

Embedding of amphiphilic molecules in the outer layer of the RBC membrane bilayer enforces high membrane curvature in some regions. However, such deformation considerably increases the shear energy of the membrane skeleton (IGLIČ and HÄGERSTRAND, 1996) so that it could be expected that at these regions the probability of local detachment of the spectrin network from the bilayer is increased (LIU *et al.*, 1989; IGLIČ *et al.*, 1995; IGLIČ and HÄGERSTRAND, 1996). Following the local detachment of the membrane skeleton from the membrane bilayer, which is indicated to be a prerequisite for microvesiculation (IGLIČ and HÄGERSTRAND, 1996), the development of a microvesicle is assumed to be a local event, meaning that perturbations of the energy state of other parts of the cell membrane which serve as a reservoir for the exogenously added amphiphilic molecules are negligible. These molecules favouring higher membrane curvature may flow to the protrusion from two reservoirs, namely from the surrounding solution and also from the surrounding membrane of the mother cell. As a consequence, the outer membrane layer area of the segment is increased with respect to the inner layer, i.e. the area difference of the segment ΔA is increased. The process continues until the shape of the skeleton-free membrane segment corresponding to the maximum possible local area difference (ΔA_{max}) is reached.

We assume that the membrane segment at any given ΔA attains an energetically favourable shape determined by the minimum of its bending energy W_b (ŽEKŠ *et al.*, 1990) while the value of ΔA at given external conditions is determined by the minimum of the total energy of the membrane (WIESE *et al.*, 1992; SVETINA and ŽEKŠ, 1996; WAUGH, 1996; IGLIČ *et al.*, 1998*a*) and also by the chemical equilibrium of the amphiphilic molecules in the system composed of the segment and its surroundings including also the extracellular solution.

2.2 *Determination of the shape of the confined bilayer segment at given area A and area difference ΔA*

In this section, the proposed microexovesicle formation mechanism is expressed by means of a mathematical model. The segment is taken to be axisymmetrical and confined to a ring of radius R_0 (Fig. 2). It is assumed that in the area of the segment the membrane skeleton is detached from the bilayer. Since the size of the vesicle is much smaller than the size of the RBC it is taken that the membrane of the parent cell in contact with the segment is flat. The shape of the axisymmetrical segment is described by the contour $z = z(x)$, where z is the coordinate along the symmetry axis and x is the distance from the axis.

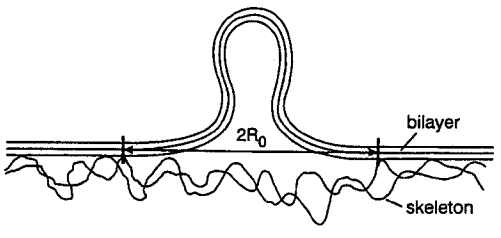

Fig. 2 *Schematic presentation of the skeleton-free axisymmetrical bilayer segment of radius R_0*

The shape of the axisymmetrical segment at given area difference of the segment (ΔA^*) is determined by minimisation of the bilayer bending energy of the segment W_b (HELFRICH, 1973):

$$W_b = \tfrac{1}{2} k_c \int (C_1 + C_2 - C_0)^2 \, dA \tag{1}$$

at fixed area (A^*) of the segment. Here, k_c is the local bending modulus of the membrane, C_1 and C_2 are the principal membrane curvatures and C_0 is the spontaneous curvature of the membrane. In the following the value $C_0 = 0$ is chosen. The integration in eqn. 1 is performed over the bilayer neutral surface area of the segment. Including the energy of the relative stretching of the membrane bilayer (EVANS, 1974; SVETINA and ŽEKŠ, 1989; MIAO *et al.*, 1994; BUKMAN *et al.*, 1996) in the determination of the shape of the segment at a given area difference of the segment ΔA^* would not change the calculated shape of the segment (SVETINA and ŽEKŠ, 1996; IGLIČ *et al.*, 1998a).

The problem of finding the shapes of a bilayer segment with minimal bending energy at a given segment area A^* and a given segment area diference ΔA^* can be mathematically formulated within the Euler–Lagrange minimisation theory (DEULING and HELFRICH, 1976; SVETINA and ŽEKŠ, 1989). Within this theory the segment shape corresponding to the minimal bending energy at a chosen area and area difference can be obtained by minimising the functional (ŽEKŠ *et al.*, 1990):

$$G = W_b - \Lambda(A - A^*) - \Omega(\Delta A - \Delta A^*) \tag{2}$$

where the Lagrange multipliers Λ and Ω are determined from the constraints for the area and area difference. Since the distance between the neutral surfaces of the bilayer leaflets (δ) is much smaller than the dimensions of the RBC, the area difference ΔA can be approximated as

$$\Delta A = \delta \int (C_1 + C_2) dA \tag{3}$$

where C_1 and C_2 are the two principal curvatures defined so that they are positive for a sphere.

In the following analysis dimensionless quantities are introduced. The unit of length is chosen to be the radius of the segment R_0. The variables x and z are redefined as follows, $x \rightarrow x/R_0$ and $z \rightarrow z/R_0$. In addition, the relative area of the segment is defined as $a = A/\pi R_0^2$, while the relative area difference is

$$\Delta a = \Delta A/\pi\delta R_0 = \int (c_1 + c_2) da \tag{4}$$

where the dimensionless curvatures are $c_1 = C_1 R_0$, $c_2 = C_2 R_0$ and $da = dA/\pi R_0^2$. The bending energy W_b and the functional G are also normalised:

$$w_b = W_b/\pi k_c = \tfrac{1}{2} \int (c_1 + c_2)^2 \, da \tag{5}$$

$$g = G/\pi k_c = w_b - \lambda(a - a^*) - \omega(\Delta a - \Delta a^*) \tag{6}$$

where $\lambda = \Lambda R_0^2/k_c$ and $\omega = \Omega h R_0/k_c$. Since our analysis of bilayer segment is restricted to axisymmetric shapes the two principal curvatures c_1 and c_2 are the principal curvature along the parallels c_p and the principal curvature along the meridians c_m (DEULING and HELFRICH, 1976). The two principal curva-

tures c_m and c_p of an axisymmetrical segment are interdependent:

$$c_m = c_p + x(dc_p/dx) \tag{7}$$

Thus the relative area element can be expressed as

$$da = (2x/(1 - (xc_p)^2)^{1/2})dx \tag{8}$$

Using the definition

$$g = \int \Gamma(x, c_p, dc_p/dx)dx \tag{9}$$

the Lagrange–Euler equation for the variational problem described can be written as

$$\frac{\partial \Gamma}{\partial c_p} - \frac{d}{dx}\left(\frac{\partial \Gamma}{\partial (dc_p/dx)}\right) = 0 \tag{10}$$

Eqn. 10 is solved numerically according to a method described in detail elsewhere (DEULING and HELFRICH, 1976; SVETINA and ŽEKŠ, 1989). The requirement that the displacement of the membrane at the boundary of the segment is zero is met by the condition $z(x = 1) = 0$. In addition, the boundary condition $c_p(x = 1) = 0$ is taken into account, assuming that the membrane is smooth at the boundary of the segment (ŽEKŠ *et al.*, 1990). The basic predictions of the model remain unchanged if the area of the skeleton-free domain is smaller than the area of the segment.

Fig. 3 shows a sequence of calculated axisymmetric shapes of the bilayer segment, simulating the development of the spherical microvesicle from the skeleton-free membrane segment. At each step of the budding process, shown in Fig. 3, the area difference is increased until the maximum value of the relative area difference of the segment Δa_{max} is reached. The shape of the segment corresponding to Δa_{max} as well the shape corresponding to the minimum Δa (Δa_{min}) are not calculated by minimisation of the bending energy, but are determined separately as described in the following section.

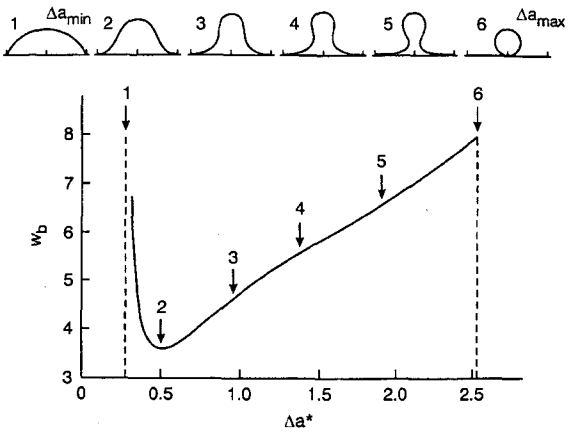

Fig. 3 *The dependence of axisymmetric shapes of the skeleton-free membrane segment and its relative bending energy (w_b) on the relative difference between the areas of the two monolayers of the segment (Δa^*) for $a^* = 1.4$. Symbol Δa_{min} denotes the minimum possible value of Δa belonging to the shape composed of a spherical section, while Δa_{max} denotes the maximum possible value of Δa that belongs to the shape composed of a sphere and a flat membrane segment*

2.3 *Shapes of the bilayer segment corresponding to the extreme relative area difference Δa*

To obtain the shapes of the skeleton-free membrane segment with extreme relative area difference Δa at the given relative area of the segment a^*, a variational problem is stated by constructing a functional

$$f = \Delta a - \lambda_\Delta(a - a^*) \tag{11}$$

The functional f may be given by

$$f = \int F(c_p, dc_p/dx, x)dx \tag{12}$$

where

$$F(c_p, dc_p/dx, x) = (2c_p + x\,dc_p/dx - \lambda_\Delta)x/(1/2(1 - (xc_p)^2)^{-1/2} \tag{13}$$

The variation of eqn. 13 is performed by solving the Euler equation

$$\partial F/\partial c_p - d/dx(\partial f/\partial (dc_p/dx)) = 0 \tag{14}$$

There are exactly two solutions to eqn. 14, namely

$$c_p = 0 \tag{15}$$

and

$$c_p = \lambda_\Delta \tag{16}$$

It follows from eqn. 7 that the solution $c_p = 0$ (eqn. 15) implies that $c_m = 0$ as well, so that this solution corresponding to the extremum of Δa represents a segment of a plane. The second solution (eqn. 16) which gives a constant value of c_p, is consistent with eqn. 7 and yields the solution $c_m = c_p = \lambda_\Delta$ representing a sphere. The shapes of the extreme Δa may therefore be composed of planar and spherical sections. In the case of the minimum possible value of Δa (Δa_{min}) at a given relative area a^* of the segment, the shape of the extreme Δa is composed of a spherical section (Fig. 3). In the case of the maximum possible value of Δa (Δa_{max}) the shape of the extreme Δa is composed of a sphere and a flat membrane segment (Fig. 3). The shapes of the extreme Δa involving more than one vesicle are not considered here.

The shapes of segments close to the shape with $\Delta a = \Delta a_{min}$ could be stabilised by proteins of conical shape distributed in the region of high bending at the edge of the segment. However, the segment shape with $\Delta a = \Delta a_{min}$ can never be reached due to its very high (infinite) bending energy (Fig. 3).

3 Results

For the extreme relative area difference the shape of the skeleton-free membrane segment can be determined from constraints on its relative area. Thus in the case of the maximum Δa (Δa_{max}) the relative radius of the spherical daughter vesicle (Fig. 3) is

$$r_v = (a^* - 1)^{1/2}/2 \tag{17}$$

while the corresponding relative maximum area difference is

$$\Delta a_{max} = 4(a^* - 1)^{1/2} \tag{18}$$

On the other hand in the case of minimum Δa (Δa_{min}) the relative radius of the spherical section composing the bud is (Fig. 3)

$$r_s = a^*/(2(a^* - 1)^{1/2}) \tag{19}$$

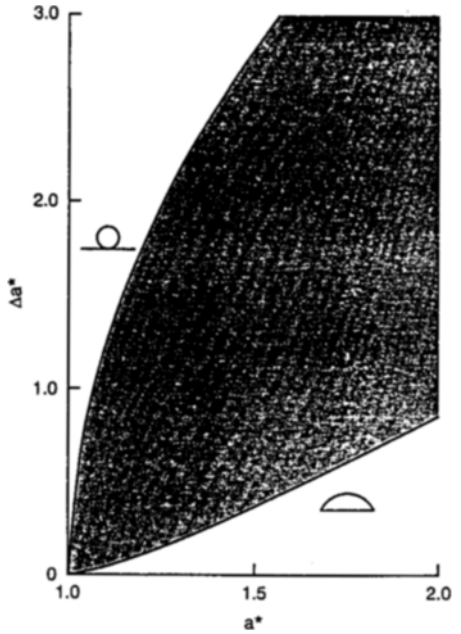

Fig. 4 *Region in the $\Delta a^*/a^*$ diagram belonging to the axisymmetrical shapes of the membrane segment. The upper line represents the maximum possible area diference of the segment at a given segment area (Δa_{max}) while the lower curve represents the minimum possible Δa (Δa_{min}) at given segment area*

while the relative height of the bud at $x = 0$ is

$$h = (a^* - 1)^{1/2} \tag{20}$$

The relative minimal area difference of the bud is

$$\Delta a_{min} = 4h - \arctan(1/(r_s - h)) \tag{21}$$

Fig. 4 shows a phase diagram of the calculated possible shapes of the skeleton-free membrane segment corresponding to the minimum bending energy in the plane of the relative segment area a^* and the relative area of the segment (Δa^*). It can be seen in Fig. 4 that the maximum possible relative area difference of the segment Δa_{max} is an increasing function of the relative area of the segment. On the basis of the results presented it can be concluded that at the given area of skeleton-free membrane segment the budding process reaches the final stage where vesiculation can start when the maximum value of the difference between the two membrane layer areas of the segment occurs.

4 Discussion and conclusions

Microvesiculation has been detected under many different experimental conditions (HÄGERSTRAND and ISOMAA, 1989) and is thought to be induced by elevation of the difference, ΔA, between the outer and inner monolayer areas of the membrane bilayer. Microvesicles shed from RBCs were normally depleted in major membrane skeletal components (HÄGERSTRAND and ISOMAA, 1994). It seems therefore that the disturbance in the interactions between the skeleton and the lipid bilayer is a general characteristics of RBC vesiculation (IGLIČ *et al.*, 1995, IGLIČ and HÄGERSTRAND, 1996). Alternatively, the depletion of membrane skeleton in RBC microexovesicles may also be the result of the formation of gaps in the skeleton network which may serve as sites where vesiculation occurs (KOZLOV *et al.*, 1990; SAXTON, 1992). Nevertheless, the basic result of this work, indicating that the final spherical shape of the RBC microexovesicle is determined solely by the condition of the maximum ΔA, does not depend on the physical mechanism leading to skeleton depletion in the budding domain.

The increase in the local area difference ΔA of the membrane segment during the budding process could occur in many different ways. For example, by intercalation of the amphiphilic molecules from the outer solution in the cell membrane (SHEETZ and SINGER 1974; ISOMAA *et al.* 1987), due to conformational changes of membrane protein band 3 induced by the change of pH (GIMSA and RIED, 1995) or due to the flow of the membrane constituent molecules to the segment from its surrounding membrane (ISRAELACHVILI *et al.*, 1976). The equilibrium value of the segment ΔA under given external conditions is determined by the minimum of the total free energy of the system (SVETINA and ŽEKŠ, 1996; IGLIČ *et al.*, 1998a) including the bending and relative stretching energy of the segment (BUKMAN *et al.*, 1996), the energy due to inhomogeneous distribution of the membrane components (LIPOWSKY, 1993; FISCHER, 1993; KRALJ-IGLIČ *et al.*, 1996) and the free energy of the amphiphilic molecules distributed in the segment and its surrounding membrane and extracellular solution (ISRAELACHVILI *et al.*, 1976).

By describing the microvesiculation as a local process it is taken that the conditions in the membrane of the parent cell change only negligibly after microvesiculation so that the energy of the membrane of the parent cell remains constant during this process (BUKMAN *et al.*, 1996). This can be justified, as the loss of the total membrane area in microvesiculation is very small. Consequently, it cannot significantly change the curvature of the parent cell membrane. Also, the number of amphiphilic molecules migrating to the regions of the membrane that eventually enclose the microexovesicles is so small that this migration cannot affect the lateral density of the membrane-embedded molecules of the parent cell.

If the area density of the membrane-embedded amphiphilic molecules is high these molecules contribute significantly to the area of the layer in which they are embedded. Within the bilayer couple principle it was established experimentally (SHEETZ and SINGER, 1974) and also confirmed theoretically (SVETINA and ŽEKŠ, 1989) that a very small (less than 1%) relative change of the two membrane leafet areas may strongly affect the membrane shape. This is a principal issue also in this work where there may be extensive changes of ΔA of the segment involved in microexovesiculation.

The process of microexovesiculation, which lies within the scope of this work, should be distinguished from the exovesiculation caused by the extreme pH values in the suspension. The formation of one or a few large (in the range of micrometres) vesicles devoid of spectrin network has been observed at extreme values of pH (LEONARDS and OHKI, 1983; IGLIČ *et al.*, 1998b). Contrary to the microexovesiculation described in the present work as a local process, the formation of larger vesicles at extreme pH should be subject to consideration of the membrane of the whole erythrocyte (IGLIČ *et al.*, 1998b).

In conclusion, it is shown in this work that an increase in the difference between the two membrane layer areas of the skeleton-free membrane segment (ΔA) in the budding process may lead to a closed spherical shape of the membrane segment with maximum possible ΔA. The calculated sequence of axisymmetric shapes of the skeleton-free membrane segment with minimal bending energy given in Fig. 3 illustrate the budding process. The final shape of the segment, composed of a spherical and a planar part, is determined solely by the condition of maximum ΔA and does not depend on the choice of membrane energy involved in the minimisation procedure.

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References

BUKMAN, D. J., YAO, J. H., and WORTIS, M. (1996): 'Stability of cylindrical vesicles under axial tension', *Phys. Rev. E*, **54**, pp. 5463–5468

DEULING, H. J. and HELFRICH, W. (1976): 'The curvature elasticity of fluid membranes', *J. Phys. France*, **37**, pp. 1335–1345

EVANS, E.A. (1974): 'Bending resistance and chemically induced moments in membrane bilayers', *Biophys. J.*, **14**, pp. 923–931

EVANS, E., and SKALAK, R. (1980) 'Mechanics and thermodynamics of biomembranes', (CRC Press, Boca Raton, FL.)

FISCHER, T. M. (1993): 'Mechanisms for determining the time scales in vesicle budding', *Phys. Rev. E*, **50**, pp. 4156–4166

GIMSA, J., and RIED, C. (1995): 'Do band 3 protein conformational changes mediate shape changes of human erythrocytes', *Mol. Membr. Biol.*, **12**, pp. 247–254

HELFRICH W. (1973): 'Elastic properties of lipid bilayers: theory and possible experiments', *Z. Naturforsch.*, **28C**, pp. 693–703

HÄGERSTRAND, H., and ISOMAA B. (1989): 'Vesiculation induced by amphiphiles in erythrocytes', *Biochim. Biophys. Acta*, **982**, pp. 179–186

HÄGERSTRAND, H., and ISOMAA B. (1994): 'Lipid and protein composition of exovesicles released from human erythrocyte following treatment with amphiphiles', *Biochim. Biophys. Acta*, **1190**, pp. 409–415

IGLIČ, A., and HÄGERSTRAND, H. (1996): 'Membrane shear elasticity and depletion of membrane skeleton in red blood cell vesicles' in CERROLAZA, M., JUGO, D. and BREBBIA, C. A. (Eds): 'Simulation modelling in bioengineering', (Computational Mechanics Publications, Southampton, Boston) pp. 109–118

IGLIČ, A., KRALJ-IGLIČ, V., and HÄGERSTRAND, H. (1998*a*): 'Stability of spiculated red blood cells induced by intercalation of amphiphiles in cell membrane', *Med. Biol. Eng. Comput.*, **36**, pp. 251–255

IGLIČ, A., HÄGERSTRAND, H., KRALJ-IGLIČ, V., and BOBROWSKA-HÄGERSTRAND M. (1998*b*): 'A possible physical mechanism of red blood cell vesiculation obtained by incubation at high pH', *J. Biomech.*, **31**, pp. 151–156

IGLIČ, A., SVETINA, S., and ŽEKŠ, B. (1995): 'Depletion of membrane skeleton in red blood cell vesicles', *Biophys. J.*, **69**, pp. 274–279

ISRAELACHVILI, J. N., MITCHELL, D. J., and NINHAM, B. W. (1976): 'Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers', *J. Chem. Soc. Faraday Trans. II*, **72**, pp. 1525–1568

ISOMAA, B., HÄGERSTRAND, H., and PAATERO, G. (1987): 'Shape transformations induced by amphiphiles in erythrocytes', *Biochim. Biophys. Acta*, **899**, pp. 93–103.

KOZLOV, M. M., CHERNOMORDIK, L. V., and MARKIN, V. S. (1990): 'A mechanism of formation of protein-free regions in the red cell membrane: the rupture of the membrane skeleton.' *J. Theor. Biol.*, **144**, pp. 347–365

KRALJ-IGLIČ, V., SVETINA, S., and ŽEKŠ, B. (1996): 'Shapes of bilayer vesicles with membrane embedded molecules', *Eur. Biophys. J.*, **24**, pp. 311–321

LEONARDS, K. S., and OHKI, S. (1983): 'Isolation and characterization of large (0.5–1.0 mm) cytoskeleton-free vesicles from human and rabbit erythrocytes', *Biochim. Biophys. Acta*, **728**, pp. 383–393

LIPOWSKY, R. (1993): 'Domain induced budding of fluid membranes', *Biophys. J.*, **64**, pp. 1133–1138

LIU, S. C., DERICK, L. H., DUQUETTE, M. A., and PALEK, J. (1989): 'Separation of the lipid bilayer from the membrane skeleton during discocyte-echinocyte transformation of human erythrocyte ghosts', *Eur. J. Cell Biol.*, **49**, pp. 358–365

MIAO. L., SEIFERT, U., WORTIS, M., and DÖBEREINER, H. G. (1994): 'Budding transitions of fluid-bilayer vesicles; the effect of area difference elasticity', *Phys. Rev. E*, **49**, pp. 5389–5407

MOHANDAS, N., and EVANS, E. (1994): 'Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects', *Annu. Rev. Biophys. Biomol. Struct.*, **23**, pp. 787–818

SAXTON, M. J. (1992): 'Gaps in the erythrocyte membrane skeleton: a stretched net model', *J. Theor. Biol.*, **155**, pp. 517–536

SEIFERT, U. (1997): 'Configurations of fluid membranes and vesicles', *Adv. in Phys.*, **46**, pp. 13–137

SHEETZ, M. P., and SINGER, S. J. (1974): 'Biological membranes as bilayer couples. A mechanism of drug-erythrocyte interactions', *Proc. Natl. Acad. Sci. USA*, **71**, pp. 4457–4461

SIKORSKI, A., and BIALKOWSKA, K. (1996): 'Interactions of spectrin with membrane intrinsic domain', *Cell. Mol. Biol. Lett.*, **1**, pp. 97–104

STECK, T. L. (1989): 'Red cell shape' in STEIN, W. and BRONNER, F. (Eds): 'Cell shape: determinants, regulation and regulatory role' (Academic Press, New York), pp. 205–246

SVETINA, S., and ŽEKŠ B. (1989): 'Membrane bending energy and shape determination of phospholipid vesicles and red blood cells', *Eur. Biophys. J.*, **17**, pp. 101–111

SVETINA, S., and ŽEKŠ, B. (1996): 'Elastic properties of closed bilayer membranes and the shapes of giant phospholipid vesicles.' in LASIC, D. D., and BARENHOLZ, Y. (Eds): 'Handbook of nonmedical applications of liposomes' (CRC Press, Boca Raton, FL), pp. 13–42

WAUGH, R. E. (1996): 'Elastic energy of curvature driven bump formation of red blood cell membrane', *Biophys. J.*, **70**, pp. 1027–1035

WIESE, W., HARBICH, W., and HELFRICH, W (1992): 'Budding of lipid bilayer vesicles and flat membranes', *J. Phys. Condens. Matter*, **4**, pp. 1647–1657

ŽEKŠ, B., IGLIČ, A., and SVETINA, S. (1990): 'Bilayer membrane models and a theoretical analysis of the vesiculation process', *Suppl. Minerva Biotechnologica*, **2**, p. 47

ZARDA P. R., CHIEN S., and SKALAK, R. (1977): 'Elastic deformations of red blood cells.' *J. Biomechanics*, **10**, pp. 211–221

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