Budding of membranes

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We have studied budding of biological membranes as an initial step leading to two important transport systems within and between cells: tunelling nanotubules that connect membrane-enclosed compartments, and microvesicles that are shed into the circulation and convey matter and information distally.

The unedrlying mechanism of the budding is based on the fact that membrane constituents by means of self-assembly create curvature field attributed to the surface they create. Therefore, the curvature of the surface and – in turn – the configuration of the consituents in this surface depend on the properties of the building blocks, the interactions between them and the interactions of the membrane constituents with the surrounding solution. Thus, budding can be initiated by changes in the surrounding solution such as addition of exogenous substances which interact with the membrane.

SPHERICAL AND TUBULAR BUDDING

Erythrocytes are a convenient system for study of the membrane budding since their shape depends primarily on the properties of the membrane. It was observed that upon addition of detergents into the erythrocyte suspension initially normal discoid erythrocyte shape underwent inward or outward undulation of the membrane to yield stomatocyte or echinocyte, depending on the type of the added detergent (Figs. 1 - 3)

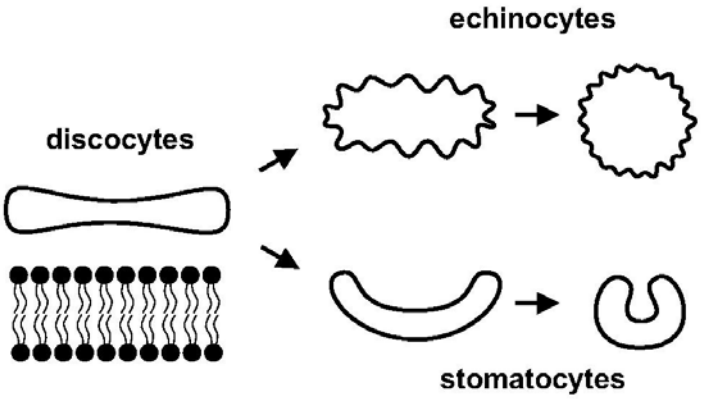


Fig.1: Scheme of the erythrocyte shape transformation.

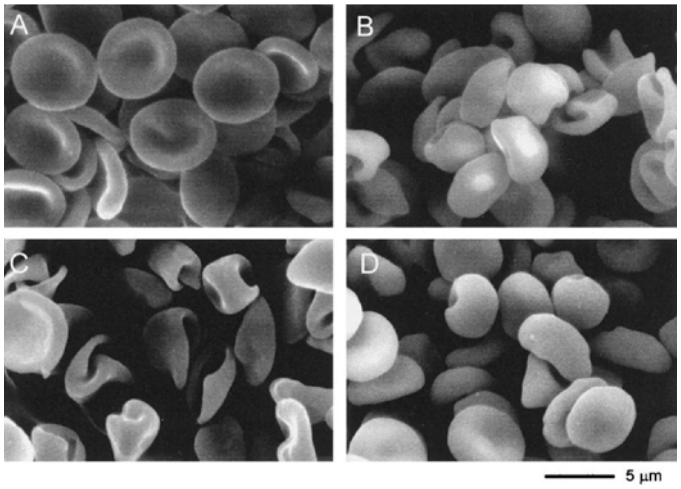


Fig.2: Discocyte-stomatocyte transformation after addition of octaethyleneglycol to the suspension of erythrocytes. From http://physics.fe.uni-lj.si/publications/pdf/3356.pdf

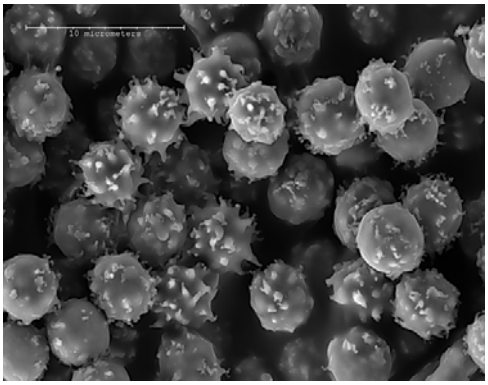


Fig.3: Echinocytes (H.Hagerstrand) .

Small buds formed on top of the echinocyte spicules, promoted by the detachment of the membrane cytoskeleton on top of the spicules. The shape of the buds was found to be rounded, sphere-like (Fig.4, lower left, Fig.5A), or elongated, tube-like (Fig.4, upper right, Fig.5B).

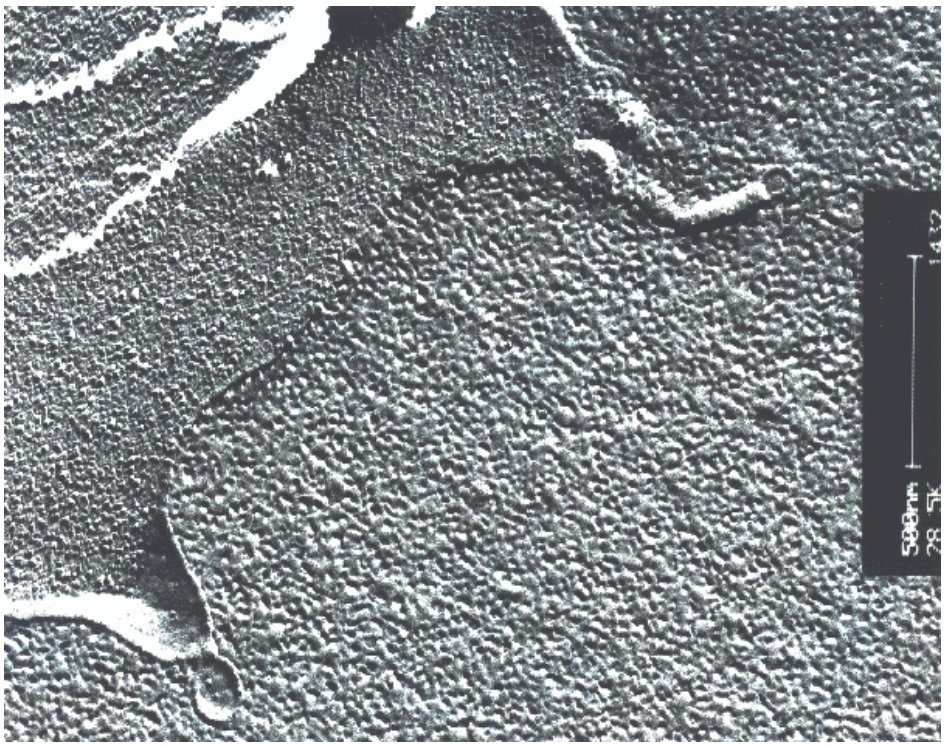


Fig.4: A freeze-fracture image of budding of the erythrocyte membrane induced by adding dodecylmaltoside to the erythrocyte suspension. From http://physics.fe.uni-lj.si/publications/pdf/KraljIglic2005EurBJ.pdf

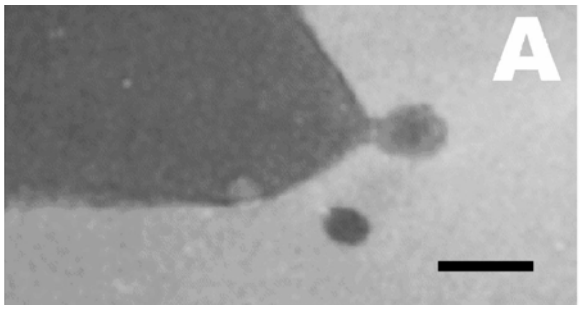


Fig.5: Spherical budding of the erythrocyte membrane on top of the echinocyte spicule induced by adding dodecylzwittergent to the erythrocyte suspension . From http://physics.fe.uni-lj.si/publications/pdf/PRE04230.pdf
Bar = 100 nm.

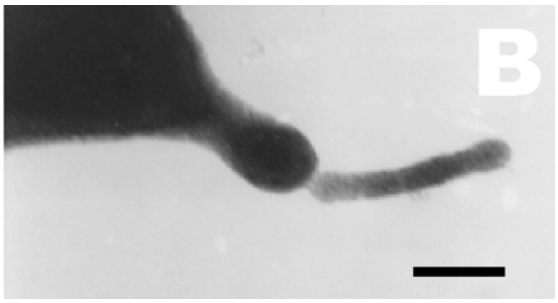


Fig.6: Tubular budding of the erythrocyte membrane on top of the echinocyte spicule induced by adding a gemini detergent dioctyldiQAS to the erythrocyte suspension . From http://physics.fe.uni-lj.si/publications/pdf/PRE04230.pdf
Bar = 100 nm.

Budding was observed also in phospholipid membranes. It was found that in electroformation, giant phospholipid vesicles are connected with a network of nanotubes. The network is torndown when the vesicles are rinsed out of the electroformation chamber while the vesicles retain its remnants in the form of long nanotubular protrusions. Fig.7 shows a POPC vesicle with long thin tubular protrusion. The protrusion developed from the nanotube in a spontaneous process.

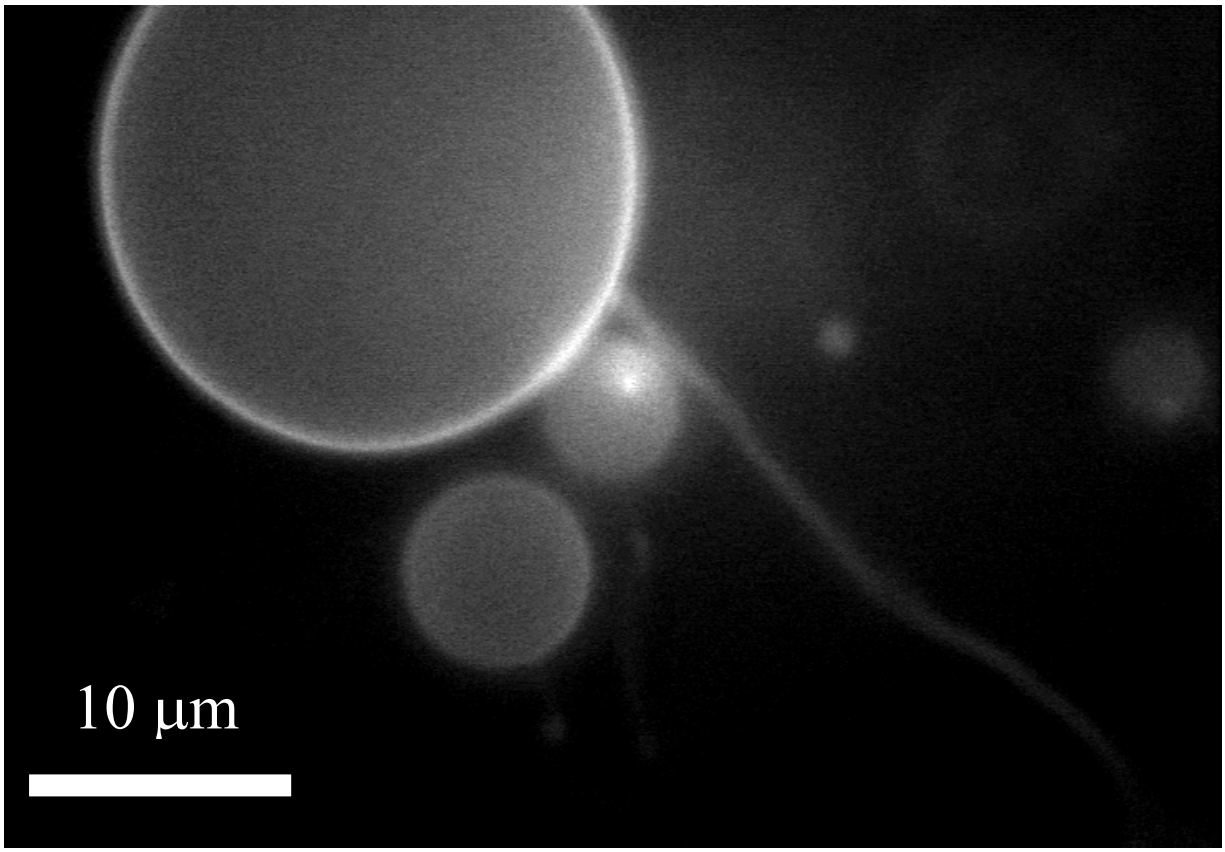


Fig.7: Giant POPC vesicle with long tubular protrusion. The length of the protrusion was about 10 diameters of the mother vesicle. A fluorescent probe NBD PC was added to POPC to enable observation under the fluorescence microscope. From http://physics.fe.uni-lj.si/publications/pdf/corgi.pdf

Long tubular protrusions were found to connect membrane-enclosed compartments in erythrocytes. Fig.8 shows erythrocyte shape transformation induced by high pH in the solution and lead to formation of two globular compartments connected by a thin tube. A vehicle formed on the tube and traveled along it.

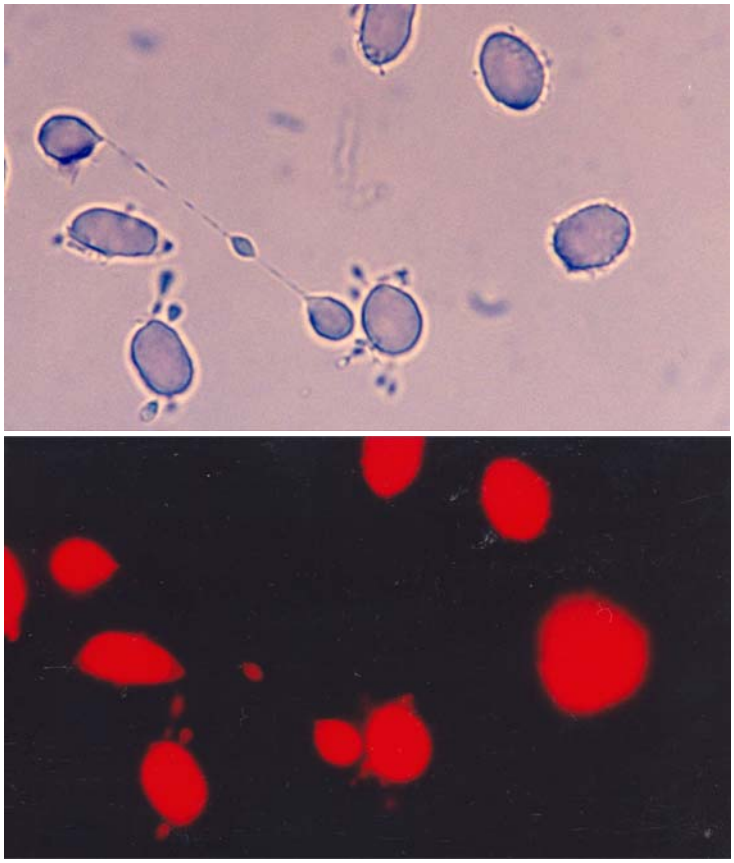


Fig.8: Two membrane-enclosed compartments connected by a tube. A gondola is noted on the tube. Erythrocytes contained fluorescent dye. From http://physics.fe.uni-lj.si/publications/pdf/PhysLettA03.pdf

A gondola was observed in phospholipid vesicles (Fig.9). It traveled along the tube and upon reaching the mother sphere delivered its contents to the mother sphere.

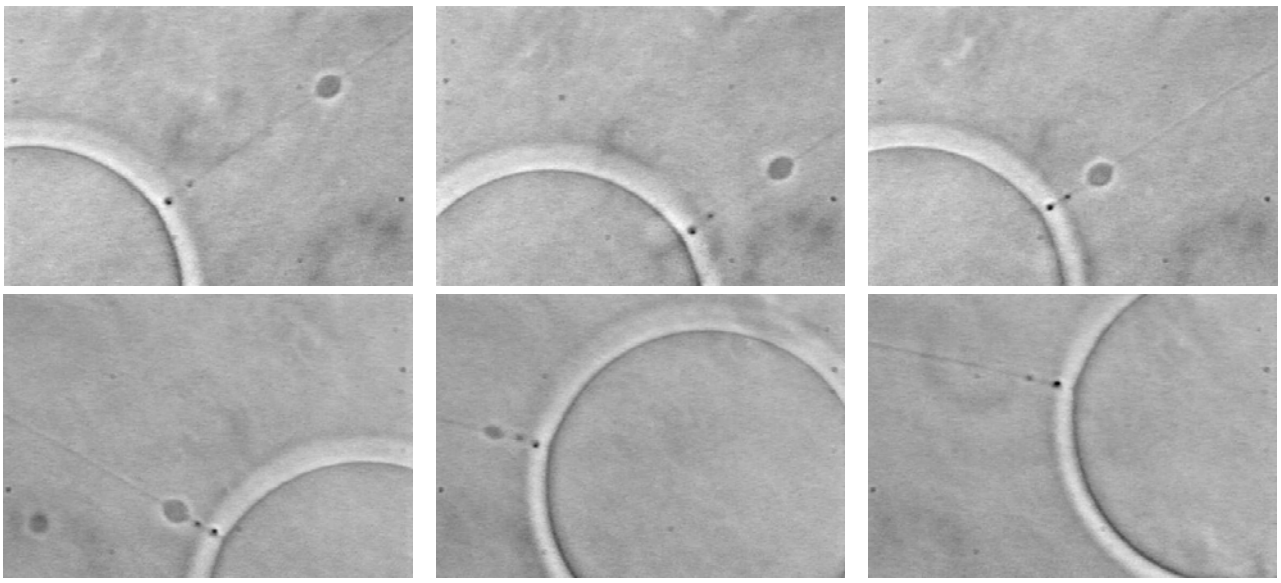


Fig.9: Travelling of the gondola along the tubular protrusion of the giant POPC vesicle. The tube was attached to the glass on the other end. From http://physics.fe.uni-lj.si/publications/pdf/PhysLettA03.pdf

The existence of long thin tubes and traveling gondolas indicated that such structures could be present also in cells where they would represent a mechanism of transport of matter and information between and within cells. It was suggested that membrane nanostructures form a subjacent pool of membraneous structures that acts as a cell infrastructure. The exsistence of nanotubular connections between cells was observed in experiments as described in http://physics.fe.uni-lj.si/publications/pdf/BasicCell.pdf and http://physics.fe.uni-lj.si/publications/pdf/Curvature.pdf and shown in (Fig.10) while the subjacent pool of membraneous structures remains largely obscure.

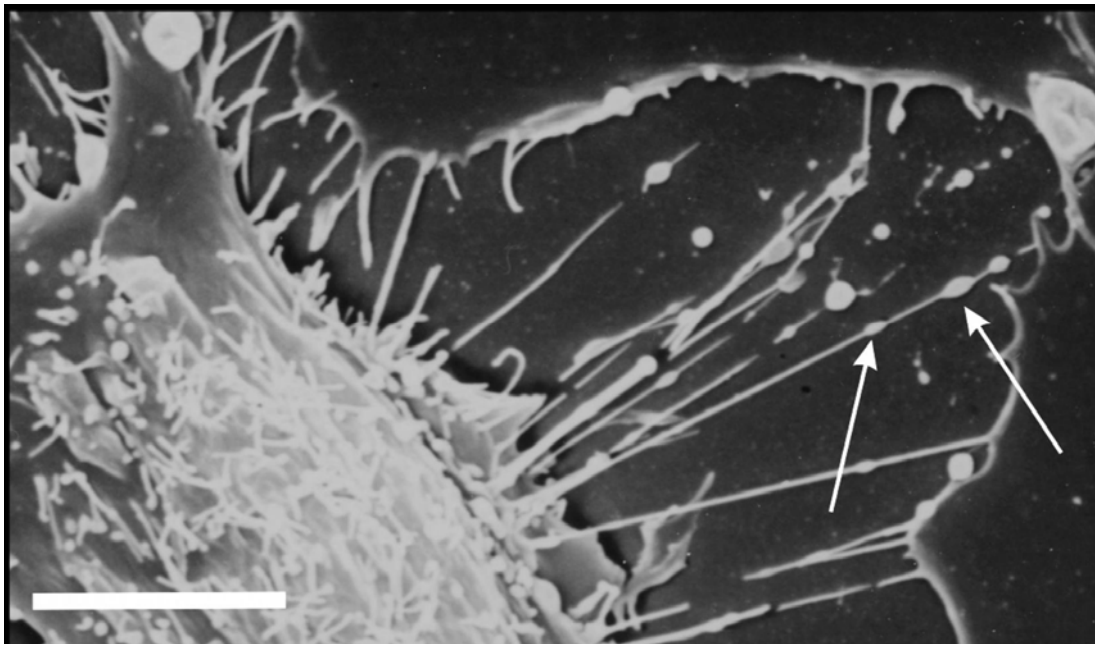


Fig.10. Tubular connections between urothelial cells. Gondolas can be observed in some connections. From http://physics.fe.uni-lj.si/publications/pdf/Curvature.pdf .

THEORETICAL DESCRIPTION

A detailed description of the physical basis of the model is given in http://physics.fe.uni-lj.si/publications/pdf/HWP2004.pdf . Briefly, membrane surface is described by its principal curvatures at a given point (Fig.11). It is assumed that a membrane constituent due to its shape prefers certain membrane curvature which we call intrinsic membrane curvature which is given by means of two principal curvatures (Fig.12). If the principal curvatures are equal, the intrinsic shape is called isotropic. If they differ, the intrinsic shape is called anisotropic.

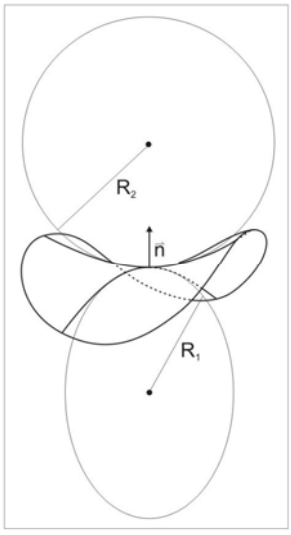
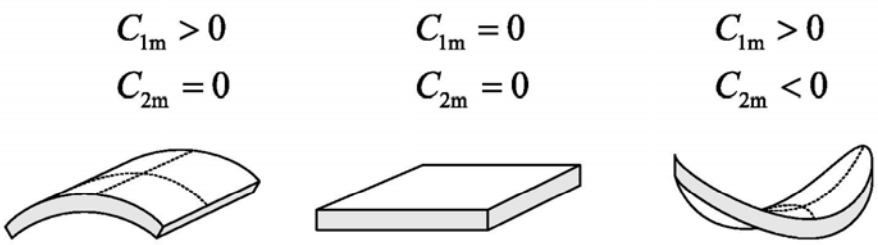


Fig.11: Principal curvatures are the inverse values of the princincipal radii of the spheres which fit the least and the most curved normal



$C_{1m}, C_{2m} \equiv$ **intrinsic (spontaneous)** principal curvatures of inclusion

Fig.12: Examples of isotropic (middle) and anisotropic (left, right) intrinsic shapes

It would cost no energy to insert a constituent into the membrane if the local principal membrane curvatures would equal intrinsic principal membrane curvatures. Due to constraints, membrane cannot attain the intrinsic curvatures at all its points. Therefore, energy is spent in order to adjust the constituent to the actual membrane curvature at its location. This energy is given by the mismatch between the intrinsic shape and the actual shape of the constituent. It is taken into account that the principal directions of the two systems are in general different so that the systems are rotated in the plane of the membrane for an angle ω. The energy of the single constituent is

$$E_i = a_0 \left\{ \frac{\bar{K}_1}{2} (\mathrm{Tr}\underline{M})^2 + \bar{K}_2 \, \mathrm{Det}\underline{M} \right\}$$

- mismatch tensor:

$$\underline{M} = \underline{R} \; \underline{C}_{\mathrm{m}} \underline{R}^{-1} - \underline{C}$$

- diagonalized curvature tensors:

$$\underline{C} = \begin{bmatrix} C_1 & 0 \\ 0 & C_2 \end{bmatrix}, \quad \underline{C}_{\mathrm{m}} = \begin{bmatrix} C_{1\mathrm{m}} & 0 \\ 0 & C_{2\mathrm{m}} \end{bmatrix}$$

- rotation matrix:

$$\underline{R} = \begin{bmatrix} \cos\omega & -\sin\omega \\ \sin\omega & \cos\omega \end{bmatrix}$$

The membrane free energy is the sum of the contributions of all the constituents. Direct interactions between constituents and entropic effects are included. To obtain the equilibrium configuration of the system (the membrane shape and lateral and orientational distributions of the constituents) free energy is minimized at relevant constraints upon the system. The minimization is performed numerically. In budding membranes it is of special interest to study the lateral and orientational density of the constituents on the bud and on the mother membrane. Fig.13 shows equilibrium shape of the budding membrane segment (black) and lateral density of membrane constituents characterized by isotropic intrinsic curvatures that are larger than the curvatures of the mother membrane.

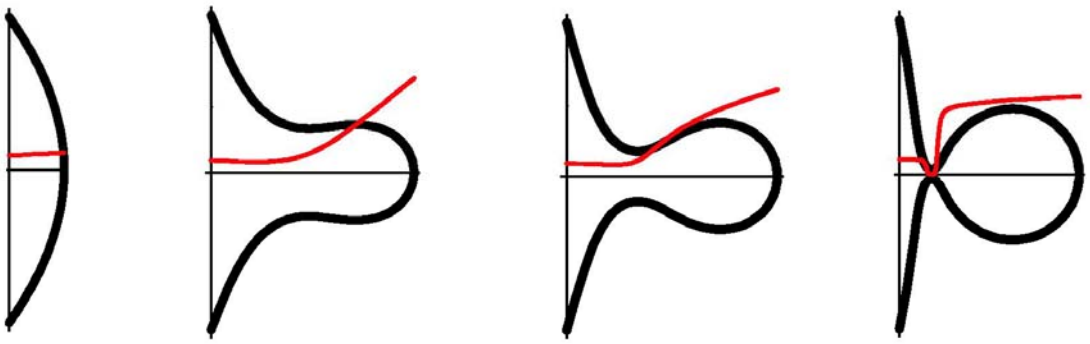


Fig.13: The calculated equilibrium membrane contour and the corresponding area covered by isotropic membrane inclusions. From http://physics.fe.uni-lj.si/publications/pdf/MMB2006.pdf .

Accumulation of certain type of constituents on the buds can be visualized in erythrocytes (Fig.14) and in cells (Fig.15).

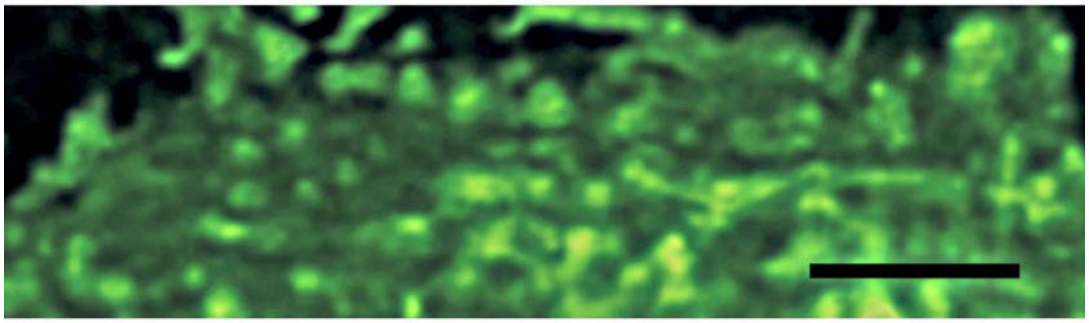


Fig.15: Accumulation of fluorescent membrane raft markers on buds in human urothelial line RT4 cells. Bar = 200 nm. From: http://physics.fe.uni-lj.si/publications/pdf/Curvature.pdf

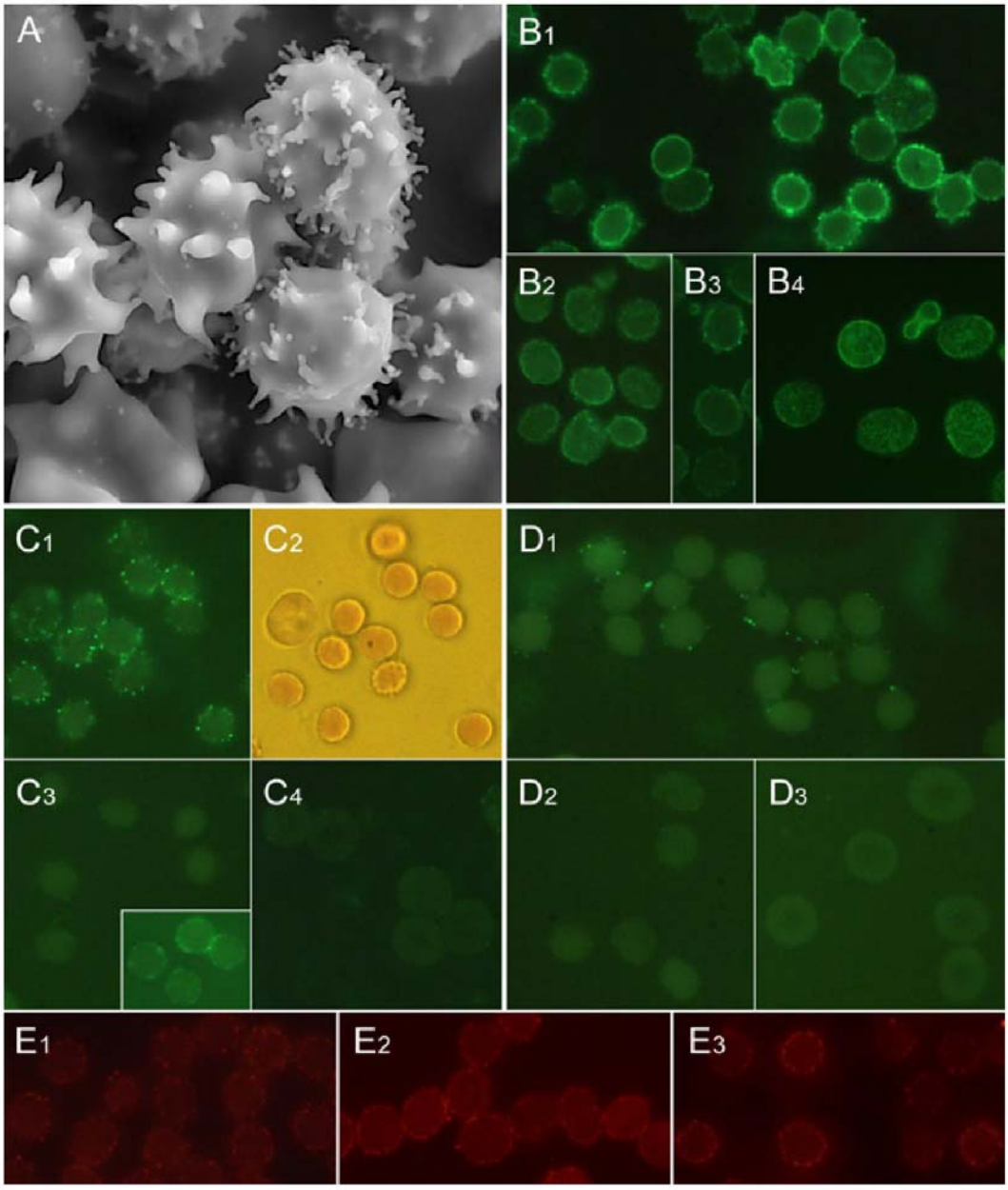


Fig.14: Accumulation of fluorescent membrane raft markers on buds in erythrocytes. From http://physics.fe.uni-lj.si/publications/pdf/MMB2006.pdf

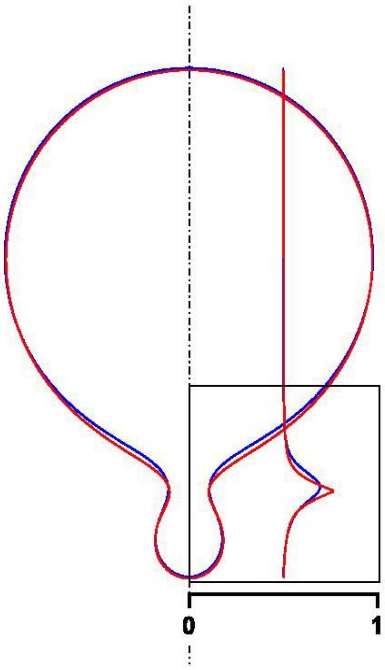


Fig.16: Calculated equilibrium shape of a phospholipid bilayer vesicle composed of one kind of phospholipid molecules. In.-plane rotation of the molecules is taken into account. The two shapes differ in the average mean curvature of the vesicle. The distribution of the orientational ordering is given in a two-state orientation model. The value 0.5 means that the two states are equally occupied. In the neck region, one of the two orientational states is considerably more favourable than the other. From http://physics.fe.uni-lj.si/publications/pdf/KraljIglic2006JSP.pdf .

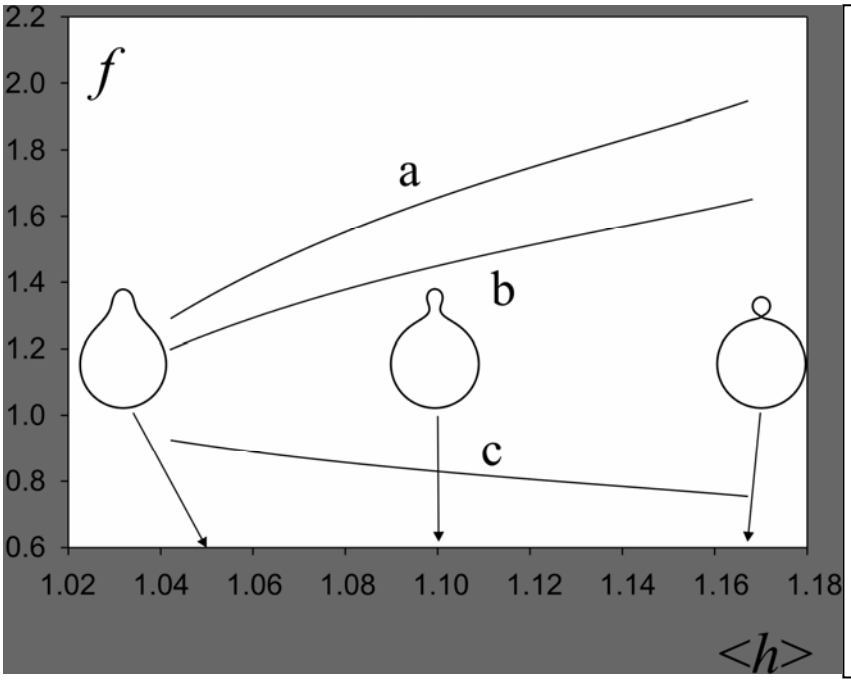


Fig.17: The free energy of the vesicle as a function of the average mean curvature of the vesicle. a: no orientational ordering, b: orientational ordering of independent constituents, c: orientational ordering and direct interactions between the constituents. It can be seen that the formation of the narrow neck is promoted (free enrgy is decreasing as the neck is formed) if the direct interactions and orientational ordering are taken into account. From http://physics.fe.uni-lj.si/publications/pdf/KraljIglic2006JSP.pdf .

Narrow necks are found to be stable in pure phospholipid systems. Fig.18 spontaneous shape transformation of a vesicle with a tubular bud into a flaccid globular vesicle. The shape oscillates around the shape with the narrow neck before it opens.

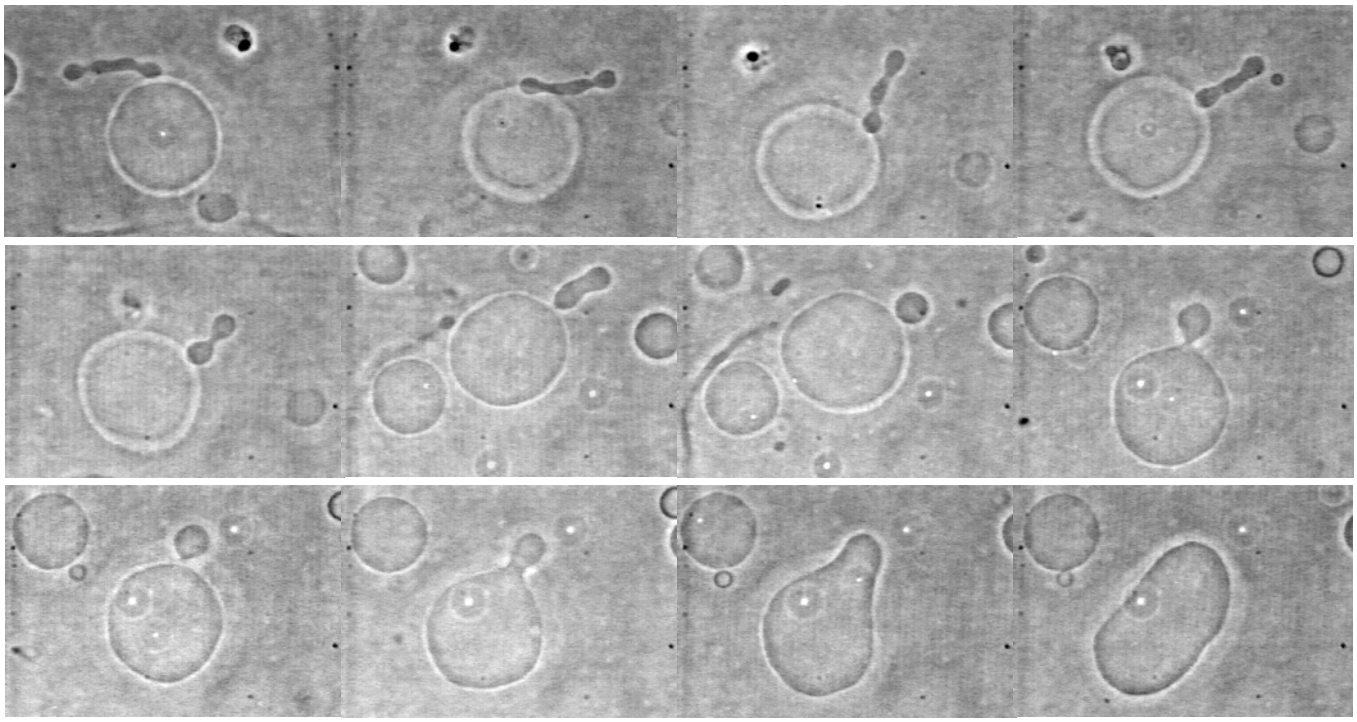


Fig.18: Spontaneous transformation of a POPC vesicle with a tubular protrusion. Bar = 10 μm.
From: http://physics.fe.uni-lj.si/publications/pdf/corgi.pdf .

SPHERICAL AND TUBULAR VESICULATION

Budding may eventually lead to the release of the vesicles from the membrane. This is a feature in erythrocytes, phospholipid vesicles and cells.

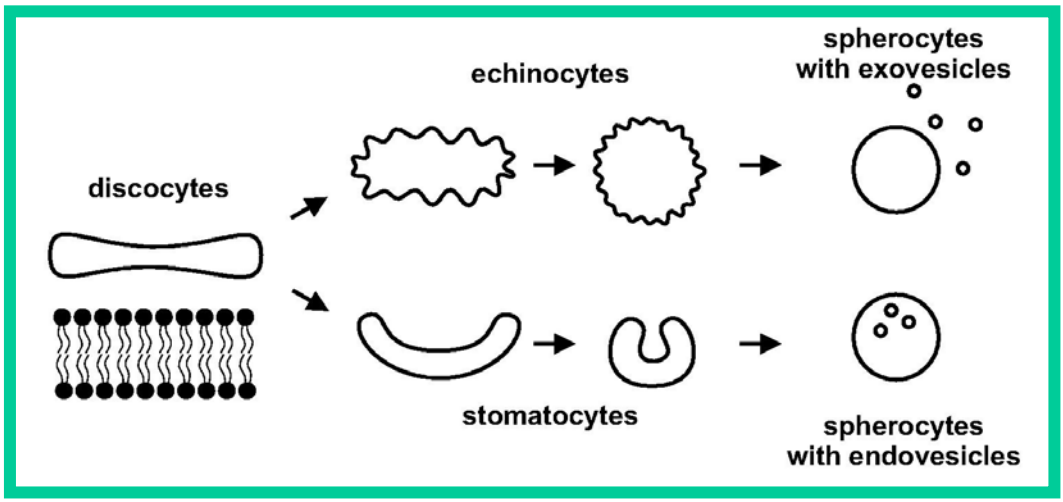


Fig.19: Following echinocytosis or stomatocytosis, erythrocytes shed endovesicles and microexovesicles that leave the mother membrane and are free to move within the vascular system.

Inward budding yields endovesicles which can be spherical or torocytic (Fig.20) while outward budding yields microexovesicles that can be spherical or tubular (Fig.21).

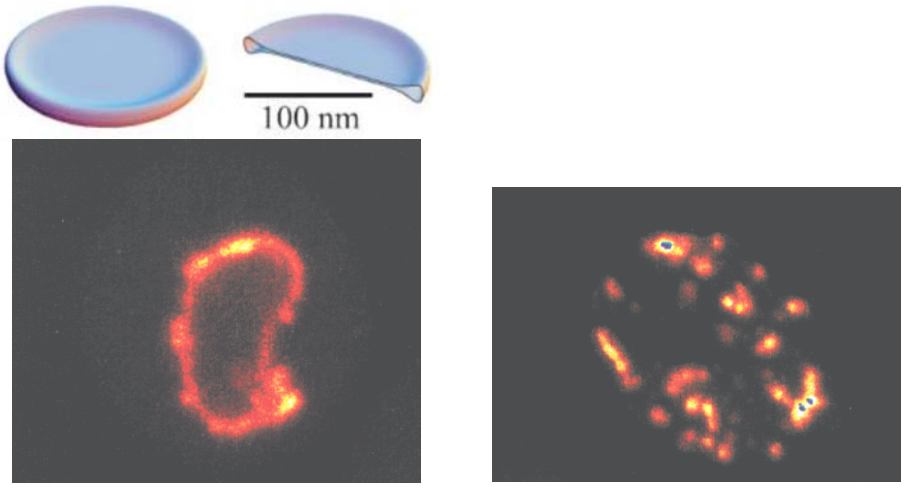


Fig.20: Torocytic endovesicles induced by addition of octaethyleneglycoldodecylether to the erythrocyte suspension (left) and spherical endovesicles induced by addition of chlorpromazine to the erythrocyte suspension (right). The contents of the endovesicles is made visible by a fluorescent dye in the solution engulfed by the buds/vesicles. From http://physics.fe.uni-lj.si/publications/pdf/3356.pdf .

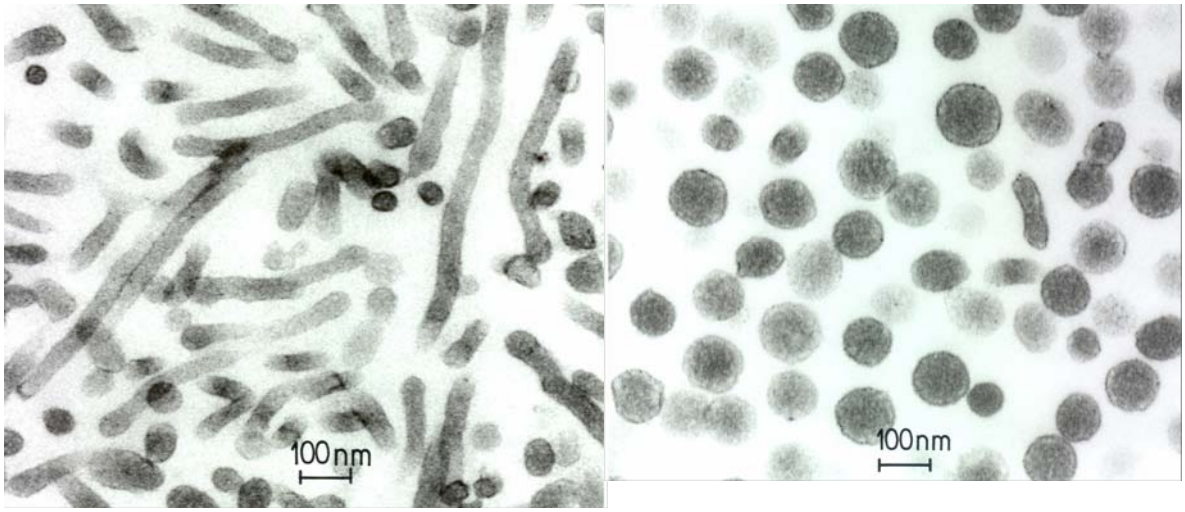


Fig.21: Tubular (left) and spherical (right) microexovesicles isolated from human blood. Tubular vesiculation was induced by addition of dodecylmaltosyde to the erythrocyte suspension while spherical budding was induced by addition of dodecylzwittergent to the erythrocyte suspension. From http://physics.fe.uni-lj.si/publications/pdf/PRE04230.pdf .

Fig.22 shows a disintegration of the bead-like protrusion of a phospholipid vesicle in phosphate buffer saline. Budding of the vesicle was induced by raising the temperature of the system.

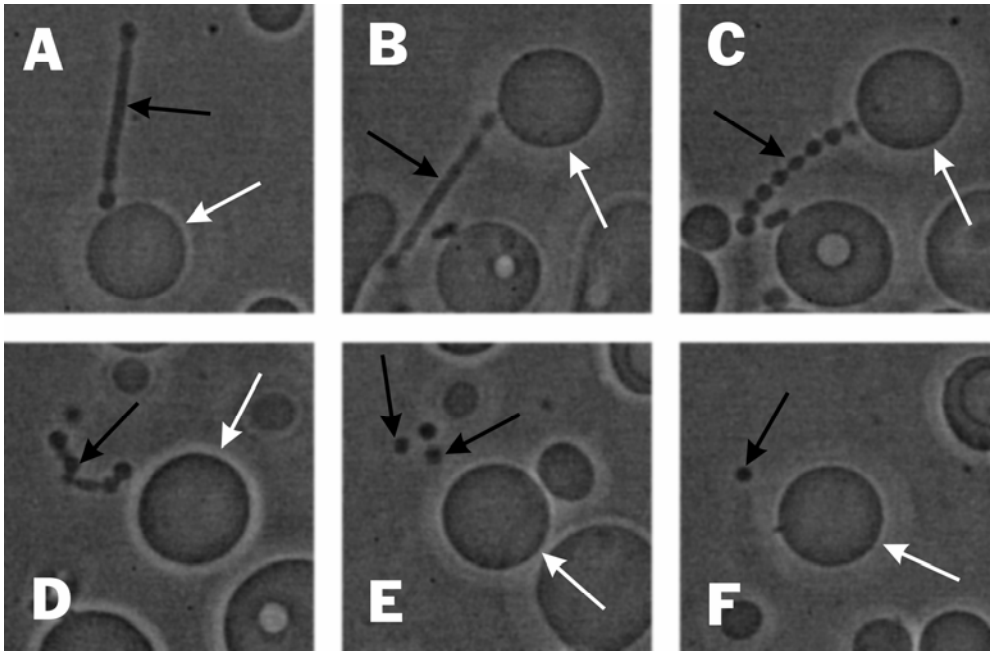


Fig.22: Upon addition of phosphate buffer saline the protrusion became bead-like, detached from the mother vesicle and disintegrated into small spherical vesicles.

The presence of serum proteins induces coalescence of the vesicles (Fig.23).

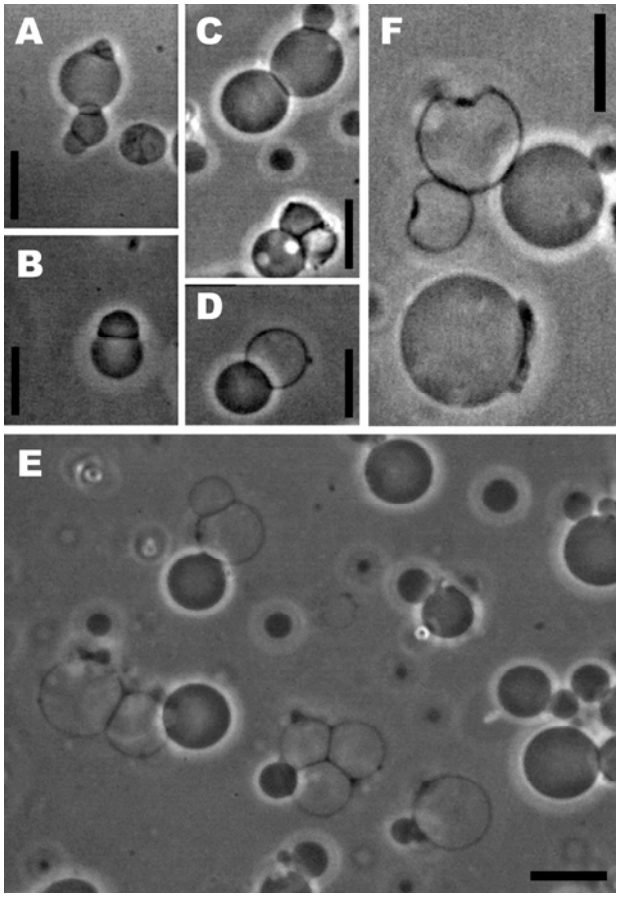


Fig.23: Effect of serum protein beta2glycoprotein I and antiphospholipid antibodies on negatively charged phospholipid vesicles. Thevesicles coalesced into complexes, lateral separation occured within the membranes while some vesicles developed pores and became ghosts. Bars = 10μm. From http://physics.fe.uni-lj.si/publications/pdf/Ambrozic2006AR.pdf .

In budding vesicles the presence of these serum proteins prevents the release of the vesicles from the mother membrane (Fig.24).

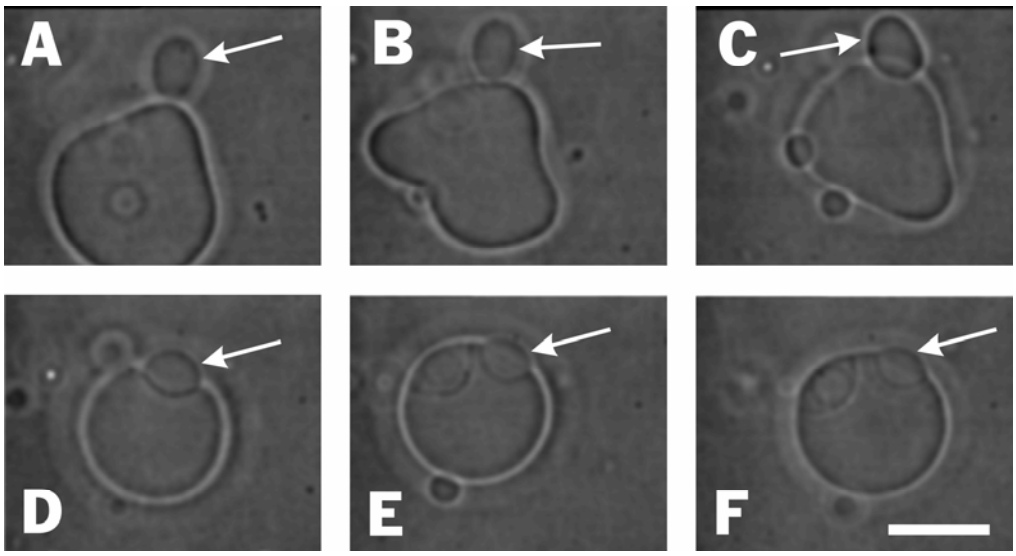


Fig.24: Budding of a phospholipid vesicle in the presence of serum protein beta2glycoprotein I in the solution. The bud coalesces with the membrane.

The effect of serum proteins on the budding of phospholipid membrane is schematically shown in Fig.25. If the vesicle buds in phosphate buffer saline, they eventually pinch off and become free to move while in the presence of certain serum proteins the buds remain attached to the mother membrane. We suggest that this is a hypothetical mechanism underlying the effect of some serum proteins and drugs by preventing the release of prothrombogenic and tumour-promoting microexovesicles from the membrane into the circulation.

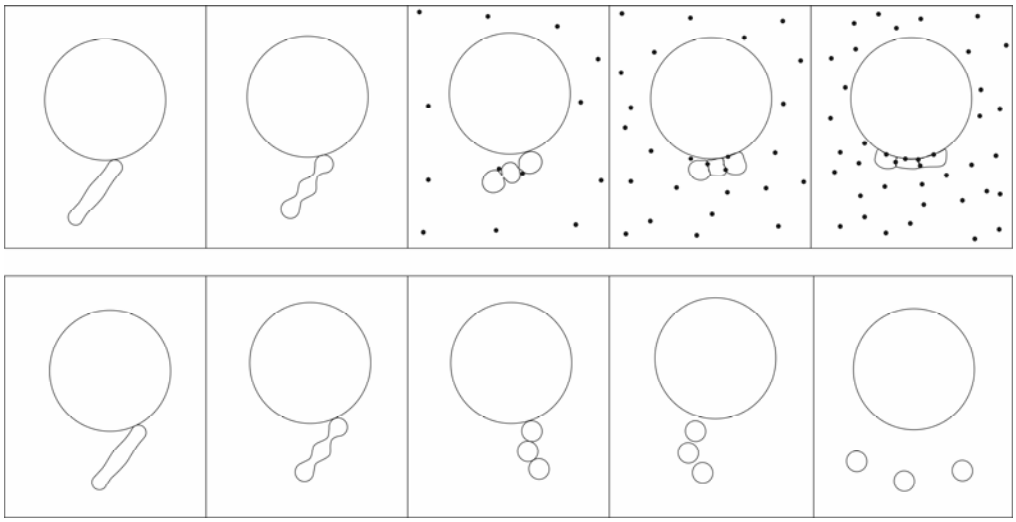


Fig.25: Upper: mediating the attractive interaction between the bud and the mother membrane prevents the release of the microvesicles into the circulation. Lower: Microexovesicles are released into the circulation to act as procoagulants and/or carriers of the tumor promoting molecules.

CONCLUSIONS

Budding and raft formation is promoted by lateral redistribution of membrane constituents, changes in average mean curvature and orienational ordering of the membrane constituents.
Anisotropic membrane structures are common in phospholipid and in cell membranes. They consitute infrastructure within and between cells through which material and information is transmitted.
The surroundings of the membrane (the solution) influences budding by promoting or preventing the release of carrier vesicles into the circulation. Budding and shedding of the microvesicles has important effects on blood coagulation and promotion of cancer.

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