The formation and coalescence of membrane microtubules

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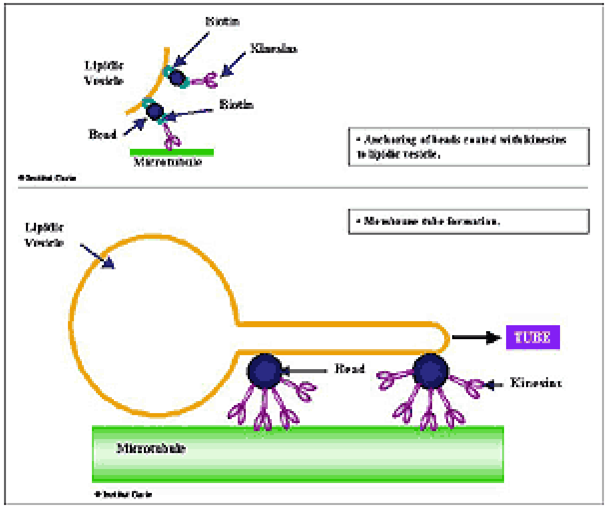
This lecture will be about some old theoretical results used to interpret some recently published experimental data

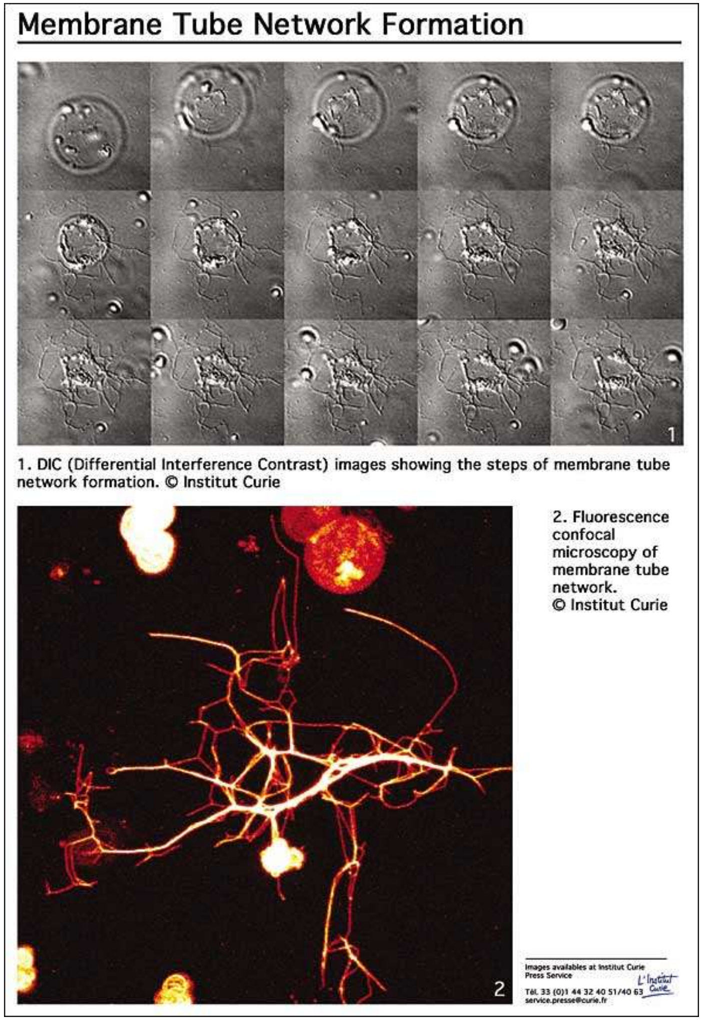
Membrane tube (tether) networks in cells and in vitro

(taken from the work of Bruno Goud and Patricia Bassereau)



• Anchoring of beads coated with kinesine to lipidic vesicle.

• Membrane tube formation.


Membrane Tube Network Formation

1. DIC (Differential Interference Contrast) images showing the steps of membrane tube network formation. © Institut Curie

2. Fluorescence confocal microscopy of membrane tube network. © Institut Curie

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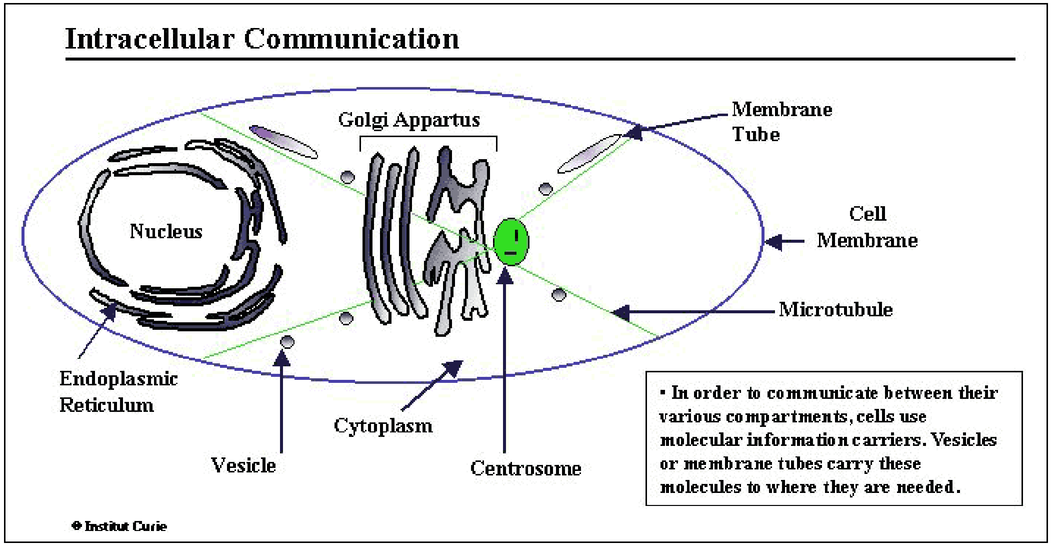
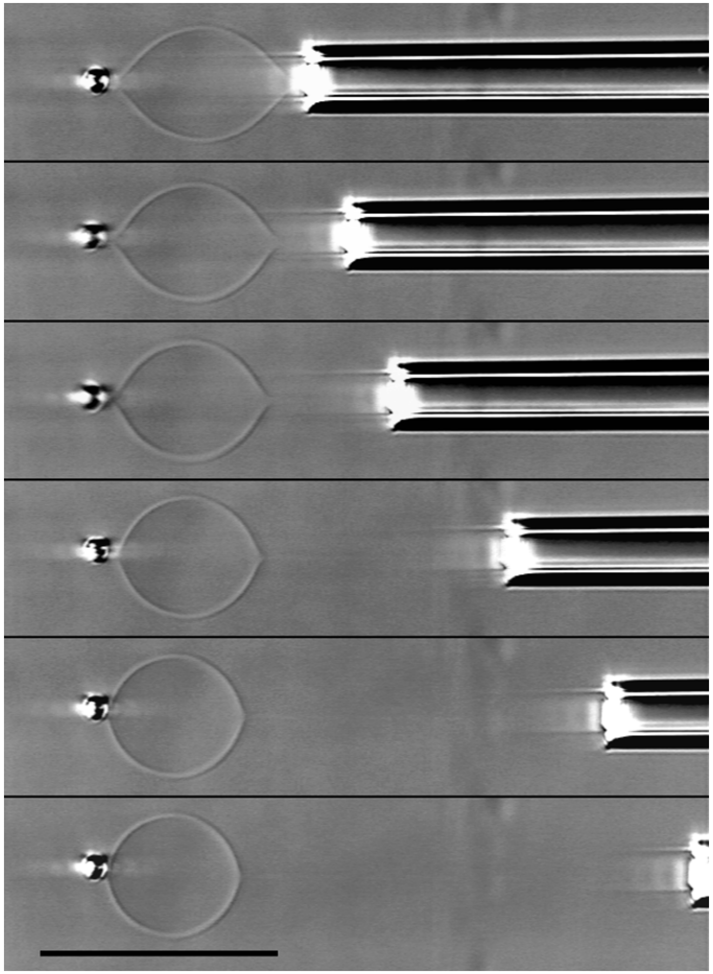
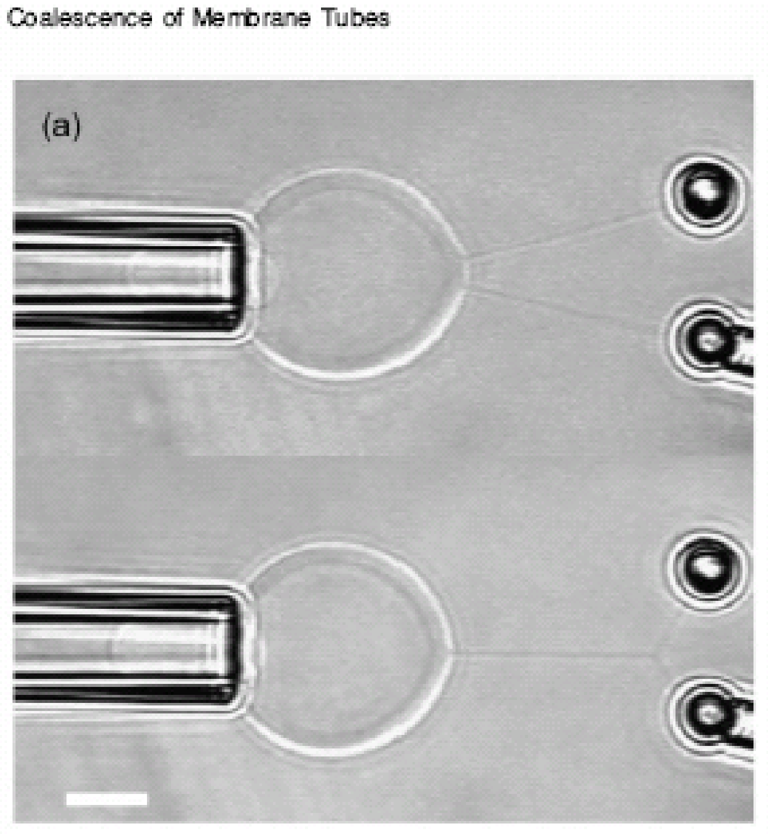

Intracellular Communication

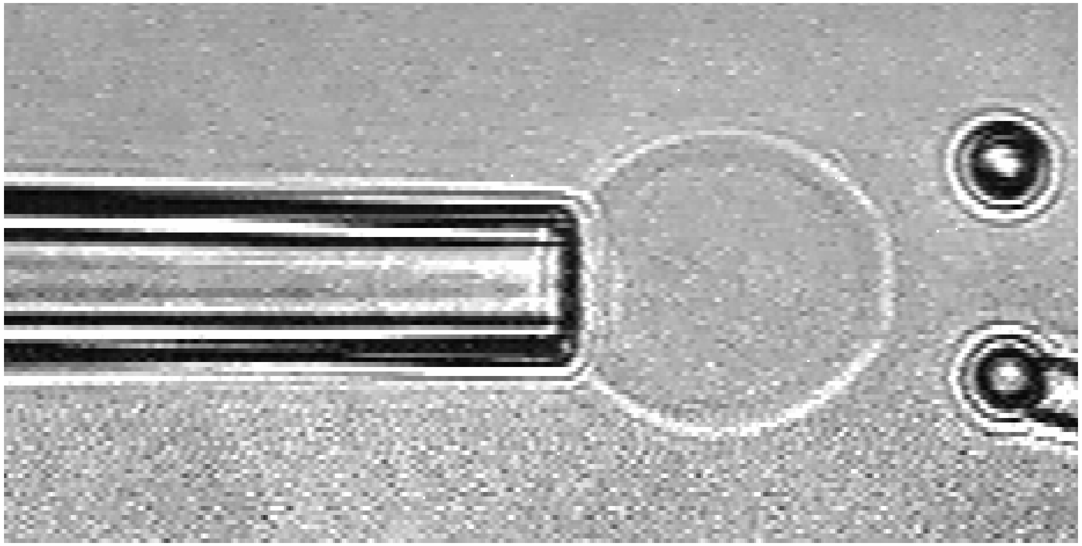
• In order to communicate between their various compartments, cells use molecular information carriers. Vesicles or membrane tubes carry these molecules to where they are needed.

Membrane tether: a thin tube obtained by pulling a vesicle apart by the force applied in a point





From Cuvelier, Derenyi, Bassereau and Nassoy, Biophys J, April 2005

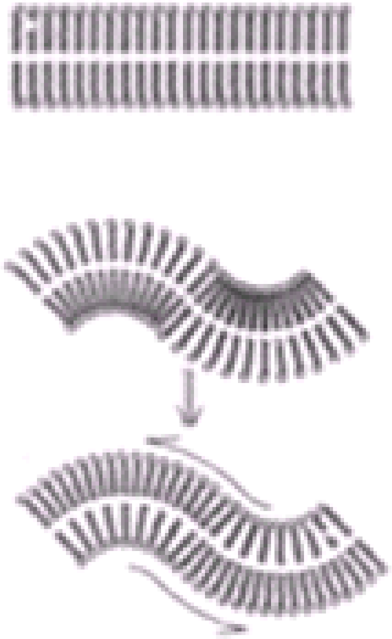


From Cuvelier, Derenyi, Bassereau and Nassoy, Biophys J, April 2005

Lecture outline

- Elastic deformational modes of lamellar membranes
- Pulling of membrane microtubules (tethers) as a method for the determination of membrane bending constants
- Classification of vesicle shapes
- Theoretical interpretation of tether formation
- Tether coalescence

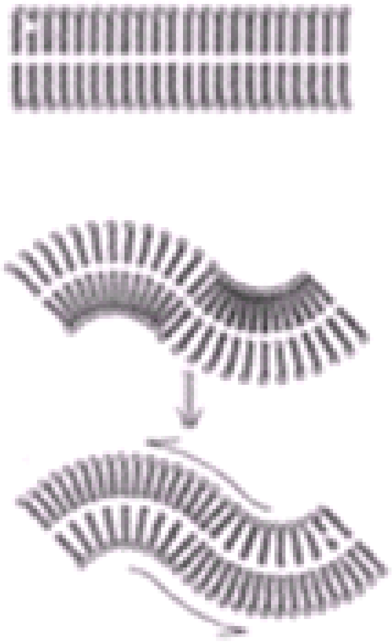
Envelopes of different vesicular objects share a common property: they are composed of layers which are in contact but laterally unconnected



connected layers

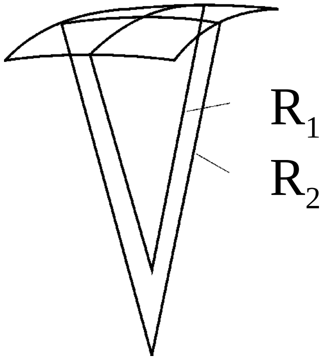
unconnected layers

Envelopes of different vesicular objects share a common property: they are composed of layers which are in contact but laterally unconnected



Elastic deformational modes of the bilayer are obtained by summing up the elastic energies of individual leaflets, and by taking into account that the areas of the two leaflets differ by

$$\Delta A = h\,\bar{C}$$



where

$$\bar{C} = \int (C_1 + C_2)\, dA$$

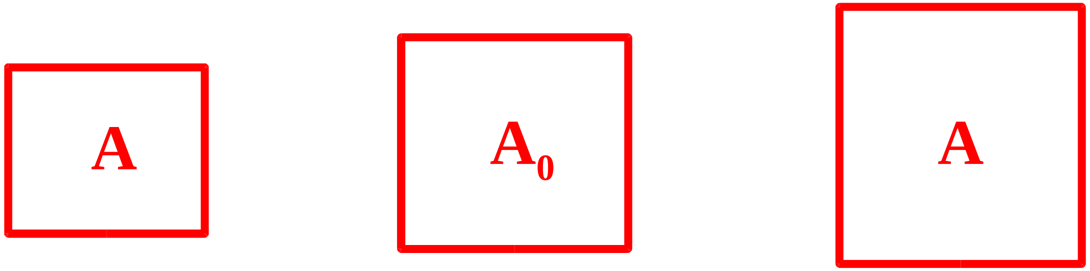
is integrated membrane curvature

h is the distance between the leaflet neutral surfaces

$C_1 = 1/R_1$, $C_2 = 1/R_2$ are principal curvatures

Deformational modes of a closed bilayer membrane

$$
\begin{aligned}
W = {} & \boxed{\frac{1}{2}\frac{K}{A_0}\left(A - A_0\right)^2 \qquad\qquad \text{area expansivity}} \\
& + \frac{1}{2}k_c \oint \left(C_1 + C_2 - C_0\right)^2 dA \qquad\qquad \text{bending (local)} \\
& + k_G \int C_1 C_2\, dA \qquad\qquad \text{Gaussian bending} \\
& + \frac{1}{2}\frac{k_r}{h^2 A_0}\left(\Delta A - \Delta A_o\right)^2 \qquad\qquad \text{nonlocal bending}
\end{aligned}
$$



Deformational modes of a closed bilayer membrane

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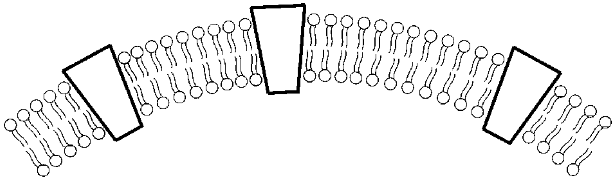
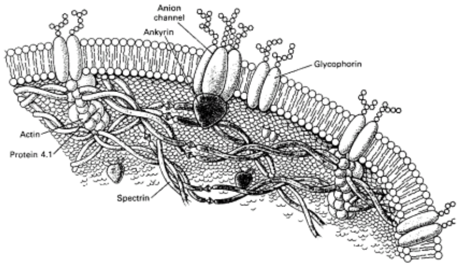
\end{aligned}
$$

Spontaneous curvature C_0 (Helfrich, 1973):





Deformational modes of a closed bilayer membrane

$$W = \frac{1}{2}\frac{K}{A_0}\left(A - A_0\right)^2$$

area expansivity

$$+\frac{1}{2}k_c\oint\left(C_1 + C_2 - C_0\right)^2 dA$$

bending (local)

$$+k_G\int C_1 C_2\, dA$$

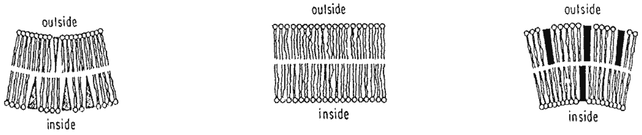
Gaussian bending

$$\boxed{+\frac{1}{2}\frac{k_r}{h^2 A_0}\left(\Delta A - \Delta A_o\right)^2 \qquad \text{nonlocal bending}}$$

Equilibrium area difference ΔA_0:

(Bilayer couple hypothesis of Sheetz and Singer, 1974)

ΔA_0 →



Essentially, vesicle shapes depend on local and nonlocal bending energy terms

$W=\frac{1}{2}\frac{K}{A_0}\left(A-A_0\right)^2$	area expansivity
$\boxed{+\frac{1}{2}k_c\oint\left(C_1+C_2-C_0\right)^2 dA}$	bending (local)
$+k_G\int C_1 C_2\, dA$	Gaussian bending
$\boxed{+\frac{1}{2}\frac{k_r}{h^2 A_0}\left(\Delta A-\Delta A_o\right)^2}$	nonlocal bending

The parameters are C_0, ΔA_0, k_r/k_c

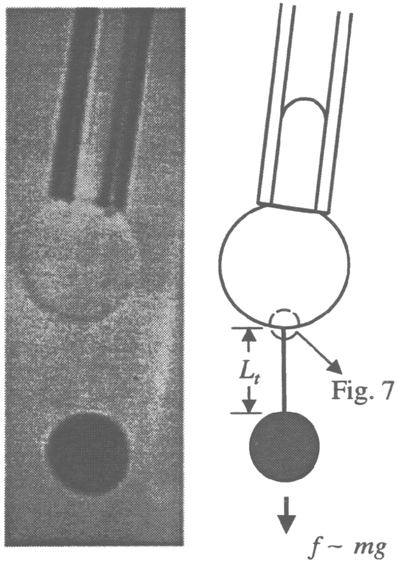
The ratio k_r/k_c for phospholipid vesicle envelopes

If the bilayer leaflets were made of a homogeneous material, we would have:

$$k_r/k_c = 3$$

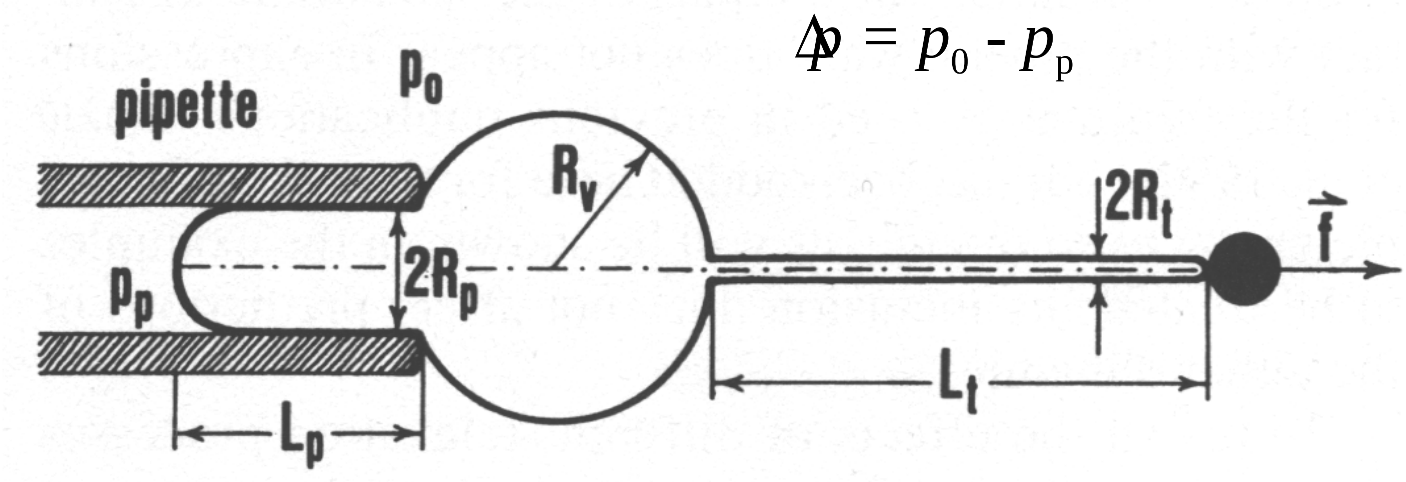
For phospholipid membranes the ratio k_r/k_c was determined by the tether pulling experiment (Božič et al., and Waugh et al., BJ 1992)

Tether pulling experiment



$$G = \frac{1}{2}\frac{k_r}{h^2 A_0}\left(\Delta A - \Delta A_0\right)^2 + \frac{1}{2}k_c \oint \left(C_1 + C_2 - C_0\right)^2 dA - fL_t - \Delta p \pi R_p^{\,2} L_p$$

$$\Delta p = p_0 - p_p$$

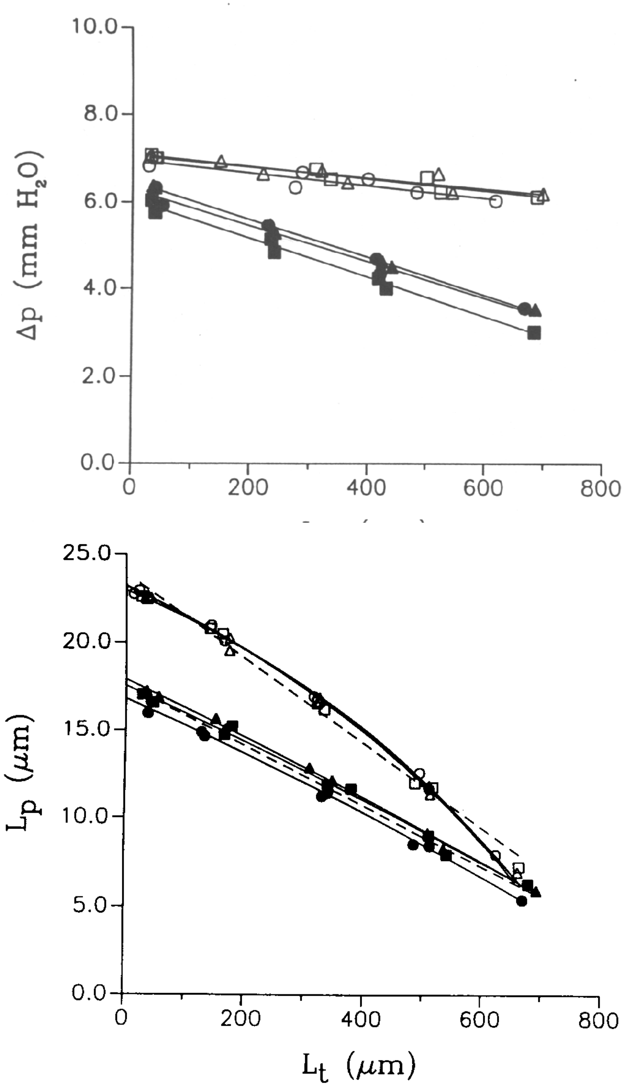


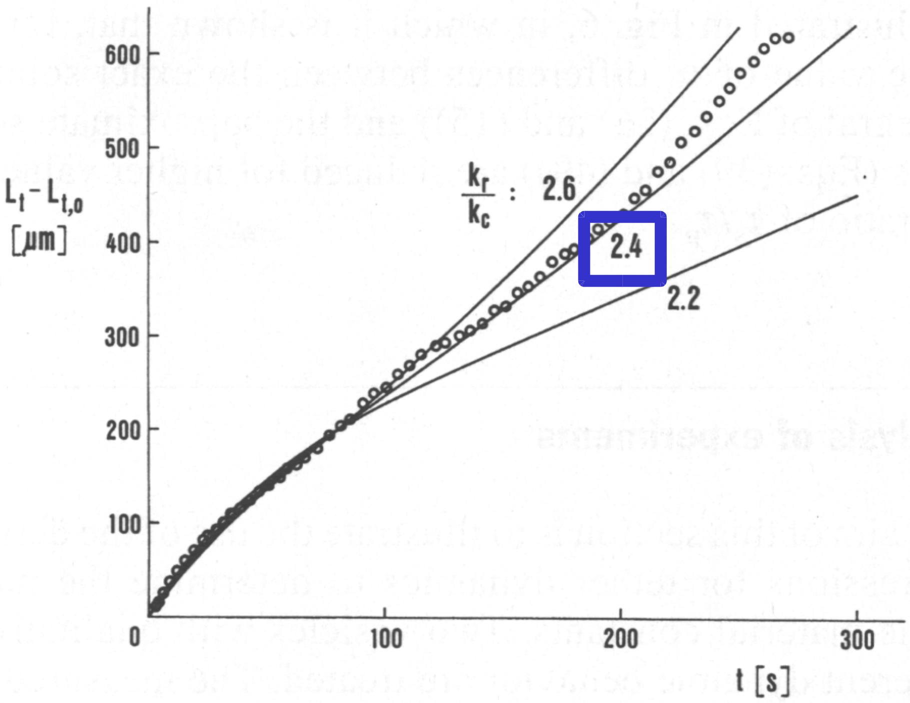
Measured parameters

(Waugh et al. BJ 1992)

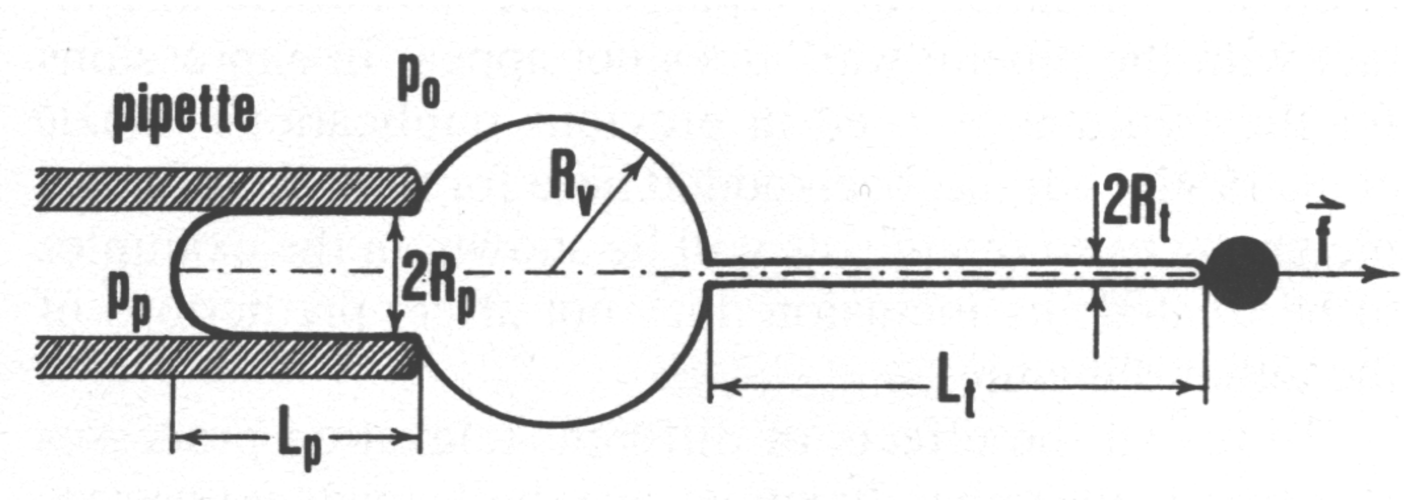
Raphael & Waugh BJ 1996

Svetina et al. Eur. Biophys. J. 1998





Is the geometrical model reliable?



To resolve this problem we calculated shapes of axially strained vesicles exactly

To introduce the spirit of these calculations I shall first shortly discuss some aspects of the shapes of free vesicles

Vesicle shapes can be conveniently classified on the basis of the **bilayer couple model** in which **$k_r/k_c = \infty$** and consequently **$\Delta A = \Delta A_0$.**

Shapes correspond to the minimum of (the local) membrane bending energy at **the constraints**

$$A = \text{const}$$

$$V = \text{const}$$

$$\Delta A = \text{const}$$

Shapes of free vesicles are characterized by only two (geometrical) parameters:

- **relative vesicle volume**

$$v = \frac{V}{V_s} = \frac{\textit{vesicle volume}}{4\pi R_s^3/3}$$

- **relative integrated membrane curvature**

$$\bar{c} = \frac{\bar{C}}{\bar{C}_s} = \frac{\textit{integrated membrane curvature}}{8\pi R_s}$$

R_s is the radius of the sphere with the same membrane area: $A = 4\pi R_s^2$

$\bar{C}_s$ is integrated membrane curvature of the sphere

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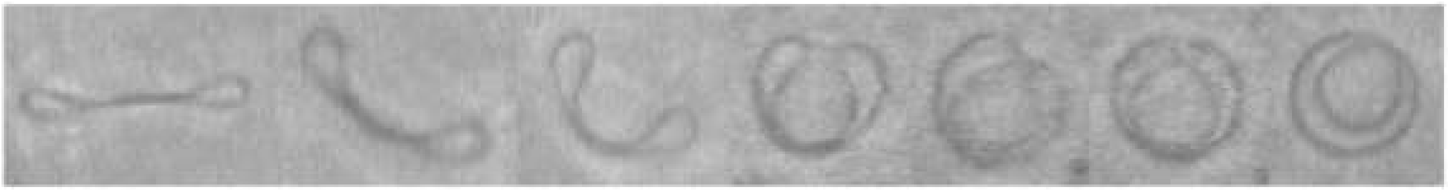
$\bar{C}_s$ is integrated membrane curvature of the sphere

Take note that $\boxed{\bar{c} = \Delta a}$ where $\Delta a = \Delta A / h 8\pi R_s$

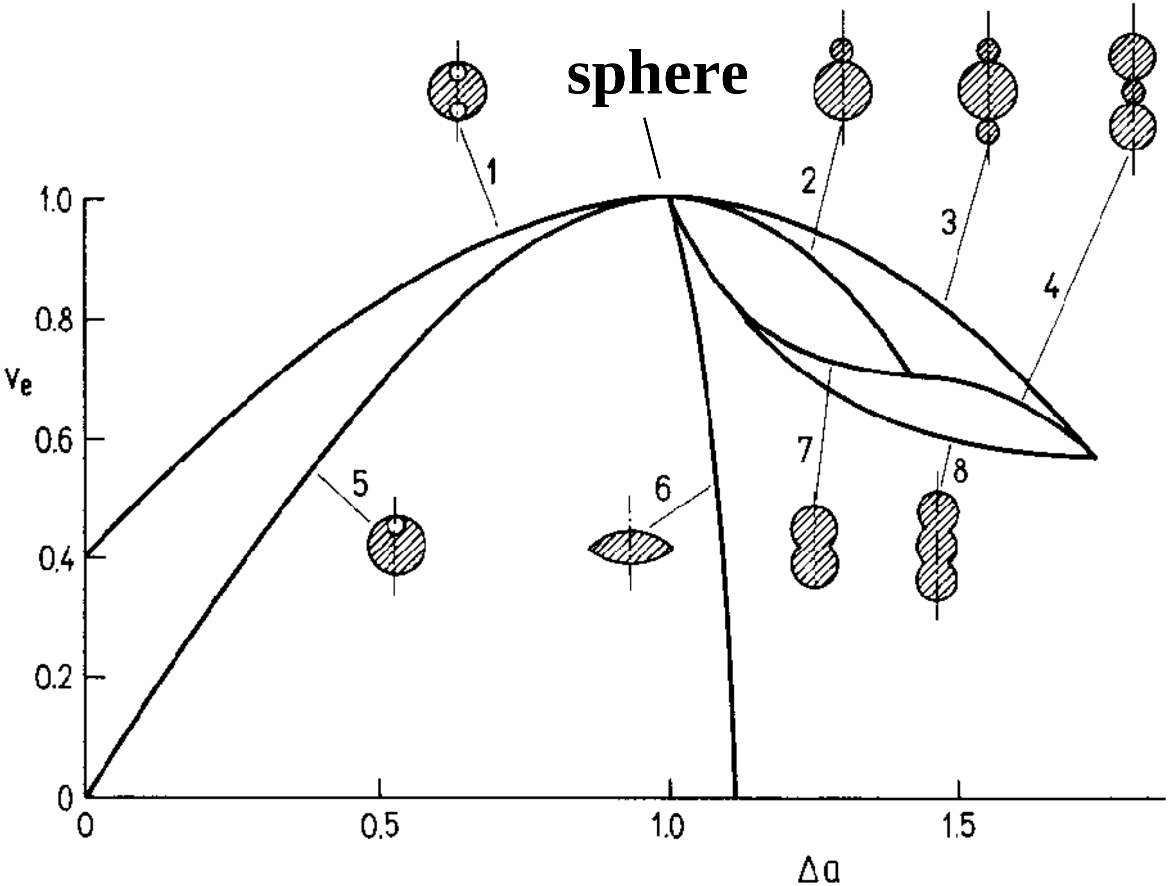
For each set of v and Δa ($\bar{c}$) values the shape can be found that has the minimum membrane bending energy

An example: comparison between observed and calculated shapes of codocytes at v = 0.3 and different Δa
(from Majhenc et al., BBA 2004)



Δa:

v - Δa phase diagram for the classification of vesicle shapes



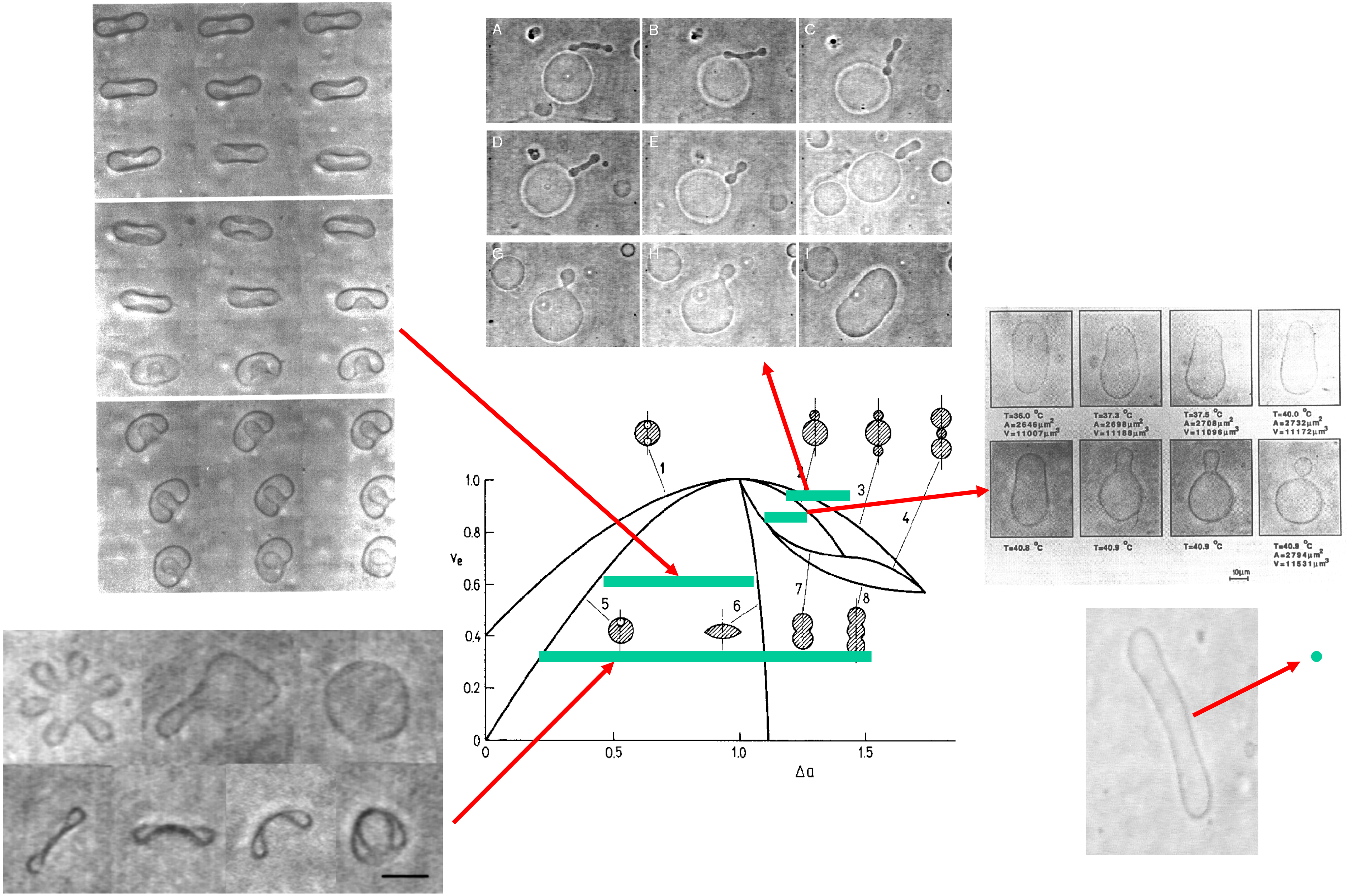
sphere

relative volume

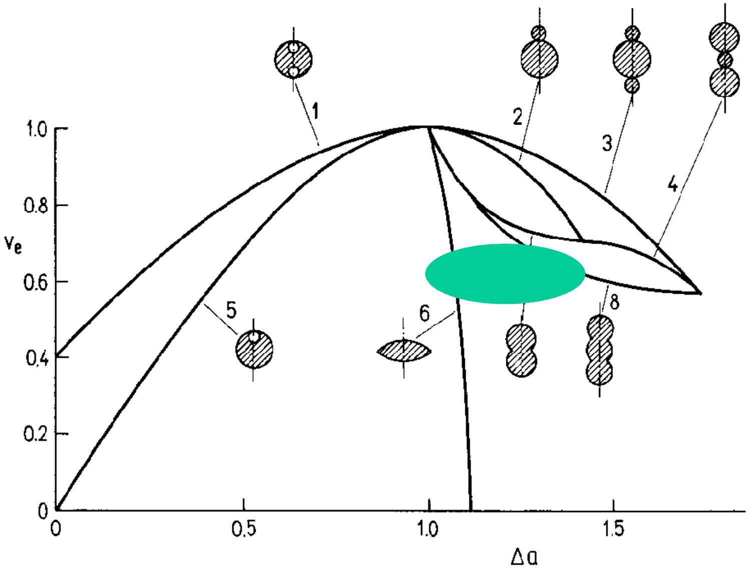
relative integral membrane curvature
relative area difference

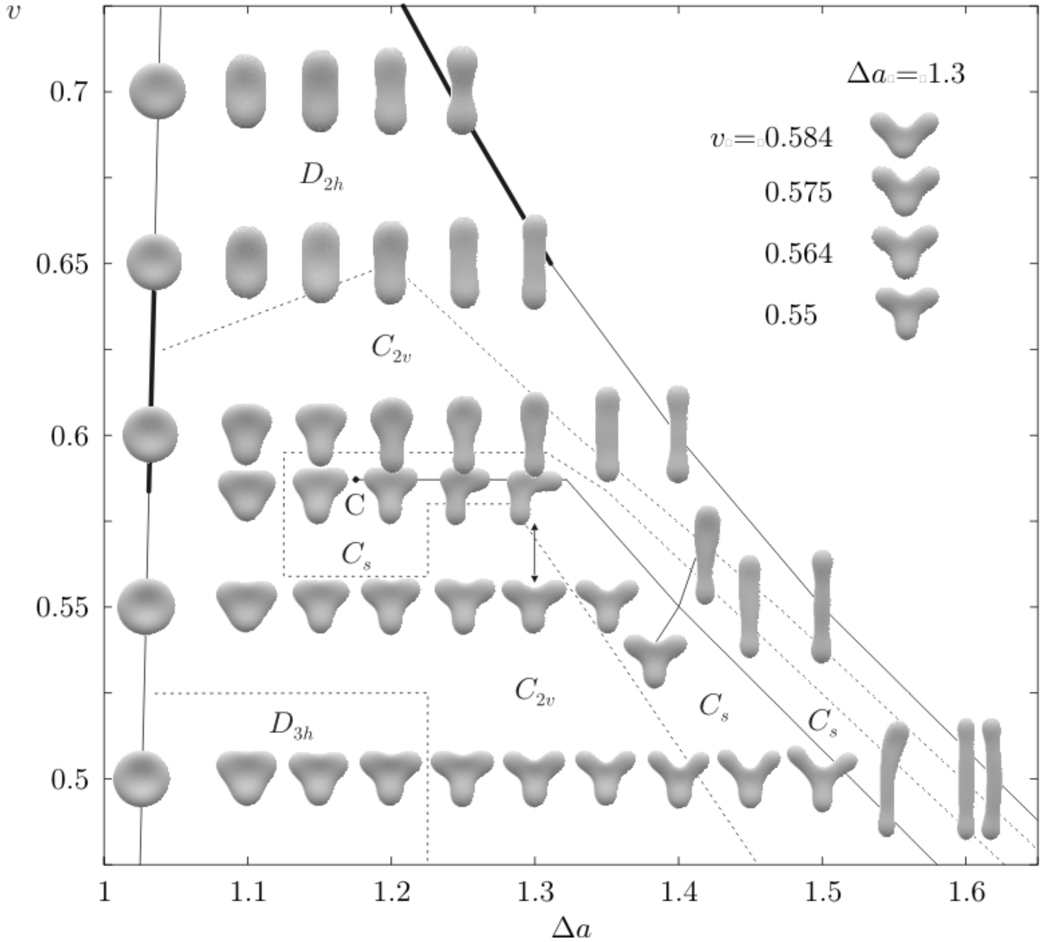
Limiting shapes are compositions of spheres or sections of spheres, with only two possible radii. They can be obtained variationally by looking at vesicles with extreme v at a given Δa

Position of various vesicle shapes in the v-Δa phase diagram

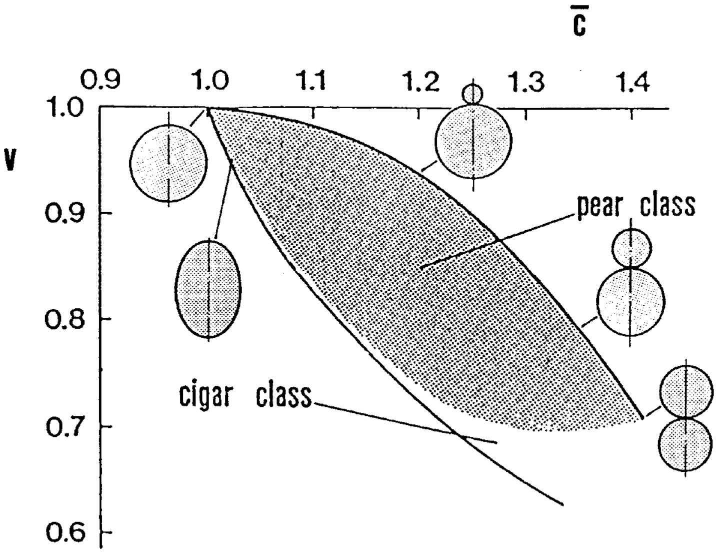


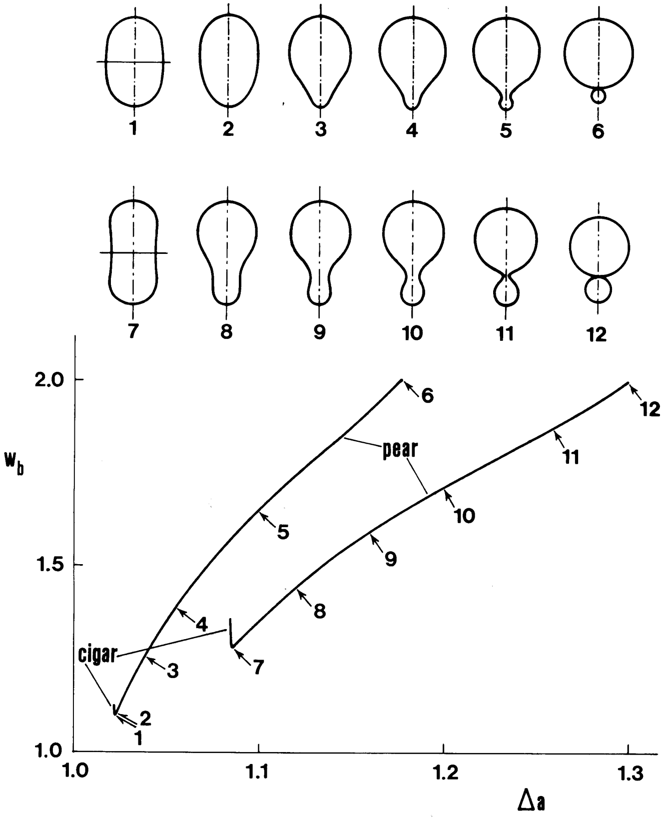
Theoretical work on non-axisymmetrical shapes in the region 0.5 < v < 0.7 (Ziherl and Svetina, Europhys. Lett. 2005)

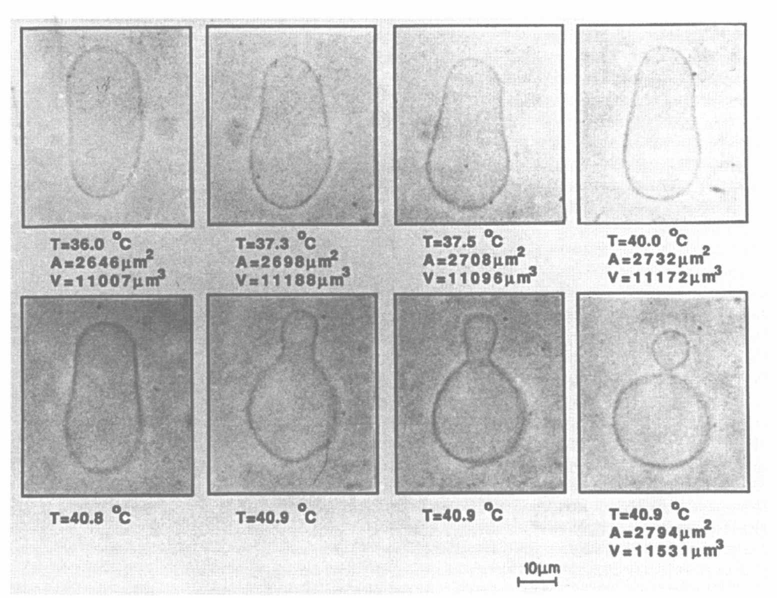




We “pulled” tethers out of the shapes of cigar and pear shape classes







v = 0.95 (left curve)

v = 0.85 (right curve)

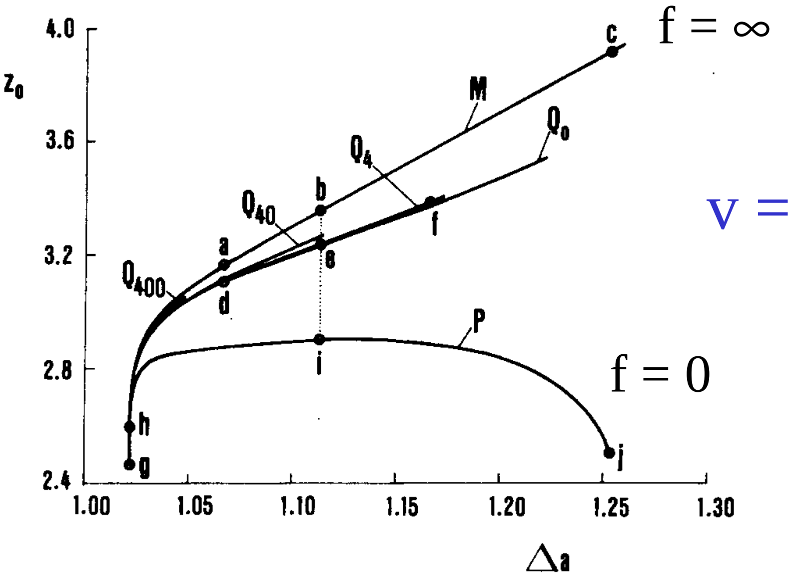
Why do tethers form?

Theoretical determination of shapes of vesicles under the effect of the axial force f (Božič et al. Phys Rev E 1997)

$$G = \frac{1}{2} k_c \oint \left(C_1 + C_2 - C_0\right)^2 dA \quad - \, f Z_0$$

V = const, A = const, ΔA = const Z_0: vesicle height

z_0 - v - Δa phase diagram:



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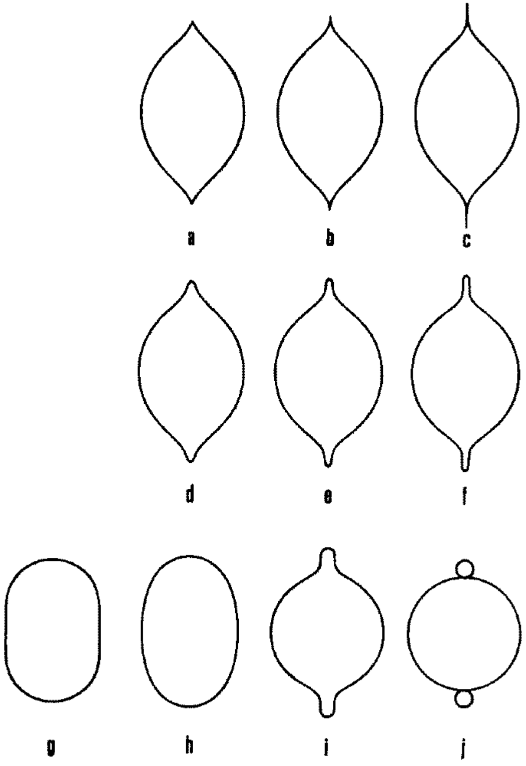
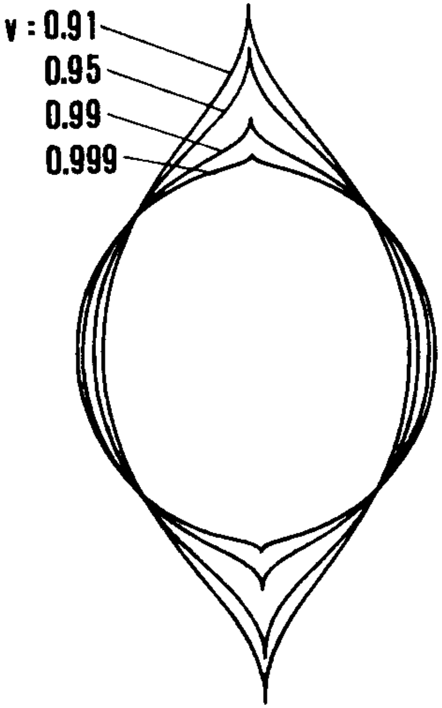
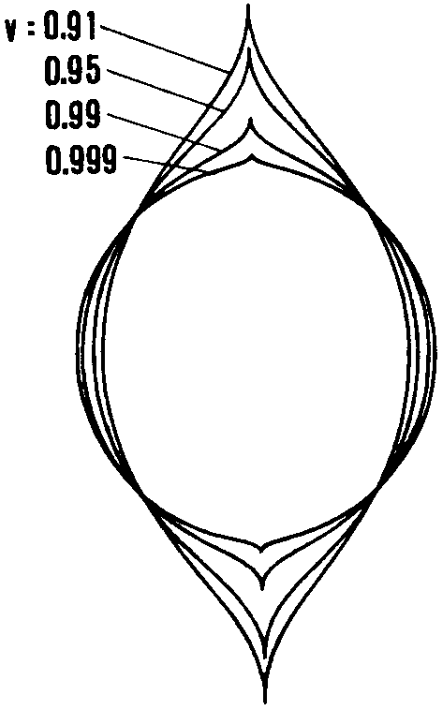
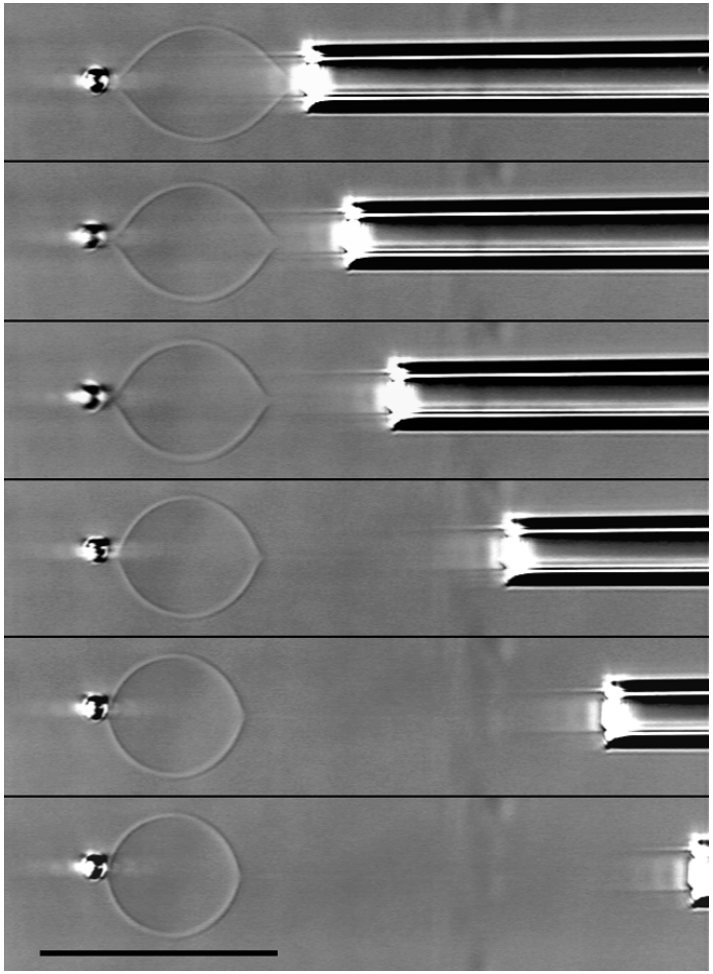


Limiting shapes are unduloids and depend on the relative volume v

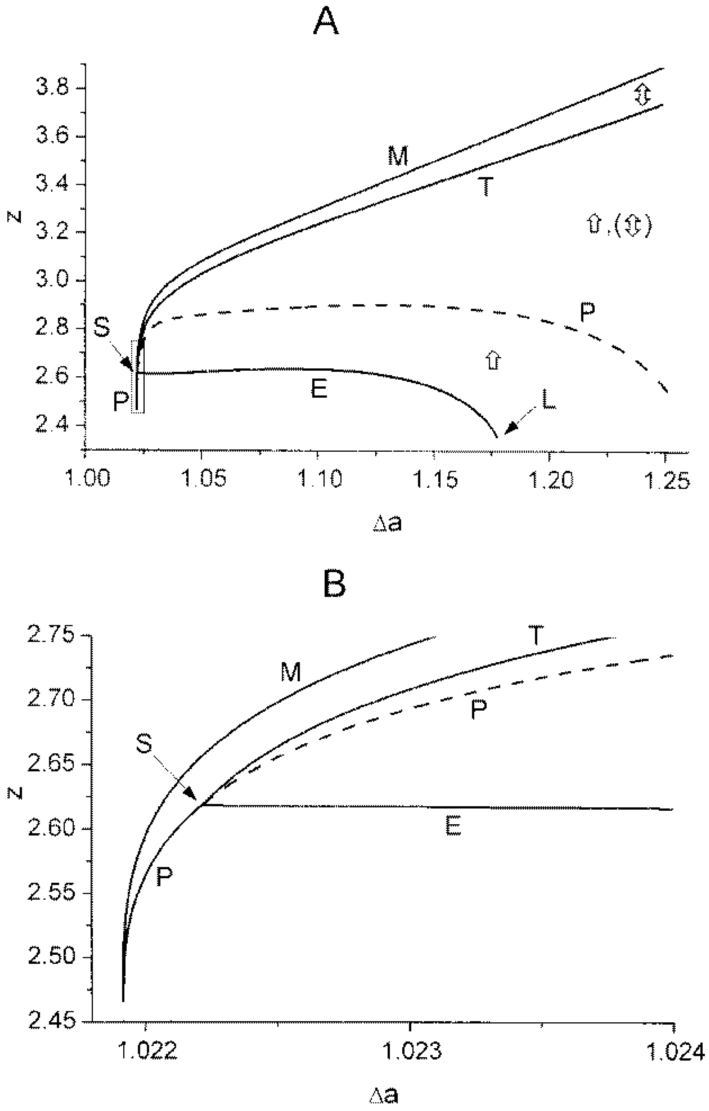


The longer is the tether, the larger is the relative volume of the bulbous part of the vesicle

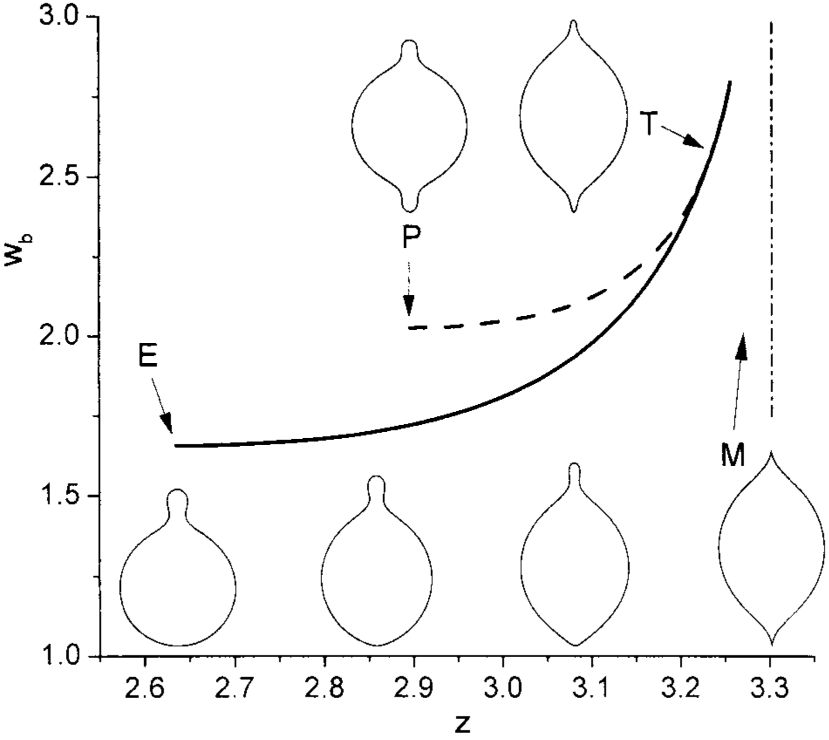




About the symmetry of axially strained vesicles (Heinrich et al. Biophys J 1999)

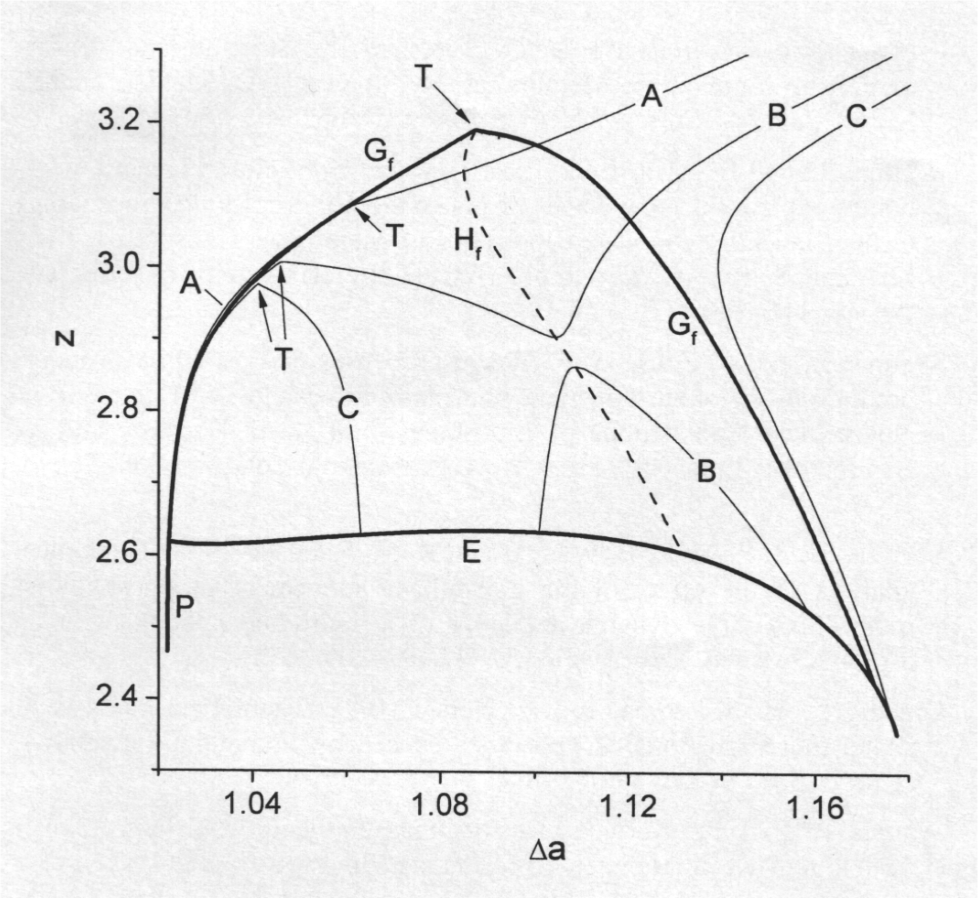


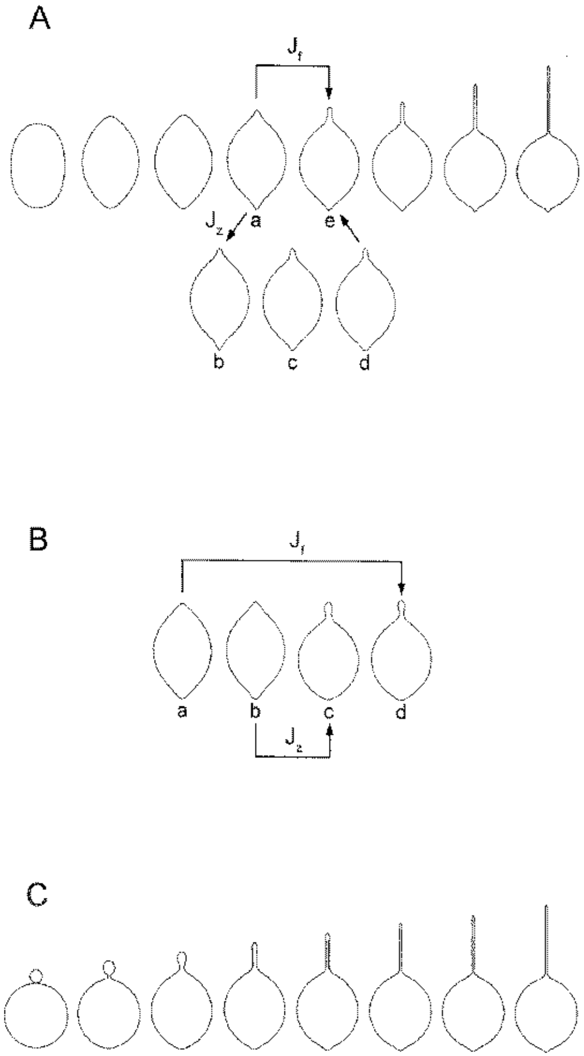
v = 0.95

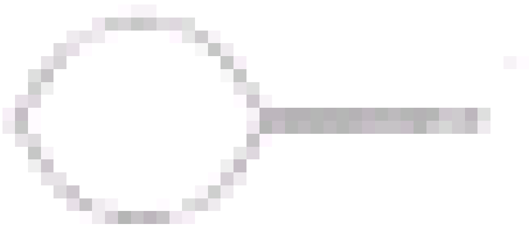


Δa = 1.10

Three shape pathways in the z - Δa phase diagram ($k_r/k_c=4$): the final shape is asymmetrical and does not depend on the initial shape





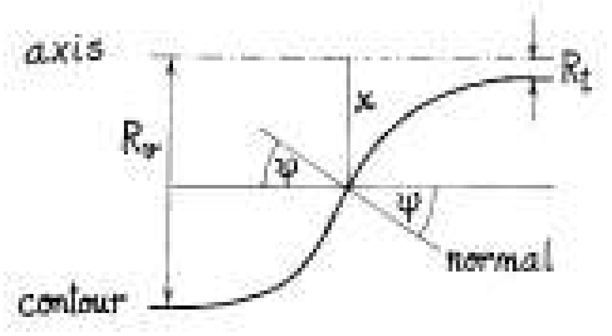


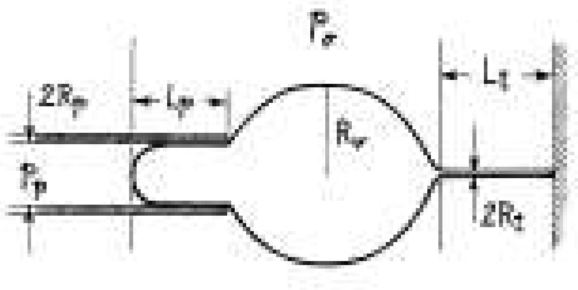
Shape consists of the main body and the tether

Main body is an unduloid, tether is a cylinder

Unduloid can be satisfactorily expressed as

$$\sin \psi = \frac{R_t}{x} + \frac{x}{R_v}$$





$$\Delta p = p_0 - p_p$$

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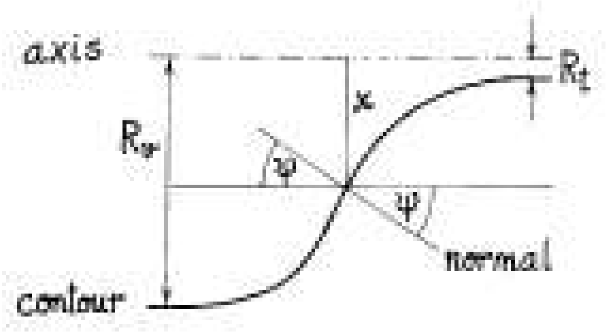
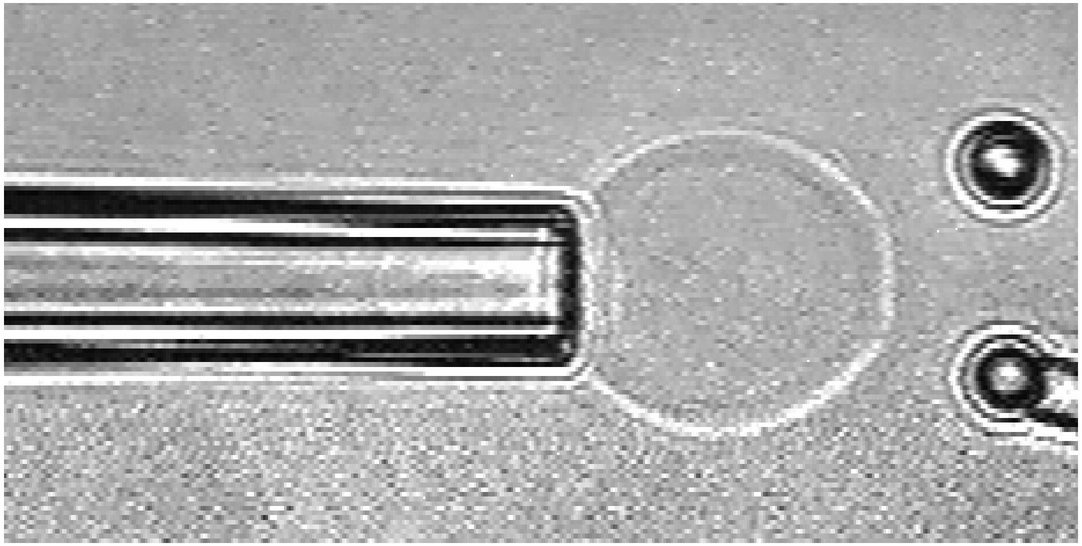


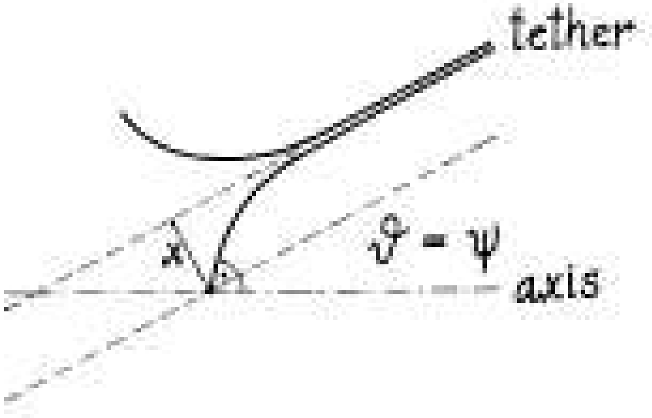


The minimum value of the angle ψ is at

$$x_c = \sqrt{R_t R_v} \qquad \sin\psi_c = 2\sqrt{\frac{R_t}{R_v}}$$

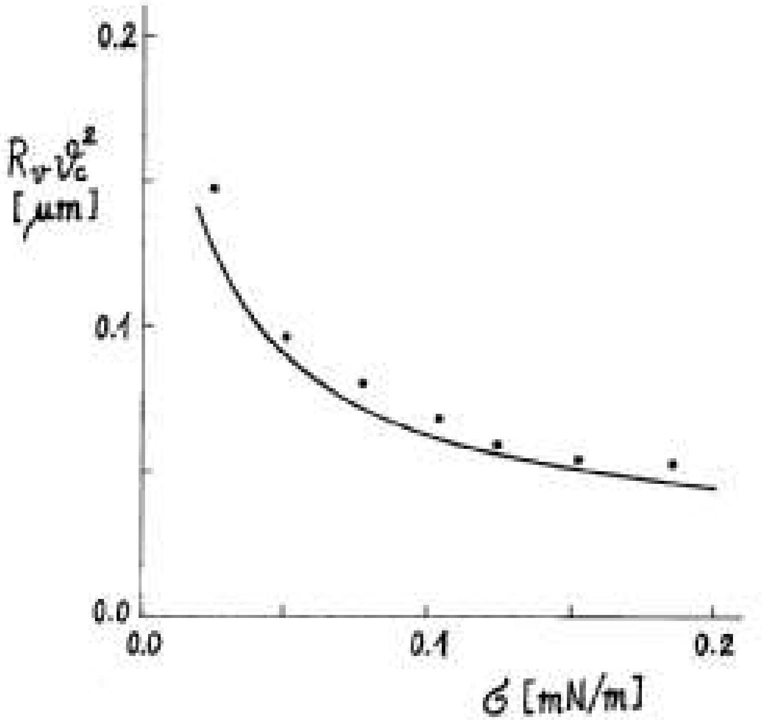
If tether radius R_t is expressed in terms of membrane tension σ we get

$$\sin\psi_c = \frac{2}{\sqrt{R_v}}\left(\frac{k_c}{2\sigma}\right)^{\frac{1}{4}}$$



Points are from Cuvelier, Derenyi, Bassereau and Nassoy, Biophys J, April 2005

The line is from the theoretically obtained expression



Conclusion

The phenomenon of tether coalescence can be consistently explained on the basis of the theory which was previously developed to explain tether formation

In the tether work participated

Ljubljana: Bojan Božič, Boštjan Žekš, Volkmar Heinrich (now in Boston)

Rochester, N.Y.: Rick Waugh, J. Song, Rob Raphael