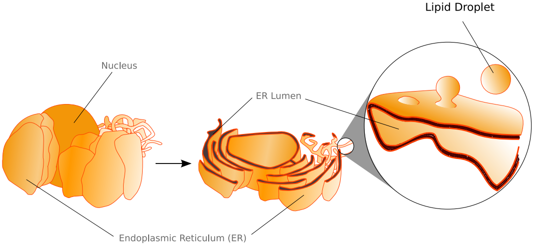
A Biophysical Model for the Biogenesis of Lipid Droplets



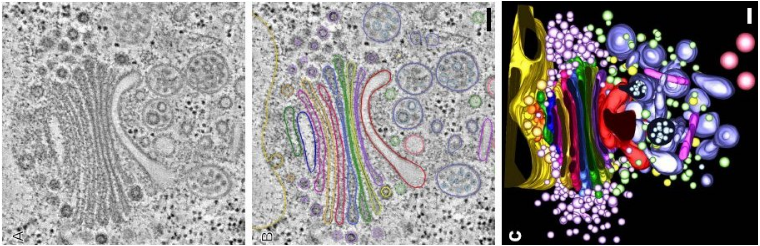
Florian Wodlei

Content

The Cell and its Organelles

The Cell

The cell is a highly complex system. A typical cell diameter is 1000 nm; a typical cell mass is 1 nanogram.



Endoplasmic Reticula (yellow). scale bars = 100nm

Endoplasmatic Reticulum

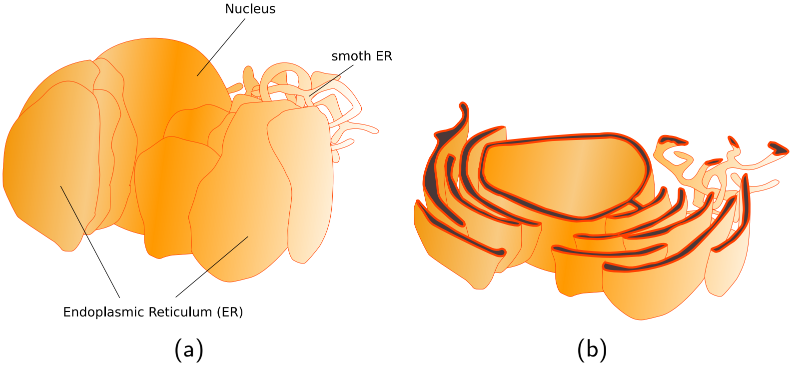
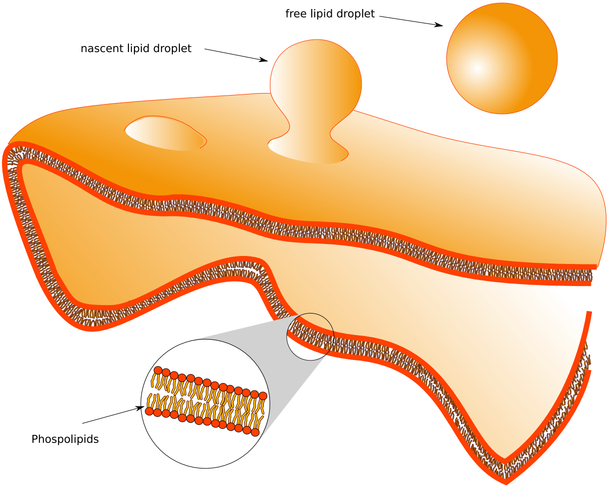
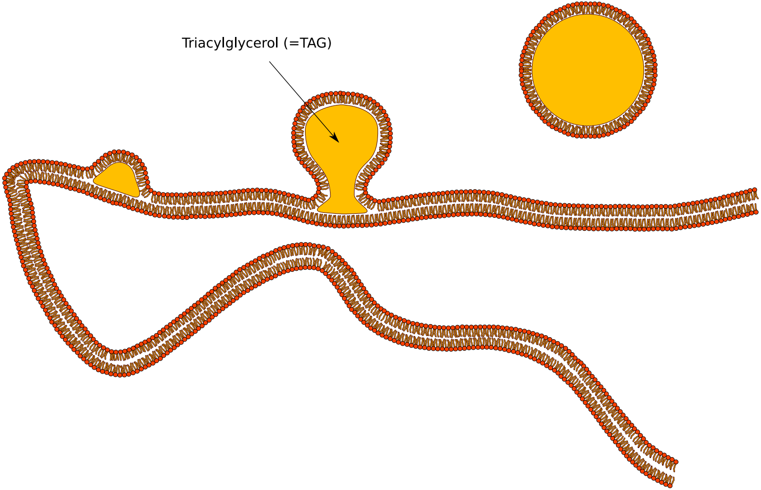


Figure: A schematic 3-dimensional sketch of the endoplasmic reticulum (ER)

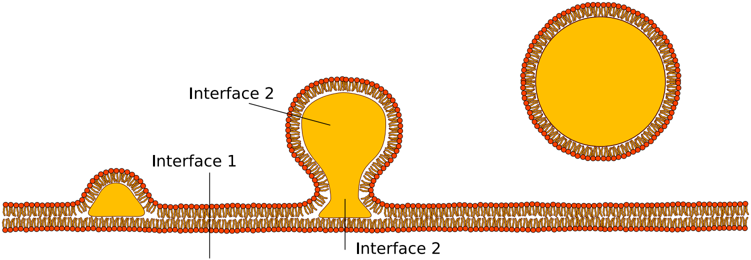
Lipid Droplet



Lipid Droplet

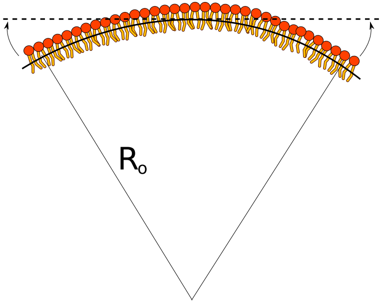


Definition of System Parameters - Interfacial Tension



Corresponding to the existing interfaces there are *interfacial tensions* (γ_1 and γ_2)

Definition of System Parameters - Curvature



Corresponding to the intrinsic curvature of the biological membrane there is the *spontaneous curvature* H_o $(= 1/R_o)$.

The Theory of Canham-Helfrich

The Canham Helfrich Theory is an elastomechanical approach towards biological membranes.

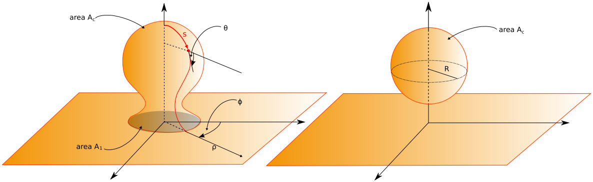
$$G = \underbrace{k_c \int dA (H - H_o)^2}_{Curvature\ \ Energy} + \underbrace{\gamma \int dA}_{Surface\ \ Energy}$$

where $H := \frac{1}{1}\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$ is the **Mean Curvature**

and H_o is the **Spontanous Curvature**

The Lipid Droplet in Terms of Cahnham-Helfrich Theory

We need now to bring this picture in physical terms.



$$G_1 = \int_{A_c}[\gamma_2 + (H - H_o)^2]dA + \int_{A_1}[\gamma_1 + (H - H_o)^2]dA + \\ + \int_{A_{ER}}[\gamma_1 + (H - H_o)^2]dA$$

$$G_0 = 4\pi R^2\left[\gamma_2 + \left(\frac{1}{R} - H_o\right)^2\right] + \int_{A_{ER}}[\gamma_1 + (H - H_o)^2]dA$$

The parametrization of the surface

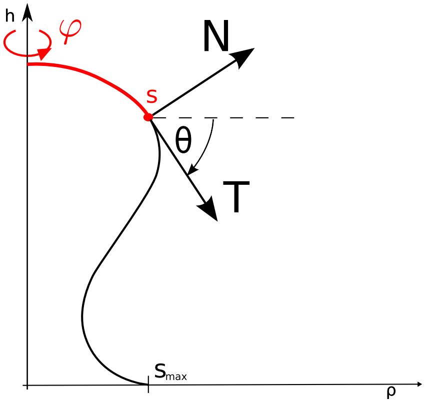


Figure: The parametrization of the surface with the coordinates (s, φ)

The parametrization of the surface

A point on the surface of the droplet is given by the vector

$$\boldsymbol{r} = \begin{pmatrix} \rho\cos\varphi \\ \rho\sin\varphi \\ h \end{pmatrix}$$

The normal vector of the surface can be calculated from $\boldsymbol{r}$ by

$$\boldsymbol{N} = \frac{\frac{\partial \mathbf{r}}{\partial \varphi} \times \frac{\partial \mathbf{r}}{\partial s}}{\left|\frac{\partial \mathbf{r}}{\partial \varphi} \times \frac{\partial \mathbf{r}}{\partial s}\right|}$$

An element of surface is given by

$$dA = \sqrt{g}\ dudv \qquad \text{where} \qquad g = det\boldsymbol{g}$$

The parametrization of the surface

We need to calculate the mean curvature H which can be calculated with the help of differential geometry

$$H = \frac{1}{2} \sum_{ij} b_{ij} g^{ij}$$

Where $\boldsymbol{b}$ is the curvature tensor given by

$$\boldsymbol{b} = \begin{pmatrix} \frac{\partial \mathbf{N}}{\partial \varphi} \cdot \frac{\partial \mathbf{r}}{\partial \varphi} & \frac{\partial \mathbf{N}}{\partial \varphi} \cdot \frac{\partial \mathbf{r}}{\partial s} \\ \frac{\partial \mathbf{N}}{\partial s} \cdot \frac{\partial \mathbf{r}}{\partial \varphi} & \frac{\partial \mathbf{N}}{\partial s} \cdot \frac{\partial \mathbf{r}}{\partial s} \end{pmatrix}$$

and $\boldsymbol{g}$ is the metric tensor given by

$$\boldsymbol{g} = \begin{pmatrix} \frac{\partial \mathbf{r}}{\partial \varphi} \cdot \frac{\partial \mathbf{r}}{\partial \varphi} & \frac{\partial \mathbf{r}}{\partial \varphi} \cdot \frac{\partial \mathbf{r}}{\partial s} \\ \frac{\partial \mathbf{r}}{\partial s} \cdot \frac{\partial \mathbf{r}}{\partial \varphi} & \frac{\partial \mathbf{r}}{\partial s} \cdot \frac{\partial \mathbf{r}}{\partial s} \end{pmatrix}$$

The parametrization of the surface

After calculation we find that

$$\boldsymbol{g} = \begin{pmatrix} \rho^2 & 0 \\ 0 & 1 \end{pmatrix} \qquad \text{and} \qquad \boldsymbol{b} = \begin{pmatrix} \rho \partial_s h & 0 \\ 0 & \partial_s^2 h \partial_s \rho - \partial_s h \partial_s^2 \rho \end{pmatrix}$$

An element of surface is then given by

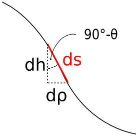
$$dA = \rho \; ds d\varphi$$

And the mean curvature H yields

$$H = \frac{1}{2}\left(\frac{\partial_s h}{\rho} + \partial_s^2 h \partial_s \rho - \partial_s h \partial_s^2 \rho \right)$$

The parametrization of the surface

Since we want to work in terms of the angle θ we are going to express H in terms of θ. From next figure one can see the relation between those quantities.



The mean curvature in terms of θ yields

$$H = \frac{1}{2}\left(\frac{\sin\theta}{\rho} + \frac{\partial\theta}{\partial s}\right)$$

We can also see that

$$\rho(s) = \int_0^s \cos\theta ds \qquad \text{and} \qquad h(s) = h_o - \int_0^s \sin\theta ds$$

The parametrization of the surface

After parametrization the free Energy yields

$$
\begin{aligned}
G_1[\theta(s); R, H_o, \gamma_1, \gamma_2] &= 2\pi \int \left[\gamma_2 + \left(\frac{\sin\theta}{2\rho} + \frac{1}{2}\frac{\partial\theta}{\partial s} - H_o\right)^2\right]\rho \, ds + \\
&+ (\gamma_2 - \gamma_1 + H_o^2)\pi\rho_{max}^2
\end{aligned}
$$

$$
G_o(R, H_o, \gamma_1, \gamma_2) = 4\pi R^2 \left[\gamma_2 + \left(\frac{1}{R} - H_o\right)^2\right]
$$

Energetical Consideration - the difference in free Energy

When is the system making the transition from the Ω state to the free state, for a given volume?

$\Rightarrow$ When the energy of the attached droplet is higher then the energy of the detached droplet!

$$\Delta G = G_1 - G_o > 0$$

Difference in free Energy

$$\begin{aligned}\Delta G[\theta(s), s; R, H_o, \gamma_1, \gamma_2] &= 2\pi \int \left[\gamma_2 + \left(\frac{\sin\theta}{2\rho} + \frac{1}{2}\frac{\partial\theta}{\partial s} - H_o\right)^2\right]\rho \; ds \\ &+ (\gamma_2 - \gamma_1 + H_o^2)\pi\rho_{max}^2 + \\ &- 4\pi R^2\left[\gamma_2 + \left(\frac{1}{R} - H_o\right)^2\right]\end{aligned}$$

Minimizing the free Energy

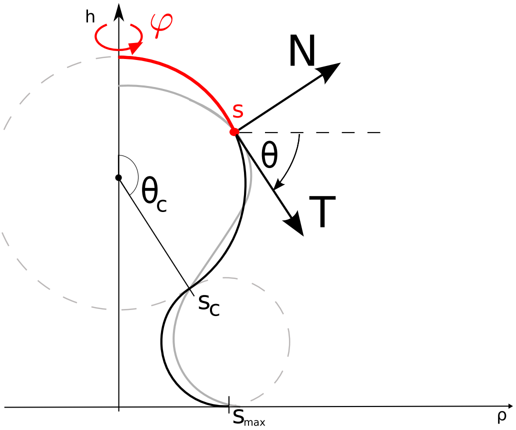
Our purpose is to find the *surface which corresponds to the minimal energy* for a given volume. That means we need to minimize the difference in free energy. That is usualy done by diffenetiation, since we have here a functional, we need to take the functional derivative

$$\Delta G[\theta(s), \theta'(s), s] ... min \qquad \Leftrightarrow \qquad \left.\frac{\delta \Delta G[\theta(s), \theta'(s), s]}{\delta \theta(s)}\right|_{V=const.} = 0$$

The resulting differential equation can only be solved nummerical

The 2-Sphere Approximation

To work still analytically we can approximate the shape of the droplet with two spheres like it is shown in next figure



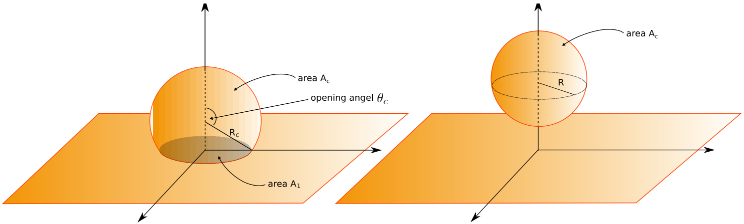
The 2-Sphere Approximation

Then the free energy yields

$$\begin{aligned}
\Delta G &= 2\pi\left\{\left[\gamma_2 + \left(\frac{\theta_c}{s_c} - H_o\right)^2\right]\left(\frac{s_c}{\theta_c}\right)^2 (1-\cos\theta_c) + \right.\\
&+ (\gamma_2 + H_o^2)\left[\frac{s_{max}}{\theta_c}\sin\theta_c(s_{max}-s_c) - \left(\frac{s_{max}-s_c}{\theta_c}\right)^2(1-\cos\theta_c)\right]\\
&- 2H_o\left[\frac{s_{max}-s_c}{\theta_c}(1-\cos\theta_c) - \frac{s_{max}}{2}\sin\theta_c\right] - (1-\cos\theta_c) + \\
&+ \left.\frac{1}{4}\left(\frac{s_{max}}{s_{max}-s_c}\right)^2 \sin^2\theta_c\ \mathcal{F}_\pm(\theta_c, s_c, s_{max})\right\} + \\
&+ (\gamma_2 - \gamma_1 + H_o^2)\pi\left(\frac{s_{max}}{\theta_c}\right)^2 \sin^2\theta_c - 4\pi R^2\left[\gamma_2 + \left(\frac{1}{R} - H_o\right)^2\right]
\end{aligned}$$

The 1-Sphere Approximation

For a first estimation we can think about that the second sphere is not very big, so that we can approximatly say $s_c \approx s_{max}$.



The 1-Sphere Approximation

Then the free Energy yields

$$\begin{aligned}\Delta G &= (\gamma_2 - \gamma_1 + H_o^2)\pi R_c^2 \sin^2\theta_c + \\ &+ 2\pi R_c^2(1-\cos\theta_c)\left[\gamma_2 + \left(\frac{1}{R_c} - H_o\right)^2\right] + \\ &- 4\pi R^2\left[\gamma_2 + \left(\frac{1}{R} - H_o\right)^2\right]\end{aligned}$$

The 1-Sphere Approximation - New variables

We are now going to introduce new variables

$$y := sin^2 \frac{\theta_c}{2} \qquad x := \frac{R}{R_c}$$

$$A_1 = \pi R_c^2 sin^2 \theta_c \overset{sin^2 \frac{\theta_c}{2} := y}{\overset{\downarrow}{=}} 4\pi R_c^2 (y - y^2)$$

$$A_c = 2\pi R_c^2 (1 - cos\theta_c) \overset{sin^2 \frac{\theta_c}{2} := y}{\overset{\downarrow}{=}} 4\pi R_c^2 y$$

The 1-Sphere Approximation

Then the free Energy yields

$$\begin{aligned}\Delta G(x,y) &= 4\pi R^2\Bigg\{\left[\gamma_2+\left(\frac{x}{R}-H_o\right)^2\right]\frac{y}{x^2}+ \\ &+ (\gamma_2-\gamma_1+H_o^2)\frac{y-y^2}{x^2}-\left(\frac{1}{R}-H_o\right)^2\Bigg\}\end{aligned}$$

The Volume Constraint

This is made by

$$V^{(1)} \stackrel{!}{=} V^{(0)}$$

Where the volume of the Ω state is

$$V^{(1)} = \frac{4\pi R_c^3}{3}\left(\frac{1}{2} + \frac{3}{4}cos\theta_c - \frac{1}{4}cos^3\theta_c\right)$$

That means

$$\frac{4\pi R_c^3}{3}\left(\frac{1}{2} + \frac{3}{4}cos\theta_c - \frac{1}{4}cos^3\theta_c\right) \stackrel{!}{=} \frac{4\pi R^3}{3}$$

As a result the constraining condition is

$$x^3 = 3y^2 - 2y^3$$

The Volume Constraint

Solving the equation

$$x^3 = 3y^2 - 2y^3$$

yields an expression for $y(x) =$

$$\frac{1}{2}\left\{1 + \cos\left[\frac{\arccos(2x^3 - 1) + 2\pi}{3}\right] + \sqrt{3}\sin\left[\frac{\arccos(2x^3 - 1) + 2\pi}{3}\right]\right\}$$

The free Energy is now only a function of the variable x

Minimizing the free Energy

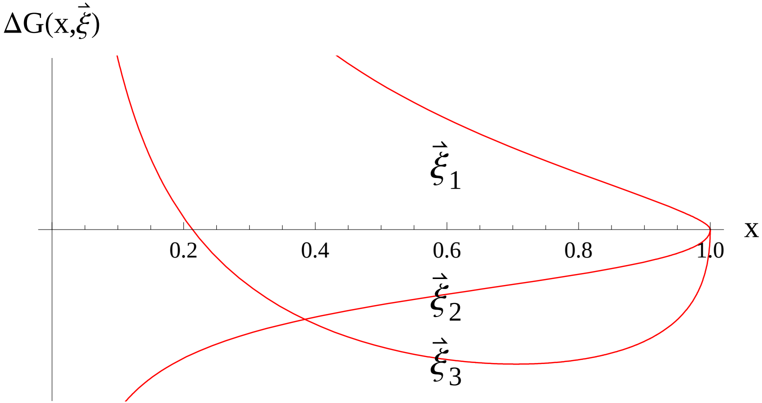
The functional dependency of the free energy ΔG upon the surface transforms in a pure dependency upon x for a given volume (here encountered by R).

$$\Delta G[\theta(s), \theta'(s), s] \qquad \longrightarrow \qquad \Delta G(x)$$

That means also that the functional derivative turns into a usual derivative

$$\left.\frac{\delta \Delta G}{\delta \theta(s)}\right|_{V=const.} = 0 \qquad \longrightarrow \qquad \left.\frac{\mathrm{d}\Delta G}{\mathrm{d}x}\right|_{V=const.} = 0$$

Minimizing the free Energy



Where the parameters $\gamma_1, \gamma_2, H_o, R$ are incorporated in the vector $\boldsymbol{\xi} = (RH_o, \sqrt{\gamma_2}R, \sqrt{\gamma_1}R) = (h_o, g_2, g_1)$

Physical Interpretation of $\Delta G(x; \xi_2)$

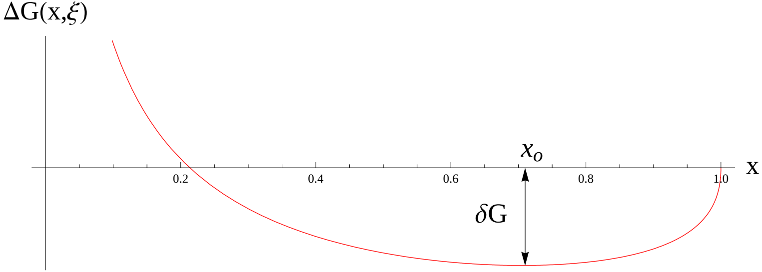


Figure: Definition of x_o, the position of the minima and δG the energy barrier

Conditions for the Case $\xi = \xi_2$

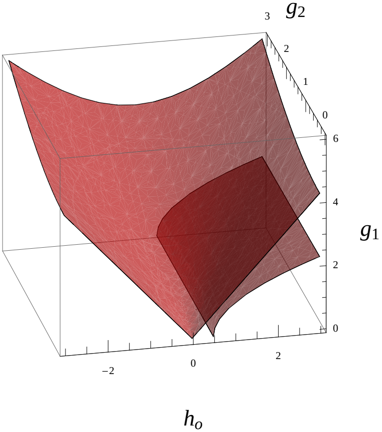
$$\lim_{x \to 0} \Delta G = \infty \qquad \text{and} \qquad \partial_x \Delta G|_{x=1} > 0$$

This results in two conditions

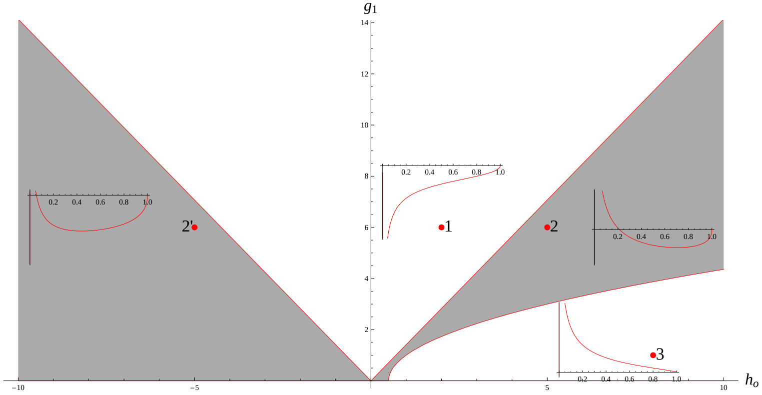
$$\begin{aligned} 1 - 2h_o + g_1^2 &> 0 \\ 2g_2^2 + 2h_o^2 - g_1^2 &> 0 \end{aligned}$$

The Parameter Space

This different behaviours of the function ΔG separates the parameter space spanned by h_o, g_2, g_1 into three regions.

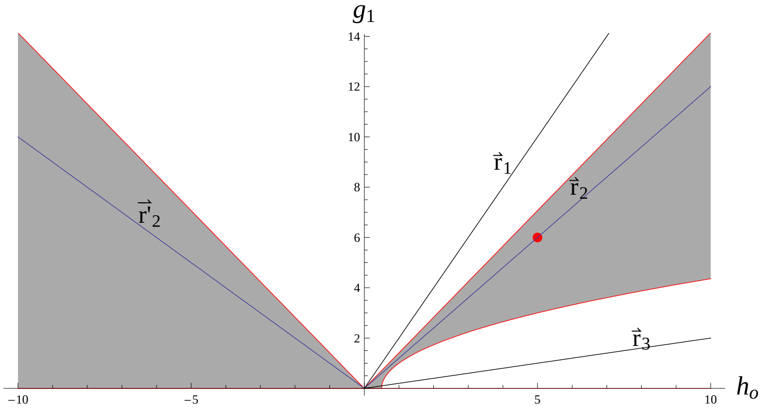


The Parameter Space



The Evolution of the Lipid Droplet

For a given set of parameters the ray ξ has a given direction.



The Evolution of the Lipid Droplet

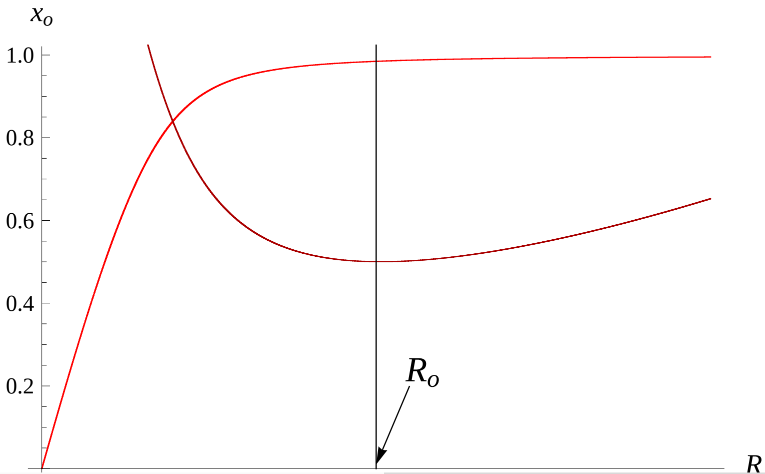
Since

$$\boldsymbol{\xi} = (H_o, \sqrt{\gamma_2}, \sqrt{\gamma_1})R$$

The evolution (increasing radius) is now the movement along such a ray

The Position of the Minima and the Energy Barriere

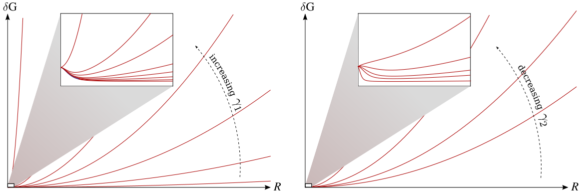
Discussion of the functions $\delta G(R)$ and $x_o(R)$



The Position of the Minima and the Energy Barriere

Discussion of the functions $\delta G(R)$ and $x_o(R)$ - γ Dependency

The Energy Barriere, i.e. the functions $\delta G(R)$ is *also* depending on γ_1, γ_2 and H_o

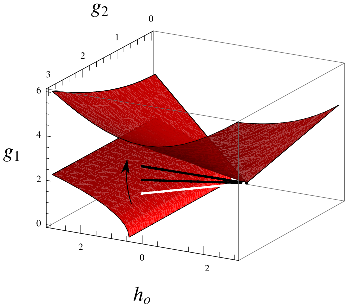


(a) (b)

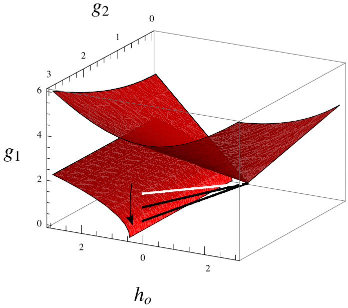
Figure: The graphs of the function $\delta G(R)$

The Position of the Minima and the Energy Barriere

Discussion of the functions $\delta G(R)$ and $x_o(R)$ - γ Dependency



(a)

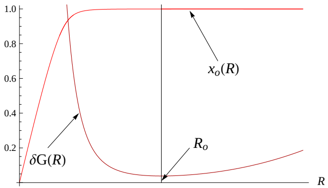


(b)

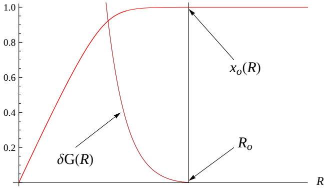
Figure: The behavior of the ray for the increase/decrease of $\gamma_{1,2}$

The Position of the Minima and the Energy Barriere

Discussion of the functions $\delta G(R)$ and $x_o(R)$ - H_o Dependency



(a) The graphs corresponding to negative H_o regime



(b) The graphs corresponding to positive H_o regime

Figure: The graphs of the function $\delta G(R)$ and $x_o(R)$

The Position of the Minima and the Energy Barriere

Conditions for the Case of interest

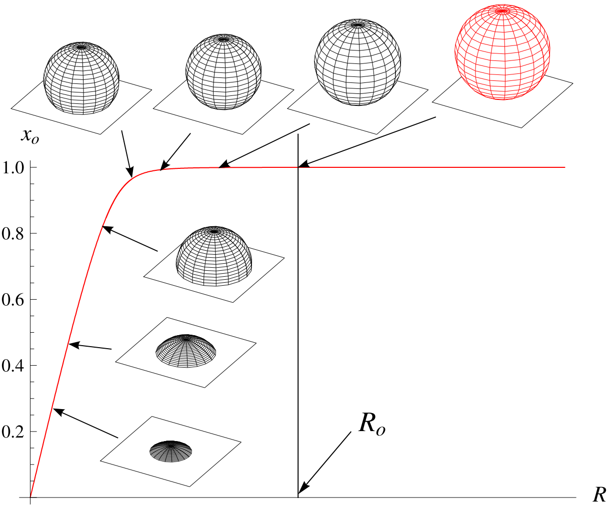
Conditions for the appearance of the minima are for H_o negative

$$H_o < 0, \qquad 2g_2^2 + 2h_o^2 - g_1^2 \gg 0, \qquad \frac{g_1}{g_2} \approx 1$$

for positive H_o the appearance of the minima occurs if

$$1 - 2h_o + g_1^2 > 0$$

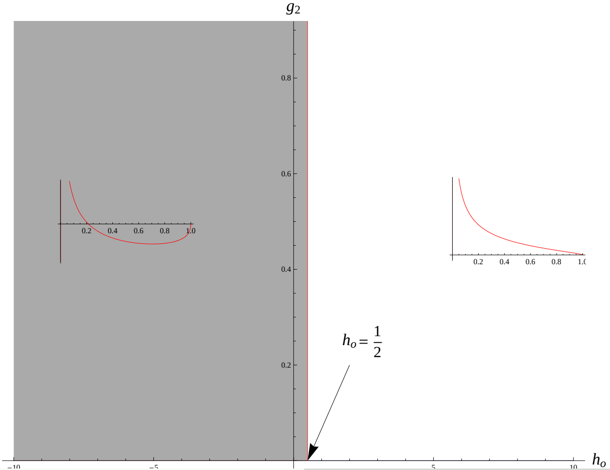
The Morphology of the Droplet Formation



Conditions for Detaching and the Determination of the Detaching Radius

In general it seems impossible to find general conditions for detaching within the whole region (II) with the methods we have used here.

Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma_1 \to 0, H_o > 0$



Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma_1 \to 0, H_o > 0$

The radius R_o for rays like r_3 is determined by the transition from region (2) to region (3) in the phase diagram. This is mathematically the intersection of the ray with the parabel, which yields the following condition

$$R_o = \begin{cases} \textit{indeterminante} & \text{if } \gamma_1 > H_o \\ \frac{H_o^2}{\gamma_1}\left(1 - \sqrt{1 - \frac{\gamma_1}{H_o^2}}\right) & \text{if } \gamma_1 \neq 0, \\ \frac{1}{2H_o} & \text{if } \gamma_1 = 0. \end{cases} \quad (1)$$

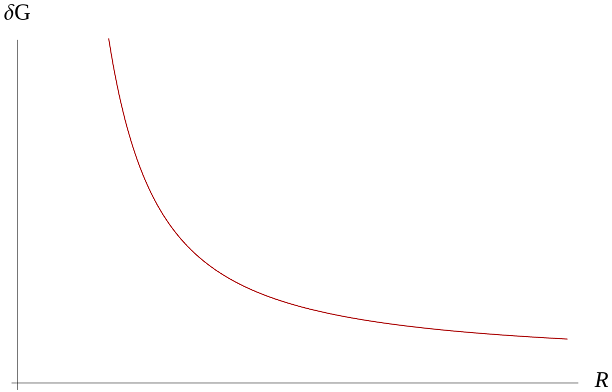
Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma_1 \to 0, H_o < 0$

For this case the situation is much more complicated, because there exist no cut with the parabel, which means mathematically x_0 can never reach 1 even if its very close to it.

Here the Energy Barriere is not zero!

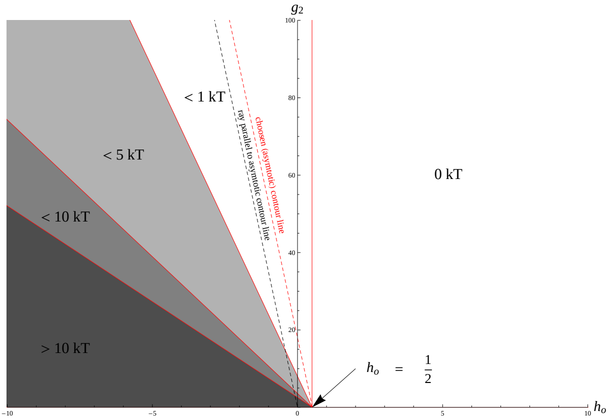
Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma_1 \to 0, H_o < 0$

The functions $\delta G(R)$ has now the following behaviour



Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma \to 0, H_o < 0$

For the whole space



Conditions for Detaching and the Determination of the Detaching Radius - The case $\gamma \to 0, H_o < 0$

This is equivalent with the intersection of the physical ray with the 1 kT contour line. This results in the following condition

$$R_o = \begin{cases} \mathit{indeterminate} & \text{if } \frac{\sqrt{\gamma_2}}{H_o} \leq K \\ \frac{D}{\sqrt{\gamma_2} - KH_o} & \text{if } \frac{\sqrt{\gamma_2}}{H_o} > K \end{cases}$$

where K and D are the slope and the g_2-intercept of the 1 kT line

Conclusion for the Spherical Cap Approximation

There is only a subset of rays which corresponds to an evolution of the attached LD towards a detachment. We found the following qualitative statements for this subset

$$\begin{cases} 2g_2^2 + 2h_o^2 - g_1^2 \gg 0, & \frac{g_1}{g_2} \approx 1 \qquad \text{for } h_o < 0 \\ 2g_2^2 + 2h_o^2 - g_1^2 > 0, & 1 - 2h_o + g_1^2 > 0 \qquad \text{for } h_o > 0 \end{cases}$$

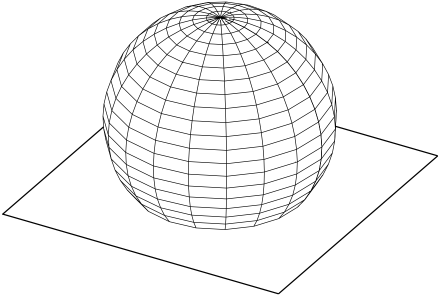
Conclusion for the Spherical Cap Approximation

for the special case $\gamma_1 \to 0$. Here we found the following conditions for the radius of detaching

$$R_o = \begin{cases} \frac{H_o^2}{\gamma_1}\left(1 - \sqrt{1 - \frac{\gamma_1}{H_o^2}}\right) & \text{if } H_o > \gamma_1 \text{ and } H_o > 0, \\ \frac{1}{2H_o} & \text{if } \gamma_1 = 0 \text{ and } H_o > 0, \\ \frac{D}{\sqrt{\gamma_2} - KH_o} & \text{if } \frac{\sqrt{\gamma_2}}{H_o} > K,\ \gamma_1 = 0 \text{ and } H_o < 0, \\ \textit{indeterminate} & \text{else.} \end{cases}$$

The Biological Case - Prediction of our Model

By taking the biologically relevant values into the spherical cap approximation we found that a nascent LD forms until it reaches a radius of 7.34 nm. Then the droplet seems to become stable, remains attached to the ER and very unlikely to detach (because of an energy barrier of 93.03 kT).



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- The main result of this work is that within the spherical cap approximation a detaching of a LD from the ER can be understood in physical terms

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- This work is a first approach towards a physical theory on the biogenesis of LD
- More knowledge about the relevant values of the biophysical parameters and the detailed metabolism of the LD will help to adopt and supports the enhancement of our model of the biogenesis of LD